MEDICAL UNIVERSITY OF GDAŃSK

FACULTY OF PHARMACY

FIRST MUG DOCTORAL SCHOOL

The use of complementary methods for exposure measurement as a part of the health risk assessment due to anti-ectoparasite veterinary drug application on household pets

Wykorzystanie komplementarnych metod szacowania ekspozycji do oceny ryzyka zdrowotnego wynikającego
ze stosowania przeciwpasożytniczych leków weterynaryjnych
u zwierząt domowych

MSc Małgorzata Wacławik

DOCTORAL PROJECT CONDUCTED AT

DEPARTMENT OF TOXICOLOGY

PROJECT SUPERVISOR:

Dr. Habil. Bartosz Wielgomas, Assoc. Prof.

GDAŃSK 2023

I would like to express my deepest gratitude towards the Head of the Department of Toxicology: Dr. Habil. Bartosz Wielgomas, Assoc. Prof., the supervisor of the following doctoral dissertation. Thank you, Professor, for the help, knowledge, patience and support that has been offered by you in great quantities every step of the way.

Małgorzata Wacławik

List of contents

1. **Abstract** 1
2. **Streszczenie** 3
3. **Abbreviations list** 5
4. **Introduction** 6
   - **4.1. Chemical properties and structures of pyrethroids** 7
   - **4.2. ADME of pyrethroids** 10
   - **4.3. Application of synthetic pyrethroids** 10
   - **4.4. Mechanism of action of synthetic pyrethroids** 11
   - **4.5. Human toxicity and health risks** 14
   - **4.6. Human biomonitoring** 15
5. **Aims of the study** 19
   - **5.1. Overview of applied methods and laboratory work** 20
6. **Results of conducted experiments** 23
   - **6.1. Method development and optimization** 23
   - **6.2. Pilot study** 23
   - **6.3. Study with planned exposure to pyrethroids** 24
   - **6.4. Cross-sectional population study** 25
7. **Conclusions** 25
8. **References** 27
9. **Personal information and achievements** 31
10. **List of manuscripts comprising the doctoral dissertation** 32

1. Abstract

Synthetic pyrethroids are substances applied in many occupational and residential settings as active agents contained in insecticidal products aimed for pest control of crops, disease vector control and veterinary treatments against ectoparasites. The widespread use of those chemicals, annually increasing in magnitude regarding amounts of employed products, results in exposure to those compounds becoming an issue of considerable concern, which has been confirmed to regard even the general population. Traditional approach to performing exposure assessment to pyrethroids involves biomonitoring, specifically, quantification of urinary pyrethroid metabolites. However, these metabolites often lack specificity for their respective parent compounds, making it challenging to pinpoint the exact source of exposure. Furthermore, the urinary levels of these metabolites can change rapidly over time due to their rapid metabolism and short biological half-life.

Many of the previous studies have indicated that the use of external parasite control products on domestic animals may be a significant source of exposure in humans, but there is a lack of clear experimental evidence confirming this hypothesis.

This doctoral project presents the successful implementation of an innovative process utilizing silicone wristbands (WBs) as novel, non-invasive, personal passive samplers for assessing non-dietary, time-weighted exposure to parent pyrethroid compounds with the focus on tracing exposure following their application on domestic animals.

After conducting an extensive literature review on the topic, a complex multi-step method for the determination of pyrethroids in wristbands was developed and optimized. The functionality of this method was later confirmed in a pilot study. In this experiment, which involved 24 volunteers, silicone wristbands proved to be a valuable tool in exposure assessment. Permethrin was detected in over 58% of the tested samples, with calculated concentrations reaching a geometric mean (GM) value of 79.64 ng/g. Additionally, the most common pyrethroid metabolite, 3-phenoxybenzoic acid (3-PBA), was quantified in 68% of the tested urine samples (GM: 0.21 ng/mL). The study also revealed a strong correlation (r_s = 0.7041, p < 0.01) between the results of urinalysis and WB analysis.

The fully functional method was applied in a planned exposure study involving a group of pet owners (n = 15). This study entailed the collection of both urine samples and silicone wristbands over a period of 5 weeks, one before and four weeks after the application of a veterinary pyrethroid-containing drug to their pets. The results of the study showed a statistically significant increase in the concentrations of urinary pyrethroid metabolites (p = 0.0429) and quantified applied permethrin in the wristbands (p = 0.003) in samples collected during the week immediately following the application of the drug.

Additionally, the repetitive collection of samples over time allowed for the investigation of exposure patterns, revealing strong consistency among members of the same households. Furthermore, the use of stationary wristbands located indoors during sampling suggested the possibility of chronic exposure to pyrethroids in households where similar products were periodically applied.

It was found that the use of veterinary drugs containing pyrethroids as active substances leads to relatively persistent contamination of residential spaces. The concentrations of metabolites in urine, four weeks after the first application in the season, were significantly higher than background levels in the period preceding the application and did not return to pre-application levels. This suggests that using these products according to the manufacturer's recommended dosage and frequency (every four weeks) may result in continuous, elevated exposure.

This observation can be considered the most significant achievement in this project, as it sheds new light on the contribution of veterinary drug applications and biocidal products in residential spaces to the overall exposure to synthetic pyrethroids in the general population.

Lastly, the developed wristband-based approach combined with urinary biomonitoring was applied in a cross-sectional population study involving a total of 85 inhabitants from Northern Poland. The most frequently detected urinary metabolite was 3-PBA (detection rate: 97.9%, GM: 0.316 ng/mL), with cypermethrin being the most frequently quantified pyrethroid in wristbands (detection rate: 59.3%, GM: 25.3 ng/g). The use of a questionnaire allowed us to identify several predictors of pyrethroid exposure: pet ownership (p = 0.0222) and a history of using veterinary products on owned pets (p = 0.0104).

Moreover, while we observed a moderate positive correlation (r_s = 0.4692, p = 0.0276) between results obtained through urinalysis and the analysis of silicone wristbands worn by participants in the entire tested population, investigating the same relationship among a sub-population of volunteers with a possible occurrence of non-dietary pyrethroid exposure revealed a stronger correlation (r_s = 0.6824, p = 0.0046). This underscores the importance and utility of silicone wristbands in distinguishing between contributions of non-dietary and dietary exposure, which cannot be achieved by biomonitoring alone.

Overall, the completion of this described doctoral project has provided innovative insight into a novel alternative exposure measurement method involving silicone wristbands. All population studies conducted as part of this project, being the first to involve wristbands in Europe, have provided information regarding exposure to synthetic pyrethroids, complementing currently published reports on the topic. Simultaneously, they have filled several knowledge gaps that will undoubtedly contribute to further advancements in the field of exposure science and health risk assessment.

2. Streszczenie

Syntetyczne pyretroidy są substancjami czynnymi w produktach służących do kontroli i zwalczania szkodników w rolnictwie, eliminacji wektorów chorób zakaźnych oraz zwalczania pasożytów zewnętrznych u zwierząt domowych – są składnikami weterynaryjnych produktów leczniczych.

Powszechne stosowanie tych związków, którego rozmiary rosną z roku na rok, powoduje, że obecnie narażenie na syntetyczne pyretroidy staje się problemem dotyczącym ogółu populacji. Tradycyjnie, ocenę narażenia na pyretoidy przeprowadza się za pomocą monitoringu biologicznego, poprzez ilościowe oznaczanie stężenia metabolitów pyretroidów w moczu. Jednak metabolity tych substancji cechuje niska specyficzność wobec związków macierzystych, co utrudnia identyfikację źródła narażenia. Ponadto stężenia metabolitów w moczu charakteryzują się znaczną zmiennością dobową ze względu na szybki metabolizm.

Celem rozprawy doktorskiej była implementacja nowej metody pomiaru ekspozycji z wykorzystaniem opasek silikonowych (WB) jako nieinwazyjnych, pasywnych próbników do oceny narażenia na syntetyczne pyretroidy.

Po przeglądzie literatury, opracowano i zoptymalizowano metodę analityczną do oznaczania pyretroidów w opaskach silikonowych. Funkcjonalność tej metody została później potwierdzona w badaniach pilotażowych. Wyniki tych badań, w których uczestniczyło 24 ochotników, wstępnie potwierdziły, że opaski silikonowe stanowią wartościowe narzędzie do oceny narażenia. Wykryto permetrynę w ponad 58% badanych próbek, a jej średnie geometryczne (GM) stężenie wyniosło 79,64 ng/g. Ponadto, kwas 3-fenoksybenzoesowy (3-PBA), który jest najpowszechniejszym metabolitem, wykryto w 68% próbek moczu (GM: 0,21 ng/ml). Zaobserwowano również silną korelację (r_s = 0,7041, p < 0,01) między stężeniami metabolitów w moczu a stężeniem permetryny w opaskach silikonowych.

W pełni zwalidowaną metodę wykorzystano w kolejnym badaniu, w którym uczestniczyło 15 właścicieli zwierząt domowych (n = 15). Od tych osób zebrano 3 losowe próbki moczu w ciągu tygodnia poprzedzającego aplikację produktu leczniczego weterynaryjnego przeznaczonego do zwalczania pasożytów zewnętrznych. Następnie, po aplikacji preparatu pobierano wielokrotnie próbki moczu przez kolejne 4 tygodnie. Uczestnicy badania nosili także opaski silikonowe tydzień przed i tydzień po aplikacji produktu leczniczego. Wyniki badań wykazały istotny statystycznie wzrost stężeń metabolitów pyretroidów w moczu (p = 0,0429) i permetryny w opaskach (p = 0,003) w próbkach pobranych w ciągu tygodnia bezpośrednio po zastosowaniu produktów zawierających syntetyczne pyretroidy.

Zaobserwowano, że istnieje wysoka zgodność w profilu ekspozycji między członkami tych samych gospodarstw domowych. Wykorzystanie i analiza stacjonarnych opasek umieszczonych w pomieszczeniach mieszkalnych podczas pobierania próbek biologicznych wskazuje na możliwość przewlekłego narażenia na pyretoidy w gospodarstwach domowych, w których regularnie stosuje się podobne produkty.

Stwierdzono, że stosowanie leków weterynaryjnych zawierających pyretroidy jako substancje czynne może prowadzić do długotrwałego występowania tych związków w pomieszczeniach mieszkalnych. Po czterech tygodniach od pierwszego zastosowania w sezonie, stężenia metabolitów w moczu były znacznie wyższe niż przed aplikacją. A zatem, stosowanie tych produktów zgodnie z dawkami i częstotliwością zalecaną przez producenta (co cztery tygodnie) może prowadzić do przewlekłego narażenia.

Ten wniosek jest kluczowym efektem projektu, ponieważ rzuca nowe światło na wpływ stosowania leków weterynaryjnych i produktów biobójczych w pomieszczeniach mieszkalnych na ogólne narażenie na syntetyczne pyretroidy w populacji generalnej.

Opracowana metoda pomiaru narażenia, wykorzystująca opaski silikonowe w połączeniu z monitoringiem biologicznym, została zastosowana w badaniach przekrojowych, w których wzięło udział łącznie 85 mieszkańców północnej Polski. Najczęściej wykrywanym metabolitem w moczu był 3-PBA (wskaźnik wykrywalności: 97,9%, GM: 0,316 ng/ml), a w opaskach: cypermetryna (wskaźnik wykrywalności: 59,3%, GM: 25,3 ng/g). Równolegle przeprowadzone ankiety pozwoliły zidentyfikować predyktory narażenia na pyretroidy, takich jak posiadanie zwierząt (p = 0,0222) i stosowanie w przeszłości produktów weterynaryjnych u zwierząt domowych (p = 0,0104).

Co istotne, wykazano umiarkowaną dodatnią korelację (r_s = 0,4692, p = 0,0276) między stężeniami metabolitów w moczu a zawartością pyretroidów w opaskach silikonowych noszonych przez uczestników w całej badanej populacji. W subpopulacji ochotników, którzy deklarowali potencjalną ekspozycję na pyretroidy (np. stosowanie środków owadobójczych), ta korelacja była jeszcze silniejsza (r_s = 0,6824, p = 0,0046). Wyniki potwierdziły przydatność opasek silikonowych do określenia udziału środowiskowych źródeł ekspozycji w ogólnym narażeniu na pyretroidy.

Realizacja tego projektu doktorskiego umożliwiła wykazanie przydatności opasek silikonowych jako narzędzia komplementarnego względem monitoringu biologicznego, do oceny narażenia na syntetyczne pyretroidy. Badania przeprowadzone w ramach projektu dostarczyły nowych informacji na temat narażenia na syntetyczne pyretroidy w wyniku stosowania leków weterynaryjnych u zwierząt domowych. Wyniki badań pomogą w uzupełnieniu brakującej wiedzy i przyczynią się do dalszego rozwoju badań nad narażeniem i oceną ryzyka zdrowotnego wynikającego ze stosowania syntetycznych pyretroidów.

3. Abbreviations

3-PBA - 3-phenoxybenzoic acid
4F-3PBA - 4-fluoro-3-phenoxybenzoic acid
ADHD – attention deficit hyperactivity disorder
ADME – acronym for: absorption, distribution, metabolism, excretion
cis-DCCA - *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
CS (syndrome) – choreoathetosis (syndrome)
CYP – cytochrome 450
DBCA - *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid
G-EQUAS - The German External Quality Assessment Scheme
GABA – gamma amino butyric acid
GC-ECD – gas chromatography with electron capture detector
GC-MS – gas chromatography – mass spectrometry
GM – geometric mean
HBM – human biomonitoring
ICC – intra-class correlation coefficient
LOD – limit of detection
LogP – partition coefficient
MW – molecular weight
SPE – solid phase extraction
trans-DCCA - *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid
WBs – (silicone) wristbands

4. Introduction

The current view on the etiology of the most common human diseases emphasizes a complex interplay between genetics and environmental factors. While genetic predisposition plays a significant role in many diseases, such as heart disease, diabetes, and cancer, it is increasingly recognized that environmental factors, including lifestyle habits, environmental pollution, and dietary habits, can influence disease risk (Rappaport 2012; Lioy and Rappaport 2011; Wild 2005). Understanding this multifactorial nature of disease causation is crucial for developing personalized and effective prevention and treatment strategies that consider both an individual's genetic makeup and their environmental exposures. The exposome is a comprehensive and evolving concept in the field of environmental health. It encompasses the totality of environmental exposures an individual encounters throughout their lifetime, including not only traditional pollutants but also lifestyle factors and socioeconomic influences. By considering the exposome, researchers aim to better understand the complex interplay between genetics and environmental factors in shaping an individual's health and susceptibility to diseases (Lioy and Rappaport 2011; Wild 2005).

Human biomonitoring plays a crucial role in exposome research by measuring the actual internal exposure of individuals to a wide range of environmental factors. It involves the assessment of various biomarkers and indicators in biological samples, such as blood, urine, and hair, to quantify the presence of environmental chemicals, toxins, and metabolites. This data provides valuable insights into an individual's cumulative exposure and allows researchers to link environmental factors to health outcomes, helping to unravel the complex relationships between environmental exposures and diseases within the exposome framework (Hartung 2023; Huber et al. 2022; Gao et al. 2022).

Pesticides are understood as a both chemically and applicatory broad group of chemicals or mixtures of chemicals possessing a single functional purpose: pest control. The global employment of those substances has increased severely in the years following the inception of their widespread production, with the biggest surge in amounts of pesticides used and manufactured being noted in the most recent years. World pesticide agricultural use in 2020 (the most recent data point available) has been estimated to be around 2,661,124 tons, amount which when compared to data regarding the subject from year 1990 (1,685,495 t), shows a 58% relative increase over only those 30 years (https://ourworldindata.org/pesticides, accessed 7.08.2023, data source: FAO – Food and Agriculture Organization of the United Nations). It should also be noted here, that this data does not include household or personal use of pesticides, which can be speculated to possibly have prominent impact on presented values if assessed.

While compounds such as arsenic and sulfur had been used for agricultural control of pests centuries ago, the process of discovery and development of pesticides has come a long way since, as numerous generations of substance groups have reached their peaks of popularity, descended from them, and in the end got banned or replaced by the next. The history of pesticides goes through the 1800s and the popularity of nicotine sulfate, times of World War II, and development of organochlorines (DDT - dichlorodiphenyltrichloroethane), to

1970s and the inception of synthetic pyrethroids as active components commonly used for widespread pest control (Ravula and Yenugu 2021).

Primary target organisms (pests) of pesticides are insects, plants, bacteria, snails, mites, nematodes, rodents, viruses, fungi and even birds, therefore providing sub-classification of pesticides into respective groups: insecticides, herbicides, bactericides, molluscicides, acaricides, nematicides, rodenticides, virucides, fungicides ad avicides.

Among pesticides, insecticides are considered to be of the highest toxicity. Following chemical subgroups can be distinguished within synthetic insecticides: organochlorines, neonicotinoids and novel butenolides, carbamates, organophosphates, phenylpyrazoles and pyrethroids.

The origin of pyrethroids begins with pyrethrum flowers (*Chrysanthemum cinerariifolium, Chrysanthemum coccineum*) having natively grown in several regions of the Balkans and Caucasus, as their flower heads having undergone pulverization ('insect powder') served as a source of chemicals of insecticidal potency. One of the first products designed with the use of said substances had been mosquito coils. In more recent times the regions where those wild plants are cultivated are: Australia, East Africa and some parts of China (Matsuo 2019). In the first half of 1900s active compounds derived from pyrethrum plants had undergone comprehensive examination, done in great amount by Staudinger and Ruzicka in 1924 (Matsuo 2019). Synthetic pyrethroids known and used in today's agriculture and widely understood pest control are chemical derivatives of its naturally occurring analogs. First ever developed synthetic pyrethroid, having 8 isomers varying in insecticidal activity was allethrin, produced by LaForge in 1949 (Laforge, Schechter, and Green 1956). Since then, the progression of research and development of other synthetic pyrethroids has yielded a group of potent, effective substances of wide range of employment that are readily available for purchase in most developed countries in varied convenient formulations, therefore creating the possibility of both occupational and non-occupational widespread exposure occurring among all age groups worldwide.

4.1. Chemical properties and structures of pyrethroids

Natural pyrethrins chemically are esters containing following moieties: acid (chrysanthemic/pyrethric), cyclopropane carboxylic acid (does not occur in all substances within the chemical group) and alcohol (Katsuda 2011; Zhu et al. 2020; Ravula and Yenugu 2021). The determination of the structure of pyrethrins initialized the process of development of their synthetic analogs – pyrethroids. Discovery of synthetic derivatives of natural pyrethrins have been achieved by experimentally modifying components building the original structures of pyrethrins. Currently about 42 substances, differing in structure, can be included in the chemical group of pyrethroids (Ravula and Yenugu 2021).

First generation of pyrethroids (e.g. tetramethrin, resmethrin) due to being prone to undergo photolysis (resulting in half-lifes as short as just few hours), presented rather low values of surface half-lifes (Spurlock and Lee 2008; Zhu et al. 2020), as a response to which a

second generation of synthetic pyrethroids had been developed, including compounds such as: bifenthrin, cyfluthrin, cypermethrin, deltamethrin, permethrin, which had been much improved in terms of environmental stability (half-lifes up to 49 weeks (Kaneko 2011; Zhu et al. 2020). Most synthetic pyrethroids readily available in various products are mixtures of isomers, with only deltamethrin occurring in a singular structural form (Rao et al. 2021). The human toxicity of pyrethroids, as well as their insecticidal action are very dependent on their stereochemistry. In general, *trans*-isomers are known to be of lower toxicity than *cis*-isomers, though usually both possess insecticidal properties (Ramchandra, Chacko, and Victor 2019).

Pyrethroids can also be differentiated into two types: type I pyrethroids, not containing α-cyano group in their formulas, substances which can cause type I poisoning syndrome, otherwise known as 'T-syndrome', and type II pyrethroids, understood as pyrethroid compounds containing α-cyano group, that are able to cause type II choreoathetosis syndrome ('CS syndrome") (Nasuti et al. 2003). (explained in more detail in section: "Mechanism of action of synthetic pyrethroids")

Synthetic pyrethroids are non-polar compounds of mostly very low vapor pressures (Laskowski 2002). Furthermore, they are known to be of rather good stability in neutral and slightly acidic pH conditions (pH 4-7), but otherwise as esters are prone to undergo the process of hydrolysis. Though the second generation of synthetic pyrethroids has been developed to be much less susceptible to sunlight, still photolysis of those chemicals can occur (Kaneko 2011) both in soils, waters, solutions as well as on surfaces. The lack of solubility of those substances in water, together with high adsorptive abilities of pyrethroids (Kaneko 2011) on one hand reduces their bioavailability to aquatic animals (Palmquist et al. 2011), but on the other, sparked a concern regarding their stability and therefore persistence in ground waters, sediments, soils and other surfaces (Palmquist et al. 2011).

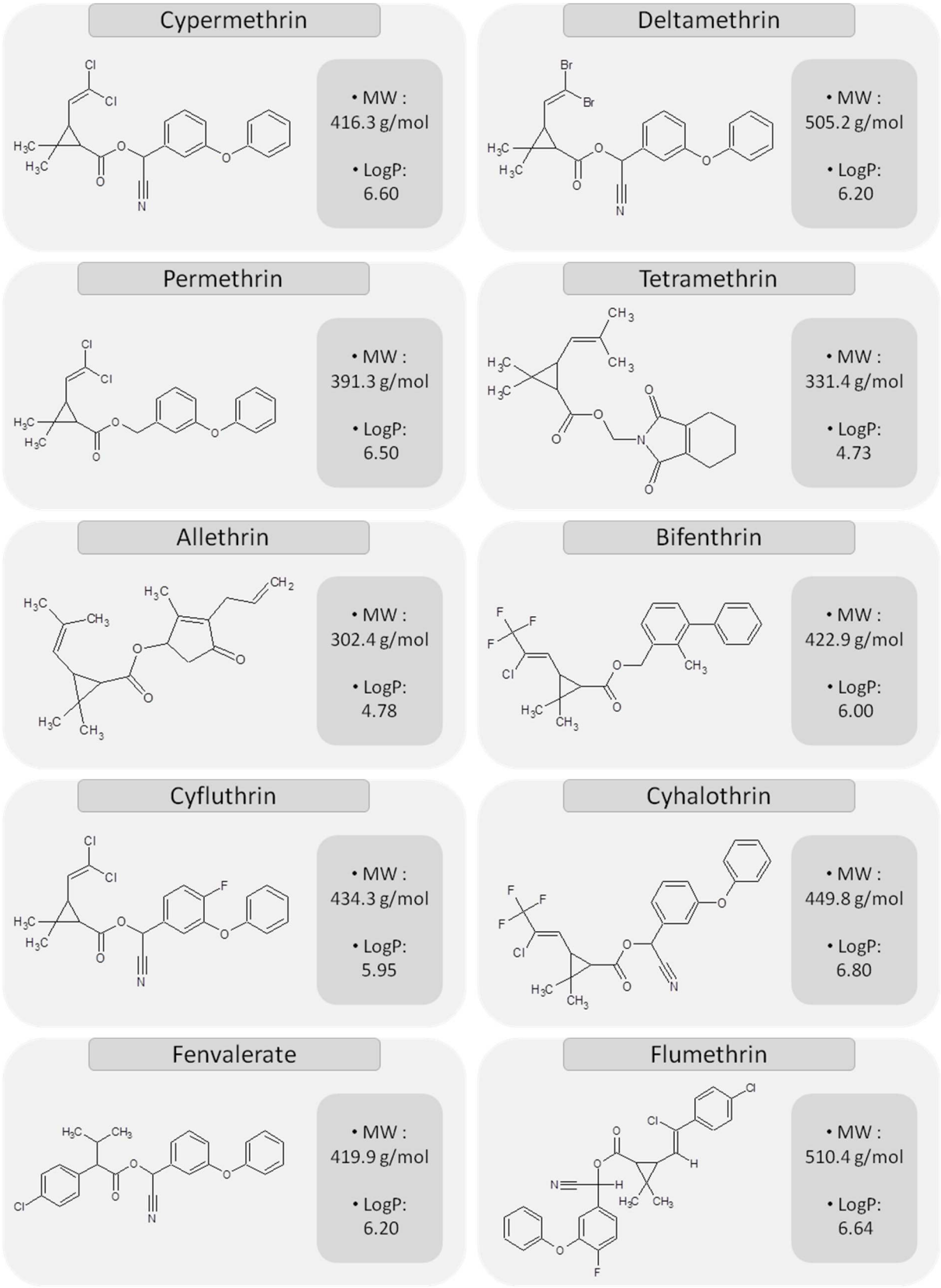


Fig. 1. Overview of structures, molecular weights (MW) and LogP values of selected synthetic pyrethroids.

4.2. ADME of pyrethroids

Synthetic pyrethroids having a broad spectrum of application both indoors and outdoors as active components of commercially available products, and with the frequency of their use increasing in the recent years, co-exposure of non-target organisms to those compounds is inescapable. The high lipophilicity of pyrethroids makes them prone to adsorb to natural surfaces such as soil, plants, water sediments (Kaneko 2011), as well as inside households, which in turn may lead to human exposure to those substances.

It is believed that pyrethroids enter human body most commonly through oral route (Kaneko 2011), either via consumption of foods (Lehmler et al. 2020) and drinks contaminated with residues of agriculturally employed chemicals of interest, or by direct hand-to-mouth reflex (mostly regarding children). Dermal absorption of synthetic pyrethroids is also plausible, however, exposure studies carried out on rats had shown this route of exposure to be of much smaller magnitude than oral pathway, which allows to assume, that the scale of dermal absorption in humans is even smaller in comparison, as human skin present lower permeability than rat skin (Kaneko 2011). Lastly, human exposure to pyrethroids may occur via inhalation (Maroni, Fait, and Colosio 1999). After being absorbed, most pyrethroids do not accumulate in internal organs, and are readily (16-24h) metabolized (Ravula and Yenugu 2021; Kaneko 2011), however some (second generation), the most lipophilic, have the tendency to build up residues in fat tissues (Kaneko 2011).

Synthetic pyrethroids undergo metabolism in two phases: phase I reactions are oxidation and cleavage of the ester bond existing in their structure, which has been investigated to be more extensive in *trans*- isomers, and are known to occur with the use of isoforms of CYP (oxidation) and carboxylesterases (ester bond cleavage), whereas phase II reactions result in formulation of mostly hydrophilic metabolites (conjugates: glucuronides, sulfates), which due to their good water solubility readily undergo excretion with urine (Ravula and Yenugu 2021; Kaneko 2011).

4.3. Application of synthetic pyrethroids

Synthetic pyrethroids have been used in varied forms as both household and agricultural pest controlling agents since their development. Currently they are considered to be the most commonly employed insecticides, as they amount to approximately 30% of global use (Lehmler et al. 2020). Various substances from within this group, or mixtures of substances, are extensively utilized in crop pest control, playing a crucial role in the efficiency of modern farming, and thus significantly contributing to global food supply stability. Perhaps one of most important applications of synthetic pyrethroids is vector control, especially in the efforts to limit/eradicate malaria related morbidity (Guessan et al. 2014). Mosquito nets treated with mixtures of insecticides including pyrethroids are most commonly employed with that aim, as the most effective approach. A medicinal employment of synthetic pyrethroids includes treatment of human lice (Lehmler et al. 2020) and scabies (trade Polish name of the drug product: *Permetryna Scabinol Forte* – for treatment of scabies, *Sora Forte* (shampoo) – lice treatment). Furthermore, pyrethroids are components of many consumer products readily

available in retail, covering assortment of insect repellents commonly utilized used against spiders, ants, wasps, hornets etc. of both indoor and outdoor residential use.

Synthetic pyrethroids play a significant role in veterinary medicine, particularly in the context of domestic animals, such as pets like dogs. Their primary function in this setting is to control and prevent infestations of ectoparasites, which are external parasites that can negatively impact the health and well-being of pets.

This group of active substances is widely used in veterinary medicine to combat a range of ectoparasites, including fleas, ticks, mites, and mosquitoes. These parasites can cause discomfort, transmit diseases, and even lead to more severe health issues for pets. One of the most common forms of synthetic pyrethroid use for domestic animals is through topical applications. These come in the form of spot-on treatments, shampoos, and sprays. Pet owners can easily apply these products to their pets' fur or skin to provide protection against parasites. Synthetic pyrethroids are highly effective in preventing and eliminating flea and tick infestations. They work by paralyzing and killing these parasites upon contact, providing both immediate relief and long-term protection. Veterinary products containing synthetic pyrethroids are user-friendly and convenient for pet owners. Spot-on treatments, for example, are simple to apply and typically provide extended protection for several weeks.

In summary, synthetic pyrethroids are a valuable tool in veterinary medicine for domestic animals, particularly in the control and prevention of ectoparasite infestations. Their ease of use, effectiveness, and relatively low toxicity to pets make them a popular choice among pet owners and veterinarians for maintaining the health and well-being of pets by safeguarding them from external parasites.

After the application of topical veterinary products containing synthetic pyrethroids on pets, there can be a certain risk of human exposure to these chemicals by transferring residue from the treated pet to the hands or clothing of the pet owner when handling the pet. In the event of direct contact with the treated pet's skin or fur, there is a possible risk of skin exposure to the active substances. Treated pets can potentially leave residues on surfaces in the environment, such as furniture, carpets, or bedding. Over time, these residues can become airborne as dust particles, particularly in areas with high foot traffic or disturbed surfaces. This dust can contain synthetic pyrethroid residues that are then inhaled. The persistence of synthetic pyrethroid residues on indoor surfaces can vary depending on the product used, the application method, and the specific formulation. Residues that remain on surfaces for extended periods may continue to pose an inhalatory exposure risk.

4.4. Mechanism of action of synthetic pyrethroids

Pyrethroids are known to have an excitatory effect on axonal cellular membranes by disturbing the physiological functioning of voltage-gated sodium channels, therefore imposing their insecticidal effect on target organisms.

The mode of action of synthetic pyrethroids focuses on inhibiting the closure of aforementioned target sites: sodium channels, therefore prolonging the influx of sodium ions through the axon membrane into the cell. The restraining effect is achieved by affecting the gating particles of the membrane proteins (Nasuti et al. 2003). This causes the increase of action potential, therefore suppressing the ability of cellular membrane to repolarize, causing

it to be perpetually depolarized, and therefore non-conducting. The effect of that is organism paralysis, commonly observed first as a 'knock-down effect' (Casida et al. 1983). The mode of action of synthetic pyrethroids has been pictorially summarized on Fig. 2.

While the general mode of action of both type I and type II of pyrethroids does not vary significantly, the magnitude of produced effect in turn, does. Type II pyrethroids are recognized to have a greater effect on sodium channels, protracting the depolarized state of cellular membranes to a more sizable extent, than type I pyrethroids (Nasuti et al. 2003). The described difference results in disparities in presented symptoms being the results of action of either of the pyrethroid types. Type I pyrethroids produce T-syndrome, usually marked by tremors, ataxia and heightened excitation, whereas striatal epilepsy (choreoathetosis), hypersensitivity and excessive salivatory activation are typically characteristic to CS syndrome, a product of action of type II pyrethroids (Nasuti et al. 2003).

Pyrethroids (mostly of type II) also possess the ability to modify the functionality of chloride channels by reducing their currents, and therefore amounting to symptomatic outcome of salivation and muscular rigidity (Forshaw, Lister, and Ray 2000). Furthermore high concentrations of synthetic pyrethroids are capable of interacting with gamma amino butyric acid (GABA) – gated chloride channels, consequently triggering seizures (Bradberry et al. 2005).

The action of synthetic pyrethroids can be further escalated by addition of synergists, nowadays generally supplementing the commercially available pyrethroid products intended for insecticidal use. Most commonly employed with that aim are either piperonyl butoxide or organophosphates (Singh et al. 2022). The first temporarily and partially inhibits the functionality of CYP mono-oxygenase enzymes responsible for metabolism of pyrethroids, while the latter obstruct pyrethroid hydrolysis (Singh et al. 2022).

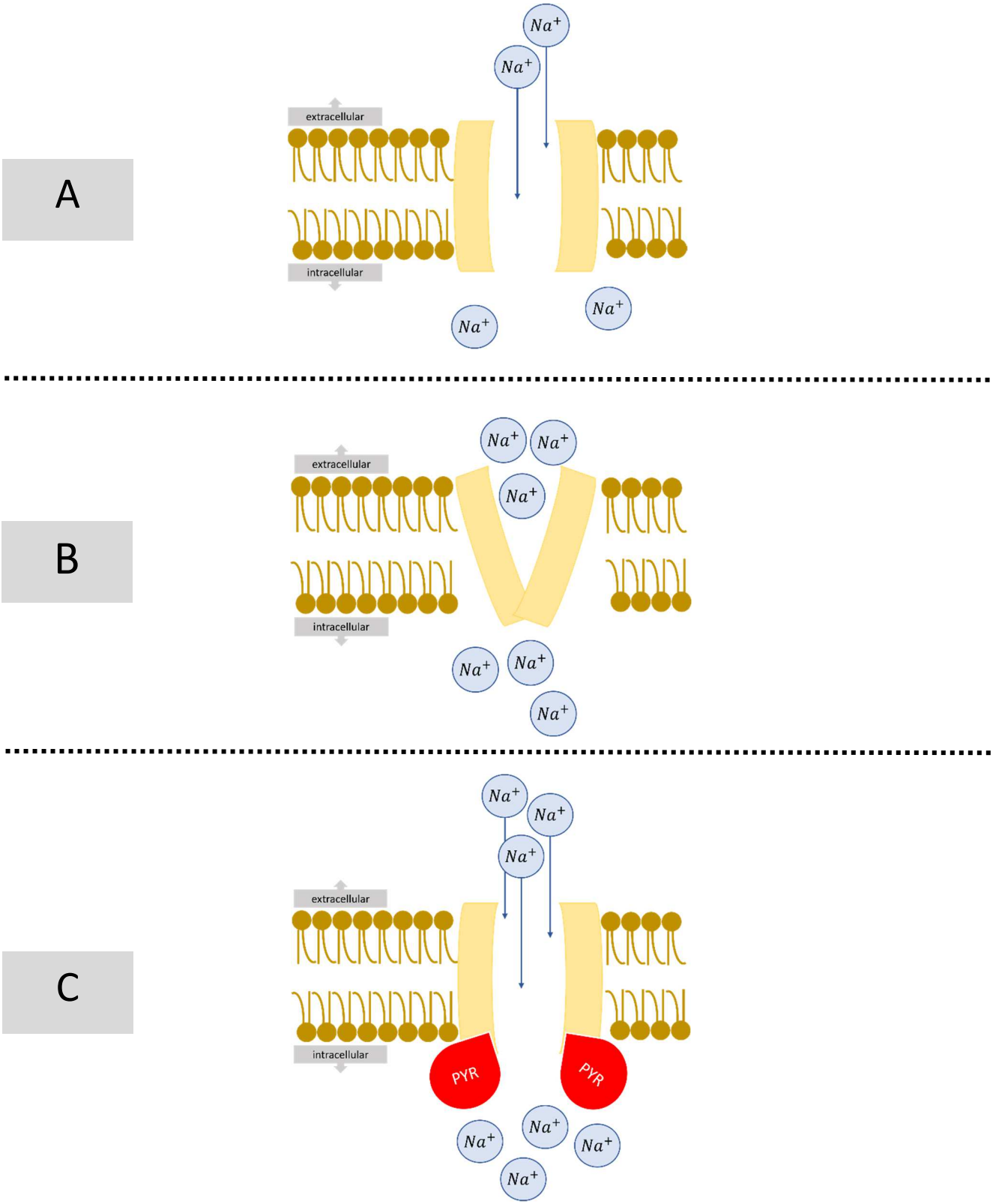


Fig. 2. Mechanism of action of synthetic pyrethroids. (A)(B) Physiologically the influx of sodium ions through axonal membrane is regulated by voltage gated sodium channels. (C) The presence of pyrethroids inhibits the regulatory function of membrane proteins, therefore preventing repolarization.

4.5. Human toxicity and health risks

In the long history of occupational and residential use of synthetic pyrethroids cases of acute poisonings are relatively rare. Most scenarios to have occurred had been the result of improper handling of product application and/or storage, deficient ventilation, or an unforeseen amble of used substance from the application side (Saillenfait, Ndiaye, and Sabaté 2015). Symptoms of acute pyrethroid poisoning are heavily dependent on the route via the exposure had taken place. Usually, short-term symptoms presented during an acute pyrethroid poisoning are: nausea and vomiting, dizziness, headaches, irritation of respiratory pathway, dermal irritation (Saillenfait, Ndiaye, and Sabaté 2015).

An issue of much larger concern, and simultaneously insufficient scientific coverage thus far, is prolonged exposure to low doses of synthetic pyrethroids. Given the frequency of both indoor and outdoor residential and occupational use of products containing pyrethroids and their environmental stability (Wolansky and Harrill 2008), the plausibility of accumulation of those substances in various microenvironments is considerable, therefore creating circumstances in which perpetual exposure to small doses of pyrethroids might occur among non-target organisms for a substantial amount of time.

Synthetic pyrethroids are believed to be endocrine disrupting chemicals (Marettova, Maretta, and Legáth 2017), as their in-vivo activity has been proven to mimic and therefore disrupt the physiological endocrine pathways, thus producing a number of negative health outcomes. One of the negative outcomes comes from pyrethroids possessing the ability to interact with estrogen receptors, which causes interferences and dysregulates the estrogenic balance. Generally, pyrethroid exposure has been connected with decreased fertility among both sexes (Radwan Michał and Jurewicz et al. 2015), with several studies providing proof of correlation existing between exposure to substances of interest and decreased sperm concentration (Ji et al. 2011). Other papers had shown significant positive association between levels of pyrethroid biomarkers quantified in biological matrices and magnitude of sperm DNA fragmentation (Ji et al. 2011), existence of morphologically defected (Jurewicz et al. 2015) or immature sperm cells. Early life exposure to synthetic pyrethroids has also been investigated and linked to causing delayed neurodevelopment among infants and young children (Shelton et al. 2014). History of parent usage of substances of interest during 1^{st} or 2^{nd} trimester had also been linked to low birth weight of their children (Hanke et al. 2003). Urinary levels of some pyrethroid metabolites have also been connected with occurrence of parent-reported behavioral problems among population of tested children (Oulhote and Bouchard 2013). Furthermore, animal studies investigating the influence of pyrethroid exposure on health outcomes have noted a decrease in gene and protein expression historically linked to onset of Parkinson's disease as a consequence of early-life administration of permethrin (Carloni et al. 2012). Other have found link between gestational exposure to deltamethrin and presence of behaviors usually interconnected with ADHD such as impulsivity and memory deficits among tested animals (Richardson et al. 2015), which given the complexity and current poor understanding of the disease onset mechanism among humans is thought-provoking. Some animal studies have also linked exposure to pyrethroids with increased fetal mortality (Ahmad, Khan, and Khan 2012).

Considering the universality of use of synthetic pyrethroids, their consequential environmental prevalence, ever increasing global usage as well as forementioned reports of

potential negative health effects developed as a result of coming into contact with these substances it is important to conduct more widespread exposure assessment studies, as well as ones targeted specifically at groups of individuals of increased proclivity to be exposed to pyrethroids, or especially susceptible to potential health effects. Among occupationally endangered famers, veterinarians or workers of factories manufacturing pyrethroid products should be specified. As for otherwise susceptible sub-populations one could consider pregnant women, children, or the elderly to be at heightened risk.

4.6. Human biomonitoring

Human biomonitoring (HBM) in its principle concerns performing a measurement of chemicals in biological matrices like urine, blood, plasma, hair or saliva, and is a commonly employed approach in assessing exposure to said chemicals (Aylward et al. 2014). Thus performed quantification provides information regarding the absorbed dose of a given chemical, regardless of the route of exposure. In case of synthetic pyrethroids, given their short half-lifes in the organism, the compounds serving most commonly as biomarkers of exposure are their urinary metabolites 3-phenoxybenzoic acid (3-PBA); 4-fluoro-3-phenoxybenzoic acid (4F-3PBA); *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA); *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*- and *trans*-DCCA, respectively). However, taking into account the rapidity of pyrethroid metabolism, and therefore the excretion rate variability of said metabolites, their concentration in a spot urine sample serves as a representation of a relatively brief period of time, only within hours from the occurrence of exposure to native compounds (Calafat et al. 2015; Koch et al. 2014; Wielgomas 2013). That fact should be considered a major drawback of this technique of exposure assessment, as sole quantification of pyrethroid metabolites at a single timepoint is very susceptible to either under or overestimation of actual exposure that had taken place, therefore creating a possibility of misclassifying it (Perrier et al. 2016). It is well documented by several authors that urinary metabolites of synthetic pyrethroids are characterized with diverse intraindividual variability described by a wide range of intra-class correlation coefficient (ICC) values assessed in different studies (Roggeman et al. 2022). Solution to that issue usually taken up by epidemiological studies is implementation of participant-burdensome collection of repeated biological samples at different timepoints. Furthermore, performing exposure assessment with the use of biomonitoring requires to possess the knowledge regarding the metabolic pathway of substances of interest, and the properties of resulting products, which are to be determined in biological matrices. It therefore can be retrodicted that analysis of a single spot sample is rarely reliable in the context of exposure analysis, and the need to perform iterative sampling can make HBM exposure measurements uneconomical, and in many cases infeasible.

Due to structural consanguinity within the group of synthetic pyrethroids, the products of their metabolism often present rather low specificity to respective parent compounds (see Fig. 3). Given that urinary pyrethroid metabolites are used as markers of exposure to parent compounds, and their levels are commonly quantified in many biomonitoring exposure studies, such quantification gives an overview to the total pyrethroid exposure that had taken place but does not offer the opportunity for a clear and specific elucidation of potential sources and routes of exposure. Furthermore, products of environmental degradation processes of

pyrethroids can produce compounds structurally similar or even identical to those being the result of human metabolism, therefore enabling for exposure to those compounds to take place as well. 3-phenoxybenzoic acid (3-PBA) is a product of environmental breakdown (Lehmler et al. 2020) of several pyrethroids, being an example of the phenomenon described earlier. Humans can be exposed to 3-PBA similarly to native compounds: via ingesting foods or indoor dust (Lehmler et al. 2020).

Employment of silicone wristbands as personal passive samplers for measurement of exposure to synthetic pyrethroids is considered a novelty of many promising prospects, as combined with traditionally carried out biomonitoring on urine samples, it supports elucidation of routes of exposure, as well as aids with determination of potential sources of exposure, due to pinpointing the specific native pyrethroid compounds the individual wearing the wristband has been in close proximity to (Wacławik, Rodzaj, and Wielgomas 2022).

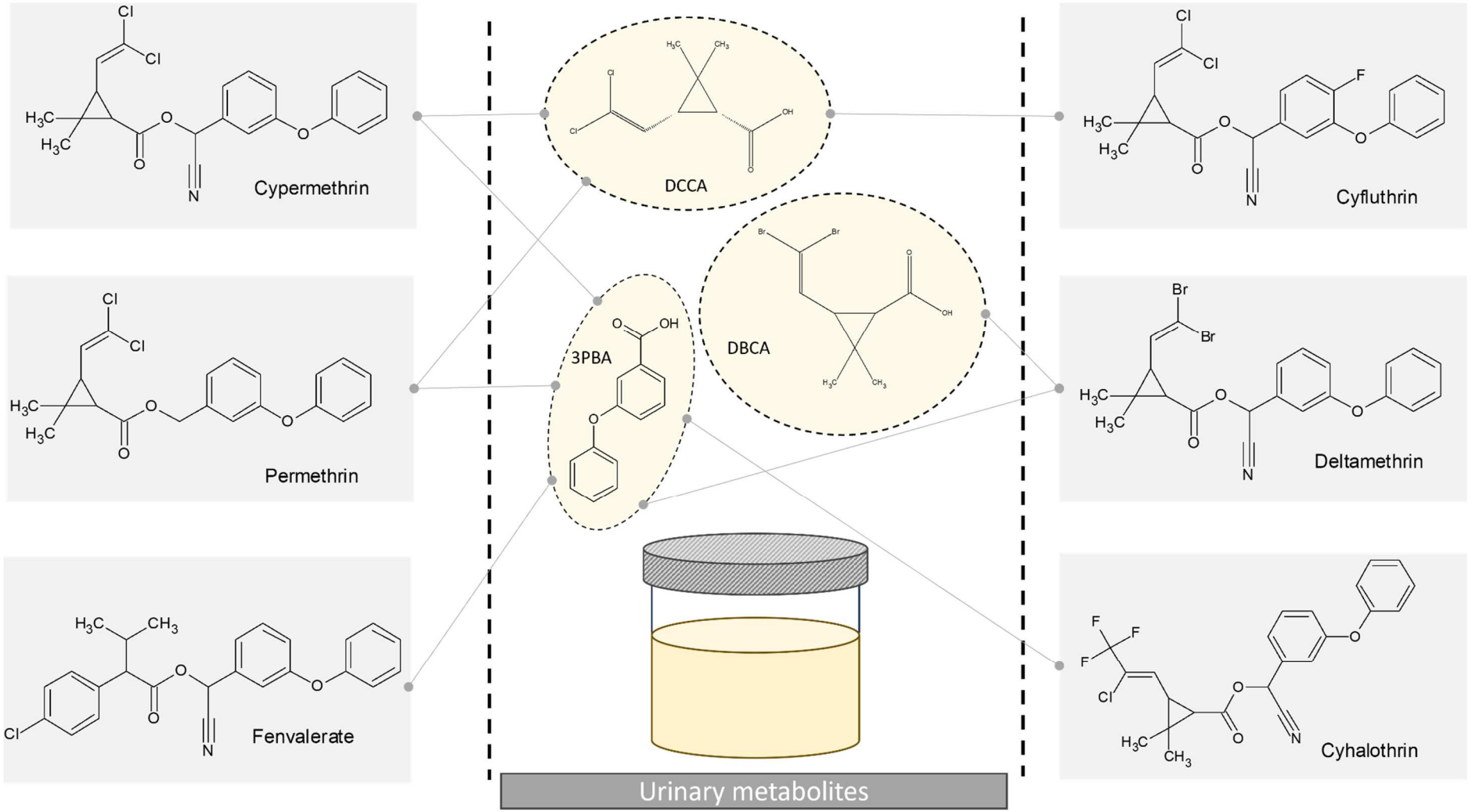


Fig. 3. Examples of native pyrethroids with their respective most commonly assessed urinary biomarkers.

5. Aims of the study

The main aim of the project was to perform exposure measurement as a part of the health risk assessment due to anti-ectoparasite drug application with the use of complementary methods of measurement. Given its complexity, the goal had been divided into a series of the following tasks:

- Conducting a thorough literature **review regarding the subject of silicone wristbands (WBs), their methodological aspects and scopes of usage has been set as the initial task to be completed prior to laboratory experiments** – the results of which had been later published – **Manuscript 1** (Wacławik, Rodzaj, and Wielgomas 2022)**.**

- **Development, optimization and validation of a method for determination of native pyrethroid compounds in silicone wristbands with the use of gas chromatography with an electron capture detector (GC-ECD)**. The functionality of the developed method had been verified by performing a pilot study on a small group of volunteers – **Manuscript 2.**

- The fully operational method has been next applied alongside biomonitoring in a study with planned exposure to insecticides contained in a veterinary anti-ectoparasitic product. The experiment has been conducted on a group of pet-owners, and aimed for **understanding the sequence of concentrations of pyrethroids and their urinary metabolites, as well as for investigation of patterns of exposure formed by analysis of both urine and wristbands and the correlation between their results** – **Manuscript 3.**

- Finally, with the aim of **elucidating potential predictors of exposure to synthetic pyrethroids, as well as to investigate the levels of their exposure**, a cross-sectional population study has been conducted with the combined use of human biomonitoring and WBs – **Manuscript 4.**

5.1 Overview of applied methods and laboratory work

Given the disparate nature of tasks listed above, varied methods were applied to each stage of the project.

- **Stage 1: Literature Review**

Conducted literature review has been focused solely on the topic of silicone wristbands, with them being the novelty of the proposed research project. At the point of the 1st year of conducting said research (2019/2020) only a limited (45) number of scientific papers regarding the topic of silicone wristbands being used in exposure assessment had been published. Search engines such as PubMed, Web of Science and Scopus had been searched for manuscripts of interest using key-phrase: "silicone wristbands". The aim of thus performed literature review was two-fold: firstly, it served as a much needed source of information regarding the applicatory aspects of employment of WBs in exposure assessment studies, that were later gathered and have been used in development of a method for determination of synthetic pyrethroids in WBs (**Manuscript 2**), and secondly, a scientific review focused on methodological and theoretical aspects considering the usefulness and functionality of silicone wristbands in exposure assessment studies had been prepared and published (**Manuscript 1**). Given the newness of said tools in passive sampling, the papers available on their topic, while insightful individually, did not contribute to presenting a comprehensive, mutually complementary set of general information or universal facts regarding the practicality of their employment for scientific purposes. Our review, being the first one published on the subject has provided an exhaustive summary of current scope of usage of WBs regarding substances detected/quantified thus far, presented variety of sample preparation procedures reported by authors, and discussed theoretical facets regarding the mechanisms of the functionality of WBs, and weighted out both the limitations and advantages of using WB-supported sample collection and analysis in exposure science. The manuscript prepared based on performed literature review is titled: "Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches" was published in the International Journal of Environmental Research and Public Health (**Manuscript 1**).

- **Stage 2 – Method Development**

The process of method development has been initiated by the establishment of stages of sample preparation: pre-exposure cleanup of silicone samplers, post-exposure cleanup, extraction and instrumental analysis. We used commercially available silicone wristbands, originally intended for promotional, fundraising, fashion, and style purposes. Therefore, for research purposes, we had to prepare them specially, including cleaning them of any factory contaminants that could interfere with further analysis and interpretation of the results.

The process of pre-exposure cleaning of commercially acquired silicone wristbands was optimized with a focus on its effectiveness, which was measured by the reduction in background noise signals in gas chromatography-mass spectrometry (GC-MS) analysis of extracts. Post-sampling cleanup of the used wristbands was implemented to remove any large-scale contaminants that might have adhered to the surface of the wristband during

the sampling period. Careful attention was given to analyte recovery during the optimization of the post-sampling cleanup step to ensure that it did not result in the loss of the substances of interest.

The process of analyte extraction (using liquid-solid extraction) involved testing various agitation methods, including simple and readily available laboratory equipment, as well as the extraction duration and the number of solvent exchanges required. The primary extract obtained from these preliminary analyses was found to require further cleanup. Different variations of dispersive solid-phase extraction and classic solid-phase extraction (SPE) techniques were tested to achieve maximum analyte recovery while simultaneously reducing background noise signals.

All analyses were performed using GC-ECD, which allowed for the selective detection of synthetic pyrethroids and the achievement of low limits of detection (LODs) for the substances of interest. This was important as the method was developed with the intention of its use in exposure assessment studies, quantifying trace amounts of analytes.

The developed and optimized method underwent validation, yielding satisfactory results that allowed for its use in a population-based study. The written summary of these experiments (**Manuscript 2**) is one of the first studies focusing extensively on the analytical and methodological aspects of silicone wristbands. It provides a comprehensive description of method development and is the only study that covers synthetic pyrethroids in full.

- **Stage 3 – Populational studies**

The first populational study has been conducted right after development of the analytical method for determination of synthetic pyrethroids in silicone wristbands and was treated as a pilot study (**Manuscript 2**) mean to confirm the utility of novel passive samplers for their employment in exposure assessment to those substances. Its conclusion has been closely followed by carrying out of two other populational studies larger in regard to number of involved participants/samples collected. A study with planned exposure (**Manuscript 3**) has been performed on a group (n = 15) of pet-owning volunteers who agreed to use a veterinary anti-ectoparasitic product containing pyrethroids on their pet, and collect both urine samples and wristbands prior to and post its application, therefore providing an opportunity to investigate thus formed patterns of exposure, to assess the correlation between results of traditionally conducted exposure assessment of exposure to synthetic pyrethroids: via biomonitoring with results of wristband analysis. The third populational study (**Manuscript 4**), launched last, carried out on a group of 85 participants, inhabitants of Northern Poland, has offered performing an assessment of exposure to synthetic pyrethroids among a cross-sectional population. Furthermore, by opposing chemical data obtained by analysis of silicone wristband extracts and urine samples with questionnaire-derived information regarding sociodemographic characteristics of participants and their daily habits, has allowed to suggest several possible predictors of exposure to synthetic pyrethroids.

Pilot study and cross sectional populational study

Sample collection, preparation and analysis has been conducted in a similar manner in both primarily launched pilot study (**Manuscript 2**), and cross-sectional

populational study (**Manuscript 4**). Volunteers taking part in these experiments were asked to collect 3 random urine samples over the course of 7 days, and to wear a wristband on the wrist of their dominant hand throughout the same week. Additionally, both studies involved participants filling out a questionnaire including questions regarding their personal characteristics, living conditions as well as daily habits. Collection of all samples in these studies had been followed by preparation and analysis of both urine samples – in order to determine the levels of urinary pyrethroid metabolites (3-phenoxybenzoic acid (3-PBA), *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA), 4-fluoro-3-phenoxybenzoic acid (4F-3PBA), *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*- and *trans*-DCCA), and *cis*-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylic acid (lambda-cyhalothric acid - BIF), and silicone wristbands – done to acquire knowledge about concentrations of native pyrethroids (cyhalothrin, permethrin, cyfluthrin, cypermethrin, deltamethrin, flumethrin) these persons have come into contact with over the 7-day long sampling period. Analysis of pyrethroid metabolites in urine has been done in accordance with established analytical method used at the Department of Toxicology for many years, reliability of which is annually confirmed by taking part in external quality control (G-EQUAS – The German External Quality Assessment Scheme). The method is based on instrumental analysis performed with the use of GC-MS. The biomonitoring of urinary metabolite concentrations in both studies had been reinforced by quantification of levels of native pyrethroid compounds in wristbands worn by study participants, with the use of previously described newly developed GC-ECD method. The collection of 3 repetitions of urine samples on 3 separate days throughout a week was supposed to minimize the effect of over and under estimation of exposure, as by establishing a median/mean value of the three acquired per participant, the result was assumed to correspond better to the actual level of average exposure that occurred within that week. Using WBs to additionally assess the levels of native pyrethroids was assumed to offer an insight into the average, time-weighted level of exposure to those compounds. The cross-sectional populational study (**Manuscript 4**) is (to our knowledge) the first including WBs to have been launched in Poland, and one of the first to additionally concern exposure to pyrethroids in Europe.

Study with planned exposure to pyrethroids

Study with planned exposure **(Manuscript 3)** to pyrethroids involved a group of 15 pet-owning volunteers, who have collected 3 random urine samples and had worn a WB on the wrist on their dominant hand throughout a week preceding the application of veterinary anti-ectoparasitic drug (containing pyrethroids) and proceeded to continue the sampling period further after it. During the first 24 hours after the drug employment the participants collected all urine samples. Next, sampling pattern required the participants to collect one urine sample a day, for the next 6 days. Additionally, single urine samples had also been collected on the 14^{th} and 28^{th} day post-application. A wristband was also to be worn throughout the first week succeeding the product application. The study was designed to provide answers to questions regarding the exposure to synthetic pyrethroids directly following their application indoors, to assess the 'baseline' exposure among pet owners, most of which have been known to apply similar products on their pet regularly and by looking into results acquired via

analysis of both types of collected samples to provide an opportunity to investigate the patterns of exposure forming in relation to the time of product employment. As an additional simultaneously carried out experiment, 'stationary' silicone wristbands were employed in living areas of the households occupied by study participants (both prior to and after the drug application) by being used as passive samplers of that microenvironment. The aim of performing such an experiment had been to assess occurrence/magnitude of prolonged exposure to pyrethroids and to investigate the migration potential of applied non-volatile chemicals in an indoor environment. Analysis of urine samples involved the previously mentioned method and similarly to other studies conducted in the project, the newly developed and optimized method for determination of native pyrethroids in silicone wristbands has been employed. The described study is the first of its kind, involving a planned exposure to the substances of interest and assessment of pyrethroid exposure performed with the use of silicone wristbands.

6. Results of conducted experiments

6.1. Method development and optimization

The developed method allows for determination of cyhalothrin, permethrin, cyfluthrin, cypermethrin, deltamethrin and flumethrin in silicone wristbands. The optimized procedure includes pre-exposure cleanup of silicone samplers performed by a series of 5 solvent washes and (which has been proven to lower the magnitude of background signal by over 90%), post-exposure rinsing of surface-bound large-scale despoilments with deionized water and isopropyl alcohol. A 15-minute-long sonication in ethyl acetate has been proven to be the most optimal way of performing extraction of analytes of interest. Thus obtained primary wristband extract after preliminary instrumental examination has been determined to undergo further purification prior to quantification of analytes of interest due to high background noise present. Solid phase extraction with use of (3% deactivated) silica gel has been chosen for this procedure, due to having produced most reproducible results, as well as maintaining high analyte recoveries. The optimized method had undergone validation in accordance with guidelines, producing satisfactory results. The limits of detection for target substances had ranged from 2 to 10 ng/g (2 ng/g – cyhalothrin, deltamethrin; 10 ng/g – permethrin, cyfluthrin, cypermethrin, flumethrin).

6.2. Pilot study

The pilot study has shown permethrin to be the most frequently (58.3%) detected native pyrethroid compound in worn silicone wristbands, with its geometric mean of concentration being 79.64 ng/g. Urinalysis revealed 3-PBA to surpass the limit of detection (0.05 ng/mL) in 68.06% of tested urine samples, therefore ranking it as the most often detected pyrethroid metabolite. The geometric means of concentrations of pyrethroid biomarkers quantified in urine ranged from 0.08 ng/mL (DBCA) to 0.21 ng/mL (3-PBA). By apposing chemical data regarding concentrations of both pyrethroid metabolites and native pyrethroids quantified in collected samples, and information regarding study participants (provided in a questionnaire), it has been noted that a declaration of performing pest control

in currently occupied living location within 5 years prior to the study is a possible exposure predictor of exposure to those substances. Similarly, higher concentrations of some of urinary metabolites (3-PBA, DBCA, *cis*- and *trans*-DCCA) and WBs permethrin had been observed in samples provided by people who declared using commercially available insecticides in their homes, owned a pet and/or declared having used veterinary drugs on it. However, given the small number of participants involved in this study, the results regarding exposure predictors are to be treated as preliminary, needed to be confirmed by a larger cross-sectional study. Additionally, strong correlation has been found between median of urinary *trans*-DCCA and WBs permethrin concentrations (r_s = 0.7041, p<0.01).

6.3. Study with planned exposure to pyrethroids

The study launched on a group of pet owners involving planned exposure to synthetic pyrethroids by application of veterinary drug products on their pets has provided numerous results in form of urinary concentrations of pyrethroid metabolites and concentrations of native pyrethroids quantified in wristbands collected both prior to and post-drug applications. The most common pyrethroid metabolite: 3-PBA has been detected in almost all (97.1%) analyzed urine samples, while DBCA had been noted to have the lowest detection rate (64.3%) among investigated metabolites. Concentrations of urinary pyrethroid biomarkers quantified in samples prior to drug application ranged from 0.096 ng/mL (GM, DBCA) and 0.729 ng/mL (GM, 3-PBA) among pyrethroid-users, and between 0.054 ng/mL (GM, *cis*-DCCA) and 0.240 ng/mL (GM, 3-PBA) among the control group (pet owners using non-pyrethroid veterinary drug product). After the drug application, the concentrations ranged from 0.090 ng/mL (GM, DBCA) to 1.948 ng/mL (GM, *trans*-DCCA) among tested individuals, and from 0.062 ng/mL (GM, *cis*-DCCA) to 0.242 ng/mL (GM, 3-PBA) in the control group. A statistically significant increase in concentrations of urinary metabolites (p = 0.0429), and wristband permethrin (p = 0.003) in samples collected during the 1st week directly following the drug application had been noted among the tested individuals, while such an observation was not made for the control group involved in the study. The patterns of exposure formed by investigation of medians of sum of pyrethroid metabolites and wristband pyrethroids had been noted to have a considerable set of similarities between members of the same households and have been heavily product and behavior dependent. In cases of some participants (members of household No. 2), who had been known to apply similar veterinary products on their pet every season in repeated doses, considerable concentrations of permethrin (range: 535.5 - 6161.6 ng/g) had been noted on wristbands worn by them prior to study-scheduled-application of insecticidal drug. The analysis of field-sampling wristbands in case of households No. 2 and No. 3 has shown concentrations of 79.03 and 60.14 ng/g, respectively during the first week post-drug application. Additionally, concentrations of urinary pyrethroid metabolites were strongly (r_s = 0.7735, Spearman's correlation, p < 0.05) associated with permethrin quantified in WBs prior to drug application, while such relationship has been qualified as very strong strongly (r_s = 0.9161, Spearman's correlation, p < 0.05) in samples collected post-drug application.

6.4. Cross-sectional population study

The cross-sectional population study has provided results regarding the magnitude of exposure to synthetic pyrethroids, by performing exposure assessment via biomonitoring supplemented by personal passive sampling of silicone wristbands. Again, unsurprisingly, 3-PBA has been the most frequently detected metabolite (detection rate: 97.9%, GM: 0.316 ng/mL), while cypermethrin has been detected most often in wristbands (detection rate: 58.8%, GM: 25.03 ng/g). Questionnaire-derived information regarding the daily habits and socio-demographic status of study participants has been apposed to the results of both urinalysis and analysis of silicone wristbands, and thus two main exposure predictors had been noted: pet ownership (p = 0.0222) and use of anti-ectoparasitic veterinary drugs on pet (p = 0.0104). results of that analysis correspond well with preliminary results of the pilot study **(Manuscript 2)**, however, it should be noted, that samples for the pilot study had been not only much less numerous, but also collected in a short period during European winter (November-December 2020), while sample collection for the cross-sectional population study has taken been much more spread out during the year (March – September 2022), and took place during tick season, which is when it is recommended to prevent/treat pet infections by using veterinary insecticides. Furthermore, a strong correlation (r_s = 0.6824, p = 0.0046) was noted between concentrations of metabolites acquired during urinalysis and results of WB analysis among participants who declared occurrence of non-dietary exposure to these compounds to be plausible. Moreover, such relation was noted to much less strong upon comparison of results among all tested participants (r_s = 0.4692, p = 0.0276).

7. Conclusions

The entirety of the doctoral project described in this dissertation in four manuscripts has amounted to development of a functional, validated method for determination of native pyrethroids in silicone wristbands **(Manuscript No. 2)**, further used to investigate the magnitude and indicate the potential sources of exposure to those compounds in a cross-sectional population study **(Manuscript No. 4)** and explored the subject of prolonged exposure to said chemicals in a first-ever study on pyrethroid involving planned exposure among a group of increased proclivity - pet owners **(Manuscript No. 3**).

The method for determination of pyrethroids in silicone wristbands has been concluded to be suitable for routine use. Given the developed protocol, it is easy to apply, as it only required readily available laboratory equipment. The pilot study demonstrated the applicability of silicone wristbands as personal passive samplers in exposure assessment studies, given that their analysis provides a set of WB-exclusive information regarding the magnitude of exposure to native pyrethroids, impossible to be determined by urinalysis alone **(Manuscript No. 2)**. Simultaneously, a strong level of correlation consistently noted between results of urinalysis and WB analysis across this project serves as proof of complementarity of employed exposure assessment methods **(Manuscripts No. 2, 3 and 4)**.

All described attempts of elucidating possible predictors of exposure to synthetic pyrethroids unanimously point to pet ownership and employment of veterinary anti-ectoparasitic drugs to be significant **(Manuscripts No. 2 and 4)**.

Studied correlations between concentrations of urinary metabolites and concentrations of native pyrethroids determined in silicone wristbands and differences in their strength between study participants potentially externally exposed to substances of interest, and those who did not declare such occurrence **(Manuscript No. 4)** can be considered strong evidence of supplementation of exposure assessment with silicone wristbands being creating an opportunity to identify and distinguish between dietary and non-dietary exposure to synthetic pyrethroids.

The study involving pet owners and planned exposure to substances of interest **(Manuscript No. 3)** had given insight into exposure measured by combination of passive WB sampling and biomonitoring in relation to veterinary drug application.

Detection of substantial amounts of permethrin on wristbands worn by some participants prior to application of the drug raises concern regarding the fate of synthetic pyrethroids in indoor spaces, as well as their durability, and therefore might be understood as a measure of occurrence of chronic exposure to these compounds **(Manuscript No. 3)**. Such phenomenon has been confirmed in our study, as concentrations of urinary pyrethroid metabolites 4 weeks after the drug application were significantly higher than median concentration of the same metabolites measured before the application. Unfortunately, sampling period covering the last week of study lacked employment of WBs, which is considered a study design limitation **(Manuscript No. 3)**.

Furthermore, detection of permethrin (active substance in veterinary drug employed in those households) in some of the field-sampling wristbands shows potential capability of distribution of this non-volatile compound indoors (most likely via suspended particles in the air) **(Manuscript No. 3)**.

Investigation of results of urinalysis and WB analysis in relation to the dose of pyrethroid compound implemented during the study did not show correlation between the values, therefore suggesting dependence of the absorbed dose on behavioral variables **(Manuscript No. 3)**.

Silicone wristbands have proven to be a very effective tool for both qualitative (identify parent compounds) and quantitative assessment of exposure to synthetic pyrethroids and can certainly complement, and in some situations, replace biomonitoring, for example, in detecting significant non-dietary exposure.

8. References

Ahmad, Latif, Ahrar Khan, and Muhammad Zargham Khan. 2012. "Pyrethroid-Induced Reproductive Toxico-Pathology in Non-Target Species." *Pakistan Veterinary Journal* 32 (1): 1–9.

Aylward, Lesa L, Evan Green, Miquel Porta, Leisa-Maree Toms, Elly Den Hond, Christine Schulz, Magda Gasull, et al. 2014. "Population Variation in Biomonitoring Data for Persistent Organic Pollutants (POPs): An Examination of Multiple Population-Based Datasets for Application to Australian Pooled Biomonitoring Data." *Environment International* 68 (July): 127–38. https://doi.org/10.1016/j.envint.2014.03.026.

Bradberry, Sally M, Sarah A Cage, Alex T Proudfoot, and J Allister Vale. 2005. "Poisoning Due to Pyrethroids." *Toxicological Reviews* 24 (2): 93–106. https://doi.org/10.2165/00139709-200524020-00003.

Calafat, Antonia M., Matthew P. Longnecker, Holger M. Koch, Shanna H. Swan, Russ Hauser, Lynn R. Goldman, Bruce P. Lanphear, et al. 2015. "Optimal Exposure Biomarkers for Nonpersistent Chemicals in Environmental Epidemiology Perspectives | Brief Communication Optimal Exposure Biomarkers in Environmental Epidemiology." *Environmental Health Perspectives* 123 (7): 166–68. https://doi.org/10.1289/ehp.7212.Hines.

Carloni, Manuel, Cinzia Nasuti, Donatella Fedeli, Maura Montani, Augusto Amici, M S Dhivya Vadhana, and Rosita Gabbianelli. 2012. "The Impact of Early Life Permethrin Exposure on Development of Neurodegeneration in Adulthood." *Experimental Gerontology* 47 (1): 60–66. https://doi.org/10.1016/j.exger.2011.10.006.

Casida, J E, D W Gammon, A H Glickman, and L J Lawrence. 1983. "Mechanisms of Selective Action of Pyrethroid Insecticides." *Annual Review of Pharmacology and Toxicology* 23 (1): 413–38. https://doi.org/10.1146/annurev.pa.23.040183.002213.

Forshaw, P. J., T. Lister, and D. E. Ray. 2000. "The Role of Voltage-Gated Chloride Channels in Type II Pyrethroid Insecticide Poisoning." *Toxicology and Applied Pharmacology* 163 (1): 1–8. https://doi.org/10.1006/taap.1999.8848.

Gao, Peng, Xiaotao Shen, Xinyue Zhang, Chao Jiang, Sai Zhang, Xin Zhou, Sophia Miryam Schüssler-Fiorenza Rose, and Michael Snyder. 2022. "Precision Environmental Health Monitoring by Longitudinal Exposome and Multi-Omics Profiling." *Genome Research* 32 (6): 1199–1214. https://doi.org/10.1101/gr.276521.121.

Guessan, Raphael N, Corine Ngufor, Andreas A Kudom, Pelagie Boko, Abibathou Odjo, David Malone, and Mark Rowland. 2014. "Mosquito Nets Treated with a Mixture of Chlorfenapyr and Alphacypermethrin Control Pyrethroid Resistant Anopheles Gambiae and Culex Quinquefasciatus Mosquitoes in West Africa" 9 (2): 1–6. https://doi.org/10.1371/journal.pone.0087710.

Hanke, Wojciech, Paul Romitti, Laurence Fuortes, Wojciech Sobala, and Marek Mikulski. 2003. "The Use of Pesticides in a Polish Rural Population and Its Effect on Birth Weight." *International Archives of Occupational and Environmental Health* 76 (8): 614–20. https://doi.org/10.1007/s00420-003-0471-4.

Hartung, Thomas. 2023. "A Call for a Human Exposome Project." *ALTEX* 40 (1): 4–33. https://doi.org/10.14573/altex.2301061.

Huber, Carolin, Rosalie Nijssen, Hans Mol, Jean Philippe Antignac, Martin Krauss, Werner Brack, Kevin Wagner, et al. 2022. "A Large Scale Multi-Laboratory Suspect Screening of Pesticide Metabolites

in Human Biomonitoring: From Tentative Annotations to Verified Occurrences." *Environment International* 168 (July): 107452. https://doi.org/10.1016/j.envint.2022.107452.

Ji, Guixiang, Yankai Xia, Aihua Gu, Xiangguo Shi, Yan Long, Ling Song, Shoulin Wang, and Xinru Wang. 2011. "Effects of Non-Occupational Environmental Exposure to Pyrethroids on Semen Quality and Sperm DNA Integrity in Chinese Men." *Reproductive Toxicology (Elmsford, N.Y.)* 31 (2): 171–76. https://doi.org/10.1016/j.reprotox.2010.10.005.

Jurewicz, Joanna, Michał Radwan, Wojciech Sobala, Kinga Polańska, Paweł Radwan, Lucjusz Jakubowski, Anna Ulańska, and Wojciech Hanke. 2015. "The Relationship between Exposure to Air Pollution and Sperm Disomy." *Environmental and Molecular Mutagenesis* 56 (1): 50–59. https://doi.org/10.1002/em.21883.

Kaneko, Hideo. 2011. "Pyrethroids: Mammalian Metabolism and Toxicity." *Journal of Agricultural and Food Chemistry* 59 (7): 2786–91. https://doi.org/10.1021/jf102567z.

Katsuda, Yoshio. 2011. "Progress and Future of Pyrethroids." In *Topics in Current Chemistry*, 314:1–30. https://doi.org/10.1007/128_2011_252.

Koch, Holger M., Lesa L. Aylward, Sean M. Hays, Roel Smolders, Rebecca K. Moos, John Cocker, Kate Jones, Nicholas Warren, Len Levy, and Ruth Bevan. 2014. "Inter- and Intra-Individual Variation in Urinary Biomarker Concentrations over a 6-Day Sampling Period. Part 2: Personal Care Product Ingredients." *Toxicology Letters* 231 (2): 261–69. https://doi.org/10.1016/j.toxlet.2014.06.023.

Laforge, F. B., M. S. Schechter, and Nathan Green. 1956. "Allethrin. Synthesis of Four Isomers of Cis-Allethrin." *Journal of Organic Chemistry* 21 (4): 455–56. https://doi.org/10.1021/jo01110a021.

Laskowski, Dennis A. 2002. "Physical and Chemical Properties of Pyrethroids." In *Rev Environ Contam Toxieol*, 174:49–170. https://doi.org/10.1007/978-1-4757-4260-2_3.

Lehmler, Hans-joachim, Derek Simonsen, Buyun Liu, and Wei Bao. 2020. "Environmental Exposure to Pyrethroid Pesticides in a Nationally Representative Sample of U.S. Adults and Children: The National Health and Nutrition Examination Survey 2007–2012." *Environmental Pollution* 267 (December): 115489. https://doi.org/10.1016/j.envpol.2020.115489.

Lioy, Paul J., and Stephen M. Rappaport. 2011. "Exposure Science and the Exposome: An Opportunity for Coherence in the Environmental Health Sciences." *Environmental Health Perspectives* 119 (11): 466–67. https://doi.org/10.1289/ehp.1104387.

Marettova, Elena, Milan Maretta, and Jaroslav Legáth. 2017. "Effect of Pyrethroids on Female Genital System. Review." *Animal Reproduction Science* 184 (March): 132–38. https://doi.org/10.1016/j.anireprosci.2017.07.007.

Maroni, Marco, Antonella Fait, and Claudio Colosio. 1999. "Risk Assessment and Management of Occupational Exposure to Pesticides." *Toxicology Letters* 107 (1): 145–53. https://doi.org/https://doi.org/10.1016/S0378-4274(99)00041-7.

Matsuo, Noritada. 2019. "Discovery and Development of Pyrethroid Insecticides." *Proceedings of the Japan Academy, Series B* 95 (7): 378–400. https://doi.org/10.2183/pjab.95.027.

Nasuti, Cinzia, Franco Cantalamessa, Giancarlo Falcioni, and Rosita Gabbianelli. 2003. "Different Effects of Type I and Type II Pyrethroids on Erythrocyte Plasma Membrane Properties and Enzymatic Activity in Rats." *Toxicology* 191 (2–3): 233–44. https://doi.org/10.1016/S0300-483X(03)00207-5.

Oulhote, Youssef, and Maryse F. Bouchard. 2013. "Urinary Metabolites of Organophosphate and

Pyrethroid Pesticides and Behavioral Problems in Canadian Children." *Environmental Health Perspectives* 121 (11–12): 1378–84. https://doi.org/10.1289/ehp.1306667.

Palmquist, Katherine, Anne Fairbrother, Johanna Salatas, and Patrick D. Guiney. 2011. "Environmental Fate of Pyrethroids in Urban and Suburban Stream Sediments and the Appropriateness of Hyalella Azteca Model in Determining Ecological Risk." *Integrated Environmental Assessment and Management* 7 (3): 325–35. https://doi.org/10.1002/ieam.162.

Perrier, Flavie, Lise Giorgis-Allemand, Rémy Slama, and Claire Philippat. 2016. "Within-Subject Pooling of Biological Samples to Reduce Exposure Misclassification in Biomarker-Based Studies." *Epidemiology* 27 (3): 378–88. https://doi.org/110.1097/eDe.0000000000000460.

Radwan Michał and Jurewicz, Joanna, Bartosz Wielgomas, Marta Piskunowicz, Wojciech Sobala, Lucjusz Radwan Pawełand Jakubowski, Wanda Hawuła, and Wojciech Hanke. 2015. "The Association between Environmental Exposure to Pyrethroids and Sperm Aneuploidy." *Chemosphere* 128: 42–48. https://doi.org/10.1016/j.chemosphere.2014.12.077.

Ramchandra, Atul M., Binila Chacko, and Peter J. Victor. 2019. "Pyrethroid Poisoning." *Indian Journal of Critical Care Medicine* 23 (C): S267–71. https://doi.org/10.5005/jp-journals-10071-23304.

Rappaport, Stephen M. 2012. "Biomarkers Intersect with the Exposome." *Biomarkers* 17 (6): 483–89. https://doi.org/10.3109/1354750X.2012.691553.

Ravula, Anandha Rao, and Suresh Yenugu. 2021. "Pyrethroid Based Pesticides – Chemical and Biological Aspects." *Critical Reviews in Toxicology* 51 (2): 117–40. https://doi.org/10.1080/10408444.2021.1879007.

Richardson, Jason R., Michele M. Taylor, Stuart L. Shalat, Thomas S. Guillot, W. Michael Caudle, Muhammad M. Hossain, Tiffany A. Mathews, Sara R. Jones, Deborah A. Cory-Slechta, and Gary W. Miller. 2015. "Developmental Pesticide Exposure Reproduces Features of Attention Deficit Hyperactivity Disorder." *FASEB Journal* 29 (5): 1960–72. https://doi.org/10.1096/fj.14-260901.

Roggeman, Maarten, Celine Gys, Anna Klimowska, Michiel Bastiaensen, Bartosz Wielgomas, Ait Bamai Yu, and Adrian Covaci. 2022. "Reviewing the Variability in Urinary Concentrations of Non-Persistent Organic Chemicals: Evaluation across Classes, Sampling Strategies and Dilution Corrections." *Environmental Research* 215 (P2): 114332. https://doi.org/10.1016/j.envres.2022.114332.

Saillenfait, Anne-Marie, Dieynaba Ndiaye, and Jean-Philippe Sabaté. 2015. "Pyrethroids: Exposure and Health Effects – An Update." *International Journal of Hygiene and Environmental Health* 218 (3): 281–92. https://doi.org/10.1016/j.ijheh.2015.01.002.

Shelton, Janie F., Estella M. Geraghty, Daniel J. Tancredi, Lora D. Delwiche, Rebecca J. Schmidt, Beate Ritz, Robin L. Hansen, and Irva Hertz-Picciotto. 2014. "Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural Pesticides: The Charge Study." *Environmental Health Perspectives* 122 (10): 1103–9. https://doi.org/10.1289/ehp.1307044.

Singh, Ashutosh, Abhishek Singh, Preeti Singh, Archana Chakravarty, Akhilesh Singh, Priti Singh, Mahendra Kumar Mishra, et al. 2022. "Insecticidal Activity, Toxicity, Resistance and Metabolism of Pyrethroids: A Review." *Science and Technology Indonesia* 7 (2): 238–50. https://doi.org/10.26554/sti.2022.7.2.238-250.

Spurlock, Frank, and Marshall Lee. 2008. "Synthetic Pyrethroid Use Patterns, Properties, and Environmental Effects." *ACS Symposium Series* 991: 3–25. https://doi.org/10.1021/bk-2008-0991.ch001.

Wacławik, Małgorzata, Wojciech Rodzaj, and Bartosz Wielgomas. 2022. "Silicone Wristbands in

Exposure Assessment: Analytical Considerations and Comparison with Other Approaches." *International Journal of Environmental Research and Public Health* 19 (4): 1935. https://doi.org/10.3390/ijerph19041935.

Wielgomas, Bartosz. 2013. "Variability of Urinary Excretion of Pyrethroid Metabolites in Seven Persons over Seven Consecutive Days—Implications for Observational Studies." *Toxicology Letters* 221 (1): 15–22. https://doi.org/10.1016/j.toxlet.2013.05.009.

Wild, Christopher Paul. 2005. "Complementing the Genome with an 'Exposome': The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology." *Cancer Epidemiology, Biomarkers & Prevention* 14 (8): 1847–50. https://doi.org/10.1158/1055-9965.EPI-05-0456.

Wolansky, M. J., and J. A. Harrill. 2008. "Neurobehavioral Toxicology of Pyrethroid Insecticides in Adult Animals: A Critical Review." *Neurotoxicology and Teratology* 30 (2): 55–78. https://doi.org/10.1016/j.ntt.2007.10.005.

Zhu, Qiuyan, Yang Yang, Zhiting Lao, Yingying Zhong, Kun Zhang, and Suqing Zhao. 2020. "Photodegradation Kinetics, Mechanism and Aquatic Toxicity of Deltamethrin, Permethrin and Dihaloacetylated Heterocyclic Pyrethroids." *Science of the Total Environment* 749: 142106. https://doi.org/10.1016/j.scitotenv.2020.142106.

Hannah Ritchie, Max Roser and Pablo Rosado (2022) - "Pesticides". Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/pesticides' [Online Resource]

9. Personal information and achievements

Name : Małgorzata Wacławik

Graduated from: Medical University of Gdańsk, Faculty of Pharmacy, Masters programme in Laboratory Medicine (2014 – 2019)

Doctoral student at the Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk (2019 – 2023).

Achievements:

- **International scientific conferences:**
    - International Conference on Chemical and Behavioral Addictions, March 9th -11th 2023, Poznań, oral presentation: "Legal and toxicological aspects of GHB versus GBL use" – **award of the 1st degree for best oral presentation by PhD Student**
    - 12 International Symposium on Biological Monitoring in Occupational and Environmental Health. Next Generation Biomonitoring, ISBM-12, June 21st – 23rd 2023, Porto (Portugal). Poster presentation: "Silicone wristband-assisted biomonitoring of pyrethroid and distribution assessment in a scheduled exposure study among pet owners".
    - International Inderdisciplinary Scientific and Training Conference of Medical University of Gdańsk, 28th – 30th September 2023, Gdynia (Poland). Oral presentation: "Silicone wristbands in exposure assessment – investigation of exposure to synthetic pyrethroids in a cross-sectional population study".
- **National scientific conferences**:
    - XIII Konferencja Szkoleniowo – Naukowa Polskiego Towarzystwa Toksykologicznego (Gdańsk, 16th – 17th September 2021, on-line) – poster presentation: „Metodyczne aspekty zastosowania opasek silikonowych jako pasywnych próbników do szacowania ekspozycji na pyretroidy" – **2nd place (ex-aequo) award for best poster presentation.**
    - National Scientific Conference: „e-Factory of Science", 10th of April 2021, on-line. Oral presentation: "Silicone wristbands: an emerging tool in human exposure assessment studies".
    - I Ogólnopolska Konferencja Naukowa Żywność i żywienie w pigułce, April 9th 2022, (on-line). Poster presentation: „Zastosowanie monitoringu biologicznego w ocenie narażenia na pozostałości wybranych pestycydów w żywności".
    - I Ogólnopolskie Forum Młodych, March 2nd 2023 (on-line). Oral presentaion: „Silicone wristbands as a biomonitoring-complementing tool for assessment of exposure to synthetic pyrehtroids (pilot study and method optimization).
- **Courses and workshops:**
    - Foundation for Advanced Education in the Sciences (FAES graduate school at NIH) – courses: (BIOF309-2) – Introduction to Python (final grade: A-), (BIOF440) – Data Visualization with Python (final grade: A+)
    - 3rd HBM-PT – Workshop on Human Biomonitoring, 18th of November 2020, HBM4EU, on-line.

10. List of manuscripts comprising the doctoral dissertation

Manuscript 1 - Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches”*.Int. J. Environ. Res. Public Health 2022, 19, 1935.

Manuscript 2 - *Małgorzata Wacławik, Dominika Skwarło, Joanna Jurewicz, Bartosz Wielgomas. „Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites – a pilot study preceded by development of cost-effective GC-ECD method”* (working title) – submission to Exposure and Health

Manuscript 3 - Małgorzata Wacławik, Wojciech Rodzaj, Joanna Jurewicz, Bartosz Wielgomas. *„Evaluation of exposure to synthetic pyrethroids among pet owners in a study with panned veterinary product application”* (working title) – submission to Journal of Hazardous Materials

Manuscript 4 - Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Comprehensive assessment of exposure to synthetic pyrethroids among inhabitants of Northern Poland via urinalysis supplemented by passive sampling with the use of silicone wristbands”* (working title) – submission to International Journal of Hygiene and Environmental Health.

Manuscript 1 - Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches”*.Int. J. Environ. Res. Public Health 2022, 19, 1935.

International Journal of
Environmental Research and Public Health

MDPI

Review

Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches

Małgorzata Wacławik †, Wojciech Rodzaj † and Bartosz Wielgomas *

Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, 107 Hallera Street, 80-416 Gdańsk, Poland; malgorzata.waclawik@gumed.edu.pl (M.W.); wojciech.rodzaj@gumed.edu.pl (W.R.)
* Correspondence: bartosz.wielgomas@gumed.edu.pl
† These authors contributed equally to this work.

Abstract: Humans are exposed to numerous potentially harmful chemicals throughout their lifetime. Although many studies have addressed this issue, the data on chronic exposure is still lacking. Hence, there is a growing interest in methods and tools allowing to longitudinally track personal exposure to multiple chemicals via different routes. Since the seminal work, silicone wristbands (WBs) have been increasingly used to facilitate human exposure assessment, as using WBs as a wearable sampler offers new insights into measuring chemical risks involved in many ambient and occupational scenarios. However, the literature lacks a detailed overview regarding methodologies being used; a comprehensive comparison with other approaches of personal exposure assessment is needed as well. Therefore, the aim of this review is fourfold. First, we summarize hitherto conducted research that employed silicone WBs as personal passive samplers. Second, all pre-analytical and analytical steps used to obtain exposure data are discussed. Third, we compare main characteristics of WBs with key features of selected matrices used in exposure assessment, namely urine, blood, hand wipes, active air sampling, and settled dust. Finally, we discuss future needs of research employing silicone WBs. Our work shows a variety of possibilities, advantages, and caveats associated with employment of silicone WBs as personal passive samplers. Although further research is necessary, silicone WBs have already been proven valuable as a tool for longitudinal assessment of personal exposure.

Keywords: biomonitoring; exposome; human exposure; silicone wristband; passive sampling; personal monitoring

check for updates

Citation: Wacławik, M.; Rodzaj, W.; Wielgomas, B. Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches. *Int. J. Environ. Res. Public Health* **2022**, *19*, 1935. https://doi.org/10.3390/ijerph19041935

Academic Editor: Andrew S. Hursthouse

Received: 17 December 2021
Accepted: 5 February 2022
Published: 9 February 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Production, use of, and exposure to chemicals are an inseparable part of technological development [1]. Natural processes, such as forest fires, can also be a source of contaminants [2]. In consequence, humans and wildlife are exposed to a myriad of pollutants that may cause negative health effects [3].

Given the diverse nature of environmental pollution sources, paired with significant knowledge gaps regarding their manner of action when in contact with a human, it is essential to gain details concerning their possible effects on human health. A fundamental step in human health risk assessment is exposure measurement [4]. Therefore, along with the growing number and diversity of synthesized chemicals, the importance of instruments that reliably assess human exposure grows. Only recognized risks can be mitigated through raising awareness and developing informed policies [5]. Although exposure assessment studies appear to be extremely valuable from a scientific point of view, the methods used to quantify exposure vary greatly. Even considering only chemical factors, so far, we do not have universal methods that would enable the assessment of exposure to substances with very diverse physico-chemical properties.

From a practical point of view, we would expect to be able to reliably estimate the average body burden by measuring the concentration of a specific substance or its degradation product/metabolite, preferably using non-invasive sampling methods. Assessment of

exposure to environmental pollutants is usually carried out either by performing human biomonitoring (HBM), which is currently considered the gold standard, or by investigating environmental media.

HBM of exposure to chemicals, based on measuring concentration of chemicals in biological matrices, such as urine, blood, or hair, is a frequently used approach [6]. Its main feature is an ability to determine the internal dose of chemicals, regardless of the route of exposure. As a result, it provides the most relevant data for risk assessment, which makes it a powerful [7] and increasingly popular technique in exposure science [8,9].

The concentration of a xenobiotic or its metabolite in the body depends on many factors, including the dose absorbed, the frequency of exposure, and the rate of biotransformation and elimination from the body [10]. For internal dose estimation based on biomarker concentration, knowledge of its pharmaco/toxicokinetics is of fundamental importance [11]. Based on their biological half-life, xenobiotics can be roughly divided into two groups: non-persistent, such as phthalate esters (PEs) and contemporary-use pesticides, which are excreted within several hours from exposure [12]; and persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and dioxins, that have biological half-lives spanning years [13]. For POPs, a single-timepoint measurement in appropriate matrix (typically blood) is sufficient for reliable exposure assessment. Non-persistent chemicals, however, often exhibit high intraindividual variability of biomarkers' concentration, warranting repeated sampling for accurate exposure estimation. To improve exposure assessment of these chemicals, simultaneous environmental sampling may be conducted [12]. Approaches providing average integrated data over a specified period of time would be particularly useful. Given the transitory nature of non-persistent organic pollutants and the scarcity of information regarding effects of emerging pollutants (both non-persistent and POPs) on human health, there appears to be a dire need for an effective methodology to be developed that would allow for reliable personalized long-term exposure assessment.

Another approach often employed in studies regarding exposure assessment is investigation of environmental media. The range of media used for such research is broad and includes various sampling methods. Environmental media most often analyzed in exposure science are water [14], soil [15], air [16], and dust [17]. Although this approach has a long use history, and throughout the years has provided science with an array of important facts, it is the personal samplers (active air samplers, hand wipes, silicone samplers) that are attracting growing interest among researchers.

Silicone samplers offer a cheap and easily accessible tool for chemically broad environmental sampling, posing as an alternative to expensive active air samplers [18,19]. Although most silicone samplers are used as personal samplers in the form of a wristband (WB) [20], some researchers employed brooches placed on the outer layer of clothing [21], strips [22], or stationary samplers, for example, in indoor [23] or outdoor [24] air monitoring. The building material of said samplers in most cases is poly(dimethylsiloxane) (PDMS), which possesses a set of attributes allowing for its implementation in exposure assessment studies regarding a wide variety of chemicals (see next section).

Considering that most data obtained in exposure assessment studies are made use of in epidemiological research, a quest for the perfect matrix and its sampling method is continuously underway. The purpose of this review is to comprehensively summarize the recent (2014–2021) advances in development of exposure assessment methods that use silicone wristbands as personal passive samplers and to compare silicone wristbands to other approaches in exposure science.

2. PDMS as a Sampler Material

PDMS is the most common silicone polymer [25]. Its long history of use in virtually all aspects of analytical chemistry—from sampling to final separation—has been extensively reviewed by Seethapathy and Górecki [26]. PDMS use is so widespread that in many papers, the terms 'PDMS' and 'silicone' are used interchangeably (e.g., Bergmann et al. [19],

Vidi et al. [27], S. Wang et al. [28]), and we follow this pattern throughout our review. One should bear in mind, however, that there are many silicone materials available [29].

The chemical formula of PDMS is $(CH_3)_3SiO[Si(CH_3)_2O]_nSi(CH_3)_3$ [26]. The number of monomeric units (n), ranging from just a few to several thousands, strongly affect the mechanical properties of the material. Short-chain PDMS are low-viscosity fluids, whereas the long-chain PDMS form solids [30], albeit an addition of filler (usually SiO_2) is needed to reinforce the structure [31]. The proportion of the filler in the final material may vary, and it affects not only the mechanical properties, but also the permeability of the material [32].

A raw silicone sampler contains oligomers that will likely interfere during the post-deployment analysis [33–35]. Indeed, in a study by Rusina et al. [29], the release of oligomers after exhaustive extraction with ethyl acetate for ten silicone rubbers was tested. In all cases, a substantial loss of mass was observed after the process (2.0–4.2%). Moreover, Anderson et al. [36] and O'Connell et al. [20] showed that improper cleaning procedure leads to high background noise in gas chromatography—mass spectrometry (GC-MS), further emphasizing the role of pre-deployment treatment of silicone samplers; see section "pre-deployment cleanup" for further discussion.

However, PDMS has a number of remarkable features that, taken together, make it an excellent material for a single-phase passive sampler. Due to a flexible backbone and the small size of methyl groups, PDMS exhibits high diffusivity, allowing many different compounds to be sequestrated [26], from air, as recently demonstrated in a series of chamber [37,38], indoor [39–41], and field studies (e.g., Bergmann et al. [19], O'Connell et al. [20]). These papers also provide theoretical background, data on PDMS-air partitioning and uptake kinetics of many compounds, and discuss other aspects of passive sampling with wristbands and other PDMS samplers as well. Although PDMS is hydrophobic in nature, it offers significant advantage in sampling moderately polar compounds compared to other popular polymers, such as low-density polyethylene [42]. Finally, silicone exhibits low reactivity [26], is affordable [29], and may be obtained in various shapes and forms, such as sheets, rods, or wristbands.

3. Emergence of Silicone Wristbands in Exposure Assessment

With the plenitude of available sampling methods, one of the emerging devices in the field is a silicone wristband. Popularized as an inexpensive fashion accessory by Lance Armstrong in the mid-2000s [43], it drew scientists' attention as a passive sampling device nearly a decade later [44]. After the first scientific paper was published [20], many works on this subject have been published in a relatively short period of time. Silicone wristbands are most commonly applied as personal passive samplers in human exposure assessment studies, and as such convey information regarding different routes of human exposure (dermal, inhalatory). Silicone wristbands offer an array of advantages as tools in personal exposure research (Figure 1).

The low cost of WB application has a considerable influence on study design, as it allows one to assemble a greater number of study participants without being overly expensive [18,45]. WBs are also non-invasive, which enhances participant compliance [46,47], as the only challenging aspect of the study that the study participants have to withstand is wearing the WBs on their wrists for the duration of sampling period. Small size and unobtrusiveness of these samplers makes this method suitable for application among sensitive populations, like the elderly, children (Figure 2), or pregnant women. The ease of deployment of those samplers also enables the sampling to be carried out by anyone, as it does not require any prior training [45,48].

If the sampler-to-skin contact during the sampling period is not prevented, WBs can provide information about both inhalatory and dermal routes of exposure [21]. This can be considered both an asset as well as a drawback, as it blends two exposure pathways, making it problematic to distinguish a source of a given chemical; however, if desired, WBs can be used as a passive air sampler only [20,49] (Figure 2). WBs also appear to be useful for analysis of metabolites excreted through skin, such as cotinine, a metabolite of

nicotine [50]. However, reports of this aspect of their usage are very scarce. Furthermore, when applied as personal samplers, WBs are carried across various microenvironments, so the chemical analysis that follows provides a time-weighted average (TWA) of several exposure episodes taking place over the duration of the experiment [45,51,52]. It is worth noting here that the determination of TWA is possible only in the linear range of uptake of substances from the surrounding environment [53], which is applicable for the semivolatile organic compounds (SVOCs) requiring at least a dozen or so days to achieve equilibrium with the wristband material. In contrast, volatile organic compounds (VOCs) quite quickly reach equilibrium with the wristband material and therefore their content in the band corresponds to the proportional concentration of the substances in the air during the last few hours of exposure [37].

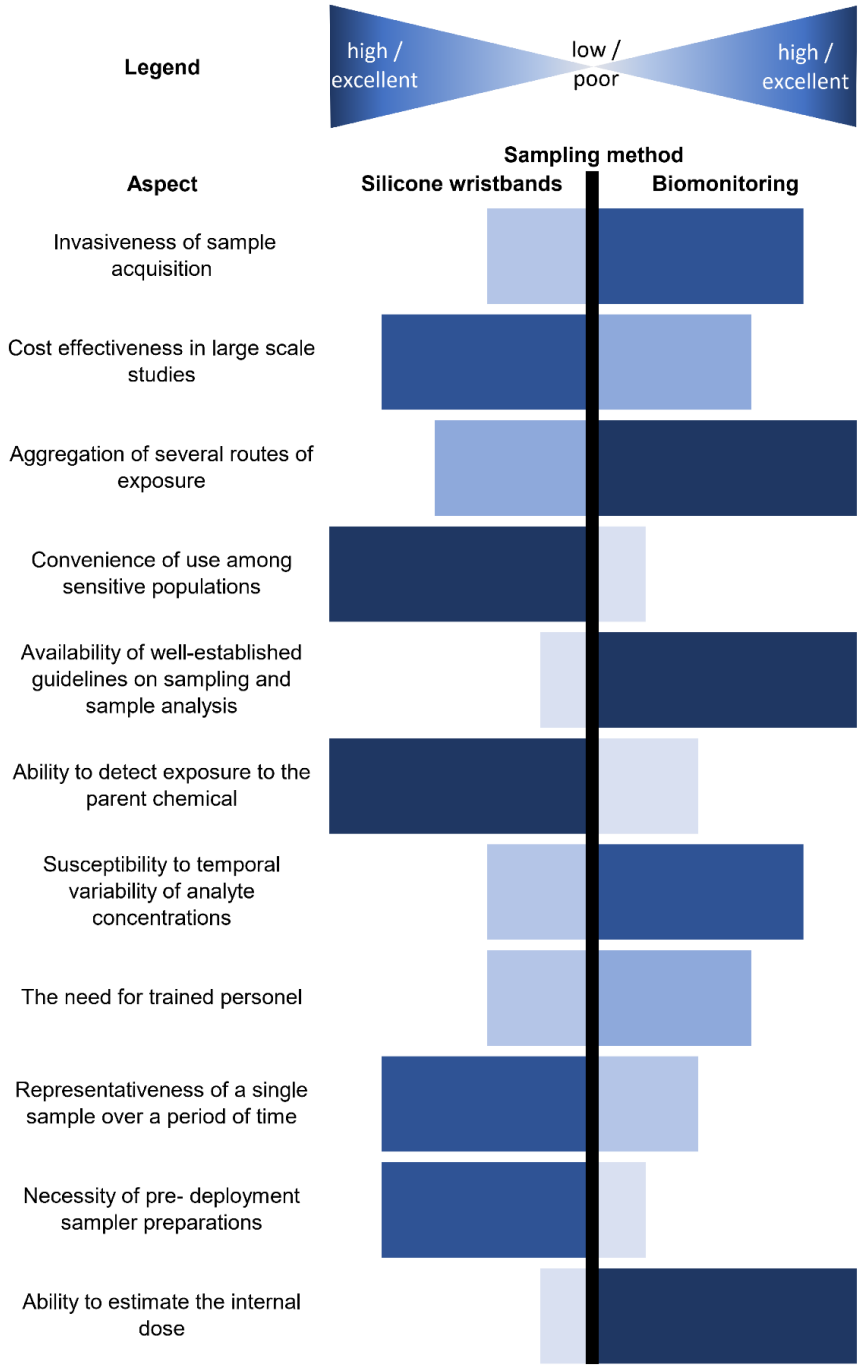


Figure 1. Comparison of attributes of exposure assessment methods with the use of WBs and biomonitoring.

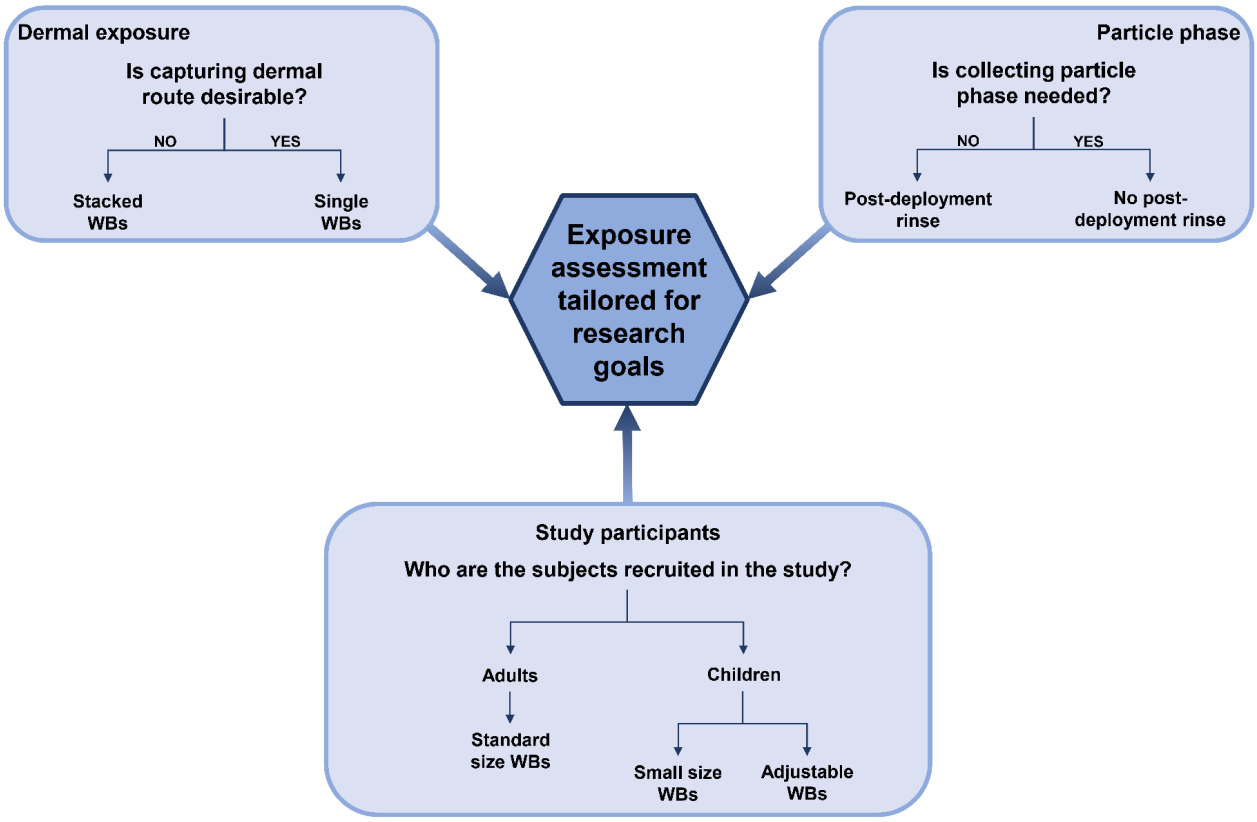


Figure 2. Possibilities of deployment of silicone wristbands (WBs). For references, see section "Emergence of silicone wristbands in exposure assessment".

4. Search Engine and Exclusion Criteria

The selection of reviewed articles was carried out using PubMed, Web of Science, and Scopus search engines. Upon searching the code-phrase: "silicone wristbands", the number of publications of interest was 53.

Excluding papers from the initial compilation was consequent to the study's methodology being described insufficiently in comparison to other research papers. This study focuses on descriptions of original research, which resulted in exclusion of review articles. The main focus of this review is set on application of silicone wristbands as personal passive samplers; therefore, experiments that included different forms of these passive samplers, such as silicone brooches, were excluded, due to consequent differences in monitored routes of human exposure. The cutoff paper publication date for our review was the 31 May 2021. The number of publications of interest post the employment of excluding factors was 45.

The vast majority of reviewed studies was carried out on various populations among inhabitants of the United States of America (>64%); other studies had been done in Europe (The Netherlands, France, Italy, Belgium), Peru, Brasil, Chile, Uruguay, Dominican Republic, Canada, Bangladesh, Senegal, and China (Table 1). Sampling timeframes described in reviewed articles varied from 2012 to 2019, and their duration from 0.3 to 34 days (for human exposure), with one study examining period lasting 161 days (exposure chamber). The median duration of a sampling period was 7 days. The largest study population consisted of 255 participants, and the least numerous had 2. A little over a half of reviewed studies examined exposures using WBs among adults (55.5%), several studies described analysis carried out on a population consisting of children (16.3%), and other explorations had been carried out on groups including both children and adults and/or adolescents. Most research concerned estimating ambient exposure among study participants (79.1%), with occupational-exposure studies being less prolific.

Table 1. The listing of sampling information regarding studies carried out with the use of silicone.

Publication Year	Sampling Year	Country *	Population	Population Age Range (<18 y.o)	*n*	Exposure Setting	Wearing Period [Days]	References
2014	NA	USA	NA	NA	<30	ambient	30	[20] †
2014	NA	USA	NA	NA	8	occupational	0.3, 1.3–1.6	[20] †
2015	2013	USA	adults	NA	50	ambient	7	[54]
2016	2015	USA	adults	NA	40	ambient	5	[51]
2016	2012/2013	USA	children	3–5	92	ambient	7	[52]
2016	2014	SEN	adults, children	NR	35	occupational	5	[55]
2017	2014	PER	adults, children	≥6	68	ambient	30–34	[19]
2017	NR	USA	adults	NA	22	ambient	2	[36]
2017	NR	USA	children	7–9	10	ambient	7	[27]
2017	2012–2013	USA	children	3–5	77	ambient	7	[56]
2018	nd	USA	adults	NA	19	ambient	21	[57]
2018	NR	USA	adults	NA	22	ambient	2	[48]
2018	2016	BEL	adults	NA	30	ambient	5	[24]
2018	2016	USA	adults	NA	30	ambient	7	[58]
2019	2017–2018	USA	adults	NA	101	ambient	7	[21]
2019	2016/2017	USA	adults	NA	10	occupational	0.83–2.08	[22]
2019	2016	BRA	adults	NA	2	ambient	3	[59]
2019	2016	USA	adults	NA	10	ambient	7	[60] †
2019	2017	USA	adults	NA	22	ambient	7	[60] †
2019	2016	USA	adolescents	14–16	97	ambient	7	[61]
2019	2008–?	USA	child-mother pairs	3–5	32	ambient	7	[62]
2019	NR	USA	adults	NA	10	ambient	7	[63]
2019	2017	USA	children	4–14	31	ambient	7,2	[64]
2019	NA	CAN, NED	NA	NA	NA	exposure chamber	1, 4, 10, 30, 50, 71, 91, 161	[38]
2019	NA	USA	NA	NA	NA	NA	7	[65]
2019	NR	NR	NR	NA	10	NR	7	[66]
2019	2016–2017	CHL	NR	NA	27	ambient	5	[45]
2019	NR	NR	NR	NA	16	ambient	18	[67]
2020	2018	URY	children	6–7.8	24	ambient	7	[68]
2020	2019	JPN	adults	NA	5	ambient	5	[69]
2020	2017	USA	adults	NA	72	occupational	1	[18]
2020	2019	USA	adults	NA	88	ambient	5	[70]
2020	2017–2018	USA	adults	NA	101	ambient	7	[47]
2020	2019	DOM	adults	NA	15	occupational	1	[71]
2020	2017–2018	USA	adults	NA	255	ambient	7	[46] †
2020	2017–2018	USA	adults	NA	20	ambient	7	[46] †
2020	2015/2016	USA	children	3–6	77	ambient	7	[72]
2020	2017–2018	USA	children	3–14	53	ambient	7, 2	[50]
2020	2018–2019	FRA	adults	NA	40	ambient	5	[28] †
2020	2018–2019	ITA	adults	NA	31	ambient	5	[28] †
2020	2018	BGD	adolescents/adults	≥14	15	occupational	1	[73]
2020	2018	USA	adults	NA	30	ambient	5	[74]
2020	2018	USA	adults	NA	17	occupational	1	[75]
2020	2017	CAN	adults	NA	45	occupational	0.3	[49]
2021	2014–2016	USA	children	3–6	27	ambient	7	[76]
2021	2018–2019	USA	children	10–17	163	ambient	7	[77]
2021	2018–2019	CHN	Child-mother pairs	≤7	47	ambient	14	[78]

Note: WBs: n—number of tested samples/participants (NR—not reported, NA—not applicable), *—in accordance with ISO 3166. †—studies described within the same paper, individual tested groups separated in this chart due to reciprocal differentiation in presented variables.

5. Chemical Analysis of Silicone Wristbands

Popularity of passive sampling with silicone WBs has increased in recent years, thanks to the seminal paper of O'Connell et al. [20]. Since then, the methodology of application of said wristbands has been evaluated, refined, and repeatedly validated in many studies carried out in diverse settings since 2014, enabling researchers to determine qualitatively and quantitatively the presence of a wide range of substances [19–21,79], such as pesticides [24], flame retardants [57,60,62,63], polycyclic aromatic hydrocarbons [18,19], or nicotine [64].

Although the majority of WBs employed in studies conducted since 2014 had been purchased from the same source (www.24hourwristbands.com, accessed on 2 December 2021), the reproducibility of performance of WBs obtained from the same or different sources has not yet been determined. Moreover, accessibility of commercially available WBs, precleaned and ready for application, is poor. These issues are definitely worth solving in the nearest future.

The laboratory procedure regarding handling of wristbands as passive samplers usually consists of several steps. In most cases, WBs require cleaning both prior to and post their deployment. The next phase of sample preparation is extraction, followed by post-extraction sample cleanup. Observed variations in conduction of pre-deployment cleanup, as well as extraction include the use of varying technologies: shakers [20,24], Soxhlet extraction sets [58,72], or vacuum ovens [61,67], as well as diverse amounts of different solvents. The extraction step, although in the technological sense is rather comparable among reviewed studies, varied across the usage of sorbents and elution solvents. A summary of methodology described in reviewed papers can be found in Table 2. Please note that the details included in each row feature a set of information drawn directly from the published paper.

5.1. Pre-Deployment Cleanup

Commercially available wristbands, usually worn as a gadget, may contain numerous impurities from raw materials, but also from their manufacturing, and thus cannot be directly used for sampling. We have not identified a single study that documented qualitatively and quantitatively the contaminants present in commercially available silicone wristbands. Due to this aspect, the bands purchased for research purposes should be properly cleaned before use.

Employment of a uniform washing step for all WBs used in the experiment results in diminished and levelized background noise observed during instrumental analysis, which is reproduced among all used samplers.

Among reviewed articles, four main approaches regarding pre-deployment cleanup were noted: Soxhlet extraction, performing an agitated wash of WBs, simple rinse or soaking WBs in solvents, and high temperature conditioning.

Most studies opted for a conventional mean of cleaning applied WBs and used Soxhlet extraction for that step. That method, although many up-to-date techniques have come out since its development, has an advantage of being robust and relatively cheap. Duration of Soxhlet extraction varied from 12 h per one cycle (with two cycles conducted) [58,70,72] to up to 3 days (per entire cleaning procedure) [73].

Other approaches substituted Soxhlet extraction with a series of agitated washes of WBs in solvents of different polarities. This technique significantly reduced the time needed to complete the procedure (in comparison to Soxhlet extraction), as the longest reported routine in total took 12.5 h and consisted of five solvent changes (each cycle took 2.5 h) [68]. The cost of applying this technique can vary heavily depending on the amount and purity of solvents used per a number of wristbands or their weight. Agitation of a wash was obtained most commonly via the use of a magnetic plate stirrer [65], an orbital (at the speed of 60–120 rotations per minute) [18,20,71], platform (60 rpm) [68], or overhead (60 rpm) shaker [24], with one study using ultrasonication for that purpose [78]. Performing an agitated wash can be considered more accessible, as it requires the use of common laboratory equipment, unlike Soxhlet extraction.

Table 2. Methodologies applied in reviewed articles (NR—not reported, Y—substances included in the study, N—substances not included in the study).

	Pre-Deployment		Post-Deployment		Extraction		Post-Extraction Sample Cleanup		Analyzed Substances									Instrumental Analysis	
Publication Year	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Instrumentation**	**Protocol**	**NBRFs**	**OPEs**	**PAHs**	**BFRs**	**PCBs**	**PEs**	**Pesticides**	**PPCPs**	**Other**		**Ref.**
2014	Agitated wash (orbital shaker)	3 × EtAc:n-hex (2.5 h), 60 rpm 2 × EtAc:MeOH (2.5 h), 60 rpm	Rinse	2 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc,100 mL, (2 h), 60 rpm	NR	NR	N	Y	Y	N	Y	Y	Y	Y	Y	GC-MS	[20]
2016	Thermal conditioning	280–300 °C (48 h)	Rinse	1 × DI water 1 × IPA	NR	2 × EtAc, 100 mL	NR	NR	N	N	N	N	N	N	Y	N	N	GC-ECD	[55]
2016	Soxhlet extraction	1 × EtAc:n-hex, (12 h) 1 × EtAc:MeOH, (12 h)	NR	NR	Soxhlet extraction	1 × n-hex:acetone, (12 h)	Syringe filter (0.2 µm PTFE) SPE cartridges (Florisil, 500 mg)	Filtration Elution: F1:n-hex (10 mL) F2:EtAc (10 mL)	N	Y	N	N	N	N	N	N	N	GC-MS	[51]
2017	Wash	3 × EtAc:n-hex 2 × EtAc:MeOH	Rinse	2 × DI water 1 × IPA	Wash	1 × EtAc, 100 mL, (12 h) 1 × EtAc, 100 mL, (2 h)	NR	NR	Y	Y	Y	Y	Y	Y	Y	Y	Y	GC-ECD, GC-MS	[19]
2017	Conditioning (vacuum oven)	300 °C, 180 min, 0.1 Torr	Rinse	2 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc, 100 mL	NR	NR	N	Y	Y	Y	Y	N	Y	Y	Y	GC-MS, GC-MS/MS, GC-µECD	[36]
2017	Soak	EtAc, n-hex, MeOH	Rinse	2 × water 1 × IPA	NR	2 × EtAc, 100 mL	SPE cartridges (C18, 500 mg)	Elution: ACN	Y	Y	N	Y	N	N	N	N	N	GC-MS	[52]
2018	NR	NR	Rinse	1 × DI water 1 × IPA	Dialysis	2 × EtAc	NR	NR	N	N	Y	N	N	N	N	N	N	GC-MS/MS	[57]
2018	Solvent exchange	3 × EtAc:n-hex 2 × EtAc:MeOH	Rinse	2 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc 100 mL, 60 rpm	NR	NR	N	N	Y	N	N	N	N	N	N	GC-MS/MS	[48]
2018	Agitated wash (overhead shaker)	1 × EtAc:n-hex, (30 min) 1 × EtAc:MeOH, (30 min):	NR	NR	Agitated wash (overhead shaker)	2 × EtAc, 40 mL, (30 min)	NR	NR	N	N	N	N	N	N	Y	N	N	LC-MS	[24]
2018	NR	NR	Rinse	2 × DI water 1 × IPA	Wash	1 × EtAc 100 mL, (12 h) 1 × EtAc, 100 mL, (2 h)	NR	NR	N	N	N	N	N	N	Y	N	N	GC-µECD	[27]
2018	Soxhlet extraction	1 × EtAc:n-hex, (12 h) 1 × EtAc:MeOH, (12 h)	NR	NR	Sonication	3 × n-hex:acetone, 10 mL	Custom SPE: Florisil (500 mg) and silica gel (12 g; F1 only)	Elution (Florisil): F1:n-hex F2:EtAc Elution (silica gel): F3:DCM:n-hex	Y	N	N	Y	N	N	N	N	N	GC-MS	[58]

Table 2. *Cont.*

	Pre-Deployment		Post-Deployment		Extraction		Post-Extraction Sample Cleanup		Analyzed Substances									Instrumental Analysis	
Publication Year	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Instrumentation**	**Protocol**	**NBRFs**	**OPEs**	**PAHs**	**BFRs**	**PCBs**	**PEs**	**Pesticides**	**PPCPs**	**Other**		**Ref.**
2019	Rinse, conditioning	Water rinse, thermal conditioning	Rinse	1 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc, 100 mL, (2 h)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	GC-MS	[67]
2019	Soxhlet extraction	1 × EtAc:n-hex, (24 h) 1 × EtAc:MeOH, (24 h)	NR	NR	Sonication	2 × n-hex:acetone, 30 mL, (2 h)	Custom SPE (neutral alumina, neutral silica gel, sulfuric acid- silica gel, sodium sulfate)	Elution: DCM (40 mL)	Y	Y	N	Y	N	N	N	N	Y	GC-MS	[21]
							Custom SPE (neutral alumina, neutral silica, Florisil, sodium sulfate)	Elution: F1:DCM (40 mL) F2:EtAc (40 mL)											
2019	NR	NR	Rinse	2 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc, 100 mL, 60 rpm	NR	NR	N	Y	Y	N	N	Y	Y	Y	Y	GC-MS	[59]
2019	Thermal conditioning (vacuum oven)	300 °C, (180 min), 0.1 Torr	Rinse	1 × DI water 1 × IPA	NR	2 × EtAc, 100 mL	SPE (C18, silica)	Elution: ACN	Y	Y	Y	Y	Y	Y	Y	Y	Y	GC-μECD, GC-MS	[61]
2019	Soak	EtAc, n-hex, MeOH	NR	NR	NR	2 × EtAc, 100 mL	SPE cartridges (C18, 500 mg)	Elution: ACN	N	Y	N	N	N	N	N	N	N	GC-MS	[62]
2019	Soxhlet extraction		Agitated wash	1 × DI water	Sonication	1 × Acetone:n-hex, 20 mL, (2 h)	Custom SPE (neutral alumina, neutral silica, Florisil, anhydrous sodium sulfate)	Elution: F1:DCM F2:EtAc	Y	Y	Y	Y	N	N	N	N	Y	GC-MS	[63]
			Rinse	1 × IPA			Custom SPE, (neutral alumina, neutral silica, acidic silica, anhydrous sodium sulfate)	elution: F3: DCM											
2019	NR	NR	Rinse	2 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc, 100 mL, (2 h), 60 rpm	-	-	N	N	Y	N	N	Y	Y	N	Y	GC-GC/ ToF-MS	[45]
2020	Soxhlet extraction	1 × EtAc (3 days)	-	-	Agitated wash (Wrist Action Shaker)	1 × ACN, 30 mL	Syringe filter (0.2 μm, Teflon)	Filtration	Y	Y	N	Y	N	N	N	N	Y	GC-MS	[49]
2020	Agitated wash (platform shaker)	3 × EtAc:n-hex, (2.5 h) 2 × EtAc:MeOH, (2.5 h), 60 rpm	NR	NR	Agitated wash (orbital shaker)	2 × EtAc, 25 mL, (2 h), 60 rpm	SPE cartridges (C18, 500 mg)	Elution: ACN	Y	Y	N	Y	Y	N	Y	N	Y	GC-MS	[68]

Table 2. *Cont.*

	Pre-Deployment		Post-Deployment		Extraction		Post-Extraction Sample Cleanup		Analyzed Substances									Instrumental Analysis	
Publication Year	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Instrumentation**	**Protocol**	**NBRFs**	**OPEs**	**PAHs**	**BFRs**	**PCBs**	**PEs**	**Pesticides**	**PPCPs**	**Other**		**Ref.**
2020	NR	NR	Rinse	1 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc, 25 mL, (24 h)	SPE cartridges (C18, 500 mg)	Elution: n-hex: DCM (4 mL)	N	N	Y	N	N	N	N	Y	N	GC-MS	[69]
2020	Agitated wash (orbital shaker)	1 × MeOH (10 min) 3 × n-hex:EtAc (1 h), 2 × MeOH:EtAc	Rinse	1 × MeOH	agitated wash (orbital shaker)	2 × 30 mL EtAc, 30 mL, (1 h)	NR	NR	N	N	Y	N	N	N	N	N	N	GC-MS	[18]
2020	Soxhlet extraction	1 × EtAc:n-hex, (12 h) 1 × EtAc:MeOH, (12 h)	NR	NR	Sonication	3 × n-hex: DCM, 10 mL, (15 min)	SPE (Florisil, 8 g)	Elution: F1:n-hex F2:EtAc	N	Y	N	N	N	N	N	N	N	GC-MS/MS	[70]
2020	Soxhlet extraction	1 × EtAc:n-hex, (24 h) 1 × EtAc:MeOH, (24 h)	NR	NR	Sonication	2 × n-hex:acetone, 30 mL, (2 h)	Custom SPE (neutral alumina, neutral silica, Florisil, sodium sulfate)	Elution: DCM	N	N	Y	N	N	N	N	N	N	GC-MS	[47]
2020	Agitated wash (orbital shaker)	2 × MeOH, (10 min), 120 rpm 2 × (1 h): n-hex:EtAc, (1 h), 120 rpm 2 × MeOH:EtAc, 120 rpm	Rinse	1 × MeOH	Agitated wash (overhead shaker)	2 × EtAc, 30 mL	NR	NR	N	N	Y	N	N	N	N	N	N	GC-MS	[71]
2020	Conditioning (vacuum oven)	300 °C, (12 h), 0.1 Torr	Rinse	2 × DI water 1 × IPA	NR	2 × EtAc, 50 mL	SPE cartridges (C18)	Eluted: ACN	N	Y	Y	Y	Y	Y	Y	Y	Y	GC-MS	[46]
2020	Soxhlet extraction	1 × EtAc:n-hex, (12 h) 1 × EtAc:MeOH, (12 h)	NR	NR	Sonication	3 × n-hex:DCM, 10 mL)	SPE cartridges (Florisil, 500 mg)	Elution: F1: n-hex F2: EtAc F3: MeOH	N	Y	N	N	N	Y	N	N	Y	GC-MS	[72]

Table 2. *Cont.*

	Pre-Deployment		Post-Deployment		Extraction		Post-Extraction Sample Cleanup		Analyzed Substances									Instrumental Analysis	
Publication Year	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Instrumentation**	**Protocol**	**NBRFs**	**OPEs**	**PAHs**	**BFRs**	**PCBs**	**PEs**	**Pesticides**	**PPCPs**	**Other**		**Ref.**
2020	NR	NR	Rinse	DI water	Sonication	2 × n-hex: acetone, 30 mL, (2 h)	Chromatography column (neutral alumina, neutral silica gel, sulfuric acid-silica gel, sodium sulfate)	Elution: DCM	Y	Y	Y	Y	N	N	N	N	N	GC-MS	[28]
							Chromatography column (neutral alumina, neutral silica gel, Florisil, sodium sulfate)	Elution:F1:DCM F2:EtAc											
2020	Soxhlet extraction	1 × pentane (3 days)	-	-	Agitated wash	ACN	SPE cartridge (Florisil, 500 mg)	Elution: EtAc	Y	Y	N	Y	N	N	N	N	Y	GC-MS	[73]
2020	Agitated wash (magnetic stir plate)	3 × EtAc:n-hex, (30 min), 60 rpm 2 × EtAc:MeOH, (30 min), 60 rpm	NR	NR	Agitated wash (magnetic stir plate)	ACN:MeOH, 20 mL, (1 h), 60 rpm	NR	NR	N	N	N	N	N	N	N	N	Y	HPLC	[65]
2020	NR	NR	NR	NR	Sonication	3 × n-hex:DCM, 10 mL	SPE (Florisil, 8 g)	Elution: F1: n-hex, F2: EtAc, F3: MeOH	Y	Y	N	Y	Y	Y	Y	N	Y	GC-MS, GC-MS/MS	[74]
2020	Agitated wash (orbital shaker)	1 × MeOH (10 min), 120 rpm 2 × EtAc:n-hex (1 h), 120 rpm 2 × EtAc:MeOH (1 h), 120 rpm	NR	NR	Agitated wash (orbital shaker)	2 × EtAc, 30 mL, (1 h), 120 rpm	NR	NR	N	N	Y	N	N	N	N	N	N	GC-MS	[75]

Table 2. *Cont.*

	Pre-Deployment		Post-Deployment		Extraction		Post-Extraction Sample Cleanup		Analyzed Substances									Instrumental Analysis	
Publication Year	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Mechanism**	**Protocol**	**Instrumentation**	**Protocol**	**NBRFs**	**OPEs**	**PAHs**	**BFRs**	**PCBs**	**PEs**	**Pesticides**	**PPCPs**	**Other**		**Ref.**
2021	Soxhlet extraction	1 × EtAc:n-hex (12 h) 1 × EtAc:MeOH (12 h)	NR	NR	Sonication	3 × DCM:n-hex	SPE cartridges (Florisil, 500 mg)	Elution: F1 F2: EtAc F3	N	N	N	N	N	N	N	Y	Y	LC-MS	[76]
2021	Rinse, conditioning	DI water, 300 °C (180 min)	rinse	1 × DI water 1 × IPA	Agitated wash (orbital shaker)	2 × EtAc	SPE (C18, silica)	Elution: ACN	N	N	N	N	N	N	Y	N	N	GC-ECD, GC-MS	[77]
2021	Sonication	3 × DCM:n-hex, (20 min)	NR	NR	Sonication	2 × DCM: n-hex, 15 mL, (20 min)	SPE cartridges (Florisil, 2 g)	Elution: 1 × n-hex 1 × EtAc	N	Y	N	N	N	N	N	N	N	LC-MS	[78]

Abbreviations: ACN, acetonitrile; BFRs, brominated flame retardants; DCM, dichloromethane; DI, deionized; EtAc, ethyl acetate; F1, F2, F3, numeration of fractions eluted (in accordance to their order of elution); IPA, isopropyl alcohol; MeOH, methanol; NBFRs, novel brominated flame retardants; n-hex, n-hexane; OPEs, organophosphate esters; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; PEs, phthalate esters; PPCPs, pharmaceuticals and personal care products; SPE, solid phase extraction.

Some studies performed the cleaning step through washing WBs in varying solvents several times [19,48,52,62], which definitely is the quickest of all described approaches.

Several studies opted for temperature conditioning of WBs as the technique of choice for performing the cleanup step. Conditioning required temperatures up to 300 °C to be achieved and held on for a time in a range of 180 min up to 48 h [36,46,55,61,67,77]. Anderson et al. [36] evaluated this cleanup method by examining the total ion chromatogram, providing pictorial evidence of its efficiency in removing prominent amounts of oligomers.

It is worth noting that one of the aspects of cleanup procedure that requires further investigation is a sufficient solvent volume/weight/number of simultaneously washed WBs ratio. Unfortunately, no study assessed the influence of the WB precleaning procedure on the target analyte uptake, its stability, or its recovery during further extraction. As noted earlier, no identification of manufacturing-related impurities in silicone material used in WBs production has been performed to date.

5.2. Post-Deployment Cleanup

During the sampling period, silicone wristbands inevitably come into contact with many materials and chemicals, both environmental (personal care products, dust, food, cleaning products, petrol, oil, and others) and human body-derived (sebum, sweat). In order to tentatively cleanse the surface of the sampler from loosely bound particulates, most of reviewed studies opted for rinsing WBs with the use of deionized water and isopropanol [19,20,48,57,59,69], whereas others opted for the use of methanol in place of isopropanol [18,71]. Finally, in some studies the surface of the sampler was not cleaned after deployment [49]. Overall, descriptions of this step of the analysis usually lack information regarding volume of used solvents or duration of this part of the protocol. Additionally, none of the available studies assessed the cleanup efficiency (e.g., amount of the analyte in rinsing solution and in the silicone matrix). No information was found in any of the publications whether the authors analyzed the rinse wash, which is the generally accepted practice for hair analysis in forensic toxicology [80].

5.3. Extraction

The sample extraction step is of utmost importance, as its efficiency, selectivity, and reproducibility will determine the amount of analytes of interest isolated from the processed matrix into the extract. This stage of sample preparation had been carried out in the reviewed research papers by washing post-exposure wristbands in a solvent. Most commonly a cycle (or series of cycles) of agitated WB wash(es) were performed, with the use of either an orbital shaker [18,20,36,45,48,59,67–69,75,77], an overhead shaker [24,71], a magnetic stir plate [65], Soxhlet extraction [51], or sonication [21,28,47,58,63,70,72,74,76,78]. The most frequently applied solvent of choice was ethyl acetate [20,24,55]. In the majority of cases, the extraction procedure corresponded a great deal with the pre-exposure WBs cleanup protocol [24,51,68], which is obviously understandable, as the aim of primary WB precleaning, before applying them in a study, is to remove contaminants, including analytes of interest, and therefore attain a blank sampling matrix to be applied in the experiment. Some studies opted for WB fragmentation upon carrying out extraction [51,58,68,70]. Extraction efficiency was evaluated throughout some reviewed studies, starting with O'Connell et al. [20], as their study confirmed the operational efficiency of extraction (90% recovery of the total amount of acenaphthalene-D_8, fluorene-D_{10}, phenanthrene-D_{10}, pyrene-D_{10}) carried out by their design (via fortification of WBs with standards) that later became a template for other studies regarding this sampling method; the spike test, however, was not done in every study. Variability of analyte levels between fortified WBs that had been evaluated in the same study has also been proven to be very satisfactory (relative standard deviation <13%), therefore validating the capability of silicone WBs to be applied in exposure assessment studies. Surrogate standards, when applied to evaluate extraction efficiency, were added either directly onto the samples before the cleanup [68], or before extraction [46], whereas

internal standards were added either before extraction [51,57], or right before analysis, directly into the prepared extract [62].

5.4. Post-Extraction Cleanup

Raw extracts attained during sample processing, in order to be useful for a chosen instrumental analysis, tend to be further purified. Among reviewed studies, the most commonly applied approach was solid phase extraction (SPE) [51,77]. This sample preparation step depends crucially on the chemical properties of analytes of interest, as the interactions between the SPE sorbent, eluent, and analysed substances determine the efficiency and selectivity of the process [81]. Most studies that opted for SPE finalized the analysis by the use of gas chromatography–mass spectrometry (GC-MS) [28,47,72]. Performing SPE prior to GC-MS is meant for separating analytes of interest into several distinct fractions, therefore avoiding coelution of substances and mutual interference during analysis. Popular SPE sorbents used among reviewed articles are: C18 [52,61,62], silica gel [58], and Florisil [58,70]. One of the reviewed articles opted for performing post-extraction cleanup (preluding SPE) of WBs via filtration with the use of 0.2 µm PTFE membrane [51] to deprive the extract of larger particles.

5.5. Other Methods

It is necessary to take notice to the research papers not listed in Table 2, regarding employment of silicone wristbands as personal passive samplers for analysis of nicotine [50,64], cotinine, and tobacco-specific nitrosamines [50]. Said studies were not included in Table 2 due to significant methodological differences from all the other studies, therefore making it inconvenient to present within our formed outline. Both studies present the use of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction technique for nicotine and cotinine analysis. QuEChERS is a quick and cheap method of sample preparation for determination of pollutants residues, e.g., pesticides [82], most common in food analysis. It is a routine dispersive SPE step consisting of single-phase extraction, liquid-liquid partitioning, and addition of salts (e.g., magnesium sulphate, sodium chloride).

6. Qualitative and Quantitative Analysis

Silicone WBs have already been shown to be suitable for analysis of a wide array of chemicals. Qualitative methods may include over 1300 analytes [79]. Moreover, a framework for unknown screening using silicone WBs and GC coupled to high-resolution mass spectrometry was recently proposed [83]. Ease of use and capturing capabilities of silicone WB make it an excellent tool for studying exposure to emerging contaminants at a personal level [48].

Quantitative analysis of silicone wristbands also may include many chemicals (Figure 3). For instance, Doherty et al. [46] quantified 199 chemicals from several classes, including pharmaceuticals and personal care products (PPCPs), pesticides, and flame retardants. In this work, compounds with logP values spread throughout over nine orders of magnitude were captured simultaneously. Notably, WBs' capabilities as a sampler allow the study of ratios between compounds of similar structure, facilitating the identification of exposure source, such as Firemaster 550 in case of OPEs [51] or secondhand tobacco smoke for nicotine and cotinine [50]. The variety of chemicals analyzed in silicone WB is depicted in Figure 3. To date, over 450 different chemicals have been quantified in silicone WBs; the full list is provided in Supporting Information 1 of Supplementary material, Table S1.

However, the use of PDMS as a sorbent material does have its limitations. To our knowledge, no study so far has quantified per- and polyfluoroalkyl substances (PFASs), an important group of emerging pollutants [84], in silicone WBs. Indeed, it has been pointed out that hydrophobic properties of PDMS make it unsuitable for sampling of perfluorooctane sulfonic acid, a well-known PFAS, in water [85]. Extraction efficiency of several other PFASs from water samples using PDMS rods was reported low as well [86]. A similar outcome may be expected for many PFASs sampled in air with a silicone WB [87].

Some (semi)volatile, non-ionic PFASs (e.g., fluorotelomer alcohols) might be an exception. However, to our knowledge, no experimental data on this matter are available to date.

Moreover, discrepancies in presentation of quantitative results exist. Some researchers use analyte mass per entire wristband (e.g., Dixon et al. [48], Xie et al. [78]), whereas others share results as analyte mass per unit mass of the wristband (usually per one gram; e.g., Hammel et al. [72], Wise et al. [74]). These differences may hinder comparisons between the studies [51]. Because wristbands of various sizes are used (e.g., Gibson et al. [62], Quintana et al. [50], Xie et al. [78]), we recommend using analyte mass per unit mass of the wristband as a more versatile approach.

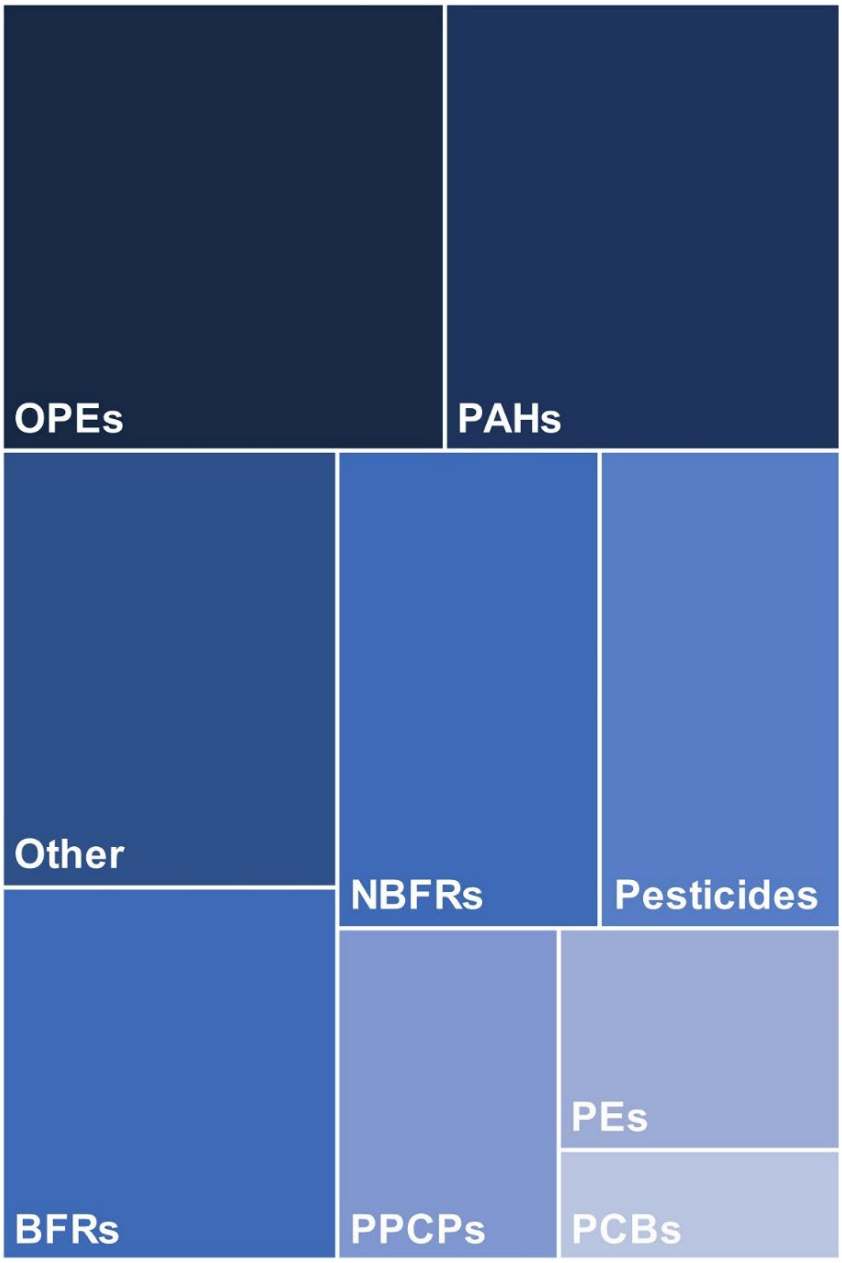


Figure 3. Groups of pollutants analyzed quantitatively in population studies using silicone wristbands. The proportions were computed after assigning a score of 1 to every group per every paper that included quantitative analysis of at least one analyte from the group. Abbreviations: BFRs, brominated flame retardants; NBFRs, novel brominated flame retardants; OPEs, organophosphate esters; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; PEs, phthalate esters; PPCPs, pharmaceuticals and personal care products.

7. Comparison of Wristbands with Other Matrices

Since the seminal work by O'Connell et al. [20] was published, several researchers conducted studies involving simultaneous collection of different biological and environmental matrices to gather more exposure data and compare silicone WBs to other means of exposure assessment. Spearman correlation coefficient (r_s) was used most frequently to determine the strength of association. Although many gaps of knowledge still remain, some remarks can already be made and are provided below.

7.1. Biological Matrices

7.1.1. Urine

Urine is an easily accessible biological matrix [88], preferred for most non-persistent chemicals [89] and representing internal exposure level [6]. Therefore, it is not surprising that urine was nearly the only biological matrix WBs were compared to (Supporting Information 2 of Supplementary material, Table S2). WBs correlated moderately well with urine in many, but not all, cases.

Urinary concentrations of 1-hydroxy- metabolites of polycyclic aromatic compounds (PAHs), namely naphthalene, phenanthrene, and pyrene, corresponded well with concentrations of parent compounds in WBs (r_s = 0.48–0.76, $p < 0.05$, Table S2). Weaker associations were found comparing these chemicals to their other metabolites or between fluorene and its metabolites [48].

Inconsistent results were obtained in studies investigating silicone WBs–urine relationship while assessing exposure to OPEs. For instance, low and statistically insignificant correlations were observed between triphenyl phosphate (TPHP) in WBs and its metabolite, diphenyl phosphate (DPHP), in urine [49,51,72,78], except for Wise et al. [74] (Table S2). DPHP, however, is not a specific (unique) metabolite of TPHP, so concurrent exposure to other OPEs possibly overshadowed the true link. Complex, route-specific, or unknown metabolism and pharmacokinetics may therefore explain to some extent limited agreement between WBs and urine [51,78]. However, if a parent compound and its specific metabolite were considered, such as tris(1,3-dichloroisopropyl) phosphate and bis(1,3-dichloroisopropyl) phosphate, respectively [90], better correlations between WBs and urine were observed, ranging from 0.43 ($p < 0.01$) [78] to 0.59 ($p < 0.0001$) [51]; however, a trend was only observed in Nguyen et al. (r_s = 0.34, p = 0.08) [49], and Wise et al. [74] reported a weak and statistically insignificant relationship (r_s = 0.24, $p > 0.05$). Tris(1-chloro-2-isopropyl) phosphate (TCIPP) and bis(1-chloro-2-isopropyl) 1-hydroxy-2-propyl phosphate (BCIPHIPP) can also be considered such a pair, with TCIPP being the parent compound detected in WBs, and BCIPHIPP the urinary biomarker [91]. To date, the correlation analyses of these analytes yield contradictory results [49,51,74], despite BCIPHIPP being frequently detected in urine and showing good reproducibility over time [92]. Dietary exposure to certain OPEs, which is not captured by WBs, may also contribute to unsatisfactory correlations with urine [78]. Further research is necessary to elucidate these discrepancies.

In general, results in WBs correlated moderately well with urinary concentrations of PPCPs or their metabolites (Table S2). Nicotine and cotinine in WBs were closely associated with urinary cotinine ($r_s > 0.84$, $p < 0.01$), establishing an exposure-response relationship [50,64]. The strength of observed association and pharmacokinetic data suggest that WBs may have also captured nicotine and cotinine excreted in sweat [50] and thereby partially reflect internal exposure. In a study focused on PPCPs exposure in children [76], PPCP concentrations in WBs were moderately associated with concentrations in urine (r_s 0.51–0.66, $p < 0.0001$), except for bisphenol A (BPA) (r_s = 0.23, $p < 0.05$). The proposed explanation was that for BPA, in contrast to other PPCPs (e.g., parabens), dietary route is a main source of exposure. In consequence, WBs were not able to capture most of the BPA participants were exposed to. As a similar phenomenon was observed in the case of TPHP [78], an OPE detected in foodstuffs [93,94], it can be speculated that low WBs-urine correlation accompanied by high abundance of metabolite/parent compound in urine

implies a dietary pathway as a main source of exposure, whereas high concentrations in both WBs and urine suggest otherwise.

Such approach was used in a study of exposure to phthalate esters (PEs) among nail salon workers [22], where high abundance of di(2-ethylhexyl) terephthalate in WBs and its metabolites in urine confirmed the occupational character of exposure, rather than dietary. This example demonstrates how data obtained with WBs can enrich a biomonitoring study. In turn, Hammel et al. [72] showed weak or moderate correlation (r_s 0.3–0.56, $p < 0.01$) between five of seven PEs with paired WBs and urine data (Table S2) among children in an ambient exposure setting.

It should be noted that several factors should be considered when evaluating correlations between these matrices. As noted earlier, silicone WBs offer a wide range of sampling timeframes, ranging from hours [20] to weeks [19] and, possibly, months, depending on study design. In turn, for many chemicals, a single urine sample reflects only recent exposure, within several hours before collection [95–99]. Therefore, continuous, fully adjustable sampling using silicone WBs should be accompanied by parallel urine collection to perform complementary, longitudinal exposure assessment. Some researchers accounted for that by pooling urine samples [51,62,74], but others collected only a single spot sample [48,50], which may have impacted the observed associations. Moreover, urinary flow is known to be variable and influenced by many short-term (e.g., hydration status) and long-term parameters, such as age and BMI [6]. Repeated sampling is known to reduce the effect of short-term variations on the urinary flow rate, therefore improving exposure assessment [89]. Nevertheless, urine is a widely used and acknowledged matrix [6], especially since exposure to nonpersistent chemicals began to attract growing attention [12]. Nearly all nationwide biomonitoring studies include urine collection [100], with the first dating back to 1970s and 1980s [101]. There is also a large body of methodological literature focusing on opportunities and caveats in urine analysis (e.g., Barr et al. [11], Faÿs et al. [102], Franklin et al. [103], Klimowska et al. [104], Meeker et al. [105], Needham et al. [106]). In contrast, WBs have been in use for exposure assessment only since 2014 [20], and no population-scale study has yet been conducted. In addition, although a few methodological papers have already been published [20,36–38], many aspects of WBs sampling need to be investigated further (see Section "Future prospects"). Additionally, urine is known to account for all routes of exposure [6], whereas WBs generally capture dermal, inhalatory, but not dietary route [21,22,72,74]. As noted earlier, however, a single WB may cover a much longer period of time than a single urine sample, which is a notable feature in longitudinal studies. Moreover, WBs are far less demanding in terms of transportation and storage conditions [20,36,55]. WBs can be therefore considered a cheaper and less burdensome alternative to urine.

7.1.2. Blood

Only two studies investigated the relationship between pollutants quantified in silicone WBs and in blood [49,58]. In Hammel et al. [58], four out of six brominated flame retardants (BFRs) detected with sufficient frequency in both matrices were moderately correlated (r_s = 0.39–0.57, $p < 0.05$) (Table S2). Associations were also observed between congeners within both matrices, identifying PentaBDE commercial mixture as a plausible source of exposure [58]. Furthermore, Nguyen et al. [49] observed a moderate association between decabromobiphenyl ether in plasma and WBs (r_s = 0.4, $p < 0.05$). These examples show that silicone WBs may be suitable for estimation of exposure not only to nonpersistent organic pollutants, as discussed earlier, but also to chemicals with long half-lives, such as BFRs [107]. However, further research is necessary to confirm these findings and investigate the WB-blood relationship in other groups of organic pollutants.

7.2. Environmental Matrices

7.2.1. Hand Wipes

We touch many objects around us with our hands [108]. Over the past decades, many chemicals have been shown to penetrate the skin barrier effectively, leading to internal exposure (e.g., Appel et al. [109], Lees et al. [110], Piotrowski [111], Weschler et al. [112]). In consequence, monitoring dermal exposure is an important element of thorough exposure assessment [113]. As both hand wipes and WBs may be used for this task, it is tempting to make a comparison between these matrices, which is provided below.

In the majority of cases, a statistically significant positive correlation between individual OPEs concentrations in WBs and in hand wipes was reported (Supporting Information 2 of Supplementary material, Table S3) [51,72]. S. Wang et al. [21] compared hand wipes and wristbands considering OPEs as a group. However, the strength of associations observed in aforementioned studies was weak to moderate, with r_s approximately 0.4 between individual OPEs (Table S3).

Levasseur et al. [76] used hand wipes and wristbands as tools for assessment of exposure to phenols in children. The r_s values, if calculated, oscillated around 0.5 (Table S3). Detection frequencies of triclosan, methylparaben, ethylparaben, and propylparaben were similar in both matrices, but sharp contrasts were observed for other chemicals, such as BPA (hand wipes and WBs, respectively: 57% vs. 100%) and butylparaben (44% vs. 95%, respectively).

Similar to OPEs, weak to moderate correlations were found between hand wipes and WBs for PEs and their alternatives (r_s = 0.24–0.42, $p < 0.05$) [72] (Table S3).

In turn, S. Wang et al. [21] investigated associations between hand wipes and WBs for more lipophilic groups of organic pollutants. Apart from OPEs, PAHs, novel brominated flame retardants (NBFRs), and polybrominated diphenyl ethers (PBDEs) were investigated. Coefficient of determination (r^2) ranged from 0.58 (PAHs) to 0.73 (PBDEs). Moreover, hand wipes and wristbands showed a very similar profile of captured chemicals.

Similarities between the results obtained using WBs and hand wipes are not unexpected, as both matrices are capable of capturing chemicals from several sources—surface contact, vapor phase, and particulates in air [21,22,114] (Figure 4). In both cases, the sampler is small, lightweight, and no power source is needed. Aggregating exposure from several sources, in addition to their low cost [21,115], makes them excellent tools for exposure assessment. Finally, despite long history in exposure assessment [116], the standardization of sample collection of hand wipes also leaves a lot to be desired [117], the key variables being the number of wipes and amount of force applied while wiping the skin [118].

The differences between these matrices, however, are even more striking (Figure 4). Although both matrices capture exposure from similar sources, their main focus appears to be different, with WBs being more effective in sampling vapor and particulate phases, and hand wipes better at reflecting dermal exposure [51]. Furthermore, sampling with hand wipes has been repeatably shown to be susceptible to hand washing, which removes many organic contaminants very effectively and may cause underestimation of exposure [119–121]. Due to this fact, participants are asked not to wash their hands for some time prior to sampling, usually an hour [121–123], but some sampling protocols require a four-hour interval since the last hand washing [120], which may be considered an inconvenience. In case of WBs, the analytes are absorbed into the polymer, so hand washing should not significantly affect the sampling, although particles on the surface may be removed in the process. Another limitation of hand wipes, partially the consequence of the previous one, is the short time window covered by a single sample and considerable influence of timing of collection [123]. As a result, numerous samples need to be collected in longitudinal exposure assessment. Considering all the characteristics stated above and the fact that concentrations in WBs often correlated better with urine as compared to hand wipes, some authors see WBs as superior to the latter [72,76].

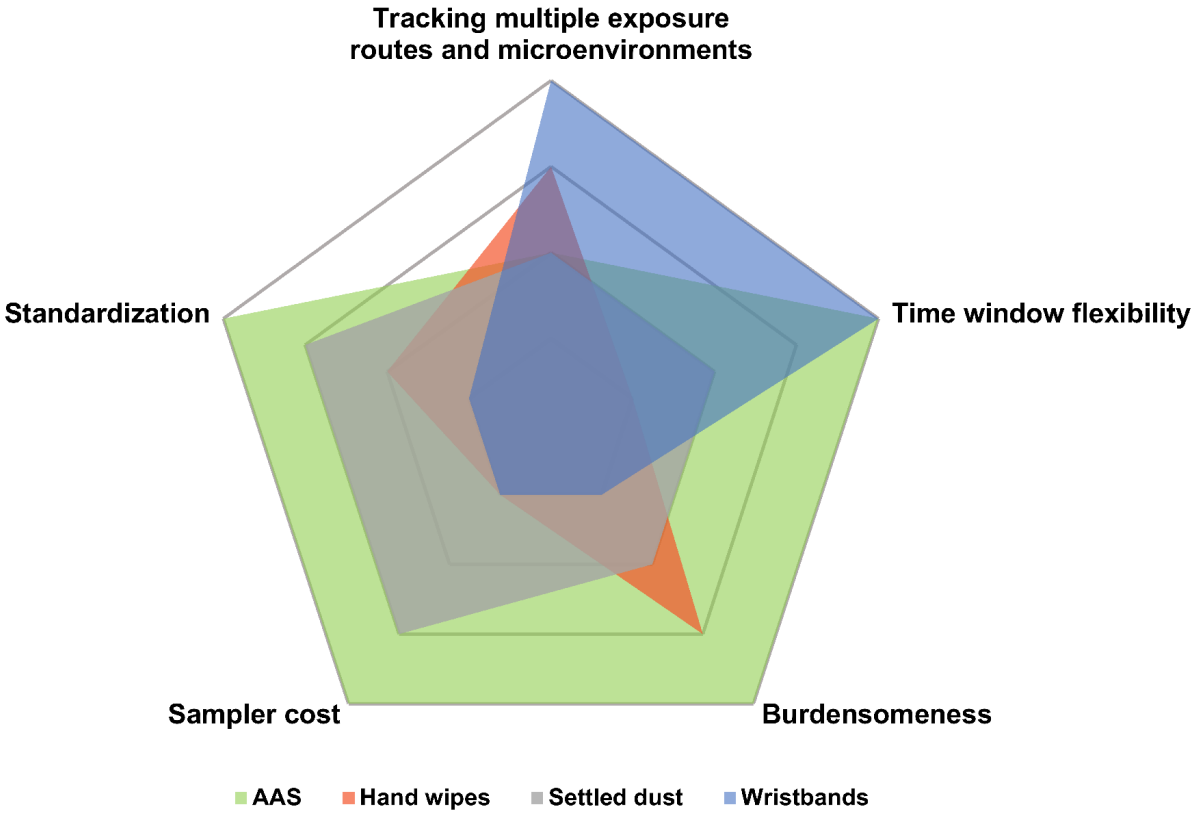


Figure 4. Comparison of key features of wristbands and other environmental media collected during exposure assessment, based on literature review and judgement of the authors. The inner pentagon represents "low/poor" value, whereas the outer one stands for "high/excellent". For discussion, see section "Comparison with other matrices". Abbreviations: AAS, active air sampling.

7.2.2. Active Air Sampling (AAS)

AAS is another useful tool in exposure assessment [124,125]. Inhalation pathway appears to be important in exposure to many pollutants [108] that can be monitored by AAS and WBs as well. The comparative discussion below limits AAS to personal sampling.

Dixon et al. [48] analyzed PAHs collected using two devices: an active air sampler (equipped with polyurethane foam (PUF) sorbent and $PM_{2.5}$ filter) and WBs, both being worn simultaneously. A number of detections of each PAH were very similar in WBs and in PUFs, but not in filters, with the notable exceptions of benzo[*b*]fluoranthene, benzo[*k*]fluorantene, benzo[*a*]pyrene, and benzo[*ghi*]perylene, which were detected frequently only in WBs and filters. For PAHs detected in 100% of WBs and PUFs, moderate and strong correlations were observed (r_s 0.47–0.71, $p \leq 0.03$; Table S3), except for pyrene. In turn, S. Wang et al. [21] compared the sum of concentrations of PAHs sequestrated in WBs and an active air sampler connected to a cartridge containing a sandwich PUF-styrene divinylbenzene copolymer (PUF/XAD/PUF), but no associations were found.

S. Wang et al. [21] also compared the total PBDEs, NBFRs, and OPEs quantitated in WBs and an AAS cartridge. No significant associations were found between these matrices for total PBDEs; however, for NBFRs and OPEs, correlations were observed (r^2 0.76 and 0.63, respectively; $p \leq 0.006$).

AAS and WBs share few similarities as personal monitors. Both approaches are capable of precise control of the temporal window covered by an individual sample [125], although AAS is more suitable for short-term studies (typically hours–days) [124,126] (see also next paragraph), whereas WBs, being a passive sampler, is utilized in long-term scenarios (usually days–weeks) [124,126,127]. As AAS samplers and WBs are worn by the subject,

both methods are useful in studies involving several microenvironments [51,52,128,129], such as home, office, and vehicle.

In many fundamental aspects, AAS and silicone WBs represent complete opposites (Figure 4). First, in contrast to WBs, AAS requires expensive, heavy, and noisy equipment [125], which may cause discomfort in participants [122], making it impractical for long-term and/or large-scale personal monitoring, especially if several subjects are to be measured simultaneously [124]. Second, AAS by design requires a power source [124] and, due to its technological sophistication [130], researchers' intervention in case of equipment failure during sample collection [48]. Third, AAS and WBs contrast sharply in the context of standardization. Ever since its first application in personal monitoring [131], AAS was closely linked to occupational exposure assessment [132,133], and numerous manuals, standards, and guidelines were published by reputable sources, such as National Institute for Occupational Safety and Health (e.g., Andrews and O'Connor [134], ASTM International [135]). To our knowledge, no such documents are available for WBs to date. Last but not least, AAS captures only inhalation exposure [124], whereas sampling with WBs includes the dermal pathway as well [21,51]. This aspect was suggested as an explanation of some differences between results obtained with AAS and WBs in both comparative studies [21,48].

7.2.3. Settled Dust

In contrast to the media discussed earlier, quantification of pollutants in settled dust is considered ambient monitoring, rather than personal [136]. Dust is a reservoir of environmental pollutants and may present exposure risk to humans, especially infants and toddlers, due to their mouthing behavior and frequent contact with the floor [137]. In all studies noted below, dust samples were collected indoors with a vacuum cleaner; therefore, the discussion that follows focuses on this method of sampling as well.

Studies assessing OPEs exposure reported few weak correlations between WBs and settled dust, in adults and children alike [72,78]. Additionally, both papers reported that concentrations in WBs better reflected internal exposure (i.e., urinary concentration of biomarkers) than in settled dust.

Concentrations of PEs in settled dust and in WBs corresponded poorly as well [72] (Table S3). Of seven correlations tested, only two weak associations were observed—for diethylphthalate ($r_s = 0.23$, $p < 0.05$) and benzylbutyl phthalate ($r_s = 0.34$, $p < 0.01$).

Modest correlations were found for the majority of PPCPs measured in WBs and settled dust by Levasseur et al. [76]. The lowest r_s was reported for butylparaben (0.23, $p < 0.05$), and the highest for triclosan (0.44, $p < 0.0001$) (Table S3). Notably, WBs correlated better with urine than settled dust within every parent compound-metabolite pair, even though study participants were children, who are more exposed to dust than other populations [76].

Some methodological aspects of the aforementioned papers should be noted. All three collected a single dust sample, and only a limited area of each household was vacuumed; this may, to some extent, account for the poor correlations observed [72,76,78]. Moreover, in case of Hammel et al. [72], different instruments were used for quantitation in WBs and settled dust. As noted earlier, two of the papers [72,76] shared the same study population.

From an exposure assessment standpoint, it is difficult to find any similarities between WBs and settled dust (Figure 4). It can be pointed out that settled dust analysis has also been criticized for insufficient standardization [117]. Indeed, many different methodologies are reported for settled dust collection via vacuuming, so even if less popular options such as wiping, brushing, or passive sampling are excluded, substantial variety remains and poses a problem for inter-study comparisons. For instance, sample collection of settled dust can be achieved through simple collection of vacuum cleaner bags from participants or vacuuming the area by researchers using household or specialized vacuum cleaners; each approach collects slightly different material. Moreover, the sample processing, especially sieving, also heavily impacts the results. Diversity of settled dust sampling methods has been reviewed in detail by Mercier et al. [17]. In contrast, a standard reference material

of indoor dust (SRM 2585) is available, which facilitates testing and comparing analytical methods between and within laboratories [138]. Moreover, a standard practice for dust collection has been published and is frequently updated [139].

The discrepancy of results described above may result from contrasting features of these matrices (Figure 4). Although the sample collection step is short, settled dust reflects average contamination from a long period of time, even several years [140]. In consequence, the temporal window covered by a settled dust sample may be difficult to control. Questionnaire data (e.g., days since last cleaning, age of a carpet) are used to estimate the time frame [141]. Moreover, humans can be exposed to dust via ingestion, inhalation (finer fractions only), and via direct contact [142], so exposure routes tracked by settled dust and WBs overlap only partially. Finally, settled dust collection via vacuuming requires cumbersome equipment that can be expensive, especially in case of specialized appliances; this poses a problem in large-scale experiments or studies investigating several microenvironments [17].

7.2.4. Other

WBs were also compared to other personal matrices, such as t-shirts [73], silicone brooches [21], or WBs worn on lapels [20,22]. A few studies investigating associations between WBs and various stationary samplers are published as well [24,47,57,143]. However, as such studies are still sparse, the reader is referred to the individual papers.

8. Future Prospects

Silicone wristbands are fairly novel sampling tools of emerging applications in exposure assessment studies. Although accessible scientific data confirm suitability of those passive samplers for such research, it should be emphasized that the content of chemicals in wristbands is considered as a semi-quantitative information, as there is no scientific ground for a fully quantitative interpretation. Further refinements and modifications are due in order to standardize methods with their employment. The first aspect of the procedure of wristband use in research that requires unifying, although has been consistent throughout studies mentioned in this review, is construction material of said samplers. Research testing conformance of wristbands coming from several disparate sources should be initiated for further validation of homogeneity and to popularize their employment in different locations around the globe.

An emergence of commercially available precleaned (and therefore prepared for prompt sampling inauguration) wristbands would be a constructive solution to the aforementioned issue.

Research regarding silicone wristbands should endeavor to achieve uniformity concerning methodology of their use. Accomplishing that will allow for more meticulous and plausible comparison of obtained findings, creating a facility for more comprehensive understanding and assessment of human exposure.

A possible prospective feature of WBs in exposure assessment studies could be amalgamating this novel sampling technique with geo-tracking of study participants either by a component of a wristband itself, or via the Global Positioning System contained within the vast majority of smartphones. Including any kind of participant trailing system in exposure assessment studies could amount to further cognition of respective environmental contribution to the overall estimated exposure depending on the time spent in each of the surroundings by the study participant, as well as the potential presence of characteristic pollutants that are to be expected in a given setting (workspace, orchard, farmland).

It would also be interesting to investigate associations between WBs and biological matrices other than urine and blood. Hair is arguably the most notable example, as it is also increasingly used in exposure assessment [144] and shares considerable similarities to WBs, such as capturing external exposure [145] and an adjustable temporal window (weeks to months) covered by a single sample [146].

Another opportunity worth considering for future method development is the application of WBs made of materials other than PDMS. Alternative building materials (or their application alongside PDMS in mixed materials passive samplers) that display different properties could potentially allow for broadening the scope of usage of wristbands for exposure assessment, as the methodology might prove to be suitable for employment for sampling further groups of substances displaying miscellaneous chemical attributes. Ionic PFASs may be a prominent example, as their hydrophilic properties prevent efficient sequestration in PDMS samplers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph19041935/s1. Table S1: List of compounds quantified in silicone wristbands in human exposure studies; Table S2: Correlations between chemicals quantified in silicone wristbands and their respective biomarkers quantified in biological matrices; Table S3: Correlations between silicone wristbands and other environmental matrices.

Author Contributions: Conceptualization, B.W., M.W. and W.R.; investigation, M.W. and W.R.; methodology, B.W., M.W. and W.R.; writing—original draft preparation, M.W. and W.R.; writing—review and editing, B.W.; visualization, M.W. and W.R.; supervision, B.W.; funding acquisition, B.W., M.W. and W.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Science Centre of Poland (grant number 2018/31/N/NZ7/02512)—Wojciech Rodzaj. Also, Wojciech Rodzaj appreciates the scholarship received from the project POWR.03.02.00-00-I035/16-00 co-financed by the European Union through the European Social Fund under the Operational Program Knowledge Education Development 2014–2020. Publication of the article was supported by the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Programme Knowledge Education Development 2014–2020. Moreover, Małgorzata Wacławik appreciates the scholarship received from the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Program Knowledge Education Development 2014–2020.

Acknowledgments: Wojciech Rodzaj would like to thank Branislav Vrana, Masaryk University, Czech Republic, for a fruitful discussion on sampling with silicone wristbands.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AAS: active air sampling; BCIPHIPP, bis(1-chloro-2-isopropyl) 1-hydroxy-2-propyl phosphate; BPA, bisphenol A; BFRs, brominated flame retardants; DPHP, diphenyl phosphate; FRs, flame retardants; GC-MS, gas chromatography-mass spectrometry; HBM, human biomonitoring; NBFRs, novel brominated flame retardants; OPEs, organophosphate esters; PAHs, polycyclic aromatic hydrocarbons; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PBFRs, polybrominated flame retardants; PDMS, poly(dimethylsiloxane); PEs, phthalate esters; PFASs, per- and polyfluoroalkyl substances; POPs, persistent organic pollutants; PPCPs, pharmaceuticals and personal care products; SPE, solid phase extraction; SVOCs, semivolatile organic compounds; PUF, polyurethane foam; QuEChERS, quick, easy, cheap, effective, rugged, and safe; TCIPP, tris(1-chloro-2-isopropyl) phosphate; TPHP, triphenyl phosphate; TWA, time-weighted average; VOCs, volatile organic compounds; WB, wristband.

References

1. UNEP. *Global Chemicals Outlook—Towards Sound Management of Chemicals*; UNEP: Geneva, Switzerland, 2013.
2. Prüss-Ustün, A.; Vickers, C.; Haefliger, P.; Bertollini, R. Knowns and unknowns on burden of disease due to chemicals: A systematic review. *Environ. Health* **2011**, *10*, 15. [CrossRef] [PubMed]
3. EEA. *Chemicals for a Sustainable Future: Report of the EEA Scientific Committee Seminar*; EEA: Luxembourg, 2018.
4. Lowry, L.K. Role of biomarkers of exposure in the assessment of human risk. *Toxicol. Lett.* **1995**, *77*, 31–38. [CrossRef]
5. Ganzleben, C.; Antignac, J.P.; Barouki, R.; Castaño, A.; Fiddicke, U.; Klánová, J.; Lebret, E.; Olea, N.; Sarigiannis, D.; Schoeters, G.R.; et al. Human biomonitoring as a tool to support chemicals regulation in the European Union. *Int. J. Hyg. Environ. Health* **2017**, *220*, 94–97. [CrossRef] [PubMed]

6. Aylward, L.L.; Hays, S.M.; Smolders, R.; Koch, H.M.; Cocker, J.; Jones, K.; Warren, N.; Levy, L.; Bevan, R. Sources of Variability in Biomarker Concentrations. *J. Toxicol. Environ. Health Part B Crit. Rev.* **2014**, *17*, 45–61. [CrossRef]
7. WHO. *Human Biomonitoring: Facts and Figures*; WHO: Copenhagen, Denmark, 2015.
8. Haines, D.A.; Saravanabhavan, G.; Werry, K.; Khoury, C. An overview of human biomonitoring of environmental chemicals in the Canadian Health Measures Survey: 2007–2019. *Int. J. Hyg. Environ. Health* **2017**, *220*, 13–28. [CrossRef]
9. Joas, A.; Knudsen, L.E.; Kolossa-Gehring, M.; Sepai, O.; Casteleyn, L.; Schoeters, G.; Angerer, J.; Castaño, A.; Aerts, D.; Biot, P.; et al. Policy recommendations and cost implications for a more sustainable framework for European human biomonitoring surveys. *Environ. Res.* **2015**, *141*, 42–57. [CrossRef]
10. Lioy, P.J. Measurement Methods for Human Exposure Analysis. *Environ. Health Perspect.* **1995**, *103*, 35–43. [CrossRef]
11. Barr, J.R.; Driskell, W.J.; Hill, R.H.; Ashley, D.L.; Needham, L.L.; Head, S.L.; Sampson, E.J.; Barr, D.B. Strategies for biological monitoring of exposure for contemporary-use pesticides. *Toxicol. Ind. Health* **1999**, *15*, 169–180. [CrossRef]
12. LaKind, J.S.; Idri, F.; Naiman, D.Q.; Verner, M.A. Biomonitoring and Nonpersistent Chemicals—Understanding and Addressing Variability and Exposure Misclassification. *Curr. Environ. Health Rep.* **2019**, *6*, 16–21. [CrossRef]
13. Porta, M.; Puigdomènech, E.; Ballester, F.; Selva, J.; Ribas-Fitó, N.; Llop, S.; López, T. Monitoring concentrations of persistent organic pollutants in the general population: The international experience. *Environ. Int.* **2008**, *34*, 546–561. [CrossRef]
14. Domingo, J.L.; Nadal, M. Human exposure to per- and polyfluoroalkyl substances (PFAS) through drinking water: A review of the recent scientific literature. *Environ. Res.* **2019**, *177*, 108648. [CrossRef] [PubMed]
15. Walsh, D.; Glass, K.; Morris, S.; Zhang, H.; McRae, I.; Anderson, N.; Alfieri, A.; Egendorf, S.P.; Holberton, S.; Owrang, S.; et al. Sediment exchange to mitigate pollutant exposure in urban soil. *J. Environ. Manag.* **2018**, *214*, 354–361. [CrossRef] [PubMed]
16. Ruiz-Jimenez, J.; Zanca, N.; Lan, H.; Jussila, M.; Hartonen, K.; Riekkola, M.L. Aerial drone as a carrier for miniaturized air sampling systems. *J. Chromatogr. A* **2019**, *1597*, 202–208. [CrossRef] [PubMed]
17. Mercier, F.; Glorennec, P.; Thomas, O.; Bot, B. Le Organic contamination of settled house dust, a review for exposure assessment purposes. *Environ. Sci. Technol.* **2011**, *45*, 6716–6727. [CrossRef] [PubMed]
18. Baum, J.L.R.; Bakali, U.; Killawala, C.; Santiago, K.M.; Dikici, E.; Kobetz, E.N.; Solle, N.S.; Deo, S.; Bachas, L.; Daunert, S. Evaluation of silicone-based wristbands as passive sampling systems using PAHs as an exposure proxy for carcinogen monitoring in firefighters: Evidence from the firefighter cancer initiative. *Ecotoxicol. Environ. Saf.* **2020**, *205*, 111100. [CrossRef] [PubMed]
19. Bergmann, A.J.; North, P.E.; Vasquez, L.; Bello, H.; Del Carmen Gastañaga Ruiz, M.; Anderson, K.A. Multi-class chemical exposure in rural Peru using silicone wristbands. *J. Expo. Sci. Environ. Epidemiol.* **2017**, *27*, 560–568. [CrossRef]
20. O'Connell, S.G.; Kincl, L.D.; Anderson, K.A. Silicone wristbands as personal passive samplers. *Environ. Sci. Technol.* **2014**, *48*, 3327–3335. [CrossRef]
21. Wang, S.; Romanak, K.A.; Stubbings, W.A.; Arrandale, V.H.; Hendryx, M.; Diamond, M.L.; Salamova, A.; Venier, M. Silicone wristbands integrate dermal and inhalation exposures to semi-volatile organic compounds (SVOCs). *Environ. Int.* **2019**, *132*, 105104. [CrossRef]
22. Craig, J.A.; Ceballos, D.M.; Fruh, V.; Petropoulos, Z.E.; Allen, J.G.; Calafat, A.M.; Ospina, M.; Stapleton, H.M.; Hammel, S.C.; Gray, R.; et al. Exposure of Nail Salon Workers to Phthalates, Di(2-ethylhexyl) Terephthalate, and Organophosphate Esters: A Pilot Study. *Environ. Sci. Technol.* **2019**, *53*, 14630–14637. [CrossRef] [PubMed]
23. Okeme, J.O.; Yang, C.; Abdollahi, A.; Dhal, S.; Harris, S.A.; Jantunen, L.M.; Tsirlin, D.; Diamond, M.L. Passive air sampling of flame retardants and plasticizers in Canadian homes using PDMS, XAD-coated PDMS and PUF samplers. *Environ. Pollut.* **2018**, *239*, 109–117. [CrossRef]
24. Aerts, R.; Joly, L.; Szternfeld, P.; Tsilikas, K.; De Cremer, K.; Castelain, P.; Aerts, J.M.; Van Orshoven, J.; Somers, B.; Hendrickx, M.; et al. Silicone Wristband Passive Samplers Yield Highly Individualized Pesticide Residue Exposure Profiles. *Environ. Sci. Technol.* **2018**, *52*, 298–307. [CrossRef] [PubMed]
25. Owen, M.J. Silicone Surface Fundamentals. *Macromol. Rapid Commun.* **2021**, *42*, 1–9. [CrossRef] [PubMed]
26. Seethapathy, S.; Górecki, T. Applications of polydimethylsiloxane in analytical chemistry: A review. *Anal. Chim. Acta* **2012**, *750*, 48–62. [CrossRef]
27. Vidi, P.A.; Anderson, K.A.; Chen, H.; Anderson, R.; Salvador-Moreno, N.; Mora, D.C.; Poutasse, C.; Laurienti, P.J.; Daniel, S.S.; Arcury, T.A. Personal samplers of bioavailable pesticides integrated with a hair follicle assay of DNA damage to assess environmental exposures and their associated risks in children. *Mutat. Res. Genet. Toxicol. Environ. Mutagenesis* **2017**, *822*, 27–33. [CrossRef]
28. Wang, S.; Romanak, K.A.; Tarallo, S.; Francavilla, A.; Viviani, M.; Vineis, P.; Rothwell, J.A.; Mancini, F.R.; Cordero, F.; Naccarati, A.; et al. The use of silicone wristbands to evaluate personal exposure to semi-volatile organic chemicals (SVOCs) in France and Italy. *Environ. Pollut.* **2020**, *267*, 115490. [CrossRef]
29. Rusina, T.P.; Smedes, F.; Klanova, J.; Booij, K.; Holoubek, I. Polymer selection for passive sampling: A comparison of critical properties. *Chemosphere* **2007**, *68*, 1344–1351. [CrossRef] [PubMed]
30. Carraher, C.E., Jr. *Introduction to Polymer Chemistry*, 4th ed.; CRC Press: Boca Raton, FL, USA, 2017; pp. 309–314.
31. Mazurek, M. Silicone Copolymer Networsk and Interpretating Polymer Networks. In *Silicon-Containing Polymers*; Jones, R.G., Ando, W., Chojnowski, J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp. 113–137.
32. Suwandi, M.S.; Stern, S.A. Transport of Heavy Organic Vapors Through Silicone Rubber. *J. Polym. Sci. Part A-2 Polym. Phys.* **1973**, *11*, 663–681. [CrossRef]

33. Brockmeyer, B.; Kraus, U.R.; Theobald, N. Accelerated solvent extraction (ASE) for purification and extraction of silicone passive samplers used for the monitoring of organic pollutants. *Environ. Sci. Pollut. Res.* **2015**, *22*, 19887–19895. [CrossRef]
34. Shahpoury, P.; Hageman, K.J. Pressurized liquid extraction of polycyclic aromatic hydrocarbons from silicone rubber passive samplers. *J. Chromatogr. A* **2013**, *1314*, 1–6. [CrossRef]
35. Smedes, F.; Booij, K. Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers. *ICES Tech. Mar. Environ. Sci.* **2012**, *52*, 20.
36. Anderson, K.A.; Points, G.L.; Donald, C.E.; Dixon, H.M.; Scott, R.P.; Wilson, G.; Tidwell, L.G.; Hoffman, P.D.; Herbstman, J.B.; O'Connell, S.G. Preparation and performance features of wristband samplers and considerations for chemical exposure assessment. *J. Expo. Sci. Environ. Epidemiol.* **2017**, *27*, 551–559. [CrossRef] [PubMed]
37. O'Connell, S.G.; Anderson, K.A.; Epstein, M.I. Determining chemical air equivalency using silicone personal monitors. *J. Expo. Sci. Environ. Epidemiol.* **2021**. [CrossRef] [PubMed]
38. Tromp, P.C.; Beeltje, H.; Okeme, J.O.; Vermeulen, R.; Pronk, A.; Diamond, M.L. Calibration of polydimethylsiloxane and polyurethane foam passive air samplers for measuring semi volatile organic compounds using a novel exposure chamber design. *Chemosphere* **2019**, *227*, 435–443. [CrossRef] [PubMed]
39. Okeme, J.O.; Saini, A.; Yang, C.; Zhu, J.; Smedes, F.; Klánová, J.; Diamond, M.L. Calibration of polydimethylsiloxane and XAD-Pocket passive air samplers (PAS) for measuring gas- and particle-phase SVOCs. *Atmos. Environ.* **2016**, *143*, 202–208. [CrossRef]
40. Sedlačková, L.; Melymuk, L.; Vrana, B. Calibration of silicone for passive sampling of semivolatile organic contaminants in indoor air. *Chemosphere* **2021**, *279*, 130536. [CrossRef]
41. Vorkamp, K.; Odsbjerg, L.; Langeland, M.; Mayer, P. Utilizing the partitioning properties of silicone for the passive sampling of polychlorinated biphenyls (PCBs) in indoor air. *Chemosphere* **2016**, *160*, 280–286. [CrossRef]
42. Allan, I.J.; Harman, C.; Ranneklev, S.B.; Thomas, K.V.; Grung, M. Passive sampling for target and nontarget analyses of moderately polar and nonpolar substances in water. *Environ. Toxicol. Chem.* **2013**, *32*, 1718–1726. [CrossRef]
43. The Observer: How a Yellow Wristband Became a Fashion Must. Available online: https://www.theguardian.com/society/2004/aug/08/cancercare.uknews (accessed on 11 April 2021).
44. Nicole, W. Wristbands for research: Using wearable sensors to collect exposure data after Hurricane Harvey. *Environ. Health Perspect.* **2018**, *126*, 1–9. [CrossRef]
45. Manzano, C.A.; Dodder, N.G.; Hoh, E.; Morales, R. Patterns of Personal Exposure to Urban Pollutants Using Personal Passive Samplers and GC × GC/ToF-MS. *Environ. Sci. Technol.* **2019**, *53*, 614–624. [CrossRef]
46. Doherty, B.T.; Pearce, J.L.; Anderson, K.A.; Karagas, M.R.; Romano, M.E. Assessment of Multipollutant Exposures During Pregnancy Using Silicone Wristbands. *Front. Public Health* **2020**, *8*, 1–13. [CrossRef]
47. Hendryx, M.; Wang, S.; Romanak, K.A.; Salamova, A.; Venier, M. Personal exposure to polycyclic aromatic hydrocarbons in Appalachian mining communities. *Environ. Pollut.* **2020**, *257*, 113501. [CrossRef] [PubMed]
48. Dixon, H.M.; Scott, R.P.; Holmes, D.; Calero, L.; Kincl, L.D.; Waters, K.M.; Camann, D.E.; Calafat, A.M.; Herbstman, J.B.; Anderson, K.A. Silicone wristbands compared with traditional polycyclic aromatic hydrocarbon exposure assessment methods. *Anal. Bioanal. Chem.* **2018**, *410*, 3059–3071. [CrossRef] [PubMed]
49. Nguyen, L.V.; Gravel, S.; Labrèche, F.; Bakhiyi, B.; Verner, M.A.; Zayed, J.; Jantunen, L.M.; Arrandale, V.H.; Diamond, M.L. Can Silicone Passive Samplers be Used for Measuring Exposure of e-Waste Workers to Flame Retardants? *Environ. Sci. Technol.* **2020**, *54*, 15277–15286. [CrossRef] [PubMed]
50. Quintana, P.J.E.; Lopez-Galvez, N.; Dodder, N.G.; Hoh, E.; Matt, G.E.; Zakarian, J.M.; Vyas, M.; Chu, L.; Akins, B.; Padilla, S.; et al. Nicotine, Cotinine, and Tobacco-Specific Nitrosamines Measured in Children's Silicone Wristbands in Relation to Secondhand Smoke and E-cigarette Vapor Exposure. *Nicotine Tob. Res.* **2020**, *23*, 592–599. [CrossRef]
51. Hammel, S.C.; Hoffman, K.; Webster, T.F.; Anderson, K.A.; Stapleton, H.M. Measuring Personal Exposure to Organophosphate Flame Retardants Using Silicone Wristbands and Hand Wipes. *Environ. Sci. Technol.* **2016**, *50*, 4483–4491. [CrossRef]
52. Kile, M.L.; Scott, R.P.; O'Connell, S.G.; Lipscomb, S.; MacDonald, M.; McClelland, M.; Anderson, K.A. Using silicone wristbands to evaluate preschool children's exposure to flame retardants. *Environ. Res.* **2016**, *147*, 365–372. [CrossRef]
53. Chen, Y.; Pawliszyn, J. Time-weighted average water sampling with a solid-phase microextraction device. *Anal. Chem.* **2003**, *75*, 2004–2010. [CrossRef]
54. Rohlman, D.S.; Syron, L.; Hobbie, K.; Anderson, K.A.; Scaffidi, C.; Sudakin, D.; Peterson, E.S.; Waters, K.M.; Haynes, E.; Arkin, L.; et al. A Community-Based Approach to Developing a Mobile Device for Measuring Ambient Air Exposure, Location, and Respiratory Health. *Environ. Justice* **2015**, *8*, 126–134. [CrossRef]
55. Donald, C.E.; Scott, R.P.; Blaustein, K.L.; Halbleib, M.L.; Sarr, M.; Jepson, P.C.; Anderson, K.A. Silicone wristbands detect individuals' pesticide exposures in West Africa. *R. Soc. Open Sci.* **2016**, *3*, 160433. [CrossRef]
56. Lipscomb, S.T.; McClelland, M.M.; MacDonald, M.; Cardenas, A.; Anderson, K.A.; Kile, M.L. Cross-sectional study of social behaviors in preschool children and exposure to flame retardants. *Environ. Health* **2017**, *16*, 1–10. [CrossRef]
57. Paulik, L.B.; Hobbie, K.A.; Rohlman, D.; Smith, B.W.; Scott, R.P.; Kincl, L.; Haynes, E.N.; Anderson, K.A. Environmental and individual PAH exposures near rural natural gas extraction. *Environ. Pollut.* **2018**, *241*, 397–405. [CrossRef] [PubMed]
58. Hammel, S.C.; Phillips, A.L.; Hoffman, K.; Stapleton, H.M. Evaluating the Use of Silicone Wristbands to Measure Personal Exposure to Brominated Flame Retardants. *Environ. Sci. Technol.* **2018**, *52*, 11875–11885. [CrossRef] [PubMed]

59. De Vecchi, R.; da Silveira Carvalho Ripper, J.; Roy, D.; Breton, L.; Germano Marciano, A.; Bernardo de Souza, P.M.; de Paula Corrêa, M. Using wearable devices for assessing the impacts of hair exposome in Brazil. *Sci. Rep.* **2019**, *9*, 1–10. [CrossRef] [PubMed]
60. Rohlman, D.S.; Donatuto, J.; Heidt, M.; Barton, M.; Campbell, L.; Anderson, K.A.; Kile, M.L. A Case Study Describing a Community-Engaged Approach for Evaluating Polycyclic Aromatic Hydrocarbon Exposure in a Native American Community. *Int. J. Environ. Res. Public Health* **2019**, *16*, 327. [CrossRef]
61. Harley, K.G.; Parra, K.L.; Camacho, J.; Bradman, A.; Nolan, J.E.S.; Lessard, C.; Anderson, K.A.; Poutasse, C.M.; Scott, R.P.; Lazaro, G.; et al. Determinants of pesticide concentrations in silicone wristbands worn by Latina adolescent girls in a California farmworker community: The COSECHA youth participatory action study. *Sci. Total Environ.* **2019**, *652*, 1022–1029. [CrossRef]
62. Gibson, E.A.; Stapleton, H.M.; Calero, L.; Holmes, D.; Burke, K.; Martinez, R.; Cortes, B.; Nematollahi, A.; Evans, D.; Anderson, K.A.; et al. Differential exposure to organophosphate flame retardants in mother-child pairs. *Chemosphere* **2019**, *219*, 567–573. [CrossRef]
63. Romanak, K.A.; Wang, S.; Stubbings, W.A.; Hendryx, M.; Venier, M.; Salamova, A. Analysis of brominated and chlorinated flame retardants, organophosphate esters, and polycyclic aromatic hydrocarbons in silicone wristbands used as personal passive samplers. *J. Chromatogr. A* **2019**, *1588*, 41–47. [CrossRef]
64. Quintana, P.J.E.; Hoh, E.; Dodder, N.G.; Matt, G.E.; Zakarian, J.M.; Anderson, K.A.; Akins, B.; Chu, L.; Hovell, M.F. Nicotine levels in silicone wristband samplers worn by children exposed to secondhand smoke and electronic cigarette vapor are highly correlated with child's urinary cotinine. *J. Expo. Sci. Environ. Epidemiol.* **2019**, *29*, 733–741. [CrossRef]
65. Zuy, Y.; Sweck, S.O.; Dockery, C.R.; Potts, G.E. HPLC detection of organic gunshot residues collected with silicone wristbands. *Anal. Methods* **2020**, *12*, 85–90. [CrossRef]
66. Rohlman, D.S.; Dixon, H.M.; Kincl, L.; Larkin, A.; Evoy, R.; Barton, M.; Phillips, A.; Peterson, E.; Scaffidi, C.; Herbstman, J.B.; et al. Development of an environmental health tool linking chemical exposures, physical location and lung function. *BMC Public Health* **2019**, *19*, 854. [CrossRef]
67. Ulrich, E.M.; Sobus, J.R.; Grulke, C.M.; Richard, A.M.; Newton, S.R.; Strynar, M.J.; Mansouri, K.; Williams, A.J. EPA's non-targeted analysis collaborative trial (ENTACT): Genesis, design, and initial findings. *Anal. Bioanal. Chem.* **2019**, *411*, 853–866. [CrossRef] [PubMed]
68. Travis, S.C.; Aga, D.S.; Queirolo, E.I.; Olson, J.R.; Daleiro, M.; Kordas, K. Catching flame retardants and pesticides in silicone wristbands: Evidence of exposure to current and legacy pollutants in Uruguayan children. *Sci. Total Environ.* **2020**, *740*, 140136. [CrossRef] [PubMed]
69. Reche, C.; Viana, M.; van Drooge, B.L.; Fernández, F.J.; Escribano, M.; Castaño-Vinyals, G.; Nieuwenhuijsen, M.; Adami, P.E.; Bermon, S. Athletes' exposure to air pollution during World Athletics Relays: A pilot study. *Sci. Total Environ.* **2020**, *717*, 137161. [CrossRef] [PubMed]
70. Reddam, A.; Tait, G.; Herkert, N.; Hammel, S.C.; Stapleton, H.M.; Volz, D.C. Longer commutes are associated with increased human exposure to tris(1,3-dichloro-2-propyl) phosphate. *Environ. Int.* **2020**, *136*, 105499. [CrossRef]
71. Caban-Martinez, A.J.; Louzado-Feliciano, P.; Santiago, K.M.; Baum, J.; Schaefer Solle, N.; Rivera, G.; Miric, M.; Perez-Then, E.; Kobetz-Kerman, E.N.; Daunert, S. Objective Measurement of Carcinogens Among Dominican Republic Firefighters Using Silicone-Based Wristbands. *J. Occup. Environ. Med.* **2020**, *62*, e611–e615. [CrossRef]
72. Hammel, S.C.; Hoffman, K.; Phillips, A.L.; Levasseur, J.L.; Lorenzo, A.M.; Webster, T.F.; Stapleton, H.M. Comparing the Use of Silicone Wristbands, Hand Wipes, and Dust to Evaluate Children's Exposure to Flame Retardants and Plasticizers. *Environ. Sci. Technol.* **2020**, *54*, 4484–4494. [CrossRef]
73. Wang, Y.; Peris, A.; Rifat, M.R.; Ahmed, S.I.; Aich, N.; Nguyen, L.V.; Urík, J.; Eljarrat, E.; Vrana, B.; Jantunen, L.M.; et al. Measuring exposure of e-waste dismantlers in Dhaka Bangladesh to organophosphate esters and halogenated flame retardants using silicone wristbands and T-shirts. *Sci. Total Environ.* **2020**, *720*, 137480. [CrossRef]
74. Wise, C.F.; Wise, C.F.; Hammel, S.C.; Herkert, N.; Ma, J.; Ma, J.; Motsinger-Reif, A.; Stapleton, H.M.; Stapleton, H.M.; Breen, M.; et al. Comparative Exposure Assessment Using Silicone Passive Samplers Indicates That Domestic Dogs Are Sentinels to Support Human Health Research. *Environ. Sci. Technol.* **2020**, *54*, 7409–7419. [CrossRef]
75. Santiago, K.M.; Louzado-Feliciano, P.; Baum, J.; Bakali, U.; Caban-Martinez, A.J. Self-reported and objectively measured occupational exposures, health, and safety concerns among fishermen: A cross-sectional Fishing Industry Safety and Health (FISH) pilot study. *Am. J. Ind. Med.* **2021**, *64*, 58–69. [CrossRef]
76. Levasseur, J.L.; Hammel, S.C.; Hoffman, K.; Phillips, A.L.; Zhang, S.; Ye, X.; Calafat, A.M.; Webster, T.F.; Stapleton, H.M. Young children's exposure to phenols in the home: Associations between house dust, hand wipes, silicone wristbands, and urinary biomarkers. *Environ. Int.* **2021**, *147*, 106317. [CrossRef]
77. Arcury, T.A.; Chen, H.; Quandt, S.A.; Talton, J.W.; Anderson, K.A.; Scott, R.P.; Jensen, A.; Laurienti, P.J. Pesticide exposure among Latinx children: Comparison of children in rural, farmworker and urban, non-farmworker communities. *Sci. Total Environ.* **2021**, *763*, 144233. [CrossRef] [PubMed]
78. Xie, Q.; Guan, Q.; Li, L.; Pan, X.; Ho, C. Exposure of children and mothers to organophosphate esters: Prediction by house dust and silicone wristbands. *Environ. Pollut.* **2021**, *282*, 117011. [CrossRef] [PubMed]

79. Dixon, H.M.; Armstrong, G.; Barton, M.; Bergmann, A.J.; Bondy, M.; Halbleib, M.L.; Hamilton, W.; Haynes, E.; Herbstman, J.; Hoffman, P.; et al. Discovery of common chemical exposures across three continents using silicone wristbands. *R. Soc. Open Sci.* **2019**, *6*, 1–16. [CrossRef] [PubMed]
80. Mantinieks, D.; Gerostamoulos, D.; Wright, P.; Drummer, O. The effectiveness of decontamination procedures used in forensic hair analysis. *Forensic Sci. Med. Pathol.* **2018**, *14*, 349–357. [CrossRef] [PubMed]
81. Żwir-Ferenc, A.; Biziuk, M. Solid Phase Extraction Technique—Trends, Opportunities and Applications. *Pol. J. Environ. Stud.* **2006**, *15*, 677–690.
82. Anastassiades, M.; Lehotay, S.J.; Stajnbaher, D.; Schenck, F.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431. [CrossRef]
83. Travis, S.C.; Kordas, K.; Aga, D.S. Optimized workflow for unknown screening using gas chromatography high-resolution mass spectrometry expands identification of contaminants in silicone personal passive samplers. *Rapid Commun. Mass Spectrom.* **2021**, *35*, 1–13. [CrossRef]
84. Wang, Z.; Dewitt, J.C.; Higgins, C.P.; Cousins, I.T. A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)? *Environ. Sci. Technol.* **2017**, *51*, 2508–2518. [CrossRef]
85. Lohmann, R.; Booij, K.; Smedes, F. Use of passive sampling devices for monitoring and compliance checking of POP concentrations in water. *Environ. Sci. Pollut. Res.* **2012**, 1885–1895. [CrossRef]
86. Villaverde-de-Sáa, E.; Racamonde, I.; Quintana, J.B.; Rodil, R.; Cela, R. Ion-pair sorptive extraction of perfluorinated compounds from water with low-cost polymeric materials: Polyethersulfone vs. *polydimethylsiloxane. Anal. Chim. Acta* **2012**, *740*, 50–57. [CrossRef]
87. Vrana, B.; (Masaryk University, Brno, Czech Republic). Personal communication, 2021.
88. Verner, M.A.; Salame, H.; Housand, C.; Birnbaum, L.S.; Bouchard, M.F.; Chevrier, J.; Aylward, L.L.; Naiman, D.Q.; Lakind, J.S. How many urine samples are needed to accurately assess exposure to non-persistent chemicals? The biomarker reliability assessment tool (BRAT) for scientists, research sponsors, and risk managers. *Int. J. Environ. Res. Public Health* **2020**, *17*, 9102. [CrossRef] [PubMed]
89. Calafat, A.M.; Longnecker, M.P.; Koch, H.M.; Swan, S.H.; Hauser, R.; Goldman, L.R.; Lanphear, B.P.; Rudel, R.A.; Engel, S.M.; Teitelbaum, S.L.; et al. Optimal Exposure Biomarkers for Nonpersistent Chemicals in Environmental Epidemiology. *Environ. Health Perspect.* **2015**, *123*, 166–168. [CrossRef] [PubMed]
90. Carignan, C.C.; McClean, M.D.; Cooper, E.M.; Watkins, D.J.; Fraser, A.J.; Heiger-Bernays, W.; Stapleton, H.M.; Webster, T.F. Predictors of tris(1,3-dichloro-2-propyl) phosphate metabolite in the urine of office workers. *Environ. Int.* **2013**, *55*, 56–61. [CrossRef] [PubMed]
91. Van den Eede, N.; Heffernan, A.L.; Aylward, L.L.; Hobson, P.; Neels, H.; Mueller, J.F.; Covaci, A. Age as a determinant of phosphate flame retardant exposure of the Australian population and identification of novel urinary PFR metabolites. *Environ. Int.* **2015**, *74*, 1–8. [CrossRef] [PubMed]
92. Bastiaensen, M.; Gys, C.; Malarvannan, G.; Fotache, M.; Bombeke, J.; Ait, Y.; Araki, A.; Covaci, A. Short-term temporal variability of urinary biomarkers of organophosphate flame retardants and plasticizers. *Environ. Int.* **2021**, *146*, 106147. [CrossRef]
93. Ding, J.; Deng, T.; Xu, M.; Wang, S.; Yang, F. Residuals of organophosphate esters in foodstuffs and implication for human exposure. *Environ. Pollut.* **2018**, *233*, 986–991. [CrossRef]
94. Poma, G.; Sales, C.; Bruyland, B.; Christia, C.; Goscinny, S.; Van Loco, J.; Covaci, A. Occurrence of Organophosphorus Flame Retardants and Plasticizers (PFRs) in Belgian Foodstuffs and Estimation of the Dietary Exposure of the Adult Population. *Environ. Sci. Technol.* **2018**, *52*, 2331–2338. [CrossRef]
95. Koch, H.M.; Lorber, M.; Christensen, K.L.Y.; Pälmke, C.; Koslitz, S.; Brüning, T. Identifying sources of phthalate exposure with human biomonitoring: Results of a 48h fasting study with urine collection and personal activity patterns. *Int. J. Hyg. Environ. Health* **2013**, *216*, 672–681. [CrossRef]
96. Li, Z.; Romanoff, L.; Bartell, S.; Pittman, E.N.; Trinidad, D.A.; McClean, M.; Webster, T.F.; Sjödin, A. Excretion Profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon metabolites after dietary exposure. *Chem. Res. Toxicol.* **2012**, *25*, 1452–1461. [CrossRef]
97. Percy, Z.; Vuong, A.M.; Ospina, M.; Calafat, A.M.; La Guardia, M.J.; Xu, Y.; Hale, R.C.; Dietrich, K.N.; Xie, C.; Lanphear, B.P.; et al. Organophosphate esters in a cohort of pregnant women: Variability and predictors of exposure. *Environ. Res.* **2020**, *184*, 109255. [CrossRef]
98. Vernet, C.; Philippat, C.; Calafat, A.M.; Ye, X.; Lyon-caen, S.; Siroux, V.; Schisterman, E.F.; Slama, R. Concentrations of Phenol Biomarkers in Pregnant Women. *Environ. Health Perspect.* **2017**, *126*, 1–12. [CrossRef]
99. Wielgomas, B. Variability of urinary excretion of pyrethroid metabolites in seven persons over seven consecutive days-Implications for observational studies. *Toxicol. Lett.* **2013**, *221*, 15–22. [CrossRef] [PubMed]
100. Choi, J.; Knudsen, L.E.; Mizrak, S.; Joas, A. Identification of exposure to environmental chemicals in children and older adults using human biomonitoring data sorted by age: Results from a literature review. *Int. J. Hyg. Environ. Health* **2017**, *220*, 282–298. [CrossRef] [PubMed]

101. Schulz, C.; Conrad, A.; Becker, K.; Kolossa-Gehring, M.; Seiwert, M.; Seifert, B. Twenty years of the German Environmental Survey (GerES): Human biomonitoring - Temporal and spatial (West Germany/East Germany) differences in population exposure. *Int. J. Hyg. Environ. Health* **2007**, *210*, 271–297. [CrossRef] [PubMed]
102. Faÿs, F.; Palazzi, P.; Hardy, E.M.; Schaeffer, C.; Phillipat, C.; Zeimet, E.; Vaillant, M.; Beausoleil, C.; Rousselle, C.; Slama, R.; et al. Is there an optimal sampling time and number of samples for assessing exposure to fast elimination endocrine disruptors with urinary biomarkers? *Sci. Total Environ.* **2020**, *747*, 141185. [CrossRef]
103. Franklin, C.A.; Muir, N.I.; Moody, R.P. The use of biological monitoring in the estimation of exposure during the application of pesticides. *Toxicol. Lett.* **1986**, *33*, 127–136. [CrossRef]
104. Klimowska, A.; Amenda, K.; Rodzaj, W.; Wileńska, M.; Jurewicz, J.; Wielgomas, B. Evaluation of 1-year urinary excretion of eight metabolites of synthetic pyrethroids, chlorpyrifos, and neonicotinoids. *Environ. Int.* **2020**, *145*, 106119. [CrossRef]
105. Meeker, J.D.; Barr, D.B.; Ryan, L.; Herrick, R.F.; Bennett, D.H.; Bravo, R.; Hauser, R. Temporal variability of urinary levels of nonpersistent insecticides in adult men. *J. Expo. Anal. Environ. Epidemiol.* **2005**, *15*, 271–281. [CrossRef]
106. Needham, L.L.; Calafat, A.M.; Barr, D.B. Uses and issues of biomonitoring. *Int. J. Hyg. Environ. Health* **2007**, *210*, 229–238. [CrossRef]
107. Thuresson, K.; Höglund, P.; Hagmar, L.; Sjödin, A.; Bergman, Å.; Jakobsson, K. Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. *Environ. Health Perspect.* **2006**, *114*, 176–181. [CrossRef]
108. Alves, A.; Kucharska, A.; Erratico, C.; Xu, F.; Hond, E.D.; Koppen, G.; Vanermen, G.; Covaci, A.; Voorspoels, S. Human biomonitoring of emerging pollutants through non-invasive matrices: State of the art and future potential. *Anal. Bioanal. Chem.* **2014**, *406*, 4063–4088. [CrossRef]
109. Appel, K.E.; Gundert-Remy, U.; Fischer, H.; Faulde, M.; Mross, K.G.; Letzel, S.; Rossbach, B. Risk assessment of Bundeswehr (German Federal Armed Forces) permethrin-impregnated battle dress uniforms (BDU). *Int. J. Hyg. Environ. Health* **2008**, *211*, 88–104. [CrossRef] [PubMed]
110. Lees, P.S.J.; Corn, M.; Breysse, P.N. Evidence for Dermal Absorption as the Major Route of Body Entry During Exposure of Transformer Maintenance and Repairmen to PCBs. *Am. Ind. Hyg. Assoc. J.* **1987**, *48*, 257–264. [CrossRef] [PubMed]
111. Piotrowski, J. Further investigations on the evaluation of exposure to nitrobenzene. *Br. J. Ind. Med.* **1967**, *24*, 60–65. [CrossRef] [PubMed]
112. Weschler, C.J.; Bekö, G.; Koch, H.M.; Salthammer, T.; Schripp, T.; Toftum, J.; Clausen, G. Transdermal uptake of diethyl phthalate and di(n-butyl) phthalate directly from air: Experimental verification. *Environ. Health Perspect.* **2015**, *123*, 928–934. [CrossRef]
113. McArthur, B. Dermal Measurement and Wipe Sampling Methods: A Review. *Appl. Occup. Environ. Hyg.* **1992**, *7*, 599–606. [CrossRef]
114. Stapleton, M.H.; Kelly, S.M.; Allen, J.G.; Mcclean, M.D.; Webster, T.F. Measurement of polybrominated diphenyl ethers on hand wipes: Estimating exposure from hand-to-mouth contact. *Environ. Sci. Technol.* **2008**, *42*, 3329–3334. [CrossRef]
115. Brouwer, D.H.; Boeniger, M.F.; Van Hemmen, J. Hand wash and manual skin wipes. *Ann. Occup. Hyg.* **2000**, *44*, 501–510. [CrossRef]
116. Durham, W.F.; Wolfe, H.R. Measurement of the exposure of workers to pesticides. *Bull. World Health Organ.* **1962**, *26*, 75–91.
117. Stapleton, H.M.; Eagle, S.; Sjödin, A.; Webster, T.F. Serum PBDEs in a North Carolina toddler cohort: Associations with handwipes, house dust, and socioeconomic variables. *Environ. Health Perspect.* **2012**, *120*, 1049–1054. [CrossRef]
118. Behroozy, A. On dermal exposure assessment. *Int. J. Occup. Environ. Med.* **2013**, *4*, 113–127. [PubMed]
119. Curwin, B.D.; Hein, M.J.; Sanderson, W.T.; Nishioka, M.G.; Buhler, W. Nicotine exposure and decontamination on tobacco harvesters' hands. *Ann. Occup. Hyg.* **2005**, *49*, 407–413. [CrossRef] [PubMed]
120. Gong, M.; Zhang, Y.; Weschler, C.J. Measurement of phthalates in skin wipes: Estimating exposure from dermal absorption. *Environ. Sci. Technol.* **2014**, *48*, 7428–7435. [CrossRef] [PubMed]
121. Liu, X.; Yu, G.; Cao, Z.; Wang, B.; Huang, J.; Deng, S.; Wang, Y. Occurrence of organophosphorus flame retardants on skin wipes: Insight into human exposure from dermal absorption. *Environ. Int.* **2017**, *98*, 113–119. [CrossRef]
122. Papadopoulou, E.; Padilla-Sanchez, J.A.; Collins, C.D.; Cousins, I.T.; Covaci, A.; de Wit, C.A.; Leonards, P.E.G.; Voorspoels, S.; Thomsen, C.; Harrad, S.; et al. Sampling strategy for estimating human exposure pathways to consumer chemicals. *Emerg. Contam.* **2016**, *2*, 26–36. [CrossRef]
123. Watkins, D.J.; McClean, M.D.; Fraser, A.J.; Weinberg, J.; Stapleton, H.M.; Sjödin, A.; Webster, T.F. Impact of dust from multiple microenvironments and diet on PentaBDE body burden. *Environ. Sci. Technol.* **2012**, *46*, 1192–1200. [CrossRef]
124. Bohlin, P.; Jones, K.C.; Strandberg, B. Occupational and indoor air exposure to persistent organic pollutants: A review of passive sampling techniques and needs. *J. Environ. Monit.* **2007**, *9*, 501–509. [CrossRef]
125. Wang, B.; Zhao, Y.; Lan, Z.; Yao, Y.; Wang, L.; Sun, H. Sampling methods of emerging organic contaminants in indoor air. *Trends Environ. Anal. Chem.* **2016**, *12*, 13–22. [CrossRef]
126. National Research Council. *Exposure Science in the 21st Century: A Vision and a Strategy*; The National Academies Press: Washington, DC, USA, 2012.
127. Nieuwenhuijsen, M. Personal Exposure Monitoring and Modeling. In *Exposure Assessment in Environmental Epidemiology*; Nieuwenhuijsen, M., Ed.; Oxford University Press: Oxford, UK, 2016; pp. 1–32.

128. Chen, Y.; Du, W.; Shen, G.; Zhuo, S.; Zhu, X.; Shen, H.; Huang, Y.; Su, S.; Lin, N.; Pei, L.; et al. Household air pollution and personal exposure to nitrated and oxygenated polycyclic aromatics (PAHs) in rural households: Influence of household cooking energies. *Indoor Air* **2017**, *27*, 169–178. [CrossRef]
129. Xu, F.; Giovanoulis, G.; Van Waes, S.; Padilla-Sanchez, J.A.; Papadopoulou, E.; Magnér, J.; Haug, L.S.; Neels, H.; Covaci, A. Comprehensive study of human external exposure to organophosphate flame retardants via air, dust, and hand wipes: The importance of sampling and assessment strategy. *Environ. Sci. Technol.* **2016**, *50*, 7752–7760. [CrossRef]
130. Król, S.; Zabiegała, B.; Namieśnik, J. Monitoring and analytics of semivolatile organic compounds (SVOCs) in indoor air. *Anal. Bioanal. Chem.* **2011**, *400*, 1751–1769. [CrossRef] [PubMed]
131. Sherwood, R.J.; Greenhalgh, D.M. A Personal air sampler. *Ann. Occup. Hyg.* **1960**, *2*, 127–132. [CrossRef] [PubMed]
132. Cherrie, J.W. The beginning of the science underpinning occupational hygiene. *Ann. Occup. Hyg.* **2003**, *47*, 179–185. [CrossRef]
133. Rappaport, S.M. Implications of the exposome for exposure science. *J. Expo. Sci. Environ. Epidemiol.* **2011**, 5–9. [CrossRef] [PubMed]
134. NIOSH Manual of Analytical Methods (NMAM). Available online: https://www.cdc.gov/niosh/nmam/ (accessed on 28 July 2021).
135. ASTM D4861-17, Standard Practice for Sampling and Selection of Analytical Techniques for Pesticides and Polychlorinated Biphenyls in Air. Available online: https://www.astm.org/d4861-17.html (accessed on 28 July 2021).
136. Weisskopf, M.G.; Webster, T.F. Trade-offs of Personal Versus More Proxy Exposure Measures in Environmental Epidemiology. *Epidemiology* **2017**, *28*, 635–643. [CrossRef]
137. Cao, Z.; Yu, G.; Chen, Y.; Cao, Q.; Fiedler, H.; Deng, S. Particle size: A missing factor in risk assessment of human exposure to toxic chemicals in settled indoor dust. *Environ. Int.* **2012**, *49*, 24–30. [CrossRef]
138. Bergh, C.; Luongo, G.; Wise, S.; Östman, C. Organophosphate and phthalate esters in standard reference material 2585 organic contaminants in house dust. *Anal. Bioanal. Chem.* **2012**, *402*, 51–59. [CrossRef]
139. ASTM D5438-17, Standard Practice for Collection of Floor Dust for Chemical Analysis. Available online: https://www.astm.org/d5438-17.html (accessed on 28 July 2021).
140. Whitehead, T.; Metayer, C.; Buffler, P.; Rappaport, S.M. Estimating exposures to indoor contaminants using residential dust. *J. Expo. Sci. Environ. Epidemiol.* **2011**, *21*, 549–564. [CrossRef]
141. Lioy, P.J.; Freeman, N.C.G.; Millette, J.R. Dust: A metric for use in residential and building exposure assessment and source characterization. *Environ. Health Perspect.* **2002**, *110*, 969–983. [CrossRef]
142. Lanzerstorfer, C. Variations in the composition of house dust by particle size. *J. Environ. Sci. Health Part A* **2017**, *52*, 770–777. [CrossRef]
143. Wang, S.; Romanak, K.A.; Hendryx, M.; Salamova, A.; Venier, M. Association between Thyroid Function and Exposures to Brominated and Organophosphate Flame Retardants in Rural Central Appalachia. *Environ. Sci. Technol.* **2020**, *54*, 325–334. [CrossRef] [PubMed]
144. Appenzeller, B.M.R. *Hair Analysis for the Biomonitoring of Human Exposure to Organic Pollutants*; Elsevier: Amsterdam, The Netherlands, 2015.
145. Appenzeller, B.M.R.; Tsatsakis, A.M. Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: State of the art, critical review and future needs. *Toxicol. Lett.* **2012**, *210*, 119–140. [CrossRef] [PubMed]
146. Fäys, F.; Hardy, E.M.; Palazzi, P.; Haan, S.; Beausoleil, C.; Appenzeller, B.M.R. Biomonitoring of fast-elimination endocrine disruptors—Results from a 6-month follow up on human volunteers with repeated urine and hair collection. *Sci. Total Environ.* **2021**, *778*, 146330. [CrossRef] [PubMed]

MSc Małgorzata Wacławik
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches"*.Int. J. Environ. Res. Public Health 2022, 19, 1935.

Which is part of my doctoral dissertation, my participation in its creation involved research conceptualization, literature investigation and preparation of the original manuscript.

My contribution in preparation of this work has been estimated to sum up to 40%.

Wacławik
(signature)

MPharm Wojciech Rodzaj
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches"*.Int. J. Environ. Res. Public Health 2022, 19, 1935.

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved research conceptualization, literature investigation and preparation of the original manuscript.

My contribution in preparation of this work has been estimated to sum up to 40%.

Wojciech Rodzaj
.....
(signature)

Bartosz Wielgomas, PhD, Assoc. Prof.
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches"*.Int. J. Environ. Res. Public Health 2022, 19, 1935.

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved project conceptualization, reviewing and editing of the original manuscript, as well as research supervision.

My contribution in preparation of this work has been estimated to sum up to 20%.

.....
(signature)

Manuscript 2 - *Małgorzata Wacławik, Dominika Skwarło, Joanna Jurewicz, Bartosz Wielgomas. „Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites – a pilot study preceded by development of cost-effective GC-ECD method”* (working title) – submission to Exposure and Health

Sumbission to: Exposure and Health

Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites – a pilot study preceded by development of cost-effective GC-ECD method

Małgorzata Wacławik[1], Dominika Skwarło[1], Joanna Jurewicz[2], Bartosz Wielgomas[1]*

[1]Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, 107 Hallera Street, 80-416 Gdańsk, Poland

[2]Department of Chemical Safety, Nofer Institute of Occupational Medicine, 8 Teresy St., 91-348 Łódź, Poland

*corresponding author: bartosz.wielgomas@gumed.edu.pl

Authors:

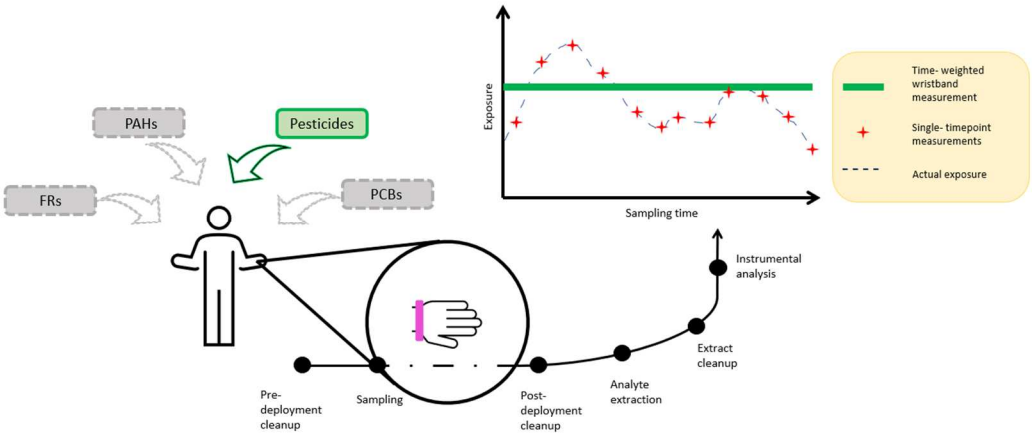
Małgorzata Wacławik –0000-0002-9288-2180

Dominika Skwarło – no ORCID number available, graduate student

Joanna Jurewicz - 0000-0001-9645-0134

Bartosz Wielgomas - 0000-0003-4245-9197

Graphical abstract:



Abstract

A novel approach to exposure assessment to synthetic pyrethroids includes involvement of silicone wristbands. This paper describes development and optimization of method for analysis of silicone wristband samples for exposure measurement of selected synthetic pyrethroids, namely: cyhalothrin, cyfluthrin, permethrin, cypermethrin, deltamethrin and flumethrin, and a subsequent pilot study, completed on (n = 24) volunteers, comprising a week-long sampling period, analysis of paired urine samples (metabolites) and wristbands (parent compounds). Permethrin was the most frequently detected (58.3%) in wristbands, its geometric mean concentration was 79.64 ng per 1 g. The most frequently detected metabolite in urine was 3-phenoxybenzoic acid (3-PBA) (68.06%). Geometric mean concentrations of urinary pyrethroid biomarkers varied from 0.21 ng/mL for 3-PBA to 0.08 ng/mL in case of *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA). Higher concentrations of urinary metabolites (3-PBA, DBCA, *cis*- and *trans*-DCCA), as well as higher concentrations of permethrin in the wristbands, were observed in study participants who declared a 5-year history of pest control in occupied buildings. In turn, the concentrations of 3-PBA, *cis*-DCCA, *trans*-DCCA, and permethrin were higher in people using insecticides in their homes. Both the ownership of pets and the use of antiparasitic veterinary drugs on them also resulted in significantly higher concentrations of metabolites in urine and permethrin in wristbands. Consistency was observed in the identification of predictors of exposure based on the concentrations of 4 metabolites in urine and the concentration of permethrin in the wristband. Thus, the silicone wristbands turned out to be a tool that allows for the qualitative and quantitative assessment of exposure and the detection of key sources of pyrethroids in a minimally invasive way. In addition, the wristbands enable the indication of the parent compound to which a given person is exposed, becoming a complementary tool to human biomonitoring for use in testing exposure to synthetic pyrethroids.

Keywords: silicone wristbands; exposure; synthetic pyrethroids; biomonitoring; method optimization

1. Introduction

The emergence of synthetic pyrethroids in place of their natural forerunners, pyrethrins, took place due to poor environmental stability of the latter, over 70 years ago (Bradberry et al. 2005). Since then, pyrethroids have enlarged their scope of utility to many areas of industry, agriculture, veterinary medicine, and forestry, mainly in pest control (Kaneko 2011). Their mechanism of action is known to be connected with modulating the action of sodium channels, which results in prolonged depolarization of nerve cells (Chrustek et al. 2018). Described mechanism is over 2000 times more effective in insect nervous systems than mammals, due to higher affinity, and thus, sensitivity of insect sodium channels to pyrethroids (Bradberry et al. 2005). Although pyrethroids are thus assumed to pose minimal threat for human health, there are several studies that indicate a possibility of these compounds to become of higher concern in the future, by presenting their potential detrimental effects on mammals and humans, (Elbetieha et al. 2001; Moniz et al. 2005). Given the widespread use of pyrethroids, it is of utmost importance that wide-scale exposure assessment studies are carried out consistently and with use of reliable, validated sampling methods. Measurement of pyrethroids metabolites concentrations in urine is considered a "gold standard" in human exposure assessment. Chemicals characterized by a short half-life, such as pyrethroids are usually measured (their metabolites) in urine, so spot sample concentration represents very brief period of time, within hours from exposure (Wielgomas 2013; Koch et al. 2014). Thus, single measurement of biomarker in biological matrix (especially urine) represents a "snapshot" of exposure. A mean of personal passive sampling, that allows for capturing a large cohort of diverse chemicals (including those metabolized rapidly) ; (Doherty et al. 2020; Wise et al. 2020) over a prolonged sampling period, therefore achieving a time-weighted average of exposure values to said chemicals (Manzano et al. 2019), are silicone wristbands. This non-invasive exposure assessment method (Bergmann et al. 2017; Dixon et al. 2019; Travis et al. 2020) has been an object of interest for many research groups since their introduction by O'Connell in 2014 (O'Connell, Steven G., Laurel D. Kincl 2014). Silicone wristbands (further: WBs) can provide information regarding both inhalatory and dermal routes of exposure (Bergmann et al. 2017; Reddam et al. 2020), and due to being easily accessible, cheap (Bergmann et al. 2017), unobtrusive and simple in use, might serve as a tool of great utility in large-scale exposure assessment studies (Manzano et al. 2019; Baum et al. 2020), especially among sensitive populations (the elderly, children, pregnant women) (Doherty et al. 2020; Travis et al. 2020). WBs being a relatively novel tool in personal passive sampling, lack standardized methodologies. Unification of said procedure should be considered the first necessary step in order to facilitate global use of WBs in exposure assessment, and to enable comparison of achieved results (Wacławik et al. 2022).

The aim of this paper is to summarize results of a pilot study concerning exposure assessment to synthetic pyrethroids with the use of silicone wristbands as personal passive samplers, compared to levels of urinary pyrethroid metabolites quantified in simultaneously collected urine samples, as well as to present results of series of experiments carried out to optimize the method of exposure assessment to synthetic pyrethroids in WBs. The aspects of sample preparation evaluated within this study involved pre-deployment cleanup of wristbands, post-deployment rinsing, extraction time, extraction method and extract cleanup procedure. The native substances quantified in WBs had been: cyhalothrin, cyfluthrin, permethrin, cypermethrin, deltamethrin and flumethrin, which authors considered to be of most importance given their versatile applications, and therefore consequently, widespread use. Selected substances are one of the most frequently found in commercially available every day-use products of insecticidal properties, which leads to an ever-pressing need to carry out a widespread exposure assessment to those compounds among the general population. The selection of analytes of interest has also been carried out with a view to upcoming studies to be carried out at our laboratory. Determining parent pyrethroid compounds in silicone wristbands could provide valuable additional information for exposure assessment and serve as a significant supplement to biomonitoring. Furthermore, investigation of patterns of substances quantified in urine and wristbands sampled simultaneously may yield new conclusions regarding routes and sources of

exposure to synthetic pyrethroids. To our knowledge, his is the first study to assess exposure to synthetic pyrethroids simultaneously using silicone wristbands and urine sample analysis.

2. Materials and methods

All silicone wristbands employed in this study have been purchased in bulk from an online vendor (www.allegro.pl), sold as an accessory used for marketing purposes or event tags (items not predefined for research purposes). For method development white wristbands have been used (avg. weight before pre-exposure cleanup: 5.05 g, avg. weight after pre-exposure cleanup: 4.86 g). Width: 12 mm, length: 20.13 cm (SD = 0.095; CV = 0.47%). Average thickness: 1.48 mm (SD = 0.19; CV = 13.05%).

Solvents used in this study involved: ethyl acetate (EtAc) (for gas chromatography MS, Supelco, Saint Louis, USA), n-hexane (Hex) (n-hexane 95% for GC, for pesticide residue analysis, POCH, Gliwice, Poland), diethyl ether (ACS grade, Sigma-Aldrich, Saint Louis, USA), methanol (MeOH) (technical grade, POCH, Gliwice, Poland), n-hexane (fraction from petroleum pure, POCH, Gliwice, Poland), 2-propanol (IPA) (for HPLC, 99.9%, Sigma-Aldrich, Saint Louis, USA), and ethyl acetate (technical grade, POCH, Gliwice, Poland). Deionized water (DI H_2O) was obtained from the laboratory water demineralizer (Hydrolab, Wiślina, Poland).

Sorbents used in described experiments included: Z-Sep Supel™QuE, Z-Sep+ Supel™QuE (Sigma-Aldrich, Saint Louis,USA), graphitized carbon black (GCB) – SampliQ Carbon SPE Bulk Sorbent (Agilent Technologies, Santa Clara, USA), primary secondary amine (PSA) (Scharlab, Barcelona, Spain), Florisil (Fluka, Buchs, Switzerland) and silica gel: pore size 60Å, 220-240 mesh particle size (Sigma-Aldrich, Saint Louis, USA).

Other reagents used in this study included: 1,1,1,3,3,3-Hexafluoro-2-propanol (Sigma- Aldrich, USA), DIC (N,N'-diisopropylcarbodiimide) (99%, Sigma Aldrich, Saint Louis, USA), potassium carbonate-anhydrous pure p.a. (POCH, Gliwice, Poland), sodium hydroxide pure p.a. (POCH, Gliwice, Poland), and hydrochloric acid (J.T. Baker, Radnor, USA).

2.1. Method development

A series of experiments was carried out do develop and to optimize the method for determination of the parent synthetic pyrethroids in WBs. The aspects of sample preparation evaluated involved: pre-deployment cleanup of wristbands, post-deployment rinsing, extraction time, extraction method and extract cleanup procedure. Respective experiments along with results obtained from them are further described in detail.

2.2. Pre-exposure cleanup

Pre-exposure cleanup is a step carried out with the aim of making commercially acquired silicone matrix more suitable for its use as passive samplers by removing surface-bound and production-originating impurities. As other studies reported (O'Connell, Steven G., Laurel D. Kincl 2014; Anderson et al. 2017) the background noise generated by these impurities, which is noticeable during instrumental analysis of an uncleaned wristband extract, can be significant, making it difficult to identify and quantify the analytes. Most research papers published between 2014 and 2022 report employing a series of WB washes in mixtures of various solvents (O'Connell, Steven G., Laurel D. Kincl 2014; Travis et al. 2020). In this study we investigated the influence of this step has on wristband-originating contaminants.

A method of preliminary washing silicone WBs consisted of placing 10 of said wristbands in 500 mL glass jars with screw-on tops (to ensure air-tightness) and performing five consecutive washes using

500 mL of different solvent mixtures as follows: three washes with ethyl acetate: hexane (1:1, v:v) followed by two washes with ethyl acetate: methanol (1:1, v:v). After each washing, resulting solvent/extract was collected. Once the solvent mixture was placed in a jar with WBs, the jar was being closed, and placed on a vortex shaker for 30 minutes (for each wash), at 900 rpm. The number of WBs placed in each jar ensured that all the samplers were submerged in the solvent mixture, while simultaneously allowed for the samplers not to conglomerate, enabling an effective cleanup to occur. The solvents used for pre-exposure cleanup were of technical grade, which ensured cost-effectiveness of the procedure.

During method development collected solvents/extracts were examined using gas chromatography coupled with mass spectrometry (GC-MS), to monitor the efficacy of cleanup procedure. The samples were analyzed in total ion current (TIC) mode and with the use of NIST mass spectral library the most prominent peaks revealed to be siloxanes. The overall effectiveness of the pre-exposure cleanup was assessed using the total peak area of the five most significant impurities (*see Fig. 1 A-B)*. Cleanup procedure allows for a significant reduction in noise area (by 99.84%). Employed cleanup procedure is effective, and yields wristbands clean enough to be used in analysis of trace amounts of substances of interest. The pre-exposure cleanup procedure we chose had additional advantages beyond its effectiveness, such as being relatively cost-effective and efficient, as multiple WBs can be prepared at the same time, and the entire process can be completed with the use of the simplest laboratory equipment. GC-MS chromatograms in *Fig. 1C* present TIC analysis of post-wash solvent mixtures pictorially displays the capability of the procedure.

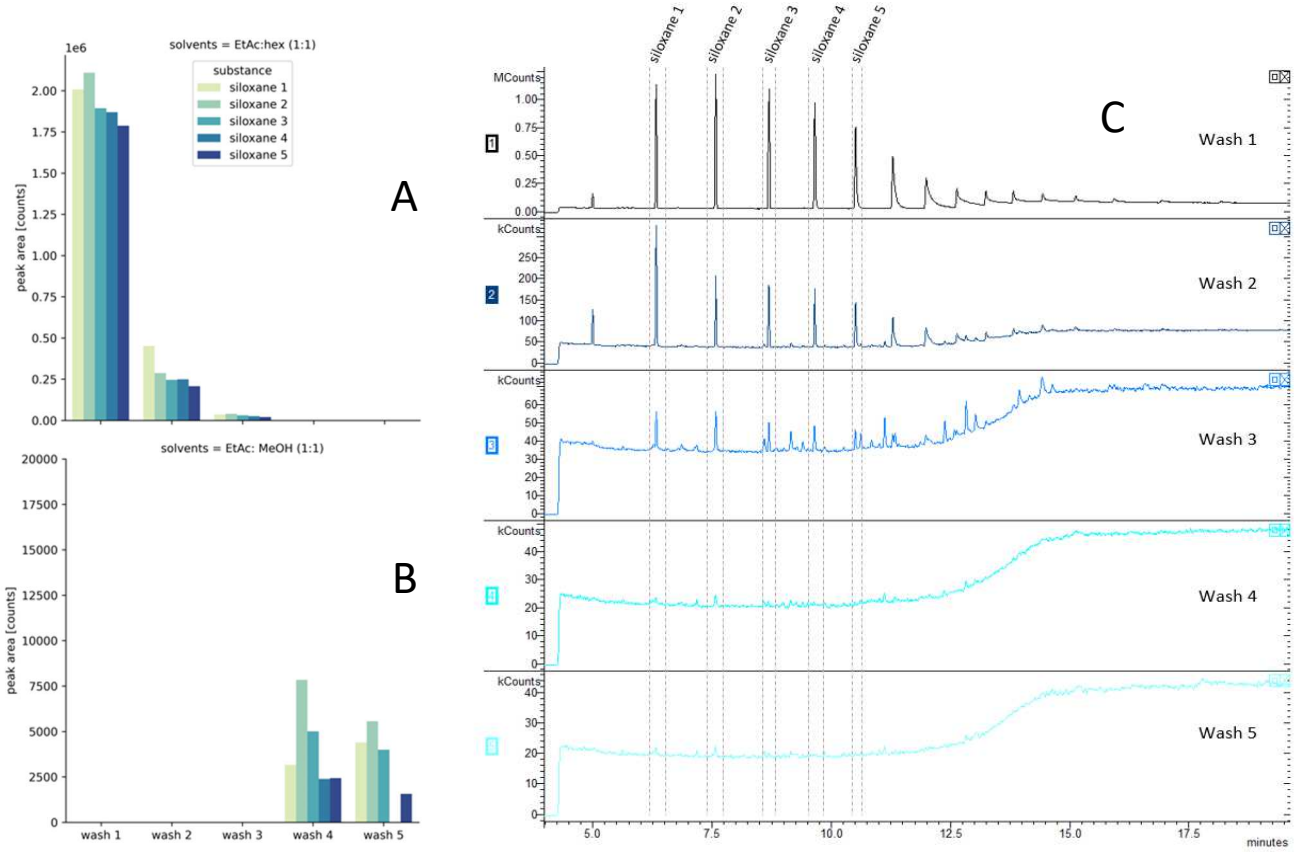


Fig. 1. Decline of peak areas for siloxanes extracted from the WB matrix via pre-exposure cleanup (A and B, please take note on the differences in y axis ranges between Fig 1. A and B). TIC chromatograms acquired via GC-MS analysis of solvent mixes used for pre-exposure cleanup of silicone wristbands (C).

2.3. Post-exposure cleanup

In principle, post-exposure cleanup is done to cleanse the surface of a previously worn wristband from contaminants such as sebum or visible residues of used toiletries. In currently available literature most studies that included the step of post-exposure wristband cleanup refer to it as 'rinsing' or 'washing' (O'Connell, Steven G., Laurel D. Kincl 2014) of WBs with either deionized water (DI H_2O) or 2-propanol (IPA), or both, as a sequence of washes. To investigate the potential influence of post-exposure cleanup on the levels of WB-absorbed analytes of interest, 0.5 g batches of pyrethroid-fortified WBs (100 ng/g WB) were rinsed either with DI H_2O, IPA, or sequentially with both, in screw-on top glass tubes (ø16mm) by applying vortex mixing. Each rinse of an individual wristband sample was timed strictly to standardize the contact time between the wristband and the solvent. After completing the 30-second wash interval, the used solvent (3 mL) was immediately drawn back from the glass tube using a pipette. After washing the concentrations of pyrethroids in wristbands were measured and the determined pyrethroids content was compared to the amount added to the WBs prior to washing and expressed as a percentage.

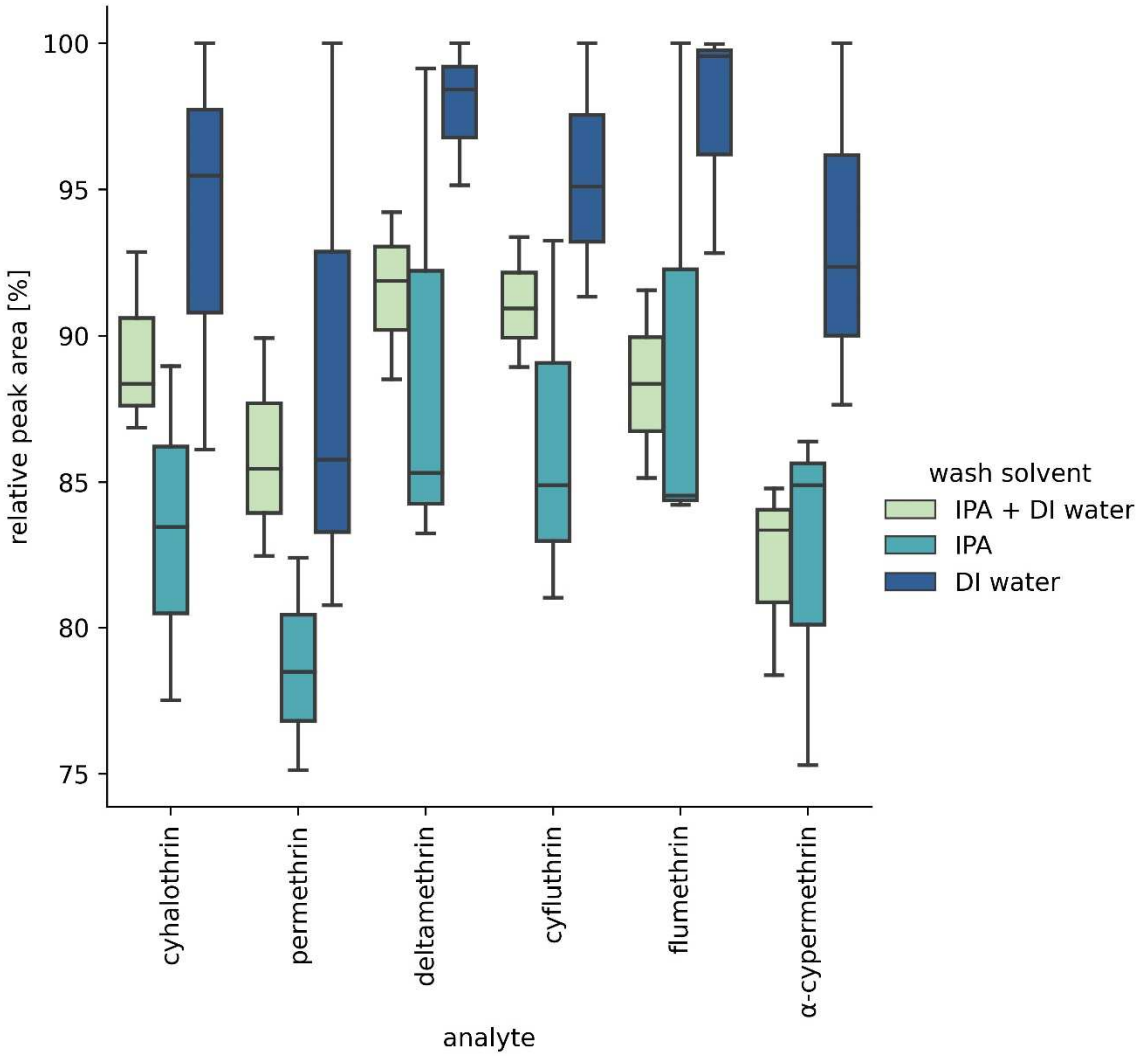


Fig. 2. Comparison of relative analyte peak areas achieved with performing varied scenarios of post-exposure silicone wristband cleanup.

Rinsing WBs with IPA in any of the two tested scenarios caused a slight reduction of signals collected during the instrumental analysis (Fig. 2), however no statistically significant differences between the tested rinsing scenarios were observed. Therefore, it can be summarized, that earlier explained post-exposure cleanup procedure does not seem to cause statistically significant analyte loss in any of the tested configurations, making its use risk-free. Since the use of post-exposure cleanup on worn WBs effectively removes visible surface contaminants without the risk of analyte loss, it has been concluded that its use would be beneficial, and so a two-step post-exposure cleanup has been added to our procedure (rinsing with IPA and deionized water).

2.4. Extraction

Choice of extraction protocol should be aimed for utilizing the most sustainable options, with taking into consideration minimalization of waste production, and energy usage. Literature regarding the use of WBs has so far presented the use of various techniques of performing that step of sample preparation. Some of the methods involved more complex approaches than others (Wacławik et al. 2022), for example Soxhlet extraction (Hammel et al. 2016), however most papers report conducting solid-liquid extraction via performing a series of solvent washes while using simple, commonly accessible laboratory hardware such as: an orbital shaker (O'Connell, Steven G., Laurel D. Kincl 2014; Baum et al. 2020), an overhead shaker (Aerts et al. 2018), a magnetic stir plate (Zuy et al. 2019), or a sonic bath (Wang et al. 2020). With the intention of keeping the favorable simplicity and cost-effectiveness of employment of WBs in research, as well as by taking into account extraction approaches presented in studies published thus far, we opted for two-fold optimization of the extraction step, by investigating different techniques (with the use of basic laboratory equipment), as well as the time needed for the process to be efficient in terms of analyte recovery.

Experiments conducted to examine the analyte recovery during solvent extraction were performed in 16×100 mm screw cap glass tubes on 0.5 g batches of WBs spiked to a concentration of 100 ng/g (each analyte). Two 30 minutes long extractions were performed with 5 mL of ethyl acetate per extraction. To facilitate rapid extraction: agitation with the use of a multitube vortex (2000 rpm), a tube roller mixer, sonication bath, or simple soaking without mixing were tested for their efficiency. The total of 10 mL of thus acquired ethyl acetate-extract has been considered a primary sample extract. Recovery of analytes was used to compare the performance of each of the techniques and all results had been compared to 'reference samples', which were blank WB extracts spiked with analytes of interest post-extraction.

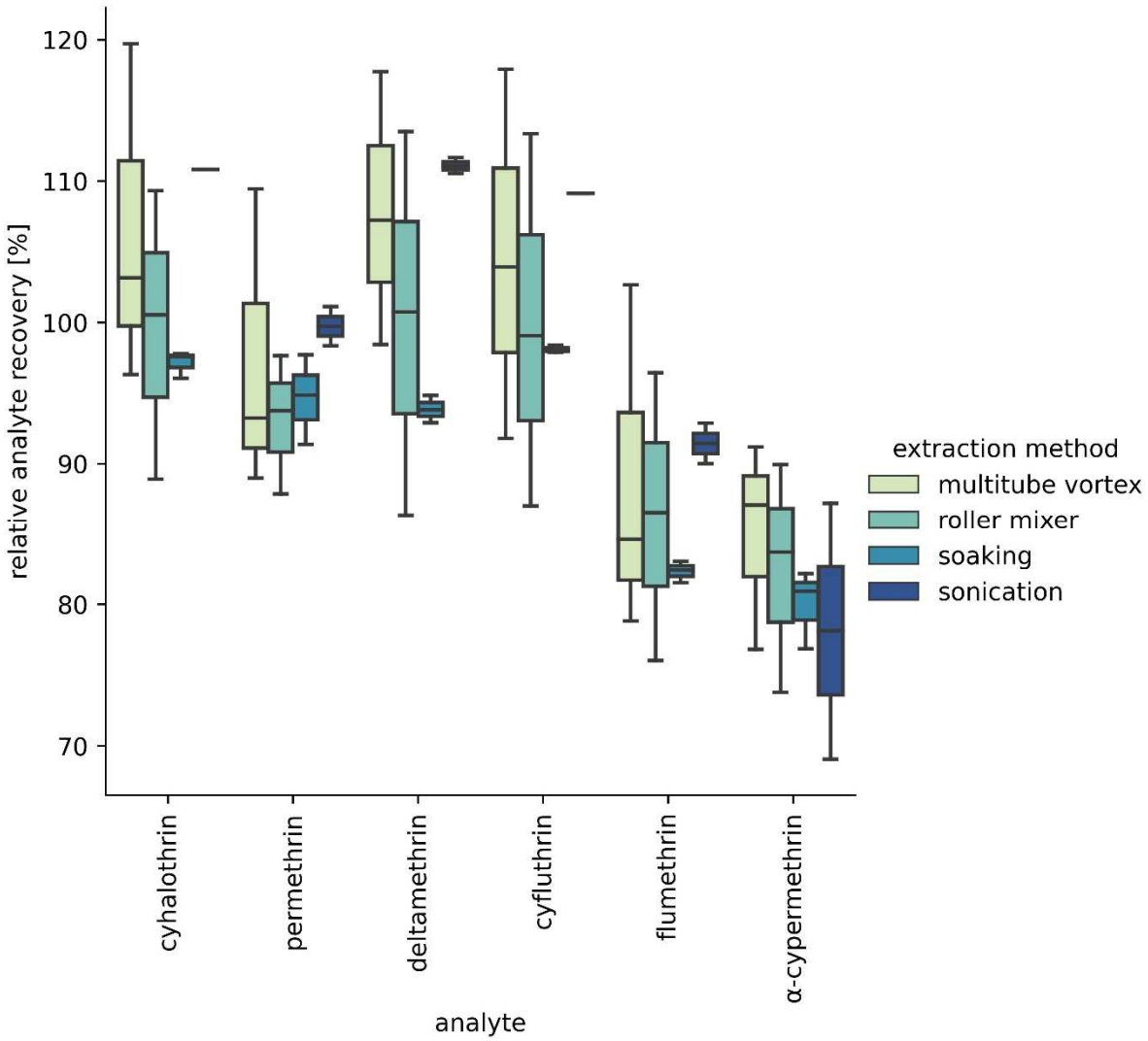


Fig. 3. Comparison of relative analyte recoveries achieved with performing varied scenarios of extraction techniques.

Relative analyte recoveries visualized in Fig. 3 allow concluding that sonication is the extraction technique producing the highest and most reproducible analyte recoveries for most of analytes of interest (CV values: 0.004% - cyhalothrin, 1.96% - permethrin, 0.01% - cyfluthrin, 0.7% - deltamethrin, 2.24% - flumethrin, 16.4% - α-cypermethrin), and thus has been proven to be the most efficient of all the tested techniques performed with the use of basic laboratory equipment.

The next step was to optimize extraction time. Individual batches of 0.5 g of WBs spiked with analytes to a concentration of 100 ng/g WB were extracted for 1, 5, 15, 30 and 60 minutes. Peak areas were used to express extraction recovery (Fig. 4.).

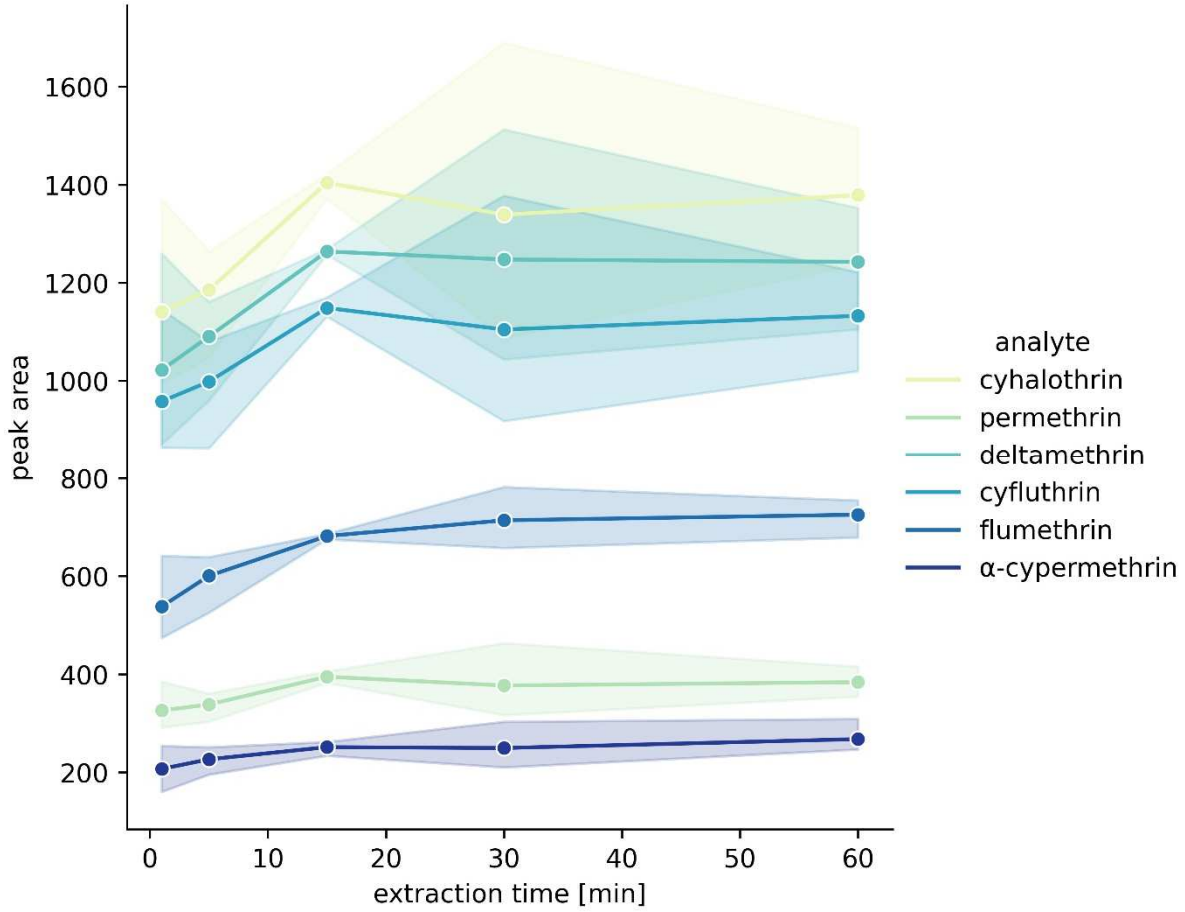


Fig. 4. Comparison of analyte peak areas achieved with performing varied extraction time intervals.

Results of this experiment had shown that the 15-minute extraction warrants maximum recovery of synthetic pyrethroids from silicone wristband matrix. Not only was recovery of analytes (compared as peak areas) for most analyzed substances (with the exception of flumethrin and cyfluthrin) the highest in case of a 15-minute interval, but the coefficients of variation for all of the tested substances were considerably lower in case of the 15-minute extraction (and were within the range of 0.48% for deltamethrin, to 3.08% for permethrin) in comparison to other extraction times.

2.5. Extract cleanup

2.5.1. Silica gel and florisil cleanup

Primary extract of silicone WBs requires at least some cleanup to remove unwanted components which might further interfere during instrumental analysis. The vast majority of published studies thus far involving silicone wristbands opted for performing solid phase extraction (SPE) as extract cleanup procedure (Hammel 2016; Arcury et al. 2021). We compared the most common and easily accessible sorbents: silica gel (at 3% and 10% of deactivation), and florisil (3% deactivation).

Custom glass chromatography columns of 10 mm internal diameter were packed with two sorbent layers starting from the bottom: respective sorbent (500 mg) and additional layer of sodium sulfate (25 mg). Columns were first conditioned with 2 mL of n-hexane and then 1 mL of solvent exchanged (to n-hexane) primary WB extract was dispensed onto conditioned column and then eluted with 4 mL of n-hexane and 4 mL of 30% diethyl ether solution in n-hexane (2nd fraction), consecutively. Hexane

fraction was discarded while 2nd fractions were collected and further evaporated under the gentle stream of nitrogen at 40°C, then reconstituted in 1 mL of n-hexane and subjected to GC-ECD analysis.

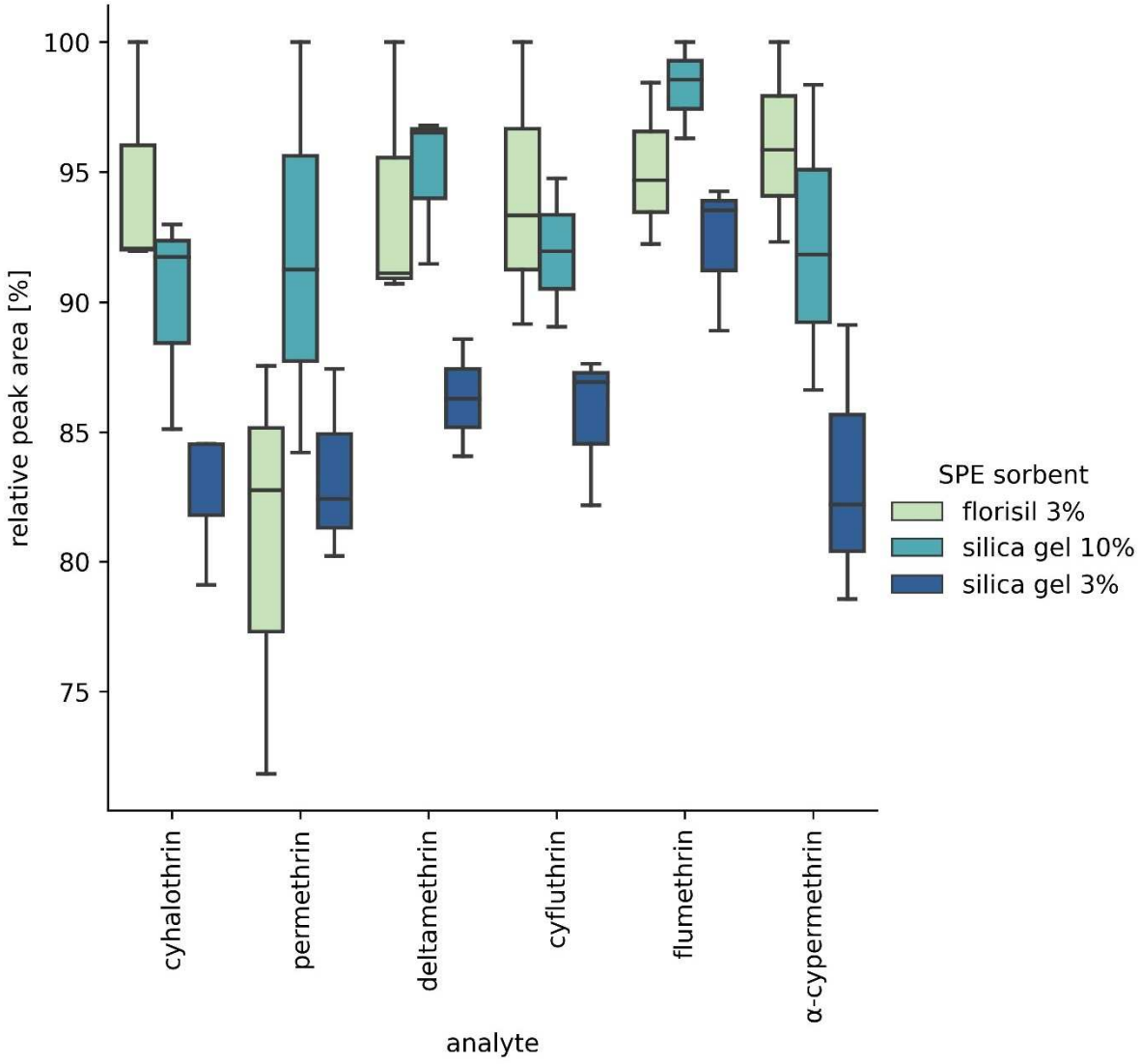


Fig. 5. Comparison of analyte peak areas achieved with performing varied scenarios of SPE cleanup of primary silicone wristband extract.

The results of the described experiment are presented on Fig. 5. The comparison of peak areas of tested analytes obtained from samples subjected to different sorbent cleanup allows to remark on the supremacy of florisil, as in case of almost all analytes of interest (except flumethrin) achieved highest signals during instrumental analysis when compared to silica gel. However, the dispersion of those results is simultaneously the widest (CVs ranging between 3.28% and 5.81%, while for 10% silica gel the range was: 1.68% to 4.70%, and from 2.60% to 3.80% for 3% silica gel). Overall, the outcome of the experiment does not allow for a clear selection of the most propitious cleanup sorbent. While the results obtained for all tested sorbents are mutually proximal leading to over 80% recovery, it would seem suitable to conclude that florisil can be considered the optimal choice, as the high peak areas and relatively low chromatographic background noise levels argue for it, with the technique being inferior to 10% silica gel employment only in terms of reproducibility, with the peak area CV values for

tested analytes still being passable. For that reason, 3% deactivated silica gel was chosen for further use.

2.5.2. Dispersive solid phase extraction (dSPE)

Dispersive solid phase extraction (dSPE) is a pretreatment technique often used for the analysis of varied compounds in matrices of diverse complexities especially in QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction technique of multiple pesticide residues in fruit, vegetables, cereals and processed products. While this technique has not yet been used in the treatment of extracts obtained from silicone wristbands, it is becoming a commonly employed approach, with sorbents required for its performance being easily accessible. For these reasons, as well as due to the simplicity of carrying out the extraction (its employment in the method involving silicone matrix would simplify the protocol even further), as well as being mindful of the substantial reduction of solvent and sorbent use (in comparison to traditional column setup), an experiment meant for evaluation of utility of dSPE in WB extract cleanup procedure was carried out.
The tested sorbents included: Graphitized Carbon Black (GCB), Primary Secondary Amine (PSA), Supel QuE Z-Sep, and Supel QuE Z-Sep+. As in the case of silica gel cleanup experiment, 1 mL of solvent exchanged (to hexane) primary standard - spiked WB extract was used. For each sample a mixture of 25 mg of tested sorbent and 75 mg of magnesium sulfate were weighed into a glass screw cap test tube, next the 1 mL of hexane sample extract was added. The samples were then vortexed for 10 minutes at 2000 rpm with the use of a multitube vortex and centrifuged at 5500 rpm for 2 minutes. Further, aliquots of 400 µL of obtained supernatant were collected into chromatographic vials and subjected to a GC-ECD analysis. The results of the described experiment have been presented on Fig. 6.

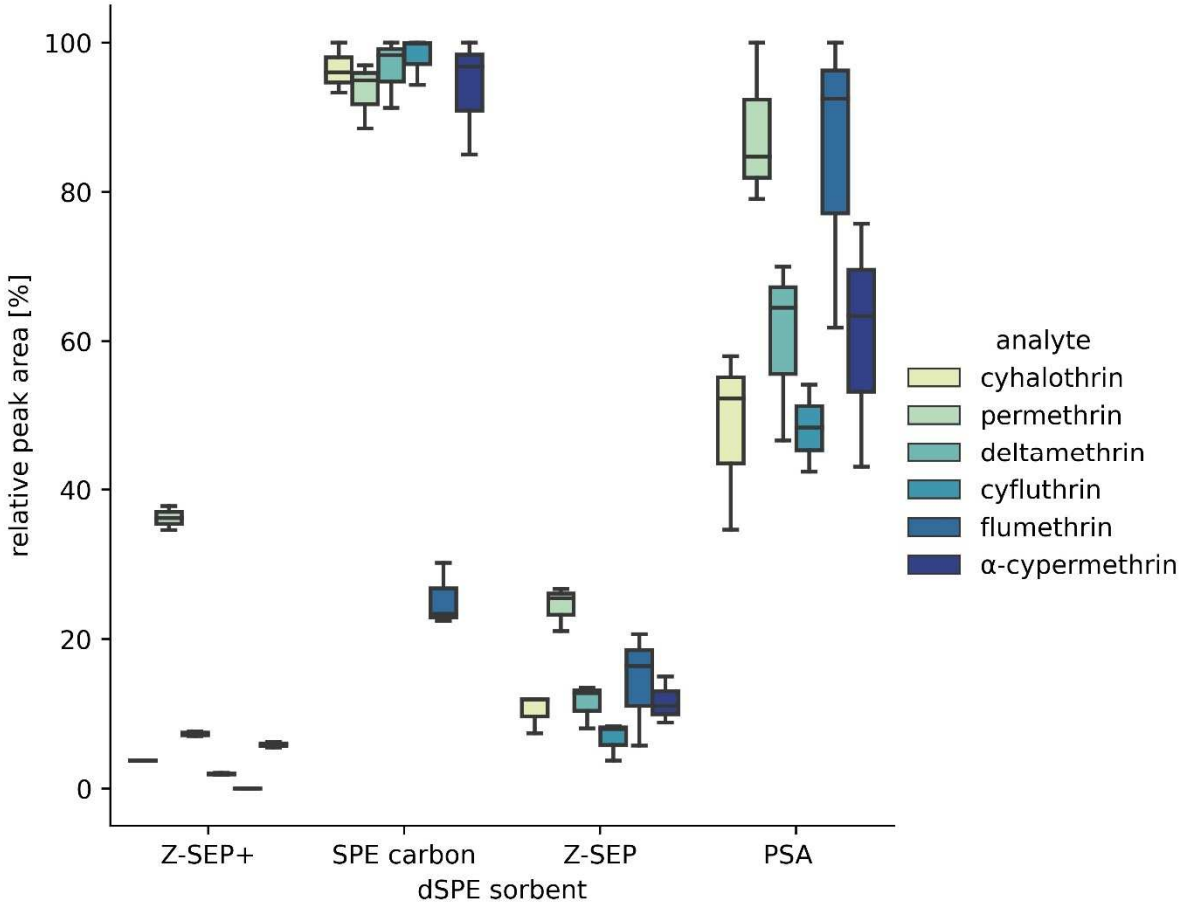


Fig. 6. Comparison of analyte peak areas achieved with performing varied scenarios of dSPE cleanup of primary silicone wristband extract.

Employment of each of tested dSPE sorbents almost in instances of all tested analytes entailed more or less considerable loss of analytes of interest. While for PSA and GCB the magnitude of this effect could be considered acceptable, results obtained with the use of Z-Sep and Z-Sep+ conclude these sorbents to be unsuitable for employment in the final method.

Evaluation of baseline noise in blank samples analyzed after dSPE with tested sorbents has shown that, otherwise promising in terms of analyte recovery, use of GCB results in very high background noise in comparison to other tested sorbents (data not shown). Ultimately, dSPE appeared to be unacceptable in terms of overall analyte recovery and cleanup efficiency and this option has been abandoned in favor of the traditional SPE.

2.6. Method Validation

Method validation had been carried out in accordance with method validation guidelines (U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) 2005). Method selectivity has been assessed by comparing signal levels between a blank and a pyrethroid fortified (32 ng/g WB) WB. Presence of interfering components has been rare, and in cases of their positive detection, their signals did not surpass 20% of the lower limit of quantification of analytes of interest. No carry-over effect has been observed. A six-point calibration curve has been prepared (range of concentrations: 10 – 400 ng/g), with each concentration level

prepared and analyzed in 3 replicates. Accuracy and precision have been investigated by analysis of series of samples at 3 concentration levels (32, 160 and 340 ng/g). More detailed information regarding the process of method validation, as well as results obtained during it, can be found in the Supplementary Information.

3. Pilot study

3.1. Aim of the Study

The aim of the pilot study was to preliminarily determine the usefulness of the developed method for quantification of synthetic pyrethroids in silicone wristbands, as well as to assess the utility of said wristbands as personal passive samplers and its comparison to the golden standard in the assessment of exposure to environmental chemicals – by performing human biomonitoring.

3.2. Study design

3.2.1. Participant recruitment

Participants have been informed of the experiment by word of mouth by the researchers and volunteered to partake. The study obtained approval of the Independent Bioethics Committee for Scientific Research of the Medical University of Gdańsk (NKBBN/536/2020, December 04, 2020). Written informed consent was obtained from all individual participants included in the study. Sample collection took place in December 2020.

3.2.2. Participant demographic

The tested population of volunteers involved a total of 24 persons of both genders (12 males, 12 females) and diverse ages (AM: 33.3 years, range: 14-65 years). Study questionnaires filled out by the participants included questions regarding basic socio-demographic information, as well as information regarding possible sources of exposure to synthetic pyrethroids. Information collected via said questionnaires are encapsulated in Table 1.

Table 1**.** Descriptive statistics of demographic and exposure related information regarding the studied population.

Factor		*n (%)*
Age		
	<20	3 (12.5)
	20-30	13 (54.27)
	>30	8 (33.3)
Gender		
	Male	12 (50)
	Female	12 (50)
Smoking		
	Yes	5 (20.83)
	No	19 (79.17)
Education level		
	Primary School	2 (8.3)
	Vocational School	2 (8.3)
	Technical School	4 (16.67)
	High School	10 (41.67)
	Higher	6 (25)
Type of inhabited area		
	Urban	19 (79.17)
	Rural	5 (20.83)
Type of housing		
	Multi-occupied house	16 (66.6)
	Detached house	8 (33.3)
Pest control history in building (within the past 5 years)		
	Yes	5 (20.83)
	I don't know	7 (29.17)
	No	12 (50)
In-house employment of commercially available insecticides		
	Yes	11 (45.83)
	I don't know	1 (4.17)
	No	12 (50)
Pet ownership		
	Yes	13 (54.17)
	No	11 (45.83)
Anti-ectoparasitic drug employment on pet		
	Yes	11 (45.83)
	No	13 (54.17)

3.2.3. Course of the study

The study design assumed the duration of sampling to be 7 consecutive days, during which study participants were asked to wear a pre-cleaned silicone wristband on the wrist of their dominant hand (except for bath/shower time- the wristband was to be temporarily taken off and placed on a clean surface). During this period, study participants were also asked to collect a total of 3 spot urine samples, with each sample collected on a separate day within the 7-day sampling period. The primary objective of the experiment was to assess exposure to synthetic pyrethroids by quantifying the levels of native pyrethroids in wristbands collected from study participants. These measurements were later compared with concentrations of urinary metabolites of pyrethroids quantified in urine samples. Furthermore, a survey mentioned earlier was conducted to preliminarily identify potential sources of exposure to the tested substances, as questions it contained involved both socio-demographic issues, as well as potential exposure to synthetic pyrethroids.

3.2.4. Sample collection, transportation, and storage

Silicone wristbands were pre-cleaned in the laboratory using a developed protocol before they were provided to the study participants, and further packaged in air-tight plastic zip-lock bags. Study participants were asked to place them in the same packaging after having worn them during the sampling period. Study participants were also equipped with screw-top cups for collecting urine samples. All urine samples collected by the study participants, as well as silicone wristbands, were to be placed in a freezer immediately after their collection, at temperatures around -18°C. Fully collected sets of samples were acquired from the participants by the researchers and transported to the laboratory, to be further stored at -20°C until analysis.

3.3. Determination of pyrethroid metabolites in urine

The method of determination of pyrethroids metabolites: 3-phenoxybenzoic acid (3-PBA); 4-fluoro-3-phenoxybenzoic acid (4F-3PBA), *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA); *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*- and *trans*-DCCA, respectively) in urine applied in this study has been in detail described elsewhere (Rodzaj et al. 2021) with slight modifications. Briefly, sample preparation involved thawing of urine samples and transferring 3 mL of each into screw-top glass test tubes (16 × 100 mm). Urine samples have been spiked with 20 µL of mixture of internal standards (*cis*-DCCA (1, carboxyl-13C2), 2-PBA (2-phenoxybenzoic acid)), 1 µg/mL in acetonitrile) and further concentrated hydrochloric acid (600 µL/ sample) was added to next undergo hydrolysis for 90 minutes in a laboratory oven at 95°C. After bringing to room temperature, 4 mL of hexane was added to the samples, which were then shaken on a multitube vortex (10 min, 2500 rpm), centrifuged (2 min, 5500 rpm), and the organic layer was transferred into a separate test tube. Extraction was repeated, and the resulting extract (around 8 mL) was again shaken with 0.5 mL of 0.1M NaOH aqueous solution. After centrifugation, the top organic layer was discarded, and 0.1 mL of hydrochloric acid and 2 mL of hexane was added to the test tube, and as previously, shaken and centrifuged. Further separation of the organic layer led to its collection and transfer to another screw-top glass test tube, to be next evaporated to dryness at 40°C, aided by the stream of nitrogen. Dry residue that remained in the test tube underwent derivatization with the use of 10 µL of 1,1,1,3,3,3-hexafluoroispropanol (HFIP), 15 µL of diisopropylcarbodiimide (DIC) and 250 µL of hexane (10 min, 2200 rpm, multitube vortex). Lastly, in order to neutralize the excess of derivatizing agents used (HFIP, DIC), 1 mL of 5% potassium carbonate solution was added, which is followed by further shaking and centrifugation of extracts. The last step of the procedure is separation of final extract from bottom layer, 170 µL of the extract is carefully collected and transferred into a glass chromatographic vial, to next undergo instrumental analysis.

Instrumental analyses of prepared sample extracts had been carried out with the use of a gas chromatograph (Varian GC-450) coupled with ion trap mass spectrometer (Varian 225-MS). Detailed description of the method, and more information regarding used hardware can be found in Supplementary Information (SI).

Concentrations of metabolites of synthetic pyrethroids in urine had undergone urine specific gravity (SG) adjustment to prevent urine dilution from influencing obtained results. Calculations have been carried out in a manner identical to one described by Rodzaj et al. 2021 (Rodzaj et al. 2021). Urinary SG of every sample had been measured with the use of a hand-held pocket-size refractometer PAL-10S (Atago Co., Tokyo, Japan). The arithmetic mean SG of the studied population was considered the reference SG in calculations.

3.3.1. Internal and external quality control

Quality control samples, at two concentrations (low concentration (LQC) = 0.25 ng/mL; high concentration (HQC) = 1.5 ng/mL) were prepared with the use of pooled physiological urine in 20 repetitions over a period of 3 weeks. Results obtained via their GC-MS analysis served for construction of control charts for each of the tested metabolites. Further, each batch of analyzed study samples has been appended with two control samples prepared at both forementioned concentrations (LQC, HQC), to ensure quality of performed analysis. For 3-PBA, DBCA, *cis*-DCCA and *trans*-DCCA the established limit of detection (LOD) was 0.05 ng/mL, with that value being 0.1 ng/mL in case of 4F-3PBA. Coefficient of variation of results obtained for control samples has been calculated and was in range between 3.43 – 17.85% for LQC samples and between 1.17 – 10.79% for HQC samples, corresponding to intra-day variability of the assay. The Department of Toxicology of Medical University of Gdańsk also successfully participates in annual German External Quality Assessment Scheme for Analyses in Biological Materials (G-EQUAS). Calibration curves had also been prepared with the use of pooled urine, in accordance with the Bioanalytical Method Validation Guidelines (U.S. Depatrment of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) 2005).

3.4. Determination of native pyrethroids in silicone wristbands

Collected wristbands were cut into small pieces (approx. 10 mm × 2 mm × 2 mm) with the use of a surgical scalpel (No. 4) with disposable blades. Separate clean blade had been used for each wristband. In this state, WBs were stored in plastic Eppendorf test tubes prior to analysis. For each analysis, 0.5 g of previously mixed WB pieces (which has been considered a singular sample throughout this study) was weighed into a glass screw-top test tube and underwent post-exposure cleanup, consisting of a single 30 second vortex-aided wash with deionized water, and a subsequent 30 second vortex wash with IPA, and were later left for 12 hours to dry. Next, 5 mL of ethyl acetate was added, and samples were extracted twice by sonication (15 min). After each extraction cycle, solvent was collected into the next glass test tube. Ten mL of obtained (primary) extract was evaporated to dryness at 40°C, under the stream of nitrogen. Dry residue was taken up to 1 mL of hexane and subjected to silica gel cleanup (0.5 g 3 % water deactivated silica gel, 3-4 mm sodium sulfate on top). Substances of interest were eluted with 4 mL of 30 % diethyl ether solution in hexane. The solvents were later again evaporated to dryness, and dry residue reconstituted in 1 mL of hexane. Final hexane extract was collected into a glass autosampler vial and underwent instrumental analysis with the use of gas chromatograph (SCION Instruments, 456-GC) with an electron capture detector (GC-ECD). The selection of GC-ECD as the technique of choice for this study was validated by its sensitivity, which in case of use of silicone wristbands had been argued to be essential for analyte detection, as preliminary studies are in most cases burdened by lack of initial knowledge regarding expected concentrations to be determined in field samples. Another asset of using GC-ECD for this study has been its high selectivity to analytes of interest. Detailed description of the method, and more information regarding used hardware can be found in SI.

3.5. Results

The analytical method was developed for the determination of 6 native pyrethroids in silicone wristbands using ultrasound-assisted extraction, silica gel clean-up of the extract and instrumental analysis by gas chromatography with an electron capture detector. The key conditions of the preparation of the wristbands before their use and the preparation for instrumental analysis were established. The use of GC-ECD significantly facilitates the availability of the method in other

laboratories, but we realize that GC-MS or GC-MS/MS may have better specificity. In our case Initial attempts of WBs extract analysis with GC-MS showed that sufficient sensitivity was not achievable, while the purity of wristband extracts was high enough for us to finally decide to use GC-ECD instead.

In the course of the study, 24 participants collected in total 24 wristbands and 72 urine samples being further analyzed respectively for native pyrethroids and their metabolites.

The population involved in the described pilot study consisted of a total of 24 participants, equally distributed between genders (50% - female, 50% - male). The average age of participants was 36, with 3 participants (12.5%) being under the age of 20 at the time of study sample collection, 13 (54.3%) persons declaring the age between 20 and 30, and 8 (33.3%) people being over the age of 30. Most of the study volunteers were non-smokers (19, 79.2%). The highest completed education level has been divided into primary school (2 people, 8.3%), vocational school (2 people, 8.3%), technical school (4 people, 16.67%), high school (10 people, 41.7%), and higher education (6 people, 25%). Most (19, 79.2%) of the study participants lived in an urban setting, with only 5 (20.8%) participants declaring their housing location to be rural. Accordingly, 16 (66.6%) persons declared to be living in a multi-family housing, with 8 participants (33.3%) assessing their housing conditions as living in a detached house. When asked about knowledge of past pest controls within the last 5 years, most (12, 50%) participants declared there had been none while 5 people (20.8%) informed that said controls had taken place in their building, with 7 people (29.2%) having no knowledge of said occurrences. Furthermore, almost half of the study participants (11, 45.8%) declared indoor usage of commercially available pesticides, 12 people (50%) said no pesticides have been used by them in-house, with just 1 person (4.2%) having no knowledge regarding that matter. Among the tested population, over half of the study participants have declared themselves to be pet owners (13, 54.2%), with 11 persons (45.8%) having no indoor-dwelling animals. Eleven persons (45.8%) have declared to use anti-ectoparasitic drugs on pets.

The normality of the distribution of analytical results had been assessed with the use of the Shapiro-Wilk test. Urinary metabolite concentration, as well as WB concentration of pyrethroids, were log-normally distributed and thus non-parametric Mann-Whitney U test was applied to compare values between groups. Descriptive statistics, as well as results of the aforementioned non-parametric analyses, are summarized in Table 1, Table 3 and Table 4.

Table 2. summarizes statistics of SG-adjusted concentrations of pyrethroid urinary biomarkers, as well as concentrations of native permethrin quantified in silicone wristbands. For urinary biomarkers detection range varied from 12.5% to 68.06% for 4F-3PBA and 3-PBA, respectively.

Table 2**.** Distribution of concentrations of urinary biomarkers of synthetic pyrethroids, and parent pyrethroids determined in silicone wristbands.

		LOD	AM (95% CI)	GM	SD	Min	P_{25}	P_{50}	P_{75}	Max	DR [%]
ng/mL	3-PBA	0.05	0.72 (0.39, 1.05)	0.21	1.40	<LOD	<LOD	0.19	0.66	8.89	68.0
	4F-3PBA	0.1	-	-	-	<LOD	<LOD	<LOD	<LOD	0.34	12.5
	DBCA	0.05	-	-	-	<LOD	<LOD	<LOD	<LOD	4.20	23.6
	cis-DCCA	0.05	-	-	-	<LOD	<LOD	0.06	0.22	3.73	34.7
	trans-DCCA	0.05	0.88 (0.47, 1.30)	0.20	1.76	<LOD	<LOD	0.15	0.73	10.12	52.8
ng/g	cyhalothrin	2	-	-	-	<LOD	<LOD	<LOD	<LOD	54.47	12.5
	permethrin	10	1105.3 (213.19, 1997.41)	79.64	2112.68	<LOD	<LOD	48.6	582.4	6673.9	58.3
	cyfluthrin	10	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	0
	α-cypermethrin	10	118.02 (20.14, 215.91)	-	-	<LOD	<LOD	<LOD	50.80	756.60	25
	deltamethrin	2	9.11 (2.08, 16.14)	-	-	<LOD	<LOD	<LOD	<LOD	51.19	20.8
	flumethrin	10	-	-	-	<LOD	<LOD	<LOD	<LOD	<LOD	0

AM (95% CI) – arithmetic mean (95% confidence interval); GM – geometric mean; SD – standard deviation; Min – minimum concentration; P_{25}, P_{50}, P_{75} - 25th, 50th, 75th percentile; Max – maximum concentration; DR [%] – detection rate; LOD – limit of detection

Pyrethroids metabolites were detected with various frequencies from 12.5 (4F-3PBA) to 68% (3-PBA). The detection rate of analyzed native pyrethroids in tested wristbands varied from 0 (flumethrin, cyfluthrin), to 58.3% for permethrin. Therefore, further statistical analyses have been conducted only on permethrin concentrations quantified in WBs, as it is the only analyte of interest to have exceeded the detection rate of 50%.

Upon data analysis, a pest control 5-year history in an occupied building led to higher urinary 3-PBA (p = 0.0001), DBCA (p = 0.0267), *cis*-DCCA (p < 0.0001), *trans*-DCCA (p < 0.0001), and WB permethrin (p < 0.0001) concentrations. Similarly, urinary concentrations of 3-PBA (p = 0.0002), *cis*-DCCA (p < 0.0001), *trans*-DCCA (p < 0.0001), native wristband-quantified permethrin (p < 0.0001) were higher when in-house deployment of commercially available insecticides took place. Geometric means of urinary 3-PBA, *cis*-DCCA, *trans*-DCCA and wristband-quantified permethrin were higher in study participants who confirmed using indoor insecticide-containing products. Over half of volunteers have declared themselves to be pet owners, with only slightly less percentage of the tested population having declared using anti-ectoparasitic veterinary products on said pets. Among pet owners, concentrations of 3-PBA (p = 0.0009), cis-DCCA (p = 0.0002), *trans*-DCCA (p = 0.0004), and permethrin (p = 0.0016) (Fig. 7A) were noticeably higher. Furthermore, the presence of detectable levels (>LOD) of permethrin in the wristband was a significant (p < 0.01) predictor of higher *trans*-DCCA concentration (Fig. 7B).

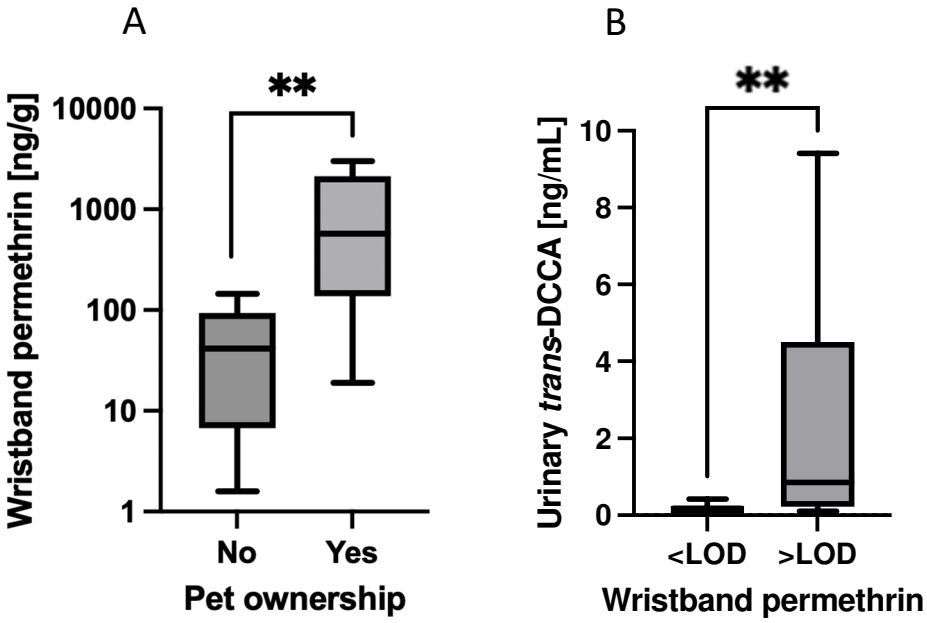


Fig. 7. Concentrations of wristband permethrin [ng/g] between pet owners and participants not owning pets (A). A statistically significant difference in permethrin concentrations has been noted (p <0.01 (✱✱)). Urinary concentrations of specific permethrin metabolite *trans*-DCCA [ng/mL] between samples with negative and positive detection of wristband permethrin (B).

Similarly, declaration of employment of forementioned anti-ectoparasitic veterinary products on pets had shown higher concentrations of the same set of substances: 3-PBA (p=0.0004), *cis*-DCCA (p < 0.0001), *trans*-DCCA (p < 0.0001), permethrin (p < 0.0001). Both in the case of pet owners, as well as among participants using veterinary drugs on pets, geometric means of concentrations of said quantified substances were noticeably higher in comparison to opposing sub-populations. Furthermore, a statistically significant association has been noted between smoking and urinary concentrations of *cis*-DCCA (p = 0.0059) and *trans*-DCCA (p = 0.0073), however, due to considerable differences in sample size between smokers and non-smokers participating in this study, this association should not perhaps be used to draw up any far-reaching conclusions regarding the matter.

Factors such as: having performed pest control in currently occupied building within the last 5 years, declaring in-house usage of commercially available insecticides, pet ownership, as well as employment of anti-ectoparasitic drugs on them have been identified as possible predictors of exposure to pyrethroids in this particular population.

Furthermore, Pearson's correlation test had been carried out between urinary concentrations of pyrethroid metabolites with detection rates exceeding 50% (3-PBA, *trans*-DCCA) and concentrations of native permethrin in silicone wristbands. The values of correlation coefficients were 0.28 and 0.44 for 3-PBA and *trans*-DCCA, respectively. Fig. 8. depicts the scattering of permethrin concentrations quantified in silicone wristbands in relation to median of urinary *trans*-DCCA concentrations calculated for each participant from the 3 samples collected during the study. The graph shows moderate concordance between the results of urinalysis and WB extract analysis. Seeing that there is some conformity throughout the results obtained for wristband and urine extracts shows that the use of silicone wristbands in exposure assessment to synthetic pyrethroids can very well serve as a

complementary tool, that provides a new array of interesting information regarding exposure to native compounds.

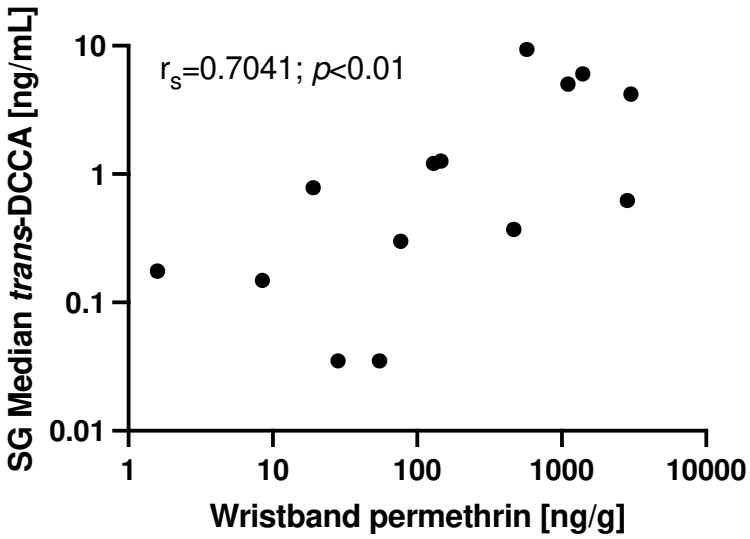


Fig. 8. Relationship between wristband permethrin concentration [ng/g] and median concentrations of urinary *trans*-DCCA [ng/mL].

Table 3. Descriptive statistics and predictors of urinary SG- adjusted concentrations (ng/mL) of pyrethroid metabolites with detection rate >50%.

	3-PBA										*trans*-DCCA									
	n (%)	**AM (95% CI)**	**GM**	**SD**	**Min**	**P25**	**P50**	**P75**	**Max**	**p-Value**	**n (%)**	**AM (95% CI)**	**GM**	**SD**	**Min**	**P25**	**P50**	**P75**	**Max**	**p-Value**
							Age										**Age**			
<20	3 (12.5)	1.88 (-0.26, 4.02)	0.51	2.78	0.04	0.04	1.07	1.97	8.89	0.8070	3 (12.5)	2.80 (0.40, 5.19)	0.77	3.12	0.04	0.04	2.57	3.40	10.12	0.4155
20-30	13 (54.27)	0.18 (0.12, 0.24)	0.11	0.19	0.04	0.04	0.13	0.25	0.90		13 (54.27)	0.20 (0.11, 0.29)	0.10	0.27	0.04	0.04	0.09	0.28	1.21	
>30	8 (33.3)	1.18 (0.56, 1.79)	0.43	1.45	0.04	0.14	0.48	1.91	5.23		8 (33.3)	1.28 (0.46, 2.09)	0.37	1.93	0.04	0.11	0.32	1.76	6.76	
							Gender										**Gender**			
Male	12 (50)	0.74 (0.32, 1.16)	0.20	1.24	0.04	0.04	0.23	0.87	5.23	0.8412	12 (50)	0.87 (0.33, 1.41)	0.19	1.60	0.04	0.04	0.14	0.75	6.76	0.6653
Female	12 (50)	0.71 (0.18, 1.24)	0.21	1.57	0.04	0.08	0.16	0.41	8.89		12 (50)	0.90 (0.24, 1.55)	0.21	1.93	0.04	0.04	0.16	0.36	10.12	
							Smoking										**Smoking**			
Yes	5 (20.83)	0.15 (0.08, 0.22)	0.10	0.13	0.04	0.04	0.13	0.23	0.44	0.0855	5 (20.83)	0.10 (0.04, 0.16)	0.07	0.10	0.04	0.04	0.04	0.14	0.38	**0.0073**
No	19 (79.17)	0.87 (0.47, 1.28)	0.25	1.54	0.04	0.04	0.25	1.07	8.89		19 (79.17)	1.09 (0.58, 1.60)	0.26	1.93	0.04	0.04	0.25	0.94	10.12	
							Type of inhabited area										**Type of inhabited area**			
Urban	19 (79.17)	0.75 (0.34, 1.15)	0.19	1.53	0.04	0.04	0.16	0.51	8.89	0.3529	19 (79.17)	1.03 (0.51, 1.55)	0.20	1.95	0.04	0.04	0.14	0.77	10.12	0.5651
Rural	5 (20.83)	0.64 (0.19, 1.09)	0.30	0.81	0.04	0.14	0.27	1.10	2.97		5 (20.83)	0.33 (0.16, 0.50)	0.20	0.30	0.04	0.10	0.23	0.48	1.01	
							Type of housing										**Type of housing**			
Multi-occupied house	16 (66.6)	0.87 (0.39, 1.34)	0.23	1.64	0.04	0.04	0.23	0.80	8.89	0.3529	16 (66.6)	1.21 (0.60, 1.81)	0.26	2.08	0.04	0.04	0.20	1.80	10.12	0.1763
Detached house	8 (33.3)	0.43 (0.14, 0.72)	0.16	0.69	0.04	0.04	0.14	0.48	2.97		8 (33.3)	0.24 (0.12, 0.35)	0.12	0.27	0.04	0.04	0.13	0.32	1.01	
							Pest control history in building (within the past 5 years)										**Pest control history in building (within the past 5 years)**			
Yes	5 (20.83)	2.36 (1.07, 3.65)	1.15	2.33	0.04	0.65	1.97	2.46	8.89		5 (20.83)	3.48 (2.07, 4.88)	2.27	2.53	0.05	2.40	3.05	3.79	10.12	
I don't know	7 (29.17)	0.19 (0.08, 0.30)	0.10	0.24	0.04	0.04	0.07	0.25	0.90	**0.0001**	7 (29.17)	0.20 (0.08, 0.33)	0.10	0.27	0.04	0.04	0.09	0.25	0.94	**<0.0001**
No	12 (50)	0.35 (0.16, 0.55)	0.15	0.58	0.04	0.04	0.16	0.31	2.97		12 (50)	0.20 (0.12, 0.28)	0.11	0.24	0.04	0.04	0.11	0.30	1.01	
							In-house employment of commercially available insecticides										**In-house employment of commercially available insecticides**			
Yes	11 (45.83)	1.37 (0.71, 2.04)	0.48	1.88	0.04	0.14	0.65	1.97	8.89		11 (45.83)	1.74 (0.91, 2.57)	0.55	2.33	0.04	0.15	0.48	2.88	10.12	
I don't know	1 (4.17)	0.18 (-0.39, 0.75)	0.10	0.23	0.04	0.04	0.06	0.44	0.44	**0.0002**	1 (4.17)	0.11 (-0.22, 0.45)	0.07	0.14	0.04	0.04	0.04	0.27	0.27	**<0.0001**
No	12 (50)	0.17 (0.11, 0.24)	0.10	0.20	0.04	0.04	0.11	0.25	0.90		12 (50)	0.16 (0.09, 0.24)	0.09	0.22	0.04	0.04	0.04	0.20	0.94	
							Pet ownership										**Pet ownership**			
Yes	13 (54.17)	1.17 (0.60, 1.75)	0.38	1.78	0.04	0.14	0.33	1.79	8.89	**0.0009**	13 (54.17)	1.49 (0.77, 2.21)	0.40	2.22	0.04	0.05	0.31	2.57	10.12	**0.0004**
No	11 (45.83)	0.19 (0.11, 0.28)	0.10	0.24	0.04	0.04	0.07	0.25	1.10		11 (45.83)	0.17 (0.09, 0.24)	0.09	0.22	0.04	0.04	0.04	0.21	1.01	
							Anti-ectoparasitic drug employment on pet										**Anti-ectoparasitic drug employment on pet**			
Yes	11 (45.83)	1.35 (0.68, 2.02)	0.46	1.89	0.04	0.14	0.65	1.97	8.89	**0.0004**	11 (45.83)	1.75 (0.93, 2.58)	0.57	2.33	0.04	0.22	0.71	2.88	10.12	**<0.0001**
No	13 (54.17)	0.19 (0.12, 0.27)	0.10	0.23	0.04	0.04	0.09	0.27	1.10		13 (54.17)	0.15 (0.08, 0.22)	0.08	0.21	0.04	0.04	0.04	0.18	1.01	

Table legend: AM (95% CI) – arithmetic mean (95% confidence interval); GM – geometric mean; SD – standard deviation; Min – minimum concentration; P25, P50, P75 - 25th, 50th, 75th percentile; Max – maximum concentration; n (%) – number of participants (percent of total population); LOD – limit of detection; Statistically significant associations are in **bold** (Mann Whitney U test, $p \leq 0.05$)

Table 4. Descriptive statistics and predictors of concentrations of parent pyrethroids quantified in silicone wristbands (ng/g).

	permethrin									
	n (%)	**AM (95% CI)**	**GM**	**SD**	**Min**	**P25**	**P50**	**P75**	**Max**	**p-Value**
					Age					
<20	3 (12.5)	3860.8 (1562.1, 6156.5)	613.8	2990.4	7.07	7.07	4901.4	6673.9	6673.9	
20-30	13 (54.3)	543.9 (-9.9, 1097.9)	45169.0	1708.9	7.07	7.07	7.07	123.5	6380.5	0.0684
>30	8 (33.3)	984.1 (396.9, 1571.4)	164.7	1390.7	7.07	32.7	167.7	2034.7	3395.9	
					Gender					
Male	12 (50)	1411.2 (565, 2257.4)	75.4	2500.9	7.07	7.07	32.7	1827.9	6673.9	0.9208
Female	12 (50)	799.4 (280.5, 1318.3)	84.1	1533.6	7.07	7.07	57.1	582.5	4901.4	
					Smoking					
Yes	5 (20.8)	30.9 (16.5, 45.4)	20.8	26.1	7.07	7.07	26.4	38.9	75.3	**0.0036**
No	19 (79.2)	1388 (788, 1987.8)	113.4	2260.3	7.07	7.07	123.5	3193.8	6673.9	
					Type of inhabited area					
Urban	19 (79.2)	1332.6 (726.5, 1938.7)	86.5	2284.3	7.07	7.07	38.9	3193.8	6673.9	0.9541
Rural	5 (20.8)	241.6 (52.2, 431)	58.1	342	7.07	7.07	58.1	260	875.6	
					Type of housing					
Multi-occupied house	16 (66.6)	1576.9 (875.7, 2278)	119.4	2414.7	7.07	7.07	81.2	3294.9	6673.9	0.2216
Detached house	8 (33.3)	162.2 (40.8, 283.5)	35.5	287.4	7.07	7.07	32.7	167.6	875.6	
					Pest control history in building (within the past 5 years)					
Yes	5 (20.8)	4909.1 (4077.9, 5740.2)	4687.7	1500.8	3193.8	3395.9	4901.4	6380.5	6673.9	
I don't know	7 (29.2)	90.9 (44.5, 137.4)	36.1	102.1	7.07	7.07	38.9	163.7	289.3	**<0.0001**
No	12 (50)	112.1 (29.6, 194.6)	23.1	243.8	7.07	7.07	7.07	66.8	875.6	
					In-house employment of commercially available insecticides					
Yes	11 (45.8)	2352.5 (1437.7, 3267.2)	457.9	2579.7	7.07	57.2	875.6	4901.4	6673.9	
I don't know	1 (4.17)	7.07 (-)	7.07	(-)	7.07	7.07	7.07	7.07	7.07	**<0.0001**
No	12 (50)	53.6 (24.8, 82.4)	19.6	84.9	7.07	7.07	7.07	57.1	289.3	
					Pet ownership					
Yes	13 (54.2)	1984.9 (1166.9, 2808.8)	195	2523.2	7.07	7.07	289.3	3395.9	6673.9	**0.0016**
No	11 (45.8)	65.7 (36.9, 94.5)	27.6	81.2	7.07	7.07	26.4	123.5	260	
					Anti-ectoparasitic drug employment on pet					
Yes	11 (45.8)	2344.5 (1427.2, 3261.9)	356.5	2587.2	7.07	7.07	875.6	4901.4	6673.9	**<0.0001**
No	13 (54.2)	56.7 (31.6, 81.8)	22.4	77.5	7.07	7.07	7.07	75.3	260	

AM (95% CI) – arithmetic mean (95% confidence interval); GM – geometric mean; SD – standard deviation; Min – minimum concentration; P25, P50, P75 - 25th, 50th, 75th percentile; Max – maximum concentration; n [%] – number of participants (percent of total population); LOD – limit of detection; Statistically significant associations are in **bold** (Mann Whitney U test, $p \leq 0.05$)

4. Discussion and conclusions

The project has amounted to a successful development and optimization of a functional analytical method for determination of 6 synthetic pyrethroids in silicone wristbands. The wrought procedure involved post-exposure cleanup of worn WBs with sequential washes with IPA and H_2O, sonication-assisted extraction of analytes of interest with ethyl acetate, purification of primary extract with solid phase extraction with silica gel, and finally instrumental analyses of wristband extracts with GC-ECD. Limits of detection achieved for respective analyzed substances are satisfactory and make the procedure to be useful in exposure assessment studies.

The approach to performing pre-exposure cleanup procedure presented in this study has proven to be very effective, as a considerable decline in silicone wristbands-derived oligomers have been removed with great efficacy. These results are in accordance with ones presented in previously described studies, that included a similarly conducted cleanup procedure (O'Connell, Steven G., Laurel D. Kincl 2014). It Is however worth noting, that O'Connell et al. have performed solvent exchanges in between five 2.5 hour long washing intervals (performed on an orbital shaker), so in comparison, cleanup procedure proffered in our study is much more time effective, while simultaneously maintains high turnout. Furthermore, while the results of thus performed washing procedure are satisfactory, it is important to mention the relatively high-level usage of solvent needed for its execution. Taking into account the resulting solvent exposure of the staff, as well as ecological matters, there certainly is room for improvement, regarding the matter. A different approach to pre-exposure silicone wristband cleanup has been reported by Anderson et al. (Anderson et al. 2017), where wristband-derived oligomers were disposed of with good efficiency by baking the samplers under vacuum. In order to provide proof of technique's utility total mass reduction of WBs, elasticity and strength properties as well as TIC and microscopic images of WBs surface have been monitored. This solution, while clearly successfully employed, has a major drawback of requiring advanced laboratory equipment, as well as professionally trained staff for its execution.

Optimized method (schematically showcased on Fig. 9.) for WB matrix sample preparation for exposure assessment to synthetic pyrethroids is suitable for routine use, in its thus optimized form does not require any advanced laboratory equipment in the process of sample preparation and is relatively easy to employ in any laboratory setting. Wristbands as personal passive samplers offer a wide array of advantages when employed in exposure assessment studies, it would be therefore beneficial to unify the protocols of their use, which would allow for comparison of results obtained in different settings.

Table 5**.** Comparison of several population studies involving assessment of urinary metabolites of synthetic pyrethroids carried out in Poland within the last 10 years.

	3-PBA		***cis*-DCCA**		***trans*-DCCA**		**DBCA**	
Ref	GM [ng/mL]	DR [%]	GM [ng/mL]	DR [%]	GM [ng/mL]	DR [%]	GM [ng/mL]	DR [%]
(Wielgomas et al. 2013)	0.32	80.0	-	8.0	-	7.0	-	11.0
(Wielgomas and Piskunowicz 2013)	0.26	82.4	-	46.0	-	46.8	-	17.1
(Jurewicz et al. 2015)	0.17	71.8	0.12	57.9	0.16	65.5	0.05	16.8
(Jurewicz et al. 2020)	0.32	66.5	0.21	32.8	0.44	34.9	0.22	19.4
(Klimowska et al. 2020)	0.27	81.0	0.22	85.4	0.36	93.9	-	44.7
(Radwan et al. 2022)	0.22	68.1	0.11	34.3	0.32	45	0.18	22.1
(Rodzaj et al. 2021)	0.22	69.0	-	36.0	0.26	76.0	-	32.0
this study	0.21	68.0	0.11	34.7	0.20	52.7	0.08	12.5

Urinary concentrations and detection rates of pyrethroid metabolites are in accordance with other studies conducted within the last 10 years in Poland (Table 5). The detection rates of 3-PBA are consistently the highest out of all pyrethroid metabolites among almost (with the exception for Klimowska 2020 (Klimowska et al. 2020), where *trans*- and *cis*-DCCA had achieved higher detection rates since the method LOD were two times lower than in the remaining studies) all reviewed studies (Wielgomas et al. 2013; Wielgomas 2013; Jurewicz et al. 2015, 2020; Klimowska et al. 2020; Rodzaj et al. 2021; Radwan et al. 2022) and range from 66.5% (Jurewicz et al. 2020) to 82.4% (Wielgomas and Piskunowicz 2013). The population geometric means of urinary concentrations of that metabolite have over the years been determined to be ranging from 0.17 ng/mL (Jurewicz et al. 2015) to 0.32 ng/mL (Jurewicz et al. 2020). Detection rates of *trans*-DCCA have been calculated to be from 34.9% (Jurewicz et al. 2020) to 93.9% (Klimowska et al. 2020), with our results being 52.78%. Geometric means of concentrations of said substance varied from 0.16 ng/mL (Jurewicz et al. 2015) to 0.44 ng/mL (Jurewicz et al. 2020).

Urinary pyrethroid metabolites have also recently been an interest of several exposure assessment studies carried out in other locations on varying populations: Czech Republic – study conducted on parent - child pairs (Šulc et al. 2022), Spain, where occupationally and environmentally exposed adults were tested (Bravo et al. 2022), New Zealand – study regarding pyrethroid exposure in children between 5 and 14 years of age (Ueyama et al. 2022; Li et al. 2022). The 3-PBA detection rates and concentrations were in some cases comparable to ours (51.8%, median 0.16 ng/mL - (Šulc et al. 2022), with several research papers reporting much higher detection rates - 91% (Bravo et al. 2022), 99.3% (Li et al. 2022) and 98% (Ueyama et al. 2022).

Other studies focused on determination of native synthetic pyrethroids in silicone wristbands have similarly reported the frequency of detection being the highest for permethrin (Arcury et al. 2021) – 49.7%, (Wise et al. 2020) – 100%, (Doherty et al. 2020) – 67%). The values of permethrin detection rates reported in forementioned studies partially allow to confirm the effectiveness of our method, as we had been able to achieve the detection rate of 58.3% in testing the general population. In a study conducted by Harley et al. (Harley et al. 2019), cypermethrin has been detected more often than any other pyrethroid compound, and the study described by Donald et al. (Donald et al. 2016) had reported the highest detection rate of deltamethrin. Concentrations of permethrin shown in other studies (Doherty et al. 2020; Wise et al. 2020) are noticeably higher than concentrations calculated in our study, however it can be explained not only by the distinctive diversity of tested populations and specific communities (rural areas, farmworkers, dog owners), but also the study locations. Our study is (to our knowledge) the first in Europe to involve the use of silicone wristbands in determination of synthetic pyrethroids among the general population.

Data analysis carried out as part of this pilot study has highlighted the fact that pet ownership appears to be a significant predictor for exposure to synthetic pyrethroids. In fact, usage of said substances indoors in various settings (on pets or as part of pest control), and at various times prior to the sample collection heavily contribute to noticeably higher concentrations of both urinary pyrethroid metabolites and native permethrin quantified with the use of silicone wristbands, thus further confirming their lengthy half-lifes within closed indoor spaces, such as an inside of a home. The pilot study demonstrated the utility of silicone wristbands as personal passive samplers for exposure assessment to synthetic pyrethroids. It's worth noting that the differences observed in concentrations of urinary pyrethroid metabolites between sub-populations, as determined via questionnaire analysis, generally align with the relationships observed between these population-dividing factors and wristband permethrin concentrations. This fact allows to conclude that analysis of silicone wristbands for native pyrethroid compounds is, and surely will further develop to be, an exposure assessment tool of great significance for health risk assessment. The correlation coefficients calculated to characterize the relationship between urinary concentrations of pyrethroid metabolites and wristband permethrin

had shown their weak (3-PBA) to moderate (*cis*-DCCA, *trans*-DCCA) association. Involvement of silicone wristbands in this pilot study has shown their utility for employment as personal passive samplers for determination of exposure to synthetic pyrethroids, as their analysis can provide unique information regarding the exposure that took place to native compounds, unable to be determined in urinalysis, while simultaneously showing correlation to results obtained via employment of the 'golden standard' – quantification of urinary metabolites of synthetic pyrethroids. This opens up the possibility of future use of wristbands in exposure assessment studies without relying solely on urinalysis.

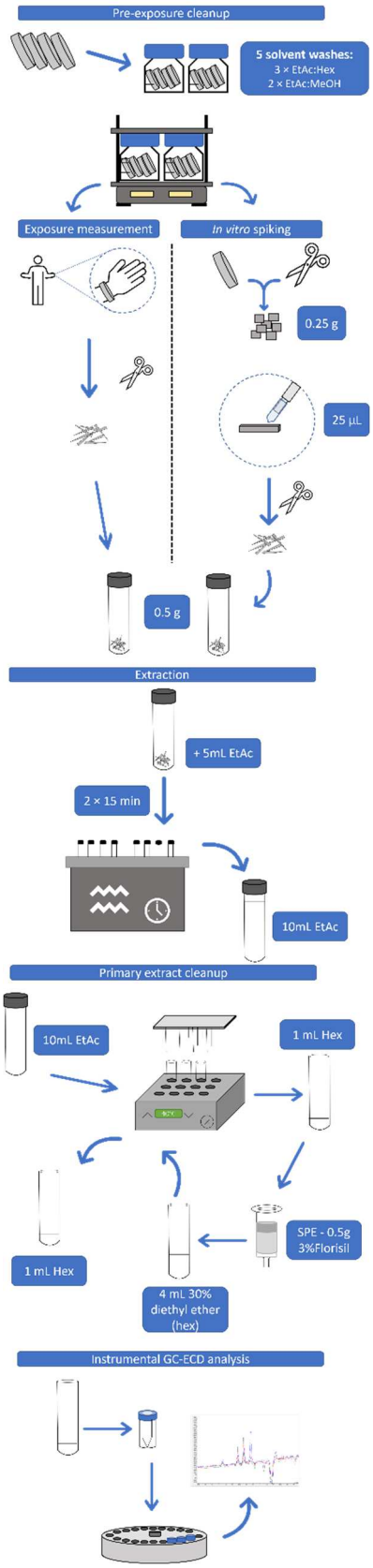


Fig. 9. Pictorial overview of optimized method for determination of synthetic pyrethroids in silicone wristbands.

5. Acknowledgements

Małgorzata Wacławik appreciates the scholarship received from the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Program Knowledge Education Development 2014–2020.

Furthermore, the authors of this manuscript give their sincere thanks to the volunteers, for their willingness to selflessly endure all the inconveniences related to participation in the study, as well as exemplary cooperation throughout it.

6. References

Aerts R, Joly L, Szternfeld P, et al (2018) Silicone Wristband Passive Samplers Yield Highly Individualized Pesticide Residue Exposure Profiles. Environ Sci Technol 52:298–307. https://doi.org/10.1021/acs.est.7b05039

Anderson KA, Points GL, Donald CE, et al (2017) Preparation and performance features of wristband samplers and considerations for chemical exposure assessment. J Expo Sci Environ Epidemiol 27:551–559. https://doi.org/10.1038/jes.2017.9

Arcury TA, Chen H, Quandt SA, et al (2021) Pesticide exposure among Latinx children: Comparison of children in rural, farmworker and urban, non-farmworker communities. Sci Total Environ 763:144233. https://doi.org/10.1016/j.scitotenv.2020.144233

Baum JLR, Bakali U, Killawala C, et al (2020) Evaluation of silicone-based wristbands as passive sampling systems using PAHs as an exposure proxy for carcinogen monitoring in firefighters: Evidence from the firefighter cancer initiative. Ecotoxicol Environ Saf 205:111100. https://doi.org/10.1016/j.ecoenv.2020.111100

Bergmann AJ, North PE, Vasquez L, et al (2017) Multi-class chemical exposure in rural Peru using silicone wristbands. J Expo Sci Environ Epidemiol 27:560–568. https://doi.org/10.1038/jes.2017.12

Bradberry SM, Cage SA, Proudfoot AT, Vale JA (2005) Poisoning due to Pyrethroids. Toxicol Rev 24:93–106. https://doi.org/10.2165/00139709-200524020-00003

Bravo N, Garí M, Grimalt JO (2022) Occupational and residential exposures to organophosphate and pyrethroid pesticides in a rural setting. Environ Res 214:1–8. https://doi.org/10.1016/j.envres.2022.114186

Chrustek A, Hołyńska-Iwan I, Dziembowska I, et al (2018) Current research on the safety of pyrethroids used as insecticides. Med 54:1–15. https://doi.org/10.3390/medicina54040061

Dixon HM, Armstrong G, Barton M, et al (2019) Discovery of common chemical exposures across three continents using silicone wristbands. R Soc Open Sci 6:. https://doi.org/10.1098/rsos.181836

Doherty BT, Pearce JL, Anderson KA, et al (2020) Assessment of Multipollutant Exposures During Pregnancy Using Silicone Wristbands. Front Public Heal 8:1–13. https://doi.org/10.3389/fpubh.2020.547239

Donald CE, Scott RP, Blaustein KL, et al (2016) Silicone wristbands detect individuals' pesticide exposures in West Africa. R Soc Open Sci 3:. https://doi.org/10.1098/rsos.160433

Elbetieha A, Da'as SI, Khamas W, Darmani H (2001) Evaluation of the toxic potentials of cypermethrin pesticide on some reproductive and fertility parameters in the male rats. Arch Environ Contam Toxicol 41:522–528. https://doi.org/10.1007/s002440010280

Hammel SC (2016) Measuring Personal Exposure to Organophosphate Flame Retardants using Silicone Wristbands and Hand Wipes. Environ Sci Technol 50:4483–4491. https://doi.org/10.1021/acs.est.6b00030.

Harley KG, Parra KL, Camacho J, et al (2019) Determinants of pesticide concentrations in silicone wristbands worn by Latina adolescent girls in a California farmworker community: The COSECHA youth participatory action study. Sci Total Environ 652:1022–1029. https://doi.org/10.1016/j.scitotenv.2018.10.276

Jurewicz J, Radwan M, Wielgomas B, et al (2015) The effect of environmental exposure to pyrethroids and DNA damage in human sperm. Syst Biol Reprod Med 61:37–43. https://doi.org/10.3109/19396368.2014.981886

Jurewicz J, Radwan P, Wielgomas B, et al (2020) Exposure to pyrethroid pesticides and ovarian reserve. Environ Int 144:0–4. https://doi.org/10.1016/j.envint.2020.106028

Kaneko H (2011) Pyrethroids: Mammalian Metabolism and Toxicity. J Agric Food Chem 59:2786–2791. https://doi.org/10.1021/jf102567z

Klimowska A, Amenda K, Rodzaj W, et al (2020) Evaluation of 1-year urinary excretion of eight metabolites of synthetic pyrethroids, chlorpyrifos, and neonicotinoids. Environ Int 145:. https://doi.org/10.1016/j.envint.2020.106119

Koch HM, Aylward LL, Hays SM, et al (2014) Inter- and intra-individual variation in urinary biomarker concentrations over a 6-day sampling period. Part 2: Personal care product ingredients. Toxicol Lett 231:261–269. https://doi.org/10.1016/j.toxlet.2014.06.023

Li Y, Wang X, Feary McKenzie J, et al (2022) Pesticide exposure in New Zealand school-aged children: Urinary concentrations of biomarkers and assessment of determinants. Environ Int 163:107206. https://doi.org/10.1016/j.envint.2022.107206

Manzano CA, Dodder NG, Hoh E, Morales R (2019) Patterns of Personal Exposure to Urban Pollutants Using Personal Passive Samplers and GC × GC/ToF-MS. Environ Sci Technol 53:614–624. https://doi.org/10.1021/acs.est.8b06220

Moniz AC, Cruz-Casallas PE, Salzgeber SA, et al (2005) Behavioral and endocrine changes induced by perinatal fenvalerate exposure in female rats. Neurotoxicol Teratol 27:609–614. https://doi.org/10.1016/j.ntt.2005.05.005

O'Connell, Steven G., Laurel D. Kincl KAA (2014) Silicone Wristbands as Personal Passive Samplers. Environ Sci Technol 3327–225. https://doi.org/dx.doi.org/10.1021/es405022f

Radwan P, Wielgomas B, Radwan M, et al (2022) Synthetic Pyrethroids Exposure and Embryological Outcomes: A Cohort Study in Women from Fertility Clinic. Int J Environ Res Public Health 19:1–10. https://doi.org/10.3390/ijerph19095117

Reddam A, Tait G, Herkert N, et al (2020) Longer commutes are associated with increased human exposure to tris(1,3-dichloro-2-propyl) phosphate. Environ Int 136:105499. https://doi.org/10.1016/j.envint.2020.105499

Rodzaj W, Wileńska M, Klimowska A, et al (2021) Concentrations of urinary biomarkers and predictors of exposure to pyrethroid insecticides in young, Polish, urban-dwelling men. Sci Total Environ 773:145666. https://doi.org/10.1016/j.scitotenv.2021.145666

Šulc L, Janoš T, Figueiredo D, et al (2022) Pesticide exposure among Czech adults and children from the CELSPAC-SPECIMEn cohort: Urinary biomarker levels and associated health risks. Environ Res 214:. https://doi.org/10.1016/j.envres.2022.114002

Travis SC, Aga DS, Queirolo EI, et al (2020) Catching flame retardants and pesticides in silicone wristbands: Evidence of exposure to current and legacy pollutants in Uruguayan children. Sci Total Environ 740:140136. https://doi.org/10.1016/j.scitotenv.2020.140136

U.S. Depatrment of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) C for BE and R (CBER) (2005) Q2 (R1): Validation of analytical procedures: text and methodology

Ueyama J, Ito Y, Hamada R, et al (2022) Simultaneous quantification of pyrethroid metabolites in urine of non-toilet-trained children in Japan. Environ Health Prev Med 27:1–8. https://doi.org/10.1265/EHPM.21-00037

Wacławik M, Rodzaj W, Wielgomas B (2022) Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches. Int J Environ Res Public Health 19:1935. https://doi.org/10.3390/ijerph19041935

Wang Y, Peris A, Rifat MR, et al (2020) Measuring exposure of e-waste dismantlers in Dhaka Bangladesh to organophosphate esters and halogenated flame retardants using silicone wristbands and T-shirts. Sci Total Environ 720:137480. https://doi.org/10.1016/j.scitotenv.2020.137480

Wielgomas B (2013) Variability of urinary excretion of pyrethroid metabolites in seven persons over seven consecutive days—Implications for observational studies. Toxicol Lett 221:15–22. https://doi.org/10.1016/j.toxlet.2013.05.009

Wielgomas B, Nahorski W, Czarnowski W (2013) Urinary concentrations of pyrethroid metabolites in the convenience sample of an urban population of Northern Poland. Int J Hyg Environ Health 216:295–300. https://doi.org/10.1016/j.ijheh.2012.09.001

Wielgomas B, Piskunowicz M (2013) Biomonitoring of pyrethroid exposure among rural and urban populations in northern Poland. Chemosphere 93:2547–2553. https://doi.org/10.1016/j.chemosphere.2013.09.070

Wise CF, Hammel SC, Herkert N, et al (2020) Comparative Exposure Assessment Using Silicone Passive Samplers Indicates That Domestic Dogs Are Sentinels To Support Human Health Research. Environ Sci Technol 54:7409–7419. https://doi.org/10.1021/acs.est.9b06605

Zuy Y, Sweck SO, Dockery CR, Potts GE (2019) HPLC detection of organic gunshot residues collected with silicone wristbands. Anal Methods 12:85–90. https://doi.org/10.1039/c9ay02305d

Funding

This work was funded by the Polish Ministry of Science and Higher Education by means of ST5 02-0005/07/514 statutory funds. Małgorzata Wacławik appreciates the scholarship received from the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Program Knowledge Education Development 2014–2020.

7. Competing interests

The authors have no relevant financial or non-financial interests to disclose.

8. Data availability

The datasets generated during and/or analyzed during the current study are not publicly available due to the protection of personal information but are available from the corresponding author on reasonable request.

9. Authors contributions

Authors contributions are being declared as follows:

Study design: B. Wielgomas, M. Wacławik
Participant recruitment: M. Wacławik. B. Wielgomas, D. Skwarło
Sample collection: M. Wacławik, B. Wielgomas, D. Skwarło
Questionnaire design: M. Wacławik, B. Wielgomas
Method optimization: M. Wacławik, B. Wielgomas
Urine analysis: M. Wacławik, D. Skwarło
Wristband analysis: M. Wacławik
Data collection and interpretation: M. Wacławik, B. Wielgomas
Preparation of the manuscript text: M. Wacławik, B. Wielgomas
Supervision of the experiment: B. Wielgomas, J. Jurewicz
Supervision of manuscript preparation: B. Wielgomas, J. Jurewicz

SUPPLEMENTARY INFORMATION

Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites– a pilot study preceded by development of cost-effective GC-ECD method

Małgorzata Wacławik[1], Dominika Skwarło[1], Joanna Jurewicz[2], Bartosz Wielgomas[1]*

[1]Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, 107 Hallera Street, 80-416 Gdańsk, Poland
[2]Department of Chemical Safety, Nofer Institute of Occupational Medicine, 8 Teresy St., 91-348 Łódź, Poland

*corresponding author: bartosz.wielgomas@gumed.edu.pl

1. Method information – determination of wristband despoilments

Gas Chromatograph	Varian GC-450			
Autosampler	CP-8400			
Injector	1177 split/splitless			
Injector temperature	290 °C			
Injector mode	Split, 1:20			
Sample injection volume	1 µL			
Column flow	1 mL/min			
Carrier gas	Helium (6.0)			
Detector	Varian 225-MS ion trap mass spectrometer			
Column	0.25mm ID, 25 µm, 30 m (ZEBRON Guardian ZB-5MSplus, Phenomenex)			
Oven program	Temp. [°C]	Rate [°C/min]	Hold [min]	Total [min]
	70.00	-	1.00	1.00
	300.00	18.00	8.00	21.78

2. Method information – determination of pyrethroid metabolites in urine

Gas Chromatograph	Varian GC-450			
Autosampler	CP-8400			
Injector	1177 split/splitless			
Injector temperature	280 °C			
Injector mode	Splitless			
Sample injection volume	2 µL			
Column flow	1 mL/min, pressure pulse (25 psi, 2 min)			
Carrier gas	Helium (6.0)			
Detector	Varian 225-MS ion trap mass spectrometer			
Column	0.25mm ID, 25 µm, 30 m (ZEBRON Guardian ZB-5MSplus, Phenomenex)			
Oven program	Temp. [°C]	Rate [°C/min]	Hold [min]	Total [min]
	60.00	-	1.00	1.00
	150.00	8.00	0.00	12.25
	280.00	30.00	5.00	21.58

3. Method information – determination of native pyrethroids in silicone wristbands

Gas Chromatograph	SCION Instruments, 456-GC
Autosampler	CP-8400
Injector	1177 split/splitless
Injector temperature	280 °C
Injector mode	Split (10:1)
Sample injection volume	1 µL
Column flow	2 mL/min, pressure pulse

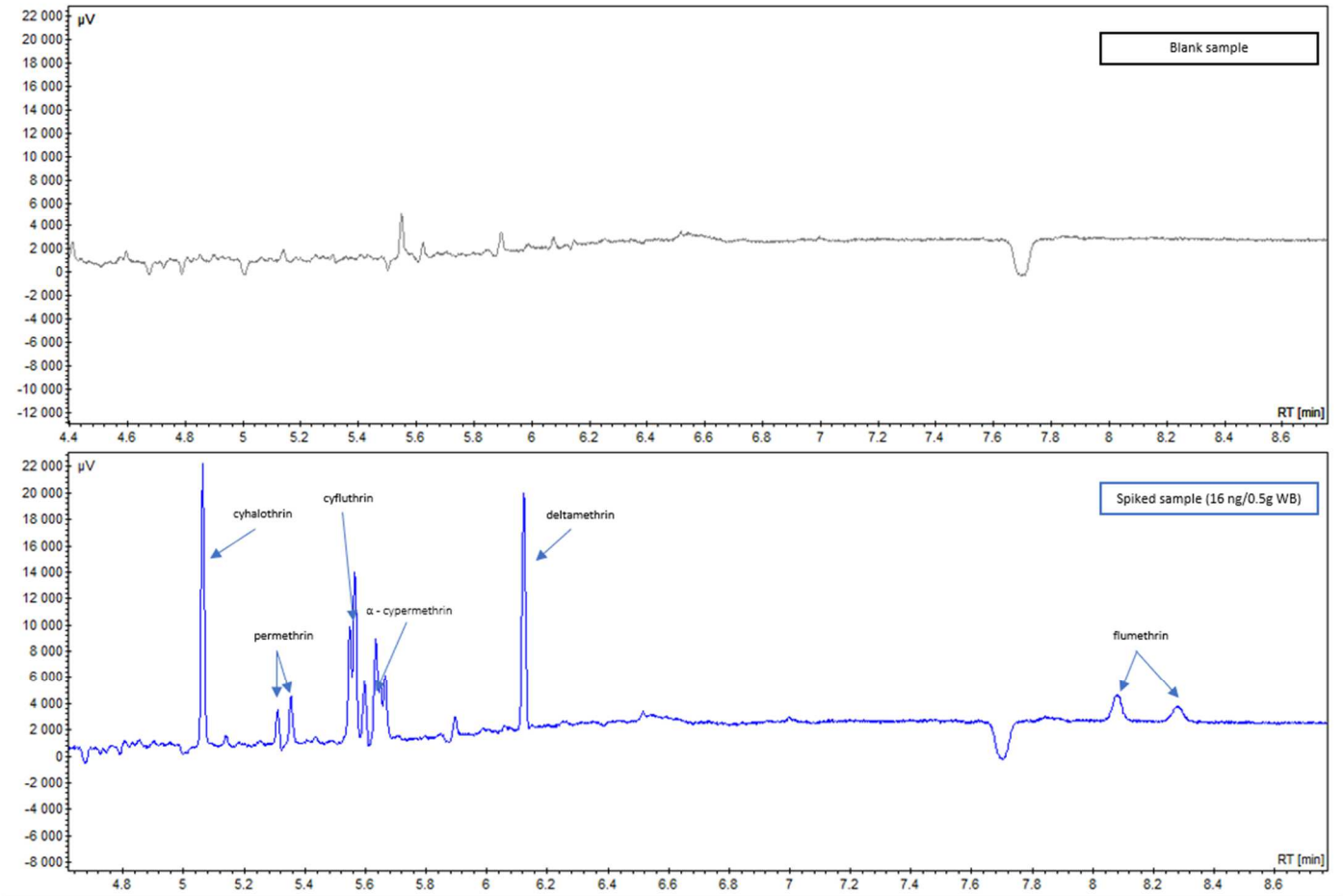
Detector	Electron Capture Detector			
Detector temperature	300 °C			
Carrier gas	Hydrogen (5.0)			
Make-up gas	Nitrogen (25 mL/min)			
Column				
Oven program	Temp. [°C]	Rate [°C/min]	Hold [min]	Total [min]
	120.00	-	1.00	1.00
	280.00	30.0	2.50	8.83

4. Cleanup sorbents preparation

Florisil and silica gel used for sample cleanup of silicone wristband extracts were prepared as follows. The wanted amount (500 g) of a sorbent (florisil or silica gel) had been weighted into a clean crystallizing dish. Next, the dish with sorbent was placed in a laboratory oven for 18 hours heated to a temperature of 140°C. Further, a calculated amount of deionized water was added to each sorbent, which was equal to either 3% of sorbent mass, (3% deactivation), or 10% of sorbent mass (10% deactivation), as the containers were placed on a horizontal roller mixer, to ensure thorough mixing of the sorbent with added water. After the addition of deionized water, the containers remained on the mixer for about an hour, after which they were ready to use.

5. Method validation:

a) Method selectivity



b) Carry-over effect

Determination of presence/magnitude of carry-over effect has been carried out by analyzing a sequence of spiked samples (200 ng/0.5g of wristband) alternately with blank wristband (WB) extracts. No carry-over effect was detected, as response of the blank samples had been lower than 20% of the response of the lower limit of quantification (LOQ).

c) Calibration curve

The prepared 6-point calibration curve consisted of points:

- 10 ng/g WB
- 20 ng/g WB
- 40 ng/g WB
- 100 ng/g WB
- 200 ng/g WB
- 400 ng/g WB

Each concentration level has been analyzed in 3 replicas. It should however be noted, that the designing of calibration curve has been slightly problematic, as very little data is available regarding expected native pyrethroid concentrations in silicone wristbands. Most information needed for this experiment had been derived from our own preliminary tests and studies regarding the matter.

Analyte	**Limit of detection [ng/g WB]**	**Equation of the calibration curve**
Cyhalothrin	2	y = 21.529x - 30.842
Permethrin	10	y = 4.158
α-cypermethrin	10	y = 2.9696x
Cyfluthrin	10	y = 15.792x + 51.123
Deltamethrin	2	y = 26.655x - 208.85
Flumethrin	10	y = 11.151x - 6.4005

d) Accuracy and precision

Three sets of Quality check (QC) samples have been prepared at three concentrations:

- QC1 – 32 ng/ g WB
- QC2 – 160 ng/g WB
- QC3 – 340 ng/g WB

Each QC concentration level has been prepared in a series of 5 samples per run, with the use of stock solutions of native pyrethroids separate from the ones used for calibration curve. Concentrations of QC samples have been designed to be spaced out within the calibration range. Each batch of QC samples have been prepared and analyzed on a different day than the rest (Day 1,2,3).

		within-run accuracy [%] - (vs. Nominal concentration value)					
		cyhalothrin	permethrin	cyfluthrin	α-cypermethrin	deltamethrin	flumethrin
Day 1	QC1	105.6	138.5	97.4	130,0	106.5	77.8
Day 2	QC1	101.7	116.8	99.7	106,2	129.9	79.9
Day 3	QC1	120.2	139.3	126.6	104,2	119.2	81.1
Day 1	QC2	94.5	123.1	113.5	118,3	76.6	89.5
Day 2	QC2	83.7	111.6	101.9	105,7	66.9	78.6
Day 3	QC2	92.5	120.4	110.9	119,5	71.1	77.6
Day 1	QC3	111.8	93.3	116.5	97,7	83.9	84.5
Day 2	QC3	100.1	107.0	110.1	110,8	74.9	79.6
Day 3	QC3	77.0	114.9	91.1	111,0	63.1	79.8

		within-run precision [%]					
		cyhalothrin	permethrin	cyfluthrin	α-cypermethrin	deltamethrin	flumethrin
Day 1	QC1	10.8	7,7	16.5	8,7	9.1	13.1
Day 2	QC1	5.3	14,2	6.6	6,5	15.3	8.0
Day 3	QC1	8.7	11,2	10.3	15,5	12.4	6.3
Day 1	QC2	14.8	4,6	13.5	3,4	8.8	9.5
Day 2	QC2	8.8	8,0	8.7	5,6	11.8	1.8
Day 3	QC2	4.6	11,4	5.6	16,3	3.6	6.2
Day 1	QC3	20.3	10,1	14.9	12,2	18.8	12.3
Day 2	QC3	11.8	7,6	12.4	11,9	16.4	14.0
Day 3	QC3	13.9	10,9	12.7	18,8	13.7	11.1

		between-run accuracy [%] - (vs. Nominal concentration value)					
		cyhalothrin	permethrin	cyfluthrin	α-cypermethrin	deltamethrin	flumethrin
Day 1	QC1	109.2	131.5	107.9	113.5	118.5	79.6
Day 2	QC1						
Day 3	QC1						
Day 1	QC2	90.2	118.4	108.8	114.5	71.5	81.9
Day 2	QC2						
Day 3	QC2						
Day 1	QC3	96.3	105.1	105.9	106.5	74.0	81.3
Day 2	QC3						
Day 3	QC3						

		between-run precision [%]					
		cyhalothrin	permethrin	cyfluthrin	α-cypermethrin	deltamethrin	flumethrin
Day 1	QC1	11.0	13.1	16.7	14.5	14.6	8.9
Day 2	QC1						
Day 3	QC1						
Day 1	QC2	11.1	8.9	10.4	11.2	9.8	9.4
Day 2	QC2						
Day 3	QC2						
Day 1	QC3	21.9	12.6	16.4	15.1	19.7	11.9
Day 2	QC3						
Day 3	QC3						

MSc Małgorzata Wacławik
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Joanna Jurewicz, Bartosz Wielgomas. *„Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites – a pilot study preceded by development of cost-effective GC-ECD method"* (working title).

Which is part of my doctoral dissertation, my participation in its creation involved performing literature review, laboratory research, data analysis and preparation of the original manuscript.

My contribution in preparation of this work has been estimated to sum up to 60%.

Wacławik
(signature)

MSc Dominika Skwarło
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> *Małgorzata Wacławik, Dominika Skwarło, Joanna Jurewicz, Bartosz Wielgomas. „Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites – a pilot study preceded by development of cost-effective GC-ECD method"* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved assistance in sample collection and analysis.

My contribution in preparation of this work has been estimated to sum up to 10%.

Skwarło Dominika
(signature)

Bartosz Wielgomas, PhD, Assoc. Prof.
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Joanna Jurewicz, Bartosz Wielgomas. *„Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites – a pilot study preceded by development of cost-effective GC-ECD method"* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved research conceptualization, reviewing and editing of the original manuscript, as well as research supervision.

My contribution in preparation of this work has been estimated to sum up to 20%.

.....
(signature)

Prof. Joanna Jurewicz
Department of Chemical Safety
Nofer Institute of Occupational Medicine
8 Teresy St., 91-348 Łódź, Poland

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Joanna Jurewicz, Bartosz Wielgomas. *„Assessment of exposure to synthetic pyrethroids with the use of silicone wristbands and biomonitoring of urinary metabolites – a pilot study preceded by development of cost-effective GC-ECD method"* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved reviewing and editing of the original manuscript, as well as research supervision.

My contribution in preparation of this work has been estimated to sum up to 10%.

Signed by /
Podpisano przez:

Joanna Katarzyna
Jurewicz

Date / Data: 2023-
10-10 07:37

.....

(signature)

Manuscript 3 - Małgorzata Wacławik, Wojciech Rodzaj, Joanna Jurewicz, Bartosz Wielgomas. *„Evaluation of exposure to synthetic pyrethroids among pet owners in a study with panned veterinary product application”* (working title) – submission to Journal of Hazardous Materials

Submission to: Journal of Hazardous Materials

Evaluation of exposure to synthetic pyrethroids among pet owners in a study with planned veterinary product application

Małgorzata Wacławik [1], Wojciech Rodzaj [1], Joanna Jurewicz [2], Bartosz Wielgomas [1]*

[1] Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, 107 Hallera Street, 80-416 Gdańsk, Poland

[2] Department of Chemical Safety, Nofer Institute of Occupational Medicine, 8 Teresy St., 91-348 Łódź, Poland

* corresponding author: bartosz.wielgomas@gumed.edu.pl

Authors:

Małgorzata (first name) Wacławik (last name) – ORCID 0000-0002-9288-2180

Wojciech (first name) Rodzaj (last name) – ORCID 0000-0002-6958-7271

Joanna (first name) Jurewicz (last name) – ORCID 0000-0001-9645-0134

Bartosz (first name) Wielgomas (last name) – ORCID 0000-0003-4245-9197

1. Abstract

Given the worldwide usage of synthetic pyrethroids and its constant increase over the recent years, possible exposure to these compounds is an issue of growing concern. Furthermore, insecticidal employment of synthetic pyrethroids as active components of veterinary anti-ectoparasitic products is a common occurrence among pet owners, which makes them a sub-population with an increased proclivity to come into contact with those compounds. In order to assess magnitude and time changeability of exposure to synthetic pyrethroids a study on 15 pet owners, with planned veterinary drug application has been launched. Urinalysis of pyrethroid metabolites has been reinforced with implementation of personal passive samplers in form of silicone wristbands, meant to provide a time-weighted average level of exposure to parent pyrethroid compounds employed in the study. Collection of multiple urine samples and wearing two separate silicone wristbands prior to and after the application of the pyrethroid-containing product, and their analysis with the use of gas-chromatography mass-spectrometry (quantification of 3-PBA (3-phenoxybenzoic acid), DBCA (*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid*, cis*- and *trans*-DCCA (*cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid in urine) and gas chromatography with an electron capture detector (determination of permethrin, cypermethrin and deltamethrin in extracts of silicone wristbands), has provided an opportunity to investigate formed patterns of exposure, that have shown numerous similarities between members of the same households. A statistically significant increase in concentrations of both urinary pyrethroid metabolites (p = 0.0429), and permethrin determined in wristbands (p = 0.003) in samples collected during a week directly following the drug application had been noted. Additionally, a very strong correlation (r_s = 0.9161, $p < 0.05$) between median concentrations of urinary metabolites and levels of wristband pyrethroid concentrations had been noted in samples collected after the drug application, with corresponding relation being strong (r_s = 0.7735, $p < 0.05$) in samples collected prior to product employment. Furthermore, concentrations measured in spot urine samples collected 4 weeks after application are higher than the levels measured initially, before product employment. The employment of silicone wristbands in tandem with biomonitoring has allowed to investigate the presence of pyrethroids in indoor environments and to assess exposure to these compounds among study participants.

Keywords: silicone wristbands, planned exposure, synthetic pyrethroids, human biomonitoring, pet owners, veterinary drugs

2. Introduction

Synthetic pyrethroids are a class of pesticides, that emerged as a more chemically stable derivatives of naturally occurring pyrethrins (Bradberry et al. 2005) and have since been employed as active components of many products dedicated for pest control of both commercial and at-home use (Fortin et al. 2008). Their use has significantly increased in the recent years (Saillenfait, Ndiaye, and Sabaté 2016). Pyrethroid-containing agents are commonly used in forestry, pest control of crops (agriculture) (Lehmler et al. 2020), lice and scabies treatments (Fortin et al. 2008), veterinary medicine (Anadón, Martínez-Larrañaga, and Martínez 2009) with their insecticidal properties also finding household applications. Currently they are considered one of the most frequently used class of pesticides. The broad array of application options of synthetic pyrethroids as well as products containing them being readily available for purchase can result in exposure taking place among varying groups in both occupational and ambient conditions, via different routes of exposure. Given, that products containing said substances are commonly used in indoor household settings, sometimes in a repetitive manner over time, together with their relatively high environmental stability (Zhu et al. 2020) they can accumulate on surfaces, leading to prolonged exposure for occupants of indoor spaces (Berger-Preiß et al. 2002; Leng et al. 2003; Yoshida, Mimura, and Sakon 2021; Al-Alam et al. 2022).

The mechanism of insecticidal action of synthetic pyrethroids relies on their interaction with sodium channels located in neuronal membranes. Their administration causes prolonged depolarization of said channels, and with that, overexcitation resulting from extensive sodium influx to the cell (Chrustek et al. 2018; Vais et al. 2001; Bradberry et al. 2005). Pyrethroid toxicity towards insects is over 2000 times greater than to mammals, mainly due to differences in sensitivity of sodium channels to these substances between the species, but also because of small body size of insects, and their relatively low body temperatures (Bradberry et al. 2005).

While low susceptibility of mammalian neurons to action of pyrethroids is considered the main advantage in evaluating the safety of their outdoor and indoor use, it is worth mentioning that studies have shown that as little as 1% of applied pesticides reach the intended target organism (Gavrilescu 2005). That, in turn leaves a conclusion that the remaining dose can be disseminated in the microenvironment and become the potential cause of exposure of non-target organisms (M. Ye et al. 2017).

The exposure to synthetic pyrethroids can occur through ingestion of contaminated water or foodstuffs, inhalatory and/or dermal route (Katsuda 2011; Kaneko 2011). Coming into contact with pyrethroids via inhalation is possible only through their transfer with dust or droplets, as due to their low volatility it is impossible for them to naturally occur as a vapor (Laskowski 2002; Yoshida, Mimura, and Sakon 2021; Al-Alam et al. 2022). Dermal route constitutes to a very small percentage of the total exposure (Katsuda 2011), however it can readily lead to oral exposure, which is an issue of exceptional importance in case of children, who are known to express higher hand-to-mouth action (Lu et al. 2006).

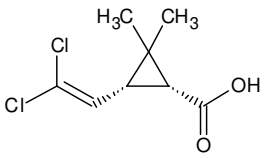
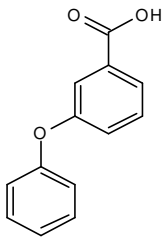
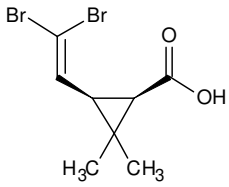
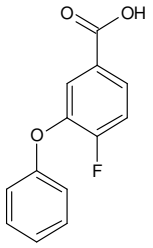
Cases of acute human poisoning due to pyrethroids are rarely noted (Bradberry et al. 2005), however, still alarmingly little is known regarding the adverse effects of chronic exposure to those compounds. Some of thus far elucidated detrimental long-term effects include negative impact on neurocognitive development among children (Shelton et al. 2014), their possession of endocrine disrupting capabilities (Marettova, Maretta, and Legáth 2017), which in turn has been linked to several issues regarding the reproductive systems of both males and females: decreasing semen quality (Jurewicz, Radwan, Sobala, et al. 2015), increased fetal mortality (Ahmad, Khan, and Khan 2012), and fertility impairments in both

genders (Radwan et al. 2014). Recently, Bao et al. (2020) suggested using a nationally representative sample of US adults, exposure to pyrethroid insecticides in the environment is linked to a higher risk of mortality due to all causes, including cardiovascular disease (Bao et al. 2020).

In view of the recently enduring increase in worldwide use of products containing synthetic pyrethroids and given rather limited state of knowledge regarding the health effects of prolonged exposure to their small doses, exposure assessment studies are of vital importance.

Exposure assessment to synthetic pyrethroids is most commonly carried out with the use of biomonitoring, a method considered to be the 'golden standard', which in this case includes measurement of levels of pyrethroid metabolites: 3-phenoxybenzoic acid (3-PBA); cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA), 4-fluoro-3-phenoxybenzoic acid (4F-3-PBA), *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid) (*cis*- and *trans*-DCCA) in urine (Table 1).

Table 1. Overview of analysed native pyrethroid substances and their respective urinary metabolites.

				
Name of compound	*cis*/*trans*-DCCA	3-PBA	DBCA	4F-3PBA
Cyhalothrin		❋		
Cyfluthrin	❋			❋
Permethrin	❋	❋		
Cypermethrin	❋	❋		
Deltamethrin		❋	❋	
Flumethrin				❋

The urinary metabolite concentrations reflect the total exposure to pyrethroids, which is essential for human health risk assessment. However, given that parent pyrethroid compounds tend to undergo rapid metabolism within human bodies, the analysis of a single spot urine sample can unveil information regarding exposure that occurred within a very limited period of time post-contact (Fortin et al. 2008) and leading to under or overestimation of average exposure. To address this limitation, one approach used for longitudinal exposure studies involves repeated urine sampling from each participant over a desired time frame. Although this approach may inconvenience participants, it ultimately provides more reliable insights into prolonged exposure to pyrethroids (Wielgomas 2013; Roggeman et al. 2022).

A relatively newly discovered method applied in exposure studies involves the use of silicone wristbands (WBs). The seminal paper describing their employment has appeared in 2014 (O'Connell, Kincl, and Anderson 2014). Since then, various approaches to using WBs for studying exposure to different compounds have been investigated. Silicone wristbands are known to possess an ability to collect/adsorb lipophilic chemicals, as they bind to the silicone polymer composing their structure. Therefore, when worn by an individual for a period of time, WBs serve as personal passive samplers and can provide information about the average exposure to substances (Manzano et al. 2019) that have been in close proximity to the wearer throughout that time. WBs offer a cost-effective (Bergmann et al. 2017; Baum et al. 2020), non-invasive (Romanak et al. 2019; Travis et al. 2020; Hammel et al. 2016) sampling method suitable for use among sensitive populations (Doherty et al. 2020; Travis et al. 2020).

One of many possible sources of exposure to synthetic pyrethroids occurring in a residential setting is employment of veterinary drug products on indoor-dwelling pets. Considering the minimum recommended doses of pyrethroids (permethrin: 50 mg/kg body weight) contained in these products, the frequency of their application (monthly), as well as the limited knowledge regarding the persistence of resulting indoor exposure, such occurrence should be thoroughly investigated.

To the best of our knowledge, there have been no experimental data published showing the extent of human exposure to synthetic pyrethroids following their use on pets using biomonitoring methods. Additionally, we wanted to assess the utility of silicone wristbands in exposure studies related to this group of chemical compounds. Our study is the first to verify the suggestions of numerous observational studies where researchers pointed to the use of veterinary parasitic drugs on pets as a strong predictor of exposure, but no one has provided experimental evidence for this until now. In this respect, our study is unique and provides valuable data. The results obtained in this study may shed new light on the human safety of using certain formulations of antiparasitic agents on pets.

3. Materials and methods

3.1. Silicone wristbands

White silicone wristbands (average: width - 12 mm, length - 200 mm, thickness - 1.48 mm, weight - 5 g) used in this study had been purchased in bulk from an online vendor (www.allegro.pl), with their intended use being an accessory for promotional purposes. Prior to employment in the described exposure assessment study, WBs had been properly cleaned by a series of solvent washes (unpublished results, Manuscript No. 2), sealed in airtight zip-lock bags, and their packaging labeled.

3.2. Solvents

Solvents used included: n-hexane (Hex) (n-hexane 95% for GC, for pesticide residue analysis, POCH, Gliwice, Poland); diethyl ether (ACS grade, Sigma-Aldrich, Saint Louis, USA); ethyl acetate (EtAc) (for gas chromatography MS, Supelco, Saint Louis, USA); methanol (MeOH) (technical grade, POCH, Gliwice, Poland); n-hexane (fraction from petroleum pure, POCH, Gliwice, Poland); ethyl acetate (technical grade, POCH, Gliwice, Poland). Water was obtained from the laboratory water demineralizer (Hydrolab, Wiślina, Poland) and 2-propanol (IPA, 2-propanol for HPLC) from VWR International (France).

3.3. Analytical standards

Standards used in the described study included native pyrethroids for wristband analysis, namely: cypermethrin (mix of isomers) (Institute of Organic Industrial Chemistry, Poland), permethrin (mix of isomers) (EPA Research, USA), beta-cyfluthrin (Institute of Organic Industrial Chemistry, Poland),

lambda-cyhalothrin (Institute of Organic Industrial Chemistry, Poland), deltamethrin (Roussel Uclaf, France), flumethrin (mix of isomers) (Sigma Aldrich, Germany). Internal standards used in urinalysis included: *cis*-DCCA 100 ug/mL in Acetonitrile-D3 (1, Carboxyl-13C2, 99%; 1-D, 97%) – purchased from Cambridge Isotope Laboratories (USA) and 2-PBA (2-phenoxybenzoic acid) – purchased from Fluka (Germany). Pyrethroid metabolites standards employed in quantitative analysis of urine samples included: 3-PBA (Lancaster, United Kingdom), *cis*-DCCA (Toronto Research Chemicals, Canada), *trans*-DCCA (Toronto Research Chemicals, Canada), DBCA (Roussel Uclaf, France) and 4F-3-PBA (Cambridge Isotope Laboratories, USA).

3.4. Other reagents/supplies

Other reagents used in sample analysis were potassium carbonate - anhydrous pure p.a. (POCH, Gliwice, Poland), sodium hydroxide pure p.a. (POCH, Gliwice, Poland); derivatizing agents - 1,1,1,3,3,3-Hexafluoro-2-propanol (Sigma- Aldrich, USA) and DIC (N,N'-diisopropylcarbodiimide) (99%, Sigma Aldrich, Saint Louis, USA); and hydrochloric acid (J.T. Baker, Radnor, USA), for hydrolysis in urinalysis. A silica gel (Sigma-Aldrich, Saint Louis, USA, pore size 60Å, 220-240 mesh particle size) was used for WBs extracts cleanup.

3.5. Gas chromatography

Listed below are specifications of instruments and chromatographic methods used for analysis of both urine and WBs with GC-MS and GC-ECD respectively.

Table 2. Summary of chromatographic conditions used in the study.

Purpose	**Urine analysis**				**Wristband analysis**			
Gas chromatograph	Varian GC - 450				SCION Instruments, 456-GC			
Autosampler	CP – 8400				CP – 8400			
Injector	1177 split/splitless				1177 split/splitless			
Injection volume	2 µL				1 µL			
Injector mode	Splitless				Split (10:1)			
Injector temperature	280 °C				280 °C			
Column	30 m, 0.25mm ID, 0.25 µm (ZEBRON Guardian ZB-5MSplus, Phenomenex)				10 m, 0.15 mm ID, 0.15 µm (VF-5ms, Agilent Technologies)			
Column flow	1 mL/min, pressure pulse (25.0 psi, 2 min)				2 mL/min			
Carrier gas	Helium (6.0)				Hydrogen (5.0)			
Make-up gas	-				Nitrogen, (25 mL/min)			
Column oven program	Temp. [°C]	Rate [°C/min]	Hold [min]	Total [min]	Temp. [°C]	Rate [°C/min]	Hold [min]	Total [min]
	60.00	-	1.00	1.00	120.00	-	1.00	1.00
	150.00	8.00	0.00	12.25	280.00	30.0	2.50	8.83
	280.00	30.00	5.00	21.58				
Detector	Varian 225-MS ion trap mass spectrometer				Ni^{63} Electron Capture Detector			

4. Study outline

4.1. Sample collection

Volunteers (persons owning at least one dog or a cat) involved in the study have been recruited by the word of mouth. Within each household, all participating residents were asked to complete a survey that included basic demographic information, habits, potential sources of pyrethroid exposure (other than pet drug application) and living conditions. Study participants were also instructed to collect urine samples, wear two wristbands, and place two wristbands in their homes over a total of 5 weeks. During the first week, which was one week before (though not necessarily immediately before) the application of veterinary ectoparasitic drugs (Fig. 1. Stage 1), participants collected a total of three random urine samples (each on a separate day) and wore a wristband on their dominant hand continuously for seven consecutive days. Additionally, a 'field' wristband was placed in each investigated household for the same week. The 'field' wristband was installed in a pre-cleaned metal screen that allowed for airflow while minimizing the risk of external contamination (e.g., touching or contact with surfaces). It was placed at a high point in the room identified by the participants as the most frequently used (which is to be understood as the room in which the participants have been spending the most time daily).
Next, the volunteers assigned a suitable day for the application of the veterinary anti-ectoparasitic drug on their pet, which was performed by one member of the household. The veterinary products (Table 3) were provided by the researchers, and the participants had the option to choose between a spot-on version of the product and a collar-type formula. Over the 24 hours following drug application, participants collected all urine samples. Drug application also marked the beginning of wearing another wristband for the next 7 days (Stage 2) and placing another 'field' wristband indoors (Fig. 1. Stage 2). During the subsequent 6 days post-application, study participants collected one urine sample daily. Additionally, on the 14th and 28th days post-application (Fig. 1. Stage 3), they collected one spot urine sample. The simplified study design is presented in **Fig. 1.**

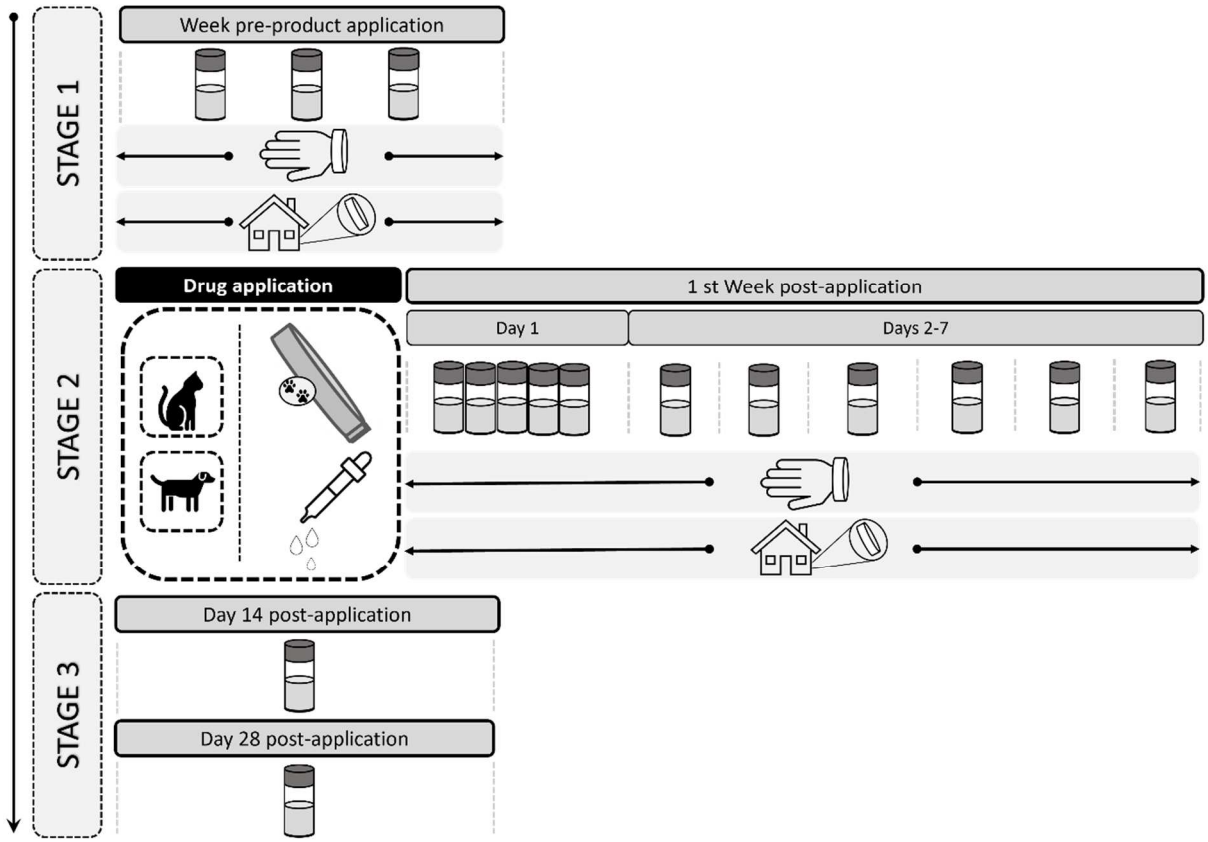


Fig. 1. The outline of sample collection over the course of the study

4.2. Study population

The studied population consisted of a total of 15 volunteers, 8 females (53.3%) and 7 males (46.7%), originating from 6 separate households, who on average were 35.27 years old (range: 15 – 63 years of age). Almost half of the study participants have declared their level of education to be 'higher' (n=7, 46.7%), with another numerous group being high school graduates (n=6, 40%), while only 2 participants (13.3%) having completed primary school at the time of the experiment. Most of the tested group have declared to be non-smokers (n=12, 80%), 2 people have admitted to be actively smoking (13.3%), while just one participant (6.7%) has declared to be a passive smoker. Study participants have also been asked about their living conditions, and so 9 people (60%) have pointed their living area to be urban, and housing to be multi-family buildings, while 6 participants (40%) have named their living locations as rural (small town/village), and have declared living in detached houses. Most (n=9, 60%) of the participants have estimated the distance between their home and farm fields to be under 150 meters, while all the other participants estimated that distance to be more than 1000 meters. It has also been inquired, if the families pets have previously undergone application of any veterinary anti-ectoparasitic topical products: 11 participants (73.3%) have confirmed such applications occurring in the past, while 4 people (26.7%) having declared never previously using any form of pesticide based anti-ectoparasitic products. Volunteers have also been asked about the use of commercially available pesticides in their households, again, 11 participants (73.3%) had confirmed using such products, while 4 people (26.7%) denied their use. The population involved in the study has been divided into two groups: participants who opted for using a veterinary drug containing pyrethroids had been considered the tested group, and those who applied products not containing (**Table 3**) pyrethroids (with active substance being fipronil instead) – the study control group (non-PYR study control). Cat owners have been assigned a

non-pyrethroid product for application by design, as felines are known to be considerably susceptible to synthetic pyrethroids, often resulting in animal poisonings of varying severity (Nebbia 2009).

4.3. Study design – specifics

Approval of the Medical University of Gdańsk Bioethics Committee for Scientific Research No. NKBBN/535/2020 on 8th of October 2020 was granted.

Table 3. Summary of information regarding the design of the study, employed veterinary drug products, pets undergoing treatment, total number of pets and previous applications of similar products (the rows are arranged according to the formula of product used).

Household No.	Product formula	Active agents	Dose per application	Pet	Total No. of pets	No. of household members	Previous applications? *
1	Spot-on	Permethrin, fipronil	3.03 g, 405.6 mg	Dog	1	4	yes
2	Spot-on	Permethrin, fipronil	2.02 g, 270.4 mg	Dog	2	5	yes
3	Spot-on	Permethrin, fipronil	1.01 g, 135.2 mg	Dog	2	2	yes
4	Spot-on	Fipronil (non-PYR, study control)	50 mg	Cat	2	2	no
5	Spot-on	Fipronil (non-PYR, study control)	50 mg	Cat	2	1	no
6	Collar	Cypermethrin, deltamethrin	1.35 g, 280 mg	Dog	1	1	yes

*It should be noted that in the described study, the application of veterinary drugs to indoor-dwelling pets has been confirmed as the first one within the calendar year. The scheduling of these applications has been adjusted to coincide with the onset of tick season in Poland to the best of our ability. The term 'Previous applications' refers to the use of similar veterinary products by study participants within the two years leading up to the study.

The frequency of applications involving repetitive doses of spot-on products in this study was recommended to be every 4 weeks until the product was depleted (3 doses per packaging), following the product's summary of characteristics.

4.4. Handling and placement of field-sampling wristbands

To assess the potential distribution of insecticidal substances from the veterinary drugs onto indoor air, a subset of silicone wristbands was prepared. These wristbands were pre-cleaned and placed inside individual aluminum cases (baskets), which had also been thoroughly cleaned ultrasonically using organic solvents. This setup was then sealed in a clean, airtight zip-lock bag and provided to the participants.

The purpose of the aluminum case was to prevent the wristband inside from coming into contact with human skin while allowing airflow through its contents. Study volunteers were instructed to hang the aluminum case (using the provided handle) in the room they identified as the "most used" during the day. This room was where most household members spent the majority of their daily time. The case

was hung at a height of approximately 2 meters, out of reach for all family members and pets. Participants were asked to keep it there for seven consecutive days.

Each household received two sets of these samplers. One set was used for a week before the veterinary drug application (concurrent with the collection of urine samples and personal silicone wristbands). The other set was hung immediately after the veterinary drug application, with a planned sampling time of 7 days.

Once the sampling period was complete, study participants were instructed to place the entire case with the wristband back in the zip-lock bag in which it was delivered, without removing the wristband from the case. The wristbands were removed from the baskets upon their arrival at the laboratory by one of the study investigators and stored at -20°C until they were analyzed.

4.5. Sample analysis

In all urine samples and wristbands collected during the study, the concentrations of synthetic pyrethroid metabolites and the concentrations of parent pyrethroids were determined accordingly. For this purpose, fully validated analytical methods subjected to continuous quality assurance and quality control procedures, were used. Synthetic pyrethroid metabolites in urine were measured after prior hydrolysis, extraction, and derivatization using HFIP and finally analyzed by GC-MS (Wielgomas and Piskunowicz 2013). Parent pyrethroids were determined in wristbands by solvent extraction, followed by cleanup of the extract using silica gel, and then subjected to instrumental analysis using GC-ECD (Manuscript No. 2). Detailed description and pictorial visualization of the sample preparation procedures regarding both urine samples and silicone wristbands can be found in Supplementary Materials.

4.6. Data handling and statistical analysis

Samples with results of any analyzed pyrethroid metabolite being below the limit of detection (LOD) were assigned a value equal to LOD/√2. To minimize the effect of urine dilution on the metabolite levels, the concentrations, which are a direct result read from the calibration curve, were corrected based on the specific gravity (urine SG), which was determined in each urine sample using a hand-held refractometer (PAL-10S, Atago Co., Tokyo, Japan). Reference values of urine SG were predetermined to be within range: 1.005 – 1.030 (Simerville, Maxted, and Pahira 2005). Samples with urine specific gravity values below the low cut-off value, or above the upper cut-off value were disqualified from further analysis. Samples with urine SG assessed to be within the reference range had their calibration curve-calculated metabolite concentrations adjusted according to the following formula:

$$C_{adjusted} = C_{calculated} \times \frac{(SG_{population\ mean} - 1)}{(SG_{sample} - 1)}$$

$C_{calculated}$ – measured metabolite concentration
$SG_{population\ mean}$ – an arithmetic mean of all urine SG assayed for samples collected in the study
SG_{sample} – measured urine specific gravity of given sample

Standard gravity (SG) adjusted concentrations of urinary pyrethroid biomarkers were used in further calculations and comparisons.

The investigation of exposure patterns required the establishment of a comparative value capable of fairly relating to the components of insecticidal products used in the study. This value ended up being the sum of urinary pyrethroid metabolite concentrations (3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA) calculated for each individual sample collected by the study participants.

For the purpose of statistically comparing concentrations between samples collected at different stages of the study using the linear multiple-timepoint sampling method (Friedman test), medians of the sums of urinary metabolite concentrations were calculated separately for samples from each stage of the study for each participant.

A comparison (Mann-Whitney-U test) of the total concentration of urinary pyrethroid metabolites between pyrethroid and non-pyrethroid users was performed based on a sum of metabolite concentrations calculated for each individual sample, following a similar approach to the investigation of pattern of exposure.

Data collection, processing and selected analyses were conducted using Microsoft 365 Excel (Microsoft Corp., Redmond, WA, USA). Statistical data analysis and visualization has been done with the aid of Statistica (TIBCO Software Inc, Palo Alto, CA, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA).

4.7. Quality control

A series of aliquots of quality control (QC) pooled urine were prepared by spiking them with analytes of interest at two concentration levels: LQC = 0.25 ng/mL (low concentration quality control) and HQC = 1.5 ng/mL (high concentration quality control). These QC samples were added in two repetitions for each concentration level to every batch of real samples, alongside blank samples. Prior to sample analysis, a series of 20 (20 LQCs, 20 HQCs) prepared QC samples were analyzed over the course of 4 weeks. This was done to construct control sheets, which later served as a comparative tool usef for ensuring the quality of assessment, as results of QC samples incorporated in sample batches were compared against these control cards (more information in Supplementary Materials).

A similar approach was implemented in the analysis of wristbands. However, due to the small number of analyzed samples, all of them underwent analysis in the single batch. Prepared QC samples were spiked at LQC – 10 ng/g, and HQC – 50 ng/g. Similarily to urinalysis, control samples spiked at these concentrations were added to the analyzed batch of wristband samples, along with suitable blanks. The results were then reviewed using the previously established control criteria.

5. Results

5.1. Urinary metabolite concentrations

Prior to numeric/statistical analysis urinary concentrations of pyrethroid metabolites quantified in collected samples underwent dilution adjustment calculations based on the sample's urine specific gravity. The calculations were carried out as previously described (Wojciech Rodzaj et al. 2021), (Manuscript No. 2). The reference value of SG for the tested population was the arithmetic mean of urine specific gravity of all tested urine samples.

The distribution of the data produced by urinalysis was assessed using the Shapiro-Wilk test, which revealed distribution did not possess the properties characteristic of a normal distribution. Given that result, all further statistical tests conducted in this study were non-parametric, and the descriptive statistics provided in Table 5 contain only suitable values.

Table 4. Detection rates of quantified urinary pyrethroid metabolites in the entire set of collected samples ('Overall'), with an additional sub-distinction of samples collected during the 1st and combined 2nd and 3rd (post-application) stages of the study.

Analyte / Detection rate [%]	3-PBA	*cis*-DCCA	*trans*-DCCA	DBCA
Overall (n)	97.1 (208)	81.3 (174)	86.4 (185)	69.6 (149)
Pre-application (n)	97.6 (41)	69.0 (29)	78.6 (33)	64.3 (27)
Post-application (n)	97.7 (168)	84.3 (145)	88.4 (152)	73.8 (125)

The basic descriptive data analysis conducted on all urinalysis results (pyrethroid and non-pyrethroid users) revealed that the pyrethroid metabolite with the highest concentrations in samples collected both prior to and post drug application was 3-PBA (prior: GM = 0.606 ng/mL, post: GM = 1.170 ng/mL). Additionally, 3-PBA was the most frequently detected biomarker in the samples collected before pyrethroid product application, with its detection rate of 97.1%. The lowest mean concentrations prior to and post application were for DBCA: 0.093 ng/mL and 0.090 ng/mL respectively. DBCA also had the lowest detection rate in samples prior to application at 64.3%. The concentrations of pyrethroid metabolites ranged between 0.035 and 85.6 ng/mL for 3-PBA, 0.035 and 38.3 ng/mL for cis-DCCA, 0.035 and 114.2 ng/mL for trans-DCCA, and 0.035 and 2.483 ng/mL for DBCA across all samples tested in the study. The consistently higher geometric means of concentrations of almost all urinary metabolites in samples collected both prior to ($GM_{3\text{-PBA}}$ = 0.729 ng/mL, $GM_{cis\text{-DCCA}}$ = 0.197 ng/mL, $GM_{trans\text{-DCCA}}$ = 0.488 ng/mL, GM_{DBCA} = 0.096 ng/mL) and after the veterinary drug product application ($GM_{3\text{-PBA}}$ = 1.858 ng/mL, $GM_{cis\text{-DCCA}}$ = 0.705 ng/mL, $GM_{trans\text{-DCCA}}$ = 1.948 ng/mL) among pyrethroid users (PYR in Table 5.) were observed (geometric mean of DBCA has been of slightly higher value among non-pyrethroid users post application – GM_{DBCA} = 0.092 ng/mL). Median values of concentrations of urinary metabolites have also been of higher value (3-PBA = 0.662 ng/mL, *cis*-DCCA = 0.157 ng/mL, *trans*-DCCA = 0.460 ng/mL, DBCA = 0.086 ng/mL) in samples collected before applying the veterinary drug by pyrethroid users in comparison to non-pyrethroid users (3-PBA = 0.210 ng/mL, *cis*-DCCA = 0.035 ng/mL, *trans*-DCCA = 0.035 ng/mL, DBCA = 0.084 ng/mL). Descriptive statistics of urinary metabolite concentrations, with subdivision to pre-application and post-application samples can be found in **Table 5**.

Table 5. Descriptive statistics of urinary concentrations [ng/mL, SG adjusted] of pyrethroid metabolites.

	APPLICATION	USER[1]	n	GM	MIN	P25	MEDIAN	P75	MAX
3-PBA	pre	PYR	35	0.729	0.035	0.362	0.662	1.726	7.785
***cis*-DCCA**	pre	PYR	35	0.197	0.035	0.052	0.157	0.709	2.245
***trans*-DCCA**	pre	PYR	35	0.488	0.035	0.109	0.460	2.268	11.25
DBCA	pre	PYR	35	0.096	0.035	0.035	0.086	0.153	1.283
3-PBA	post	PYR	133	1.858	0.035	0.697	1.485	5.256	85.58
***cis*-DCCA**	post	PYR	133	0.705	0.035	0.180	0.755	2.962	38.30
***trans*-DCCA**	post	PYR	133	1.948	0.035	0.456	2.326	8.824	114.1
DBCA	post	PYR	133	0.090	0.035	0.035	0.077	0.161	2.483
3-PBA	pre	non-PYR	7	0.240	0.076	0.163	0.210	0.569	0.688
***cis*-DCCA**	pre	non-PYR	7	0.054	0.035	0.035	0.035	0.146	0.172
***trans*-DCCA**	pre	non-PYR	7	0.063	0.035	0.035	0.035	0.218	0.345
DBCA	pre	non-PYR	7	0.079	0.035	0.035	0.084	0.143	0.164
3-PBA	post	non-PYR	39	0.242	0.035	0.194	0.302	0.447	0.829
***cis*-DCCA**	post	non-PYR	39	0.062	0.035	0.035	0.056	0.102	0.332
***trans*-DCCA**	post	non-PYR	39	0.088	0.035	0.035	0.075	0.169	0.714
DBCA	post	non-PYR	39	0.092	0.035	0.060	0.095	0.140	0.360

[1] – distinction between control group (non-PYR users), and participants that have applied pyrethroid containing product (PYR)
GM – geometric mean
n – number of samples
P_{25} – 25th percentile
P_{75} – 75th percentile
MIN – minimal value
MAX – maximum value

Given the noticeable differences observed in the mean concentrations of each of the metabolites between samples collected before and after application, with almost (with the exception of DBCA) all tested biomarkers presenting higher mean concentrations in samples collected during days following the veterinary drug application (Table 3. and Fig. 4.). We divided the study into three periods for concentration comparisons: 'pre-application', 'week 1 post-application', and 'weeks 2-4 post-application'. The only statistically significant difference ($p = 0.0429$) in metabolite concentrations was observed in pyrethroid users when comparing metabolite levels obtained before applying any products with levels quantified in samples collected during the first week following the application (as shown in Fig. 4A.).

By documenting a significant increase in exposure during the first week post-application, based on the trend in metabolite concentration changes in urine (Fig. 7.), the key question became whether using permethrin-based products in accordance with the manufacturer's recommended minimum dosage and frequency leads to achieving exposure levels equivalent to pre-application levels before the next dose is administered. To address this question, we directly compared the median sum of pyrethroid metabolite concentrations in the urine of PYR users before application and in the fourth week after application (the last urine samples in the sampling period, Fig. 4B.).

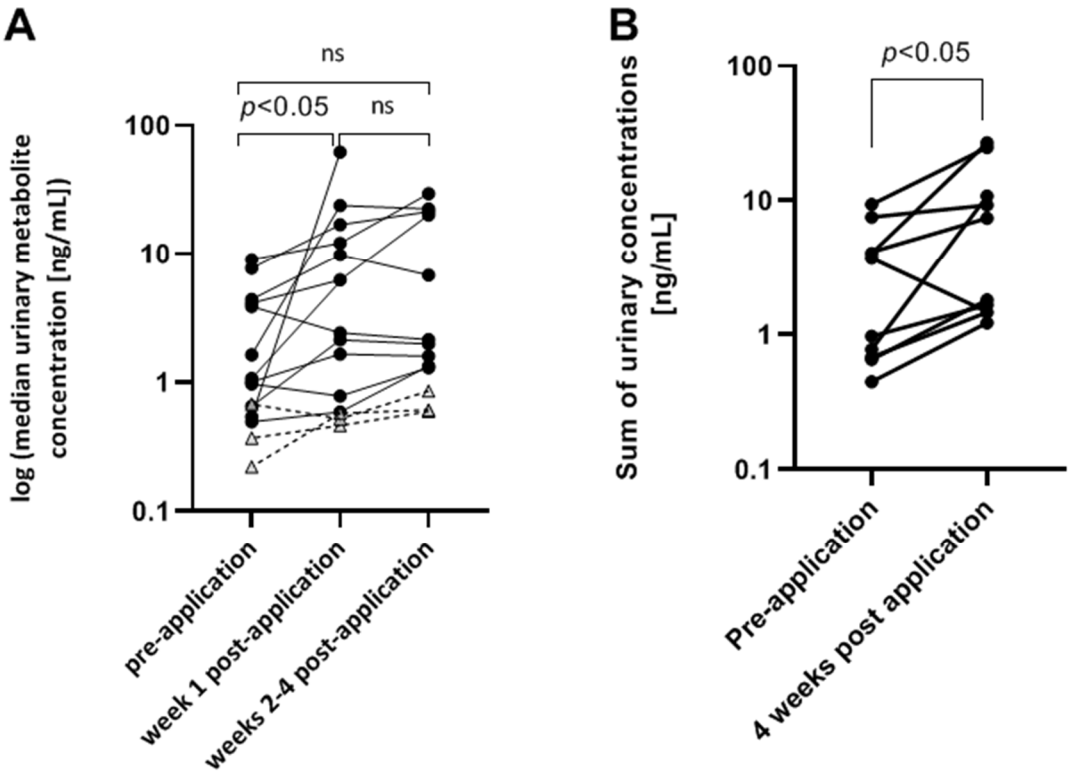


Fig. 4. Time trends of median of sum of urinary pyrethroid metabolite concentrations observed prior to and post drug application in pyrethroid users (●) and - pyrethroid users and non-pyrethroid users (Δ) (A) and direct comparison (B) between pre-application median of sum of urinary metabolite concentrations and 4 weeks after application. ns – not significant, p>0.05; *- statistically significant difference, p<0.05. The concentrations of metabolites in urine did not undergo statistically significant changes in non-pyrethroid users (Δ).

5.2. Concentrations of native pyrethroids in WBs

In the first stage of the study, a total of 15 wristbands were collected. In the second stage, 14 wristbands were collected because one person lost his/her wristband during the sampling period. The analysis of silicone-wristband extracts revealed that permethrin was the most frequently detected substance in bands collected both before drug application (detection rate: 46.7%) and after (notably, with a detection rate of 78.6%). Cyhalothrin and cyfluthrin were not detected in any of the tested samples. Cypermethrin was detected in 4 samples collected in stage 1 of the study (26.7%) and in 4 samples collected in stage 2 (28.6%). Deltamethrin was quantified in 2 samples both before (13.3%) and after (14.3%) veterinary drug application. Flumethrin was only detected in one sample from stage 1 (6.7%) and was not found in any of the samples collected during stage 2 of the study.
Similarly to the results of urinalysis, the distribution of pyrethroid concentrations quantified in wristband extracts did not exhibit characteristics of a normal distribution. Therefore, nonparametric statistical tests were employed for further data analysis.

Significantly higher pyrethroid concentrations ($p = 0.003$) were found in the wristbands worn after the application of veterinary drugs on pets (see Fig. 5.).

While the detection of native pyrethroids present in the veterinary products used by the study participants in their wristbands was expected, some of the target substances were also detected in wristbands worn prior to the administration of the anti-ectoparasitic drugs. For instance, permethrin was found in wristbands worn by inhabitants of Household No. 2 and No. 3. Cypermethrin was detected in wristbands worn by two members of Household No. 1 and two from Household No. 2. Deltamethrin was found in wristbands worn by one member of Household No. 4 and one from Household No. 3, and flumethrin was detected in one wristband worn by a member of Household No. 2. To further investigate these findings, we utilized questionnaire data obtained from the volunteers to trace possible sources of exposure, as outlined in the "Interpretation of results in relation to questionnaire data" section.

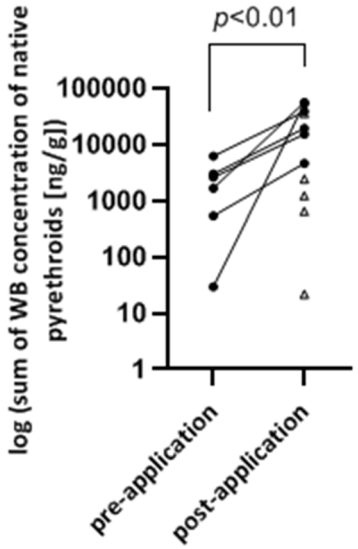


Fig. 5. Sum of concentrations of native pyrethroids in wristbands prior to and post application among all participants who have employed pyrethroid-containing product during the study. (Δ – wristbands collected prior to application from those individuals did not yield detectable pyrethroid concentrations)

As mentioned before, given the study design involving household using either pyrethroid or non-pyrethroid veterinary products, exposure to synthetic pyrethroids among members of households 4 and 5 was considered a study control (non-PYR) in the general overview of the pyrethroid-focused experiment. Consistent with the aforementioned division of the tested study volunteers into pyrethroid users and non-pyrethroid users, corresponding further data analysis had been performed, results of which are pictured below (Fig. 6.)

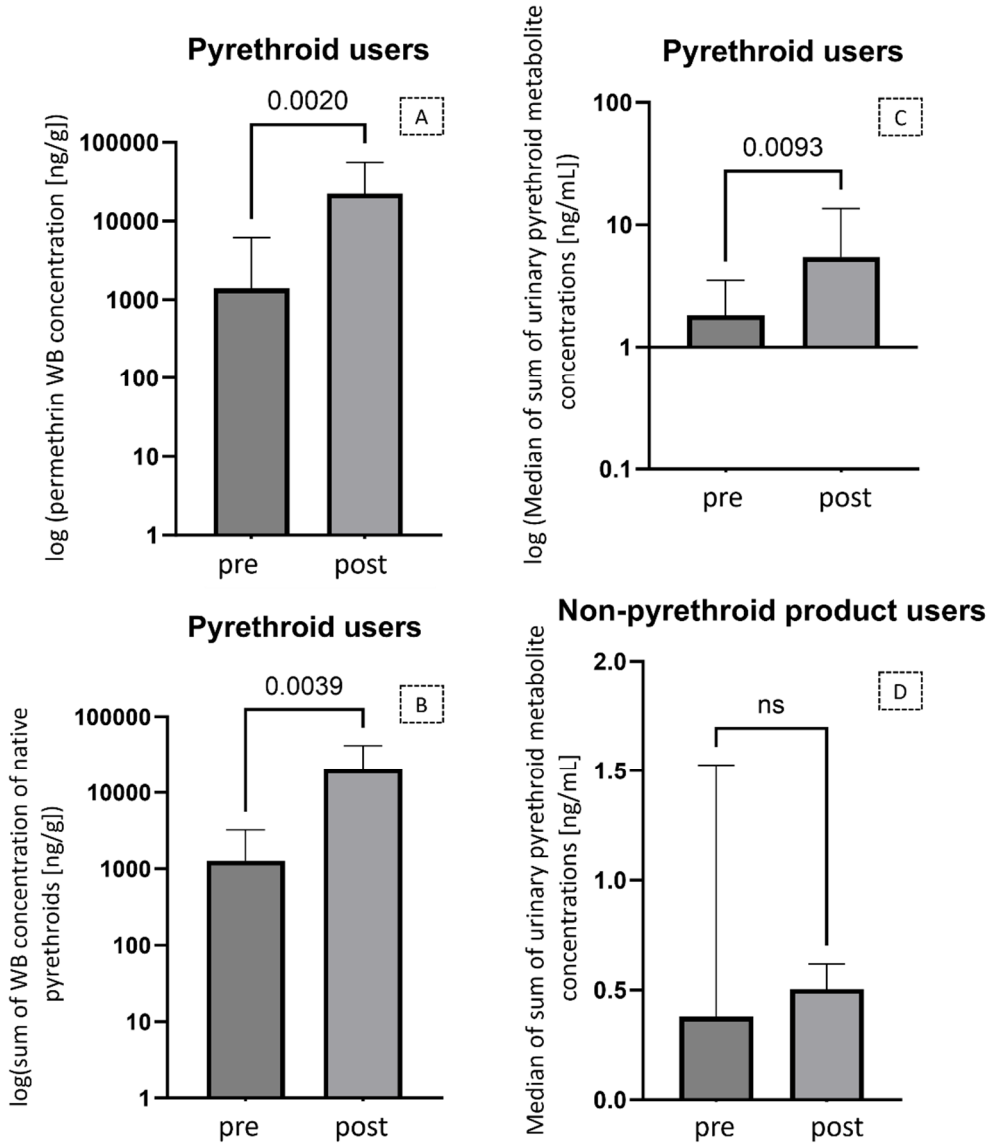


Fig. 6. Comparison of permethrin WB concentrations and urinary metabolite concentrations prior to and post application among pyrethroid (A, B and C) and non-pyrethroid (D) users (Mann-Whitney-U test). None of the pyrethroids analyzed were detected in WB in non-pyrethroid users prior and post application.

The analysis of samples collected by study participants in both groups, obtained via urinalysis and wristband analysis, revealed a statistically significant difference in the median sum of urinary metabolite concentrations between samples collected during stage 1 of the study and those collected in stages 2 and 3 among pyrethroid users (p = 0.0093). However, no such significance was observed among non-pyrethroid users. Additionally, a significant difference in the concentrations of permethrin detected on wristbands was found (p = 0.0020) among pyrethroid users when comparing its concentrations between samples collected before and after drug application.

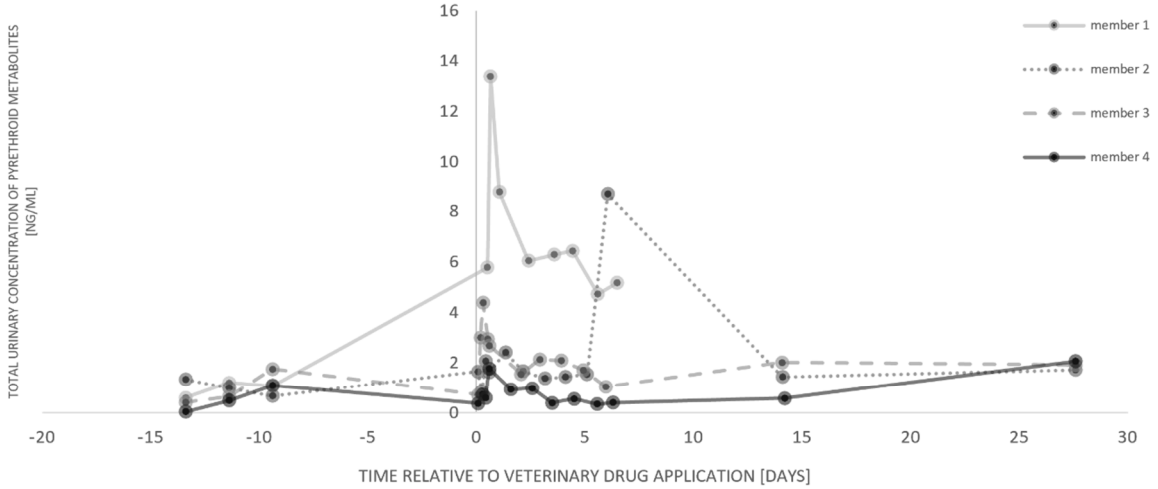
5.3. Investigation of concentration patterns

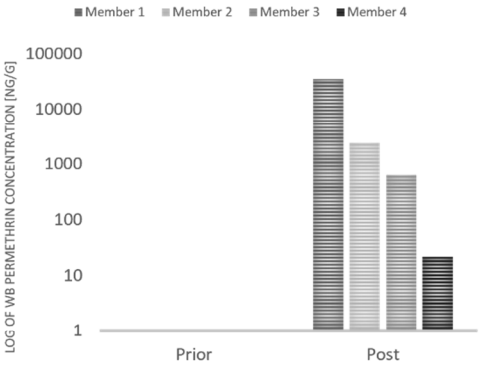
One of the primary objectives of this study was to investigate exposure patterns by monitoring both the concentrations of urinary pyrethroid biomarkers and the concentrations of native pyrethroids obtained from wristbands collected and worn before and after the application of veterinary drugs. This investigation aimed to provide a preliminary assessment of exposure patterns and the magnitude of exposure that these matrices can reveal.

In Fig. 7., a series of graphs (two for each participant) displays consecutively quantified concentrations at specific time points relative to the time of drug application. It's important to note that the bar graphs illustrating native pyrethroids detected on the wristbands depict only the substances contained in the product used. Detection of other native pyrethroids in either of the worn wristbands at this point has been omitted.

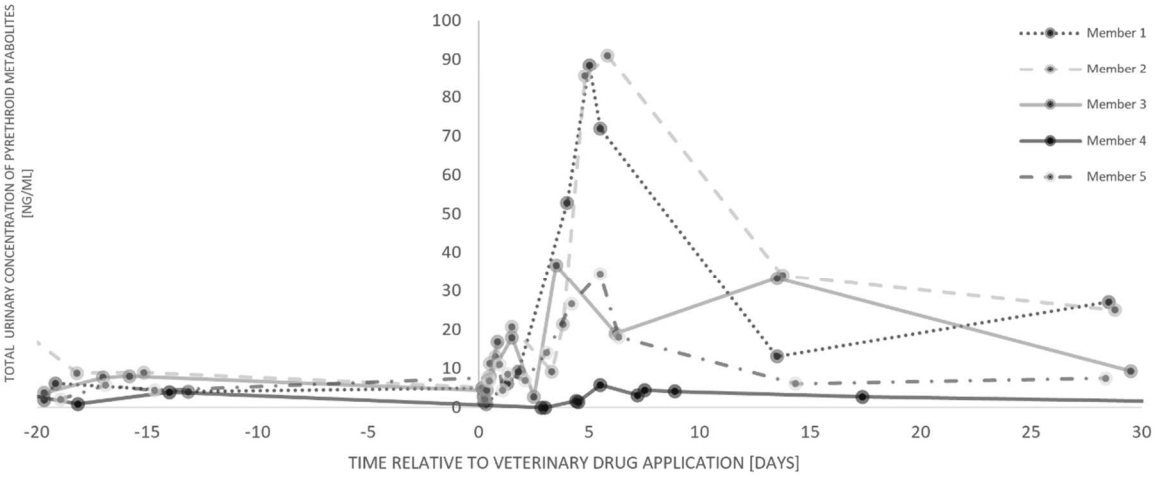
5.4. Relationship between applied pyrethroid dose and resulting exposure

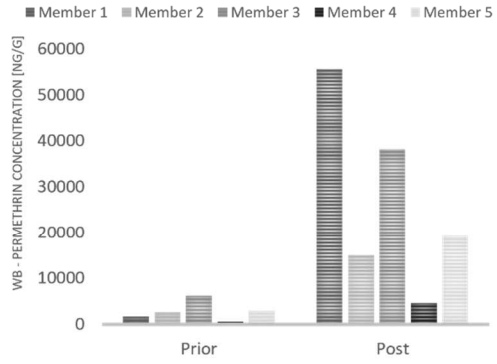
A correlation between the applied dose of pyrethroids among pyrethroid users involved in the study and the difference in 3-PBA concentrations between stage 2 and stage 1 (1^{st} week post-application vs pre-application) had shown a weak correlation between the values (r_s = -0.399, p = 0.1977) of no statistical significance, thus proving that the magnitude of exposure resulting in use of veterinary drug products had little connection to the applied dose, and most likely is heavily dependent on behavioral variables.



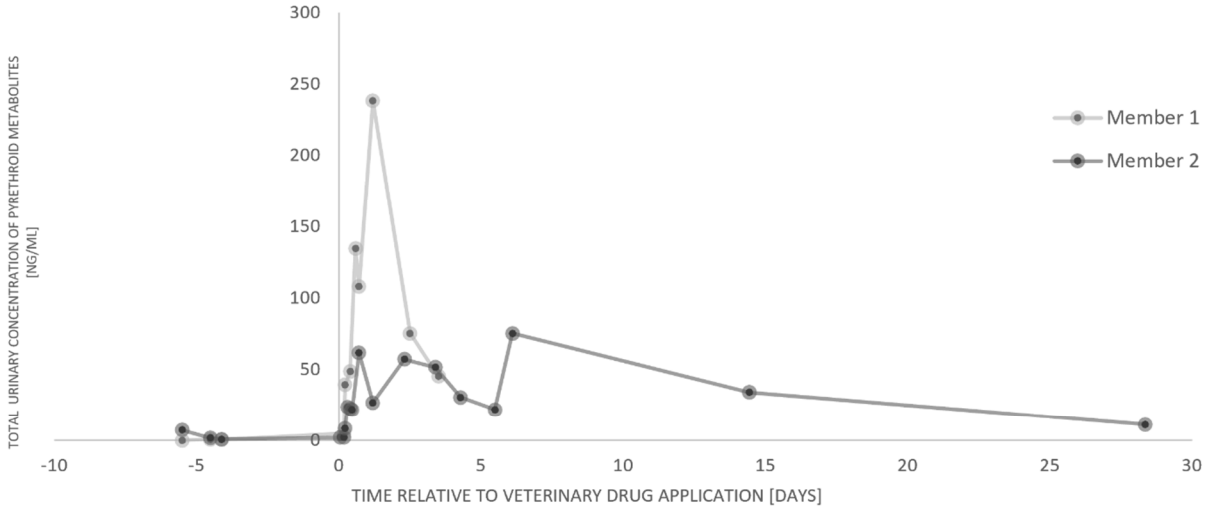


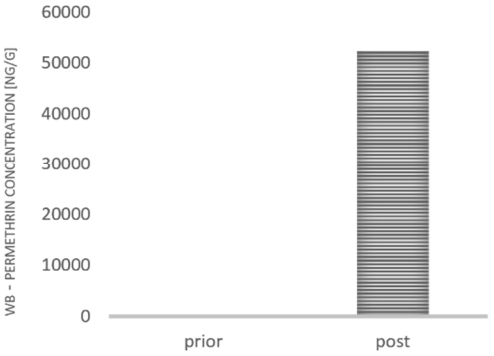
Household No.1



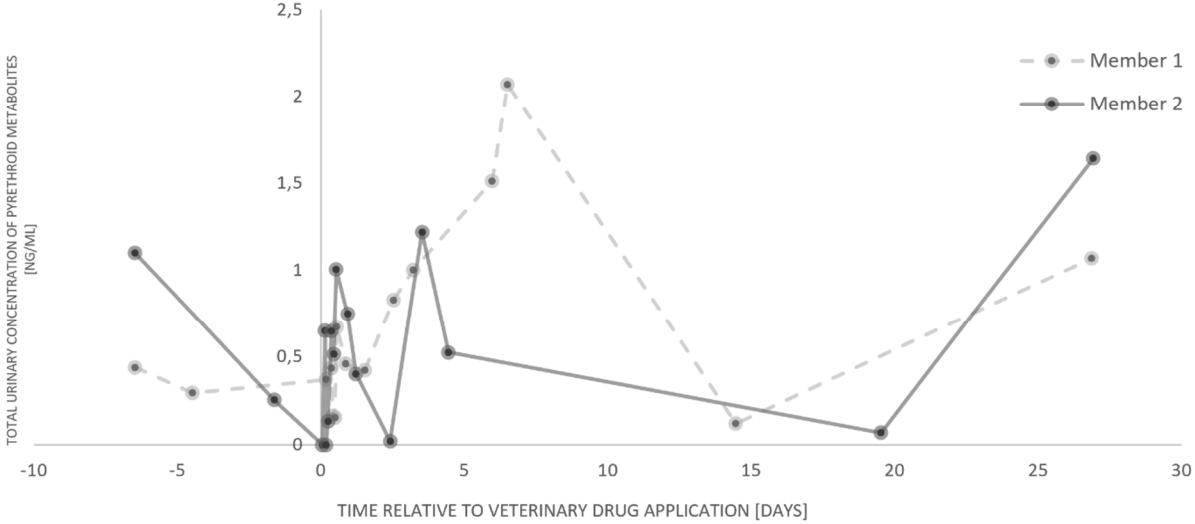


Household No.2





Household No.3*



Household No.4

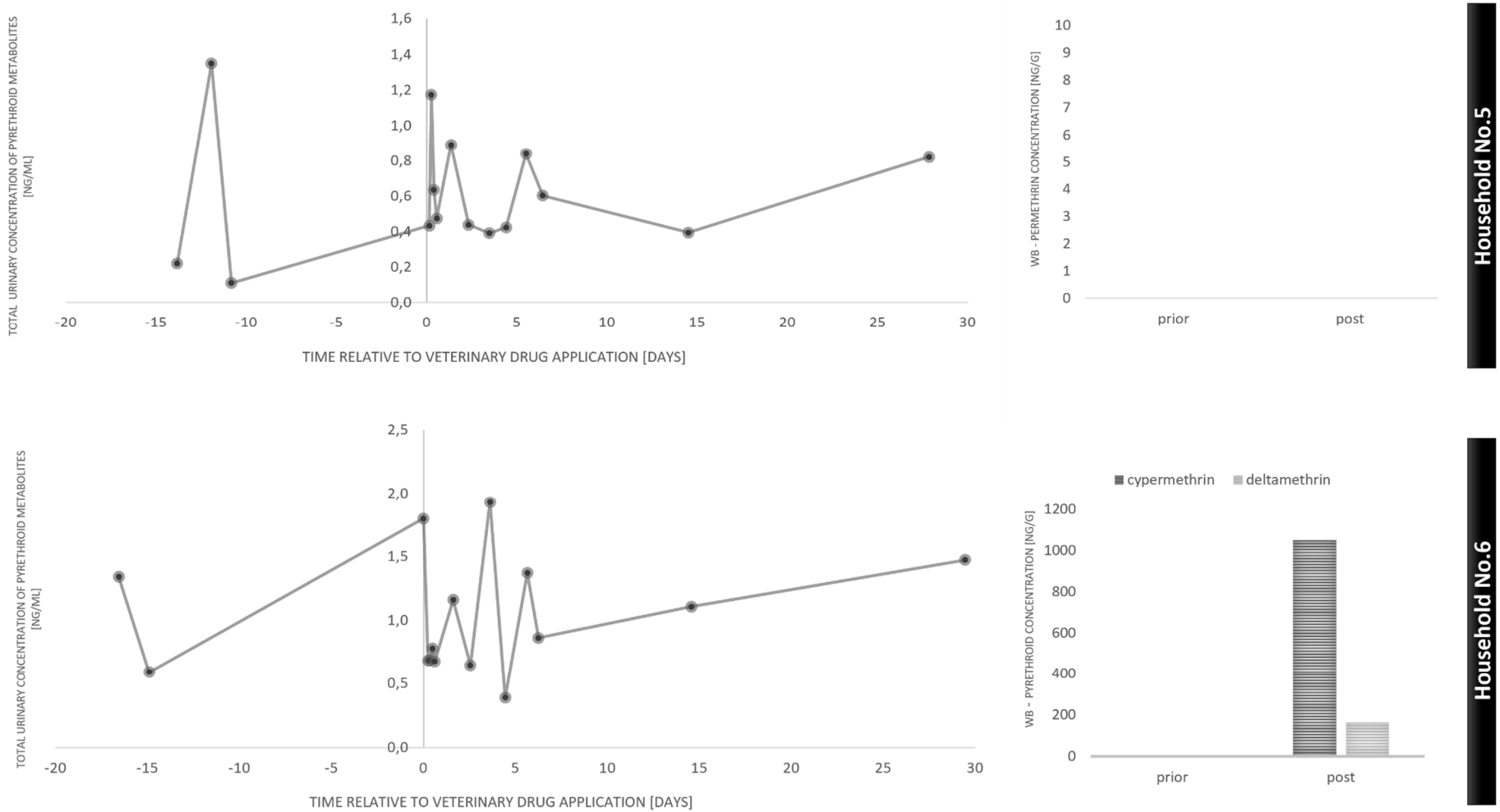


Fig. 7. Urinary concentrations of pyrethroid metabolites and wristband native pyrethroids quantified in samples collected from study participants throughout the entire course of the study. * - results of WB analysis in household No. 3. are shown only for member 1, as member 2 lost the wristband worn during stage 2.

5.5. Relationship between WB pyrethroids and urinary metabolites.

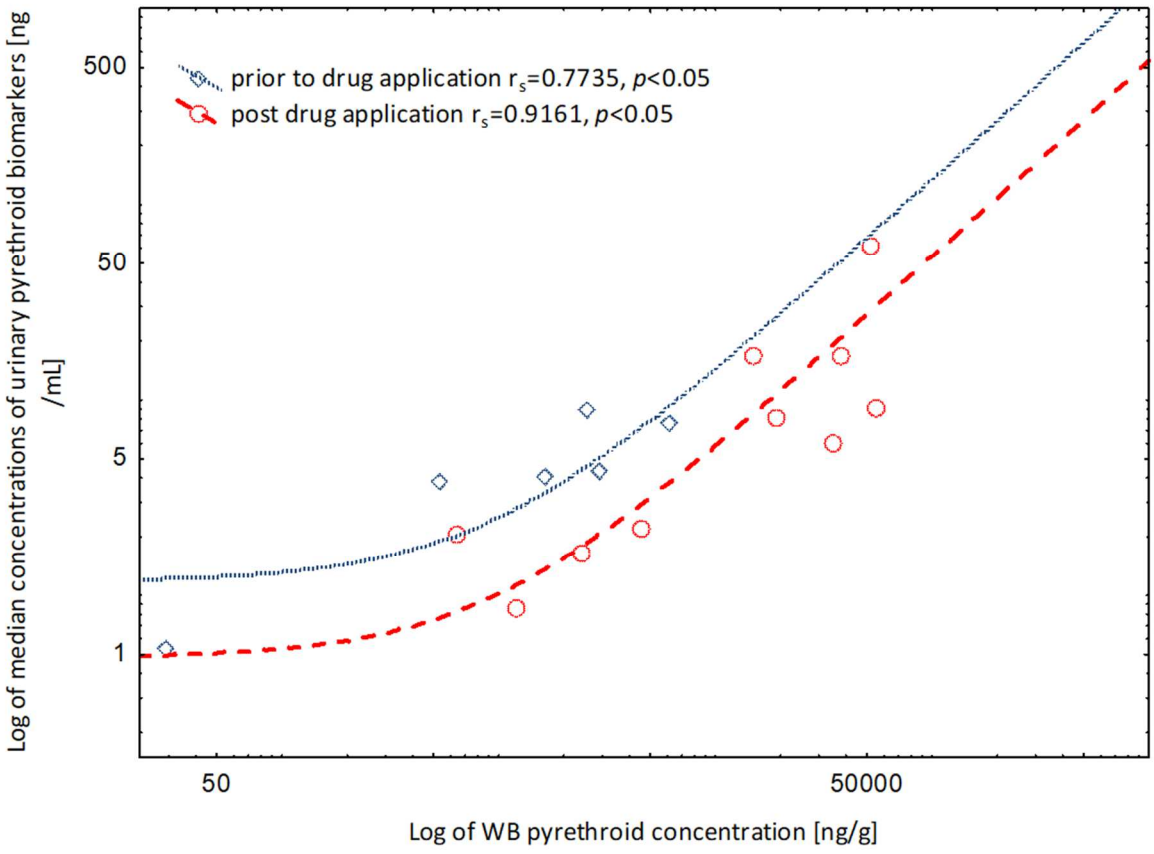


Fig. 8. Correlation coefficients for analysis of samples collected pre and post drug application. The blue dotted line and blue shapes (◇) represent samples collected prior to drug application, the red dashed line and red shapes (○) represent samples collected after the drug application.

Concentrations of urinary metabolites were strongly (r_s = 0.7735, Spearman's correlation, p < 0.05) correlated with WB pyrethroids prior to drug application, and very strongly (r_s = 0.9161, Spearman's correlation, p < 0.05) (Schober and Schwarte 2018) post drug application.

5.6. Pyrethroids in field-sampling wristbands

The analysis of field-sampling wristbands was conducted following the same procedures as those used for personal wristbands. Notably, no pyrethroids were detected in any of the field-sampling wristbands installed in the households during the week preceding the drug application. However, in households No. 2 and No. 3, permethrin was quantified in wristbands at concentrations of 79.03 and 60.14 ng/g, respectively, during the first week following the drug application.

6. Interpretation of results in relation to questionnaire data

Investigation of patterns created (Fig. 7.) by the urinary concentrations of pyrethroid metabolites quantified in urine samples collected over time has given an opportunity to sum up that the formed patterns are presenting similarities between household members, and clearly are dependent on the

formula of the product used (though it should be noted, that only one collar-type product has been involved in the study).

6.1. Household No. 1

With the exception of member No. 2, almost all members of family No. 1 exhibit patterns that align with our study model. These patterns remain consistent throughout – a noticeable increase in the sum of urinary metabolite concentrations is observed in the initial urine collections immediately following the drug application, followed by a subsequent decline in the following days. Notably, family member No. 1 had the highest recorded urinary concentrations among all the housemates, likely attributed to this individual being the one who applied the drug.

Family member No. 2's exposure pattern is less clear. There is a slight increase in metabolite concentrations immediately after application, but a more substantial peak is observed on the 6th day post-application. This phenomenon was investigated, but no extraordinary activities or changes in pet contact were reported. One potential explanation could be an additional incident of pyrethroid exposure during that time.

Wristband analysis for all household occupants consistently showed undetectable concentrations of permethrin before the drug application. Subsequently, significant amounts of permethrin (ranging from 21.6 to 35135.2 ng/g) were recorded in the wristbands worn during the 7 days after the application. According to the questionnaire date the use of insecticides on the pet (imidacloprid and flumethrin) occurred a year prior using a collar-type product. Detectable concentrations of cypermethrin were found in wristbands worn by two household members. Although the specific exposure event leading to this detection remains unidentified, both individuals regularly engage in agricultural activities. Therefore, it is concluded that past use of crop-protecting insecticides may have been the likely source of their exposure.

6.2. Household No. 2

The analysis of urinary metabolite concentration time trends among members of household No. 2 showed remarkable consistency. In most cases, concentrations peaked on the 6th and 7th days after the drug application, contrary to the expected peak on the 1st day. This deviation is likely due to the drug being administered over the weekend, leading to increased interaction with the pet during that time. However, this is a plausible explanation and hasn't been further investigated or confirmed.

Similar to household No. 1, wristband analysis post-application revealed high concentrations of permethrin (range: 4574.3 – 55556.8 ng/g), in line with the family's use of a spot-on product containing permethrin. Surprisingly, substantial permethrin concentrations were also quantified on wristbands worn prior to drug application (range: 535.5 - 6161.6 ng/g). This was assumed to result from either accidental exposure to pyrethroids during that time (considering urinary metabolite concentrations recorded at that time, this is unlikely), or prolonged exposure to permethrin. Analysis of field-sampling wristbands hung in the household, relative comparisons of average sum of metabolite concentrations (prior to drug application) to other study participants, and questionnaire data indicating repetitive applications of similar veterinary drugs on the family pet over the last 5 years, with the most recent

occurring over 6 months prior to the study launch, support the latter theory. Permethrin was likely present in the indoor spaces occupied by family members even before the scheduled drug application.

6.3. Household No. 3

The patterns of exposure revealed by the analysis of urinary metabolite concentrations in the collected urine samples show similar trends to those observed among members of household No. 1. Unfortunately, family member No. 2 lost the wristband worn immediately after the drug application before the sampling period concluded, creating a data gap. However, an analysis of both wristbands worn by family member No. 1 indicates detectable concentrations of permethrin both before and after the drug application (similar to household No. 2). This finding was further investigated in light of questionnaire-derived data, which provided an explanation in the form of a previous application of a veterinary drug with a similar composition a little over 6 months before the start of this study.

6.4. Households No. 4 and No. 5

In these households, insecticidal product that does not contain pyrethroids was used on the cats. Due to the exceptional toxicity of pyrethroids to cats, they are replaced with other antiparasitic substances such as fipronil or imidacloprid. This fact is additionally important from the perspective of our project, as it rules out the use of pyrethroids in these households in previous years, making those participants a well-suited control group.
The patterns observed in the urinary analysis, as well as the absence of detectable pyrethroid concentrations on the wristbands worn during both sampling periods, are as expected and understandable.

6.5. Household No. 6

The occupant of household No. 6 chose to use a collar-type product for the convenience of application and the comfort of his/her dog. According to the composition of the administered product, detectable amounts of deltamethrin and cypermethrin were noted upon analysis of wristbands worn during the week following the drug application. No detectable concentrations of pyrethroids were quantified on wristbands worn during the week prior to the drug application, even though past usage of collar-type products (exceeding a year) had been declared. Additionally, no detectable concentrations were found on field-sampling wristbands placed indoors during both sampling periods.

The presence of permethrin on field-sampling wristbands in households No. 2 and No. 3 has provided evidence of indoor air transfer of pyrethroids, likely through suspended particles (household dust). However, relatively low concentrations in field wristbands in comparison to personal wristbands indicate that inhalation exposure is quantitatively marginal.

7. Discussion

There is an increasing number of epidemiological reports indicating that exposing people to environmental concentrations/doses of pyrethroids, much lower than those used in standard toxicological animal studies, can possibly lead to serious health consequences. Among these, the negative impact on reproductive health (X. Ye and Liu 2019; Ahmad, Khan, and Khan 2012; Saillenfait, Ndiaye, and Sabaté 2016; Jurewicz, Radwan, Wielgomas, et al. 2015; Lu et al. 2006; Radwan et al. 2014) and neurobehavioral development disorders (Pitzer et al. 2022) are primarily mentioned. Therefore,

understanding the actual sources of exposure and exposure pathways to synthetic pyrethroids is incredibly important.
Numerous cross-sectional observational studies have indicated the use of anti-parasitic preparations in domestic animals as one of the significant sources of human exposure to synthetic pyrethroids, based on exposure levels measured by metabolite concentrations in urine (Wise et al. 2022; 2020; Morgan 2015; W. Rodzaj et al. 2021). However, there was no experimental evidence to confirm this hypothesis. Therefore, we decided to investigate how exposure levels change in the period starting one week before scheduled drug application and ending 4 weeks after the application. We used both human biomonitoring to determine metabolite concentrations in urine and silicone wristbands to measure the concentrations of parent compounds to assess exposure. While other studies involving wristbands, with similar outlines of sample collection (prior to and post exposure) have already been introduced, like a study on Dominican Republic Firefighters conducted by Alberto J Caban-Martinez et al. (Caban-Martinez et al. 2020), where polycyclic aromatic hydrocarbons had been quantified, our study (to our best knowledge) is the first one involving planned exposure to pyrethroids and a combined biomonitoring and WBs sampling.

The provisional acceptable daily intake (ADI) of permethrin has been established at 0.01 mg/kg body weight (Committee for Veterinary Medicinal Products (CVMP) 1998), which translates to 600 µg/day for an individual with an average body mass of 60 kg. A single application of commercially available veterinary drug products in this study (as shown in Table 2) introduces a dose (1.01-3.03 g) that exceeds the ADI by a factor of 1600 to 5000 times, depending on the specific product used, into an indoor environment. This is a worrying finding, especially when one considers that the spot-on product application, as recommended by producers, is to be repeated every 4 weeks throughout the tick season, which in Europe can last up to 6 months.

It is worth emphasizing that the ADI value was determined based on studies in animals, where the critical effect was adaptive changes in the liver (Committee for Veterinary Medicinal Products (CVMP) 1998) and might not take into account more sensitive health effects indicated in epidemiological research.

In several earlier publications based on biomonitoring, it was suggested that the use of pesticides in residential settings is a strong predictor of exposure for individuals living in the same household (Bäumer and Baynes 2021; Wise et al. 2022). Few studies have focused on observing the fate of pesticides after application in residential areas or on pets. Those that are available suggest that the use of products to control fleas, lice, ticks, or bedbugs leads to persistent contamination of residential spaces and chronic exposure of the residents to these residues. A particularly vulnerable group is children, who, due to hand-to-mouth activity, are likely to be more exposed than adults, as indicated by other studies (Li et al. 2022).

Further research is needed to assess the extent of exposure and the accumulation potential of pesticides after the application of different forms of veterinary drugs (spot-on or collar). Although in our study, only one household used a collar, the exposure measurement results, both based on metabolite concentrations in urine and the concentration of parent compounds in the collar, indicate that indeed the collar, due to the relatively slow kinetics of active substance release, does not lead to a sudden release into the microenvironment.

The detection of considerable amounts of permethrin in some extracts of wristbands from participants worn before the scheduled drug application raises the question about the long-term stability of

pyrethroids in indoor spaces. This persistence in combination with repeated administration of consecutive doses of veterinary drugs might significantly impact the possibility and occurrence of prolonged exposure for the occupants of these spaces.

Interestingly, the correlations we observed between the concentration of pyrethroids in the wristband (Fig. 8.) and urinary metabolites are very similar to those noted by Wise et al. (2022) (Wise et al. 2022). In general, the concentration of permethrin in the wristband at 10,000 ng/g corresponded in both cases to the concentration of metabolites in urine (3-PBA or *trans*-DCCA) at around 10 ng/mL. Our study differs from the aforementioned one in that ours took place under relatively controlled exposure conditions. We knew what drug products, at what dose and what form – spot-on or collar, and when they were used in domestic animals, and we also monitored exposure in the same participants before and after the application of the preparation. It is also worth to mention, we used wristbands from a different source than Wise et al. (Wise et al. 2022), which, although based solely on the agreement between the correlations described above (wristband pyrethroid vs. urinary metabolites), confirms that silicone wristbands can be a relatively universal and readily available tool for exposure assessment, leading to obtaining consistent and reproducible results.

We expected an increase in exposure immediately after the application of products on pets, and generally, this did occur in most cases where spot-on products were used. Interestingly, we observed no correlation between the amount of active substance applied and the level of exposure, as indicated by metabolite concentrations in urine or permethrin levels in wristbands. Therefore, it can be presumed that behavior and hygiene habits (such as hand washing frequency and vacuuming frequency) have a predominant influence on exposure. These factors were beyond our control in this study but should be considered in planning future research.

Despite the relatively similar profiles of changes in metabolite concentrations in urine from the time of application to four weeks afterward, taking into account the aforementioned behavioral factors and, for example, time spent indoors, measuring exposure based on a single urine sample may introduce a significant bias in exposure classification.

It turns out that the concentrations measured in spot urine samples 4 weeks after application are still higher than the background levels measured in the initial stage of the study.

It can be summarized, that the study has provided alarming results at times, as the investigation of results of most 'notorious' pyrethroid users of tested individuals (Household No.1) has shown that urinary median 3-PBA concentrations among 3 out of 5 household members quantified in samples collected in stage 1 of the study had values (1.81; 4.81; 1.63 ng/mL) surpassing the value of 95th percentile of 3-PBA concentrations noted in Polish general population (1.241 ng/mL) (Wielgomas and Piskunowicz 2013), which may serve as an additional confirmation of chronic exposure taking place in their household due to repetitive employment of veterinary insecticides.

The investigation of patterns of exposure by analysis of both urinary metabolite concentrations, as well as wristband extracts had proven that topically applied veterinary products, which are by default not meant to be absorbed, but rather take insecticidal action directly on the skin surface (Bäumer and Baynes 2021) seem to be a substantial source of exposure to pyrethroids due to their lateral transportation occurring between pets and owners.

8. Study strengths and limitations

To the best of our knowledge, our study is the first assessment of human exposure to pyrethroids that combines biomonitoring and silicone wristband analysis in an investigation with planned exposure to the substances of interest.

The study's design, which includes a scheduled veterinary drug product application, has allowed us to monitor the resulting exposure, which was partly controlled (known chemical composition of drug product, time of application and dose of active substance), yielding unique and interesting results. The utilization of field-sampling has provided valuable insights, pointing to the possibility of chronic exposure to low doses of synthetic pyrethroids occurring in indoor spaces, either through air transfer or direct contact with surfaces.

Additionally, we used validated methods and the implementation of rigorous quality control procedures, ensured the credibility of the obtained results. In the case of urinalysis, this credibility is further reinforced by our laboratory's annual participation in the German External Quality Assessment Scheme for Analyses in Biological Materials (G-EQUAS).

One notable limitation of this study is the relatively small number of participants involved. However, it should be emphasized that even with the convenience of wristband-based sampling, the required number of urine collection points and the overall 5-week duration of the sampling period were highly inconvenient and tiresome for the volunteers. In hindsight, the authors acknowledge that a more detailed set of information regarding the daily habits of study participants would have been a valuable addition to the data obtained throughout the experiment. Finally, we did not take into account in our analysis the surface area of the living space, which determines the degree of "dilution" of the active substance in the enclosed environment.

9. Final Conclusions

The analysis of changes in metabolite concentrations in urine and the presence of parent compounds in silicone wristbands indicates that individuals living in households where pyrethroids have been applied to pets experience a significant increase in exposure in the first few days, lasting up to weeks after application. From our observations, it also appears that pyrethroids can accumulate in indoor environments, especially with repeated applications of antiparasitic agents on pets. Preliminarily, we can also infer that small amount of pyrethroids, likely after settling on household dust particles, may be absorbed through respiratory pathways. However, due to the limited number of measurements, confirmation of this phenomenon is necessary.

Albeit with a small number of cases, we demonstrated that spot-on products are responsible for significantly higher exposure compared to collar-form products. The lack of a significant correlation between the size of the administered dose of the active substance clearly indicates that behavioral factors are primarily responsible for the absorbed dose.

Finally, silicone wristbands have proven to be a highly effective tool for both qualitative (identifying parent compounds) and quantitative assessment of exposure to synthetic pyrethroids. They can certainly complement, and in some situations, even replace biomonitoring methods, especially for detecting significant non-dietary exposure, as seen in this study.

10.Acknowledgments

Małgorzata Wacławik appreciates the scholarship received from the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Program Knowledge Education Development 2014–2020.

Furthermore, the authors of this manuscript give their sincere thanks to the volunteers, for their willingness to selflessly endure all the inconveniences related to participation in the study, as well as exemplary cooperation throughout it.

11.References

Ahmad, Latif, Ahrar Khan, and Muhammad Zargham Khan. 2012. "Pyrethroid-Induced Reproductive Toxico-Pathology in Non-Target Species." *Pakistan Veterinary Journal* 32 (1): 1–9.

Al-Alam, Josephine, Alexandre Sonnette, Olivier Delhomme, Laurent Y. Alleman, Patrice Coddeville, and Maurice Millet. 2022. "Pesticides in the Indoor Environment of Residential Houses: A Case Study in Strasbourg, France." *International Journal of Environmental Research and Public Health* 19 (21). https://doi.org/10.3390/ijerph192114049.

Anadón, A., M. R. Martínez-Larrañaga, and M. A. Martínez. 2009. "Use and Abuse of Pyrethrins and Synthetic Pyrethroids in Veterinary Medicine." *Veterinary Journal* 182 (1): 7–20. https://doi.org/10.1016/j.tvjl.2008.04.008.

Bao, Wei, Buyun Liu, Derek W. Simonsen, and Hans Joachim Lehmler. 2020. "Association between Exposure to Pyrethroid Insecticides and Risk of All-Cause and Cause-Specific Mortality in the General US Adult Population." *JAMA Internal Medicine* 180 (3): 367–74. https://doi.org/10.1001/jamainternmed.2019.6019.

Baum, Jeramy L.R., Umer Bakali, Chitvan Killawala, Katerina M Santiago, Emre Dikici, Erin N Kobetz, Natasha Schaefer Solle, Sapna Deo, Leonidas Bachas, and Sylvia Daunert. 2020. "Evaluation of Silicone-Based Wristbands as Passive Sampling Systems Using PAHs as an Exposure Proxy for Carcinogen Monitoring in Firefighters: Evidence from the Firefighter Cancer Initiative." *Ecotoxicology and Environmental Safety* 205 (December): 111100. https://doi.org/10.1016/j.ecoenv.2020.111100.

Bäumer, Wolfgang, and Ronald Baynes. 2021. "Surface Distribution of Pyrethroids Following Topical Application to Veterinary Species: Implications for Lateral Transport." *Journal of Veterinary Pharmacology and Therapeutics* 44 (1): 1–10. https://doi.org/10.1111/jvp.12907.

Berger-Preiß, Edith, Karsten Levsen, Gabriele Leng, Helga Idel, Dorothee Sugiri, and Ulrich Ranft. 2002. "Indoor Pyrethroid Exposure in Homes with Woollen Textile Floor Coverings." *International Journal of Hygiene and Environmental Health* 205 (6): 459–72. https://doi.org/10.1078/1438-4639-00181.

Bergmann, Alan J., Paula E. North, Luis Vasquez, Hernan Bello, Maria del Carmen Gastañaga Ruiz, and Kim A. Anderson. 2017. "Multi-Class Chemical Exposure in Rural Peru Using Silicone Wristbands." *Journal of Exposure Science and Environmental Epidemiology* 27 (6): 560–68. https://doi.org/10.1038/jes.2017.12.

Bradberry, Sally M, Sarah A Cage, Alex T Proudfoot, and J Allister Vale. 2005. "Poisoning Due to Pyrethroids." *Toxicological Reviews* 24 (2): 93–106. https://doi.org/10.2165/00139709-200524020-00003.

Caban-Martinez, Alberto J, Paola Louzado-Feliciano, Katerina M Santiago, Jeramy Baum, Natasha Schaefer Solle, Geovanny Rivera, Marija Miric, Eddy Perez-Then, Erin N Kobetz-Kerman, and

Sylvia Daunert. 2020. “Objective Measurement of Carcinogens Among Dominican Republic Firefighters Using Silicone-Based Wristbands.” *Journal of Occupational & Environmental Medicine* 62 (11): e611–15. https://doi.org/10.1097/JOM.0000000000002006.

Chrustek, Agnieszka, Iga Hołyńska-Iwan, Inga Dziembowska, Joanna Bogusiewicz, Marcin Wróblewski, Anna Cwynar, and Dorota Olszewska-Słonina. 2018. “Current Research on the Safety of Pyrethroids Used as Insecticides.” *Medicina (Lithuania)* 54 (4): 1–15. https://doi.org/10.3390/medicina54040061.

Committee for Veterinary Medicinal Products (CVMP). 1998. “Permethrin, Summary Report (1).” Vienna: Springer Vienna.

Doherty, Brett T., John L. Pearce, Kim A. Anderson, Margaret R. Karagas, and Megan E. Romano. 2020. “Assessment of Multipollutant Exposures During Pregnancy Using Silicone Wristbands.” *Frontiers in Public Health* 8 (September): 1–13. https://doi.org/10.3389/fpubh.2020.547239.

Fortin, Marie Chantale, Michèle Bouchard, Gaétan Carrier, and Pierre Dumas. 2008. “Biological Monitoring of Exposure to Pyrethrins and Pyrethroids in a Metropolitan Population of the Province of Quebec, Canada.” *Environmental Research* 107 (3): 343–50. https://doi.org/10.1016/j.envres.2008.03.002.

Gavrilescu, M. 2005. “Fate of Pesticides in the Environment and Its Bioremediation.” *Engineering in Life Sciences* 5 (6): 497–526. https://doi.org/10.1002/elsc.200520098.

Hammel, Stephanie C., Kate Hoffman, Thomas F. Webster, Kim A. Anderson, and Heather M. Stapleton. 2016. “Measuring Personal Exposure to Organophosphate Flame Retardants Using Silicone Wristbands and Hand Wipes.” *Environmental Science & Technology* 50 (8): 4483–91. https://doi.org/10.1021/acs.est.6b00030.

Jurewicz, Joanna, Michał Radwan, Wojciech Sobala, Kinga Polańska, Paweł Radwan, Lucjusz Jakubowski, Anna Ulańska, and Wojciech Hanke. 2015. “The Relationship between Exposure to Air Pollution and Sperm Disomy.” *Environmental and Molecular Mutagenesis* 56 (1): 50–59. https://doi.org/10.1002/em.21883.

Jurewicz, Joanna, Michał Radwan, Bartosz Wielgomas, Wojciech Sobala, Marta Piskunowicz, Paweł Radwan, Michał Bochenek, and Wojciech Hanke. 2015. “The Effect of Environmental Exposure to Pyrethroids and DNA Damage in Human Sperm.” *Systems Biology in Reproductive Medicine* 61 (1): 37–43. https://doi.org/10.3109/19396368.2014.981886.

Kaneko, Hideo. 2011. “Pyrethroids: Mammalian Metabolism and Toxicity.” *Journal of Agricultural and Food Chemistry* 59 (7): 2786–91. https://doi.org/10.1021/jf102567z.

Katsuda, Yoshio. 2011. “Progress and Future of Pyrethroids.” In *Topics in Current Chemistry*, 314:1–30. https://doi.org/10.1007/128_2011_252.

Laskowski, Dennis A. 2002. “Physical and Chemical Properties of Pyrethroids.” *Reviews of Environmental Contamination and Toxicology* 174: 49–170. https://doi.org/10.1007/978-1-4757-4260-2_3.

Lehmler, Hans-joachim, Derek Simonsen, Buyun Liu, and Wei Bao. 2020. “Environmental Exposure to Pyrethroid Pesticides in a Nationally Representative Sample of U.S. Adults and Children: The National Health and Nutrition Examination Survey 2007–2012.” *Environmental Pollution* 267 (December): 115489. https://doi.org/10.1016/j.envpol.2020.115489.

Leng, Gabriele, Ulrich Ranft, Dorothee Sugiri, Wolfgang Hadnagy, Edith Berger-Preiß, and Helga Idel. 2003. “Pyrethroids Used Indoors - Biological Monitoring of Exposure to Pyrethroids Following an Indoor Pest Control Operation.” *International Journal of Hygiene and Environmental Health*

206 (2): 85–92. https://doi.org/10.1078/1438-4639-00199.

Li, Yan, Xianyu Wang, Jean Feary McKenzie, Andrea ’t Mannetje, Soo Cheng, Chang He, Janet Leathem, et al. 2022. “Pesticide Exposure in New Zealand School-Aged Children: Urinary Concentrations of Biomarkers and Assessment of Determinants.” *Environment International* 163 (April): 107206. https://doi.org/10.1016/j.envint.2022.107206.

Lu, Chensheng, Dana B. Barr, Melanie Pearson, Scott Bartell, and Roberto Bravo. 2006. “A Longitudinal Approach to Assessing Urban and Suburban Children’s Exposure to Pyrethroid Pesticides.” *Environmental Health Perspectives* 114 (9): 1419–23. https://doi.org/10.1289/ehp.9043.

Manzano, Carlos A., Nathan G. Dodder, Eunha Hoh, and Raul Morales. 2019. “Patterns of Personal Exposure to Urban Pollutants Using Personal Passive Samplers and GC × GC/ToF-MS.” *Environmental Science and Technology* 53 (2): 614–24. https://doi.org/10.1021/acs.est.8b06220.

Marettova, Elena, Milan Maretta, and Jaroslav Legáth. 2017. “Effect of Pyrethroids on Female Genital System. Review.” *Animal Reproduction Science* 184 (March): 132–38. https://doi.org/10.1016/j.anireprosci.2017.07.007.

Morgan, Marsha K. 2015. “Predictors of Urinary Levels of 2,4-Dichlorophenoxyacetic Acid, 3,5,6-Trichloro-2-Pyridinol, 3-Phenoxybenzoic Acid, and Pentachlorophenol in 121 Adults in Ohio.” *International Journal of Hygiene and Environmental Health* 218 (5): 479–88. https://doi.org/10.1016/j.ijheh.2015.03.015.

Nebbia, Carlo. 2009. “Pyrethroids Are Not the Most Appropriate Remedy for Tackling Fleas and Ticks in Cats, and May Be Dangerous for Fish Too.” *The Veterinary Journal* 182 (1): 1–2. https://doi.org/10.1016/j.tvjl.2009.05.017.

O’Connell, Steven G., Laurel D. Kincl, and Kim A. Anderson. 2014. “Silicone Wristbands as Personal Passive Samplers.” *Environmental Science & Technology* 48 (6): 3327–35. https://doi.org/10.1021/es405022f.

Radwan, Michał, Joanna Jurewicz, Bartosz Wielgomas, Wojciech Sobala, Marta Piskunowicz, Paweł Radwan, and Wojciech Hanke. 2014. “Semen Quality and the Level of Reproductive Hormones after Environmental Exposure to Pyrethroids.” *Journal of Occupational and Environmental Medicine* 56 (11): 1113–19. https://doi.org/10.1097/JOM.0000000000000297.

Rodzaj, W., M. Wileńska, A. Klimowska, E. Dziewirska, J. Jurewicz, R. Walczak-Jędrzejowska, J. Słowikowska-Hilczer, W. Hanke, and B. Wielgomas. 2021. “Concentrations of Urinary Biomarkers and Predictors of Exposure to Pyrethroid Insecticides in Young, Polish, Urban-Dwelling Men.” *Science of the Total Environment* 773. https://doi.org/10.1016/j.scitotenv.2021.145666.

Rodzaj, Wojciech, Malwina Wileńska, Anna Klimowska, Emila Dziewirska, Joanna Jurewicz, Renata Walczak-Jędrzejowska, Jolanta Słowikowska-Hilczer, Wojciech Hanke, and Bartosz Wielgomas. 2021. “Concentrations of Urinary Biomarkers and Predictors of Exposure to Pyrethroid Insecticides in Young, Polish, Urban-Dwelling Men.” *Science of The Total Environment* 773 (June): 145666. https://doi.org/10.1016/j.scitotenv.2021.145666.

Roggeman, Maarten, Celine Gys, Anna Klimowska, Michiel Bastiaensen, Bartosz Wielgomas, Ait Bamai Yu, and Adrian Covaci. 2022. “Reviewing the Variability in Urinary Concentrations of Non-Persistent Organic Chemicals: Evaluation across Classes, Sampling Strategies and Dilution Corrections.” *Environmental Research* 215 (P2): 114332. https://doi.org/10.1016/j.envres.2022.114332.

Romanak, Kevin A., Shaorui Wang, William A. Stubbings, Michael Hendryx, Marta Venier, and Amina Salamova. 2019. “Analysis of Brominated and Chlorinated Flame Retardants, Organophosphate Esters, and Polycyclic Aromatic Hydrocarbons in Silicone Wristbands Used as Personal Passive Samplers.” *Journal of Chromatography A* 1588 (6): 41–47. https://doi.org/10.1016/j.chroma.2018.12.041.

Saillenfait, Anne-Marie, Dieynaba Ndiaye, and Jean-Philippe Sabaté. 2016. “The Estrogenic and Androgenic Potential of Pyrethroids in Vitro. Review.” *Toxicology in Vitro* 34 (August): 321–32. https://doi.org/10.1016/j.tiv.2016.02.020.

Schober, Patrick, and Lothar A. Schwarte. 2018. “Correlation Coefficients: Appropriate Use and Interpretation.” *Anesthesia and Analgesia* 126 (5): 1763–68. https://doi.org/10.1213/ANE.0000000000002864.

Shelton, Janie F., Estella M. Geraghty, Daniel J. Tancredi, Lora D. Delwiche, Rebecca J. Schmidt, Beate Ritz, Robin L. Hansen, and Irva Hertz-Picciotto. 2014. “Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural Pesticides: The Charge Study.” *Environmental Health Perspectives* 122 (10): 1103–9. https://doi.org/10.1289/ehp.1307044.

Simerville, Jeff A., William C. Maxted, and John J. Pahira. 2005. “Urinalysis: A Comprehensive Review.” *American Family Physician* 71 (6): 1153–62.

Travis, Steven C., Diana S. Aga, Elena I. Queirolo, James R. Olson, Mónica Daleiro, and Katarzyna Kordas. 2020. “Catching Flame Retardants and Pesticides in Silicone Wristbands: Evidence of Exposure to Current and Legacy Pollutants in Uruguayan Children.” *Science of the Total Environment* 740: 140136. https://doi.org/10.1016/j.scitotenv.2020.140136.

Vais, Horia, Martin S. Williamson, Alan L. Devonshire, and Peter N.R. Usherwood. 2001. “The Molecular Interactions of Pyrethroid Insecticides with Insect and Mammalian Sodium Channels.” *Pest Management Science* 57 (10): 877–88. https://doi.org/10.1002/ps.392.

Wielgomas, Bartosz. 2013. “Variability of Urinary Excretion of Pyrethroid Metabolites in Seven Persons over Seven Consecutive Days—Implications for Observational Studies.” *Toxicology Letters* 221 (1): 15–22. https://doi.org/10.1016/j.toxlet.2013.05.009.

Wielgomas, Bartosz, and Marta Piskunowicz. 2013. “Biomonitoring of Pyrethroid Exposure among Rural and Urban Populations in Northern Poland.” *Chemosphere* 93 (10): 2547–53. https://doi.org/10.1016/j.chemosphere.2013.09.070.

Wise, Catherine F., Stephanie C. Hammel, Nicholas J. Herkert, Maria Ospina, Antonia M. Calafat, Matthew Breen, and Heather M. Stapleton. 2022. “Comparative Assessment of Pesticide Exposures in Domestic Dogs and Their Owners Using Silicone Passive Samplers and Biomonitoring.” *Environmental Science and Technology* 56 (2): 1149–61. https://doi.org/10.1021/acs.est.1c06819.

Wise, Catherine F, Stephanie C Hammel, Nicholas Herkert, Jun Ma, Alison Motsinger-Reif, Heather M Stapleton, and Matthew Breen. 2020. “Comparative Exposure Assessment Using Silicone Passive Samplers Indicates That Domestic Dogs Are Sentinels To Support Human Health Research.” *Environmental Science & Technology* 54 (12): 7409–19. https://doi.org/10.1021/acs.est.9b06605.

Ye, Ming, Jeremy Beach, Jonathan W. Martin, and Ambikaipakan Senthilselvan. 2017. “Pesticide Exposures and Respiratory Health in General Populations.” *Journal of Environmental Sciences (China)* 51: 361–70. https://doi.org/10.1016/j.jes.2016.11.012.

Ye, Xiaoqing, and Jing Liu. 2019. “Effects of Pyrethroid Insecticides on Hypothalamic-Pituitary-

Gonadal Axis: A Reproductive Health Perspective." *Environmental Pollution* 245: 590–99. https://doi.org/10.1016/j.envpol.2018.11.031.

Yoshida, Toshiaki, Mayumi Mimura, and Naomi Sakon. 2021. "Estimating Household Exposure to Moth Repellents P-Dichlorobenzene and Naphthalene and the Relative Contribution of Inhalation Pathway in a Sample of Japanese Children." *Science of the Total Environment* 783: 19310–24. https://doi.org/10.1016/j.scitotenv.2021.146988.

Zhu, Qiuyan, Yang Yang, Zhiting Lao, Yingying Zhong, Kun Zhang, and Suqing Zhao. 2020. "Photodegradation Kinetics, Mechanism and Aquatic Toxicity of Deltamethrin, Permethrin and Dihaloacetylated Heterocyclic Pyrethroids." *Science of the Total Environment* 749: 142106. https://doi.org/10.1016/j.scitotenv.2020.142106.

Evaluation of exposure to synthetic pyrethroids among pet owners in a study with planned veterinary product application – Supplementary Materials

Małgorzata Wacławik[1], Wojciech Rodzaj[1], Joanna Jurewicz[2], Bartosz Wielgomas[1]*

[1]Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, 107 Hallera Street, 80-416 Gdańsk, Poland

[2] Department of Chemical Safety, Nofer Institute of Occupational Medicine, 8 Teresy St., 91-348 Łódź, Poland

*corresponding author: bartosz.wielgomas@gumed.edu.pl

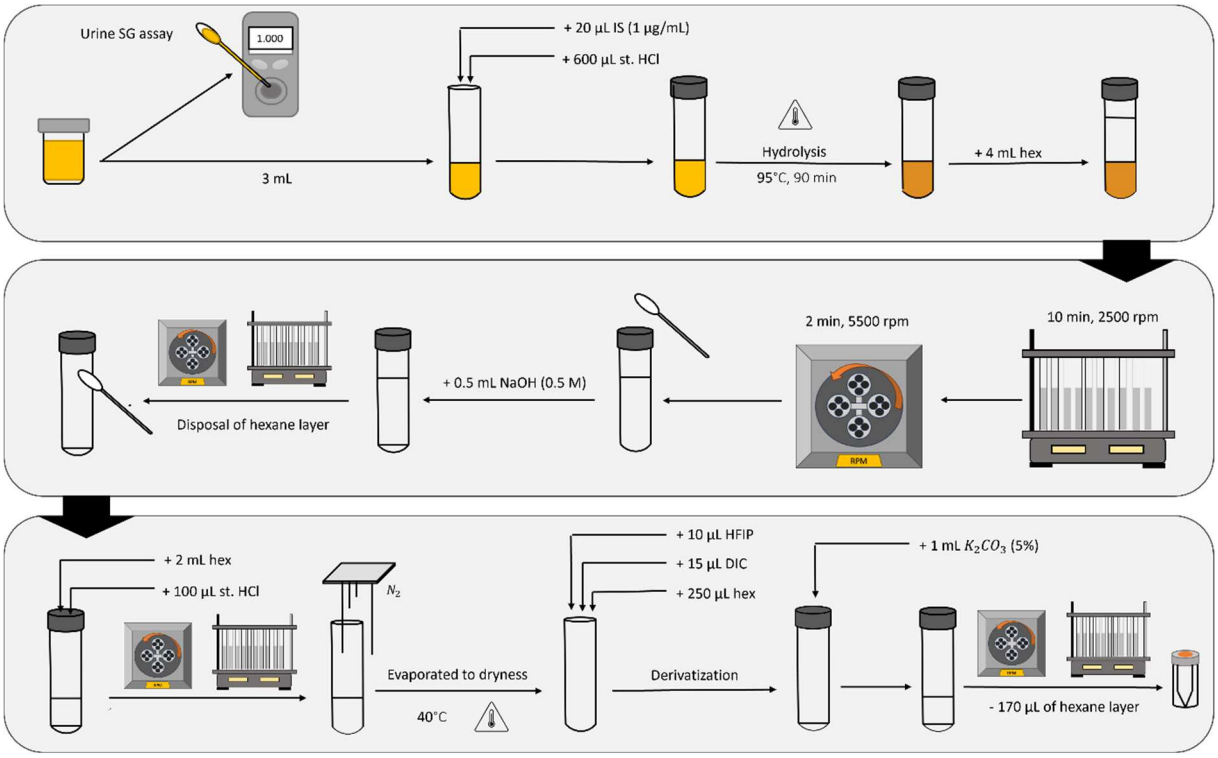
1. Sample analysis
    a. Urine analysis
        i. Sample storage

All urine samples collected by the study participants have been stored in a freezer (-18°C) both at the volunteers' homes, and at the Department of Toxicology after their transfer there.

        ii. Sample preparation procedure

Thawed urine samples had first undergone refractometric assay of urine specific gravity (Urine SG) with the use of hand-held refractometer PAL-10S (Atago Co., Tokyo, Japan). Samples with urine SG exceeding 1.035 g/mL and below 1.003 g/mL have been excluded from further analyses. A sample of 3 mL of thawed urine has been pipetted into a screw-top test tube (ø 16 mm). Next, 20 µL of an internal standards (IS) solution (2-PBA (2-phenoxy benzoic acid), *cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-*DCCA) 100 ug/ml in Acetonitrile-D3 (1, Carboxyl-13C2, 99%; 1-D, 97%)) is being added, which has been followed by addition of 600 µL of concentrated hydrochloric acid (HCl). The contents of the test tube are then being vortexed for 3 seconds and placed in a laboratory oven heated to 95°C, where they undergo hydrolysis for 90 minutes. Next, the samples have been taken out of the oven, and placed on a benchtop, allowing them to cool down to room temperature. Next, 4 mL of hexane has been added to the hydrolyzed sample, which next has been subjected to multi-tube vortex mixing (2500 rpm, 10 min) and centrifugation (5500 rpm, 2 min). The organic top layer has been transferred to a new test tube, the extraction was repeated, and extracts collected in both steps had been pooled. Then 0.5 mL of 0.1M sodium hydroxide solution has been added to the combined extracts, with the formulated mixture having again undergone vortex mixing and centrifugation, as previously, with the exception of next having discarded the top layer. Further, 100 µL of concentrated HCl, and 2 mL of hexane have been added to the remaining contents of the test tube. The sample than again has been subjected to vortex mixing and centrifugation (same as in previous steps). The top layer has then been transferred to a new test tube, placed in a dry bath (40°C), under a stream of nitrogen, and evaporated to dryness. The dry residue

has later been derivatized with the use of 10 µL of 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) and 15 µL of N,N'-Diisopropylcarbodiimide (DIC). Along with derivatizing agents 250 µL of hexane has been added. The extracts had undergone derivatization at room temperature, for 10 minutes, by being vortexed at 2000 rpm. Next, the excess of derivatizing agents had been neutralized by adding 1 mL of 5% potassium carbonate solution. The mixture again has undergone vortex mixing (2500 rpm, 10 min), and centrifugation (5500 rpm, 2 min), after which finally 170 µL of top hexane layer has been transported to a chromatographic vial and subjected to instrumental analysis. The pictorial summary of the described procedure can be found on SM – Fig. 1.



SM - Fig.1. Pictorial description of the urine sample preparation procedure.

b. Wristband analysis

i. Collection and storage

Pre cleaned (Manuscript No. 2) WBs have been provided to the study participants in clear zip-lock bags, to which the samplers were to be returned after the sampling period, and in such packaging were to be stored at home in a freezer, and later transported to the University and stored there (-18°C) upon analysis.

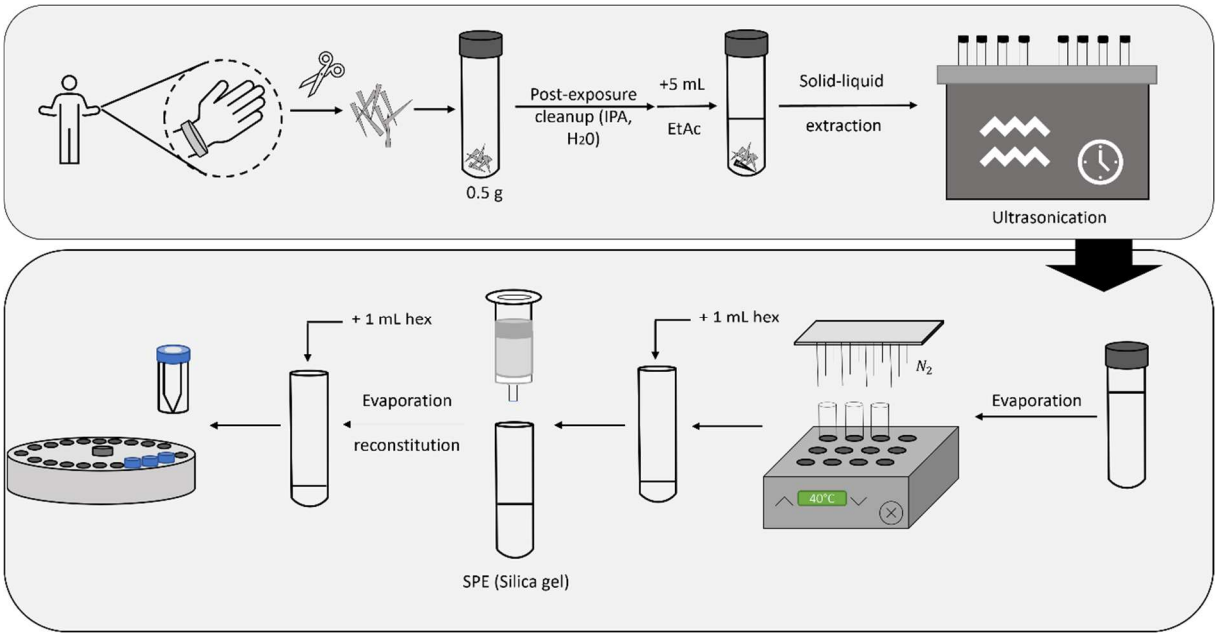
ii. Post-exposure cleanup

Post-exposure cleanup of silicone wristbands has been carried out in accordance with previously described procedure (Wacławik et al. 2023,

pending publication, Component No.2). Briefly, 0.5 g of weighted WB for analysis has been rinsed with small (approx. 2.5 mL) volumes of IPA and water, consecutively. After each rinse, the solvent was drawn up from the WB pieces and discarded. The samples were then left under the fume hood to dry completely overnight. Dry WB pieces were then subjected to the remaining sample preparation steps.

iii. Sample preparation

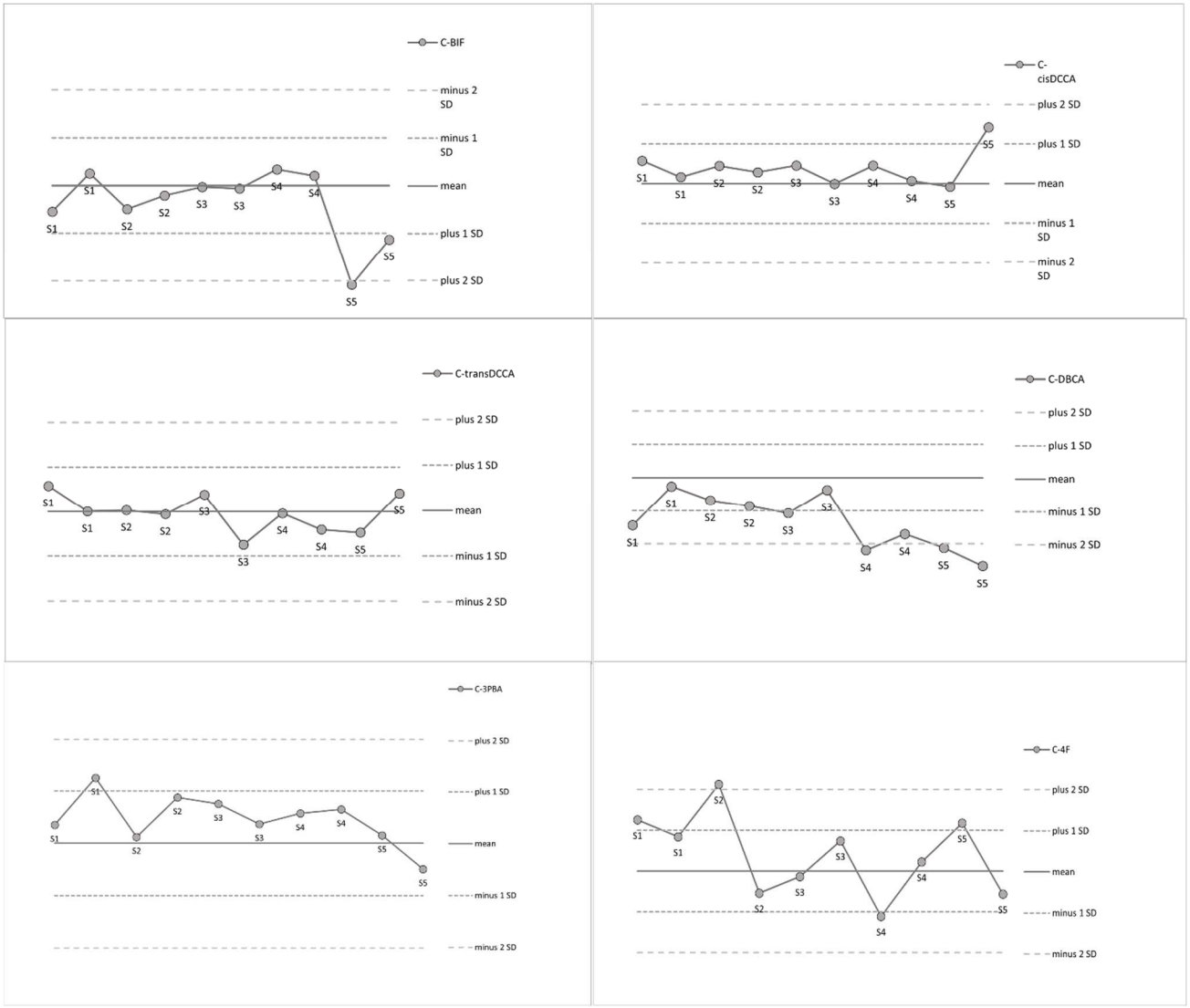
The procedure of sample preparation for analysis of silicone wristbands with the aim of quanfitying levels of native pyrethroids has been described in detail elswhere (Manuscript No.2). Briefly: prior to sample analysis, each of the participant's wristabnds has been cut up into small pieces with the use of a surgical disposable blade, homogenized by mixing up the resulting pieces and stored in 15 mL plastic falcon tubes in a freezer (-18°C). Homogenized WB (0.5 g) has been brought to room temperature and weighted to a screw-top test tube (ø 16 mm), and subjected to solid-liquid extraction by addition of 5 mL od ethyl acetate, aided by ultrasonication for 15 minutes, after which the liquid was transferred to a new test tube. This primary extraction was repeated twice, extracts obtained in both repetitions collected to the same test tube and mixed. Next, thus obtained primary extract has been evaporated to dryness in a dry bath (40°C), under a stream of nitrogen. The dry residue was reconstituted to 1 mL of hexane, which next has undergone solid phase extraction (SPE) in glass reusable columns, with the use of 3% deactivated silica gel, and a small layer (3-5 mm) of added sodium sulfate on top. The column has been conditioned with 2 mL of hexane, prior to sample extract transfer. The elution of analytes of interest has been conducted with a total of 4 mL of 30% solution of diethyl ether in hexane. The product of thus conducted secondary extraction was again evaporated to dryness (dry bath, 40°C, stream of nitrogen). The dry residue of the secondary extract was reconstituted in 1 mL of hexane, transferred into a chromatographic vial and subjected to instrumental analysis with the use of GC-ECD. The pictorial visualization of described procedure is presented on SM - Fig.2.



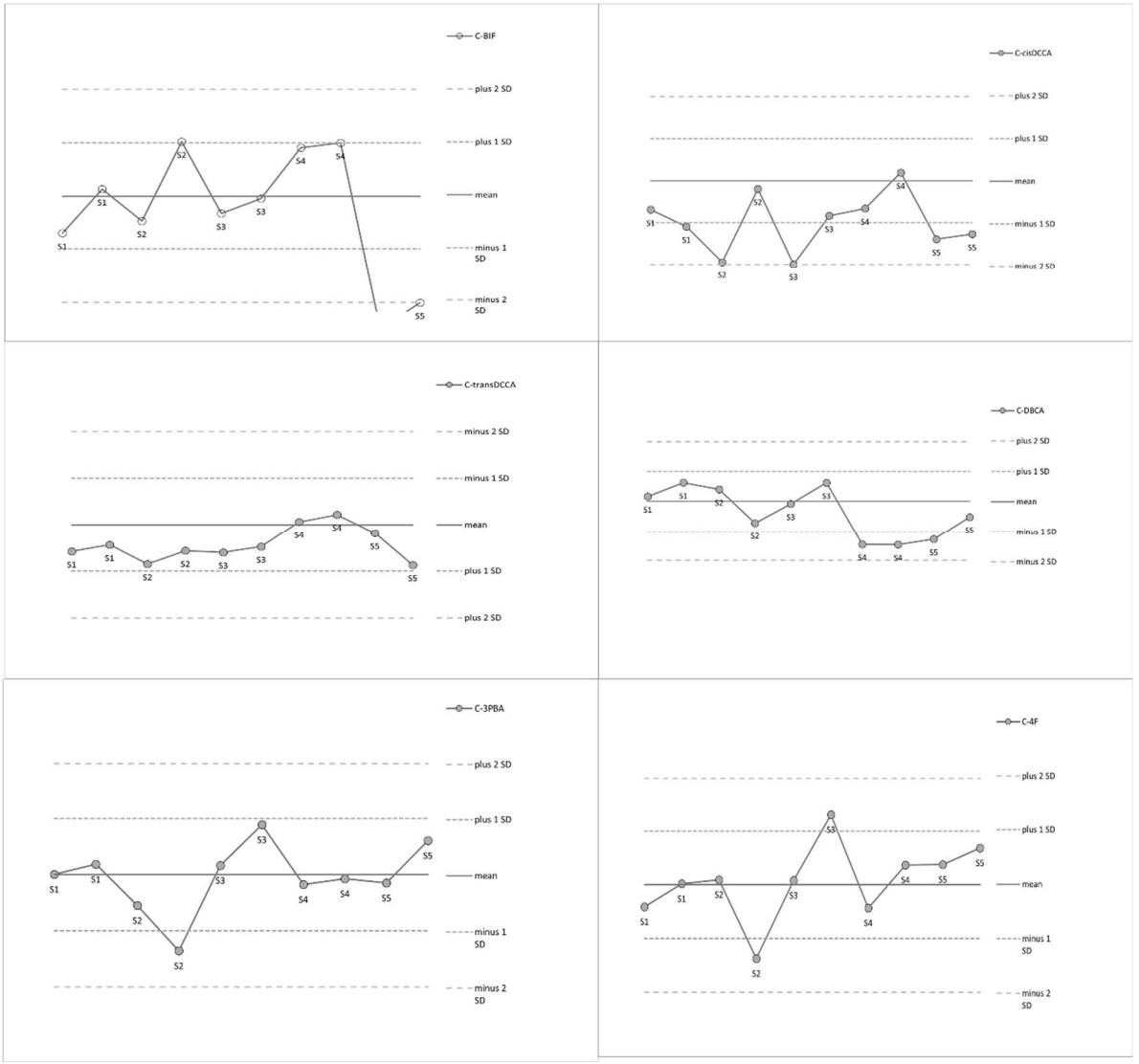
SM - Fig.2. Pictorial summary of the wristband sample preparation protocol.

2. Quality control

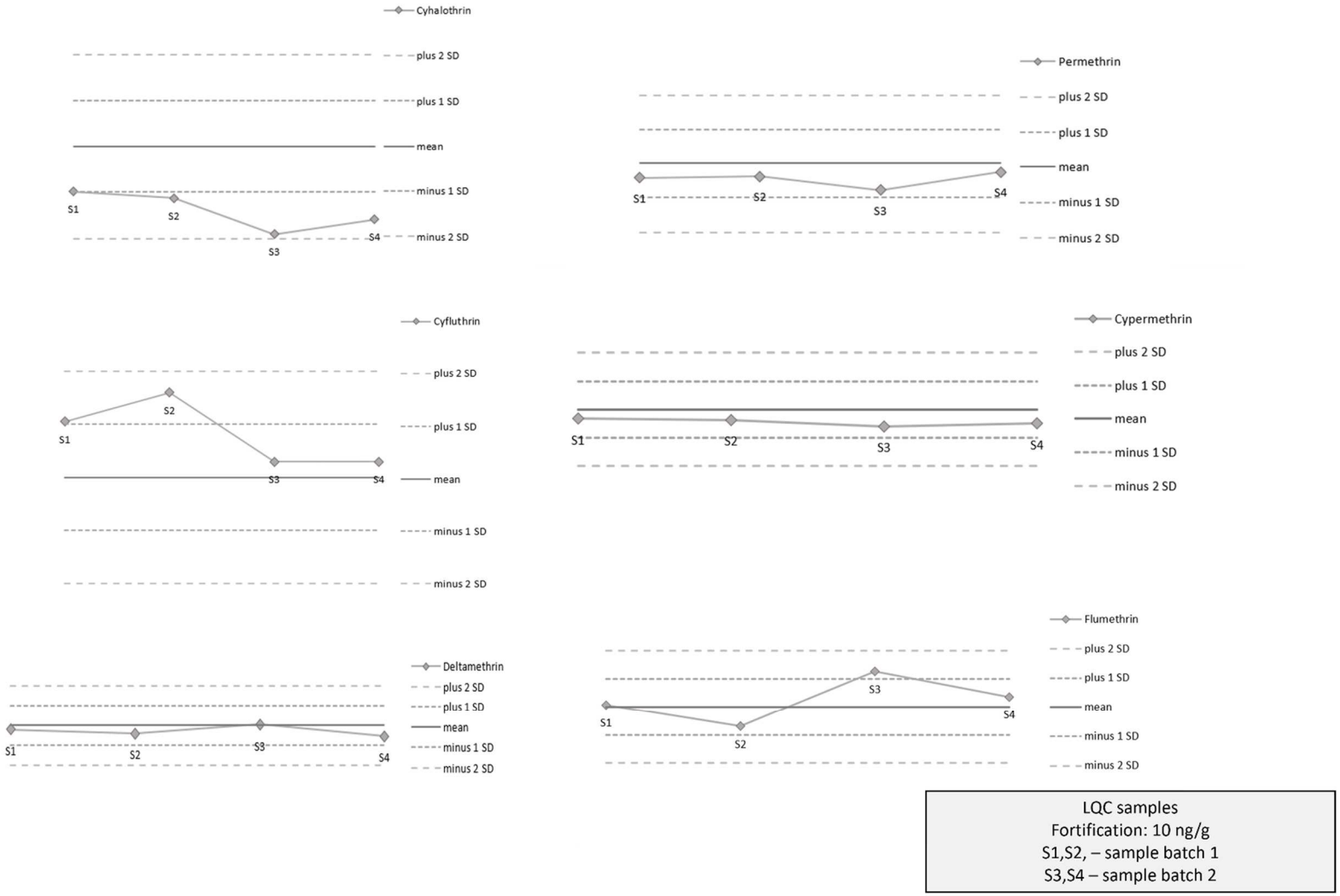
As briefly described in section: 'Quality control' of the manuscript, control samples at two levels of concentration have been added in two repetitions to each analyzed batch of samples. Concentrations of control samples for urinalysis had been: 1.5 ng/mL (HQC) and 0.25 ng/mL (LQC). In wristband analysis, the concentrations of spiked control samples had been 10 ng/g and 50 ng/g in LQC and HQC samples, respectively. The Westgard's rule of excluding the analyzed sample batch employed in the study was: $\mathbf{2_{2s}}$ for both quality control of urine analysis and ensuring quality of wristband analyses. The control charts formulated during urinalysis can be found on figures **3** (LQCs) and **4** (HQCs), and results of wristband analysis on figures **5** (LQCs) and **6** (LQCs).



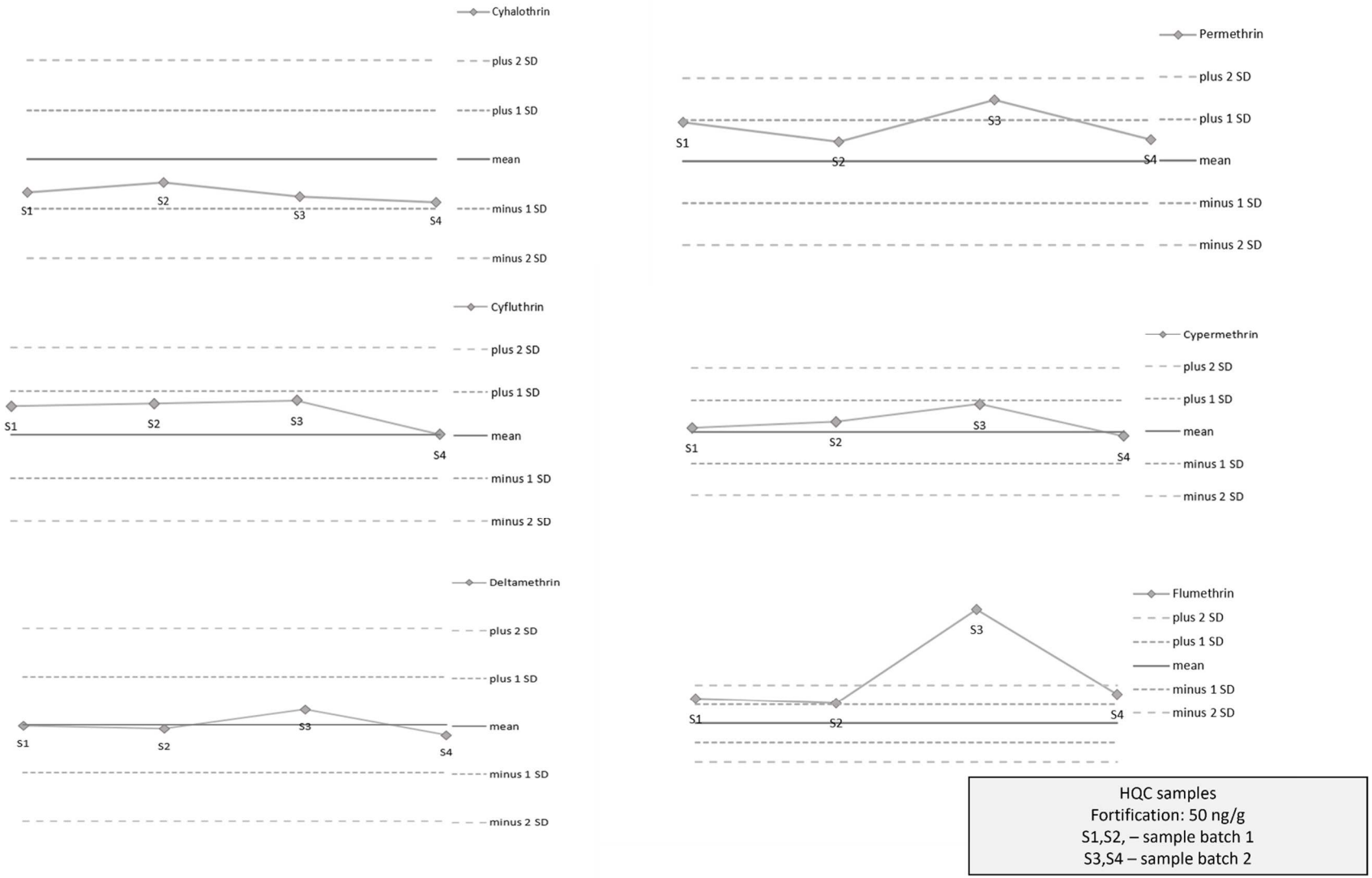
SM – Fig. 3. Urinary pyrethroid metabolites quality control charts: S1, S2, S3, S4, S5 – quality control samples for sample batch No.: 1,2,3,4,5, respectively (2 per sample batch). LQC samples.



SM – Fig. 4. Urinary pyrethroid metabolites quality control charts: S1, S2, S3, S4, S5 – quality control samples for sample batch No.: 1,2,3,4,5, respectively (2 per sample batch). LQC samples.



SM – Fig. 5. Quality control charts for assessment of pyrethroids in silicone wristbands (LQC samples).



SM – Fig. 6. Quality control charts for assessment of pyrethroids in silicone wristbands (HQC samples).

MSc Małgorzata Wacławik
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Wojciech Rodzaj, Joanna Jurewicz, Bartosz Wielgomas. *„Evaluation of exposure to synthetic pyrethroids among pet owners in a study with planned veterinary product application."* (working title).

Which is part of my doctoral dissertation, my participation in its creation involved performing literature review, laboratory research, data analysis and preparation of the original manuscript.

My contribution in preparation of this work has been estimated to sum up to 55%.

Wacławik
(signature)

MPharm Wojciech Rodzaj
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Wojciech Rodzaj, Joanna Jurewicz, Bartosz Wielgomas. *„Evaluation of exposure to synthetic pyrethroids among pet owners in a study with planned veterinary product application."* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved assistance with sample collection and establishing the design of the study.

My contribution in preparation of this work has been estimated to sum up to 15%.

Wojciech Jerzy Rodzaj

Elektronicznie podpisany przez Wojciech Jerzy Rodzaj
Data: 2023.10.13 09:41:28 +02'00'

.....

(signature)

Prof. Joanna Jurewicz
Department of Chemical Safety
Nofer Institute of Occupational Medicine
8 Teresy St., 91-348 Łódź, Poland

Gdańsk, 11.10.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Wojciech Rodzaj, Joanna Jurewicz, Bartosz Wielgomas. *„Evaluation of exposure to synthetic pyrethroids among pet owners in a study with panned veterinary product application”* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved reviewing and editing of the original manuscript, as well as research supervision.

My contribution in preparation of this work has been estimated to sum up to 10%.

Signed by /
Podpisano przez:

Joanna Katarzyna Jurewicz

Date / Data: 2023-10-13 10:45

.....

(signature)

Bartosz Wielgomas, PhD, Assoc. Prof.
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 14.10.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Wojciech Rodzaj, Joanna Jurewicz, Bartosz Wielgomas. *„Evaluation of exposure to synthetic pyrethroids among pet owners in a study with planned veterinary product application."* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved research conceptualization, reviewing and editing of the original manuscript, as well as research supervision.

My contribution in preparation of this work has been estimated to sum up to 20%.

Podpisano elektronicznie
Bartosz Wielgomas
2023-10-14

.....

(signature)

Manuscript 4 - Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Comprehensive assessment of exposure to synthetic pyrethroids among inhabitants of Northern Poland via urinalysis supplemented by passive sampling with the use of silicone wristbands”* (working title) – submission to International Journal of Hygiene and Environmental Health.

Submission to: International Journal of Hygiene and Environmental Health

Comprehensive assessment of exposure to synthetic pyrethroids among inhabitants of Northern Poland via urinalysis supplemented by passive sampling with the use of silicone wristbands

Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas*

Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, 107 Hallera Street, 80-416 Gdańsk, Poland

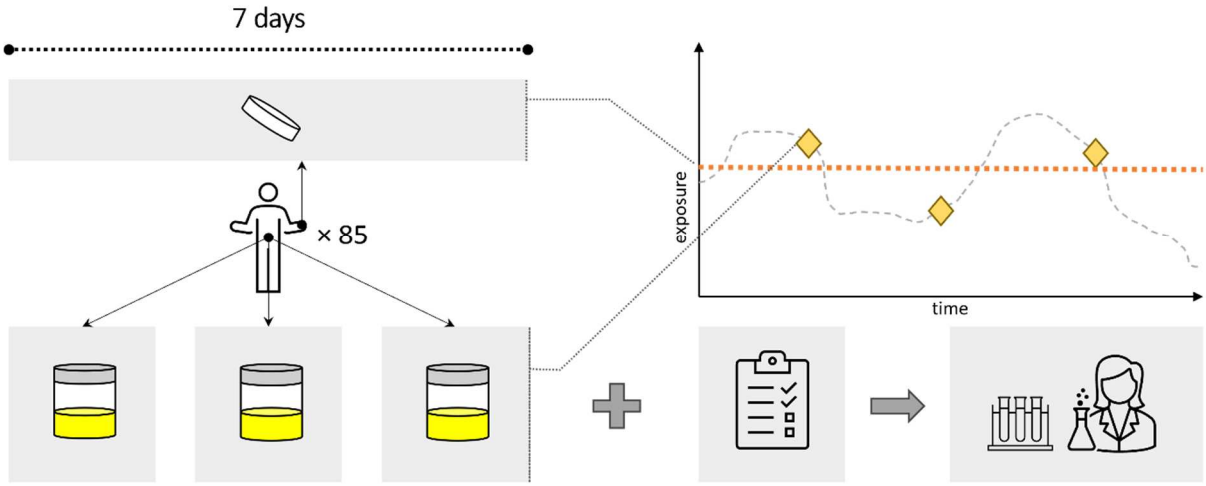
*corresponding author: bartosz.wielgomas@gumed.edu.pl

Małgorzata Wacławik - 0000-0002-9288-2180

Dominika Skwarło - no ORCID available

Bartosz Wielgomas - 0000-0003-4245-9197

Graphical abstract:



Abstract:

The study described aimed to assess exposure to synthetic pyrethroids in a convenient sample of Northern Poland's residents (n = 85). This was achieved by quantifying six pyrethroid metabolites in urine samples: 3-phenoxybenzoic acid (3-PBA), *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA), 4-fluoro-3-phenoxybenzoic acid (4F-3PBA), *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*- and *trans*-DCCA), and *cis*-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylic acid (lambda-cyhalothric acid - BIF). These were analyzed in urine samples collected in three repetitions over the course of one week.

In addition to traditional biomonitoring, we determined the levels of native pyrethroids (cyhalothrin, permethrin, cypermethrin, deltamethrin, flumethrin) in extracts from silicone wristbands worn by the study participants for the same 7-day period. The most frequently detected urinary metabolite was 3-PBA, found in 97.9% of the tested urine samples (GM = 0.316 ng/mL). In silicone wristbands (WBs), cypermethrin was detected in 58.8% of the tested samplers (GM = 25.3 ng/g).

By analyzing the chemical data alongside information gathered from questionnaires filled out by the study volunteers, we identified pet ownership (p = 0.0222) and the use of anti-ectoparasitic veterinary drugs on pets (p = 0.0104) as potential predictors of exposure to pyrethroids.

Furthermore, a strong positive correlation (r_s = 0.6824, p = 0.0046) was observed between the results of urinalysis and WB analysis among individuals who reported possible non-dietary exposure (e.g., use of pest control products) to these compounds. This relationship was found to be less pronounced when comparing results among all study participants (r_s = 0.4692, p = 0.0276), providing evidence that wristbands are capable of distinguishing between dietary and non-dietary exposure to pyrethroids.

Keywords: exposure assessment, silicone wristbands, biomonitoring, synthetic pyrethroids, passive sampling, Northern Poland, cross-sectional

1. Introduction

Synthetic pyrethroids are insecticides commonly used in pest control of field crops and orchards, as tools for controlling the malaria vector population of mosquitos, as well as active substances in a variety of consumer-ready products meant for indoor and outdoor use (Guessan et al. 2014; Lehmler et al. 2020; Bradberry et al. 2005). While acute toxicity to synthetic pyrethroids is a rare occurrence among humans (Bradberry et al. 2005), the emergence of negative health effects of longitudinal exposure to those substances is still being heavily studied. Current research points to synthetic pyrethroids being connected to reproductive health issues due pyrethroid hormone-like activity (Marettova, Maretta, and Legáth 2017), low birth weight among children of exposed parents (Hanke et al. 2003), as well as advent of ADHD-like behaviors (Richardson et al. 2015). Given improved environmental stability of synthetic pyrethroids, when compared to naturally occurring pyrethrins (which preluded its synthetic derivatives) (Zhu et al. 2020; Katsuda 2011), as well as universality and frequency of their use, it is likely for pyrethroids to linger and accumulate in microenvironments, therefore potentially leading to prolonged exposure. With their worldwide production and employment increasing rapidly, additionally reinforced by significant literature shortages regarding the subject in recent years, emergence of exposure assessment studies among varied populations are of high importance.

Traditionally, exposure assessment to synthetic pyrethroids is carried out through quantification of urinary levels of their metabolites *3-phenoxybenzoic acid (*3-PBA), *cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid* (DBCA), *4-fluoro-3-phenoxybenzoic acid* (4F-3-PBA), *cis*- and *trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis*- and *trans*-DCCA) and *cis*-3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylic acid (*lambda-cyhalothric acid* - BIF). However, given the relatively low specificity most of the analyzed metabolites present in relation to respective parent compounds, as well as taking into consideration their rapid metabolism, and thus high excretion variability of their concentrations, this method when performed on a single spot sample can represent only exposure that occurred in close time proximity to the sample collection (Calafat et al. 2016), and does not offer an opportunity to elucidate the native compound to which the exposure had occurred, therefore making identification of potential sources of exposure more challenging.

A novel method in exposure assessment studies, which aids the traditional approach in aspects mentioned above is the use of silicone wristbands (WBs) as personal passive samplers. The use of silicone wristbands worn for some time, gives an opportunity to investigate the average exposure that had taken place over that time, not only to the native compounds, but also to the products of their environmental degradation, which often vary in toxicity, and to which humans are exposed alongside parent compounds. The employment of WBs offers a cheap, non-invasive (Baum et al. 2020; Bergmann et al. 2017) alternative to biomonitoring, which due to being very convenient in sampling results in high participant compliance (Wacławik, Rodzaj, and Wielgomas 2022).

The aims of this study had been exposure assessment to synthetic pyrethroids among inhabitants of Northern Poland via biomonitoring complemented with employment of silicone wristbands, investigation of potential sources of exposure to those substances and evaluation of usefulness and practicality of usage of WBs for their assigned purpose.

2. Materials and methods

Technical grade ethyl acetate, n-hexane (fraction from petroleum pure) and technical grade methanol (MeOH), all from POCH (Gliwice, Poland) were used in pre-exposure cleanup of silicone wristbands. Furthermore, n-hexane (Hex) (n-hexane 95% for GC, for pesticide residue analysis, POCH, Gliwice, Poland); diethyl ether (ACS grade, Sigma-Aldrich, Saint Louis, USA); ethyl acetate (EtAc) (for gas chromatography MS, Supelco, Saint Louis, USA), 2-propanol (IPA, 2-propanol for HPLC, VWR International, France) have been used. Additionally, water used in this study was prepared by the laboratory water demineralizer (Hydrolab, Wiślina, Poland). Other chemical reagents/supplies employed in sample preparation were: 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma- Aldrich, USA), N,N'-diisopropylcarbodiimide (DIC) (99%, Sigma Aldrich, Saint Louis, USA), hydrochloric acid (J.T. Baker, Radnor, USA), potassium carbonate - anhydrous pure p.a. (POCH, Gliwice, Poland) and sodium hydroxide pure p.a. (POCH, Gliwice, Poland). Solid phase extraction of primary wristband extracts had been carried out with the use of silica gel (Sigma-Aldrich, Saint Louis, USA, pore size 60Å, 220-240 mesh particle size).

Analytical standards used in the described study included native pyrethroids for wristband analysis, namely: cypermethrin (mix of isomers) (Institute of Organic Industrial Chemistry, Poland), permethrin (mix of isomers) (EPA Research, USA), beta-cyfluthrin (Institute of Organic Industrial Chemistry, Poland), lambda-cyhalothrin (Institute of Organic Industrial Chemistry, Poland), deltamethrin (Roussel Uclaf, France), flumethrin (mix of isomers) (Sigma Aldrich, Germany). Internal standards used in urinalysis included: *cis*-DCCA 100 µg/mL in acetonitrile-D3 (1, Carboxyl-13C2, 99%; 1-D, 97%) – purchased from Cambridge Isotope Laboratories (USA) and 2-PBA (2-phenoxybenzoic acid) – purchased from Fluka (Germany). Pyrethroid metabolites standards employed in quantitative analysis of urine samples included: 3-PBA (Lancaster, United Kingdom), *cis*-DCCA, *trans*-DCCA, BIF (Toronto Research Chemicals, Canada), 4F-3-PBA (Cambridge Isotope Laboratories, USA) and DBCA (Roussel Uclaf, France).

The batch of silicone wristbands used in this study had been a part of a larger shipment, which also included WBs employed in previously described studies (Manuscripts No. 2,3), briefly: purchased wristbands were white in colour, on average 12 mm wide, and 200 mm long, with the mean value of their thickness being 1.48 mm. On average, each wristband weighted around 5 g. All the wristbands used in the study had been purchased via an online vendor: www.allegro.pl. The original application of thus sold WBs had been promotional, thus prior to their employment in the described study WBs had undergone previously described (Manuscript No. 2) pre-exposure cleanup procedure.

3. Study outline

Approval of the Medical University of Gdańsk Bioethics Committee for Scientific Research No. NKBBN/536/2020 on 4th of December 2020 was granted. The study was conducted on a convenient sample of 85 volunteers who were residents of Northern Poland. Before participating, they provided written consent to confirm their willingness to take part in the study. Exclusion criteria included open wounds, rash and/or irritation around the wrist of the dominant hand, and kidney failure. Participation of individuals under 18 years of age required permission from their legal guardian. The study involved collecting three random urine samples on three separate days within one week, while simultaneously wearing a silicone wristband on their dominant hand for the same seven consecutive days. Additionally, the study volunteers were required to complete a questionnaire, providing information about their lifestyle, occupation, residential situation, and other relevant details (see Table 1). All containers, vials, and paperwork were labeled clearly before distribution to facilitate easy sampling by the participants. The study kits were prepared, delivered to, and collected from the volunteers by the research team.

Urine samples collected on the designated days were placed in a freezer (-18°C) and stored there until they were transported to the laboratory of the Department of Toxicology, Medical University of Gdańsk, Poland.

Silicone wristbands were provided to the study participants in transparent, clean, labeled zip-lock bags, which were to be returned at the end of the 7th day of the sampling period. The wristbands were also stored in a freezer until they were collected by the research team. The study volunteers were advised to wear the silicone wristband on the wrist of their dominant hand for as much time during the day as possible, including during bathing/showering and sleeping, unless it caused discomfort.

The sample collection for this study took place between April 2022 and December 2022. Study participants were also asked about the regular dwelling location of their pets, and all pet owners confirmed that their pets lived in the same indoor space as the human occupants. Additionally, the participants were inquired about any recent lice treatment within the last 6 months, but none of the volunteers in the study reported suffering from this condition.

4. Sample analysis

4.1. Determination of pyrethroid metabolites in urine

The detailed description of procedure used for sample preparation for determination of pyrethroid metabolites in human urine has been in detail described elsewhere (Manuscripts No. 2 and 3). Gas chromatography-mass spectrometry (GC-MS) (Varian 450 GC, equipped with autosampler: CP-8400, a split/splitless injector 1177 and Varian 225-MS ion trap mass spectrometer) was employed. Matrix matched calibration curve with two internal standards was used for quantitative interpretation. Limits of detection for tested analytes had been set at concentration: 0.05 ng/mL for *cis*-DCCA, *trans*-DCCA, 3-PBA and DBCA, and at 0.1 ng/mL for 4F-3PBA and BIF (Table. 1).

4.2. Urine Specific Gravity

Since the extent of urine dilution can significantly impact the measured analyte concentrations (Cone et al. 2009), a correction procedure was implemented. Before undergoing the preparation procedure for analysis, each sample was subjected to a refractometric assay to determine urine specific gravity (SG). The measurement of urine SG was conducted using a handheld refractometer (PAL-10S, Atago Co., Tokyo, Japan), with deionized water as the reference (with an SG value of 1.000).

Samples with a urine SG value exceeding 1.030 or falling below 1.005 (Simerville, Maxted, and Pahira 2005) were excluded from further analyses. As a result, in some cases, only 2 out of the 3 samples collected by a participant were used in the data analysis. For samples with urine SG values in the range of 1.005 to 1.030, their urinary metabolite concentrations were adjusted using the following formula:

$$C_{adjusted} = C_{measured} \times \frac{(SG_{population\ mean} - 1)}{(SG_{sample} - 1)}$$

$C_{measured}$ -analyte concentration measured by instrumental method
$SG_{population\ mean}$ - an arithmetic mean of all urine SG assayed for samples collected in the study
SG_{sample} - measured urine specific gravity of given sample

SG adjusted urinary biomarker concentrations were further included in data analyses.

4.3. Determination of native pyrethroids in silicone wristbands

The method employed in this study for sample preparation and analysis of silicone wristbands has been developed, validated and described in previous manuscripts (Manuscripts No. 2 and 3), briefly: 1 g of fragmented and then homogenized silicone wristband had been weighted into a test tube, subjected to solid-liquid ultrasonic bath-assisted extraction with ethyl acetate which has been repeated twice. He pooled primary extracts were evaporated to dryness at 40°C under a stream of nitrogen, and the dry residue was reconstituted in 1 mL of hexane. The hexane extract was further purified by solid phase extraction (SPE) using 3% deactivated silica gel. The analytes of interest were eluted using a 30% diethyl ether solution in hexane. The extract was evaporated to dryness once again, and the dry residue dissolved in 1 mL of hexane, which was subjected to instrumental analysis with the use of gas chromatography with electron capture detector (GC-ECD) (456-GC SCION Instruments, equipped with CP-8400 autosampler, 1177 split/splitless injector).

4.4. Quality control

Similarly to a previously described study, (Manuscript No. 3) prior to analysis of urine samples included in the study, a series of 20 repetitions of quality control samples at two levels of concentration (low concentration quality control – LQC = 0.25 ng/mL, high concentration quality control – HQC = 1.5 ng/mL) had been prepared. Their preparation and instrumental analysis have been spread out over a 3-week period, the results of which allowed for formulation of control charts for assessment of concentration of each of the tested pyrethroid metabolites.

To each set of 50 urine samples collected during the study, additional 4 QC samples (2 HQC's and 2 LQC's) had been added. Quality of results of LQC and HQC samples for each sample batch was assessed with the use of constructed control charts, thus ensuring assessment quality control, as if proven necessary, a preparation and analysis of sample batch had been repeated.

Likewise, a corresponding procedure had been carried out for the wristband analysis, as quantification of native pyrethroid concentrations (cyhalothrin, cyfluthrin, cypermethrin, permethrin, deltamethrin, flumethrin) in a series of control samples (LQC – 10 ng/g, HQC – 50 ng/g) served for construction of control charts later used for quality assessment of control samples (1 LQC, 1 HQC per sample batch) added to respective batches of participants samples (3 batches in total).

4.5. Data handling and statistical analyses

Medians of concentrations of a maximum of 3 urine samples from the same participant were calculated. If only 2 samples were collected, mean of those values was computed. Participants who had provided only one or none of the urine samples, or who had lost their wristband, they were excluded from the study. In cases of both urinalysis results, as well as results of wristband analysis, concentrations below the limit of detection (see Table 3.) were assigned values equal to $LOD/\sqrt{2}$ (Hornung and Reed 1990).

Differences in concentrations between sub-groups of participants formed by variables obtained via questionnaire (Tables 1. and 2.) were investigated by performing either Mann-Whitney-U test (for dichotomous variables) or Kruskall-Wallis test (for variables with more than 2 answer choices). For both, the *p*-value threshold had been $p < 0.05$. Statistical analyses and calculations as well as data visualization had been performed with the use of Microsoft 365 Excel (Corp., Redmond, WA, USA),

Statistica (TIBCO Software Inc, Palo Alto, CA, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA)

5. Results

In total, 247 urine samples and 85 silicone wristbands were collected by the study participants and analyzed further for 6 metabolites and 5 parent pyrethroids, respectively. Four metabolites (3-PBA, DBCA, *cis*- and *trans*-DCCA) were found in more than 50% urine samples while specific metabolites of bifenthrin and cyhalothrin (BIF and 4F-3-PBA) were detected in 12.1 and 17.3% of the samples, respectively (Table 1). Only cypermethrin was present in more than 50% of wristbands. The urinary metabolite profile is consistent with the profile of parent compounds detected in the wristbands, as the most frequently detected metabolites in urine (3-PBA, DBCA, *cis*- and *trans*-DCCA) are common metabolites of the most frequently detected parent compounds (permethrin, cypermethrin and deltamethrin) in the wristbands. In turn, specific metabolites of flumethrin and cyhalothrin (4F-3-PBA and BIF) are detected relatively rarely (Table 1).

Table 1. Limits of detection (LOD) and detection rates of tested substances. In case of urinalysis, results of metabolites detected in over 50% of the analyzed samples were further analyzed, whereas in case of wristband analyses, native pyrethroids that have achieved detection rate exceeding 25% were subjected to further statistical analysis (**in bold**).

Analyte	*Specimen*	*LOD (urine – ng/mL; wristband – ng/g)*	*Detection rate [%]*
3-PBA	Urine	0.05	**97.9**
4F-3-PBA	Urine	0.1	17.3
BIF	Urine	0.1	12.1
DBCA	Urine	0.05	**88.7**
cis-DCCA	Urine	0.05	**52.0**
trans-DCCA	Urine	0.05	**60.1**
Cyhalothrin	Wristband	2	20.0
Permethrin	Wristband	10	**28.2**
Cypermethrin	Wristband	10	**58.8**
Deltamethrin	Wristband	2	**28.2**
Flumethrin	Wristband	10	9.4

The normality of distributions of quantified analyte concentrations was investigated, none of which had been confirmed, therefore further data analysis had been carried out with the use of nonparametric test.

The highest concentrations (geometric means and maximum values) were observed for 3-PBA and cypermethrin and permethrin in urine and wristbands, respectively.

Table 2. Summary of descriptive statistics of concentrations of analytes tested in the study in urine (SG adjusted ng/mL) and wristbands (ng/g)

	n	*GM*	*Minimum*	*P_{25}*	*Median*	*P_{75}*	*Maximum*
3-PBA	247	0.316	<LOD	0.173	0.305	0.591	16.2
DBCA		0.136	<LOD	0.068	0.119	0.219	5.005
cis-DCCA		0.071	<LOD	0.035	0.035	0.122	4.445
trans-DCCA		0.116	<LOD	0.035	0.073	0.290	17.9
Permethrin	85	14.0	1.592	7.070	7.070	7.070	6586.7
Cypermethrin		25.03	7.070	7.070	24.09	47.2	1456.5
Deltamethrin		2.773	1.410	1.410	1.410	7.537	132.2

GM – geometric mean
Min – minimal value
P_{25} – 25th percentile
P_{75} – 75th percentile
Max – maximum value

5.1. Study population

The study was conducted with a group of 85 volunteers residing in Northern Poland. The participants had an average age of 32.8 years (ranging from 8 to 69 years) and an average body weight of 70.9 kg. Information from the questionnaires completed by the volunteers is summarized in Table 1. In some cases, missing information resulted in the displayed results not totaling to 100%. One participant lost their wristband during the sampling period and was consequently excluded from the study. Additionally, 9 urine samples were excluded from further analysis due to urine specific gravity values falling outside the assigned range (as described in Section 5.2). In these cases, the two remaining urine samples for each of the 9 participants were subjected to further analysis.

For some survey questions, positive responses led to more specific questions being asked. For example, participants who reported using pharmaceuticals during the study were further questioned about the names/types of the medications. This collected information was later used to assess possible sources of exposure. Participants who declared ownership of pets were asked about their habits related to their animals. All pet owners stated that their pets remain indoors for most of the day. Of the pet owners, 31 participants (64.6%) confirmed using veterinary anti-ectoparasitic drugs on their pets in the past, while 16 (33.4%) claimed they had never done so. When asked about the average number of hours spent at home, responses ranged from 8 to 24 hours, with an average value of 14.2 hours. Only 3 study participants lived alone at the time of the study, while the majority shared their living space with at least one other person. The study participants were also asked about past lice and/or scabies treatments, and all of them reported never having undergone such treatments.

Table 3. Study population characteristics extracted from participants questionnaires, descriptive statistics of sums of urinary metabolite concentrations (SG adjusted ng/mL) and results of statistical comparisons.

	n (%)	GM (95% CI)	Min	P_{10}	P_{25}	Median	P_{75}	P_{90}	Max	*P value*[a]
Gender										
Male	33 (38.8)	0.91 (-0.19 – 4.27)	0.28	0.45	0.53	0.81	1.36	2.05	36.92	0.616
Female	52 (61.2)	0.95 (0.45 – 2.88)	0.36	0.48	0.60	0.71	1.55	2.15	32.14	
Dominant hand										
Right	79 (92.9)	0.97 (0.70 – 3.10)	0.28	0.45	0.57	0.77	1.53	2.15	36.92	0.137
Left	6 (7.1)	0.62 (0.49 – 0.79)	0.51	0.51	0.53	0.57	0.81	0.82	0.82	
Level of acquired education										
Primary Education	3 (3.5)	1.37 (-2.38 – 5.99)	0.78	0.78	0.78	0.89	3.75	3.75	3.75	0.350
High School	23 (27.1)	0.82 (0.72 – 1.18)	0.36	0.43	0.51	0.81	1.23	1.60	2.23	
Technical College	15 (17.6)	0.83 (0.64 – 1.28)	0.46	0.53	0.56	0.64	1.45	2.05	2.09	
Vocational School	1 (1.2)	-	0.39	0.39	0.39	0.39	0.39	0.39	0.39	
Higher Education	42 (49.4)	1.06 (0.39 – 4.91)	0.28	0.46	0.61	0.76	1.65	2.20	36.92	
Usage of insecticides in workplace										
Yes	3 (3.5)	0.99 (-1.08 – 3.74)	0.31	0.48	0.31	1.45	2.23	2.23	2.23	0.162
No	77 (90.6)	0.95 (0.64 – 3.10)	0.28	0.31	0.60	0.28	1.38	2.09	36.92	
Usage of hand creams during study sample collection										
Yes	36 (42.4)	0.82 (0.75 – 1.24)	0.36	0.41	0.53	0.65	1.35	2.15	3.75	0.218
No	49 (57.6)	1.04 (0.47 – 4.34)	0.28	0.46	0.60	0.82	1.53	2.13	36.92	
Frequency of bathing/showering										
Once a day	71 (83.5)	0.98 (0.67 – 3.34)	0.28	0.46	0.54	0.78	1.56	2.15	36.92	0.728
Multiple times a day	5 (5.9)	0.64 (0.39 – 0.94)	0.38	0.38	0.61	0.61	0.73	0.99	0.99	
Less frequently	8 (9.4)	0.82 (0.49 – 1.35)	0.48	0.48	0.63	0.70	1.09	2.02	2.02	
Pet ownership										
Yes	48 (56.5)	1.08 (0.52 – 4.48)	0.30	0.51	0.60	0.83	1.59	2.23	36.92	0.103
No	37 (43.5)	0.78 (0.73 – 1.11)	0.28	0.39	0.52	0.71	1.20	1.79	2.65	
Use of veterinary products on pets[b]										
Yes	31 (64.6)	1.27 (0.30 – 6.45)	0.45	0.51	0.59	0.95	2.09	2.23	36.92	0.256
No	16 (33.3)	0.80 (0.66 – 1.13)	0.31	0.48	0.61	0.74	1.36	1.65	1.68	
Number of owned pets										
None	37 (43.5)	0.78 (0.73 – 1.11)	0.28	0.39	0.52	0.71	1.20	1.79	2.65	0.262
One	34 (40)	1.13 (0.25 – 5.86)	0.31	0.51	0.60	0.76	1.51	2.23	36.92	
More than one	14 (16.5)	0.99 (0.78 – 1.52)	0.46	0.51	0.57	0.92	1.68	2.05	2.23	
Presence of animals in workplace										
Yes	19 (22.4)	0.84 (0.66 – 1.51)	0.28	0.31	0.52	0.64	1.60	2.20	3.75	0.431
No	62 (72.9)	0.99 (0.58 – 3.63)	0.36	0.48	0.59	0.77	1.51	2.13	36.92	
Terrain surrounding inhabited location										
Rural	24 (28.2)	0.84 (0.74 – 1.18)	0.31	0.53	0.59	0.76	1.30	1.65	2.23	0.782
Urban	59 (69.4)	1.01 (0.60 – 3.81)	0.28	0.43	0.56	0.77	1.59	2.20	36.92	
Housing conditions										
Detached house	29 (34.1)	0.73 (0.66 – 0.99)	0.31	0.39	0.54	0.66	0.95	1.45	2.23	0.069
Multi-family house	56 (65.9)	1.07 (0.63 – 4.01)	0.28	0.48	0.58	0.88	1.66	2.20	36.92	
Population density of living area										
Big city	51 (60)	0.94 (0.93 – 1.35)	0.28	0.48	0.56	0.84	1.60	2.13	3.75	0.328
Town/Suburbs	18 (21.2)	0.98 (-1.00 – 9.92)	0.31	0.36	0.46	0.65	0.99	32.14	36.92	
Village/Small Town	16 (18.8)	0.89 (0.73 – 1.23)	0.53	0.57	0.64	0.80	1.30	1.53	2.23	
Relative distance of fields from the inhabited area										
>1000 m	68 (80)	0.97 (0.63 – 3.42)	0.28	0.45	0.57	0.77	1.45	2.15	36.92	0.605

150 m < x <1000 m	10 (11.8)	0.77 (0.47 – 1.38)	0.31	0.42	0.54	0.62	1.24	2.01	2.23	
< 150 m	6 (7.1)	0.81(0.41 – 1.39)	0.48	0.48	0.60	0.66	1.45	1.53	1.53	
Relative distance of orchards from the inhabited area										
>1000 m	74 (87.1)	0.95 (0.65 – 3.21)	0.28	0.46	0.57	0.76	1.45	2.09	36.92	
150 m < x <1000 m	7 (8.2)	0.96 (0.51 - 1.70)	0.54	0.54	0.57	0.91	1.65	2.23	2.23	0.120
< 150 m	1 (1.2)	2.23	2.23	2.23	2.23	2.23	2.23	2.23	2.23	
Duration of inhabitancy of current living location										
Less than a year	14 (16.5)	0.80 (0.60 – 1.29)	0.48	0.48	0.51	0.58	1.38	2.02	2.05	
More than one year	35 (41.2)	0.93 (0.87 – 1.29)	0.38	0.45	0.61	0.82	1.51	2.13	2.65	0.424
More than five years	36 (42.3)	1.01 (0.21 – 5.52)	0.28	0.39	0.58	0.69	1.49	2.20	36.92	
Performance of pest indoor control within last 5 years										
Yes	9 (10.6)	2.89 (-2.19 – 20.19)	0.75	0.75	0.81	2.20	3.75	36.92	36.92	
No	47 (55.3)	0.77 (0.73 – 0.99)	0.31	0.46	0.56	0.66	1.20	1.53	2.15	**0.004**
"I don't know"	29 (34.1)	0.92 (0.85 – 1.39)	0.28	0.41	0.53	0.84	1.60	2.13	2.65	
Indoor use of commercially available insecticides										
Yes	25 (29.4)	1.03 (0.91 – 1.57)	0.45	0.54	0.63	0.95	1.60	2.20	3.75	0.162
No	59 (69.4)	0.91 (0.47 – 3.69)	0.28	0.41	0.53	0.71	1.36	2.05	36.92	
Source of drinking water										
Bottled water	22 (25.9)	0.86 (0.75 – 1.22)	0.36	0.43	0.64	0.79	1.35	1.56	2.23	
Tap filtered water	40 (47.1)	0.89 (0.85 – 1.33)	0.28	0.45	0.53	0.83	1.56	2.03	3.75	
Tap water (public outlet)	21 (24.7)	1.12 (-0.53 – 8.72)	0.45	0.54	0.59	0.71	1.36	2.13	36.92	0.993
Tap water (private well)	2 (2.3)	1.08 (-9.48- 12.23)	0.52	0.52	0.52	1.38	2.23	2.23	2.23	
Practicing a defined diet										
Yes	8 (9.4)	1.29 (0.89 – 2.04)	0.54	0.54	0.78	1.59	2.11	2.23	2.23	0.067
No	77 (90.6)	0.91 (0.62 – 3.08)	0.28	0.45	0.56	0.71	1.36	2.09	36.92	

*[a] – p-value of Mann-Whitney U test (for dichotomous variables) or Kruskal-Wallis test (for variables with more than 2 answer choices) – statistically significant results in **bold**.*

[b] – Statistical analysis has been carried out on a sub-population of pet owners.

LOD – Limit of detection

GM - geometric mean

Min – minimal value

P_{10} – 10th percentile

P_{25} – 25th percentile

P_{75} – 75th percentile

P_{90} – 90th percentile

Max – maximum value

Table 4. Study population characteristics extracted from participants questionnaires, descriptive statistics of wristband permethrin concentrations and results of statistical analysis of differences between their levels.

	n (%)	GM	Min	P_{10}	P_{25}	Median	P_{75}	P_{90}	Max	*p* [a]
Gender										
Male	33 (38.8)	15.95	6.63	<LOD	<LOD	<LOD	<LOD	169.49	6586.73	0.930
Female	52 (61.2)	13.36	<LOD	<LOD	<LOD	<LOD	7.95	143.21	6386.41	
Dominant hand										
Right	79 (92.9)	15.10	<LOD	<LOD	<LOD	<LOD	10.89	169.48	6586.73	0.184
Left	6 (7.1)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
Level of acquired education										
Primary Education	3 (3.5)	38.02	<LOD	<LOD	<LOD	<LOD	1099.10	1099.10	1099.10	0.926
High School	23 (27.1)	13.93	<LOD	<LOD	<LOD	<LOD	16.20	145.31	309.27	

Technical College	15 (17.6)	10.90	<LOD	<LOD	<LOD	<LOD	<LOD	38.91	169.48	
Vocational School	1 (1.2)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
Higher Education	42 (49.4)	15.44	<LOD	<LOD	<LOD	<LOD	<LOD	165.14	6586.73	
Usage of insecticides in workplace										
Yes	3 (3.5)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.377
No	77 (90.6)	13.96	<LOD	<LOD	<LOD	<LOD	86.38	165.14	6586.73	
Usage of hand creams during study sample collection										
Yes	36 (42.4)	12.35	<LOD	<LOD	<LOD	<LOD	<LOD	143.21	1099.10	0.715
No	49 (57.6)	15.95	<LOD	<LOD	<LOD	<LOD	8.83	169.48	6586.73	
Frequency of bathing/showering										
Once a day	71 (83.5)	14.23	<LOD	<LOD	<LOD	<LOD	8.83	143.21	6586.73	0.939
Multiple times a day	5 (5.9)	14.20	<LOD	<LOD	<LOD	<LOD	<LOD	230.74	230.74	
Less frequently	8 (9.4)	16.54	<LOD	<LOD	<LOD	<LOD	76.19	309.27	309.27	
Pet ownership										
Yes	48 (56.5)	15.30	<LOD	<LOD	<LOD	<LOD	7.95	169.48	6586.73	0.977
No	37 (43.5)	13.13	<LOD	<LOD	<LOD	<LOD	<LOD	165.14	450.57	
Use of veterinary products on pets [b]										
Yes	31 (64.6)	23.36	<LOD	<LOD	<LOD	<LOD	38.90	419.17	6586.73	**0.010**
No	16 (33.3)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	
Number of owned pets										
None	37 (43.5)	13.13	<LOD	<LOD	<LOD	<LOD	<LOD	165.14	450.57	0.932
One	34 (40)	14.77	<LOD	<LOD	<LOD	<LOD	<LOD	86.38	6586.73	
More than one	14 (16.5)	16.65	<LOD	<LOD	<LOD	<LOD	38.91	169.48	419.17	
Presence of animals in workplace										
Yes	19 (22.4)	15.09	<LOD	<LOD	<LOD	<LOD	16.20	230.75	1099.1	0.744
No	62 (72.9)	13.78	<LOD	<LOD	<LOD	<LOD	<LOD	145.31	6586.73	
Terrain surrounding inhabited location										
Rural	24 (28.2)	12.22	<LOD	<LOD	<LOD	<LOD	<LOD	86.38	419.17	0.664
Urban	59 (69.4)	15.63	<LOD	<LOD	<LOD	<LOD	10.89	230.75	6586.73	
Housing conditions										
Detached house	29 (34.1)	10.25	<LOD	<LOD	<LOD	<LOD	<LOD	38.91	419.17	0.281
Multi-family house	56 (65.9)	17.01	<LOD	<LOD	<LOD	<LOD	13.54	230.75	6586.73	
Population density of living area										
Big city	51 (60)	14.18	<LOD	<LOD	<LOD	<LOD	10.89	165.14	1099.1	0.751
Town/Suburbs	18 (21.2)	18.14	<LOD	<LOD	<LOD	<LOD	<LOD	6386.4	6586.73	
Village/Small Town	16 (18.8)	11.27	<LOD	<LOD	<LOD	<LOD	<LOD	38.91	419.17	
Relative distance of fields from the inhabited area										
>1000 m	68 (80)	14.74	<LOD	<LOD	<LOD	<LOD	7.95	169.48	6586.73	0.875
150 m < x <1000 m	10 (11.8)	13.66	<LOD	<LOD	<LOD	<LOD	<LOD	252.77	419.17	
< 150 m	6 (7.1)	12.41	<LOD	<LOD	<LOD	<LOD	37.62	38.91	38.91	
Relative distance of orchards from the inhabited area										
>1000 m	74 (87.1)	14.94	<LOD	<LOD	<LOD	<LOD	<LOD	165.14	6586.73	0.251
150 m < x <1000 m	7 (8.2)	10.44	<LOD	<LOD	<LOD	<LOD	8.83	86.38	86.38	
< 150 m	1 (1.2)	419.17	419.17	419.17	419.17	419.17	419.17	419.17	419.17	
Duration of inhabitancy of current living location										
Less than a year	14 (16.5)	14.98	<LOD	<LOD	<LOD	<LOD	16.20	169.48	230.75	0.914
More than one year	35 (41.2)	12.73	<LOD	<LOD	<LOD	<LOD	<LOD	165.14	450.57	
More than five years	36 (42.3)	15.75	<LOD	<LOD	<LOD	<LOD	8.98	143.21	6586.73	
Performance of pest indoor control within last 5 years										

Yes	9 (10.6)	93.14	<LOD	<LOD	<LOD	10.89	1099.1	6586.73	6586.73	**0.007**
No	47 (55.3)	8.67	<LOD	<LOD	<LOD	<LOD	<LOD	25.52	86.38	
"I don't know"	29 (34.1)	18.03	<LOD	<LOD	<LOD	<LOD	76.67	230.75	450.57	
Indoor use of commercially available insecticides										
Yes	25 (29.4)	17.22	<LOD	<LOD	<LOD	<LOD	35.09	165.14	1099.1	0.302
No	59 (69.4)	13.39	<LOD	<LOD	<LOD	<LOD	<LOD	169.48	6586.73	
Source of drinking water										
Bottled water	22 (25.9)	10.90	<LOD	<LOD	<LOD	<LOD	8.83	37.62	165.14	0.703
Tap filtered water	40 (47.1)	12.77	<LOD	<LOD	<LOD	<LOD	<LOD	157.39	1099.1	
Tap water (public outlet)	21 (24.7)	20.81	<LOD	<LOD	<LOD	<LOD	16.20	450.57	6586.73	
Tap water (private well)	2 (2.3)	54.44	<LOD	<LOD	<LOD	213.1	419.17	419.17	419.17	
Practicing a defined diet										
Yes	8 (9.4)	20.11	<LOD	<LOD	<LOD	8.98	80.75	419.17	419.17	0.124
No	77 (90.6)	13.81	<LOD	<LOD	<LOD	<LOD	<LOD	165.14	6586.73	

*[a] – p-value of Mann-Whitney U test (for dichotomous variables) or Kruskal-Wallis test (for variables with more than 2 answer choices) – statistically significant results in **bold**.*
[b] – Statistical analysis has been carried out on a sub-population of pet owners.
LOD – Limit of detection
GM - geometric mean
Min – minimal value
P_{10} – 10th percentile
P_{25} – 25th percentile
P_{75} – 75th percentile
P_{90} – 90th percentile
Max – maximum value

All of the answers received to questions mentioned above (Table 1.) had been computed into variables suitable for numerical analysis.

Due to a significant number of results falling below the limit of quantification, we chose to use the sum of individual metabolite concentrations as a measure of exposure magnitude. This approach is primarily aimed at identifying general and highly significant trends or predictors, rather than relying on chance findings. In selected cases, additional confirmatory analyses were conducted for the more specific metabolites of permethrin and cypermethrin, namely *cis*-DCCA, as previous studies have indicated permethrin and cypermethrin as dominant contributors to the overall exposure. Variables with dichotomic answers had been subjected to Mann-Whitney U test analyses, while variables with more than two possible answer options had undergone statistical analysis by Kruskal-Wallis one way ANOVA. The analyses that turned out to provide a statistically significant comparison result are shown on Fig. 1.

The extensive analyses of relationships between analyte concentrations and potential predictors had yielded some interesting results. The investigation of connection between participants' usage of hand creams during the study and concentrations of synthetic pyrethroids in wristbands had shown a statistically significant linkage between cream employment and sum of pyrethroid WB concentrations (p = 0.0347) (Fig. 1E).

Nine individuals reported using pest control treatments in their indoor living areas within 5 years prior to this study. The group that had used these treatments had higher median concentrations of total urinary pyrethroid metabolites (p=0.0005) (Fig. 1A), median urinary *cis*-DCCA concentration (p=0.0068)

(Fig. 1C.), wristband concentration of permethrin (p=0.0013) (Fig. 1D.), and wristband total pyrethroid concentration (p=0.0122) (Fig. 1B.) compared to the rest of the study population.

Pet ownership was identified as a significant predictor of urinary *cis*-DCCA concentrations (p=0.0222) (Fig. 1F.), as well as past usage of veterinary anti-ectoparasitic products on owned pets in relation to permethrin WB concentration (p=0.0104) (Fig. 1G.).

6. Correlation between results of urinalysis and wristband analysis

Medians of urinary 3-PBA concentrations obtained for each set of three urine samples provided by each participant had shown moderate (Schober and Schwarte 2018) positive correlation (r = 0.4692, p = 0.0276) with detectable wristband permethrin concentrations (n = 25) (Fig. 2.)

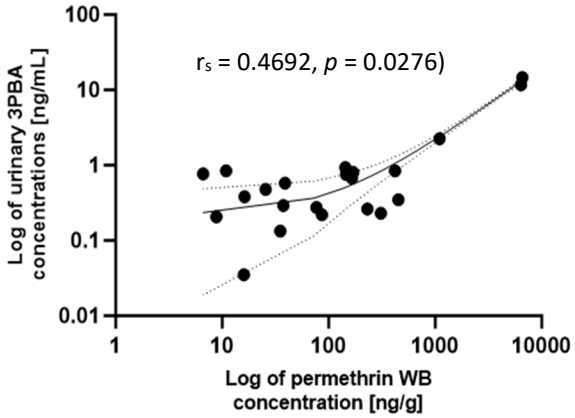


Fig. 2. Medians of urinary 3-PBA concentrations against WB permethrin concentrations (only permethrin positive wristbands were included). Dotted line represents the regression bands.

Additionally, considering the questionnaire responses concerning potential non-dietary ('external') exposure to synthetic pyrethroids, the correlation between the values described above was separately examined in subpopulations of participants who owned a pet, reported using commercially available indoor insecticides, or had conducted pest control treatments in their homes within the 5 years leading up to the study ('externally exposed'). This was contrasted with those who did not report such exposures ('no declared external exposure'). The data analysis revealed a moderate correlation (of greater magnitude than observed in the entire tested population) between results obtained via urinalysis and WB analysis (r = 0.6824, p = 0.0046) for participants who had acknowledged activities potentially increasing their likelihood of contact with pyrethroids. Conversely, it indicated that the analyzed values exhibited a negligible level of correlation (Schober and Schwarte 2018), (r = 0.000, p > 0.999) among those participants who did not (Fig. 3 A-B., respectively).

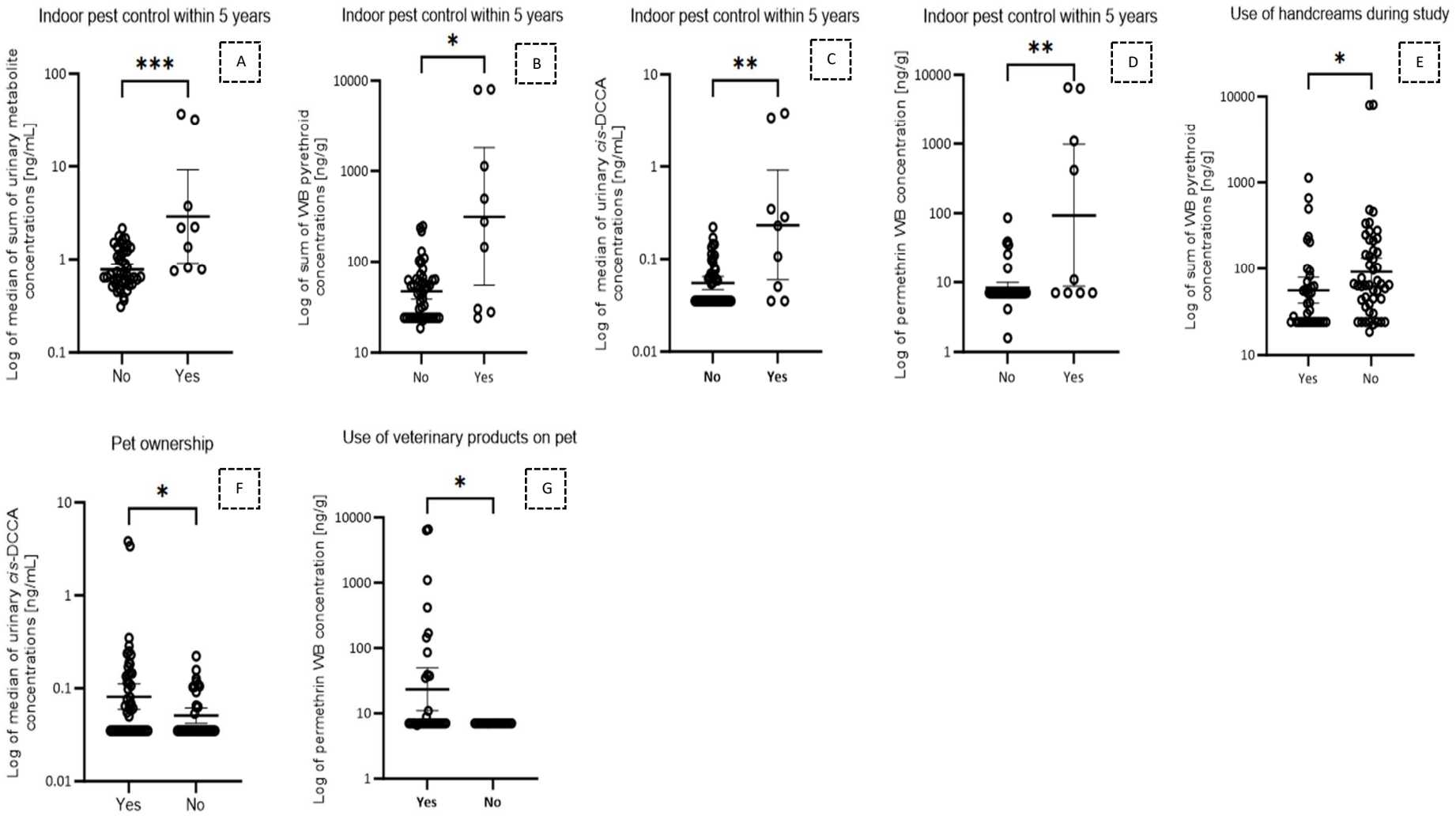


Fig. 1. Major predictors of exposure to pyrethroids (*p*-value threshold 0.05, Mann-Whitney-U test).

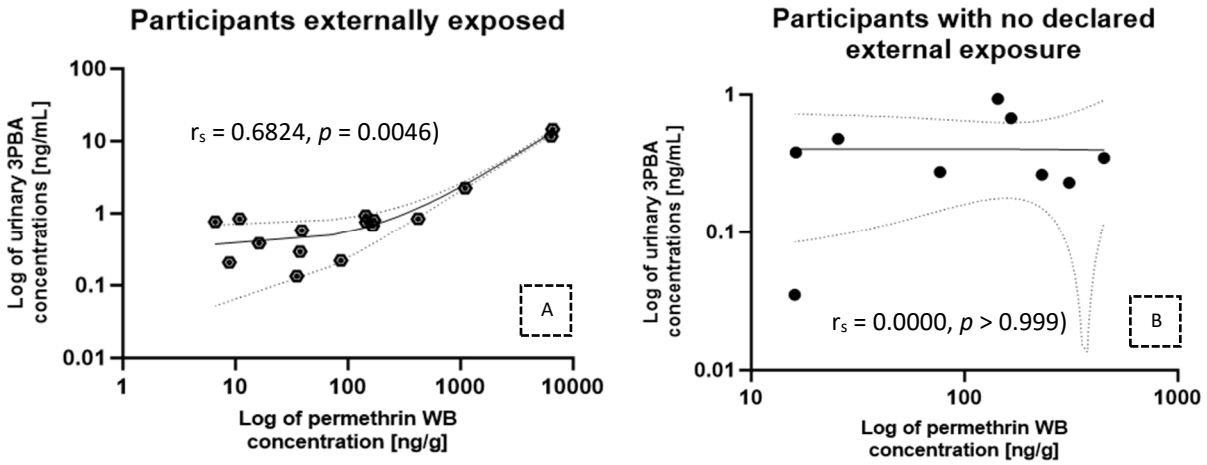


Fig. 3. Correlations between the medians of urinary 3-PBA concentrations and WB permethrin concentrations (with concentrations >LOD) among study participants with a potentially increased predisposition for pyrethroid exposure (n = 16) (A) and study participants who declared no external exposure (n = 9) (B). Dotted lines represent the regression bands.

Additionally, the same relationship has been examined among concentrations obtained from samples collected by study participants who have declared to owning a pet at the time of the study. A strong (Schober and Schwarte 2018) correlation has been noted (r_s = 0.7143, p = 0.0079) (Fig. 4.).

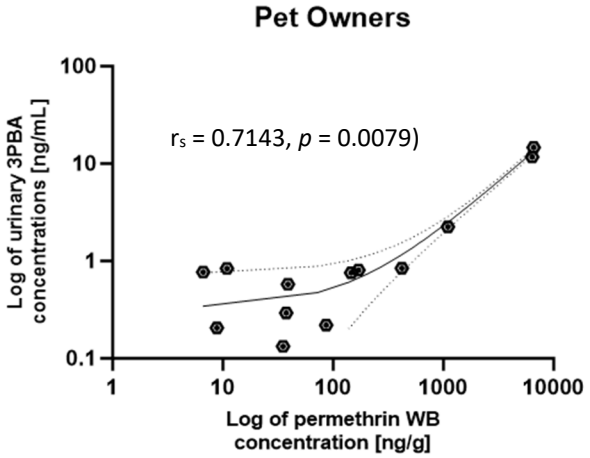


Fig. 4. Correlation between medians of urinary 3-PBA concentrations and WB permethrin concentrations among pet owners (n=14) involved in the study.

7. Discussion

7.1. Comparison to other studies

Over the last ten years several studies have investigated the topic of pyrethroid exposure among varied populations in different locations across the globe. A compact overview of exposure assessments to these substances has been provided in **Table 5.**

Table 5. Comparative overview of several chosen representative studies involving assessment of urinary pyrethroid metabolites represented by 3-PBA performed in Poland and other locations.

	Reference	Population	Det. rate [%]	GM [ng/mL]
Poland	(Wielgomas, Nahorski, and Czarnowski 2013)	Northern PL dwelling	80.0	0.32
	(Wielgomas and Piskunowicz 2013)	Northern PL dwelling	82.4	0.26
	(Radwan et al. 2015)	Males, patients of infertility clinic	-	0.17
	(Jurewicz et al. 2020)	Females, patients of infertility clinic	66.5	0.32
	(Klimowska et al. 2020)	Northern PL dwelling	81.0	0.27
	(Rodzaj et al. 2021)	Males, urban dwelling	69.0	0.22
	This study	**Northern PL dwelling**	**97.9**	**0.32**
Other	(Hu et al. 2020)	Males	100.0	-
	(Health Canada 2015)	General population	100.0	0.53
	(Li et al. 2022)	School-aged children	99.3	1.30
	(Šulc et al. 2022)	Parent-child pairs	51.8	0.16

The pyrethroid metabolite with the highest detection rates across the great majority of manuscripts assessing exposure to synthetic pyrethroids is 3-PBA, the least specific of all urinary metabolites. Its detection rates consistently surpassing 50% of tested samples allows for its concentration to be a fair comparative value representing the generality of exposure. In our study, the detection rate of 3-PBA is notably higher than in other recent studies conducted in Poland and comparable to those carried out

abroad (see Table 5). The geometric mean of 3-PBA concentrations in the population tested in this study is very similar to those reported in previous studies conducted in Poland, which were 0.32 ng/mL (Wielgomas, Nahorski, and Czarnowski 2013; Jurewicz et al. 2020), among a geographically uniform population and a group of female patients at an infertility clinic, respectively. An advantage of this study is the collection of three urine samples from each participant at different time points during the same week. This approach allows for a more time-weighted assessment of exposure to synthetic pyrethroids compared to a single analysis of a spot urine sample, which provides only a snapshot of exposure due to rapid pyrethroid metabolism (Calafat et al. 2016). In contrast, a much higher geometric mean of 3-PBA concentrations (1.30 ng/mL) was observed in a population of New Zealand school-aged children (Li et al. 2022). This discrepancy can be attributed to children's natural tendency to transfer contaminants from their hands or fingers directly to their mouths. The differences in 3-PBA levels between the study involving school-aged children (Li et al. 2022) and the one described here are likely multifactorial but can be partly attributed to the limited inclusion of children in our study, as only two participants were below 18 years of age. This limitation should be considered when evaluating the overall exposure of the population.

While there is limited literature on the use of silicone wristbands for exposure assessment in general, there are even fewer studies that involve the quantification of synthetic pyrethroids using this sampling method. The pyrethroid detection rates in our study are somewhat consistent with the results of other population-based studies. Cypermethrin, as in the study by Harley et al. (Harley et al. 2020) was the most frequently quantified native pyrethroid among the study participants (Table 3). The detection rate of cypermethrin (59.3%) surpassing that of permethrin (29.1%) was somewhat unexpected, as permethrin is the most commonly used pyrethroid insecticide in commercially available products, and it typically dominates in detection in most wristband-based studies (Arcury et al. 2021; Wise et al. 2020; Doherty et al. 2020). In contrast, other studies have reported deltamethrin as the most frequently detected pyrethroid (Donald et al. 2016), while the detection rate of that compound in our study was 30.2 %.

In some instances, comparing the pyrethroid concentrations obtained between studies has proven to be challenging. This is because not all previously referenced papers reported population means or medians for these concentrations, and some studies calculated *cis*- and *trans*-permethrin levels separately, while our study investigated the combined sum of both isomers. For example, the average permethrin concentration among adolescent farmworkers (Harley et al. 2020) was much higher (154 ng/g) than the value observed in our study (GM = 14 ng/g). It's important to note that each of the referenced studies focused on very specific populations for exposure assessment, such as child farmworkers (Arcury et al. 2021), pet owners (Wise et al. 2020), pregnant women (Doherty et al. 2020), farmworker community adolescent girls (Harley et al. 2020), farming individuals (Donald et al. 2016). These unique population characteristics could explain the discrepancies in the detection and quantified levels of synthetic pyrethroids between these studies and the one described here.

7.2 Potential exposure predictors

Analyzing questionnaire-derived data with concentrations of analyzed substances has offered an opportunity to investigate which of the daily habits/ life characteristics the participants have been asked about might be predictors of exposure to synthetic pyrethroids. As described earlier in the Results section, our data analysis has led to some interesting findings.

The performance of indoor pest control treatments in residential areas within the five years preceding the study was identified as a significant predictor of wristband permethrin concentration, *cis*-DCCA urinary concentrations, as well as the total exposures measured by the sum of medians of urinary

metabolite concentrations and the sum of pyrethroid WB concentrations. On the other hand, the indoor usage of insecticidal products was not confirmed to be a significant predictor of exposure among the studied population, which contradicts the findings of previous studies (Rodzaj et al. 2021). However, it is essential to keep in mind that not all the pesticide-based products used in residential settings contain pyrethroids. They could just as well be neonicotinoids or repellents, such as DEET.

The use of hand creams during the study period was revealed to be a significant predictor of lower wristband pyrethroid concentration. Although we conducted further investigations, including an examination of the specific products used by participants as declared in the questionnaire, we currently do not have a clear explanation for this observation.

Pet ownership has turned out to be a statistically significant factor in relation to urinary concentrations of *cis*-DCCA, which is somewhat in accordance with other similar studies, as Rodzaj et al. (Rodzaj et al. 2021) has reported 'ownership of at least one dog' to be statistically significant in relation to urinary *trans*-DCCA and 3-PBA concentrations, but the study simultaneously did not report statistical significance between those values and pet ownership directly. Pet ownership, however, should not be understood as the primary exposure predictor, as exposure to pyrethroids in that group most likely stems from usage of veterinary products on said pets.

Similarly, usage of veterinary insecticides on said pets among the sub-population of pet owners in our study has been significantly linked to elevated concentrations of permethrin quantified on participants wristbands, and while it did not find support in statistical analysis of urinary metabolite concentrations, it should be considered a risk factor for exposure to pyrethroid insecticides based on our results. With reference to that fact, elevated 3-PBA levels among veterinary products-using pet owners had also been recorded in a study on rural and urban populations of northern Poland (Wielgomas and Piskunowicz 2013), and in a recent study among young urban-dwelling men (Rodzaj et al. 2021), where a relationship between urinary *trans*-DCCA concentrations and use of veterinary insecticides was found, thus further reinforcing our finding.

Data analysis of our study did not report participants gender or age to be statistically significant factors in regards to exposure to synthetic pyrethroids, and results of numerous similar analyses in other studies (Morgan et al. 2016; Rodzaj et al. 2021; Li et al. 2022), are in accordance with our findings.

Type of terrain surrounding the inhabited area whether it was urban or rural, did not emerge as a significant predictor of exposure in our study. While some studies (Wielgomas and Piskunowicz 2013) did not find a correlation between urinary 3-PBA concentrations in adults living in either rural or urban environments, statistically significant relationships were observed when investigating children and their parents with respect to this variable.

Several other variables that did not appear to be significant predictors of exposure in our study include dominant hand, the number of owned pets, duration of living in current location (at the time of the study), the presence of animals in the workplace, the use of insecticides in the workplace, and the place of residence (Table 1).

Interestingly, following a defined diet in this study was found to be a significant predictor of exposure only in relation to urinary DBCA concentrations (p = 0.0075, Mann-Whitney-U test). It's important to note that only a small number of participants followed a defined diet (n = 8), and the specifics of these diets varied among participants, including vegetarian, vegan, gluten-free, and dairy-free diets. As a result, the significance of this finding should be considered limited overall. However, deltamethrin was found in 86% of duplicate diet samples in the study performed on 35 healthy consumers from the

region of Wageningen, Netherlands (Nijssen et al. 2023). Dietary deltamethrin correlated well with urinary DBCA concentration in this population.

7.3 Correlation between results of urinalysis and WB analysis

To the best of our knowledge, this is the first cross-population study in Europe to combine silicone wristbands and human biomonitoring for assessing exposure to synthetic pyrethroids.

Examination of correlation between urinary pyrethroid metabolite concentrations and results obtained via analysis of silicone wristbands had shown some compelling results. While the correlation coefficient (Spearman's correlation) between WB permethrin concentrations [ng/g] and medians of urinary 3-PBA concentrations (calculated for a set of 3 urine samples collected by each of the participants, unless excluded due to urine dilution rate being out of accepted range) among all participants involved in the study had shown moderate degree of correlation between said values ($r = 0.4692$). The much higher values of correlation coefficients obtained in a corresponding analysis in isolated sub-populations of pet owners and 'externally exposed' participants ($r = 0.7143$ and 0.6824, respectively) provide strong evidence of silicone wristbands being able to capture and elucidate non-dietary exposure to synthetic pyrethroids, which is further reinforced by the values of *cis*-DCCA and permethrin concentrations being uncorrelated ($r = 0.000$) among people with no suspicion of being exposed to synthetic pyrethroids by owning a pet, using commercially available insecticides indoors or performing pest-control treatments in their homes within 5 years' time prior to study. This finding also shows that even though dietary source of pyrethroids is believed to be the leading cause of exposure to those compounds (Lehmler et al. 2020), the impact of non-dietary exposure might be of considerable significance even among non-occupationally exposed individuals. Results obtained in this study decidedly prove that employment of silicone wristbands in exposure assessment studies to synthetic pyrethroids might aid the process of specification of routes of exposure to those compounds, as well as in estimating the contribution of non-dietary vs. dietary routes exposure in the total exposure to pyrethroids.

8. Study limitations

The authors acknowledge that one of the study limitations is lack of questionnaire-provided information regarding the surface of the living area of the home occupied by study participants, as, if known, it would provide an additional interesting dimension to currently obtained result and would possibly allow for more insightful conclusions to be drawn from this study.

9. Conclusions

Based on the results presented in this study, we conclude that the strong predictors of exposure to synthetic pyrethroids in the studied population are primarily the use of veterinary anti-parasitic drugs on domestic animals and the indoor use of products containing these ingredients for insect control. This study has highlighted silicone wristbands as a useful, minimally invasive tool for exposure assessment. Our findings demonstrate that wristbands can support human biomonitoring both qualitatively and quantitatively. Thanks to the wristbands, it is possible to identify the parent compound responsible for exposure, which cannot be determined solely based on the presence of less specific metabolites in urine. Additionally, wristbands are likely to selectively assess exposure to pyrethroids from non-dietary sources.

10. Acknowledgments

Małgorzata Wacławik appreciates the scholarship received from the project POWR.03.02.00-00-I014/17-00 co-financed by the European Union through the European Social Fund under the Operational Program Knowledge Education Development 2014–2020.

Furthermore, the authors of this manuscript give their sincere thanks to the volunteers, for their willingness to selflessly endure all the inconveniences related to participation in the study, as well as exemplary cooperation throughout it.

11. References

Arcury, Thomas A, Sara A Quandt, Jennifer W Talton Ms, Winston Salem, North Carolina, Haiying Chen, Taylor J Arnold Ma, Kim A Anderson, Richard P Scott, and Stephanie S Daniel. 2021. "Pesticide Exposure among Latinx Child Farmworkers in North Carolina," no. February: 602–19. https://doi.org/10.1002/ajim.23258.

Baum, Jeramy L R, Umer Bakali, Chitvan Killawala, Katerina M Santiago, Emre Dikici, Erin N Kobetz, Natasha Schaefer Solle, Sapna Deo, Leonidas Bachas, and Sylvia Daunert. 2020. "Evaluation of Silicone-Based Wristbands as Passive Sampling Systems Using PAHs as an Exposure Proxy for Carcinogen Monitoring in Firefighters: Evidence from the Firefighter Cancer Initiative." *Ecotoxicology and Environmental Safety* 205 (December): 111100. https://doi.org/10.1016/j.ecoenv.2020.111100.

Bergmann, Alan J., Paula E. North, Luis Vasquez, Hernan Bello, Maria del Carmen Gastañaga Ruiz, and Kim A. Anderson. 2017. "Multi-Class Chemical Exposure in Rural Peru Using Silicone Wristbands." *Journal of Exposure Science and Environmental Epidemiology* 27 (6): 560–68. https://doi.org/10.1038/jes.2017.12.

Bradberry, Sally M, Sarah A Cage, Alex T Proudfoot, and J Allister Vale. 2005. "Poisoning Due to Pyrethroids." *Toxicological Reviews* 24 (2): 93–106. https://doi.org/10.2165/00139709-200524020-00003.

Calafat, Antonia M., Matthew P. Longnecker, Holger M. Koch, Shanna H. Swan, Russ Hauser, Lynn R. Goldman, Bruce P. Lanphear, et al. 2016. "Response to 'Comment on "Optimal Exposure Biomarkers for Nonpersistent Chemicals in Environmental Epidemiology."'" *Environmental Health Perspectives*. https://doi.org/10.1289/ehp.1611282.

Cone, Edward J., Yale H. Caplan, Frank Moser, Tim Robert, Melinda K. Shelby, and David L. Black. 2009. "Normalization of Urinary Drug Concentrations with Specific Gravity and Creatinine." *Journal of Analytical Toxicology* 33 (1): 1–7. https://doi.org/10.1093/jat/33.1.1.

Doherty, Brett T., John L. Pearce, Kim A. Anderson, Margaret R. Karagas, and Megan E. Romano. 2020. "Assessment of Multipollutant Exposures During Pregnancy Using Silicone Wristbands." *Frontiers in Public Health* 8 (September): 1–13. https://doi.org/10.3389/fpubh.2020.547239.

Donald, Carey E., Richard P. Scott, Kathy L. Blaustein, Mary L. Halbleib, Makhfousse Sarr, Paul C. Jepson, and Kim A. Anderson. 2016. "Silicone Wristbands Detect Individuals' Pesticide Exposures in West Africa." *Royal Society Open Science* 3 (8).

https://doi.org/10.1098/rsos.160433.

Guessan, Raphael N, Corine Ngufor, Andreas A Kudom, Pelagie Boko, Abibathou Odjo, David Malone, and Mark Rowland. 2014. “Mosquito Nets Treated with a Mixture of Chlorfenapyr and Alphacypermethrin Control Pyrethroid Resistant Anopheles Gambiae and Culex Quinquefasciatus Mosquitoes in West Africa” 9 (2): 1–6. https://doi.org/10.1371/journal.pone.0087710.

Hanke, Wojciech, Paul Romitti, Laurence Fuortes, Wojciech Sobala, and Marek Mikulski. 2003. “The Use of Pesticides in a Polish Rural Population and Its Effect on Birth Weight.” *International Archives of Occupational and Environmental Health* 76 (8): 614–20. https://doi.org/10.1007/s00420-003-0471-4.

Harley, Kim G, Kimberly L Parra, Jose Camacho, Asa Bradman, James E S Nolan, Chloe Lessard, Kim A Anderson, et al. 2020. “Community : The COSECHA Youth Participatory Action Study,” 1022–29. https://doi.org/10.1016/j.scitotenv.2018.10.276.Determinants.

Health Canada. 2015. “Fifth Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measurest Survey Cycle 5 (2016-2017).” 2015. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/environmental-contaminants/human-biomonitoring-environmental-chemicals.html?utm_campaign=not-applicable&utm_medium=vanity-url&utm_source=canada-ca_biomonitoring.

Hornung, Richard W, and Laurence D Reed. 1990. “Estimation of Average Concentration in the Presence of Nondetectable Values.” *Applied Occupational and Environmental Hygiene* 5 (1): 46–51. https://doi.org/10.1080/1047322X.1990.10389587.

Hu, Yi, Yan Zhang, Angela Vinturache, Yiwen Wang, Rong Shi, Limie Chen, Kaili Qin, Ying Tian, and Yu Gao. 2020. “Effects of Environmental Pyrethroids Exposure on Semen Quality in Reproductive-Age Men in Shanghai, China.” *Chemosphere* 245. https://doi.org/10.1016/j.chemosphere.2019.125580.

Jurewicz, Joanna, Paweł Radwan, Bartosz Wielgomas, Michał Radwan, Anetta Karwacka, Paweł Kałużny, Marta Piskunowicz, Emila Dziewirska, and Wojciech Hanke. 2020. “Exposure to Pyrethroid Pesticides and Ovarian Reserve.” *Environment International* 144 (August): 0–4. https://doi.org/10.1016/j.envint.2020.106028.

Katsuda, Yoshio. 2011. “Progress and Future of Pyrethroids.” In *Topics in Current Chemistry*, 314:1–30. https://doi.org/10.1007/128_2011_252.

Klimowska, Anna, Katarzyna Amenda, Wojciech Rodzaj, Malwina Wileńska, Joanna Jurewicz, and Bartosz Wielgomas. 2020. “Evaluation of 1-Year Urinary Excretion of Eight Metabolites of Synthetic Pyrethroids, Chlorpyrifos, and Neonicotinoids.” *Environment International* 145. https://doi.org/10.1016/j.envint.2020.106119.

Lehmler, Hans-joachim, Derek Simonsen, Buyun Liu, and Wei Bao. 2020. “Environmental Exposure to Pyrethroid Pesticides in a Nationally Representative Sample of U.S. Adults and Children: The National Health and Nutrition Examination Survey 2007–2012.” *Environmental Pollution* 267 (December): 115489. https://doi.org/10.1016/j.envpol.2020.115489.

Li, Yan, Xianyu Wang, Jean Feary McKenzie, Andrea ’t Mannetje, Soo Cheng, Chang He, Janet Leathem, et al. 2022. “Pesticide Exposure in New Zealand School-Aged Children: Urinary Concentrations of Biomarkers and Assessment of Determinants.” *Environment International* 163 (April): 107206. https://doi.org/10.1016/j.envint.2022.107206.

Marettova, Elena, Milan Maretta, and Jaroslav Legáth. 2017. “Effect of Pyrethroids on Female Genital System. Review.” *Animal Reproduction Science* 184 (March): 132–38. https://doi.org/10.1016/j.anireprosci.2017.07.007.

Morgan, Marsha, Paul Jones, Jon Sobus, and Dana Boyd Barr. 2016. “Predictors of Urinary 3-Phenoxybenzoic Acid Levels in 50 North Carolina Adults.” *International Journal of Environmental Research and Public Health* 13 (11). https://doi.org/10.3390/ijerph13111172.

Nijssen, R, A Lommen, H van den Top, R van Dam, C Meuleman-Bot, M Tienstra, P Zomer, et al. 2023. “Assessment of Exposure to Pesticides: Residues in 24 h Duplicate Diets versus Their Metabolites in 24 h Urine Using Suspect Screening and Target Analysis.” *Analytical and Bioanalytical Chemistry*, September. https://doi.org/10.1007/s00216-023-04918-x.

Radwan, Michał, Joanna Jurewicz, Bartosz Wielgomas, Marta Piskunowicz, Wojciech Sobala, Paweł Radwan, Lucjusz Jakubowski, Wanda Hawuła, and Wojciech Hanke. 2015. “The Association between Environmental Exposure to Pyrethroids and Sperm Aneuploidy.” *Chemosphere* 128: 42–48. https://doi.org/10.1016/j.chemosphere.2014.12.077.

Richardson, Jason R., Michele M. Taylor, Stuart L. Shalat, Thomas S. Guillot, W. Michael Caudle, Muhammad M. Hossain, Tiffany A. Mathews, Sara R. Jones, Deborah A. Cory-Slechta, and Gary W. Miller. 2015. “Developmental Pesticide Exposure Reproduces Features of Attention Deficit Hyperactivity Disorder.” *FASEB Journal* 29 (5): 1960–72. https://doi.org/10.1096/fj.14-260901.

Rodzaj, Wojciech, Malwina Wileńska, Anna Klimowska, Emila Dziewirska, Joanna Jurewicz, Renata Walczak-Jędrzejowska, Jolanta Słowikowska-Hilczer, Wojciech Hanke, and Bartosz Wielgomas. 2021. “Concentrations of Urinary Biomarkers and Predictors of Exposure to Pyrethroid Insecticides in Young, Polish, Urban-Dwelling Men.” *Science of The Total Environment* 773 (June): 145666. https://doi.org/10.1016/j.scitotenv.2021.145666.

Schober, Patrick, and Lothar A. Schwarte. 2018. “Correlation Coefficients: Appropriate Use and Interpretation.” *Anesthesia and Analgesia* 126 (5): 1763–68. https://doi.org/10.1213/ANE.0000000000002864.

Simerville, Jeff A., William C. Maxted, and John J. Pahira. 2005. “Urinalysis: A Comprehensive Review.” *American Family Physician* 71 (6): 1153–62.

Šulc, Libor, Tomáš Janoš, Daniel Figueiredo, Ilse Ottenbros, Petr Šenk, Ondřej Mikeš, Anke Huss, and Pavel Čupr. 2022. “Pesticide Exposure among Czech Adults and Children from the CELSPAC-SPECIMEn Cohort: Urinary Biomarker Levels and Associated Health Risks.” *Environmental Research* 214 (May). https://doi.org/10.1016/j.envres.2022.114002.

Wacławik, Małgorzata, Wojciech Rodzaj, and Bartosz Wielgomas. 2022. “Silicone Wristbands in Exposure Assessment: Analytical Considerations and Comparison with Other Approaches [Dokument Elektroniczny].” *Int. J. Environ. Res. Public Health* 19 (nr 4, art. ID 1935): s. 1-28. https://doi.org/10.3390/ijerph19041935.

Wielgomas, Bartosz, Wacław Nahorski, and Wojciech Czarnowski. 2013. “Urinary Concentrations of Pyrethroid Metabolites in the Convenience Sample of an Urban Population of Northern Poland.” *International Journal of Hygiene and Environmental Health* 216 (3): 295–300. https://doi.org/10.1016/j.ijheh.2012.09.001.

Wielgomas, Bartosz, and Marta Piskunowicz. 2013. “Biomonitoring of Pyrethroid Exposure among Rural and Urban Populations in Northern Poland.” *Chemosphere* 93 (10): 2547–53. https://doi.org/10.1016/j.chemosphere.2013.09.070.

Wise, Catherine F, Stephanie C Hammel, Nicholas Herkert, Jun Ma, Alison Motsinger-Reif, Heather M

Stapleton, and Matthew Breen. 2020. “Comparative Exposure Assessment Using Silicone Passive Samplers Indicates That Domestic Dogs Are Sentinels To Support Human Health Research.” *Environmental Science & Technology* 54 (12): 7409–19. https://doi.org/10.1021/acs.est.9b06605.

Zhu, Qiuyan, Yang Yang, Zhiting Lao, Yingying Zhong, Kun Zhang, and Suqing Zhao. 2020. “Photodegradation Kinetics, Mechanism and Aquatic Toxicity of Deltamethrin, Permethrin and Dihaloacetylated Heterocyclic Pyrethroids.” *Science of the Total Environment* 749: 142106. https://doi.org/10.1016/j.scitotenv.2020.142106.

Comprehensive assessment of exposure to synthetic pyrethroids among inhabitants of Northern Poland via urinalysis supplemented by passive sampling with the use of silicone wristbands. – Supplementary Materials

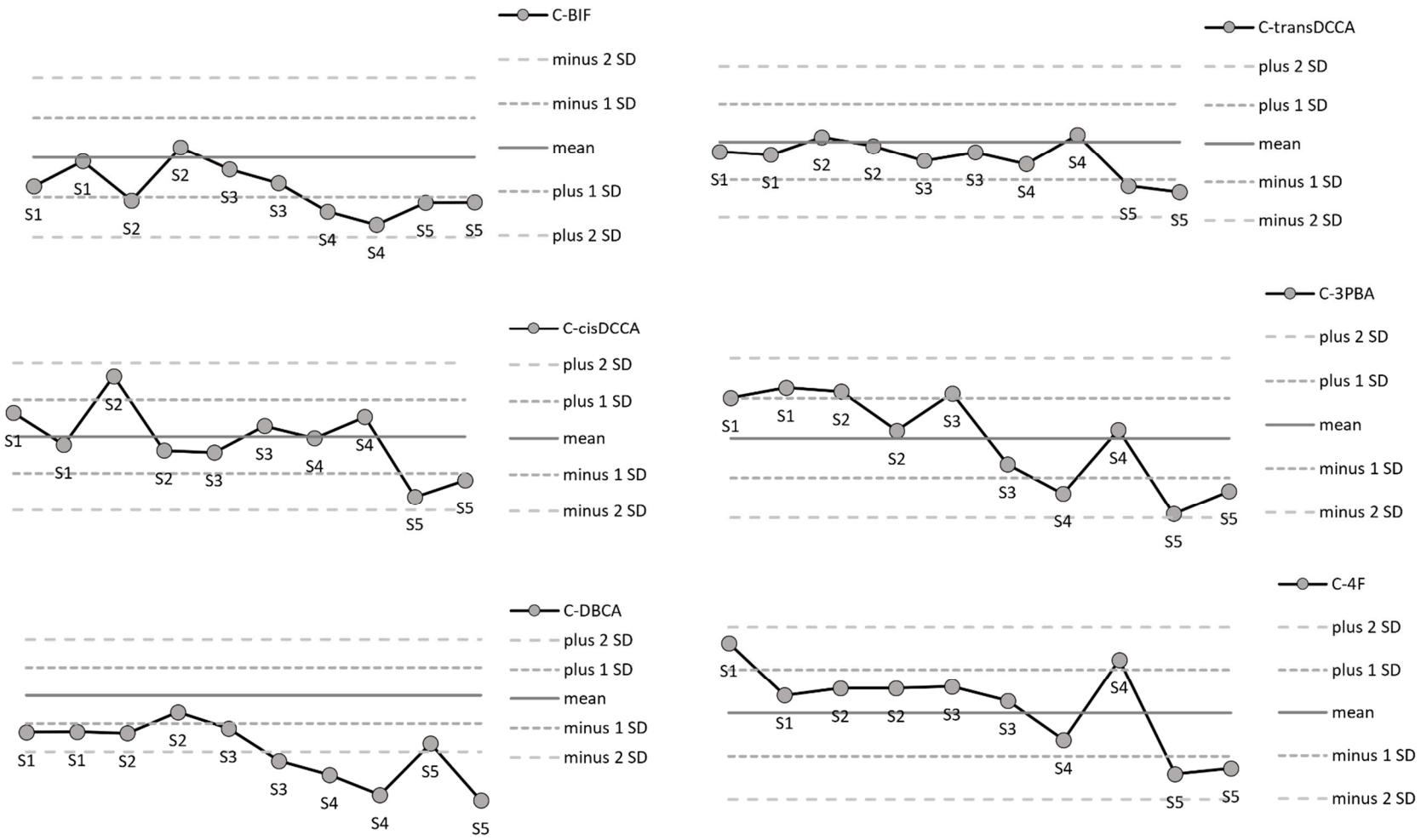
Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas,

Department of Toxicology, Faculty of Pharmacy, Medical University of Gdańsk, 107 Hallera Street, 80-416 Gdańsk, Poland

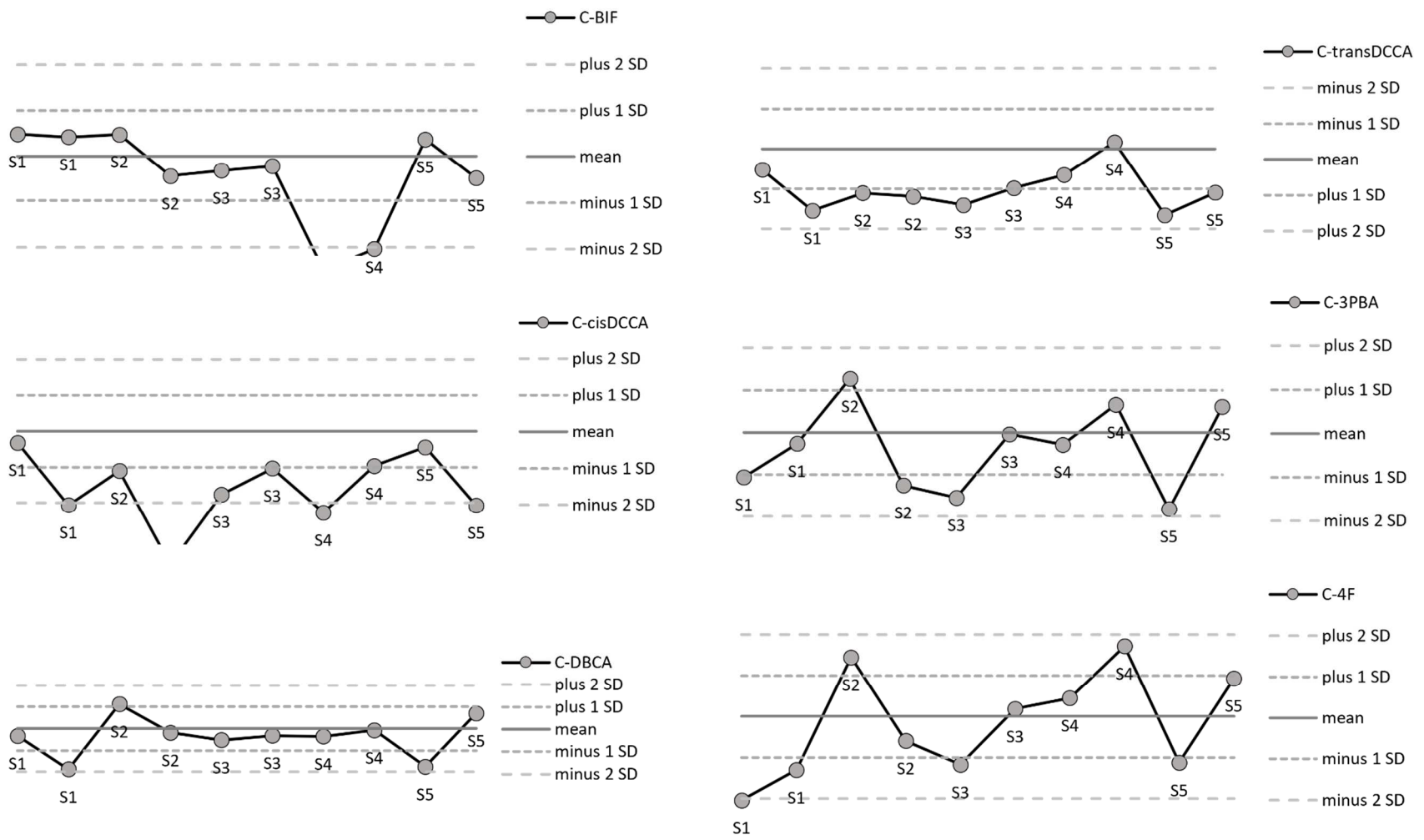
*corresponding author: bartosz.wielgomas@gumed.edu.pl

1. Quality control

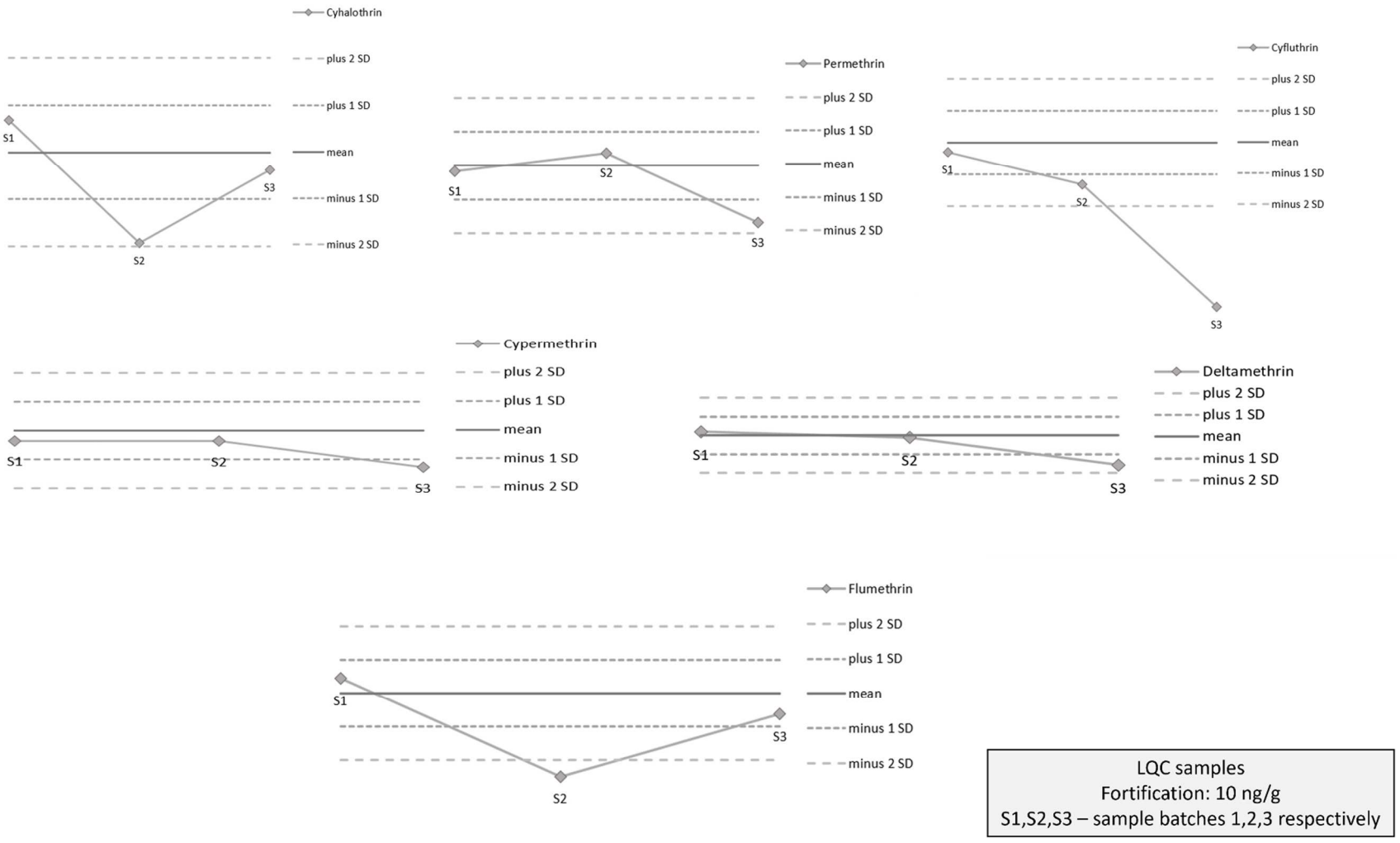
As briefly described in section: 'Quality control' of the manuscript, control samples at two levels of concentration have been added in two repetitions to each analyzed batch of samples. Concentrations of control samples for urinalysis had been: 1.5 ng/mL (HQC) and 0.25 ng/mL (LQC). In wristband analysis, the concentrations of spiked control samples had been 10 ng/g and 50 ng/g in LQC and HQC samples, respectively. The Westgard's rule of excluding the analyzed sample batch employed in the study was: $\mathbf{2_{2s}}$ in quality control of urine analysis, with an additional criteria being, that the occurrence must be noted for at least two analytes in the same batch simultaneously. For wristband analysis, a criteria of exclusion set was: $\mathbf{1_{2s}}$, again, occurring for at least two analytes simultaneously. The control charts formulated during urinalysis can be found on figures **1** (LQCs) and **2** (HQCs), and results of wristband analysis on figures **3** (LQCs) and **4** (LQCs).



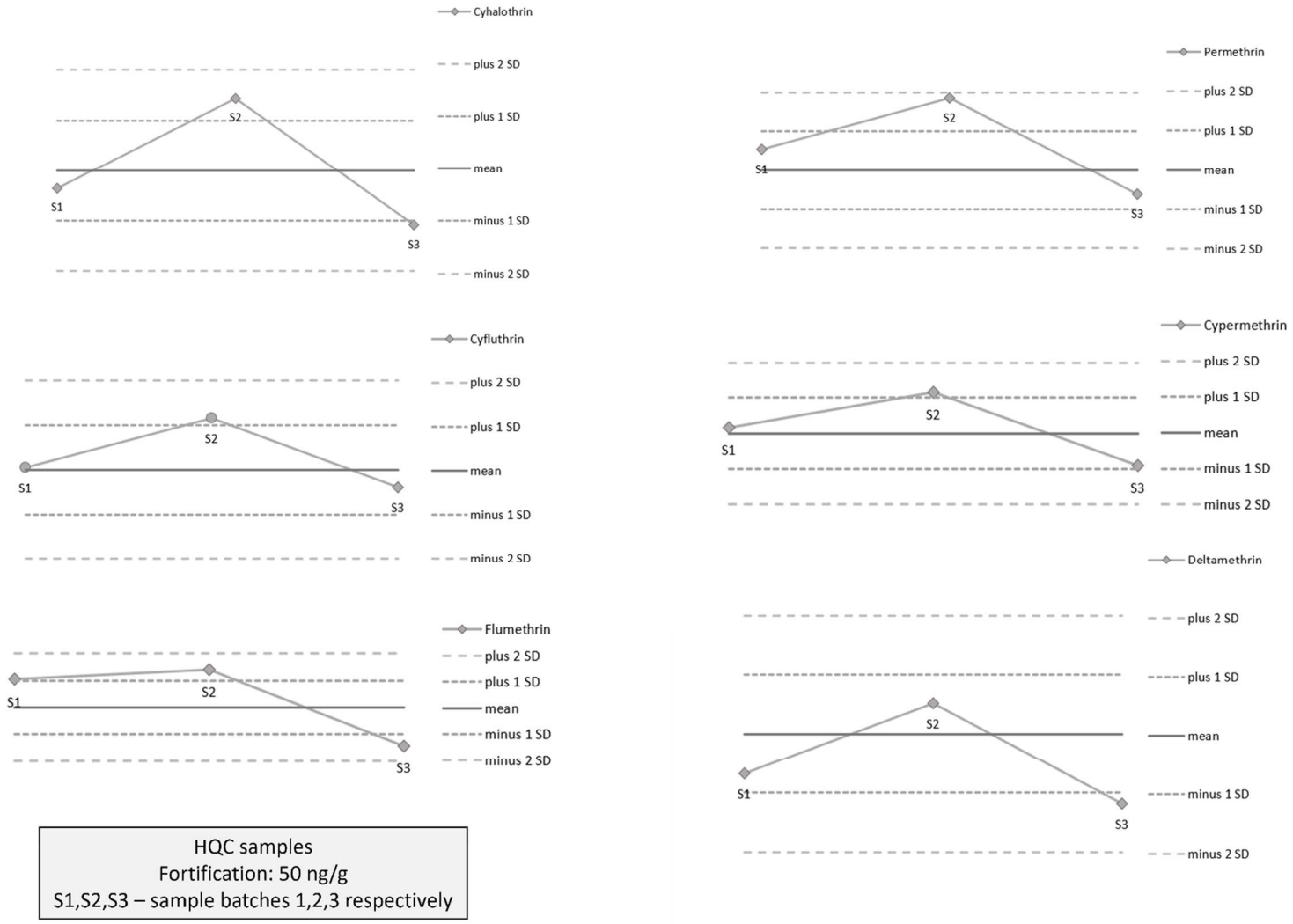
SM – Fig. 1. Urinary pyrethroid metabolites quality control charts: S1, S2, S3, S4, S5 – quality control samples for sample batch No.: 1,2,3,4,5, respectively (2 per sample batch). LQC samples.



SM – Fig. 2. Urinary pyrethroid metabolites quality control charts: S1, S2, S3, S4, S5 – quality control samples for sample batch No.: 1,2,3,4,5, respectively (2 per sample batch). LQC samples.



SM – Fig. 3. Quality control charts for assessment of pyrethroids in silicone wristbands (LQC samples).



SM – Fig. 4. Quality control charts for assessment of pyrethroids in silicone wristbands (HQC samples).

MSc Małgorzata Wacławik
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Comprehensive assessment of exposure to synthetic pyrethroids among inhabitants of Northern Poland via urinalysis supplemented by passive sampling with the use of silicone wristbands"* (working title).

Which is part of my doctoral dissertation, my participation in its creation involved performing literature review, laboratory research, data analysis and preparation of the original manuscript.

My contribution in preparation of this work has been estimated to sum up to 65%.

Wacławik
(signature)

MSc Dominika Skwarło
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> *Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas: „Comprehensive assessment of exposure to synthetic pyrethroids among inhabitants of Northern Poland via urinalysis supplemented by passive sampling with the use of silicone wristbands"* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved assistance in sample collection and analysis.

My contribution in preparation of this work has been estimated to sum up to 15%.

Skwarło Dominika
(signature)

Bartosz Wielgomas, PhD, Assoc. Prof.
Department of Toxicology
Medical University of Gdańsk
Al. Gen. J. Hallera 107, 80-416 Gdańsk

Gdańsk, 11.09.2023

Statement

I hereby declare that as the co-author of the following work:

> Małgorzata Wacławik, Dominika Skwarło, Bartosz Wielgomas. *„Comprehensive assessment of exposure to synthetic pyrethroids among inhabitants of Northern Poland via urinalysis supplemented by passive sampling with the use of silicone wristbands."* (working title).

Which is part of doctoral dissertation of MSc Małgorzata Wacławik, my participation in its creation involved research conceptualization, reviewing and editing of the original manuscript, as well as research supervision.

My contribution in preparation of this work has been estimated to sum up to 20%.

.....
(signature)