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Silicone Wristband Passive Samplers Yield Highly

Individualized Pesticide Residue Exposure Profiles

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ABSTRACT

Monitoring human exposure to pesticides and pesticide residues (PRs) remains crucial for informing public health policies, despite strict regulation of plant protection product and biocide use. We used 72 low-cost silicone wristbands as noninvasive passive samplers to assess cumulative 5-day exposure of 30 individuals to polar PRs. Ethyl acetate extraction and LC-

MS/MS analysis were used for the identification of PRs. Thirty-one PRs were detected of which 15 PRs (48%) were detected only in worn wristbands, not in environmental controls. The PRs included 16 fungicides (52%), 8 insecticides (26%), 2 herbicides (6%), 3 pesticide derivatives (10%), 1 insect repellent (3%), and 1 pesticide synergist (3%). Five detected pesticides were not approved for plant protection use in the EU. Smoking and dietary habits that favor vegetable consumption were associated with higher numbers and higher cumulative concentrations of PRs in wristbands. Wristbands featured unique PR combinations. Our results suggest both environment and diet contributed to PR exposure in our study group. Silicone wristbands could serve as sensitive passive samplers to screen population-wide cumulative dietary and environmental exposure to authorized, unauthorized and banned pesticides.

INTRODUCTION

Pesticides are widely used in agriculture, industry, and in the home environment as plant protection products and as biocidal active substances to control weeds, fungi and pests. Strict international policies and regulations with respect to market approval and maximum residue limits have been implemented and enforced by authorities to minimize risk of pesticide use. However, whether or not exposure to pesticide residues (PRs) in the environment, food and water forms a potential threat for human health remains debated.¹ The chronic and acute exposure, including periconceptional, pre- and perinatal exposure, to pesticidal and biocidal active substances and their residues, are increasingly associated with a number of adverse health outcomes, such as Parkinson's disease,² prostate and liver cancer,^{3,4} and reduced sperm quality in adults;⁵ cough,⁶ respiratory problems,⁷ leukemia,^{8,9} brain tumors,¹⁰ and neurobehavioral

disorders in children;¹¹ and neurodevelopmental disorders,^{12,13} low birth weight,¹⁴ and congenital disorders in infants.^{15,16} Many of these epidemiological exposure studies, however, have limitations.¹⁷ Actual exposures are hard to measure as the surrogate metrics of exposure, such as reported days or frequency of pesticide use, provide insufficiently precise information about effective exposure. Whereas there is no “gold standard” for exposure,¹⁸ there is a need for improving exposure estimation to discriminate surrogate from actual exposures.

Possible routes of entry of pesticides and their derivatives are transdermal absorption, inhalation and oral ingestion.¹⁹ Occupational exposure and the handling of items that have been treated with pesticides (e.g. cut flowers,^{20,21} plant cultivation infrastructure and materials²²) increase the probability of uptake and subsequent acute or chronic adverse health effects^{15,23}. Such exposure can be minimized by using personal protective equipment,^{22,24,25} by implementing engineering controls during handling,²⁵ by using proper ventilation,²⁶ and by reducing or avoiding pesticide use.^{1,27} Environmental exposure to contaminated water, soil or air from runoff, dust and spray drift generated by domestic or agricultural pesticide applications is also an important pathway of exposure to PRs.^{1,16,28} At the level of the general population, residential pesticide use,^{8,10,29} contact with contaminated household surfaces,³⁰ and notably diet^{1,29,31-35} have been identified as important exposure pathways to PRs. Short-term and cumulative dietary exposure to a number of PRs have been shown to be a public health concern,^{36,37} in particular for children.³⁴ PR screening in food and feed and monitoring of human exposure to PRs therefore play vital roles in safeguarding public health.³⁸

Human biomarker monitoring, for instance urinary biomarker measurements using liquid chromatography (LC) and mass spectrometry (MS),^{29,38,39} is the principal method to monitor human exposure to PRs. Urine, sweat, saliva, breast milk, nails and hair are suitable noninvasive

human matrices for pesticide exposure monitoring.^{19,40-42} Less intrusive methods include wastewater sampling for population-wide exposure assessment⁴³ and (personal) passive sampling devices (PSDs). Recently polyethylene, polyurethane foam and silicone matrices have been proposed as passive samplers for environmental^{44,45} and individual assessment of exposure to persistent organic pollutants, pesticides, brominated diphenyl ethers, organophosphate flame-retardants and other potentially harmful substances.⁴⁶⁻⁵³

In this cross-sectional, noninterventional study, we tested the suitability of low-cost commercially available silicone wristbands as personal passive sampling devices to assess personal exposure to LC-amenable pesticide residues. We hypothesized that PR spectra detected in the wristbands would reflect the participants' exposure to pesticide residues, which is determined by their pesticide use, outdoor and indoor environmental exposure and personal food choices.

MATERIALS AND METHODS

Study Design. Study participants were recruited from the Department of Earth and Environmental Sciences of the University of Leuven, Leuven, Belgium ($n = 30$; SI Table S1) in September 2016. The protocol for a cross-sectional, noninterventional study was reviewed and approved by the Social and Societal Ethics Committee of the University of Leuven (SMEC protocol number G-2016 09 636). Participants were assured confidentiality of personal information, anonymity and the possibility of withdrawing from the study at any time without the need for justification. All participants were adults and gave written prior informed consent.

Participants agreed to wear a silicone wristband for 5 days, to place a second wristband near their residence, and to complete questionnaires.

Wristband Cleaning. Adult-size (200 mm L $\times$ 12 mm W $\times$ 2 mm T; weight 5.33 g, SD = 0.10 g) silicone wristbands with embossed serial numbers were purchased (pdc Healthcare, Nivelles, Belgium; wristbands.com). The wristbands were cleaned using two successive extractions in an overhead shaker (Reax 2, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) to remove potentially present analytes of interest and compounds that could cause chromatographic interference. The first extraction of 30 minutes with 1:1 ethyl acetate/hexane (v/v) was followed by a second extraction of 30 minutes with 1:1 ethyl acetate/methanol (v/v). After the extractions, the wristbands were dried under a nitrogen stream, and stored in a dark place in labeled, neutral glass vials of 60 ml with screw closure. This cleaning method was adapted from the procedure described by O'Connell et al.⁴⁶ to minimize the consumption of organic solvents.

Wristband Exposure. Participants wore the wristbands continuously for a 5-day period (starting on the Monday morning of a work week) during all daily activities. Stationary silicone wristbands were placed by the participants on a location outside their residence (protected from rain) and served as controls of the residential outdoor environment. Participants recorded start and stop time of the wristband exposure in a log book. Additional stationary control bands were placed in the main building of the department where the participants were recruited ($n = 4$, occupational environment controls) and in Heverleebos-Meerdaalwoud, a high-value mixed deciduous forest 3 km south of Leuven ($n = 8$, clean air environment controls). The purpose of the stationary control bands was to identify airborne PRs. Six cleaned wristbands were kept in their vials in the laboratory at room temperature to serve as blanks. At the end of the 5-day

period, participants placed the control and worn wristbands in their original vials and kept the vials refrigerated at 4–6°C. The wristband exposure time (95% confidence interval) was 84.3–88.2 hours. In the laboratory, returned vials were stored at –20°C awaiting chemical analysis.⁵⁰

Questionnaires. Participants provided three types of background information needed to interpret the results: daily outdoor exposure information, demographic data and personal data. To characterize the intensity of outdoor exposure, participants recorded on a daily basis information on their whereabouts and travels. For each travel the participants recorded start and end point addresses, start and end time, and type of transportation used. This information was later used to calculate total time spent moving outdoors, and by using Google Maps routes, total distance traveled (km). Participants completed a short questionnaire to provide demographic data and information on their living environment, smoking habits and occupational or domestic exposure to pesticides. After the experiment, participants provided information on pet ownership and treatment, the number of showers taken during the 5-day period, and general dietary choices (organic vs. conventional and vegetarian vs. conventional).

Wristband Extraction. For detection of pesticide residues, a tandem mass spectrometry (MS/MS) protocol was developed, simplifying the protocol from O’Connell et al.⁴⁶ To extract pesticides, 40 ml of ethyl acetate was added to the vial containing the wristband. Vial, wristband and extraction solvent were shaken mechanically during 30 min by an overhead shaker (Reax 2®, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). This procedure was repeated two times with the same volume and time. The ethyl acetate phase (120 ml) was evaporated with a sample concentrator at 40°C (Genevac Ltd, Ipswich, UK) and under a nitrogen stream until the volume was reduced to 200 µl. To prepare the final extract, the raw extract was

spiked with 200 μ l internal standard solution (Oxfendazole, a broad spectrum veterinary anthelmintic compound, at 100 ng/ml), then diluted with 400 μ l mobile phase A (water/methanol (90/10 v/v) containing 5 mM ammonium acetate) and adjusted to 1 ml with methanol. An aliquot (500 μ l) was transferred in a filtration vial prior to injection into the UHPLC-MS/MS system.

Wristband Chemical Analysis. Based on production, maximum residue levels in food and frequency of detection, LC-MS/MS systems have a wider scope and better sensitivity to detect pesticide residues than GC-MS systems.^{54,55} Therefore, the screening was focused on LC-amenable residues of polar plant protection products and biocides. UHPLC-MS/MS analyses were performed on an UPLCTM system (Waters, Milford, MA) coupled to a Quattro PremierTM mass spectrometer (Waters, Milford, MA) following the protocol of Hanot et al.⁵⁶ Five microliter of sample was injected onto a reverse-phase ACQUITYTM UPLC BEH C18 column (1.7 μ m, 2.1 $\times$ 100 mm) (Waters, Milford, MA). The mobile phases were composed of water/methanol (90/10 v/v) (phase A) and methanol/water (90/10 v/v) (phase B) both containing 5 mM ammonium acetate. The chromatographic separation was performed at a flow rate of 0.45 ml min⁻¹ with an elution gradient starting with 99.9% of mobile phase A and linearly evolving until 0.1% in 10 min and maintained during two minutes. The column was re-equilibrated for the next injection during 3 min with 99.9% of mobile phase A. Three UHPLC runs were necessary to acquire two transitions for each of 200 pesticides. For the two first runs the sample was ionized with an electrospray probe in the positive mode; for the third run the negative mode was used. Selected quantities of 200 LC-amenable pesticide residues were added just before the vial filtration. The linearity range of the method was 0.4–100 ng g⁻¹ wristband. In all cases, good linearity was achieved with correlation coefficients > 0.99. The limit of quantification (LOQ) was 0.2 ng g⁻¹ wristband.

Quality Control. To ensure reliability of results, a blank and a spiked sample were analysed in each of 5 batches. For the spiked sample (analytical quality control sample), a multi-residue solution was spiked on wristband, which was allowed to dry for 1 h, and then analysed. Multi-residue solutions containing 200 LC-amenable pesticide residues were prepared in methanol according to the European quality criteria for pesticide residue analysis in food and feed.⁵⁷ Wristbands were spiked by spraying small droplets directly on the wristbands following the procedure of Toumi et al.²⁰ The spike was calculated to reach an average concentration of about 2 ng g⁻¹ wristband for each LC-amenable pesticide residue (equivalent to a concentration of 10 ng of pesticide residue on a wristband of 5 g). No pesticides were detected in the blank samples with a concentration above LOD, proving an absence of pesticides in the cleaned wristbands themselves, except for the insect repellent N,N-diethyl-meta-toluamide (DEET). The concentration of DEET was similar in all the blank samples (0.5 ng g⁻¹ wristband). This background value was subtracted from the detected DEET values for all samples. Detections below LOQ were assigned the value of ½ LOQ (0.1 ng g⁻¹) to enable statistical analyses.

Statistical Analyses. The frequencies of pesticide residue detection were compared between worn and stationary wristbands using chi-square tests for independent samples. The cumulative concentration of PRs ($\log\sum\text{PR}$) was calculated as the log₁₀-transformed sum of the concentrations of all PRs (ng g⁻¹) detected in each wristband ($\log\sum\text{PR} = \log_{10}[\sum\text{PR}_i + 1]$; the +1 component of the transformation was included to transform total concentrations < 1 ng g⁻¹ to positive values), with and without DEET. Differences in the average number of PRs detected and differences in $\log\sum\text{PR}$ between worn and stationary wristbands were assessed using Kruskal-Wallis one-way analysis of variance. Generalized linear models were used to estimate the effects of environmental and demographic variables on the number of PR detected and $\log\sum\text{PR}$

(without DEET). For the number of PRs, a Poisson log-linear model was used, with sex, smoking habit and food choices as factors and age, BMI, number of showers and total distance traveled as covariates. Estimated marginal means and 95% confidence intervals were obtained and pairwise comparisons were made for the significant ($p < 0.05$) factors. A linear model with the same factors and covariates was used for $\log \sum \text{PR}$. To identify groups with similar PR profiles, individual PR profiles (based on $\log_{10}[\text{PR}+1]$ -transformed values) were clustered into $k = 2-6$ groups using the Sørensen distance measurement. The most informative number of groups was determined using Indicator Species Analysis.⁵⁸ Nonmetric multidimensional scaling (NMS) based on the Sørensen distance measure was then used to place individual participants and PR profile groups in a 2-dimensional ordination space. Clustering and ordination were conducted using PC-ORD 5.0 for Windows (MjM Software, Gleneden Beach, OR). All other statistical analyses were performed in IBM SPSS Statistics 20 (IBM Corporation, Armonk, NY).

RESULTS AND DISCUSSION

All participants ($n = 30$, 100%) completed the exposure experiment, returned the worn wristbands and controls, and completed the questionnaires. The study population comprised 16 males (53%) and 14 females (47%). At the time of the survey, participants were on average 32.6 years old (range 24–72 years) and had a normal weight (average BMI 22.8, range 18.5–28.3). Participants spent on average 8.8 hours in traffic during the 5-day period (range 2.8–17.7 hours) and covered on average a distance of 251 km (range 31.5–711.3 km) (Table S1). No occupational or accidental exposure to pesticides was reported. Four participants reported to have used pet care products (spot on flea treatment and flea collars) that contained a number of

221 antiparasitic agents that included fipronil, methoprene, selamectine, imidacloprid and
222 flumethrine (methoprene, selamectine and flumethrine were not included in the LC-MS/MS
223 screening).

224 **PRs in Wristbands.** In total, 31 LC-amenable pesticide residues were detected in the 72
225 deployed wristbands. These PRs included 16 fungicides (52%), 8 insecticides (26%), 2
226 herbicides (6%), 1 insect repellent (3%), 3 pesticide derivatives (10%) and 1 pesticide synergist
227 (3%) (Table 1). Five substances which are not approved for plant protection use in the EU were
228 detected: fipronil (and its desulfinyl photoproduct and sulfone metabolite),^{59,60} carbendazim,
229 bendiocarb, tolylfluanid (via the metabolite DMST) and ethirimol (Table 1). Carbendazim and
230 ethirimol are also degradation products of thiophanate-methyl and bupirimate, respectively, and
231 those compounds are approved for plant protection use in the EU. Five fungicides and 4
232 insecticides had the dual use of plant protection product and biocidal active substance (BAS)
233 (Table 1). The insect repellent N,N-diethyl-meta-toluamide (DEET) was detected in 93% of all
234 worn bands and in 81% of the stationary controls. The high prevalence of DEET in the controls,
235 even in controls that were placed in forest with high ecological value, indicates that DEET is a
236 pervasive contaminant in the environment, not only in water,^{61,62} but also in aerosols.^{49,63} Apart
237 from DEET, the PRs with the highest prevalence were the pesticide synergist piperonyl butoxide
238 (PBO; 63% of the worn bands, 9.5% of the controls), the broad spectrum fungicide azoxystrobin
239 (47% of the worn bands, 2% of the controls) and the fungicide and parasiticide thiabendazole
240 (TBZ) (40% of the worn bands, 2% of the controls). PBO is added to pesticide formulations to
241 increase their effectiveness, in particular pyrethrin and pyrethroid insecticides (not included in
242 our screening), and has been detected in personal and indoor residential air⁶ and in PSDs.⁴⁹
243 Prenatal exposure to PBO has been associated with negative health outcomes in children.^{6,12} The

fungicides azoxystrobin and TBZ are widely used as plant protection products and as biocidal active substances to enhance preservation of film, fiber, leather, rubber, polymerized materials, wood and construction material. Environmental exposure, via aerosols, dust or direct contact with treated materials and/or food items (e.g. TBZ on orange, lemon and other citrus peels), may therefore be partially responsible for the detection of these compounds.

Nine PRs had a significantly higher prevalence in worn wristbands than in controls (all $p < 0.05$; Table 1). No PR was only detected in controls and PRs detected in controls were always also detected in matched worn wristbands (Table 1). Conversely, 15 PRs (48%) were detected only in worn wristbands and not in controls. Four of these were residues of compounds that are also used as biocidal active compounds (3 insecticides and 1 fungicide) and environmental exposure via direct contact or via aerosols in indoor environments that were visited by the participants but not covered by our set of controls (e.g. shops and public places) may have contributed to the exposure to these substances (e.g. bendiocarb insecticide). The other 11 compounds only detected in worn wristbands are or were only used as plant protection products (3 insecticides and 8 fungicides). These PRs may have been picked up by the wristbands after direct contact with treated food items (e.g. imazalil on citrus fruit), or they may have entered the wristbands via sweat. PR exposure monitoring via sweat is uncommon, mainly because estimating the rate of perspiration and the quantification of sweat are difficult and make sweat a less attractive medium than urine.¹⁹ Nevertheless, a number of studies confirms that perspiration eliminates contaminants from the human body, including organochlorinated pesticides.^{64,65} Sweat testing using sweat patches is a validated method for continuous monitoring of opiates and other illicit drug abuse,⁶⁶ and sweat patches have also been used to detect pesticides in the sweat of rural children.⁶⁷ Sweat patches have a wider detection window in terms of time compared to

urine tests,⁶⁸ and offer a cumulative estimate of exposure.⁶⁹ Likewise, the silicone wristbands used in this study offered a 5-day cumulative estimate of pesticide exposure, and the results of the screening suggest that silicone PSDs directly worn on the skin may also capture ingested contaminants, differing from earlier PSD studies.⁴⁹

PR Diversity and Concentration. The number of different compounds found on wristbands was significantly higher in worn wristbands than in controls (Kruskal-Wallis $\chi^2=34.61$; $p < 0.001$; Table 2). In the worn wristbands, there were in total 163 detections of 31 different compounds. In the stationary controls, there were 37 detections of 12 compounds for the residential outdoor environment, 9 detections of 6 compounds in the office environment and 9 detections of 2 compounds in the forest environment. The cumulative concentrations $\log\Sigma\text{PR}$ with and without DEET were significantly higher in wristbands worn by participants than in stationary controls (with DEET: Kruskal-Wallis $\chi^2 = 42.25$; $p < 0.001$; without DEET: Kruskal-Wallis $\chi^2 = 31.67$; $p < 0.001$; Table 2). Worn wristbands integrated exposure to PRs over a larger area than stationary controls, came into contact with materials and were exposed to human sweat. The higher number of compounds and higher concentrations detected in worn wristbands are thus most likely the result of the interaction between an area and/or surface sampling effect and the personal diet.

Variables Affecting PR Diversity. Smoking and passive exposure to smoke, organic or mixed organic diet choice and mixed vegetarian diet choice were associated with higher numbers of PRs and higher cumulative concentrations $\log\Sigma\text{PR}$ (without DEET) in wristbands (all $p < 0.05$, see Table 3 and Table S2). The effects of the covariates age ($p < 0.001$) and total distance traveled during the 5-day period ($p = 0.001$) were statistically significant for the number of PRs

but the linear associations between these covariates and the number of PRs were weak and related to the presence of only few observations in the highest age and distance traveled classes. These results suggest that the area sampling effect mentioned above is possibly more limited and that diet could play a more important role in determining PR diversity in worn wristbands.

Exposure to tobacco smoke has earlier been related to PR exposure,⁷⁰ and the dietary intake of fruits and vegetables has been associated with urinary concentrations of chlorpyrifos metabolites,⁷¹ pyrethroid metabolites and organophosphate metabolites such as dialkyl and diethyl phosphates (DAP, DEP).^{29,33-35} The relative higher consumption of fruit and vegetables by persons opting for organic and/or vegetarian food choices may thus increase PR exposure, but the health benefits of including fruit and vegetables in the diet outweigh the potential negative effects.⁷² Population-wide assessment studies in Belgium and France have shown that the total dietary exposure to most PRs is between 4 and 100 times lower than the acceptable daily intake (ADI).^{73,74} Also, diets that include organic and/or vegetarian food choices are often strongly correlated with other indicators of healthy lifestyle.⁷⁵

Individual PR Profiles in Worn Wristbands. Five participants (17%) returned wristbands that contained no PRs, except for the insect repellent DEET (Figure 1). All other worn wristbands (83%) featured highly individualized plant protection product and biocide exposure profiles, each wristband characterized by a unique combination of detected PRs (Figure 1). There were four groups of wristbands with unique PR exposure profiles (i.e. excluding those only containing DEET) that showed similar PR composition within groups. These groups were well separated in a two-dimensional ordination space (Figure 2; 71% of the original variance explained). The dimensions in the ordination, which are expected to represent environmental or behavioral gradients that influence the PR profiles (e.g. place of residence, occupation, socio-

economic background and diet), were not associated with participant background data measured in this study, most likely because of the limited socio-economic variability within our sample. Nevertheless, distances between individual points are proportional to dissimilarities between individual PR profiles and the observed pattern, without overlapping PR profile points, illustrates the highly individualized PR profiles detected in the wristbands, despite the high similarity in occupation and socio-economic status of our participants. The four groups differed significantly in terms of PR load, i.e. average number of PRs (Kruskal-Wallis $\chi^2 = 22.59$; $p < 0.001$) and $\log\sum\text{PR}$ (without DEET: Kruskal-Wallis $\chi^2 = 24.98$; $p < 0.001$) (Table S3).

Fipronil Group. The insecticide fipronil (IV = 95.4; $p < 0.001$) and the fipronil metabolite fipronil sulfone (IV = 100.0; $p < 0.001$) were significant indicators for a first cluster of 8 wristbands with high PR load (many compounds, relatively high concentrations). Fipronil is used in domestic pest control products including spot-on pet care products and the use of such products may result in the accumulation of fipronil and its derivatives in indoor and outdoor dust.^{76,77} Direct contact with treated pets can cause short-term, acute exposure to fipronil and other insecticides,³³ but there is no evidence for significant long-term secondary exposure from pets to residents via the household environment.^{78,79} Fipronil was detected along with desulfynil-fipronil in the wristbands of three participants in this group and with imidacloprid in the wristbands of two participants in this group. Desulfynil-fipronil is not a human metabolite but an environmental degradation product formed under the influence of sunlight,⁸⁰ and imidacloprid is used in flea collars. The presence of pet care compounds suggests environmental (domestic) exposure to fipronil in four cases. Three of those four participants indeed reported having used spot-on pet care products containing fipronil (Eliminall® spot on for cats, fipronil 50 mg; and FrontLine Combo® spot on for cats, fipronil 10% w/v) or imidacloprid (Seresto flea collar,

imidacloprid 1,25 g and flumethrine 0,56 g). The presence of fipronil along with its metabolite fipronil sulfone but without the environmental degradation product desulfinyl-fipronil suggests that dietary exposure may have contributed to the exposure to fipronil in four other participants in this group. In July 2017 fipronil was detected in eggs and egg-derived products in the European food chain following the illicit use of fipronil in poultry farms (an event known to the broad public as the 2017 fipronil eggs contamination, egg crisis or European tainted-egg scandal)⁸¹. Published studies with rat and mice models showed that fipronil induces oxidative damage to the kidneys and the brain,⁸² could be neurotoxic to humans,⁸³ and its toxic degradates are persistent and may bioaccumulate in fish.⁸⁴

Other Groups. The fungicide fludioxonil (IV = 65.8; p = 0.011) was a significant indicator of a second cluster of five wristbands with high PR load (fludioxonil group). Fludioxonil is widely used as a postharvest fungicide to control molds in fruits and vegetables and consumers are exposed to fludioxonil through contact with treated food items and consumption of processed fruits and vegetables.^{85,86} In France, fludioxonil belongs to the group of pesticides to which the population is commonly exposed through food consumption⁸⁷. In-vitro studies have shown that fludioxonil has carcinogenic and genotoxic effects, in particular in pesticide mixtures.^{87,88} Apart from DEET, PBO and one herbicide, all other compounds in this group were fungicides, including TBZ, dimethomorph, imazalil, metrafenone and pyrimethanil. The fungicide boscalid (IV = 60.3; p = 0.012) was a significant indicator for a third cluster of three wristbands with relatively low PR load (boscalid group). A fourth cluster of nine wristbands, a group without significant indicators, also had a relatively low PR load, especially when compared to the fludioxonil and fipronil groups (Table S3). In this last group fipronil was detected in two

wristbands, but without metabolite or photoproduct. The other compounds included a variety of fungicides and insecticides but at low concentrations and with low detection frequencies.

Perspectives for Further Application. Our results suggest that both environment and diet contributed to PR exposure in our study group, which was a convenience sample with relative small size. To draw inference at the population level, further studies with a large number of participants are recommended, for instance in existing cohorts or in the framework of a population-wide health survey. Silicone wristbands are able to pick up contaminants from air, water, materials and skin. Depending on the objectives of future studies, wristbands could be deployed in direct contact with the skin to include contaminants that leave the body via the skin after inhalation, skin contact or ingestion, or they could be deployed in ways that exclude direct skin contact, e.g. attached to the outer clothing⁴⁶, to focus on environmental exposure. Silicone wristbands could therefore serve as sensitive passive samplers to screen population-wide cumulative dietary and/or environmental exposure to authorized, unauthorized and banned pesticides.

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Author Contributions

RA designed the experiment, secured ethical clearance, analysed the data and drafted and revised the manuscript. LJ, PS and KT performed the chemical analyses. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

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406
407 ABBREVIATIONS

408 LC-MS/MS, liquid chromatography tandem-mass spectrometry; NMS, nonmetric
409 multidimensional scaling; PR, pesticide residue; PSDs, passive sampling devices; UHPLC, ultra
410 high performance liquid chromatography.

411
412 SUPPORTING INFORMATION AVAILABLE

413 Table S1. Characteristics of the study population

414 Table S2. Association between participant characteristics and cumulative concentration of
415 pesticide residues in silicone wristbands worn for five days

416 Table S3. Number and cumulative concentration of residues of plant protection products and
417 biocidal active substances in five groups of silicone wristbands worn for 5 days

418 This information is available free of charge via the Internet at <http://pubs.acs.org>.

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Figure 1. Concentrations (ng g^{-1}) of LC-amenable pesticides (including metabolites, synergists and repellents) detected above quantification limit ($\text{LOQ} = 0.2 \text{ ng g}^{-1}$) in silicone wristbands worn by 30 volunteers (IDs on Y axis) for one working week, illustrating highly individualized pesticide exposure profiles. Pesticide residues belonged to the following functional groups: R, insect repellent; S, pesticide synergist; F, fungicide; I, insecticide; Mi, insecticide metabolite; Mf

696 fungicide metabolite. Active substances not approved for plant protection use at Belgian and/or
697 EU level are printed in bold. A three-color scale is used to indicate lowest values (green), 50
698 percentile (yellow) and highest values (red) for the concentrations of DEET and for the
699 concentrations of all other PRs combined.

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711 TABLES

712 **Table 1. Prevalence of Residues of Plant Protection Products and Biocidal Active**
 713 **Substances (BAS) in Silicone Wristbands Worn for 5 Days and in Stationary Controls**

compound	worn by	controls			type	CAS nr.
	participants	residence	office	forest		
	<i>n</i> =30	<i>n</i> = 30	<i>n</i> = 4	<i>n</i> = 8		
azoxystrobin	14*	0	0	1	fungicide/BAS	131860-33-8
bendiocarb	1 ^W	0	0	0	insecticide/BAS	22781-23-3
boscalid	7* ^W	0	0	0	fungicide	188425-85-6
carbendazim	2 ^W	0	0	0	fungicide/BAS	10605-21-7
cyazofamid	1 ^W	0	0	0	fungicide	120116-88-3
DEET	28	22	4	8	insect repellent	134-62-3
dimethomorph 1	7* ^W	0	0	0	fungicide	110488-70-5
dimethomorph 2	7*	0	1	0	fungicide	110488-70-5
DMST	1	2	0	0	metabolite	66840-71-9
ethirimol	1 ^W	0	0	0	fungicide	23947-60-6
fipronil	10*	2	0	0	insecticide/BAS	120068-37-3
fipronil, desulfinyl-	3	1	0	0	metabolite	205650-65-3
fipronil sulfone	8*	1	0	0	metabolite	120068-36-2

fludioxonil	8*	1	1	0	fungicide/BAS	131341-86-1
fluopyram	2 ^w	0	0	0	fungicide	658066-35-4
imazalil	3 ^w	0	0	0	fungicide	35554-44-0
imidacloprid	3 ^w	0	0	0	insecticide/BAS	138261-41-3
mepamipyrin	1 ^w	0	0	0	fungicide	110235-47-7
methoxyfenozide	2 ^w	0	0	0	insecticide	161050-58-4
metrafenone	4	0	1	0	fungicide	220899-03-6
oxadiazon	1	0	1	0	herbicide	19666-30-9
piperonyl butoxide (PBO)	19*	3	1	0	synergist	51-03-6
pirimicarb	1	1	0	0	insecticide	23103-98-2
prosulfocarb	1	1	0	0	herbicide	52888-80-9
pyraclostrobin	4	1	0	0	fungicide	175013-18-0
pyrimethanil	3 ^w	0	0	0	fungicide	53112-28-0
pyriproxifen	1 ^w	0	0	0	insecticide/BAS	95737-68-1
tebuconazole	5	1	0	0	fungicide/BAS	107534-96-3
tebufenozide	1 ^w	0	0	0	insecticide	112410-23-8
thiabendazole (TBZ)	12*	1	0	0	fungicide/BAS	148-79-8
thiacloprid	2 ^w	0	0	0	insecticide	111988-49-9

* Prevalence statistically significantly higher in worn wristbands than in stationary controls, Pearson chi-square $p <$

0.05

W Residue only detected in worn wristbands.

Substances in bold are not approved for plant protection use in Belgium.

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Table 2. Number and Cumulative Concentration of Residues of Plant Protection Products and Biocidal Active Substances in Silicone Wristbands Worn for 5 Days and in Stationary Controls

		worn by	controls		
		participants	residence	office	forest
		<i>n</i> = 30	<i>n</i> = 30	<i>n</i> = 4	<i>n</i> = 8
N(PR)	median	6.5 ^a	1 ^b	3 ^{ab}	1 ^b
	IQR	6	0	6	0
	range	1-13	0-5	1-9	1-2
ΣPR (ng g ⁻¹)	median	125.5 ^a	1.6 ^b	9.7 ^{ab}	2.7 ^b
with DEET	IQR	267.2	9.2	35.5	2.3
	range	9.1-2217.8	0-145.9	6.1-51.3	1.0-4.7
ΣPR (ng g ⁻¹)	median	12.3 ^a	0 ^b	0 ^{ab}	0 ^b
without DEET	IQR	65.8	0	33.3	0
	range	0-222.6	0-68.9	0-44.4	0-0.1

N(PR): Number of LC-amenable pesticides (including metabolites, synergists and repellents) detected above quantification limit (LOQ = 0.2 ng g⁻¹)

ΣPR: Cumulative concentration (ng g⁻¹) of LC-amenable pesticides (including metabolites and synergists with and without DEET) detected above quantification limit (LOQ = 0.2 ng g⁻¹)

723 Different superscript letters indicate significant pairwise differences ($p < 0.05$).

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726 **Table 3. Association between Participant Characteristics and the Number of Pesticide**
727 **Residues in Silicone Wristbands worn for 5 Days**

Variable	N(PR)	95% CI for EMM		p
	EMM	lower limit	upper limit	
Sex				0.703
female	6.0	4.4	8.0	
male	5.6	4.2	7.4	
Smoking habit				0.014
non-smoker	4.2 ^a	3.3	5.6	
passive exposure	7.1 ^b	5.4	9.4	
smoker	6.5 ^{ab}	4.1	10.3	
Organic food choice				0.014
organic diet	8.4 ^a	4.9	14.2	
mixed diet	6.3 ^a	4.9	8.0	
conventional diet	3.7 ^b	2.6	5.3	
Vegetarian food choice				0.002
vegetarian diet	4.2 ^a	2.8	6.4	

mixed diet	8.8 ^b	6.8	11.5
conventional diet	5.2 ^a	3.5	7.8
Age			<0.001
BMI			0.332
Number of showers			0.054
Exposure (kms)			0.001

Estimated marginal means (EMM) based on a Poisson log-linear model for the number of PRs (AIC = 161.666, likelihood ratio chi-square 37.50, $p < 0.001$). Covariates appearing in the model were evaluated at age = 32.6 years, BMI = 22.8, number of showers taken = 2.2 and traffic exposure = 251 km. For factors with a significant effect ($p < 0.05$), different superscript letters indicate significant pairwise differences.