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Pyrethroids in private homes
Accumulation and human exposure

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1. Preface and acknowledgements

The present investigation,"Pyretroids in private homes" was a survey elucidating the exposure of inhabitants when their houses or flats were treated with synthetic pyrethroids against bed bugs.
The project was financed by the Danish Environmental Protection Agency (Miljøstyrelsen) and was carried out in the period April 2015 to November 2019 in a collaboration between Department of Agroecology, Aarhus University (Karl-Martin Vagn Jensen, Inge S. Fomsgaard, Kirsten Heinrichson), Cimex consult (Ole Kilpinen, former senior scientist Aarhus University) and Environmental Medicine, Department of Public Health, University of Southern Denmark (Helle Raun Andersen).
The project was coordinated by Karl-Martin Vagn Jensen until his retirement June 2017, after which Ole Kilpinen took over the role as coordinator.

The project would like to acknowledge all the anonymous hosts who, for a couple of tickets to the cinema, gave us the opportunity to collect samples in their houses as well as providing the project with blood and urine samples. For gentle and careful taking of blood samples we would like to thank biotechnicians Irene B. Hansen. We also thank the companies Rentokil Initial A/S, Glostrup and Mortalin A/S, Haslev, who coordinated their insect control in private homes with us. Without their involvement, the project could not have been completed.
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The advisory group and the Danish Environmental Protection Agency have had no influence on the presentation and interpretation of the results and the report's conclusion.

2. Sammenfatning

I Danmark bruges for nærværende udelukkende bekæmpelsesmidler fra gruppen af syntetiske pyrethroider til bekæmpelse af væggelus. De syntetiske pyrethroider er stabile forbindelser, hvorfor relativ langsom nedbrydning må forventes under normale forhold. Samtidig er der i mange lande konstateret nedsat følsomhed for pyrethroider blandt væggelus. Det betyder, at der ofte må behandles gentagne gange, hvilket fører til risikoen for akkumulering og øget eksponering af beboerne.

Denne undersøgelse havde til mål at dokumentere, hvorvidt der i praksis er risiko for akkumulering af pyrethroider i forbindelse med bekæmpelse af væggelus og samtidig belyse i hvor høj grad beboerne bliver eksponeret for pyrethroider.

Undersøgelsen bestod af to dele. En horisontal undersøgelse med et antal lokaliteter hvor der blev behandlet en eller flere gange inden for de foregående 6 måneder, dog ikke den sidste måned. Der blev indsamlet støvprøver ved hjælp af støvsuger og eksponeringsprøver ved, at gå rundt i et behandlet lokale i 10 minutter med en ren bomuldssok på den ene fod. Samtidig blev der indsamlet urinprøver fra beboerne og disse blev analyseret for pyrethroid metabolitter. Alle medvirkende blev udspurgt om deres vaner, samt hvilken information de havde modtaget fra skadedyrsbekæmperne. Resultaterne viste ret store forskelle på, hvordan skadedyrsbekæmperne informerer om hvordan beboerne skal forholde sig for at undgå eksponering. Den typiske forholdsregel var, at man skal undgå at gå ind i de behandlede rum i ½-2 timer efter behandlingen. Ellers er der ikke væsentlige forholdsregler og som regel ingen instruktioner om, hvordan man til slut fjerner behandlingen igen.

Generelt blev der fundet klart mest pyrethroid i støvprøver. Mængderne varierede naturligvis meget, da det også var meget variabelt, hvor meget der havde været behandlet (fra 1-10 behandlinger indenfor det seneste år). I nogle tilfælde blev der fundet et meget stort indhold af pyrethroid og ofte flere forskellige aktivstoffer i samme prøve. Det mest ekstreme tilfælde var 7,5 mg deltamethrin + 3 mg lambda-cyhalothrin + 46,5 mg permethrin i støvprøven fra støvsugning af ét behandlet lokale. Det viste sig således, i et bioassay med insekticid følsomme fluer, at helt op til 189 dage efter en behandling døde disse, når de blev eksponeret på gulvet. Det var ikke muligt at se nogen tydelig sammenhæng mellem den mængde af pyrethroid der findes i omgivelserne og den mængde pyrethroid metabolit der findes i urinprøverne. Dette tolkes derhen, at beboernes adfærd har meget stor indflydelse på, i hvor høj grad de eksponeres for pyrethroid. Selvom der er meget pyrethroid i omgivelserne, bliver man ikke nødvendigvis eksponeret for den, hvis man f.eks. ikke er særlig aktiv i hjemmet. Det er også meget sandsynligt, at pyrethroidet er klumpet fordelt, da man ofte kun sprøjter de steder der erfaringsmæssigt er relevante. Det betyder at der i visse områder er meget og andre områder kun lidt aktivt stof.

Undersøgelsens anden del var en longitudinal undersøgelse, hvor et antal beboere blev fulgt i en periode, hvor der blev behandlet mod væggelus. De samme typer prøver som første del blev indsamlet, men herudover blev der også udtaget blodprøver. Igen var der ret stor variation i hvor meget pyrethroid der kunne genfindes og der var ikke nogen tydelig sammenhæng mellem mængden af pyrethroid og tiden siden sidste behandling. I de fleste tilfælde var niveauerne stadig høje på den sidste indsamlingsdag. Det vurderes derfor som meget sandsynligt, at der kan ske akkumulering af pyrethroid ved gentagne behandlinger, som det også blev set i det horisontale delprojekt. Heller ikke her var det muligt, at finde nogen tydelig sammenhæng mellem mængden af pyrethroid i omgivelserne og mængden af pyrethroid metabolit i urinen. Nogle beboere havde tydeligvis været udsat for store mængder pyrethroid, som set af mængden af nedbrydningsprodukter i urinen. Som i første del, har beboernes adfærd i boligen

sandsynligvis stor betydning for, hvor meget de eksponeres. Det lykkedes kun af finde en pyrethroid metabolit i blodprøverne og det var ikke muligt at se nogen tydelig sammenhæng med de andre målte parametre.

Forsøg på at udregne hvor meget pyrethroid beboerne har været udsat for, ud fra mængden af metabolit i urinen, viser, at der godt kan være situationer, hvor ADI overskrides for visse pyrethroider. Det er muligt at sådanne overskridelser er midlertidige og den væsentligste bekymring i den sammenhæng er da også, hvis det sker i særligt følsomme perioder, såsom for små børn eller gravide. Det er meget klart ud fra dette studie, at det er helt nødvendigt, at man foretager studier i "felten" for at få et realistisk mål for den virkelige eksponering.

Undersøgelsen har ført til en stribe anbefalinger i forhold til initiativer, som kan kvalificere skadedyrsbekæmpere til at reducere risikoen for, at beboerne eksponeres for bekæmpelsesmidler. Herudover også anbefalinger i forhold til registrering af bekæmpelsesmidler og behov for fremtidige undersøgelser. Her er der brug for flere og større undersøgelser, som kan løse de praktiske problemer, der er identificeret i denne undersøgelse. Der er brug for undersøgelser som kan klarlægge i flere detaljer, hvor meget beboerne eksponeres for pyrethroider i forbindelse med skadedyrsbekæmpelse. Særligt bør der være fokus på de effekter som kan opstå når man samtidig eksponeres for flere forskellige syntetiske pyrethroider. Der er også brug for at udvikle bekæmpelsesmetoder og procedurer, der kan reducere beboernes eksponering for insekticider generelt.

3. Summary

In Denmark, only pesticides from the group of synthetic pyrethroids are currently used to control bedbugs. The synthetic pyrethroids are stable compounds, so relatively slow degradation is expected under normal conditions. At the same time, decreased sensitivity to pyrethroids among bedbugs has been found in many countries. This means that treatment must often be repeated, leading to the risk of accumulation and increased exposure of the residents.

The purpose of this study was to document whether in practice there is a risk of accumulation of pyrethroids through bedbug control programs and at the same time to elucidate the extent to which the residents are exposed to pyrethroids.
The study consisted of two parts, a horizontal study with a number of sites that had been treated one or more times within the last 6 months, but not the last month. Vacuum samples and exposure samples were collected by walking around a treated room for 10 minutes with a clean cotton sock on one foot. At the same time, urine samples were collected from the residents and these were analysed for pyrethroid metabolites, all contributors were asked about their habits, and what information they had received from the pest control operators. The results showed quite large differences in how pest control operators inform how residents should behave in order to avoid exposure. The typical precaution was to avoid entering the treated room for ½-2 hours after the treatment, otherwise there were no essential precautions and usually no instructions on how to finally remove the treatment again.

In general, most pyrethroid was found in dust samples. The quantities vary, of course, as it was also very variable how much had been treated (from 1-10 treatments within the past year). In some cases, there was a very high content of pyrethroid and often several different active substances in the same sample. The most extreme case was 7,5 mg deltamethrin + 3 mg lambda-cyhalothrin + 46,5 mg permethrin in the dust sample from vacuuming one treated room. Thus, in a bioassay with insecticide sensitive flies, it was found that up to 189 days after a treatment, these died when exposed to the floor. It was not possible to see any clear correlation between the amount of pyrethroid present in the environment and the amount of pyrethroid metabolite present in the urine samples. This was interpreted to mean that the behaviour of the residents has a great influence on the extent to which they are exposed to pyrethroid. Although there is a lot of pyrethroid in the environment, you are not necessarily exposed to it if you are, for example, not very active in the home. It is also very likely that the pyrethroid is lumpy distributed as it is usually only sprayed on places that are relevant in experience, so that in some areas there is a lot and other areas only little active substance.

The second part of the study was a longitudinal study in which a number of residents were followed during a period of treatment for bedbugs. The same samples were collected, but in addition blood samples were also taken. Again, there was quite a large variation in the amount of pyrethroid found and no clear correlation was seen between the amount of pyrethroid and the time since the last treatment. In most cases the levels were still high on the last day of collection and therefore it was considered very likely that pyrethroid accumulation can be achieved by repeated treatments, as was also seen in the horizontal part. Here, too, it was not possible to find any clear correlation between the amount of pyrethroid in the environment and the amount of pyrethroid metabolite in the urine. Some residents had obviously been exposed to large amounts of pyrethroid, as seen by the amount of metabolites in the urine. As in the first part, the behaviour of the residents in the dwelling is likely to have a great impact on how much they are exposed. It was only possible to find one pyrethroid metabolite in the blood samples and it was not possible to see any clear correlation with the other measured parameters.

The study has led to a series of recommendations in relation to initiatives aimed at qualifying pest control operators to reduce the risk of occupant exposure. In addition, identification of complex conditions related to the registration of pesticides and the need for future studies. Here, more and larger studies are needed that can solve the purely practical problems identified in this study. Studies are needed to clarify in detail how much the residents are exposed to pyrethroids in connection with pest control, especially the effects that can occur when exposed to many different synthetic pyrethroids. There is also a need to develop control methods and procedures that can reduce residents' exposure to insecticides.

4. Introduction

In many countries, including Denmark, the most widely used insecticides for pest control in private homes are from the group of synthetic pyrethroids. These synthetic pyrethroids were developed with the aim of being persistent, so that they can remain effective over prolonged periods of time. The rationale for making them persistent is that this will allow insects to become exposed over time through natural roaming and eventually die. The persistence of the products depends both on the relevant active ingredients (a.i.) and the precise formulation of the product. For example, some products are formulated as micro capsules, where the a.i. is concealed within small capsules, that protects it from degradation. At the same time, these micro capsules are so small and fragile, that the pest insects will pick them up and break them, so that they are exposed to large doses of the a.i.

In Denmark, there are in practise no limits in the number of treatments that may be carried out over a certain period, or in the number of different products that can be applied either simultaneously or one after another. It is quite common that different pest control companies are involved in cases of problematic pest infestations, but there is no regulation concerning transfer of information between the companies about the treatment history.

The most common treatment practise is to spray the pest control products with a low-pressure spray on specific areas in the dwellings. In the case of bed bug infestations, this means lower parts of the bed, under and around the bed, along base boards, and other areas where the bed bugs are expected to hide. It is usual procedure not to spray directly on the top side of the bed. Thus, a very heterogenous coverage is to be expected.

It seems likely that accumulation would take places over time when it is necessary to carry out multiple treatments. Besides this, the same synthetic pyrethroids that are used against bed bugs are also used against pest insects in other settings that might end up in human exposure. This could be from impregnation of carpets, treatment of pets for fleas and ticks, treatment against head lice, residues in food, etc. Thus, the risk of accumulation is relevant if the compounds persist over longer time in the relevant environment.

4.1.1 Bed bugs and resistance

Bed bugs are a growing concern in many countries of the world, including Denmark. Though there are no good data showing this, many pest control companies report a growing number of cases with beg bug infestations. This could have several reasons, such as increased travelling, changes in pesticide application methods against other pest insects, but probably most importantly to phasing out of pesticides due to concern over their human toxicological effects, and to the development of resistance against the remaining insecticides (Boase 2007).
Resistance synthetic pyrethroids have been documented in several countries (Moore and Miller 2006; Romero et al. 2007), including Denmark (Kilpinen et al. 2011). This is clearly of concern as all products registered for bed bug control in Denmark belong to the synthetic pyrethroids (See Appendix 1).
Resistance to pyrethroids are usually caused by a mutation in the gene that codes for the target site of pyrethroids (the Na-channel in nerve cells). These mutations are widespread in many insect populations due to the intensive and sole use of pyrethroids for pest control. It is the general recommendation (WHO 2006) that whenever possible, treatments should change between a.i.'s with different modes of action to prevent the development of resistance. In the case of indoor treatments against pest insects the pest control operators (PCOs) are in the situation where only products belonging to the synthetic pyrethroids, all with very similar mode of action, are available. Resistance might result in the necessity for treating over a longer period

with more treatments. This is of concern, not only with regard to further resistance development but also because of accumulation of a.i. in an environment where humans including children, are at high risk of close contact.

4.1.2 Human exposure

In a previous pilot study (Jensen et al. 2015) it was documented that quite high levels of pyrethroid could be found weeks or even months after treatments against bed bugs. This is consistent with studies from other countries (Berger-Preiss et al. 2002, Leng et al. 2003, Leng and Gries 2005) where pyrethroids could be detected for up to one year after treatment (Leng et al. 2005).

The synthetic pyrethroids are also widely used in the agricultural sector which means that humans also are exposed to synthetic pyrethroids through food intake (Clayes et al. 2011). In fact, this is generally considered the most common exposure route (Juraske et al. 2009), but with the growing evidence that there is a substantial risk of exposure in case of bed bug treatments, the combined exposure is highly relevant to investigate.

Pyrethroids are lipophilic compounds that are absorbed through the gastrointestinal surface, in the lungs and probably to some extend over the skin. It is not well known how much is taken up over the skin. Pyrethroids are nerve poisons that affects the Na-channels of the nerve cells. When a pyrethroid binds to the Na-channel it will remain open for a longer time, leading to prolonged stimulation and activation of the nervous system. Insects usually die from overstimulation of the nervous system.

There are only a few reports of acute poisoning of humans by pyrethroids, and knowledge is lacking about potential long-term damages after prolonged exposure to low doses. The human nervous system is most vulnerable to neurotoxic damages during fetal development and in the first years after birth (Grandjean and Landrigan 2014). Experimental studies have shown that animals exposed to pyrethroids during the fetal period or right after birth have permanent damages to their brains (Bjorling-Poulsen et al. 2008). This could be lowered motor functioning and behavioral changes in mice exposed to cyfluthrin (Soni et al. 2011) or in rats exposed to deltamethrin (Lazani et al. 2001). Further, offspring of mice orally exposed to deltamethrin during gestation and lactation showed several ADHD-like features, including hyperactivity, impulse-like behaviours, and deficits in working memory and attention. (Richardson et al., 2015).

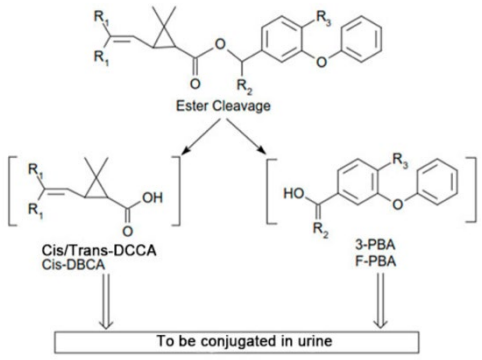
In humans, maternal urinary concentrations of pyrethroid metabolites in pregnancy were associated with ADHD-symptoms in their children at 3 years-of-age in a recent Danish study (Dalsager et al., 2019). Similar associations were seen in other studies (Furlong et al., 2017; Viel et al., 2017) and a recent Spanish study found a link between the use of pyrethroids in private homes during pregnancy and psychomotor development of children 14-months of age (Llop et al. 2013). Besides, childhood pyrethroid exposure (child urinary concentrations of pyrethroid metabolites) has also been associated with impaired cognitive functions, especially verbal and memory functions (Viel et al., 2015) and increased risk of behavioural problems (Oulhote and Bouchard, 2013; Viel et al., 2017). Whether long-term exposure to low doses of pyrethroids is contributing to the observed increase in ADHD and other neurobehavioural problems or to correlations between insecticide exposure in general and the development of neurological diseases such as Autism (Shelton et al. 2014) or Parkinson's disease (Baltazar et al. 2014) is not yet known.

In addition to neurotoxic effects, several pyrethroids have endocrine disrupting properties in experimental studies. The pyrethroid fenvalerate caused increased gonadotropins and a decline in testosterone in male rats (Mani et al., 2002). Exposure of pregnant rats to low doses of deltamethrin affected genital development of male pups in the form of smaller testes and epidi-

dymides and effects on later sperm count (Andrade et al. 2002; Ben Slima et al. 2012). Further, exposure to deltamethrin throughout gestation and lactation caused shorter ano-genital distance (AGD) in male offspring (Kilian et al., 2007) indicating insufficient androgen action. Similar effects were seen for lambda-cyhalothrin in mice (Al-Sarar et al. 2014). In accordance with these findings, several recent epidemiological studies have raised concerns about potentially adverse effects on sperm quality and sperm DNA, reproductive hormones, and pregnancy outcome (Saillenfait et al., 2015). Hence, population representative urinary concentrations of pyrethroid metabolites have been associated with reduced semen quality (Meeker et al., 2008), higher serum concentrations of FSH and LH, and lower inhibin B, and testosterone (Meeker et al., 2009) and sperm aneuploidy (Radwan et al., 2015). Among Chinese women, urinary concentrations of pyrethroid metabolites were significantly associated with increased risk of primary ovarian function (POI) (Li et al., 2018).

4.1.3 Pyrethroid metabolism

During metabolism of synthetic pyrethroids, the central ester bond is cleaved, leading to formation of 3-phenoxybenzoic acid (3-PBA) and in parallel with more specific, often halogenated, metabolites.



These metabolites are conjugated - mainly in the liver, and mainly as glucuronides - and excreted in urine, where they can be measured and used as a biomarker for internal exposure. The relatively few population studies on pyrethroids and health effects is probably related to relatively low detection frequency of pyrethroid metabolites in urine samples in former studies (Barr et al. 2010). However, use of pyrethroids has been increasing during the last decade because they have replaced insecticides with high acute toxicity, such as organophosphate and carbamate insecticides, in biocides and also, to some degree, as insecticides in agriculture. Improved analytical methods and the increasing use of pyrethroids means that a much larger proportion of the population now have measurable urinary concentrations (Oulhote and Bouchard 2013; Trunnelle et al. 2014(CDC, 2015; Dalsager et al., 2019)). The diet is considered the main source of pyrethroid exposure for the majority of the Danish population, but because of increased need for treatment of homes against bed bugs and other household pests, exposure from this source will likely also increase. Several studies, mainly from the US, have demonstrated that residential use of pyrethroids lead to enhanced internal exposure evidenced by high correlations between pyrethroid concentrations measured in homes (dust wipe samples) and urinary concentrations of pyrethroid metabolites (Morgan 2012; Trunnelle et al. 2014). In homes that are frequently treated, it has been estimated that the exposure from this source exceed the exposure from the diet (Morgan 2012).

Very little knowledge exists about the toxicokinetics and metabolism of pyrethroids in humans. Soderlund et al (2002) summarized that cypermethrin is absorbed to a much greater extent orally than dermally and that the half-life after oral exposure to pest control operators was less

than 1 day. In a poisoning case with permethrin, the concentration of the trans isomer was below the plasma detection limit after 25 hours, while the cis isomer could still be detected after 10 days (Gotoh, 1998). Among workers who were exposed to a number of pyrethroids, urine metabolites from Phase I conversion (hydrolysis and oxidation) were the same as earlier reported in rodents. The occurrence of Phase I and Phase II metabolites (the latter being conjugations with amino acids, glucoronic acid or sulfate) in plasma is not known. Most published animal experiments are generally carried our as single-dose trials. Leng et al (2003) intended to investigate the content in the bloodstream of pyrethroids. Non-specific equipment was used for the study and it was performed with only one dose, which could be the reason for not detecting pyrethroids in the bloodstream. There is a need to investigate the presence of Phase I and Phase II metabolites in the bloodstream of humans who have been continuously exposed to pyrethroids.

4.2 Aim of the study

The purpose of this study was to investigate pyrethroid exposure levels, by using human biomonitoring, among residents in private homes following treatments against e.g. bed bugs. It was considered especially important not to intervene in any way with the normal procedure of the PCO's treatment regime, or with the residents' activities and behaviour after treatment, in order to get a realistic picture of the exposure situation. Synthetic pyrethroids are rather persistent in the indoor environment, they are used for many different applications in private homes and often multiple treatments are necessary to overcome the infestation (e.g. bed bugs). Therefore, it was also the aim to investigate the accumulation of pyrethroids in the homes following multiple applications by analyses of dust samples.

To fulfil the research aims, we used a two-way approach: A horizontal study, where locations were investigated one month or later after they had been treated against bed bugs and a longitudinal study, where locations were sampled several times before, during and after a treatment procedure. In the horizontal study, untreated (for at least one month) locations were included as controls.

In both approaches, dust samples were collected from the homes to get a measure of how persistent these compounds were in this environment and to estimate the external exposure level for the residents. Besides, blood and urine samples from the residents were collected and analysed for pyrethroid metabolites to estimate how much were taken up, i.e., the internal exposure level. It was not the aim of the present study to investigate potential toxic effects in the residents, only to document human exposure and potential accumulation in the home environment after multiple treatments as well as the time frame for continued exposure after treatment.

5. Materials and methods

5.1 Study design and sample collection

Most sampling methods applied here were also applied in an earlier study (Jensen et al. 2015), but additional methods to collect urine and blood samples were added to the protocol.

5.1.1 Horizontal study

Participants were selected based on the criteria that their homes had been treated against bed bugs within the last 6 months, and that at least one month had passed since last treatment. There were no criteria regarding type of treatment or number of treatments. Most of the participants were found by the help of professional pest control operators (PCO) who had carried out the treatments, but some were also found after advertising on the internet. Residents living in homes that had not been treated with pyrethroids within 1 month were included as controls.

A single visit was made at each location. Prior to the visit, the participant had been informed that a urine sample would be collected during the visit. At each location a suitable room (mostly the bedroom) that had been treated was selected. In this room, samples were collected. The room was finally measured up to calculate the area of exposure and the participants were asked to deliver a urine sample.

During the visit, the participants were asked to fill in a questionnaire regarding dietary habits and food preferences (i.e. organic or non-organic), if they had any pets, carpets in the home, frequency of vacuum cleaning or if there were other possible sources of pesticide exposure. The participants were also asked about the treatments that had been carried out, as well as the information they had received from the PCO. Whenever possible, information was collected about the products that had been used by the PCOs but such information had not always been given to the resident.

5.1.2 Longitudinal study

In the longitudinal study, the first sample collection had to occur before the first treatment. This planning was a major challenge because when a bed bug infestation is identified, treatment usually takes place within a day of two. Thus, time was very short to make arrangements and the PCOs were reluctant to get involved in the study. Thus, after a considerable delay it was decided to include also locations that had been treated previously in order to get an acceptable number of participants.

The aim was to collect samples before treatment and then again on day 1, 14 and 28 after treatment. However, the usual treatment regime against bed bugs, is to make 2-3 treatments with 1-2 weeks in between. Thus, the sampling protocol had to be adjusted according to the individual treatment regimens that the PCO carried out. This means that repeated measurements were made from the same persons after several treatments.
It was considered acceptable to collect urine and blood samples from two persons at the same location if possible.

5.1.3 House fly bioassay

In the longitudinal study a housefly bioassay was also carried out. Two different strains of house flies, *Musca domestica*, L., were used. The insecticide-susceptible standard reference strain WHO-SRS was received in 1988 from the Department of Animal Biology, University of Pavia, Italy. The insecticide resistant strain 381 zb was collected on a farm in Denmark in 1978 and maintained and selected continuously at the Danish Pest Infestation Laboratory. Resistance against permethrin was identified in 1979 and against dimethoate in 1983. The strain shows resistance against other synthetic pyrethroids and to various other organophosphate compounds and to DDT.

TABLE 1. LD50 and LD95 measured in the two fly-strains WHO-SRS and 381zb against deltamethrin and R/S λ-cyhalothrin. The resistance factor R/S is given as well.

	WHO-SRS LD_{50} 95% Fiducial Limits	381zb LD_{50} 95% Fiducial Limits	WHO-SRS LD_{95} 95% Fiducial Limits	381zb LD_{95} 95% Fiducial Limits
Deltamethrin	0.0000987 $7.936*10^{-6}$-0.00026	0.11307 NA	0.00637 0.00231-0.08884	2.82041 NA
λ-cyhalothrin	0.0000693 $3.628*10^{-7}$-0.00028	0.05791 0.00568-0.55438	0.00424 0.00144-0.08158	0.81837 0.16932-168793
R/S Deltamethrin	R/S_{50} = 1146		R/S_{50} = 443	
R/S λ-cyhalothrin	R/S_{95} = 836		R/S_{95} = 193	

Flies was anaesthetized with CO_2 and transferred to Petri dishes (diameter 60mm). In each locality to be tested with the fly-bioassay 12 Petri dishes with 10 flies each was used: 6 with WHO-SRS flies, 6 with 381zb flies. A piece of carton paper was moved in between the two parts of the petri dish, the lit was then removed and dish with the flies covered by the carton paper was placed on the floor with the carton paper towards the floor. Gently the carton paper was pulled out forcing the flies to sit directly on the floor. The flies were exposed on the floor for 30 minutes. Afterwards the carton paper was gently pushed back underneath the Petri dish and the lit was put back on.

When arriving at the laboratory the 10 flies from a Petri dish were anaesthetized and transferred to a 155 ml plastic cup containing sugar and a cotton plucked tube of water and closed with a lid with a mesh. The number of dead flies was counted after 6, 24, 48, and 72 hours. It is well known that resistant flies exposed to low doses of especially synthetic pyrethroids may be mori-bund, but then regain their vitality after some hours which is the reason for counting them four times. It turned out that control mortality was rising between 48 and 72 hours and therefore 48 h was chosen for further calculations. The Petri dishes with the control flies were subjected to the same procedures as the test dishes, except that they were not exposed to treated surfaces.

The housefly bioassay was not applied in the longitudinal study due to problems of producing houseflies on time, when test locations had to be visited at very short notice, usually within 24 hours.

5.1.4 Sample collection

5.1.4.1 Dust and sock samples

During each visit, one of the collectors walked around in the room for ten minutes wearing a "standard" hospital sock (100% cotton from Max Mortensen & Co, Odense, Denmark) on top of a plastic foot cover on one foot. The aim was to walk on the entire floor surface in the room, where the habitants would normally contact the treated surfaces, without moving any furniture or other items in the room. The collector walked for exactly 10 minutes. After collection, but before analysis, the lower part of the sock as indicated by the structure of the sock, was cut off, to give a standard area of exposure to be analyzed.

FIGURE 1. Sock sampling where a person is walking for ten minutes with a test sock.

After the "sock" sampling the entire room was vacuumed, with a special vacuum equipment, that allowed dust samples to be collected. This equipment consisted of an Electrolux "Heavy duty separator ZE001N" mounted on a custom vacuum cleaner. The principle behind the heavy-duty separator is a cyclone that separates all dust from the air. The Heavy-duty separator was modified to a allow a plastic container (520 ml) to be mounted in a way that allowed a dust to be collected in the plastic container and after the vacuuming the plastic container could be closed tightly.

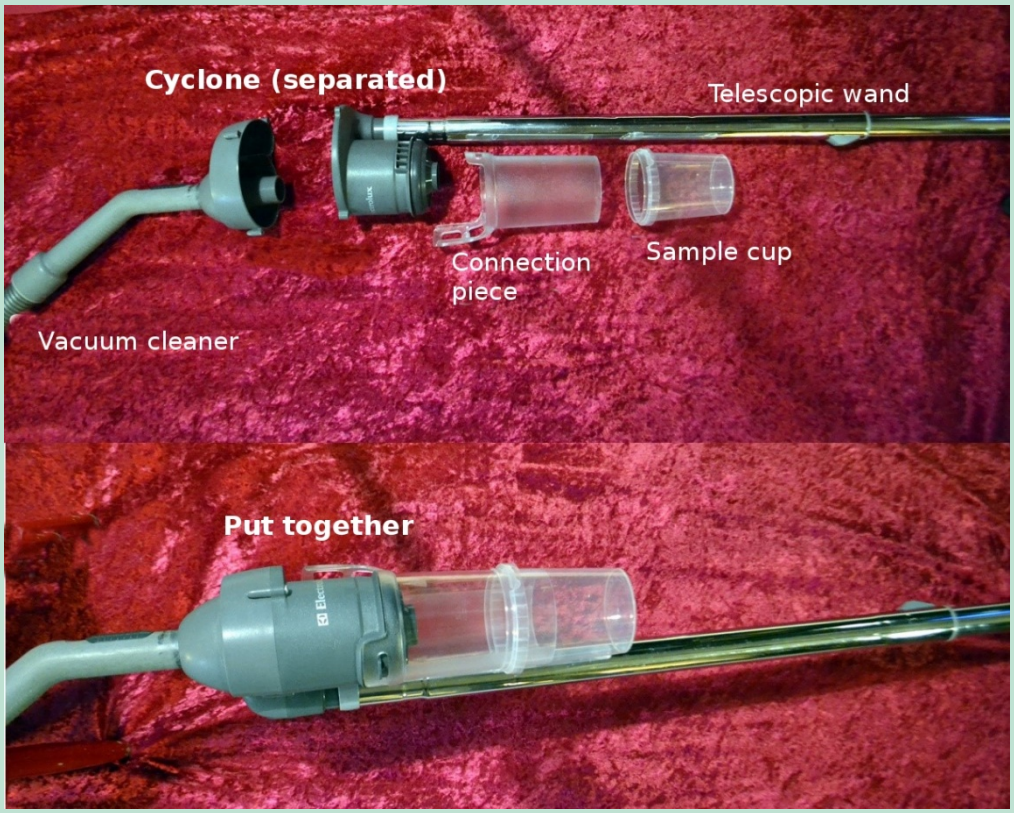


FIGURE 2. Heavy-duty separator

Both sock and vacuum samples were stored in the freezer (-18°C) until analysis.
All vacuuming equipment that could contaminate subsequent samples, was cleaned in a 10% Deconex solution for at least 24 hours before being used again.

5.1.4.2 Urine samples

Participants were informed prior to sampling that they should make a 100ml urine sample before or during the visit. If possible, this should be morning urine. Urine samples were stored at -18°C until analysis.

5.1.4.3 Blood samples

In the longitudinal study participants were asked to deliver a blood sample at each visit, except on day 28 past treatment. Blood samples were taken by a biomedical laboratory scientist during the visit. Samples were kept cold and within 24 hours, centrifuged and the plasma stored at -20°C until analysis.

5.1.5 Pyrethroid concentration in dust and sock samples

Dust and sock samples were analyzed for the pyrethroids deltamethrin, λ-cyhalothrin, permethrin, and cyfluthrin in the cross-sectional study. In the longitudinal study, samples were also analyzed for etofenprox as this a.i. was introduced on the marked during the project. Cyfluthrin was included in the analysis despite it originally being excluded from the project. From an earlier project the component parameters were already in the LC-MS/MS and responses to cyfluthrin was found for several samples.

Each dust or sock sample was extracted in the sample container by adding 100 ml acetonitrile and extracting first for 15 minutes in ultra-sonic bath, followed by 30 minutes of shaking. 1 ml of the extract was filtered through a 0.22µ filter. 25µl of the filtrate was injected in the LC-MS/MS. The content of the samples was calculated on basis of an external standard curve with concentrations from 0.24 ng/ml to 500 ng/ml in the Analyst software. For cyfluthrin, no standard curve was available but from a previous project it was possible to calculate a standard curve manually in excel. The LOD for cyfluthrin was 6µg per dust/sock sample.

The recovery percentage of the method was tested on basis of blank samples spiked with 1.6µg, 16µg and 160µg per dust/sock sample. The results showed recovery between 80% and 120%, which is acceptable according to Eurachem guidelines. Results were not corrected according to recovery. Detection limits for deltamethrin and λ-cyhalothrin was 1µg per dust/sock sample and 0.5µg per dust/sock sample, respectively.

Samples were analyzed by means of tandem mass spectrometry (LC-MS/MS) consisting of a binary pump (Agilent 1260 Infinity), autosampler (Agilent 1260 HIP) and AB Sciex- mass spectrometer (3200 Q-trap).

Compounds were separated chromatographically at 25°C on a BDH Hypersil C18 column (250x2.1mm; 5µ) from Thermo Scientific, with the eluent A (1% MeOH with 20mM ammonia acetate) and eluent B (90% MeOH with 20mM ammonia acetate). Flow speed of the mobile phase was 0.2 ml/min. Temperature of the autosampler was 15°C. In the horizontal study the gradient was: 0-2 min, 10%-100% B; 2-17 min, 100% B; 17-18 min, 100%-10% B; 18-26 min, 10% B. In the longitudinal study the gradient was: 0-2 min, 10%-100% B; 2-22 min, 100% B; 22-23 min, 100%-10% B; 23-30 min, 10% B. Blanks were run between samples to avoid carry-over between samples.

5.1.6 Pyrethroid metabolite concentration in urine samples

Urine samples were analyzed for the generic pyrethroid metabolite, 3-PBA (3-phenoxybenzoic acid) representing exposure to most pyrethroids including cypermethrin, deltamethrin, permethrin, lambda-cyhalothrin, tau-fluvalinate, esfenvalerate, and fenpropathrin. In addition, the following, more specific, pyrethroid metabolites were included: cis- and trans-DCCA (3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid) representing exposure to the cis- and trans-isomers of permethrin, cypermethrin, and cyfluthrin; 4-F-3-PBA (4-fluoro-3-phenoxybenzoic acid), a specific metabolite of cyfluthrin; cis-DBCA (cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid), a specific metabolite of deltamethrin; CPBA (4-chloro-alpha-isopropyl benzene acetic acid), metabolite of esfenvalerate and fenvalerate; and ClF3CA (chloro-3-fluoro-vinylcyclo-propane carboxylic acid) as metabolite of bifenthrin and cyhalothrin. The latter two metabolites were only included in WP2.

The analyses were performed by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS), after addition of isotope-labeled internal analogues of the compounds (except CPBA and ClF3CA) as internal standards, deglucuronidation and solid-phase extraction (SPE), according to a slightly modified version of the method described by Davis et al. (2013). SPE was performed manually using Oasis HLB (60 mg) cartridges. The LC-MS/MS system consisted of an Accela 1200 pump, a CTC PAL autosampler, and a TSQ Vantage Mass Spectrometer (Thermo Scientific, San José, CA). The separation was performed on a Kinetex C18 (50 x 2.1 mm; 2.6 µm) column (Phenomenex, Torrance, CA), operated at 30°C. Spectrophotometric determination of urinary creatinine concentrations was conducted on a Konelab 20 Clinical Chemistry Analyzer, using a commercial kit (Thermo, Vantaa, Finland). All analyses were performed at Environmental Medicine, University of Southern Denmark (SDU).

TABLE 2. Pyrethroid metabolites measured in urine samples

Metabolite	Abbreviation	LOD ng/ml	Pesticide
3-phenoxybenzoic acid	3-PBA	0.03	Common metabolite of most pyrethroids, e.g,: cypermethrin, deltamethrin, permethrin, lambda-cyhalothrin, etofenprox, tau-fluvalinate, esfenvalerate, fenpropathrin, (but not cyfluthrin or bifenthrin)
4-fluoro-3-phenoxybenzoic acid	4-F-3-PBA	0.2	cyfluthrin
cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	Cis-DCCA	0.5	cis-permethrin, cis-cypermethrin, cis-cyfluthrin
trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	Trans-DCCA	0.4	trans-permethrin, trans-cypermethrin, trans-cyfluthrin
cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid	Cis-DBCA	0.5	deltamethrin
4-chloro-alpha-isopropyl benzene acetic acid	CPBA	0.2	esfenvalerate, fenvalerate
chlorotrifluorovinylcyclo-propane carboxylic acid	CF3CA	0.1	bifenthrin and cyhalothrin

LOD: Limit of detection

5.1.7 Pyrethroid metabolites in blood samples

Plasma samples in the longitudinal study were analyzed for two possible metabolites of deltamethrin and λ-cyhalothrin: 3-phenoxybenzoic acid (3-PBA) (CAS nr 3739-38-6) and 3-(4-hydroxy)phenoxybenzoic acid (CAS nr 35065-12-4), and for a possible metabolite of deltamethrin: (1-R-cis)decamethrinic acid (cis-DBCA) (CAS nr 53179-78-5).

5.1.7.1 Sample preparation

Protein precipitation. Plasma samples that had been stored at -20°C were thawed and placed on crushed ice. 4x650 µl (four subsamples due to centrifuge capacity) were pipetted into 2 ml Eppendorf tubes. 1.3 ml ice cold MeOH was added to each tube. The tubes were shaken thoroughly and stored at -20°C, overnight. On the following day the samples were shaken thoroughly again and left to heat to 20°C. They were then centrifuged at 15000 rpm for 10 minutes. The supernatant from the 4 subsamples were decanted into 50 ml Flacon tubes and added 17 ml Milli Q water.

SPE-cleaning. The SPE was Oasis MCX 6cc 500mg (Part no: 186000776 from Waters) equipped with a connection and a 20 ml plastic syringe (without plunger) mounted as reservoir. The SPE was conditioned with 10ml tetrahydrofuran (THF) : acetonitrile (CAN) (1:1), 10ml MeOH and 3 ml Milli Q water dripping though without suction (slight suction in the beginning). The sample was added and left to drip through. Shortly after the SPE was washed with 2ml Milli Q water (slight suction) followed by 1 ml MeOH, where after it was left under suction for 30 minutes. The analytes were eluted with 2 ml THF:ACN (1:1). First 1 ml, with suction. Two minutes later another 1 ml, which was left to drip through. Followed by 2-3 minutes of suction, to be sure that it was completely dry. The eluate was collected in 2ml volumetric flask and adjusted to 2 ml with THF:ACN (1:1) and shaken carefully.

Dilution. The extract was diluted 1:2 with Milli Q water before injecting 50µl into LC-MS/MS. The content of the samples was calculated based on an external matrix-matched standard curve with concentration between 0.00975 ng/ml and 1.25 ng/ml swine plasma.

5.1.7.2 Quantitative analysis of blood samples

Samples were analyzed by means of tandem LC-MS/MS like dust and sock samples. Compounds were separated at 30°C on a Kintex EVO C18 100A column (150 xx 2.1 mm; 2.6µ) from Phenomenex with the eluent A (20mM acetic acid in Milli Q water) and eluent B (20 mM acetic acid in acetonitril). Flow of the mobile phase was 0.2 ml/min. Temperature in autosampler was 15°C. Blanks were run between samples to avoid carry-over between samples.

The recovery percentage of the method was tested on basis of blank samples with addition of 0.1 ppb and 1 ppb of the three compounds to be analyzed. Recovery was between 50% and 70%, which is slightly low according to the Eurachem guidelines. However, the guideline suggests not to compensate for recovery at this level. Limit of detection (LOD) was 0.0075 ng/ml for 3-PBA, 0.06 ng/ml for 3-(4-hydroxy)phenoxybenzoic acid and 0.12 ng/ml for cis-DBCA. Limits of detection were determined based on visual estimation of signal to noise ratio, for the lowest possible standard for each of the three metabolites.

5.1.7.3 QTRAP-based identification of additional metabolites

Data-dependent acquisition methods were used for analysis of the extracts with the purpose of identifying metabolites, related to the analyzed compounds, but for which no standards were available. EMS-IDA-EPI and MRM-IDA-EPI methods were applied.

5.2 Statistics

Since cyfluthrin is metabolized into 4F-3-PBA (but not 3-PBA) the molar sum of 3-PBA and 4F-3-PBA was calculated and used as indicator for the combined exposure to pyrethroids. Differences in urinary metabolite concentrations between groups were tested using Mann-Whitney U-test. Bivariate correlations between urine concentrations and blood and dust concentrations were analyzed by Spearmans rho.

6. Results

6.1 Horizontal study

15 treated and 10 untreated locations were included in the study. Besides that, seven collections (treated) from the longitudinal study could be included in the horizontal study as the conditions meet the criteria (at least one month since last treatment).

6.1.1 Questionnaire

15 persons at the treated locations in the horizontal study answered the full questionnaire. Most questions were on other likely sources of pyrethroid exposure, but some questions focused on which information the PCO had given about how the residents should behave to avoid exposure to the applied pyrethroid and if they were given restrictions to avoid affecting the efficacy of the treatment. Table 3 shows the results of these questions. The answers are quite diverse, but the general picture was that the residents were recommended to stay out of the treated room until the treated area was dry, which could be from 30 minutes to 3 hours. The most general recommendation concerning the efficacy of the treatment was to avoid vacuuming/cleaning for two weeks, which could be until next treatment, and that the residents should occupy the room, to induce the bed bugs to come out.

TABLE 3. Answers to the question "What information did you receive concerning your own exposure to the treatment?" in the horizontal study. Answers are subsequently divided into information concerning resident exposure and efficacy of the treatment.

Code	Resident exposure	Efficacy of treatment
140416-1	No precautions	No cleaning
150916-1	No precautions	Less vacuuming for 14 days
150916-2	Residents should use room	
280916-1	Residents should not change room for sleeping	
111016 -1	Put plastic under the sheets	No vacuuming for some days
121016 - 1	Wash clothing at 60°C or freeze it	
131016 - 1	No precautions	Nothing
131016 - 2	Stay out of room immediately after treatment	Residents should use room
251016 - 1	Avoid cleaning	
251016 - 2	It is a safe product and relatively harmless to humans	
271016 - 1	No information, had to go to work after treatment	
161216 -1	Stay out of room for 30 min	
240217 -1	Stay out of room for 1 hour	
100317 -1	Avoid walking in bare feets	No cleaning until just before next treatment
100317 -2	Stay out of room for 2 hours	

Besides these answers (Table 3-4) three reports of adverse effects on the residents were reported. The effects were:

- Red skin and swellings around the eyes (290916-1)
- Burning sensation in hands and feet after some treatments (251016-1)

- Discomfort. Small blisters and wounds (100317-2)

TABLE 4. Answers to questions relating to known pest infestations and the treatments.

Code	Known pest infestation	Duration of pest infestation	No of treatments last year	Treatments by residents
140416-1	Bed bugs	8 mo	8-10	No
150916-1	Bed bugs	6-12 mo	3	2 x cyfluthrin (from PCO); 1 x deltamethrin (from PCO); 1 x pyrethrin (I+II)
150916-2	Bed bugs	36 mo	>10	2 x unknown (from PCO)
280916-1	No	-	4	No
111016 -1	No	-	2	1 x silicone (flea product)
121016 - 1	Bed bugs	2 mo	3	No
131016 - 1	No	-	1	No
131016 - 2	No	-	1	No
251016 - 1	No	-	4	No
251016 - 2	Bed bugs	16 mo	3	1 x permethrin powder (foreign product bought in DK)
271016 - 1	No	1.5 mo	2	No
161216 -1	Bed bugs	6 mo	5-6	No
240217 -1	Bed bugs	18 mo	3 (3 last mo)	No
100317 -1	No	-	2	No
100317 -2	No	-	1	No

Table 4 shows the answers relating to whether a pest infestation was known or only under suspicion and treatments that had been done either by the PCO or by the residents themselves. Only in seven out of 15 cases, was there a known infestation of bed bugs. In the remaining eight cases, the locations were treated only on suspicion of bed bugs. Six of the seven known infestations had lasted six months or more. In two cases, the PCO had handed out pyrethroid products for supplementary use by the residents.

6.1.2 Pyrethroid concentration in sock and dust samples

Table 5 and table 6 show the amount of pyrethroids found in sock and dust samples in the horizontal study, at the treated and the untreated locations, respectively. As expected, the variation is very high. Some locations had been treated up to 10 times in the preceding year, whereas others had been treated only once. The time since last treatment also varied from 23 to 189 days.
Overall, much more insecticide was found in the dust samples, as compared to sock samples (Table 5 and 6). One sock sample (240217-1) contained quite high amount of λ-cyhalothrin. Even after 189 days (sample 271016-1), pyrethroids could still be measured in both sock and dust samples.
At one location (140416-1), where very high levels of pyrethroids were found, the samples were also analyzed for etofenprox, as the PCO informed that this had been used. In the dust sample 9, 380 µg were found, but it was not detected in the sock sample. In this location, at

least 8 treatments had been carried out the preceding year and the PCO had given up on eradicating the bed bugs

FIGURE 3. Heavily infested location (140416-1) with both bed bugs and coleoptera larvae

TABLE 5. Pyrethroids found in sock and dust samples, treated locations. LOD: Limit of detection.

			Sock (µg/sample)					Dust (µg/sample)			
	No. Treatments last Year	Days p.t.	Deltamethrin	λ-cyhalothrin	Permethrin	Cyfluthrin	Dust (g)	Deltamethrin	λ-cyhalothrin	Permethrin	Cyfluthrin
LOD			**1**	**0.5**	**0.5**	**6**		**1**	**0.5**	**0.5**	**6**
140416-1	8	54	4	<LOD	6	<LOD	12.5	7,550	3,050	46,500	<LOD
150916-1	3	45	19	<LOD	<LOD	8	9.7	1,500	3	16	382
150916-2	10	62	50	<LOD	12	116	7.8	103	2	73	516
280916-1	4	125	<LOD	<LOD	<LOD	<LOD	6.1	4	38	6	<LOD
111016 -1	2	115	<LOD	94	<LOD	<LOD	5.2	1	206	<LOD	<LOD
121016 - 1	3	47	<LOD	94	<LOD	<LOD	5.3	2	475	<LOD	<LOD
131016 - 1	1	38	<LOD	9	<LOD	<LOD	4.7		47	<LOD	<LOD
131016 - 2	1	52	<LOD		<LOD	<LOD	5.8	14	1	6	<LOD
251016 - 1	4	35	<LOD	55	<LOD	<LOD	4.7	2	75	3	<LOD
251016 - 2	3	35	<LOD	53	177	<LOD	5.6		105	1,820	9
271016 - 1	2	189	<LOD	82	<LOD	6	5.3	3	66	22	13
161216 -1	5	92	<LOD	5	<LOD	6	7.4	8	122	28	110
240217 -1	3	23	9	2,805	<LOD	46	12.4	56	4,700	3	94
100317 -1	2	39	<LOD	44	<LOD	<LOD	5.7	2	92	2	<LOD
100317 -2	1	44	<LOD	33	<LOD	<LOD	9.7	1	206	2	<LOD
070118*	3	30	<LOD	<LOD	<LOD	<LOD	7.5	1	6	<LOD	<LOD
010518A*	2	29	<LOD	<LOD	<LOD	<LOD	4.7	2	<LOD	1	67
010518B*	2	29	<LOD	<LOD	<LOD	<LOD	4.7	2	<LOD	1	67
240518A*	3	51	12	<LOD	<LOD	5	4.9	54	<LOD	1	77
240518B*	3	51	12	<LOD	<LOD	5	4.9	54	<LOD	1	77
150918A*	3	33	<LOD	36	<LOD	<LOD	4.8	6	584	1	<LOD
150918B*	3	33	<LOD	36	<LOD	<LOD	4.8	6	584	1	<LOD

* locations from the longitudinal study also included in the horizontal study

TABLE 6. Pyrethroids found in sock and dust samples, untreated control locations.

			Sock (µg/sample)					Dust (µg/sample)			
	No. Treatments last Year	Days p.t.	Deltamethrin	λ-cyhalothrin	Permethrin	Cyfluthrin	Dust (g)	Deltamethrin	λ-cyhalothrin	Permethrin	Cyfluthrin
071016 -1	0	-	<LOD	<LOD	<LOD	<LOD	6.0	8	1	5.4	<LOD
071016 -2	0	-	<LOD	<LOD	<LOD	<LOD	6.7	2	1	<LOD	<LOD
071016 -3	0	-	<LOD	<LOD	<LOD	<LOD	6.0	2	5	<LOD	<LOD
311016 -1	0	-	<LOD	<LOD	<LOD	<LOD	7.3	1	4	21.4	<LOD
311016 -2	0	-	<LOD	<LOD	<LOD	<LOD	5.0	<LOD	3	2.1	<LOD
171116 -1	0	-	<LOD	<LOD	<LOD	<LOD	6.9	1	2	21.7	<LOD
171116 -2	0	-	<LOD	<LOD	<LOD	<LOD	6.1	<LOD	1	1.6	<LOD
181116 -1	0	-	<LOD	<LOD	<LOD	<LOD	5.4	4	1	5.7	<LOD
020217 -1	0	-	<LOD	<LOD	<LOD	<LOD	11.4	5	1	7.6	<LOD
020217 -2	0	-	<LOD	<LOD	<LOD	<LOD	5.4	<LOD	1	1.7	<LOD

6.1.3 House fly bioassay

There was no correlation between 381zb-mortality and days after treatment (Spearman's rank correlation, p-value = 0.9413), nor between WHO-SRS-mortality and days after treatment (Spearman's rank correlation, p-value = 0.3774). Mortality was always higher in WHO-SRS than in 381zb, but there was no correlation between the difference in mortality and days after treatment (Spearman's rank correlation, p-value = 0.301). The data (Table 7) illustrates that, at all the test localities, there were areas with enough a.i. to control the susceptible flies. The longest registration of insecticidal activity against the flies was 189 days (271016-1)

TABLE 7. Fly mortality measured 48 hours after 30 minutes exposure on the floor in a treated or untreated flat/house. Mostly the inhabitants did not know which product the PCO had used but based on chemical analysis of a.i. higher than 5µg per dust sample is given as D=deltamethrin, L=λ-cyhalothrin, P=permethrin, C=cyfluthrin, and E=etofenprox.

	Host	Susceptible WHO-SRS-48H Mort. %	Resistant 381zb-48h Mort. %	A.i.*	Days after last treatment
Treated	240217-1	100.0 ± 0.0	30.0 ± 52.0	D/L/P/C	23
	251016-1	33.3 ± 57.7	0.0 ± 0.0	D/L/P	35
	251016-2	69.7 ± 39.1	3.3 ± 5.8	L/P/C	35
	131016-1	10.0 ± 0.0	23.3 ± 15.3	L	38
	100317-1	50.0 ± 36.1	0.0 ± 0.0	D/L/P	39
	100317-2	43.3 ± 11.5	3.3 ± 5.8	D/L/P	44
	150916-1	100.0 ± 0.0	17.3 ± 15.5	D/L/P/C	45
	121016-1*	77.7 ± 38.7	52.0 ± 50.1	D/L	47
	131016-2	36.7 ± 55.1	14.0 ± 12.2	D/L/P	52
	140416-1*	66.7 ± 57.7	10.0 ± 10.0	D/L/E	54
	150916-2	100.0 ± 0.0	44.0 ± 19.7	D/L/P/C	62
	161216-1	73.3 ± 46.2	0.0 ± 0.0	D/L/P/C	92
	111016-1	100.0 ± 0.0	3.3 ± 5.8	D/L	115
	280916-1	30.3 ± 19.5	0.0 ± 0.0	D/L/P/C	125
	271016-1	100.0 ± 0.0	3.3 ± 5.8	D/L/P/C	189
Untreated	071016-1	0.0 ± 0.0	0.0 ± 0.0	-	-
	071016-2	0.0 ± 0.0	6.7 ± 5.8	-	-
	071016-3	26.7 ± 20.8	23.3 ± 11.0	-	-
	311016-1	40.0 ± 34.6	17.0 ± 11.3	D/L/P	-
	311016-2	0.0 ± 0.0	0.0 ± 0.0	-	-
	171116-1	3.3 ± 5.8	0.0 ± 0.0	D/L/P	-
	171116-2	0.0 ± 0.0	0.0 ± 0.0	-	-
	181116-1	0.0 ± 0.0	0.0 ± 0.0	-	-
	020217-1	0.0 ± 0.0	0.0 ± 0.0	-	-
	020217-2	0.0 ± 0.0	0.0 ± 0.0	-	-

6.1.4 Pyrethroid metabolites in urine samples

A high dust content of deltamethrin at the two first locations was reflected in a high urinary concentration of the specific deltamethrin metabolite, cis-DBCA and a high content of cyfluthrin was related to high urinary concentrations of 4F-3-PBA (Table 8). Besides these associations, no clear relation was seen between urinary concentrations of specific urinary pyrethroid metabolites and the concentration detected in dust samples, but in general, locations with relatively high dust content of deltamethrin, λ-cyhalothrin, and/or permethrin were related to higher urinary concentrations of the generic pyrethroid metabolite, 3-PBA.

Overall, the median concentrations of pyrethroid metabolites were not significantly different between treated and untreated locations, except for cis-DCCA and 3-PBA, expressed by g creatinine, which were higher in untreated locations. However, the maximum concentrations were markedly higher in treated locations for most metabolites (Table 9). A max value of 16 µg/L for cis-DCCA indicate a high dermal/inhalation exposure since after oral exposure, the parent compounds are mainly excreted as trans-DCCA (Cote et al., 2014).

TABLE 8. Concentrations (µg/L) of pyrethroid metabolites in urine samples. For creatinine corrected values see Appendix 3.

		3-PBA (µg/L)	4-F-3-PBA (µg/L)	cis-DCCA (µg/L)	trans-DCCA (µg/L)	cis-DBCA (µg/L)
	LOD	**0.03**	**0.2**	**0.5**	**0.4**	**0.4**
Treated	140416-1	4.08	<LOD	<LOD	4.44	0.80
	150916-1	0.60	3.11	15.64	4.12	0.52
	150916-2	0.30	2.62	<LOD	1.35	<LOD
	280916-1	0.16	<LOD	<LOD	<LOD	<LOD
	111016 -1	0.56	<LOD	<LOD	<LOD	<LOD
	121016 - 1	0.78	<LOD	<LOD	<LOD	<LOD
	131016 - 1	3.21	<LOD	<LOD	<LOD	<LOD
	131016 - 2	0.27	<LOD	<LOD	<LOD	<LOD
	251016 - 1	0.22	<LOD	<LOD	<LOD	<LOD
	251016 - 2	4.46	<LOD	<LOD	1.56	<LOD
	271016 - 1	0.29	<LOD	<LOD	1.14	<LOD
	161216 -1	0.09	0.29	<LOD	0.54	<LOD
	240217 -1	1.16	0.21	<LOD	2.75	<LOD
	100317 -1	7.66	<LOD	<LOD	<LOD	<LOD
	100317 -2	0.17	<LOD	<LOD	<LOD	<LOD
	070118	1.48	<LOD	<LOD	0.76	<LOD
	010518A	2.55	0.56	<LOD	2.31	0.78
	010518B	0.13	<LOD	<LOD	<LOD	<LOD
	240518A	0.11	<LOD	<LOD	<LOD	<LOD
	240518B	0.23	<LOD	1.06	<LOD	<LOD
	150918A	0.17	<LOD	<LOD	<LOD	<LOD
	150918B	0.2	<LOD	<LOD	<LOD	<LOD
Untreated	140416-1	0.49	<LOD	2.88	<LOD	<LOD
	150916-1	a	a	a	a	a
	150916-2	0.26	<LOD	<LOD	0.98	0.57

280916-1	0.44	<LOD	<LOD	<LOD	<LOD
111016 -1	0.26	<LOD	<LOD	<LOD	<LOD
121016 - 1	0.24	<LOD	<LOD	0.77	<LOD
131016 - 1	0.20	<LOD	<LOD	0.40	<LOD
131016 - 2	a	a	a	a	a
251016 - 1	0.23	<LOD	<LOD	<LOD	<LOD
251016 - 2	1.37	<LOD	<LOD	<LOD	<LOD

[a] Missing urine samples

TABLE 9. Median concentrations of pyrethroid metabolites in urine samples collected from residents in treated or untreated locations.

	Treated locations			Untreated locations		
	%	Median (max)		%	Median (max)	
	N>LOD	µg/L	µg/g creatinine	N>LOD	µg/L	µg/g creatinine
Trans-DCCA	41	0.32 (4.44)	0.42 (3.17)	38	0.38 (0.98)	0.53 (2.81)
Cis-DCCA	9	0.35 (16.00)	0.39 (11.47)**	13	0.35 (3.00)	0.65 (1.26)
Cis-DBCA	14	0.16 (0.80)	0.19 (0.57)	13	0.17 (0.57)	0.21 (1.63)
4F-3-PBA	27.3	0.14 (3.11)	0.17 (4.86)	0	<LOD	
3-PBA	100	0.30 (7.66)	0.42 (3.94)	100	0.26 (1.37)	0.60 (0.95)
Σ 3-PBA + 4F-3-PBA*		-	3.33 (23.53)		-	3.91 (6.12)

* mmol/g creatinine; **p<0.05

6.2 Longitudinal study

6.2.1 Pyrethroids in sock and dust samples

As in the horizontal study, there were relatively high variations in the amount of pyrethroid measured in sock and dust samples and most were found in the dust samples (Table 10). In some cases, pyrethroids were also found before treatment started because the locations had been treated before. There was no clear picture in the amount found in the days following treatments. In most cases the levels were still high at the last collection day. In one case (150918A+B) where the room was treated with λ-cyhalothrin, high levels were found in both sock and dust samples two days after treatment.

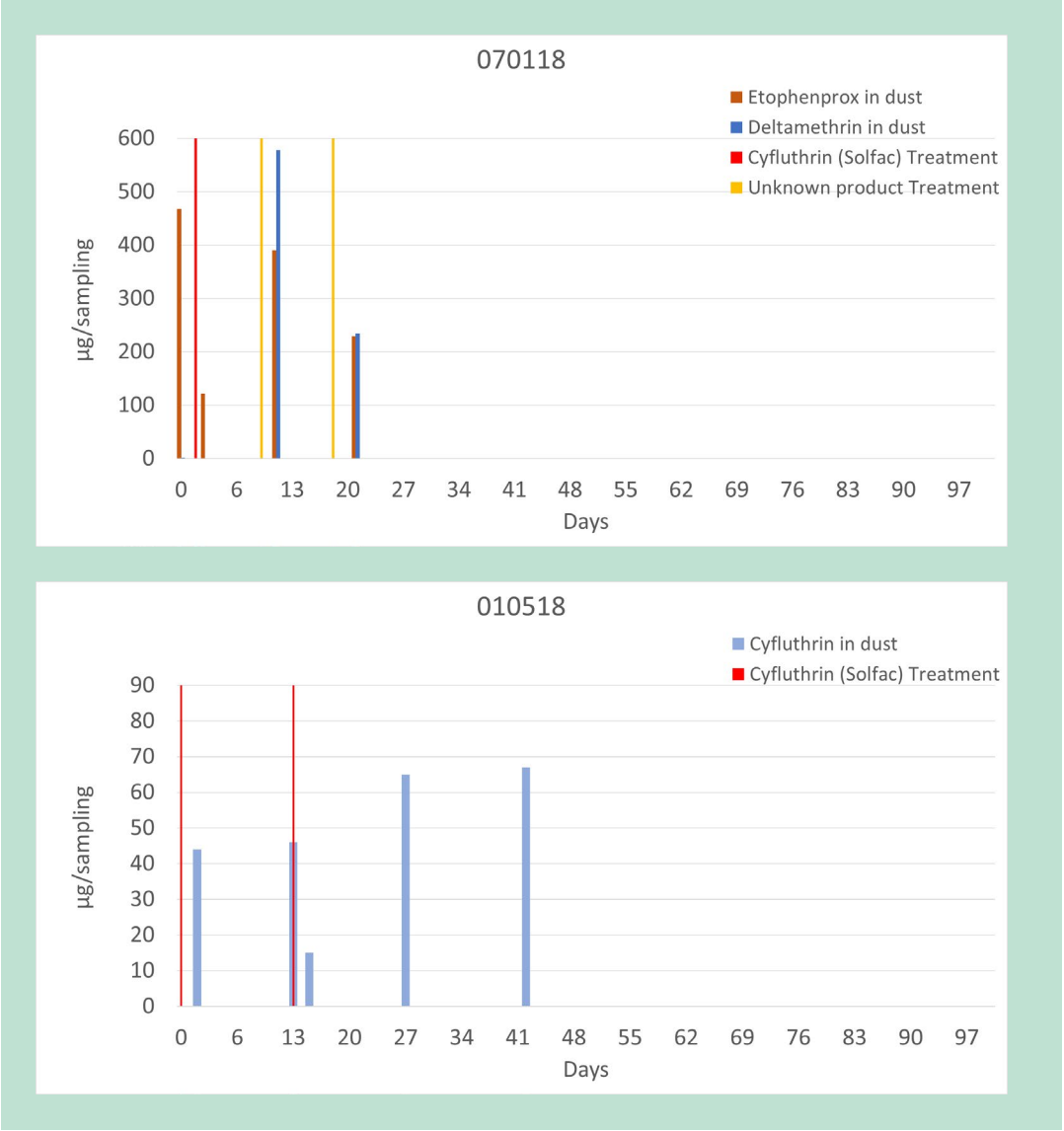


FIGURE 4. Illustration of treatments (vertical lines) and pyrethroids measured in dust samples at the two first locations in the longitudinal study.

FIGURE 5. Illustration of treatments (vertical lines) and pyrethroids measured in dust samples at the two last locations in the longitudinal study.

TABLE 10. Pyrethroids found in sock and dust samples.

		Sock (µg/sample)					Dust sample (µg/sample)					
Location	**Day p.t.**	**Deltamethrin**	**λ-cyhalothrin**	**Permethrin**	**Cyfluthrin**	**Etofenprox**	**Dust (g)**	**Deltamethrin**	**λ-cyhalothrin**	**Permethrin**	**Cyfluthrin**	**Etofenprox**
070118	**-1**	<LOD	<LOD	<LOD	<LOD	2	7.5	1	6	<LOD	<LOD	468
	1	<LOD	<LOD	<LOD	<LOD	13	5.1	<LOD	2	<LOD	<LOD	122
	10/2	287	<LOD	<LOD	<LOD	15	6.5	578	2	8	<LOD	390
	20/12	71	<LOD	<LOD	<LOD	10	7.2	234	2	2	<LOD	229
	45/37	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
010518A+B	**0**	<LOD	<LOD	<LOD	<LOD	<LOD	4.6	1	<LOD	<LOD	<LOD	1
	1	<LOD	<LOD	<LOD	35	<LOD	4.3	1	<LOD	<LOD	44	1
	13/0	<LOD	<LOD	<LOD	<LOD	<LOD	5	5	<LOD	2	46	1
	14/1	<LOD	<LOD	<LOD	14	<LOD	4.6	1	<LOD	<LOD	11	<LOD
	27/14	<LOD	<LOD	<LOD	<LOD	<LOD	4.9	1	<LOD	<LOD	65	1
	42/29	<LOD	<LOD	<LOD		<LOD	4.7	2	<LOD	1	67	<LOD
240518A+B	**0**	<LOD	<LOD	<LOD	7	<LOD	4.9	24	1	1	333	1
	1	5	<LOD	<LOD	5	<LOD	4.7	87	1	1	195	<LOD
	15	2	<LOD	<LOD	<LOD	<LOD	5.8	166	1	2	269	1
	25/1	18	<LOD	1	14	<LOD	4.3	76	4	7	148	<LOD
	38/14	18	<LOD	<LOD	7	<LOD	4.8	211	1	2	248	<LOD
	64/51	12	<LOD	<LOD	5	<LOD	4.9	54	<LOD	1	77	<LOD
150918A+B	**-2**	<LOD	1	<LOD	<LOD	<LOD	4.2	1	11	<LOD	<LOD	<LOD
	2	12	2,274	<LOD	<LOD	<LOD	4.8	13	1,080	1	<LOD	<LOD
	16/-1	<LOD	50	<LOD	<LOD	<LOD	4.5	7	572	1	<LOD	<LOD
	20/3	<LOD	62	<LOD	<LOD	<LOD	4.4	3	476	1	<LOD	<LOD
	36/19	<LOD	23	<LOD	<LOD	<LOD	4.5	3	648	<LOD	<LOD	<LOD
	50/33	<LOD	36	<LOD	<LOD	<LOD	4.8	6	584	1	<LOD	<LOD

[a] Missing sock and dust sample

6.2.2 Pyrethroid metabolites in urine samples

TABLE 11. Individual concentrations of pyrethroid metabolites in urine samples collected from residents in the longitudinal study at different days after treatment

		Urine (µg/g creatinine)						mmol/g creatinine
Location	Day p.t.	Trans-DCCA	Cis-DCCA	Cis-DBCA	CF3CA	4F-3-PBA	3-PBA	3-PBA+ 4F-3-PBA
070118	-1	0.43	0.18	0.10	0.16	0.04	0.83	4.03
	1	0.24	<LOD	0.07	0.08	0.115	3.25	15.65
	10/2	0.63	<LOD	0.42	0.47	<LOD	2.55	12.17
	20/12	1.65	<LOD	0.87	0.18	<LOD	1.90	11.27
	45/37	0.26	0.07	0.12	0.15	0.08	1.84	8.95
010518A/ 010518B	0	0.17/ 0.08	0.14/ 0.07	0.39/ 0.03	0.09/ 0.02	<LOD/ <LOD	0.29/ 0.05	2.35/ 0.44
	1	0.44/ 0.12	1.74/ 0.06	0.12/ 0.03	0.10/ 0.17	0.13/ 0.02	3.25/ 0.11	2.27/ 1.02
	13/0	1.41/ 0.28	0.13/ 0.37	0.07/ 0.04	0.14/ 0.09	0.24/ 0.04	1.30/ 0.27	7.12/ 1.45
	14/1	1.44/ 0.23	<LOD/ 0.15	0.18/ 0.09	0.08/ 0.15	0.29/ 0.07	1.52/ 0.34	8.34/ 1.89
	27/14	0.23/ 0.09	0.33/ 0.10	0.15/ 0.05	0.16/ 0.07	0.17/ 0.07	0.28/ 0.09	2.03/ 0.72
	42/29	1.41/ 0.03	0.17/ 0.16	0.48/ 0.11	0.23/ 0.04	0.34/ 0.09	1.56/ 0.07	8.73/ 0.70
240518A/ 240518B	0	0.40/ 0.17	0.32/ 0.16	0.36/ 0.04	0.07/ 0.06	0.16/ 0.15	0.02/ 0.19	0.79/ 1.53
	1	0.47/ 0.22	0.15/ 0.12	0.54/ 0.07	0.07/ 0.04	0.28/ 0.21	0.10/ 0.19	1.70/ 1.65
	15	0.39/ 0.15	0.23/ 0.09	0.64/ 0.11	0.14/ 0.17	0.13/ 0.10	0.21/ 0.18	1.53/ 1.27
	25/1	0.37/ 0.15	0.39/ 0.29	0.23/ 0.29	0.15/ 0.06	0.24/ 0.16	0.20/ 0.24	1.97/ 1.80
	38/14	0.52/ 0.14	0.21/ 0.46	0.29/ 0.10	0.17/ 0.05	0.11/ 0.13	0.23/ 0.22	1.53/ 1.58
	64/51	0.59/ 0.19	0.57/ 0.93	0.27/ 0.27	0.11/ 0.08	0.10/ 0.15	0.20/ 0.20	1.38/ 1.62
150918A/ 150918B	-2	5.50/ 0.07	2.50/ <LOD	3.26/ 0.02	<LOD/ 0.05	5.43/ <LOD	7.51/ 0.09	58.43/ 0.83
	2	22.43/ 0.07	25.38/ 0.05	32.16/ 0.01	<LOD/ 0.04	23.19/ 0.02	40.19/ 0.11	287.49/ 0.56
	16/-1	0.28/ 0.78	0.22/ 0.19	0.04/ 0.80	0.04/ 0.62	0.02/ 0.01	0.10/ 0.69	0.53/ 3.25
	20/3	0.17/ 1.98	0.29/ 0.21	<LOD/ 0.80	0.26/ 0.43	<LOD/ <LOD	0.26/ 1.11	2.59/ 5.68
	36/19	0.77/ 2.16	0.71/ 6.24	0.29/ 0.15	1.06/ 2.27	<LOD/ <LOD	1.18/ 1.12	7.24/ 8.38
	50/33	0.25/ 0.36	0.73/ 0.19	<LOD/ 0.04	0.18/ 0.36	<LOD/ 0.17	0.27/ 0.24	2.22/ 1.84

Table 11 shows the individual urinary concentrations of pyrethroid metabolites in relation to days after treatment. No clear pattern was seen between days post treatment and urinary pyrethroid metabolite concentrations. In locations with two residents, a marked difference in urine concentrations was often seen.

6.2.3 Pyrethroid metabolites in blood samples

The only pyrethroid metabolite that could be detected in the blood plasma was 3-PBA. Comparing the levels of 3-PBA in urine samples and in the blood plasma shows a significant correlation coefficients (spearmans rho) of 0.74 (see figure 6 and Table 14).

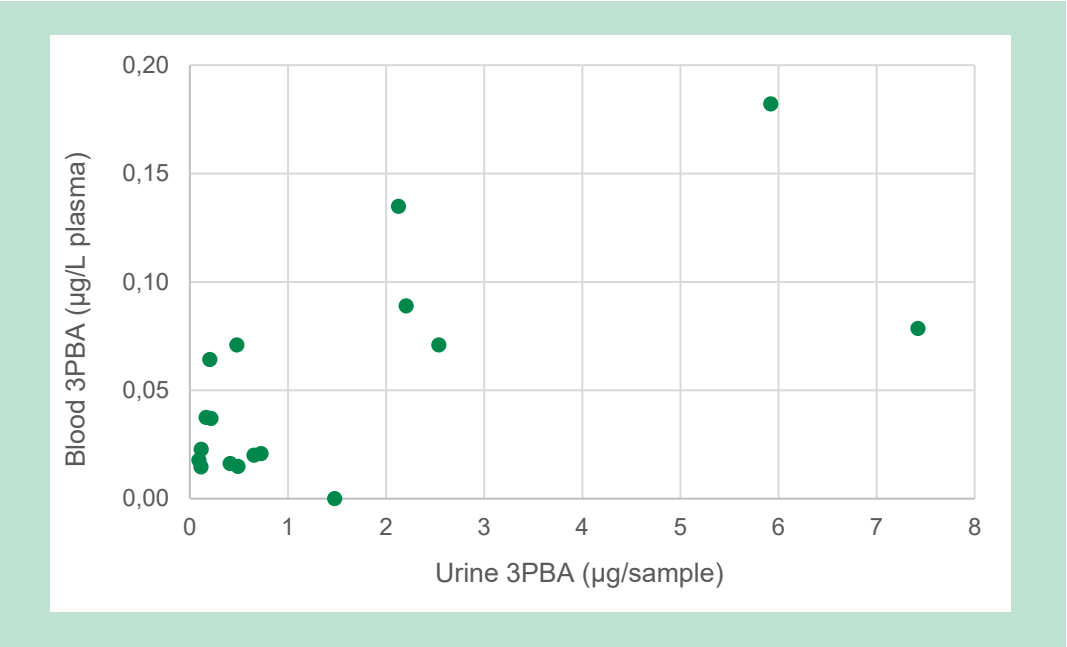


FIGURE 6. Blood 3-PBA in relation to Urine 3-PBA, excluding the outlier 150918A (see section 3.3).

The two other pyrethroid metabolites that were included in the quantitative analysis 3-(4-hydroxy)phenoxybenzoic acid and (1-R-cis)decamethrinic acid (cis-DBCA) were not detected. The QTRAP-based attempts to identify further metabolites did not succeed. EMS-IDA-EPI are broad screenings in which sensitivity is low. MRM-IDA-EPI analyses are substantially more sensitive and can be directed towards the search for instance theoretical conjugates of known metabolites. The MRM-IDA-EPI analyses unfortunately did not give results either. The concentrations probably were too low.

6.3 Combined data from horizontal and longitudinal studies

One individual (code number 150918A) had much (>10 fold) higher urinary concentrations of several PYR-metabolites (Table 8) both two days before, and especially, two days after treatment. On the same days, very high levels were also seen in the sock dosimeter as well as in the dust samples from this locality (Table 10). Thus, this location was contaminated before treatment. Therefor data for the urine samples collected before treatment from the two individuals in this location were excluded from further data analyses to give a more correct picture.

TABLE 12. Detection frequency of pyrethroid metabolites in urine samples collected among residents before/no treatment and after treatment

Metabolite	Percentage of samples above limit of detection		
	All (n=62)	Treated (n=50)	Untreated (n=12)
3-PBA	98.4	100	91.7
Trans-DCCA	41.9	44.0	33.3
Cis-DCCA	11.3	12.0	8.3
4F-3-PBA	24.2	28.0	8.3
Cis-DBCA	14.5	16.0	8.3
CPBA*	0	0	0
CF3CA*	59.0	62.9	25.0

*only analyzed in WP2

CPBA was not detectable in any samples. All other specific pyrethroid metabolites were detected more frequently in urine samples from treated locations than untreated locations. The common metabolite, 3-PBA, was detectable in all urine samples from treated locations and all except one sample from untreated locations.

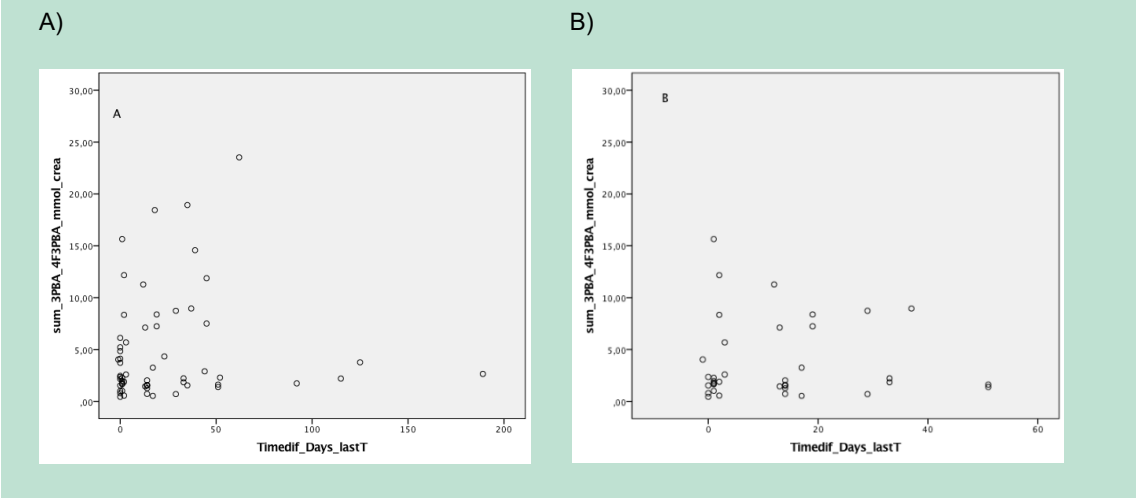


FIGURE 7. Association between urinary concentration of the total pyrethroid metabolite concentration estimated by the molar sum of 3-PBA and 4F-3-PBA and number of days after last pyrethroid treatment. A) shows data from both the horizontal (WP1) and the longitudinal (WP2) studies. B) shows data from the horizontal study separately. One sample with a very high content (287 mmol/g creatinine) at day 2 after treatment in the longitudinal study is not shown

The highest concentrations of the molar sum of 3-PBA and 4F-3-PBA was observed within the first two weeks after treatment but some samples were still relatively high 40-50 days after last treatment (Figure 7).

TABLE 13. Concentrations of pyrethroids metabolites in blood and urine samples collected from residents in untreated homes and at different time points after pyrethroid treatment.

		Median (maximum)			
Metabolite	unit	untreated	1-3 days after treatment	4-14 days after treatment	15-60 days
Blood					
n		7	14	9	11
3-PBA	µg/L plasma	<LOD (0.02)	0.01 (0.18)	<LOD (0.07)	0.02 (0.14)
Urine					
n		13	14	15	14
Sum of 3-PBA and 4F-3-PBA	mmol/g creatinine	2.46 (6.12)	2.12 (287.5)	2.02 (18.44)	2.59 (18.93)
3-PBA	µg/g creatinine	0.36 (0.95)	0.30 (40.2)	0.28 (3.80)	0.34 (3.94)
Trans-DCCA	µg/g creatinine	<LOD (2.81)	0.31 (22.4)	<LOD (2.16)	<LOD (3.02)
Cis-DCCA	µg/g creatinine	<LOD (1.26)	<LOD (25.4)	<LOD (6.23)	<LOD (11.47)
4F-3-PBA	µg/g creatinine	<LOD (0.50)	<LOD (23.2)	<LOD (0.73)	<LOD (2.28)
Cis-DBCA	µg/g creatinine	<LOD (1.63)	<LOD (32.2)	<LOD (0.87)	<LOD (0.56)
CF3CA	µg/g creatinine	<LOD (0.16)*	0.12 (0.47)	0.16 (2.27)	0.14 (0.36)**

*n=5, **n=7

TABLE 14. Correlation coefficients (spearmans rho) between the generic pyrethroid metabolite, 3-PBA in blood and urine, specific urinary metabolites of cis- and trans forms of permethrin, cy-permethrin, cyfluthrin (trans- and cis DCCA), deltamethrin (cis-DBCA), and cyhalothrin and bifenthrin (CF3CA) and concentrations of pyrethroids measured in dust samples collected by the sock method or by vacuum.

	Blood 3-PBA	Urine 3-PBA	Trans-DCCA	Cis-DCCA	Cis-DBCA	CF3CA
Blood 3-PBA	**1.00**	**0.59**	0.29	-0.05	-0.01	0.23
Deltamethrin sock	-0.09	-0.09	0.15	0.06	0.25	-0.10
Deltamethrin dust	-0.08	-0.27	0.15	0.18	0.26	-0.02
Deltamethrin per g dust	-0.09	-0.29	0.13	0.16	0.26	0.00
λ-cyhalothrin, sock	0.29	0.19	0.13	0.06	-0.02	**0.36**
λ-cyhalothrin, dust	**0.34**	0.22	0.22	**0.33**	0.23	**0.37**
λ-cyhalothrin, g dust	0.29	0.19	0.18	0.29	0.20	**0.32**
Permethrin, sock	-0.19	0.12	0.17	0.07	0.17	-0.10
Permethrin, dust	0.05	-0.13	0.15	0.18	0.24	0.02
Permethrin per g dust	-0.00	-0.14	0.14	0.15	0.23	0.09
Etofenprox sock	**0.46**	**0.33**	0.10	-0.10	0.07	0.18
Etofenprox dust	0.12	-0.02	-0.16	**-0.33**	-0.21	-0.02
Etofenprox g dust	0.05	-0.08	-0.21	**-0.40**	-0.17	0.05
Sum in sock	0.31	0.23	0.24	0.10	0.16	**0.37**
Sum in dust	**0.37**	0.17	0.27	0.28	0.26	**0.42**
Sum per g dust	**0.36**	0.17	0.26	0.28	0.27	**0.42**

Bold indicate $p < 0.05$, shadow cells indicate where positive correlations would be expected

No clear pattern was found in the correlation analysis (Table 14). In some cases, significant correlations were found but this was not consistent. There was a significant correlation between 3-PBA in urine and blood and the specific λ-cyhalothrin metabolite, CF3CA, was significantly correlated with λ-cyhalothrin in dust samples.

7. Discussion

7.1 Information by PCO to residents

The most general information was that the residents should stay out of the room until the treatment had dried. This period varied between 30 minutes to 3 hours. The challenge in this connection is that it is generally accepted that it is important that the treated rooms are in use by the residents, because otherwise the bed bugs will remain in their hiding places. Furthermore, vacuuming and cleaning the treated rooms is considered problematic for the efficacy of the treatment and, in most cases, it was a general recommendation that there should be no vacuuming and cleaning for approximately two weeks, post treatment.

In a few cases the PCO had informed the residents that they should avoid walking bare footed or that they should wear socks and shoes for the whole day. One PCO also stated that the residents should wash their hands and avoid eye rubbing, Otherwise, there were no precautions with regard to unattended exposure.

Interestingly, only about half the cases had positively identified bed bugs (or any other pest) before treatment was initiated. It was our impression in several cases that the problem was not bed bugs, but possibly something else. Clearly, it is not possible to make a safe identification that there are **no** bed bugs. Anyway, more focus on evaluating the signs and symptoms of bed bugs before initiating a treatment could lower the risk of resident being unnecessarily exposure to insecticides.

Previous studies have shown that many bed bug populations in Denmark (Kilpinen et al 2011), as in rest of the world (Moore and Miller 2006, Romero et al 2007), have a lowered sensitivity to pyrethroids. In the present study there were several cases where it was necessary to treat multiple times over prolonged periods and still the infestations were not overcome. The synthetic pyrethroids are supposedly quite persistent but in many cases the bed bug populations are still not eradicated, and repeated treatments over several weeks are necessary. Thus, it seems likely that the efficacy in practice is not as good as it should be, probably due to the reduced sensitivity of the bed bug. Thus, it might be worth considering to recommend that treated rooms are cleaned thoroughly, two weeks after treatment. Here it was reported that the PCOs recommended not cleaning for two weeks, but they gave no direct information about what to do after these two weeks.

7.2 Persistence of pyrethroids

Although high concentrations were obviously observed right after treatment in some cases, there were no clear relationship between time since treatment and the concentration of pyrethroids found in the local environment. Even after months quite high concentrations were observed. This is a clear indication that residents are living with a potentially continuous exposure risk. Even though it could not be measured directly in the present investigation, the presence of pyrethroids long after treatment, makes it clear that repeated treatments may result in accumulation of pyrethroids in private homes. In the few cases where the PCO had treated many times, extremely high concentrations were found (up to 46 mg a.i. in a dust sample). Mostly there is no clear advice on the product labels to inform the PCO how often they may repeat the treatment with the same or another product. One product on the Danish marked says

that treatment can be repeated 11 times per year, while others says nothing at all or that a repeated treatment can be necessary in some severe cases.

7.3 House fly bioassay

In WP 1 flies were used to identify contamination with insecticidal substances in the flats or houses. Two important observations could be drawn from this, first that the susceptible flies were affected in all treated residences from the shortest (23 days) to the longest period (189 days) after treatment. Concerning the resistant strain, the picture varied, but only in one residence did the mean mortality exceed 50%. These low mortalities, even after relatively excessive treatments indicate that the potential for further selection for resistance is enhanced even half a year after the last treatment.

7.4 Urinary pyrethroid metabolites

A wide variation in urinary concentrations of pyrethroid metabolites was observed among residents from homes treated with pyrethroids. Although the median concentrations were in general not higher than among residents from untreated homes, some individuals from treated locations had markedly higher concentrations. Such a variation was expected, since the internal exposure will depend on many factors, such as type of activities carried out in the home and residence time after treatment, the interior of the dwelling (e.g. carpets) and ventilation.
Our results are in accordance with results obtained among farm workers exposed occupationally to pyrethroids, showing excretion profiles related to working task and number of entries in treated areas and rapid elimination between exposure periods. Thus, maximum urinary concentrations in most workers were reached 18-32 hours after onset of an exposure episode (Ferland et al., 2015; Ratelle et al., 2016).These studies were based on voids of all urine collected during three working days while we have used spot urine samples, which only reflect a relatively short period of time and, thus, induce more variation.

The advantage of using biomonitoring to assess exposure is that it provides an integrated measure of the internal exposure through all exposure routes. Pyrethroids are rather lipophilic substances shown to be very stable in house dust (Leng et al., 2005) and, dependent on how the products are formulated, they can be absorbed through the skin, airways or the mouth (orally). The use of pyrethroids indoors in the home can therefore result in a direct but complex exposure of the residents via different routes. The absorption rates of pyrethroids depend on the route of exposure and product formulation. At least for permethrin and cypermethrin, the absorption rate was reported to be highest after inhalation and lowest through the skin (Cote and Bouchard, 2018). Thus, inhalation is likely the major exposure route after indoor use of pyrethroids, but oral and dermal exposure may also contribute.

After absorption, pyrethroids are metabolised and excreted in urine and faeces within a few days. However, after prolonged exposure, pyrethroids can build up in fatty tissue and remain in the body for longer time (European Food Safety Authority, 2011) as also predicted from toxicokinetic modelling (Cote et al., 2014).

Besides their use as biocides for indoor insect control, pyrethroids are among the most used insecticides in agriculture in Denmark and worldwide. Hence, residues in food is another important exposure source for the general population and the most likely explanation why residents in homes that were not treated with pyrethroids, have detectable concentrations of metabolites in urine. While the dietary exposure is assumed to be rather low but continuous, the exposure level from residential pest control will fluctuate in relation to treatment periods and individual activities and behavior leading to more variation in urine concentrations (Morgan et

al., 2016; Wielgomas, 2013). To get a more precise picture of the internal exposure profile after treatment would require collection of more repeated urine samples before and especially after treatment as well as access to detailed information on which products were used and the quantities used. However, it turned out to be more difficult than assumed to collaborate with the PCO companies regarding recruitment of participants and to obtain the relevant information. This is a clear limitation of this study, but nevertheless our study demonstrates high exposure levels among some residents after treatment. Hence, the 95 percentiles of the pyrethroid metabolite concentrations observed in this study were considerably higher than among pregnant women (Odense Child Cohort, OCC) from the general Danish population assumed mainly to be exposed from residues in the diet (Table 15). The 95 percentiles in this study, were also higher than in the other European studies, probably because the samples in those studies were not collected directly in connection with indoor pyrethroid treatment.

7.5 Pyrethroid metabolites in blood samples

Measuring pyrethroid metabolites in blood samples is less well establishing than measuring on urine samples. The present results indicated that it could be relevant to included measures on 3-PBA in blood samples in future screening studies, but much more data is needed in order to evaluate the relationship between exposure levels and metabolite levels in the blood.

7.6 Exposure levels – comparison with regulatory safety levels

Oral exposure levels of pyrethroids (and other pesticides) are regulated via Acceptable Daily Intake, ADI, values established from experimental studies. To enable comparison with these "safety doses", an estimated dose can be calculated from urinary metabolite concentrations:

ED = $CU \times V \times \frac{1}{fu}$, where ED is the estimated daily dose (µg/kg/day), *CU* is the urine concentration of the metabolite (µg/L), *V* is the human daily excretion volume of urine (1,7 L/day for adults ≅ 0,024 L/kg/day if body weight is 70 kg), and f_u is the fraction of the metabolite excreted in urine relative to the total dose (µg), of the parent pyrethroid.

This calculation is normally used to estimate the dose after oral exposure via the diet (Aylward et al., 2018). Hence, the estimated dose reflects the oral dose of the parent compound, that would give rise to the urinary concentration used in the calculation. The f_u-values are calculated from the molar excretion fraction (f_m).

$fu = fm \times \frac{MWum}{MWp}$, where MW_{um} and MW_p are molar weights of the metabolite and the parent pyrethroid, respectively.

For 3-PBA, f_m has been estimated to 9% and the corresponding f_u-values are 0.04 for cyhalothrin and deltamethrin, and 0.05 for permethrin (Aylward et al., 2018). Using the 95-percentile for 3-PBA found in this study (Table 15), EDs for deltamethrin, λ-cyhalothrin, and permethrin can be estimated.

Deltamethrin and λ-cyhalothrin:
ED = 7.05 µg/L x 0.024 L/kg bw/day x 1/0.04 = 4.23 µg/kg bw/day = 0.004 mg/kg bw/day.

Permethrin:
ED = 3.38 µg/kg bw/dag = 0,003 mg/kg bw/dag.

For comparison, the current ADI values in the EU are:
Deltamethrin: 0.01 mg/kg bw/day,

λ-cyhalothrin: 0.0025 mg/kg bw/day,
Permethrin: 0.05 mg/kg bw/day

Hence, if the 95-percentile concentration of 3-PBA of 7.05 µg/L urine was formed solely from λ-cyhalothrin, the ADI would be exceeded. EDs based on concentrations of the more specific metabolites require available f_u or f_m-values of these metabolites.

ADI values for pyrethroids are based on studies in rodents after oral exposure and define the dose an individual can (presumably) be exposed to on a daily basis over the entire life span without causing adverse health effects. Comparison with ADI-values, as indication of health risk, after indoor use of pyrethroids has some limitations. First, an internal exposure level achieved after dermal and inhalation exposure routes may be more harmful than after oral exposure because first-pass metabolism in the liver is bypassed. Thus, more of the active substance can reach target organs before being metabolised. Secondly, sensitive experimental models for endocrine disruption and disturbance of neurodevelopment have not yet been included in regulatory testing of pesticides used for establishment of the ADI-values. Thus, the ADI-values may not protect vulnerable population groups, such as pregnant women and young children, against such effects. Accordingly, pyrethroids at exposure levels occurring in the general population have been associated with neurobehavioral effects (Dalsager et al., 2019; Furlong et al., 2017; Viel et al., 2017) and adverse effects on reproductive outcome (Li et al., 2018; Meeker et al., 2009; Saillenfait et al., 2015). So, even though the high exposure levels seen for some individuals in this study is likely temporary it might be of concern if exposure occurs during vulnerable periods in fetal life and/or childhood.

TABLE 15. Urinary concentrations of pyrethroid metabolites in different studies. The values are volume-based concentrations (µg/L) in spot urine samples.

Study, country	Population	Sampling year	N	Metabolite	LOD	%>LOD	50th pct (median)	95th pct	Indoor use**	Ref
This study, DK	All participants	2018	32 (64 urine samples)	3-PBA	0,03	98,4	0,36	7,05	Yes, for 43 samples (67%)	
				4F3-PBA	0,2	25,0	<LOD	2,51		
				Cis-DCCA	0,5	12,5	<LOD	2,77		
				Trans-DCCA	0,4	42,2	<LOD	3,78		
				Cis-DBCA	0,5	15,6	<LOD	0,98		
OCC, DK	Pregnant women	2010-12	1195	3-PBA	0,03	93,9	0,20	2,18		Dalsager et al. (2018); Dalsager et al. (2019)
				4F3-PBA	0,2	0,1	<LOD	<LOD		
				Cis-DCCA	0,5	2,7	<LOD	<LOD		
				Trans-DCCA	0,4	12,1	<LOD	1,34		
				Cis-DBCA	0,5	2,6	<LOD	<LOD		
PELAGIE, France	Children, 6 y	2009-12	245	3-PBA	0,008	63	0,02	0,20	yes, 33% of the families	Glorennec et al. (2017)
				4F3-PBA	0,003	16	<LOD	0,02		
				Cis-DCCA	0,067	64	0,09	0,49		
				Trans-DCCA	0,01	95	0,22	1,75		
				Cis-DBCA	0,067	84	0,20	1,12		
Elfe, France	Pregnant women	2011	1077	3-PBA	0,004	100	0,36	1,89	Yes, 46% of the families	Dereumeaux et al. (2018)
				4F3-PBA	0,005	17,8	<LOD	0,02		
				Cis-DCCA	0,003	100	0,16	0,91		
				Trans-DCCA	0,006	100	0,26	2,29		
				Cis-DBCA	0,005	100	0,23	1,38		
Poland	Adults (N=190) and children (N=184)	2012	374	3-PBA	0,1	82,4	0,25	1,24	Half rural and half urban population	Wielgomas and Piskunowicz (2013)
				Cis-DCCA	0,1	46,0	<LOD	0,89		
				Trans-DCCA	0,1	46,8	<LOD	1,00		
				Cis-DBCA	0,1	17,1	<LOD	0,50		
Germany	Adults without known pyrethroid exposure, 26-58 y	2012	38	3-PBA	0,01	100	0,22	1,79		Schettgen et al., 2016)
				4F3-PBA	0,01	5	<LOD	<LOD		
				Cis-DCCA	0,01	100	0,08	0,57		
				Trans-DCCA	0,01	100	0,17	0,92		
				Cis-DBCA	0,01	80	0,04	0,28		
				ClF3CA	0,01	90	0,04	0,98		
				CPBA		40	<LOD	0,08		
NHANES, USA	Adults, 20-59 y	2009-10	1309	3-PBA	0,1		0,39	6,95		CDC (2015)

				4F3-PBA	0,1		<LOD	<LOD		
				Trans-DCCA	0,6		<LOD	5,88		
				Cis-DBCA	0,6		<LOD	<LOD		
SUPERB, USA	Children, 2-8 y	2007-09	83	3-PBA	0,6	60	0,75	4.69	Yes	Trunnelle et al. (2014b)
	Adults, 18-57y		90	3-PBA	0,6	64	0,82	9.44		
MICASA, USA Farmer families	Children, 2-8 y	2009	103		0.1	78	1,93	7,36	Yes, 43%	Trunnelle et al. (2014a)
	Mothers, 23-52 y		105			82	1,63	8,24		
CHMS, Canada	6-79 y	2007-09	5604	3-PBA	0.05	99.4	0,22	2,90		Ye et al. (2015)
				4F3-PBA	0,03	42,4	<LOD	0,08		
				Cis-DCCA	0,03	98,5	0,07	0,94		
				Trans-DCCA	0,05	99,6	0,17	2,50		
				Cis-DBCA	0,02	47,5	<LOD	0,07		
VHEMBE, South Africa	Pregnant women	2012-13	695	3-PBA		100	0,70	2,38 [a]	Yes, pyrethroids applied for mosquito control	Eskenazi et al. (2018)
				4F3-PBA		12,5	<LOD	0,01 [a]		
				Cis-DCCA		100	0,30	1,03 [a]		
				Trans-DCCA		100	0,34	1,48 [a]		
				Cis-DBCA		100	0,22	1,12 [a]		

** confirmed indoor use of pyrethroids prior to sampling, [a] 90th pct

8. Perspectives

This study demonstrates enhanced exposure levels among residents living in homes treated with synthetic pyrethroids. A wide variation in urinary concentrations of pyrethroid metabolites was observed among residents from homes treated with pyrethroids. Although the median concentrations were in general not higher than among residents from untreated homes, some individuals from treated locations had markedly higher concentrations. Such a variation was expected, since the internal exposure will depend on among other things, the activities that individuals are carrying out in the home.

Although high concentrations of pyrethroids in the surroundings were observed right after treatment in some cases, there were no clear relationship between time since treatment and the concentration of pyrethroids found in the local environment. Even after months quite high concentrations were observed. This is a clear indication that residents are living with a potentially continuous exposure risk. Even though it could not be measured directly in the present investigation, the presence of pyrethroids long after treatment, makes it clear that repeated treatments may result in accumulation of pyrethroids in private homes.

Despite the large variation seen in the present investigation it is clear that there are problematic situations where residents are exposed to relatively high doses of insecticides and often for several weeks following treatment. This raises a number of questions to be dealt with in the future.

One aim of the questionnaire was to identify variation in guidance given by the PCO's. It was revealed that there is a need for an initiative to make a qualified guidance for PCO's doing treatment in private houses. Some of the focus points should be:

1. How much and in which form must a customer be informed about the process around a treatment?
2. When and how must the house be cleaned, especially when a thorough cleaning can be performed as an endpoint to the treatment?
3. How can the PCO ensure that the suggested treatment is relevant in the circumstances i.e. are the pest species identified correctly or identified at all?
4. Are there any special precautions that parents to small children should know of and likewise with pregnant women?

In relation to registration of pest control products, an important problem has been identified, namely that by shifting PCO and product, no one have knowledge about the amount of biocide distributed on the premises at any given time. This was clear as several cases of multiple a.i. residues were observed following long treatment sequences.

5. How can the regulating authorities secure that the label or other instructions not only claim the gross number of treatments allowed per year with one product, but also clearly states the maximum number of repeated treatments with different products, as well as the interval between treatments?
6. How to ensure that succeeding PCO companies get information about the previous treatment history as well as knowledge about how to use such information?

This investigation with its aim to identify the exposure of occupants to insecticide contaminated houses turned out to be heavily affected by the ambition not to interfere with neither the PCO's work nor the occupant's daily life during the treatment. This turned out to be extremely compli-

cated, as coordination between all the participants, as well as all sorts of delays made it difficult to obtain consistent data collection. Nevertheless, the investigation clearly showed that the concept is correct and therefore emphasizes that such studies must be done.

Concerning future research there are several points of interest:

7. What is the risk for the residents under practical exposure situations as found in this project? It is not one product and one registration that people meet in the real world, it is a conglomerate of products, doses, and residues. Thus, it is not the registration of one product and the toxicological data behind that specific product that is relevant, but the whole conglomerate of products and formulations, which is not the responsibility of one single company.
8. It was not possible to find correlations between pyrethroids in the environment and pyrethroid metabolites in urine and blood samples, due to the large variation. Thus, more controlled studies are needed with more focus on controlled exposure risk. Paying the PCO and the participants for any inconvenience could make it easier to run a consistent data collection. Also, a larger number of participants is needed to overcome the large variation observed and all relevant a.i.'s must be included
9. How can resistance problems be bypassed in a situation as the one described here, where all available a.i. attack the same mechanism in the insects and therefore no product working on alternative resistance mechanisms can be suggested? This is meant in the broadest possible way, including new a.i., new formulations, physical control methods, reliable monitoring of pests etc.
10. The present study implies that there is an accumulation of insecticides in close vicinity of humans, but as the study was designed as a screening, much more detailed studies on people living in treated homes is needed to quantify the risk.

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Appendix 1. Bed bug products

TABLE 16. Products registered for treatment against bed bugs in Denmark as of 1/6 2019 (Danish Environmental Agency)

Product	A.i.	CAS-nr.	Concentration	Registration date
Fenox	etofenprox	80844-07-1	300 g/l	27-10-2017
K-Othrine SC 25	deltamethrin	52918-63-5	25 g/l	01-08-2017
Demand 10 CS *	lambda-cyhalothrin	91465-08-6	100 g/l	22-10-1998
Linie Kemi RTU Proff	esbiothrin	260359-57-7	0,4 g/l	12-02-2015
	permethrin	52645-53-1	2,4 g/l	
Linie Kemi RTU Xtra	permethrin	52645-53-1	24 g/kg	27-02-2009
	esbiothrin	260359-57-7	4 g/kg	
Material Shop Insektspray	pyrethrin I and II	8003-34-7	2,5 g/kg	13-10-2015
	permethrin	52645-53-1	2 g/kg	
Ny Bonus Myremiddel	permethrin	52645-53-1	2,4 g/l	20-02-2009
	esbiothrin	260359-57-7	0,4 g/l	
Perma Forte B	permethrin	52645-53-1	15 g/kg	14-10-2015
	pyrethrin I and II	8003-34-7	5 g/kg	
Tanaco RTU Xtra	permethrin	52645-53-1	2,4 g/kg	27-02-2009
	esbiothrin	260359-57-7	0,4 g/kg	
Trinol Nr. 810 Insektmiddel Original	pyrethrin	8003-34-7	3 g/kg	16-10-2015
	permethrin	52645-53-1	10 g/kg	
Trinol nr. 810 myremiddel	esbiothrin	260359-57-7	0,4 g/l	25-01-2012
	permethrin	52645-53-1	2,4 g/l	
Solfac 50 EW**	cyfluthrin		50 g/l	21-12-2009

* Use and possession of Demand has been prohibited since 28/9 2018
** approval expires 21,12,2019

Appendix 2. Description of test localities

Appendix 2.1 Horizontal study

TABLE 17. Treated locations. Tested person is mentioned first among residents.

	Residents	Pets	Home	Carpet co-verage	Diet (ca %)
140416-1	Male (76)	dog	House, >3 rooms	75-100%	0%
150916-1	Female (>60), Male	-	House, >3 rooms	75-100%	0%
150916-2	Female*, male, child	Cat	House, 2 rooms	0%	80%
280916-1	Female, 2 children, 2 tenants	Cat	House, >3 rooms	0%	80%
111016 -1	Male (36), Female, 2 children	-	Apartment, >3 rooms	0%	80%
121016 - 1	Male (34)	-	Apartment, 2 rooms	0%	100%
131016 - 1	Female	-	Apartment, 3 rooms	0%	60%
131016 - 2	Female, 2 females	-	Apartment, 3 rooms	0-25%	20%
251016 - 1	Male (41)	-	Apartment, 3 rooms	0%	0%
251016 - 2	Female (30), Male, 3 children	-	Apartment, >3 rooms	0%	0%
271016 - 1	Female (>62) Male	Cat	House, >3 rooms	0%	100%
161216 -1	Female (20), female	-	Apartment, 2 rooms	0%	20%
240217 -1	Male (69)	-	House, >3 rooms	0-25%	60%
100317 -1	Male, female, 2 children	-	House, >3 rooms	0%	100%
100317 -2	Female (38)	Dog	Apartment, 1 room	0-25%	100%
70118	Female	-	Apartment, 1 room	0%	0%
010518A	Female (27), 2 Male	Cat	Apartment, 3 rooms	0%	60%
010518B	Male (25), Female, male	Cat	Apartment, 3 rooms	0%	40%
240518A	Female, male	-	House, >3 rooms	25-50%	20%
240518B	Male, female	-	House, >3 rooms	25-50%	20%
150918A	Female (32), male, 2 children	-	Apartment, >3 rooms	50-75%	100%
150918B	Male (36), female, 2 children	-	Apartment,> 3 rooms	50-75%	100%

* Female pregnant during the treatment period and pregnant again at the sampling date.

TABLE 18. Untreated control locations. Tested person is mentioned first among residents.

	Residents	Pets	Home	Carpet co-verage	Diet (ca %)
140416-1	Female (27), male	-	Apartment, 3 rooms	0-25%	20%
150916-1	Male (42), 2 children	-	Apartment, >3 rooms	0%	60%
150916-2	Male (45), female	Cat	Apartment, >3 rooms	0%	100%
280916-1	Female (40), 2 children	-	Apartment, >3 rooms	25-50%	40%
111016 -1	Female (43), female	Hamster	Apartment, >3 rooms	0%	0%
121016 - 1	Male (44), female	-	House, >3 rooms	0-25%	100%
131016 - 1	Female (32)	Dog	Apartment, >3 rooms	0-25%	100%
131016 - 2	Female (56)	Dog	Apartment, >3 rooms	0%	100%
251016 - 1	Male (42)	Dog	Apartment, 2 rooms	0-25%	80%
251016 - 2	Female (36)	-	Apartment, 2 rooms	0-25%	80%

Appendix 2.2 Longitudinal study

Test persons	Residents in flat/house	Locality description
150918A, female. 150918B, male.	1 adult male 1 adult male 1 boy (age 7) 1 girl (age 3)	Apartment in block from 1954, 94 m² area, and 4 rooms. Clean and orderly flat. Cupboard was emptied and all close stored in closed plastic bags to avoid spreading of bedbugs. Wall to wall carpet in flat except bedroom. The Bedroom was the room used for collection

Diet Approx. organic in %.		Other risks of exposure
Fruit	100%	08-09-2018: Family visit a home with a dog. Status for flea or tick treatment on dog not known.
Vegetables	100%	
Milk	100%	
Meat	100%	
Cereal	100%	

Active ingredient	Product	Amount used	Treatment date	Sampling date.	Day after first treat-ment
λ-cyhalothrin	Demand 10CS	2 L	03-09-2018		0
				15-09-2018	12
λ-cyhalothrin	Demand 10CS	1 L	17-09-2018		14
				19-09-2018	16
				04-10-2018	31
λ-cyhalothrin	Demand 10CS	¼ L	05-10-2018		32
				08-10-2018	35
				24-10-2018	51
				07-11-2018	65

Test persons	Residents in flat/house	Locality description
240518A, female. 240518B, male.	1 adult male 1 adult female	House build in 1972. Area 130 m², 4 rooms. Clean and orderly house. Female slept in the treated bedroom Wall to wall carpet in bedroom and wooden floor in the living room.

Diet Approx. organic in %.		Other risks of exposure
Fruit	20%	A sofa in the living room was treated with an unknown deltamethrin based product.
Vegetables	20%	
Milk	100%	
Meat	20%	
Cereal	20%	

Active ingredient	Product	Amount used	Treatment date	Sampling date.	Day after first treatment
Cyfluthrin	Solfac	2L	08-05-2018		-16
Cyfluthrin	Solfac	3L	24-05-2018	24-05-2018	0
					0
				25-05-2018	1
				07-06-2018	14
Deltamethrin	K-othrine	2L	18-06-2018		25
				19-06-2018	26
				02-07-2018	39
				08-08-2018	76

Test persons	Residents in flat/house	Locality description
010518A, female. 010518B, male	1 adult male 1 adult female	Apartment in block from 1967, 78 m², and 3 rooms. Shared flat. Cat living in. Wooden floors, no carpets.

Diet Approx. organic in %.		Other risks of exposure
Fruit	100%	One year ago, the cat was treated for fleas. Host reckon that there has not been any no other risk for pesticide exposure.
Vegetables	100%	
Milk	100%	
Meat	20%	
Cereal	20%	

Active ingredient	Product	Amount used	Treatment date	Sampling date.	Day after first treatment
				01-05-2018	0
Cyfluthrin	Solfac*	?	01-05-2018		
				02-05-2018	1
			14-05-2018		
Cyfluthrin	Solfac*	?		14-05-2018	13
				16-05-2018	15
				28-05-2018	27
				12-06-2018	42

*Claimed to be K-othrine, but must be Solfac based on the analysis.

Test persons	Residents in flat/house	Locality description
070118A	1 adult male 3 adult females	Apartment in block from 1935, 58m², and 2 rooms. Shared flat. In need of some maintenance. Male is not permanent residents. He has another flat in which a major problem with bedbugs has been identified

Diet Approx. organic in %.		Other risks of exposure
Fruit	20%	Use of imported products from eastern Europe - unidentified A.i. The flat was treated against *Attagenus spp* in November and again in December 2017 with an unknown product.
Vegetables	20%	
Milk	20%	
Meat	20%	
Cereal	20%	

Active ingredient	Product	Amount used	Treatment date	Sampling date.	Day after first treatment
				07-01-2018	-1
Etofenprox	Fenox		08-01-2018		
				09-01-2018	1
Unknown	Unknown		16-01-2018		
				18-01-2018	10
Unknown	Unknown		25-01-2018		
				28-01-2018	20
				22-02-2018	45

Appendix 3. Urine pyrethroid metabolites

TABLE 19. Concentrations (creatinine corrected, µg/g creatinine) of pyrethroid metabolites in urine samples of the horizontal study.

		3-PBA (µg/g)	4-F-3_PBA (µg/g)	cis-DCCA (µg/g)	trans-DCCA (µg/g)	cis-DBCA (µg/g)
Treated	140416-1	0.33	<LOD	<LOD	0.36	0.06
	150916-1	0.05	0.26	1.30	0.34	0.04
	150916-2	0.06	0.55	<LOD	0.28	<LOD
	280916-1	0.05	<LOD	<LOD	<LOD	<LOD
	111016 -1	0.04	<LOD	<LOD	<LOD	<LOD
	121016 - 1	0.16	<LOD	<LOD	<LOD	<LOD
	131016 - 1	0.43	<LOD	<LOD	<LOD	<LOD
	131016 - 2	0.04	<LOD	<LOD	<LOD	<LOD
	251016 - 1	0.02	<LOD	<LOD	<LOD	<LOD
	251016 - 2	0.45	<LOD	<LOD	0.16	<LOD
	271016 - 1	0.04	<LOD	<LOD	0.17	<LOD
	161216 -1	0.01	0.03	<LOD	0.06	<LOD
	240217 -1	0.09	0.02	<LOD	0.21	<LOD
	100317 -1	0.35	<LOD	<LOD	<LOD	<LOD
	100317 -2	0.04	<LOD	<LOD	<LOD	<LOD
	070118	0.09	<LOD	<LOD	0.05	<LOD
	010518A	0.18	0.04	<LOD	0.16	0.05
	010518B	0.01	<LOD	<LOD	<LOD	<LOD
	240518A	0.02	<LOD	<LOD	<LOD	<LOD
	240518B	0.02	<LOD	0.10	<LOD	<LOD
	150918A	0.03	<LOD	<LOD	<LOD	<LOD
	150918B	0.03	<LOD	<LOD	<LOD	<LOD
Untreated	140416-1	**0.02**	**<LOD**	**0.11**	**<LOD**	**<LOD**
	150916-1	[a]	[a]	[a]	[a]	[a]
	150916-2	**0.08**	**<LOD**	**<LOD**	**0.32**	**0.18**
	280916-1	**0.07**	**<LOD**	**<LOD**	**<LOD**	**<LOD**
	111016 -1	**0.07**	**<LOD**	**<LOD**	**<LOD**	**<LOD**
	121016 - 1	**0.10**	**<LOD**	**<LOD**	**0.31**	**<LOD**
	131016 - 1	**0.03**	**<LOD**	**<LOD**	**0.06**	**<LOD**
	131016 - 2	[a]	[a]	[a]	[a]	[a]
	251016 - 1	**0.04**	**<LOD**	**<LOD**	**<LOD**	**<LOD**
	251016 - 2	**0.11**	**<LOD**	**<LOD**	**<LOD**	**<LOD**

[a] Missing urine samples

Pyrethroids in private homes - Accumulation and human exposure

In Denmark, only pesticides from the group of synthetic pyrethroids are currently used to control bedbugs. The synthetic pyrethroids are stable compounds, so relatively slow degradation is expected under normal conditions. At the same time, decreased sensitivity to pyrethroids among bedbugs has been found in many countries. This means that treatment must often be repeated, leading to the risk of accumulation and increased exposure of the residents.

The purpose of this study was to document whether in practice there is a risk of accumulation of pyrethroids through bedbug control programs and at the same time to elucidate the extent to which the residents are exposed to pyrethroids.

In general, most pyrethroid was found in dust samples. The most extreme case was 7,5 mg deltamethrin + 3 mg lambda-cyhalothrin + 46,5 mg permethrin in the dust sample from vacuuming one treated room. Thus, in a bioassay with insecticide sensitive flies, it was found that up to 189 days after a treatment, these died when exposed to the floor. It was not possible to see any clear correlation between the amount of pyrethroid present in the environment and the amount of pyrethroid metabolite present in the urine samples. This was interpreted to indicate that the behaviour of the residents has a great influence on the extent to which they are exposed to pyrethroid.

In the second part of the study a number of residents were followed during a period of treatment for bedbugs. The same samples were collected, but in addition blood samples were also taken. Again, there was quite a large variation in the amount of pyrethroid found and no clear correlation was seen between the amount of pyrethroid and the time since the last treatment. In most cases, the levels were still high on the last day of collection and therefore it was considered very likely that pyrethroid accumulation can be achieved by repeated treatments, Here, too, it was not possible to find any clear correlation between the amount of pyrethroid in the environment and the amount of pyrethroid metabolite in the urine. Some residents had obviously been exposed to large amounts of pyrethroid, as seen by the amount of metabolites in the urine. As in the first part, the behaviour of the residents in the dwelling is likely to have a great impact on how much they are exposed. It was only possible to find one pyrethroid metabolite in the blood samples and it was not possible to see any clear correlation with the other measured parameters.

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