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Exposure science and occupational health: insights from ISES 2022

Edited by
Susana Viegas, Urs Schlüter, M. Elizabeth Marder, Marina Almeida Silva and Brian Curwin

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Exposure science and occupational health: insights from ISES 2022

Topic editors

Susana Viegas — New University of Lisbon, Portugal
Urs Schlüter — Federal Institute for Occupational Safety and Health, Germany
M. Elizabeth Marder — California Environmental Protection Agency, United States
Marina Almeida Silva — Centro de Investigação em Saúde e Tecnologia, Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Portugal
Brian Curwin — National Institue for Occupational Safety and Health, United States

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EDITED AND REVIEWED BY
Paolo Vineis,
Imperial College London, United Kingdom

*CORRESPONDENCE
Marina Almeida-Silva
✉ marina.silva@estesl.ipl.pt

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Editorial: Exposure science and occupational health: insights from ISES 2022

Marina Almeida-Silva[1,2]*, Susana Viegas[3], Brian Curwin[4], M. Elizabeth Marder[5] and Urs Schlüter[6]

[1]H&TRC-Health & Technology Research Center, ESTeSL-Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Lisbon, Portugal, [2]OSEAN-Outermost Regions Sustainable Ecosystem for Entrepreneurship and Innovation, Funchal, Portugal, [3]Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA National School of Public Health, NOVA University Lisbon, Lisbon, Portugal, [4]National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention (CDC), Cincinnati, OH, United States, [5]California Department of Public Health, Sacramento, CA, United States, [6]Unit 4.I.4 Exposure Assessment, Exposure Science, Division 4 Hazardous Substances and Biological Agents, Federal Institute for Occupational Safety and Health (BAuA), Dortmund, Germany

KEYWORDS

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Editorial on the Research Topic
Exposure science and occupational health: insights from ISES 2022

Introduction

The annual meeting of the International Society of Exposure Science – from exposure to human health: new developments and challenges in a changing environment – took place from 25 to 29 September 2022 in Lisbon, Portugal. The aim of the conference was to promote information sharing and facilitate discussion on exposure sciences and related fields in the context of the changing environment, especially how we – as exposure scientists - can better understand and respond to the complex and multidisciplinary issues in exposure and environmental health through sciences and policies. This research topic now presents insights on exposure science and occupational health that were presented during the ISES 2022 conference covering all aspects of occupational exposure science. The ISES 2022 conference resulted in many abstracts being submitted (more than 450 submissions) describing the findings of new research on exposure science. The final participation numbers were 421 in-person attendees and 35 virtual attendees. It should be mentioned that 93 (20%) attendees were students or new researchers. The main geographical origins of our attendees were North America (42%) followed by Europe (39%).

Modern exposure science is rooted in the industrial hygiene and radiation health physics practices of the last century, and exposure science continues to play an important role in occupational health. Today, an individual may encounter a wide range of agents that directly or indirectly result in some form of adverse effect or harm. Generally referred to as "stressors," these agents can be chemical, physical, biological, or psychosocial, as well as mixtures thereof. Exposure science is the distinct discipline that encompasses the study of receptors and their behaviors related to contact with such stressors, the nature and extent of such contact, and the fate of these stressors over space and time.

The scientific articles published under the scope of this Research Topic "*Exposure Science and Occupational Health: Insights from ISES 2022*" cover various aspects of occupational and environmental exposures.

In recent research spanning from small business beauty salons in Arizona to hospitals in the Czech Republic and Slovakia underscores the urgent need for stricter workplace safety measures (Bláhová et al.; Ramírez et al.). These studies offer valuable insights into how chemicals used in everyday occupational tasks—ranging from antineoplastic drugs in hospitals to hair products in salons—pose serious risks to human health. Despite this, regulatory frameworks and practical protective guidelines are often fragmented or outdated.

One particularly poignant study examines the contamination of surfaces in hospitals and pharmacies by antineoplastic drugs (ADs) commonly used in cancer treatment. These drugs are essential for patient care but represent a substantial risk to healthcare workers due to their carcinogenic and mutagenic properties (Bláhová et al.). The study analyzed over 2,200 samples in healthcare facilities and recommended technical guidance values (TGVs) for managing this contamination. This is a crucial step toward minimizing the occupational exposure of healthcare personnel, but the broader challenge remains—how do we balance life-saving drugs with workplace safety? The authors proposed setting contamination limits at 100 pg/cm² in most healthcare settings, but the complexity of these environments means that even low-level contamination in areas like staff rooms can lead to unintended exposure. The "no-threshold effects" of genotoxic drugs complicate establishing safe exposure limits, making prevention and meticulous monitoring indispensable. Here, prevention might take the form of enforcing cleaning protocols and limiting access to highly contaminated areas.

Equally concerning is the exposure of workers in beauty salons to VOCs, as highlighted in a study from Tucson, Arizona (Ramírez et al., also Lothrop et al. "*Studying full-shift inhalation exposures to volatile organic compounds (VOCs) among Latino workers in very small-sized beauty salons and auto repair shops*"). Beauty salons, a multibillion-dollar global industry, expose workers to harmful chemicals found in hair and beauty products, which volatilize and contaminate the air during routine tasks such as hair styling or nail treatments. This research emphasizes how salon workers—largely women of color with limited health insurance—bear the brunt of these exposures, which are often higher than for the average population.

This study found that VOC exposure levels vary significantly between salons due to differences in ventilation, product usage, and services offered. Salon workers frequently experience reproductive issues, respiratory problems, and skin disorders, yet few regulations govern these settings. Like the hospital contamination study, this research underscores the need for stronger regulatory action, particularly as these exposures can result in long-term health issues.

In another example of chemical exposure, a study on neonicotinoid insecticides—commonly used in household settings on plants and pets—illustrates how pervasive chemical exposure is in modern life (Wrobel et al.). This research focuses on two widely used insecticides, acetamiprid and imidacloprid, and their presence in the urine of volunteers after household use. Although the study concludes that exposure levels are well below acceptable limits, it highlights the importance of biomonitoring to detect and manage human exposure to hazardous chemicals. Regular users of these chemicals, such as professional gardeners or pet care workers, are particularly vulnerable to cumulative exposure. As the study suggests, more detailed research is needed to fully assess the risks to those who use these chemicals regularly in professional context.

Also, fields that are typically understudied receive attention. The publication by Dietz et al. evaluates systematically the scientific literature about the relevance of oral exposure in workplaces concluding that oral exposure is considered as potentially contributing (123 studies) or explicitly relevant (80 studies). The exposure of firefighters at fire training facilities and of employees at respiratory protection and hose workshops was examined by biomonitoring (Koslitz et al.). Polycyclic aromatic hydrocarbons were found in a fivefold increase in mean for firefighters. However, levels in workshop employees were found to be low, with the majority of urine samples yielding concentrations below the limit of quantification.

Different methods of exposure assessment are reported. Exposure modeling was used in two studies (Aachimi et al.; Hahn et al.), biomonitoring is reported in two studies (Koslitz et al.; Wrobel et al.) and workplace air monitoring is reported in three studies (Sabic et al.; Lothrop et al.; Ramírez et al.). Most of the studies evaluated occupational exposure to different chemicals. However, also microbiological contamination at workplaces is described (Viegas, Eriksen et al.; Viegas, Dias et al.).

The studies on spray processes (Sabic et al.; Hahn et al.), including the use of volatile solvents in industrial painting, bring another layer to the occupational exposure discussion. Real-time monitoring of solvent evaporation during spray application and drying processes showed that secondary exposure during drying often exceeded the initial exposure from spraying. This finding has significant implications for industries like manufacturing and construction, where workers may not realize that drying paints or solvents continue to release harmful chemicals into the air long after application. These studies call for the refinement of existing exposure models and the development of better predictive tools for workplace safety. For example, the current models used for estimating airborne concentrations during spray processes often overestimate or underestimate actual exposure levels. Enhanced, real-time data collection and more accurate exposure models are essential to creating safer work environments.

These studies collectively raise awareness about the silent dangers posed by everyday exposures at workplaces, offering a clarion call for stronger policies, better monitoring systems, and above all, a commitment to worker safety across all industries.

Author contributions

MA-S: Conceptualization, Writing – original draft, Writing – review & editing. SV: Conceptualization, Validation, Writing – review & editing. BC: Conceptualization, Methodology, Validation, Writing – review & editing. MM: Writing – review & editing. US: Conceptualization, Methodology, Validation, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Susana Viegas,
New University of Lisbon, Portugal

REVIEWED BY
Antonio Baldassarre,
University of Florence, Italy
Matteo Creta,
National Health Laboratory, Luxembourg
Cristina Sottani,
Scientific Clinical Institute Maugeri (ICS Maugeri), Italy

*CORRESPONDENCE
Luděk Bláha
✉ blaha@recetox.muni.cz

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Proposals of guidance values for surface contamination by antineoplastic drugs based on long term monitoring in Czech and Slovak hospitals and pharmacies

Lucie Bláhová[1], Luděk Bláha[1]*, Lenka Doležalová[2], Jan Kuta[1] and Tereza Hojdarová[1]

[1]RECETOX, Faculty of Science, Masaryk University, Brno, Czechia, [2]Masaryk Memorial Cancer Institute, Brno, Czechia

Introduction: The exposures to hazardous antineoplastic drugs (AD) represent serious risks for health care personnel but the exposure limits are not commonly established because of the no-threshold effects (genotoxic action, carcinogenicity) of many ADs. In this study, we discussed and derived practically applicable technical guidance values (TGV) suitable for management of AD risks.

Methods: The long-term monitoring of surface contamination by eight ADs was performed in pharmacies and hospitals in the Czech Republic and Slovak Republic in 2008–2021; in total 2,223 unique samples were collected repeatedly in 48 facilities. AD contamination was studied by LC-MS/MS for cyclophosphamide, ifosfamide, methotrexate, irinotecan, paclitaxel, 5-fluorouracil and gemcitabine and by ICP-MS for total Pt as a marker of platinum-based ADs.

Results: The study highlighted importance of exposure biomarkers like 5-fluorouracil and especially carcinogenic and persistent cyclophosphamide, which should be by default included in monitoring along with other ADs. Highly contaminated spots like interiors of laminar biological safety cabinets represent a specific issue, where monitoring of contamination does not bring much added value, and prevention of staff and separated cleaning procedures should be priority. Rooms and surfaces in health care facilities that should be virtually free of ADs (e.g., offices, kitchenettes, daily rooms) were contaminated with lower frequency and concentrations but any contamination in these areas should be carefully examined.

Discussion and conclusions: For all other working places, i.e., majority of areas in pharmacies and hospitals, where ADs are being prepared, packaged, stored, transported, or administered to patients, the study proposes a generic TGV of 100 pg/cm^2. The analysis of long-term monitoring data of multiple ADs showed that the exceedance of one TGV can serve as an indicator and trigger for improvement of working practices contributing thus to minimizing of unintended exposures and creating a safe work environment.

KEYWORDS

hazardous drugs, surface contamination, antineoplastic drugs, monitoring, technical guidance values

1. Introduction

A growing number of oncology patients as well as new types of therapy applications (1) leads to increasing use of antineoplastic drugs (ADs). In 2020, more than 19 million new cases of cancer were diagnosed (2). The therapeutic benefits of ADs with carcinogenic, mutagenic, and teratogenic properties outweigh the risks for patients but they represent a risk for health care workers. The long-term occupational exposures have been associated with adverse health outcomes including reproduction toxicity or cancer (3, 4). Acute adverse health effects in such as skin rashes and hair loss have been also reported (5, 6).

Occupational exposures of health care staff to ADs may occur in pharmacies and hospitals through direct dermal contact, inhalation, accidental ingestion, or indirectly via surfaces contaminated by ADs during their preparation, handling or administration to patients (3, 7). To minimize the occupational exposure and achieve maximum product safety, the preparation of ADs is regulated. Preparation of ADs is usually done in laminar or negative pressure boxes (3). In some countries, including Czech Republic, closed systems such as biohazard safety cabinets (BSC) are required by national regulation for AD preparation (8). However, other processes in handling of ADs are often less controlled and may lead to serious occupational exposures of nurses as well as sanitary staff (cleaning of contaminated floors or desktops/tables, handling and washing of contaminated beddings). Recently, exposures to ADs in home care settings have also been documented (9, 10).

The risks of hazardous medicinal products have recently been addressed by authorities around the world. The European Union updated in 2022 the 2004/37/EC Directive on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (Directive (EU) 2022/431),[1] and a detailed Guidance for the safe management of hazardous medicinal products at work was published in 2023 by the EU Agency for Safety and Health at Work (OSHA).[2] In parallel, the European Trade Union Institute (ETUI) and the European Biosafety Network (EBN) released the updated list of hazardous medicinal products based on the Regulation (EC) 1272/2008 on the classification, labeling and packaging (CLP).[3] The ongoing EU Partnership on Risk Assessment of Chemicals PARC (https://www.eu-parc.eu/) also runs the initiative on pan-European evaluation of ADs occupational risks. Also in the USA, the National Institute for Occupational Safety and Health released a detailed document on managing exposures to hazardous drugs in 2023 (11).

While some occupational exposure limit values have been provided in the EU for 58 industrial carcinogens, mutagens and reprotoxic substances (Annex III of EU Directive 2004/37/EC),[4] no official limits for surface contamination by hazardous medicinal products have been established yet. Correspondingly, national regulations or protocols in health care facilities usually follow the "as low as reasonably achievable" principle (ALARA) to assure low occupational exposures (12). Nevertheless, despite the existing guidelines and prevention measures, monitoring studies still report AD contamination in health care facilities, and the external exposures were confirmed by detection of ADs or their metabolites in urine or blood of health care workers as well as family members of oncology patients (13–18).

Monitoring of contamination in workplaces is an important tool in risk management of these hazardous compounds (3, 10, 12, 19–22), and the standardized wipe sampling of the surfaces is the most broadly used approach to detect contamination by ADs (3). Monitoring results allow to prioritize hot spots, identify major sources, routes of release of ADs during handling, compare situations among health care facilities and positive results often trigger implementation of remedial, and preventive measures (3).

Nowadays, about 100 chemically diverse ADs with various mechanisms of action are used in cancer chemotherapy (3). The consumption of different ADs differ, and some compounds are highly relevant exposure markers with respect to their use and properties such as environmental persistence despite of cleaning procedures (23). For example, in the Czech Republic, about eight ADs are applied intra venously in large quantities including cyclophosphamide (CP), platinum-based drugs (Pt), 5-fluorouracil (FU), paclitaxel (PX), gemcitabine (GEM), irinotecan (IRI), ifosfamide (IF) and methotrexate (MET). These 8 ADs form 50% or more of the AD applications prepared in individual hospitals (9, 10). In agreement with other studies, this shows high importance of few ADs, namely CP, FU and Pt-based drugs as representative markers of occupational exposures (3, 15, 23–25).

The exposure levels in different pharmacy and hospital places may differ by orders of magnitude reaching up to hundreds ng/cm^2 (documented e.g., for CP and FU) or tens ng/cm^2 (Pt-based drugs) (15, 25–27). Most commonly, ADs are analyzed in wipe samples from the floors, desktops or various handles (26). Some sites, such as interior of laminar flow boxes are naturally highly contaminated due to open handling of ADs, lower levels are being found at other sites such as storage rooms, outpatient clinics etc. (21, 25, 28–34). On the other hand, these areas, where the procedures and staff are much less controlled represent higher risk to health care workers, e.g., via transdermal absorption (35, 36).

As mentioned above, individual occupational exposure limits for AD in work environment are not commonly established because of the "no-threshold effects" (genotoxic action of many ADs) and poorly understood links with adverse health effects in workers (3, 32). However, for practical reasons, risk managers seek for recommendations such as threshold guidance values (TGV) or hygiene guidance value (HGV). These have been proposed by some authors based on long-term monitoring data sets, e.g., as the 75th, 90th or 95th percentiles of the detected contamination (27, 30, 31, 34, 37, 38). Exceedance of TGVs (or HGVs) indicates that the exposures are not properly controlled and may trigger implementation of measures. Alternatively, a Dutch study (39) suggested a "traffic-light" model for CP considering correlations between the CP levels in the urine of healthcare workers and corresponding surface contamination. This study suggested that surface concentrations of CP < 0.1 ng/cm^2 might be considered relatively safe ("green"), while CP values above 10 ng/cm^2 are

1 https://eur-lex.europa.eu/eli/dir/2022/431/oj

2 https://osha.europa.eu/en/publications/guidance-safe-management-hazardous-medicinal-products-work

3 https://www.europeanbiosafetynetwork.eu/wp-content/uploads/2022/10/The-ETUIs-list-of-hazardous-medicinal-products-HMPs_2022.pdf

4 https://echa.europa.eu/carcinogens-mutagens-oels

not acceptable and calls for immediate action (39). Numerically similar guidance values 0.1 ng/cm^2 for CP and other ADs were suggested further by Connor et al. (20), Kiffmeyer et al. (31), Crul and Simons-Sanders (40), and Korczowska et al. (32) and this value was also highlighted in a document from the European Biosafety Network commenting on amendments of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens.[5]

Based on these evidences, national organizations continue to release recommendations for handling of hazardous drugs in health care sector (3) but debates on guidance values are still open and other important factors such as combined exposures to ADs mixtures remain to be addressed.

The aim of the present study was to exploit a long-term monitoring data of AD contamination in pharmacies and hospitals in the Czech Republic and Slovakia to propose and discuss practically applicable technical guidance values (TGV). Our research shows that different TGVs may be relevant for different specific areas and places within health care facilities, and we discuss three categories. First, the strongly controlled areas where ADs are prepared (AD preparatory rooms). Second, other places in hospitals and pharmacies, where basic personal protective equipment is used such as storage, transport, administration to patients. Third, places expected to be without major contamination such as offices, daily rooms or kitchenettes. The TGVs derived in the present study support evidence-based and tailored risk management as well as benchmarking of surface AD contamination.

2. Materials and methods

2.1. Material

Analytical standards and solvents were obtained from Toronto Research Chemicals (TRC) or Sigma-Aldrich, British Pharmacopeia Chemical Reference Substances (BPCRS), Analytika (Czech Republic), Merck, and Biosolve BV. More details are provided in Supplementary material. Quality control sample for validation of extraction was prepared in methanol. Field blanks were regularly provided by participating hospitals.

2.2. Methods

2.2.1. Design of the monitoring programme

Monitoring program in the Czech Republic runs since 2008 with Slovak Republic added since 2018. It is organized in campaigns two times per year by RECETOX Center at Masaryk University. As of 11/05/2021 (November) the database used for the present paper contained total $N = 9190$ analyses (data points) covering period 2008–2021. This represented $N = 2{,}223$ unique samples, collected repeatedly in $N = 48$ different pharmacies and/or hospitals. During 2008–2014 only CP and Pt contamination was measured. In 2015, monitoring was further extended with FU, and since 2018–2019 eight ADs (Pt, CP, FU, PX, IF, IRI, GEM, MET) are covered in our monitoring with validated sampling and analytical procedures (21).

Hospitals and pharmacies are invited to voluntarily participate in monitoring, the costs are jointly covered by health care facilities and research grant projects of RECETOX. Participants are provided with standardized sampling kits (described below) and organize own wipe-sampling of surfaces according to the instructions and video manual (https://muni.cz/go/e00d53). Sampling is recommended at the end of a working day or before the next shift, usually before routine daily cleaning in hospitals but the actual sampling strategies reflect needs and decisions of individual participants. The collected wipe-samples are shipped by courier to RECETOX laboratories being responsible for further sample processing, instrumental measurements, and data analyses. The results from each campaign are provided to individual participant, and the participant data are compared with the overall statistics of the annual monitoring. This allows detailed comparing (ranking) of individual hospital/pharmacy within national-wide data. The AD handling procedures at various participants follow generic regulatory recommendations but they cannot be fully harmonized with respect to specific hygiene protocols in different health care providers in Czechia and Slovakia.

2.2.2. Wipe sampling and sample extraction

Surface wipe samples were obtained by standardized procedure (10, 21, 41). Surfaces samples from the pre-marked spots (30 × 30 cm) were obtained with moistened swabs (20 mM acetate buffer, pH 4) and stored at −20°C until extraction. The area of irregular surfaces (such as handles or phones) that could not be marked was calculated after dividing it into regular shapes (e.g., triangles, rectangles, circles) followed by summing up of individual areas. Field blanks (only moistened swab) and quality controls (swab spiked with quality control mixture; CP 3.6 ng/mL, Pt 3.6 ng/mL, FU 7.2 ng/mL, and PX 4.6 ng/mL) were extracted by sonication (45 min; 25 mL of 20 mM acetate buffer pH 4), centrifuged, and the supernatant was used for analyses of organic ADs by LC-MS/MS. For Pt, 0.4 mL aliquot of the supernatant was diluted with 2 ml of 3% hydrochloric acid and analyzed by ICP-MS.

The recoveries of the wipe and extraction procedures from different surfaces were validated in our previous studies (21, 41). Briefly, for CP, Pt, FU mean recovery for all tested surfaces was > 90%. For other monitored compounds - PX, IF, GEM, IRI, MET - mean recoveries were 80, 94, 94, 96, 47%, respectively, for the stainless-steel surface, and 67, 92, 88, 47, 26 %, respectively, for the benchtop material (see Supplementary Table 3 for details).

2.2.3. Instrumental analyses of ADs

Liquid chromatography/tandem mass spectrometry, LC-MS/MS Agilent 1200 coupled with Agilent 6410 Triple-Quad MS was used for analyses of CP between 2008 and 2015 (21). Since 2015, Waters Acquity LC chromatograph (Waters, Manchester, UK) and Xevo TQ-S quadrupole mass spectrometer (Waters, Manchester, UK) were used for multitarget analyses of cyclophosphamide CP, 5-fluorouracil FU, paclitaxel PX, irinotecan IRI, ifosphamide IF, methotrexate MET, and gemcitabine GEM using a recently described multitarget method (41). Analytes were detected in both

5 https://www.europeanbiosafetynetwork.eu/wp-content/uploads/2019/03/Amendments-to-CMD3-and-implications-1.pdf

positive and negative ion modes using tandem mass spectrometry. Settled parameters – i.e., collision energy, cone voltage, retention time as well as the lower limit of quantification, LLOQ, the lowest amount of analyte taken from a known area – 900 cm^2 - in the sample matrix that can be repeatedly quantified (the signal to noise ratio > 10) are presented in Supplementary Table 1. Data were processed by MassLynxTM software (Waters, Manchester, U.K) and corrected to isotopically labeled standards (CP D4; FU 15N2 13C; PX D5; IRI D10; GEM 13C15N2; MET D3). The results of contamination were reported as picograms of AD per square centimeter of the tested surface (pg/cm^2).

Inductively coupled plasma/mass spectrometry, ICP-MS used Agilent 7500ce or 7700x ICP-MS systems (Agilent Technologies Inc., Japan) for the analyses of total Pt concentration as a marker of Pt-based ADs (21, 41). Quantification was based on external calibration (194Pt and 195Pt isotopes) with the correction of signal drift and non-spectral interferences on internal standards (185Rh and 209Bi). Results are reported as pg of Pt per square centimeter of surface.

Although the sensitivity of the measurements of long-monitored substances (such as CP and Pt) improved during years because of new instruments, we decided to use the originally derived limits of quantification throughout the present study. This allowed us to assure consistency when comparing frequencies of contamination.

2.2.4. Data analyses

The analyses were done in Microsoft Excel and GraphPad (Boston, MA, USA) and included stratification of original contamination data into categories based on different places of sampling followed by visualization and calculation of basic statistics such as mean, median, min-max, standard deviation, etc.

3. Results and discussion

The present study investigated surface contamination in 40 pharmacies (N = 1,277 samples) and 43 hospitals (N = 946). In addition to this data set, monitoring covered also 17 patient homes (N = 133), three retirement houses (N = 19), and 2 hospices (N = 10) (9) but the data are not considered in the present paper.

From total N = 2,223 samples collected in hospitals and pharmacies (field blanks excluded), the most frequently sampled areas were desktops/tables and shelfs (N = 1,025) and floors (N = 716). Other types of collected samples included interiors of the BSCs, touch displays, handles, fridge doors, outpatient clinic chairs, phones, toilets, etc. The number of yearly AD preparations in participating hospitals varied and hospitals were categorized according to final report of European Commission (42). The monitoring covered small hospital units without own preparation of ADs (N = 5), hospitals with low number AD preparations per year (max 5 000; N = 17), medium size hospitals with max 15,000 preparation per year (N = 8) and large specialized oncology centers (N = 18) preparing between 15 000 – 58 000 applications of ADs per year.

The most frequently prepared drugs during 2018–2019 were FU (3 300 preparations per year, median within large specialized oncology hospitals), Pt based drugs (median 2 800 preparations), PX (median 1 004), CP (median 936), GEM (median 732), IRI (median 660), IF and MET (both median of 120 preparations per year) (For detail see Supplementary Table 2).

Table 1 and Figure 1 show occurrence and contamination by six ADs, i.e., CP and Pt (covering years 2008–2021), FU (2015–2021) and IF, GEM and PX (2018, 2019–2021). The two ADs included in our monitoring - IRI and MET (since 2018) - were only rarely detected with generally low concentrations (Table 1), and they were excluded from follow-up data analyses.

The data were first categorized by main areas with different working regimes (pharmacies, hospital patient areas, offices), and specific sites within these areas. Figure 1 shows the trends in contamination during the years. In Figure 1, specific sites within an area (i.e., within pharmacy and within hospital) were pooled for simplicity, and the most recent situation is highlighted (data collected during early 2008–2017 years are pooled and compared with individual years 2018, 2019, 2020, and 2021).

As apparent, Table 1 clearly shows that ADs were most frequently detected (and often in high concentrations – see 75th and 90th percentile concentrations in Table 1) on surfaces within interiors of BSCs where ADs are being openly handled and prepared for patients. This is in an agreement with other recent studies, where the highest obtained concentrations (up to 9.27 ng/cm^2 of FU) in BSCs were reported by Sottani et al. (23). Comparably, in Canadian study, maximum contaminations were observed on the floor in front of the BSC (CP up to 120 ng/cm^2) (19). BSCs thus may serve as an important source of contamination for other hospital areas. Namely in situations when cleaning staff is not well-trained and may spread the contamination from BSCs (23). However, under standard conditions, BSCs are likely to pose lower occupational risk because they are closed under-pressure systems, which minimizes potential impact on pharmacy staff, which is commonly well educated and uses extensive personal protective equipment. Contamination of BSCs thus represents a separate issue with respect to exposure scenario, and data of BSCs contamination were excluded and not used for further discussions of TGVs in hospitals.

As predicted, the results clearly showed that areas, where AD contamination should be virtually avoided (offices, kitchenettes, daily rooms) were, indeed, generally less contaminated. In the offices and related areas, only about 20% of samples were positive for few ADs such as Pt, CP and GEM (see Table 1).

Nevertheless, the overall frequencies of occurrence (Table 1) in pharmacies (BSCs excluded) and hospitals were comparable for most ADs and showed high detection rates namely for Pt, CP, GEM and FU (with overall more than 50% of samples positive). Percent positivity (i.e., % above LLOQ) is a useful parameter to characterize contamination, namely when LLOQs of the analytes are within the same range (43), which was the case also in the present study (see details on LC-MS/MS method in Supplementary Table 1). High positivity in our monitoring is comparable to another recent study from Italy that showed 44% positives in pharmacies and 59% in patient care units for CP, FU, GEM and Pt (23). Importance of carcinogenic CP as a major indicator of surface contamination is

TABLE 1 Contamination of different areas (pharmacies, hospital, offices) and specific sites by six ADs in the Czech Republic.

Pt			N (2008–21)	% >LLOQ	75th per.	90th per.	FU	N (2015–21)	% >LLOQ	75th per.	90th per.
	Pharmacy	BSC	74	91%	138	744		88	92%	5 602	19 458
		Work area	486	75%	6	21		352	50%	73	329
		Other	262	53%	2	9		111	19%	<LLOQ	18
	Hospital	WC and outpatient clinic	310	94%	145	679		360	45%	126	596
		Patient and nurse room nurse room	335	77%	13	91		256	46%	143	820
	Office	Office and daily room	133	26%	0.2	1		164	6%	<LLOQ	<LLOQ
CP			N (2008–21)	% >LLOQ	75^{th} per.	90^{th} per.	**PX**	N (2016-21)	% >LLOQ	75^{th} per.	90^{th} per.
	Pharmacy	BSC	99	93%	992	3 428		73	53%	35	180
		Work area	600	69%	54	197		287	15%	<LLOQ	7
		Other	321	36%	4	61		88	2%	<LLOQ	<LLOQ
	Hospital	WC and outpatient clinic	400	83%	199	840		315	53%	87	518
		Patient and nurse room	274	53%	12	93		225	17%	<LLOQ	12
	Office	Office and daily room	182	21%	<LLOQ	7		130	2%	<LLOQ	<LLOQ
GEM			*N* (2019–21)	% >LLOQ	75th per.	90th per.	**IRI**	N (2018–21)	% >LLOQ	75th per.	90th per.
	Pharmacy	BSC	38	92%	420	1 825		57	65%	60	628
		Work area	125	81%	30	164		226	20%	<LLOQ	8
		Other	37	43%	2	9		74	5%	<LLOQ	<LLOQ
	Hospital	WC and outpatient clinic	121	65%	117	743		275	24%	<LLOQ	33
		Patient and nurse room	78	35%	5	19		198	10%	<LLOQ	<LLOQ
	Office	Office and daily room	52	21%	<LLOQ	3		108	2%	<LLOQ	<LLOQ
IF			*N* (2018–21)	% >LLOQ	75th per.	90th per.	**MET**	N (2018–21)	% >LLOQ	75th per.	90th per.
	Pharmacy	BSC	57	75%	104	237		57	23%	<LLOQ	17
		Work area	226	53%	20	84		226	4%	<LLOQ	<LLOQ
		Other	74	46%	6	38		74	1%	<LLOQ	<LLOQ
	Hospital	WC and outpatient clinic	275	13%	<LLOQ	2		275	4%	<LLOQ	<LLOQ
		Patient room and nurse room	198	37%	3	47		198	12%	<LLOQ	3
	Office	Office and daily room	108	14%	<LLOQ	3		108	0%	<LLOQ	<LLOQ

N, number of samples; %>LLOQ, percentage of positive samples; 75th and 90th percentiles, contamination levels in pg/cm^2. Pt, platinum drugs; CP, cyclophosphamide; GEM, gemcitabine; IF, ifosfamid; FU, 5-fluorouracil; PX, paclitaxel; IRI, irinotecan; MET, methotrexate.

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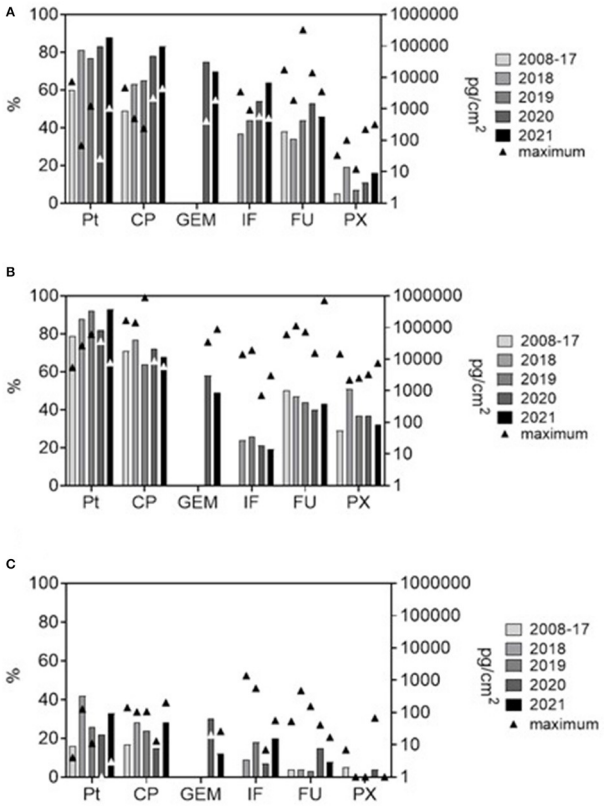

FIGURE 1
Frequencies of positive samples (%, bars; left Y-axis) and maximum contamination levels (pg/cm^2, triangle symbols; right Y axis, log scale) for six antineoplastic drugs from the long-term monitoring. **(A)** pharmacies (excluding internal contamination of biological safety cabinets), **(B)** hospital areas (outpatient clinics and patient rooms), and **(C)** offices (offices, staff daily rooms, kitchenettes etc).

confirmed also in recent studies from Canadian hospitals (19) or France (44).

Importantly, our data showed differing time trends. While apparent declines in % positives over the time were observed in hospitals (Figure 1B), there was an opposite trend of increasing positivity in pharmacies (Figure 1A). Further, there were specific differences between hospitals and pharmacies for PX (higher % positive in hospitals) or IF (more frequently found in pharmacies; Table 1). Although decreasing contamination might be expected with regards to long term recognition of the problem and implementation of remedial measures (19, 28, 32, 39), this is not generally confirmed in all reports. Similarly to the present long-term study, variable and non-systematic trends were also reported for FU contamination in Italian hospitals and pharmacies (23) or for GEM, CP and PX in oncology centers in Canada (43). This variability could be related to complexity of health care services including factors such as workload, cleaning regime, national regulatory requirements, solubility of individual drugs, their metabolization or degradation, etc. (https://ec.europa.eu/social/main.jsp?langId=en&catId=89&newsId=10564&furtherNews=yes&).

As a next step, we analyzed data from 2018–2021 to capture the most recent contamination, and, correspondingly, to derive TGVs reflecting the current situation.

Considering different levels of protection of staff in pharmacies and hospitals, areas were categorized into three groups. First, (i) the AD preparation areas, i.e., the isolated room within a pharmacy, where ADs are being prepared for patients, and staff is well protected (usage of whole body coveralls, goggles, face masks and durable gloves). As described above, data on the inner contamination of BSC were excluded. Second, (ii) other AD handling areas such as delivery and storage areas, dispatch rooms in pharmacies as well as outpatient clinics or patient rooms in hospitals including toilets. Within this second category, certain level of staff protection is usually required and used, typically medical gloves. The third category were (iii) the offices, daily rooms, kitchenettes etc., where workers do not use any protective equipment.

Figure 2 presents the aggregated 2018–2021 data of contamination, and several generic conclusions could be derived. First, the contamination in areas (i) AD preparation and (ii) other AD handling does not substantially differ, the ranges of contamination for most ADs overlap, the 25th-75th quantile range is between 1 and 100 pg/cm^2. Some specific differences, such as higher PX contamination in hospitals, were discussed above. For the category (iii) offices, contamination was lower with maxima exceptionally exceeding 100 pg/cm^2. Nevertheless, data revealed AD contamination even in these areas that are used by completely unprotected staff, and periodic monitoring should be recommended to check potential exposures. Any contamination in this category (iii) offices (i.e., surface concentrations above LLOQ) should call for case by case examination and implementation of corresponding measures. Overall, this analysis shows that separate technical guidance values (TGVs) might relevant for different areas corresponding to different exposure scenarios of workers.

With regards to previously derived TGVs, authors used different approaches but a value of 100 pg/cm^2 (0.1 ng/cm^2) was repeatedly suggested (20, 31, 32, 39, 40). Table 2 shows the comparison of this threshold with the contamination of (i) AD preparation and (ii) AD handling areas in Czechia and Slovakia. In Table 2, data are additionally categorized to tables and working desktops (i.e., spots commonly touched by hands, i.e., higher risk for workers), and the floors (lower risk of direct contact for most of the workers). The exceedance of 0.1 ng/cm^2 threshold ranged between 2% of samples from all surfaces (see Pt in category (i) AD preparatory rooms) to 25% exceedance for FU in both (i) AD preparatory and (ii) other AD handling areas. The most frequent exceedances were – in both categories of areas – observed at FU followed by CP and PX. More specifically, within the (i) AD preparatory rooms (upper part of the table), the most contaminated were packaging desktops and transfer carriages (FU and CP followed by GEM and IF). On the contrary, in the (ii) other AD handling areas, threshold was mostly exceeded on the floors, specifically under the administration IV poles in outpatient clinics and around the toilets (FU and CP followed by Pt, GEM and PX).

Another derivation of TGVs considers statistical analyses and percentiles based on monitoring data. The exceedance of certain

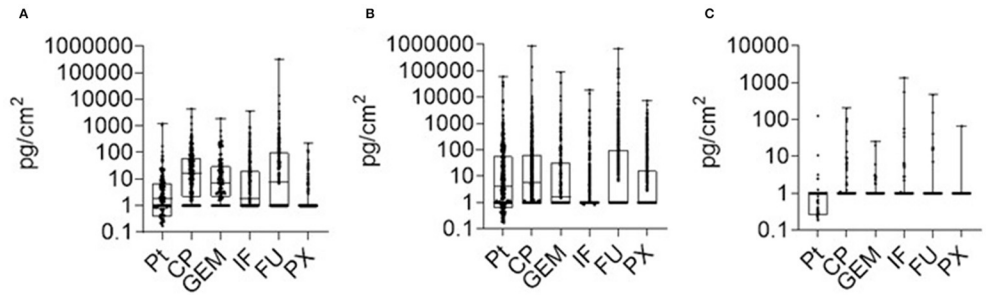

FIGURE 2
AD contamination (2018–2021) in three categories of areas within health care facilities. **(A)** preparation AD areas (inner parts of BSC excluded); **(B)** other AD handling areas, **(C)** offices and daily rooms. Data show median (line) with 25–75 percentile range (box) and minimum-maximum range.

TABLE 2 Exceedance of 100 pg/cm^2 threshold originally suggested for CP by Sessink (39) in hospital and pharmacy samples in (i) AD preparation areas, and (ii) other AD handling and drug administration areas.

	All surfaces		Tables		Floors	
	N	%>100 pg/cm^2	*N*	%>100 pg/cm^2	*N*	%>100 pg/cm^2
AD preparation areas						
Pt	184	2%	128	1%	27	4%
CP	238	15%	168	17%	35	9%
GEM	125	12%	86	14%	17	0%
IF	226	9%	160	11%	32	3%
FU	238	25%	168	25%	35	9%
PX	238	18%	168	18%	35	3%
Other AD handling areas						
Pt	446	20%	214	4%	157	42%
CP	553	20%	257	8%	201	36%
GEM	236	18%	102	8%	89	30%
IF	547	5%	254	4%	199	7%
FU	553	25%	257	22%	201	27%
PX	553	15%	257	4%	201	27%

Data from 2018 to 2021 monitoring; samples from biohazard safety cabinets were excluded.

value, such as 90th percentile, indicates that the sample is among the top 10% highest contaminated, which calls for immediate investigation and remedial actions. From the management perspective, a single TGV (75th, 90th or 95th percentile) is another approach and two TGV levels were also discussed in the literature. For example, Schierl et al. (27) reported monitoring of 102 pharmacies in Germany and proposed that contamination of FU and Pt below the 50th percentile indicates a good working practice, while the values higher than 75th percentile called for adaptation of working procedures.

Detailed analysis of percentiles of our monitoring data is shown in Figure 3 and Table 3. The 90th percentile for all ADs was found to be highly variable in different years which is expected for higher percentiles (e.g., compare Figure 3), while the 75th percentile was more stable in time, and, it thus appeared to be more suitable for derivation of a threshold for the workers and their possible exposure to ADs.

Detailed statistics (Table 3) show that within the (i) AD preparatory rooms, the 75th percentile was in most cases below the suggested 100 pg/cm^2. For the second category - (ii) other AD handling areas - contamination of floors was higher with the 75th percentiles exceeding the 100 pg/cm^2. Desktops/tables and "other" spots (such as door handles) had lower 75th percentiles ranging from <LLOQ to 95 pg/cm^2 for all six ADs. Similar observations of higher floor contamination with 75th percentiles exceeding the 100 pg/cm^2 threshold were also reported by other authors such as Hedmer et al. (37) for CP and IF contamination in Sweden or Labrèche et al. (38) for FU in Canada. Similar conclusions were recently published by Dugheri et al. (45) who observed higher floor contamination (compared to desktops), and suggested the new surface exposure level of 100 pg/cm^2 (with the exception of bathrooms).

Although the floor contamination by ADs is high, direct exposures via skin contact for most of the health care workers is less

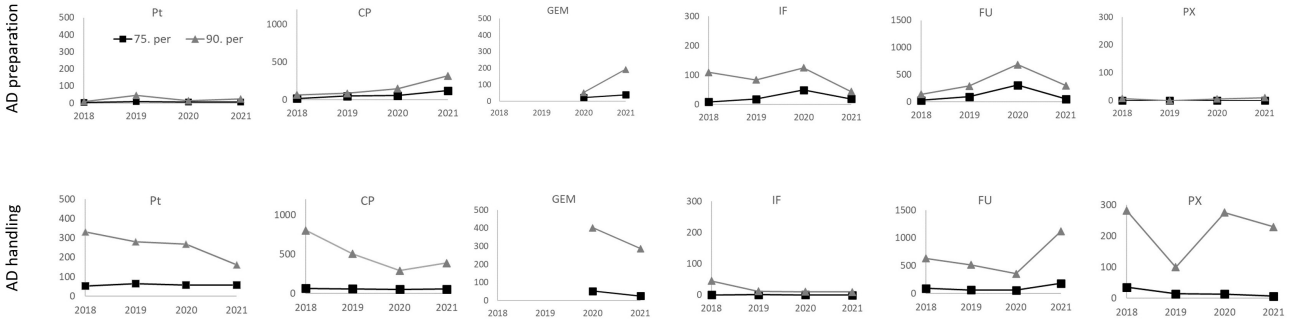

FIGURE 3
Surface contamination (pg/cm^2; 75th and 90th percentiles) by 6 antineoplastic drugs from the 2018–2021 monitoring. Results for two categories of areas with differing protection level, i.e., (i) AD preparation areas - upper panels, and (ii) other AD handling areas - lower panels. Results for biological safety cabinets, BSCs, are excluded.

TABLE 3 Statistics for surface contamination (pg/cm^2) by six antineoplastic drugs from the 2018–2021 monitoring.

		AD preparation areas				Other AD handling areas			
		N	75th per.	90th per.	95th per.	*N*	75th per.	90th per.	95th per.
Pt	Tables	128	7	21	26	214	5	26	83
	Floors	27	3	8	17	157	199	747	4 078
	Other	29	8	27	649	75	57	277	830
CP	Tables	168	62	172	262	257	15	69	211
	Floors	35	47	113	170	201	322	976	2 639
	Other	35	58	173	402	95	35	162	1 535
GEM	Tables	86	34	186	241	102	6	60	133
	Floors	17	3	5	7	89	146	690	1 530
	Other	22	53	88	161	45	28	105	542
IF	Tables	160	19	105	209	254	<LLOQ	7	28
	Floors	32	22	49	80	199	2	31	146
	Other	34	14	34	84	94	<LLOQ	67	561
FU	Tables	168	96	350	815	257	63	445	1 072
	Floors	35	20	50	142	201	137	783	1 661
	Other	35	211	477	1 606	95	95	1 870	6 183
PX	Tables	168	<LLOQ	7	16	257	<LLOQ	9	42
	Floors	35	<LLOQ	<LLOQ	18	201	117	592	1 196
	Other	35	<LLOQ	7	12	95	25	478	1 256

Results are shown for two areas with differing protection level, i.e., (i) AD preparation areas, and (ii) other AD handling and drug administration areas and further categorized for Tables, Floors and Other specific spots (door handles, phones, PDA displays, etc).

likely (37). However, this route of exposure is of specific concern for hospital cleaning staff (38), which should be properly trained how to remain protected, and how to avoid spread of ADs from highly contaminated places such as floors or interiors of biosafety cabinets (44). Although decreasing of the floor contamination may be theoretically achievable, e.g., by repeated applications of strong oxidation cleaning products (41), it is challenging and highly demanding considering common hospital practices.

Finally, a potential effort how to better protect health care workers might be using of TGVs that are annually updated based on periodic contamination monitoring. These could further be "tailored" for different places (e.g., floors vs. desktops) or different ADs (AD-specific TGV). Correspondingly, our study suggests that TGVs for floors should be higher than 100 pg/cm^2 for some ADs so it can be realistically achieved. Such a detailed approach is, however, not very practical for regular hygiene management as many different trigger values might bring uncertainty and confusion. Having one TGV is further supported from our monitoring data, where the 75th percentile for two most important contaminants (i.e., CP and FU) was sufficiently

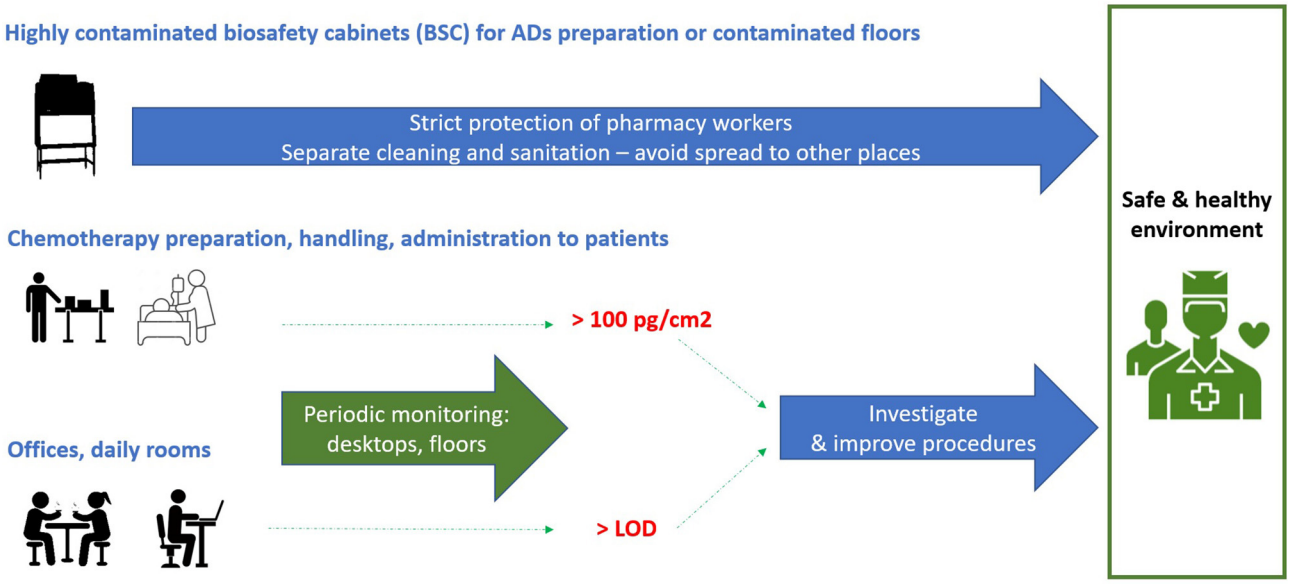

FIGURE 4
Overview of the main findings and practical recommendations.

high to serve also as a trigger for other ADs, i.e., Pt, PX, IF and GEM.

4. Conclusions

The thorough analysis of the long-term monitoring data of AD contamination in Czech and Slovak hospitals revealed following conclusions and recommendations summarized also in Figure 4.

First, it confirmed high relevance of traditional exposure biomarkers such as CP and FU (19). Especially, CP is frequently detected in high concentrations, it is persistent on surfaces (41, 46, 47), and represents a long-term concern considering its carcinogenicity (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2022:088:TOC).

Second, highly contaminated spots, namely interiors of laminar biological safety cabinets (BSC) or flow boxes but also contaminated floors represent a major and separate issue. This should be specifically handled by implementing careful cleaning procedures that are separated from other areas preventing thus potential spread of AD contamination. Cleaning and prevention are priority, and monitoring of AD contamination in BSC interiors does not bring much added value, it might be recommended only case by case.

Third, hospital and pharmacy areas that should be virtually free of AD contamination, i.e., offices, kitchenettes, daily rooms, etc., are indeed less contaminated. However, staff is usually not protected in these areas at all, and periodic monitoring should be performed. Any positive contamination by ADs (i.e., samples >LLOQ) should call for immediate examination and adaptation of preventive measures.

Fourth, for the areas in pharmacies and hospitals, where ADs are being prepared, stored, transported and administered to patients, periodic monitoring is needed. A single value of 100 pg/cm^2 could be suggested as a generic TGV based on the long-term monitoring data of many studies. For most ADs and most exposure situations, this value is close to the 75th percentile (the samples with contamination >100 pg/cm^2 are among the top 25% contaminated). A TGV of 100 pg/cm^2 is thus a "warning" or "trigger" value that calls for investigation and improvement of practices, which may be considered during the implementation of new regulations such as the EU Directive 2022/431.

In conclusion, long-life exposures of health care staff to ADs represent a major issue, and routine monitoring along with implementation of proper measures help to implement the "as low as reasonably achievable" principle (ALARA) (12) minimizing thus occupational risks. Challenging problems that require research attention are the take-home anticancer therapies (48), veterinary clinics or research facilities that might contribute to spread of AD contamination to other environments such as patient homes (9).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LBlaho contributed to the study design, validated methods, performed analyses of organic drugs, collected data, performed statistical analyses, and drafted the manuscript. JK developed methods, analyzed PT in studied samples, and contributed to manuscript writing. LD contributed to the design of the study, sampling, and manuscript writing. TH contributed to

sample extractions, analyses, and to manuscript writing. LBlaho contributed to the study design, method development, data processing, interpretation, and manuscript writing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1235496/full#supplementary-material

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EDITED BY
Sophie Ndaw,
Institut National de Recherche et de Sécurité pour la prévention des maladies professionnelles et des accidents du travail (INRS), France

REVIEWED BY
Ted W. Simon,
TedSimon LLC, United States
Melanie Gorman Ng,
Independent Researcher, New Westminster, Canada

*CORRESPONDENCE
Marlene Dietz
✉ Dietz.Marlene@baua.bund.de

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The relevance of oral exposure in the workplace: a systematic review and meta-analysis

Marlene Dietz[1,2]*, Wiebke Ella Schnieder[1,3], Urs Schlüter[1] and Anke Kahl[2]

[1]Unit 4.I.4 Exposure Assessment, Exposure Science, Division 4 Hazardous Substances and Biological Agents, Federal Institute for Occupational Safety and Health (BAuA), Dortmund, Germany, [2]America Chair of Occupational Safety, School of Mechanical Engineering and Safety Engineering, University of Wuppertal, Wuppertal, Germany, [3]Environmental Monitoring and Forensic Chemistry, Hamm-Lippstadt University of Applied Sciences, Hamm, Germany

Introduction: The inclusion of all relevant exposure routes in the exposure assessment is essential for the protection of workers. However, under European chemical regulations but also for workplace risk assessments according to occupational safety and health (OSH) requirements, the quantitative assessment of oral exposure is usually neglected assuming good occupational hygiene. In contrast, several studies point to the importance of unintentional ingestion in the workplace. To our knowledge, there is no systematic analysis of the extent of this exposure route.

Methods: Therefore, the aim of this study was to assess systematically the current knowledge on the relevance of occupational oral exposure using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) method. Five electronic databases and nine institutional websites were searched for all publications on the relevance. The data were extracted into a concept matrix. In the subsequent meta-analysis, the identified conclusions on the relevance were analyzed. In addition, the measurement methods or modeling approaches that were described for occupational oral exposure were determined as well as the potentially relevant workplaces and substances.

Results: In total, 147 studies were included in this analysis that contain a general or several, differentiated assessments of the relevance of occupational oral exposure. Nine of these studies assessed this exposure route as irrelevant. However, 123 studies considered oral exposure as potentially contributing and 80 studies explicitly identified it as relevant. 78 and 94 of the publications described modeling and measurement approaches, respectively. The workplaces frequently identified as potentially or explicitly relevant were other indoor, other industrial or recycling workplaces. Analogously, metals, dust and powders or pesticides were the most frequently investigated substance groups.

Discussion: As several studies assessed occupational oral exposure as relevant in the context of different workplaces and substances, further investigation of this exposure route is needed. This systematic review and meta-analysis serve as a basis for further development of feasible assessment methods for this route of exposure.

KEYWORDS

inadvertent ingestion, oral exposure, occupation, workplace, overall exposure, PRISMA

1 Introduction

The assessment of all contributing exposure pathways is fundamental for the protection of workers. To date, European chemical regulations (1, 2) or workplace risk assessments under OSH (3) have focused on the assessment of inhalation and dermal exposure. However, oral exposure in the workplace may be a third contributing exposure pathway and depends on several aspects. First, it is influenced by the workers behavior and their compliance with hygiene practices (4–6). For example, the occurrence of oral exposure may depend on the frequency of hand washing during a shift (7). Second, exposure sources such as spray deposition or contaminated surfaces, objects or skin serve as starting points for oral exposure (5, 8). Third, other influences such as the transfer efficiency of substances between hand and mouth (6, 9, 10) and fourth, the nature of a substance such as metals versus infectious agents influence the formation of oral exposure (11).

However, there are different perspectives on oral exposure in the workplace. The REACH Regulation states in its guidance document R.14 that oral exposure only needs to be considered in specific cases. It specifies that compliance with good occupational hygiene practices is usually sufficient to address oral exposure and that no quantitative assessment of unintentional ingestion is needed (12).

In contrast to this, Cherrie et al. estimated that 15.6% of the total UK working population is exposed by inadvertent ingestion (11). In addition, studies comparing external and internal exposure of workers, hint on a potential relevance of occupational oral exposure. In these studies, dermal and inhalation exposure measurements and biomonitoring were performed in parallel. For example, Beattie et al. examined exposure to nickel and hexavalent chromium exposure in the electroplating industry and found that the corresponding biomonitoring levels could not be explained by inhalation and dermal exposure levels, only (13). The authors concluded that ingestion might contribute to the total exposure of workers. In addition, Karita et al. studied the exposure of workers in a lead refinery and demonstrated a positive correlation between external facial or nail exposure and blood lead levels ($r = 0.730$ and $r = 0.590$, respectively) (14). However, the dermal uptake of lead and the inhalation uptake of the present particle sizes were negligible.

Consequently, oral exposure in the workplace could contribute significantly to the total exposure of workers. As total exposure needs to be considered for the overall protection of workers, the aim of this systematic review was to investigate the relevance of oral exposure in the workplace by examining published studies.

2 Methods

To determine the current state of knowledge and to comprehensively identify publications on the relevance of oral exposure in the workplace, a systematic literature review was conducted using the following PRISMA-based (15) method. No review protocol was defined and the review was not registered.

2.1 Information sources and search strategies

The five databases selected as information sources were Web of Science, PubMed, COCHRANE, bergischbib, and Deutsche Nationalbibliothek. Since many project reports are only available on the corresponding institutional websites, nine websites of institutes performing potentially relevant research were added as summarized in Table 1.

Since the focus of this literature search was to identify publications on the relevance of occupational oral exposure, all searches included the topics "occupation", "oral", and "exposure" as a base search. This base search strategy was specified on the basis of four different subject areas, which all can be used to draw conclusions about oral exposure. This approach resulted in four search strategies, all of which included the base search strategy and addressed the relevance of oral exposure in the workplace from different perspectives:

1 Direct statements on the relevance of oral exposure in the workplace
2 Conclusions based on estimates or modeling approaches
3 Conclusions based on measurements
4 Statements based on activities or substances for which the occurrence of oral exposure was considered relevant in advance.

In the authors' experience, the database on the relevance of occupational oral exposure to liquids is limited. Therefore, to ensure comprehensive inclusion of liquid-specific literature on the relevance of oral exposure and to verify this lack of research, liquid-specific information was specifically included in the search by combining the first three general search fields with a liquid-specific term. The fourth search field was not further specified as it already covers substance or activity specific publications.

For each of the liquid-specific and general search fields, search terms and corresponding synonyms were identified independently by two of the authors (MD, WS). The results were discussed and combined into applicable search strings by consensus.

For the evaluation of the developed search strings, 15 already known publications were selected that refer to the relevance of oral exposure in the workplace. To test whether the search strings were able to identify these known publications, the search strings were then applied to the Web of Science and PubMed databases, as these contain eight and eleven of the selected evaluation publications, respectively. By analyzing the obtained search results, the search strings were iteratively improved and specified to maximize the number of evaluation publications covered and to minimize the number of irrelevant publications. The developed search strings identified eight of eight available known publications in Web of Science. In PubMed ten of eleven available publications were identified by the search strings. More information on the evaluation is documented in Supplementary Tables S1, S2. The final seven search strings are included in Table 2.

The applicability of these search strings to websites was limited because some websites do not necessarily allow complex search strings. Therefore, simplified search strings were used on websites, as documented in Supplementary Table S3. The date of the last search is also documented in this table.

TABLE 1 Overview of included databases and institutional websites with corresponding URL.

Information source	Database	Website	URL
Bergischbib	x		http://www.bergischbib.de/
COCHRANE	x		https://www.cochranelibrary.com/advanced-search
Deutsche Nationalbibliothek	x		https://katalog.dnb.de
PubMed	x		https://pubmed.ncbi.nlm.nih.gov/advanced/
Web of Science	x		https://www.webofscience.com
Federal Institute for Occupational Safety and Health (BAuA)		x	https://www.baua.de/DE/Angebote/Publikationen/Publikationen_node.html
United States Environmental Protection Agency (EPA)		x	https://www.epa.gov/nscep (advanced search)
Health and Safety Executive (HSE)		x	https://www.hse.gov.uk/pubns/
Institute of Occupational Medicine (IOM)		x	https://www.iom-world.org/research/online-library/
National Institute for Occupational Safety and Health (NIOSH)		x	https://www2a.cdc.gov/nioshtic-2/advsearch2.asp
Organisation for Economic Co-operation and Development (OECD)		x	https://www.oecd-ilibrary.org/
National Institute for Public Health and the Environment (RIVM)		x	https://www.rivm.nl/en/recentpublications
Netherlands Organisation for Applied Scientific Research (TNO)		x	https://repository.tno.nl/islandora/search/
World Health Organization (WHO)		x	https://apps.who.int/iris/

2.2 Study selection

For the study selection, criteria were defined for the study population (P) and the study outcome (O). Since a two-step selection process was used, the criteria were defined for a title and abstract and a full text screening level. For both screening levels, the study population P had to be workers. This excluded the most common information on oral exposure of children. At the title and abstract level, the outcome O was sufficiently covered by the description of a study design that generally allows conclusions on the relevance of occupational oral exposure. This was narrowed down at the full text screening level, where the outcome O had to be a specific conclusion on the relevance of occupational oral exposure. In accordance with the four search fields, it was not specified whether this is stated directly or inferred from estimates, measurements or substance- or activity-specific information.

Only studies with a publication date between 2000 and 2023 were included in order to reasonably limit the number of studies identified with respect to the extensive search strings. Except for the databases Deutsche Nationalbibliothek and bergischbib, where mainly German language publications are included and where additional analogous German search strings were used, publications had to be in English.

Search results were evaluated against the defined population and outcome criteria. Each assessment was performed by one author, with two of the authors (MD, WS) working in parallel. To ensure reliable assessments within and between these two authors, a consistency check was performed both for the application of the title and abstract criteria and the full text criteria. Both authors applied the criteria to the same sample of 50 (title and abstract) or 10 (full text) randomly selected publications. Then, the authors' decisions on the population and outcome criteria were compared, discussed and concluded by consensus. If necessary, a further result of this discussion could be an adoption of the population and outcome criteria. The comparison of the ratings was formalized by the calculation of Cohen's kappa, which assesses the consistency of the ratings taking into account the consistency that would be expected by chance (16).

During the screening process the publications were sorted according to the occurrence of the keywords "occupation", "worker", "workplace", "occupational exposure", "ingestion", and "oral exposure".

2.3 Data extraction and data analysis

A concept matrix was used to extract relevant data from the included publications. This table contains detailed concepts, each representing possible information from the publications (17). For example, "Oral exposure in the workplace is irrelevant because of good hygiene practices" would be a detailed categorization of information from one or more publications. Using this concept, a comprehensive matrix was created by filling in all included publications. The advantage of this method is the very detailed and structured processing of qualitative or quantitative information.

Preparing the final concept matrix, the qualitative information on the relevance of oral exposure in the workplace was extracted into the course categories "irrelevant," "potentially relevant" and "relevant." Separately, the categories "conclusion based on modeling" or "conclusion based on measurements" were assessed to evaluate the source of information. This process was performed in parallel by two of the authors (MD, WS).

Subsequently, one of the authors (MD) developed concepts that are more detailed. A distinction was made between whether a conclusion was drawn in the publication reviewed or whether the publication reviewed included this conclusion as a citation. In the second case, it was checked whether the primary publication is included in this PRISMA study and, if so, the citing study was not counted as a new statement on the relevance of occupational oral

TABLE 2 Applied seven search strings.

No.	Field	Liquid	String
-	*Base term*	n.a.	(Occupa* OR Job OR Employ* OR Profession* OR Worker OR Workplace OR Industr*) AND (Oral* OR Ingest* OR inadvertent OR incidental OR perioral* OR peri-oral*) AND (Expos* OR Intake OR Uptake OR Ingest*)
1	1	No	*Base term* AND (Relevan* OR Importan* OR Significan* OR Critical* OR Essential*)
2	2	No	*Base term* AND (Estimat* OR Calculat* OR Assess* OR Evaluat* OR Rate OR Rating OR Model* OR Explor* OR Measur* OR Monitor*)
3	3	No	*Base term* AND (Biomonitor* OR Total body burden OR Bio-monitor* OR (dermal AND inhalative AND (biomonitor* OR bio-monitor*)))
4	1	Yes	*Base term* AND (Relevan* OR Importan* OR Significan* OR Critical* OR Essential*) AND (liquid OR fluid OR liquor OR Solution OR spray)
5	2	Yes	*Base term* AND (Estimat* OR Calculat* OR Assess* OR Evaluat* OR Rate OR Rating OR Model* OR Explor* OR Measur* OR Monitor*) AND (liquid OR fluid OR liquor OR Solution OR spray)
6	3	Yes	*Base term* AND (Biomonitor* OR Total body burden OR Bio-monitor* OR (dermal AND inhalative AND (biomonitor* OR bio-monitor*))) AND (liquid OR fluid OR liquor OR Solution OR spray)
7	4	n.a.	(Oral* OR Ingest* OR inadvertent OR incidental OR perioral* OR peri-oral*) AND (Expos* OR Intake OR Uptake) AND (spray* OR paint* OR pesticide OR weld* OR print* OR lead OR electroplat*) AND (Occupa* OR Job OR Employ* OR Profession* OR Worker OR Workplace)

In the search strategies, the term "*Base term*" needs to be replaced by its definition from the first row.

exposure. If the primary publication was not included in this PRISMA study, the citing study identified during this review remained in the evaluation. This procedure avoids overweighting the conclusions of single studies. Due to this approach and the extensive search strategies and databases/websites, no further publications were identified based on the references of included studies.

Based on this categorization, the frequency of different conclusions on oral exposure in the workplace and the possibly underlying modeling or measuring approaches were analyzed. In particular, the dependencies between the workplace, the substance group and the relevance rating were investigated. Furthermore, modeling and measurement approaches underlying the conclusions were disclosed. The risk of a bias was discussed qualitatively.

2.4 Software

During the progress of the searches, the software EndNote X9 (18) was used for literature management. CADIMA 2.2.4.2 (19) was used for the automatic removal of duplicates, which was refined manually to also delete for example the same publications with differently abbreviated journal names. Additionally, the consistency checks, the title and abstract and the full text screening were performed with CADIMA as supportive software. The development of the concept matrix was carried out in Excel 2016 (20) and the further evaluation was performed in R 4.2.3 (21).

3 Results

3.1 Study selection and included studies

Cohen's kappa was used to evaluate the agreement of authors in identifying the publications during the consistency checks for title and abstract and full text screening. The obtained values were 0.627 and 0.814 which can be considered as substantial agreement and almost perfect agreement according to Landis and Koch (22). Therefore, neither the title and abstract criteria nor the full text criteria needed to be adjusted.

By applying the described methodology, a total of 77,739 publications were identified, 10,403 of which resulted from the liquid-specific search strategies 4–6. This highlighted the limited database for liquids compared to solids and in the following liquid-specific and non-specific publications were considered together. After removing duplicates, 30,527 records remained. Since this number could not be fully screened by the authors at the title and abstract level, the first 8,638 (28.3%) publications were examined, after the publications were sorted according to their relevance as described in the methods. Throughout the title and abstract screening process, fewer and fewer relevant publications were identified. Therefore, it was assumed that most relevant publications have been screened. Of the 8,638 records, 8,278 were excluded because of negative ratings of the population and outcome criteria in 6,525 and 6,646 cases, respectively. Of these, 4,893 records were negative for both, population and outcome criteria. 360 publications were identified for consideration at the next screening stage. After the title and abstract screening, the full texts of these remaining 360 publications were all screened, resulting in the inclusion of 147 publications. From the excluded records, 43 and 154 publications did not fulfil the population and outcome criteria, respectively. 30 records did neither fulfil population nor outcome criteria. This screening process was summarized in the form of a flowchart in Figure 1, which additionally documents the reasons for exclusion. A list of the included studies with the extracted information for the workplace, the substance group and the categorization of relevance is provided in Supplementary Table S4.

Detailed categories were developed to extract and summarize the information from the 147 included publications. In addition,

information on the substance and the workplace was extracted in the form of detailed categories as summarized in Figure 2. Where categories overlapped, the most descriptive category was chosen to provide the most accurate picture. For example, agricultural work would be categorized as "Agriculture" rather than "Other outdoor workplaces". If more than one category was needed to specify, e.g., the substance, those categories were selected. For example, an investigation of metal-contaminated dusts would result in the categories "Metals" and "Dust / powder".

Thus, 147 publications made statements about the relevance of oral exposure in the workplace. As shown in Figure 3A, 94 and 78 of these statements were based on monitoring or modeling approaches, respectively. In 123 of the 147 publications, the authors concluded that oral exposure could potentially be relevant for the total exposure of workers. This statement was narrowed down in 80 publications to the conclusion that oral exposure was a definite contributor. Only the authors of nine publications concluded that occupational oral exposure was not relevant. Since in this evaluation publications can be classified into several categories (e.g., when statements are concretized for specific workplaces) the sum of all entries in this evaluation was larger than 147.

3.2 Conclusions on oral exposure in the workplace

The conclusions on the relevance of occupational oral exposure were examined in some more detail here (see also Figure 3B). Of the 80 publications that considered occupational oral exposure to be relevant, 53 considered it to be the main exposure pathway and 31 considered it to be at least one of the contributing pathways next to dermal and/or inhalation exposure. The authors of nine publications mentioned oral exposure as an occupational exposure pathway but considered the investigated situation as irrelevant for oral exposure: one publication due to compliance with good hygiene practices, describing the use of plasticizers in the production of polymers (23); eight due to the specific work considered in these cases (e.g., applications in agriculture or laboratories).

3.3 Identification of relevant workplaces

In the following, the results on the relevance of oral exposure were related to types of workplaces. The aim was to identify workplaces where oral exposure should be considered because of its relevance. This was done by calculating how often each conclusion applied to a workplace. For example, the nine publications that concluded that occupational oral exposure was not relevant described 10 workplaces, two of these were pest control workplaces. By calculating this proportion for all relevance statements and workplaces, Figure 4A was obtained.

As shown in Figure 4A, the energy sector, laboratory workplaces and pest control workplaces had the highest proportion of statements concluding that occupational oral exposure was irrelevant. However, the significance of the results for irrelevant workplaces was limited because this assessment was based on only 10 different conclusions and a 20% evaluation result corresponded to only two underlying conclusions.

When assessing (potential) relevance, energy, laboratory, metalworking, other outdoor workplaces, polymers, weapons or workshops were less frequently named in the context of (potentially) relevant workplaces. In contrast to this, other indoor workplaces,

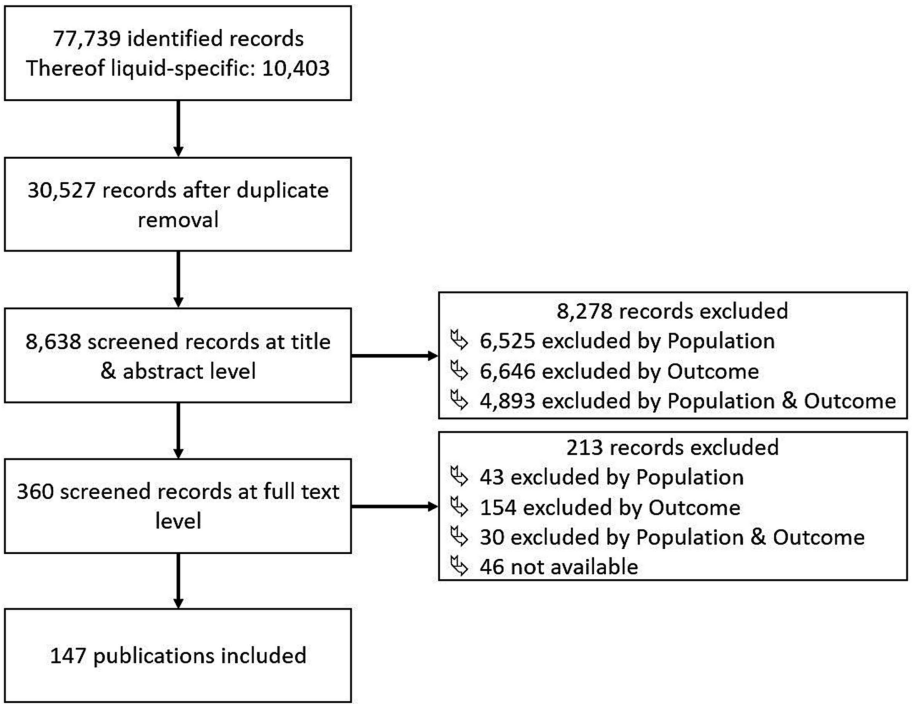

FIGURE 1
Flowchart including number of identified liquid-specific records and negative ratings for population and outcome criteria.

other industrial workplaces and recycling workplaces were stated (potentially) relevant in over 10% of the corresponding evaluations. Thus, these workplaces were most frequently identified as relevant for occupational oral exposure.

3.4 Identification of relevant substances

Since for future assessments not only workplaces but also relevant substances have to be identified, an analogous evaluation of the conclusions on the relevance of occupational exposure and the different substance groups was carried out. Here, for example two of the 10 irrelevant conclusions applied to metals. Figure 4B shows the evaluation results of the different conclusions for substances.

In contrast to the workplace-specific evaluation, the differences between irrelevance and (potential) relevance evaluations were limited in the substance specific-evaluation. This indicated that the current state of knowledge did not allow generalizing conclusions about the relevance of individual substance groups. Instead, Figure 4B shows that the focus of recent research was on dusts and powders, metals and pesticides. Therefore, it was not possible to consider liquids separately for the relevance of occupational oral exposure on this data basis.

3.5 Underlying model and measurement approaches

Because some of the search strategies focused on conclusions about the relevance of occupational oral exposure based on modeling or monitoring, the different underlying approaches were summarized to provide an aggregated view of the information sources.

Figure 5A illustrates that most calculation-based conclusions used a constant ingestion rate to estimate occupational oral exposure. Other common modeling approaches included the contact frequency, the corresponding contact surface, contaminations and compartments. The description of the transfer of contaminations between compartments was also an underlying concept. Less frequently, the personal behavior and calculations based on biomonitoring results were documented. In particular, the frequency of hand washing was not considered in any of the publications. Hand washing could lower the oral exposure by reducing the previous dermal exposure of the hands. Therefore, personal behavior and biomonitoring evaluations were summarized under the term "Others".

In Figure 5B, a similar overview is prepared for the underlying measurement approaches.

Corresponding to the data used in the identified modeling approaches, the most common measurement approaches described the investigation of surface contaminations, dermal exposure measurements, biomonitoring and air monitoring. Consequently, the most frequently documented approaches can be used for parallel measurements, as described in the introduction. Less frequently described were undernail scrapings ($n = 1$), saliva analysis ($n = 2$) or exhaled breath condensate analysis ($n = 3$), which were partially summarized under the category "Biomonitoring". In addition, soil sampling, behavioral observations and analysis of contaminations on PPE or clothing were documented. Furthermore, 30 publications concluded on the relevance of occupational exposure without modeling or measurements, i.e., based on the judgment of the author.

Both, the overview on modeling and measurement approaches showed that complex information and considerable effort are currently required to investigate the oral exposure for specific workplaces. There are no standardized methods for measurement or modeling.

3.6 Analysis of bias

Bias can occur in publications in several ways. One is the omission of certain aspects or information, which can occur for various reasons, such as the intentional non-reporting of assumptions or non-significant results. However, the omission or neglect of relevant issues due to a lack of critical questioning of current guidelines or general practice can also lead to incomplete assessments and thus to bias in publications and the resulting meta-analysis.

With regard to this review, as a quantitative assessment of occupational oral exposure is not yet required, e.g., under REACH, oral exposure was not likely to be considered as a potentially contributing route in many publications. In particular, workplaces where oral exposure is not of concern according to the current state of knowledge, were not further investigated for this exposure pathway. According to this assumption, many publications that would conclude irrelevance could not be found with the applied search strategies because they did not mention the oral exposure pathway. Furthermore, the number of publications which concluded irrelevance may not reflect all associated workplaces, especially since the current standard is not to quantify occupational oral exposure.

This might explain the distribution of included publications, as the number of studies on irrelevance ($n = 9$) was smaller than the number of studies on potential relevance ($n = 123$) or relevance ($n = 80$) which might therefor be a consequence of bias. However, there may be other cases where occupational oral exposure is not relevant due to adherence to good hygiene, irrelevance of oral exposure due to specific workplaces or other yet unidentified reasons. Therefore, there is a risk of bias, in particular publication bias.

4 Discussion

Since the overall exposure of workers needs to be considered for a comprehensive assessment of worker exposure, this systematic review focused on the potential relevance of occupational oral exposure as a route of exposure that has not been the focus of research in the past.

In the course of the review, 147 publications were identified that contained conclusions on the relevance of oral exposure in the workplace. When examining the detailed categories in this review, both conclusions on the relevance and the irrelevance of occupational oral exposure were identified. However, the publications concluding a (potential) relevance of occupational oral exposure outnumbered those concluding no relevance. In particular, studies in which occupational oral exposure was deemed irrelevant might not mention this route of exposure in the resulting publications.

Still, the results of this review indicate that oral exposure may contribute significantly to the overall occupational exposure. According to the results of this review, this depends both on the workplaces and activities. In addition, the dependency on different substance groups was also investigated in this review. However, the

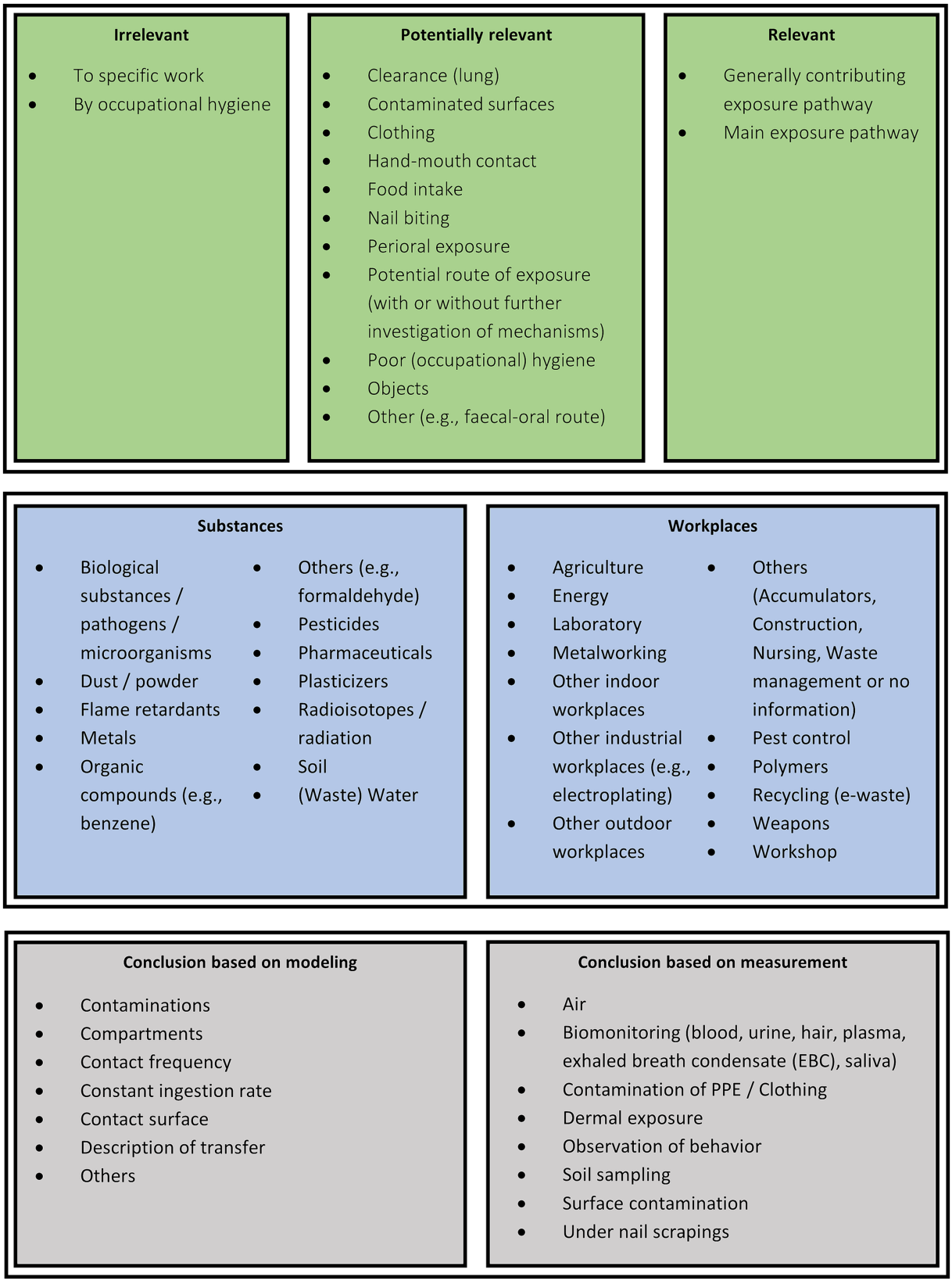

FIGURE 2
Overview of detailed categories for relevance, substances, workplaces and conclusions based on models or measurements.

database did not allow the identification of relevant or irrelevant substance groups here. Instead, it was only possible to identify groups of substances that have been more frequently focused on in the past.

The review also summarized the underlying modeling and measurement approaches. The most common modeling approaches included a constant ingestion rate, contact frequencies, or descriptions

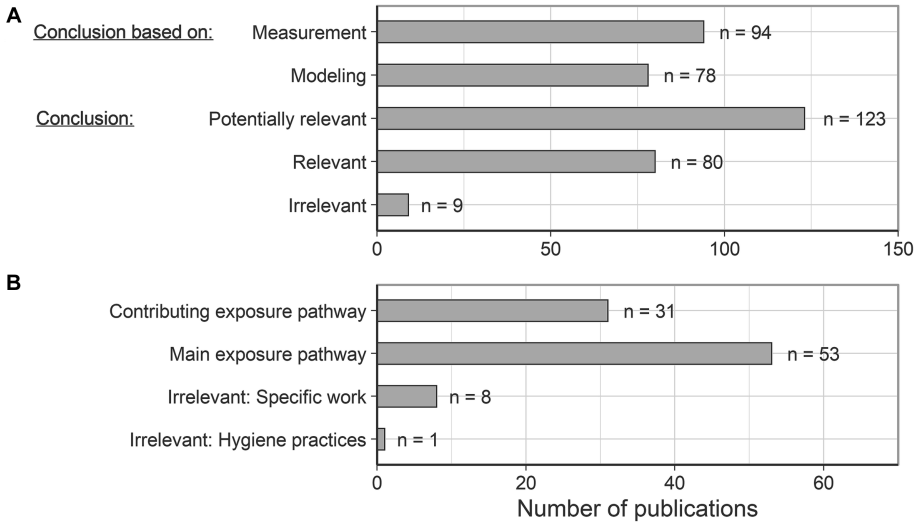

FIGURE 3
(A) Overview of the information bases and conclusions of the included publications on occupational oral exposure. **(B)** Detailed investigation of different conclusions on the relevance of occupational oral exposure.

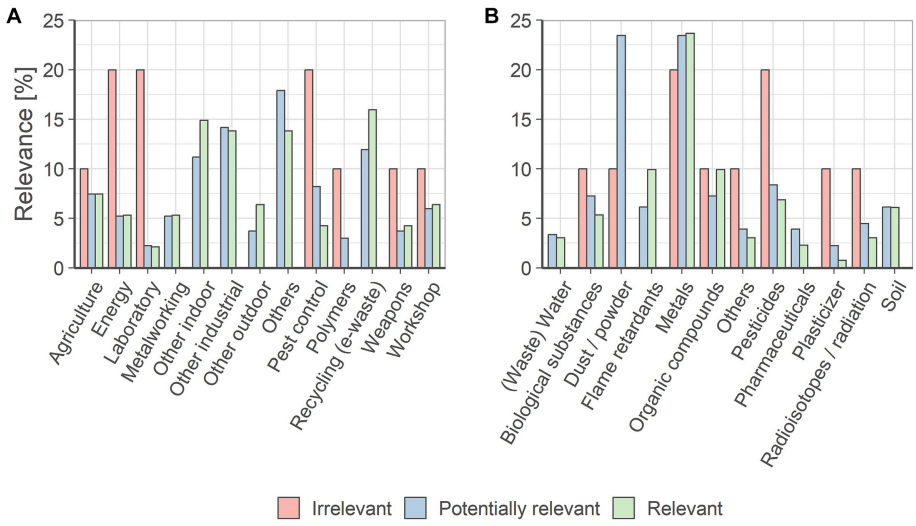

FIGURE 4
(A) Evaluation of the statements on the relevance of occupational oral exposure specific to workplaces. The percentage of relevance relates the respective workplace-specific relevance conclusion to the total number of this conclusion. **(B)** Evaluation of the statements on the relevance of occupational oral exposure specific to substance groups. The percentage of relevance relates the respective substance-specific relevance conclusion to the total number of this conclusion.

of the transfer. Measurements mostly investigated surface or air contaminations and biomonitoring. This shows that there are several different non-standardized approaches to occupational oral exposure assessment that are complex in their data collection requirements and are therefore complex in use.

4.1 Limitations and strengths

Due to the extensive search strings and the resulting number of publications identified, this systematic literature review was limited by the exclusion of studies published before the year 2000 and by the limitation of study inclusion by publication language (English and German). In addition, publications were only assessed by one author during the title and abstract and full text screening. However, the consistency between the authors and within the authors was improved by the consistency checks and the following discussion of criteria and assessments. In addition, the data extraction was not validated by a second author. Instead, the data extraction was performed in small steps starting with a qualitative extraction of relevant information into course categories to ensure a minimization of errors.

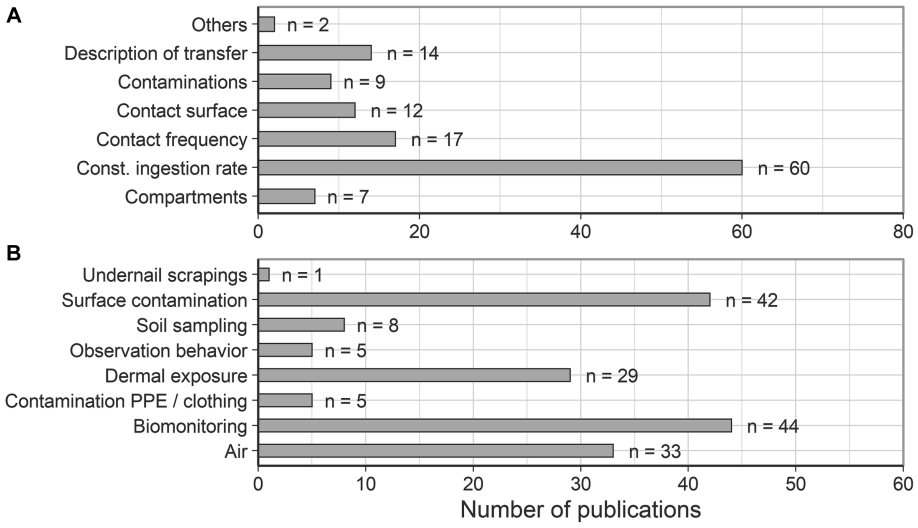

FIGURE 5
(A) Summary of modeling approaches underlying the conclusions on the relevance of occupational oral exposure. **(B)** Summary of measurement approaches underlying the conclusions on the relevance of occupational oral exposure.

A strength of this systematic literature review was its differentiated search strategies, which included different study designs and strategies for drawing conclusions about oral exposure in the workplace, including direct statements as well as conclusions drawn from measurement or modeling approaches. In addition, the search not only included five databases but also websites of research institutes that can provide research reports, which are not covered by databases resulting in a comprehensive enhancement of the knowledge on the relevance of occupational oral exposure to date. Moreover, the sorting of records according to keywords allowed a comprehensive screening process and the identification of the most relevant publications from a large initial dataset. Finally, the developed concept of detailed categories in the concept matrix allowed a detailed and interpretable reflection of the current state of knowledge regarding the relevance of occupational oral exposure.

5 Conclusion

To the authors' knowledge, this is the first systematic literature review on the relevance of oral exposure (to chemicals) in the workplace. It showed that occupational oral exposure can be relevant. So far, this has been documented mainly for other indoor workplaces, other industrial workplaces or recycling. However, an analogous identification of relevant substance groups is not yet possible due to a limited database, especially for liquids. Recent research has focused on the substance groups of metals, dusts and powders, and pesticides.

In order to comprehensively protect workers in terms of their overall exposure, the next step is to specify the conditions of its occurrence with respect to workplaces and substance groups, in particular liquids. Since current approaches to modeling and measurement of occupational oral exposure require complex information and considerable effort, simplified and standardized modeling and measurement approaches are needed for the future assessment of occupational oral exposure.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MD: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. WS: Formal analysis, Investigation, Writing – review & editing. US: Conceptualization, Supervision, Writing – review & editing. AK: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1298744/full#supplementary-material

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EDITED BY
Susana Viegas,
New University of Lisbon, Portugal

REVIEWED BY
Hakan Tinnerberg,
University of Gothenburg, Sweden
Peter E. J. Baldwin,
Health and Safety Executive, United Kingdom

*CORRESPONDENCE
Nathan Lothrop
✉ lothrop@arizona.edu

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Studying full-shift inhalation exposures to volatile organic compounds (VOCs) among Latino workers in very small-sized beauty salons and auto repair shops

Nathan Lothrop[1]*, Flor Sandoval[2], Imelda Cortez[2], Rietta Wagoner[1], Nicolas Lopez-Galvez[1], Kimberly Parra[1], Ann Marie Wolf[2], Betsy C. Wertheim[3], Carolina Quijada[1], Amanda Lee[1], Stephanie Griffin[1], Melanie Bell[1], Scott Carvajal[1], Maia Ingram[1] and Paloma Beamer[1]

[1]Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ, United States, [2]Sonora Environmental Research Institute, Inc., Tucson, AZ, United States, [3]University of Arizona Cancer Center, University of Arizona, Tucson, AZ, United States

Background: One in every 200 US jobs is in a beauty salon or auto repair shop, where workers are regularly exposed to volatile organic compounds (VOCs) that may cause a range of short- and long-term health issues. In these shops, Latino workers are overrepresented and lack culturally and linguistically appropriate industrial hygiene resources. This leaves a gap in knowledge on inhalation exposures to VOCs in this hard-to-reach and ubiquitous worker population.

Objective: Our goal was to recruit hard-to-reach, predominantly Spanish-speaking workers in beauty salons and auto repair shops and monitor total VOC inhalation exposures for over entire work shifts, with minimal impact on workers, clients, and business.

Methods: We developed and refined measurement and exposure assessment methods for personal and area full-shift VOC inhalation exposures.

Results: With minimal participant loss, we measured over 500 h of real-time, personal VOC exposures and recorded activities and other exposure factors for 47 participants, while also documenting chemical inventories and quantifying indoor area concentrations of specific VOCs among 10 auto repair shops and 10 beauty salons.

Conclusion: Lessons learned from our study can assist future studies of inhalation exposures in other hard-to-reach occupational populations.

KEYWORDS

occupational health, community health workers, CBPR, exposure assessment, air pollution

1 Introduction

Inhalation exposures to volatile organic compounds (VOCs) result in well-documented, often irreversible, health effects, including asthma (1, 2), cardiovascular disease (3), cancer (4, 5), adverse birth outcomes (6), and cognitive and neurological symptoms (7). Workers in beauty salons and auto repair shops are exposed to VOCs every day, yet they are almost completely unstudied in the United States (US) with some international studies (8, 9). Worker VOC exposures in *beauty* salons, which offer hair services in addition to other cosmetic work (e.g., nails), have only been studied using area monitors for select VOCs and post-shift urinalysis, which may be complicated by dermal exposure (10). In auto *repair* shops, which do mechanical repairs as well as auto body work, only a handful of specific VOCs in a small subset of work activities have been investigated in the US (11, 12). Meanwhile, one in every 200 US jobs is in a *beauty* salon or auto *repair* shop (13), and this does not include 264,600 self-employed beauty *salon* workers (0.2% of all jobs) (14). Further, *beauty* salons and auto *repair* shops employ over 150% more total workers (13) than the mutually exclusive, well-studied *nail* and auto *body* shops.

While VOC exposure risks are present in all beauty and auto repair shops, they are more acute in small businesses (<100 employees), which are less likely to hire industrial hygiene (IH) consultants (15, 16). Approximately 232,608 (>53%) beauty salon and 304,817 (77%) auto repair workers are employed in shops with <20 employees (17). Latino workers are over-represented in the small business workforce (18) in low-wage jobs with increased risk of occupational injury (19). Meanwhile, they are less likely to trust government agencies to ask for assistance (20), and there are few linguistically and culturally appropriate occupational health materials (21). Further, Latino beauty salon workers in the US use products and processes that are different from other ethnicities (22). Together, this has resulted in a critical gap in exposure knowledge about a sizeable portion of the US workforce.

Like previous studies with hard-to-reach occupational populations (20, 23, 24), our team utilized a collaborative community-academic partnership approach between the Sonora Environmental Research Institute, Inc. (SERI) and the University of Arizona (UA) to measure inhalation exposures to VOCs in predominantly Spanish-speaking workers in beauty salons and auto repair shops in southern metropolitan Tucson, Arizona. We developed methods to discretely measure personal total VOC exposures in real-time for the entire shift and document worker activities and other exposure factors and chemical inventories, all while minimizing impact on worker behaviors, business profitability, and client or customer comfort.

2 Materials and methods

2.1 Overview

SERI *promotoras* or community health workers recruited participants from beauty salons and auto repair shops, which were visited up to two times each. These bicultural and bilingual (Spanish and English) *promotoras* are largely from the community they work with, and have long-established trust and rapport with the small business community and with these specific trades (25). On the initial site visit, the *promotora* facilitated introductions of participants and UA staff. The *promotora* completed a site survey to assess relevant exposure factors, such as ventilation, and a chemical inventory of products at the business. During each visit, UA study personnel measured participants' personal, real-time total VOC exposures for their whole shift, while recording exposure factors, including activity (e.g., bleaching hair, cleaning brakes) and any nearby activity, the room or location, and ventilation conditions. While inhalation personal protective measures were recorded, these were almost non-existent. To measure specific VOCs in the shop, air samples were collected at least once using an evacuated canister. The study took place from June through November 2018.

2.2 Recruitment

Study participants were recruited door-to-door at local businesses by SERI *promotoras* from the study area as described previously (26). *Promotoras* first obtained written permission from the business owner or manager to recruit at the shop. If an owner or manager was not present, the *promotoras* would revisit the shop when convenient. In each participating shop, the goal was to recruit owners, managers, or workers who expected to be at the shop most of the day, to monitor personal VOCs for four shifts per shop, which could include any combination of single or multiple measurements of each participant. This would be completed for 10 shops in each business sector (i.e., beauty and auto repair), for a total of 40 shifts per sector.

Participants had to be ≥18 years of age, able to speak and read Spanish or English, and expect to be employed at the shop for ≥3 months. The last requirement was instituted to ensure follow-up with each participant to have their sampling results reported back to them. Upon consenting, *promotoras* administered participant demographic and background surveys. Personnel completed all verbal and written communications in the participant's language of choice (i.e., Spanish or English), and scheduled site visits for VOC monitoring at the business' convenience. Study subjects were not compensated for their time but would receive their sampling results. All consent was obtained in writing. This study was approved by the University of Arizona Human Subjects Protection Program.

2.3 VOC monitoring site visits

2.3.1 Measuring personal VOC exposures

Real-time monitoring of total VOC exposure was conducted using the ppbRAE 3,000 (RAE Systems, Inc., San Jose, CA), which can detect over 3,000 different VOCs with precision of one part per billion (ppb) with accuracy of 10 to 2,000 ppm: ±3% at calibration point. While the equipment representative suggested we bump test and calibrate monitors before each visit at the individual businesses, the study team was concerned this would add time to the site visit and present complicated liability issues, thereby reducing business recruitment and acceptance. Instead, in the hour before each site visit, UA staff bump tested and calibrated the ppbRAE monitors in a designated fume hood at the UA, as well as performing other diagnostic checks, all as per manufacturer instructions. Each monitor was 'bumped' or tested for accuracy with 100 parts per million (ppm) concentration of isobutylene. While possible to 'translate' a concentration in isobutylene to another VOC, it is impossible to know

what chemical and what proportion during sampling. If a monitor failed the built-in accuracy criteria, it would lock until being successfully calibrated at 0, 100, and 1,000 ppm isobutylene concentrations. Each monitor's pump was tested by blocking the flow of the running monitor, confirming it alarmed, and then restarting the device. A check of monitor lamp contamination was performed by cupping a hand around the probe without blocking the flow. If the concentration rose above 500 ppb and did not return to 0 ppb within 10 s, the monitor's lamp was cleaned.

After we completed our first two sampling visits, we found the monitor's internal clock drifted approximately 3 min every 24 h, making it difficult to link concentrations to recorded activities. To remedy this, we updated monitor time to Arizona Mountain Standard Time before every visit. To determine the monitor logging interval, we tested how a temporary source of VOCs (e.g., a burst of hair spray) would be logged at time intervals of 5, 10, 20, 40, and 60 s by all 7 study monitors. The 20 s logging interval was chosen as it both avoided increased 'noise' as found in smaller intervals, and still captured defined spikes, which were blunted or lost in larger intervals. All monitor alarm sounds were muted during sampling activities to increase comfort of participants and clients, and only visual cues were used.

On the initial site visit, UA personnel would not enter the shop until the SERI *promotora* arrived to introduce them to the business owner and workers. To make the business and participants feel more comfortable, at least one of the same bilingual and bicultural field staff attended all site visits for each shop. After introductions, a ppbRAE was turned on for each participant and run outdoors for two minutes to obtain an ambient background concentration to correct for in later analysis. Study personnel fitted participants at their convenience with the monitor. Participants were given the option to wear the monitor either on a belt or in a sling backpack carried over one shoulder. Other wearable sampler setups (e.g., a leg holster, a 2-strap backpack) were developed and tested with the input of study team members with a range of education and work backgrounds, including those with relevant first- and secondhand work experience and friends, relatives, and acquaintances in these trades. Ultimately, it was decided that the belt and sling backpack were the most comfortable; most convenient to adjust; least esthetically objectionable for beauty salon workers; least likely to get caught on a moving part, a hazard for auto repair work; and most cost-effective to replace. To be easily identified by study staff, each monitor was color-coded with a matching silicone-coated snap-bracelet attached to the belt or pack (Figure 1). The participant could adjust how they wore the monitor as often they wanted, usually with the help of study staff.

The ppbRAE inlet was extended via tubing to collect air within the breathing zone of each participant (i.e., < 0.3 m radius of mouth and nose). The sampling train inlet was secured with an alligator clip to the lapel or apron to allow for movement without the train getting caught on nearby equipment, coworkers, or clients. As recommended by the monitor manufacturer, we used Versilon SE-200 fluorinated ethylene-propylene lined tubing (Saint-Gobain, Courbevoie, France) with two in-line filters with 0.3-micron pore sizes (one at the start and one at the end of the sampling train) to prevent debris or liquid from entering and subsequently damaging the monitor. All connections were done with twist-off, interlocking parts so that tubing length could be quickly changed without tools to reduce workflow disruption. While study staff pilot-tested sampling setups in both simulated and real-world scenarios, it was not discovered until after the first two sampling visits that tubing between the monitor probe tip and the first in-line filter would kink when participants would bend or kneel, which would result in a flow fault and loss of data. Subsequently, the section of tubing was fed through a 10 mm diameter spring to greatly reduce these incidents.

During sampling, study personnel viewed a handheld EchoView Host (RAE Systems, Inc., San Jose, CA), which showed the concentration and alarm status of all active ppbRAE monitors. To protect both participants and study personnel from excessive VOC exposures, we set a 15-min short-term exposure limit alarm of 1,000 ppm, as we were unaware of what VOCs we were measuring or their relative mixture. If a unit went into a short-term exposure limit alarm, all field staff were instructed to leave the building for fresh air

FIGURE 1
Participants wearing the ppbRAE monitor on a belt and in a sling backpack.

and return when the alarm stopped. The owner or manager was made aware of this potential situation during recruitment. When the participant left the site property before the end of their shift (e.g., lunch break), they left the monitor running with field staff until returning. Concentrations recorded during this time off-site are redacted in later analysis (Moreno Ramírez et al., Unpublished). This was done to avoid having to recalibrate the monitor in the field. At the end of the shift, the monitor was turned off upon the participant removing it.

2.3.2 Identifying key exposure factors

During each site visit, study staff observed and recorded participants' activities and relevant information that could influence their VOC exposures for the duration of their shift. Based on the team's experience in studying real-time, micro-level activities of children for exposure modeling (i.e., location, activity and intensity, surface) (27, 28), we developed paper activity logs to record exposure factors relevant to beauty and auto shops. Standardized menus of common activities (e.g., shampooing, changing oil), rooms where activities occurred, and ventilation conditions (e.g., ceiling fan, open window) were created based initially on SERI *promotora* experience and topical literature, and were updated with SERI and UA field experience (Supplementary Tables S1, S2). Staff mapped out rooms and ventilation setups or scenarios on paper at outset of the initial visit and used for all subsequent activity records at the site.

The activity log was set up such that participants could only complete a single activity in a single room at a time, but they could be affected by multiple ventilation conditions and nearby activities. A nearby activity was defined as any activity within 5 m of the participant, as based on how far a release of a pressurized spray released could be detected by a monitor. Changes in exposure factors were recorded down to the second. Whenever the participant changed activity, room, or ventilation scenario, or there was an activity occurring near the observed participant, a new line in the activity log would be created, with the start time marked. Later, the end time of activities would be inferred by the start time of the next. As possible, notes on product use and type were also taken (e.g., applying Brand X hair dye). Any changes in exposure factors, including quick changes between tasks common in beauty salons, lasting <10 s (e.g., sprayed brake cleaner for 3 s) were recorded in notes without beginning a new entry.

Study staff remained in the customer area or other location predesignated by the manager and did not follow participants when they left the area or were out of view. This was both to minimize participant workflow disruption, but also for field staff safety. When participants were out of view, no assumption could be made about their activity, room, or ventilation condition, and this was recorded as "out of view." When the circumstances allowed, study staff inquired with participants about activities that were "out of view" to retroactively update these entries. The end of the participant's work shift was recorded when the monitor was taken off and powered down. After the visit, hand-written activity logs were transcribed into a REDCap (REDCap, Vanderbilt University, Knoxville, TN) database by UA staff.

2.3.3 Inventorying chemicals

To catalog chemicals in the shop during the first site visit for measuring exposures, a *promotora* photographed all products in use or in storage, as shown by the manager or workers. Images were captured and organized on an electronic tablet in a web-based REDCap form. Each image contained multiple products to improve efficiency by minimizing the number of photos. Study personnel also found containers were refilled with products or chemicals different than labeled or not labeled at all. To avoid potential contact with unknown chemical residues, study personnel did not touch product containers unless the shop manager gave explicit permission, and thus did not interrupt workflow. Additionally, study personnel were instructed not to enter any auto paint storage or use facility due to risk of acute diisocynate exposure, despite the risk of compromised image quality. After the conclusion of the first visit, study staff entered each product from the images into a database, along with transcribed chemical ingredient names, Chemical Abstracts Service (CAS) numbers, and amounts by volume from the product's most recent Safety Data Sheet (SDS).

When the SDS was unavailable, ingredients were transcribed directly from matching images of the product label found on the vendor's website. Ingredients were cataloged by CAS number. When a unique chemical had multiple CAS numbers, we chose the CAS number shared by CAS, PubChem, and the most recent exposure limit documents from the National Institute of Occupation Safety and Health and the American Conference of Government Industrial Hygienists. Additionally, ingredients with no CAS number, such as "Perfume" or "Proprietary" were also entered into this database. If a product was a variant or part of a line of items (e.g., different hair dye or auto oil viscosities) we documented these as a single product, unless it had different SDS chemical ingredients, in which case a distinct product was recorded for each distinct SDS.

2.3.4 Measuring specific VOCs in shops

In order to quantify specific VOCs during site visits, we obtained time weighted average concentrations for 73 specific VOCs using a Summa canister and the US Environmental Protection Agency TO-15 analysis method (29). In each shop, a 6 L Summa canister (TestAmerica, Phoenix, AZ) was started at the beginning of the first participant's shift. Summa canisters are evacuated vessels (~29.9 mm Hg) made of specially treated stainless steel that are designed to passively collect whole air samples once the valve is opened. The intake flow controller rate was selected so that 6 L of air would be sampled over the expected length of participant shifts, as communicated by the shop's participants beforehand (typically 8–12 h), to avoid a scenario in which too little air was sampled, which would increase the risk of undetected VOC concentrations. After the desired collection time, the valve was closed, and the canister sent to a laboratory for analysis of the contents.

With input from the manager, in a conversation often facilitated by the *promotora*, we placed each Summa canister in the room with the most expected activity on the floor in a location that would not interrupt workflow. If allowed, additional Summa canister samplers were set up in areas that would remain closed for large parts of the day for specific activities, such as waxing rooms in beauty salons or paint booths in auto repair shops. When allowed, an additional Summa sample was collected during the second site visit to determine between-day variability. Locations of samplers were marked on the aforementioned site map. Canister sampling ended when the last worker wearing a monitor concluded their shift for the day. Duplicate samples (i.e., two adjacent canisters) were taken

in 10% of shops. After sampling, Summa canisters were stored at room temperature until being transported via TestAmerica courier within 1 week of sampling to the TestAmerica facility in Phoenix, AZ.

3 Results

3.1 Recruitment and characteristics of study subjects

Promotoras enrolled 10 of 15 (67%) beauty and 11 of 23 (43%) auto shops they visited. One of the auto shops completed recruitment paperwork but left the study before sampling. In recruited beauty salons, 9 of 10 (90%) eligible owners and 14 of 25 (56%) workers consented to air sampling, while in auto shops, 6 of 10 (60%) eligible owners and 18 of 24 (75%) workers participated. Unlike in beauty salons, auto shop owners were eligible but seldom physically in the shop to participate in sampling. To ensure sufficient data quality and detail, two personal monitoring participants (one per field staff) were scheduled for each per day, necessitating two monitoring/sampling visits in all shops.

One auto shop requested only one sampling day, such that four workers were monitored at the same time by two field staff. In one auto shop, when the field staff arrived for the second visit, the owner said his shop was withdrawing from the study because his two participating workers had complained about workflow disruptions experienced on the first visit. When study staff asked the participants themselves what could be done differently, the owner answered for them, saying they were no longer in the study. The 24 auto repair participants were nearly all male, predominantly Latino, and 40 years old on average, while the 23 beauty salon participants were nearly all female (91%), all Latino, and slightly older (mean age 46.5 years) (Table 1). On average, auto repair work shifts were about an hour and a half shorter than beauty salons (5.8 vs. 7.2 h) as recorded during site visits.

TABLE 1 Characteristics of participants by shop type.

		Beauty salons ($N = 10$)	Auto repair shops ($N = 10$)
		n (%) or Mean ± SD	*n* (%) or Mean ± SD
Age (years)		46.5 ± 9.31	40.0 ± 13.7
Gender	Female	21 (91%)	0 (0%)
	Male	2 (9%)	23 (96%)
	Refused	0 (0%)	1 (4%)
Ethnicity	Latino	23 (100%)	18 (75%)
	Not Latino	0 (0%)	6 (25%)
Race	White	20 (87%)	21 (88%)
	Refused	3 (13%)	3 (12%)
Shop role	Owner/Manager	9 (39%)	6 (25%)
	Worker	14 (61%)	18 (75%)
Shift length (hours)		7.2 ± 1.5	5.8 ± 2.3

SD, standard deviation.

3.2 Measuring personal total VOC exposures

While wearing the VOC monitor, every participant changed from the belt to the backpack or vice versa at least once per shift. Salon participants seldom changed how it was worn (e.g., changing the shoulder for the backpack or shifting the monitor on the belt), yet auto shop workers did this often, especially lying down or contorting themselves to complete a repair. In these cases, field staff would help them reorient the monitor and the sampling train, including swapping out different lengths of tubing as needed to allow them to move freely. In addition, auto repair workers noted the backpack was hot to wear, which was not surprising given they do not work in climate-controlled spaces and sampling took place in summer and fall. Due to personal ergonomic issues, 1 of 24 auto repair and 2 of 23 beauty shop participants had to take off the backpack and hang it nearby for a portion of their shift. The only short-term exposure limit alarm occurred at an auto shop, but in this particular shop, the owner had barred study staff from entering the repair floor at any point. As a result, the participant was only alerted after they left the repair floor. Participant time weighted averages (TWAs) of total VOCs were higher in beauty salons (geometric mean = 2,035 ppb) compared to auto repair shops (832 ppb) (Supplementary Figure S1). In addition, the inter-quartile range of TWAs was smaller for auto repair subjects (2,884 ppb) versus beauty salon workers (4,116 ppb).

3.3 Identifying key exposure factors

In beauty salons, we recorded 277 total hours of activity data, while in auto repair shops, we logged 243 h (Table 2). Beauty participants changed activities, rooms, or ventilation conditions an average of 7.4 times/h, compared to 4.9 times/h for auto workers. In auto repair shops, we could not view or identify participant activity nearly 30% of the time, compared to just 11% in salons. When activity identification was possible, the most common activities in auto shops were mechanical repair (26% of time), administration (15%), and going on break (13%), while fluid services and cleaning (2%) were the least. In beauty salons, hair styling/cutting was the most frequent activity (37% of time), followed by going on break (18%) and hair processing (12%), while the least were cleaning (7%) and skin care (3%). While no nail technicians consented to air sampling, one was active in a salon for a portion of the visit. In auto shops, nearby activities occurred approximately 7% of the time, compared to 25% in beauty shops (i.e., predominantly hair styling/cutting and hair processing). Ventilation conditions varied widely; auto repair shops had 24 unique combinations, yet the most common scenarios involved an open door (40% of time) or local exhaust (35%) (Supplementary Table S3). Among salons, there were 19 distinct ventilation combinations, with participants working in a scenario with central HVAC 80% of the time (Supplementary Table S4).

3.4 Inventorying chemicals

We inventoried 304 total products or product variants from all businesses; of these, 293 were unique in brand, name, and variation (e.g., red vs. blue hair dye), with 114 (39%) in auto and 179 (61%) in

TABLE 2 Activity definitions and durations in hours, ranked from most to least frequent, by shop type.

Auto repair shops		
Activity	General definition	Hours (%)
Unknown	Not observed or reliably deduced	70.0 (29%)
Mechanical repair	No active chemical use; no body work (e.g., rotate tires)	63.0 (26%)
Administration	Work aside from vehicle repair (e.g., talk on phone)	35.5 (15%)
Break	Not working (e.g., lunch)	31.6 (13%)
Painting, body or collision repair	Repairing or painting auto body	20.4 (8%)
Cleaning parts	Cleaning parts by any means (e.g., spray brake cleaner)	11.6 (5%)
Fluid services	Draining or replacing fluids (e.g., oil change)	5.62 (2%)
Cleaning	Cleaning shop itself	5.36 (2%)
All	--	243 (100%)

Beauty salons		
Activity	General definition	Hours (%)
Hair styling/ cutting	Working on hair without any chemical	103 (37%)
Break	Not working (e.g., lunch)	51.2 (18%)
Hair processing	Working with any chemical on hair	32.6 (12%)
Administration	Work aside from beauty activities (e.g., talk on phone)	32.3 (12%)
Unknown	Not observed or reliably deduced	29.8 (11%)
Cleaning	Cleaning shop itself	20.2 (7%)
Skin care	Non-hair beauty work (e.g., waxing, nails)	7.74 (3%)
All	--	277 (100%)

beauty shops. Of these 293 unique products, 73 (25%) had no ingredient information, most of which were beauty products ($n = 61$), including 9 products imported from Mexico. Products with product naming and ingredient information comprised 536 chemicals, of which only 323 (60%) were uniquely identifiable with CAS numbers. We found that in both types of shops, specialty products used on a per-client or per-repair basis were not regularly in stock and obtained only 24–48 h prior to a scheduled appointment, which likely left many specialty products out of the chemical inventory. No shops had up-to-date chemical or product inventories available.

3.5 Measuring specific VOCs in shops

We utilized 16 Summa canisters in auto repair shops (13 in repair/overhaul areas and 3 in paint booths), with 3 shops measured on 2 different days, and 15 Summa canisters in beauty salons in the main work area, with 5 shops measured on 2 different days. No shops refused a sampler on the first visit, nor when the study team requested sampling additional locations or days. In auto shops, 31 unique chemicals were detected, and the most frequent was acetone ($n = 16$ samples), toluene (16), ethylbenzene (14), and xylene (14). Similarly, 31 unique chemicals were detected in beauty shops, and the most common were 2-propanol ($n = 15$ samples), acetone (13), and toluene ($n = 11$). Among detected chemicals, no concentrations were greater than relevant American Conference of Government Industrial Hygienists Threshold Limit Values (Supplementary Tables S5, S6).

4 Discussion

While other studies have investigated VOC exposures in US beauty salons via area monitoring (10) or personal sampling in auto repair shops for a small subset of activities (11, 12), we recruited a hard-to-reach population to study full-shift, inhalation exposures to VOCs in 20 very small auto repair shops and beauty salons among predominantly Spanish-speaking workers in southern metropolitan Tucson, AZ with the aid of *promotoras*. We measured real-time, personal, total VOC exposures and recorded second-by-second activities and other exposure factors (e.g., ventilation) for 47 participants, while also documenting chemical inventories and quantifying indoor area concentrations of specific VOCs. Innate differences between sectors and their work were borne out by participant demographics, work pace and duration, shop size and layout, and product regulations, which subsequently influenced how effectively we could identify personal exposure factors and the availability of product chemical information.

During recruitment, *promotoras* were more successful in enrolling beauty salons (67%) compared to auto shops (43%), yet participation rates were below that of previous work in similar shops (25). However, participation by workers within each business was higher in this study than other occupational health and exposure studies of Latino occupational populations (30, 31). This success speaks to the partnership with SERI *promotoras* and their ability to connect with participants as community liaisons, as shown in other settings (32). Further, no participants themselves explicitly dropped from the study, likely because of the relationships built by *promotoras* and the study's discrete methods for assessing VOC exposures.

Acceptability of methods was demonstrated by no participants asking to leave the study, though some participants had to take off the monitor for a portion of their shift (3 of 47 total participants) to avoid aggravating previous injuries. Further, it was common to adjust the monitor setup, which would sometimes require study personnel help. Auto workers did this often, especially when changing positions to fit in or under a vehicle, while stylists did so but far less frequently. Given that our study is the first to complete real-time personal total VOC monitoring for entire work shifts in these US populations notably in auto repair, we found that our personal monitoring setups worked well but typically required study personnel to assist with adjustments. More pilot testing and input from those in the trades would benefit both the comfort of participants and data quality, while reducing staff time spent adjusting setups.

Generally, beauty shop workers changed tasks more often than mechanics, as evidenced by changes in activity notation per hour. It was common for stylists to move between multiple clients with different processes in multiple areas, while mechanics often focused on a single task for extended periods. One likely reason is the simultaneous presence of multiple customers in beauty shops, as compared to auto shops, which did not have a client actively waiting.

Alternatively, compared to stylists in air-conditioned beauty shops, auto workers commonly work in temperatures of 38° C during this time of year (33) without climate control, potentially resulting in slower activity (34). In addition, moving quickly between rooms and tasks is more possible in small beauty shops, which were no more than 175 m^2 in size, compared to auto shops which almost always encompassed multiple buildings, including one shop with its own salvage yard which had a total area of 5,240 m^2.

As a result of layout differences, we had difficulty identifying activities in auto shops (29% were not observed), due to limited sight lines and that participants often worked in areas unsafe for study staff to enter (e.g., paint booth). The most extreme example was when one auto shop manager forbade field staff from entering the work area to watch participants minutes after the first site visit began. Field staff stayed in the customer waiting area which was in a different building, and would ask participants about their activities when they left the work area on breaks. Not surprisingly, the proportion of unknown activities ranged from 33–99% for participants in this shop. Limited identification of auto repair activities was also in part due to mechanics often working on the opposite side of, inside, or underneath a vehicle. As such, study staff may have felt less comfortable interrupting or talking with workers to ask about previous tasks. In comparison, only 11% of all activity in beauty shops was unknown.

While recording activities and exposure factors on paper forms offered the chance for noting precise detail that might be important, we found that transposing into REDCap was time consuming due to the transposer often needing to confirm detailed handwritten notes. Given the uncertainty of the breadth of tasks and products, the requisite detail and entry speed, and available application development expertise, creating a digital entry system was not feasible at the time. Future endeavors would benefit from creating a digital entry system as used previously to document similar levels of activity detail from recorded video (27, 28). While video recording would benefit data entry accuracy, it was not feasible for this vulnerable population. A SERI *promotora* with significant experience in working with such shops said it would cause owners, managers, and workers to more likely avoid the study because of privacy and liability concerns.

In creating chemical inventories by photographing products in the store and transposing them into a database later, we saved time during documentation at the site visit but expended much more staff effort in database creation. Unlike other studies, which found and used SDS records in shops to calculate exposures (35), we never found updated versions in auto shops (as required by local ordinance) nor in salons. While this may speak to the degree of local fire code enforcement (36) or a knowledge gap among some owners, it also makes it clear that relying on such records alone is not enough for future studies. While most SDSs for auto products listed identifiable chemicals, one in four beauty products either had no listed chemical ingredients or contained unidentifiable compounds, such as "Fragrance."

Companies cannot be forced to disclose "trade secrets" under the Fair Packaging and Label Act if the chemical is deemed non-toxic and used solely in cosmetics (37). Unsurprisingly, all salons had at least one product with unidentifiable chemical ingredients, which carries an important lesson about the potential unknown exposures in the less-regulated arena of beauty salon products. Future research and public health will benefit from California's Assembly Bill 2,775, which requires professional cosmetics sold in the state after July 1st, 2020 to list all chemical ingredients (38). This will likely affect products sold throughout the US, given California is 12% of the US by population (39), which should result in more informative ingredient lists.

Interestingly, while VOCs were the exposure of interest because of their known acute and chronic health effects, formaldehyde (a VOC not measured by any of our monitors) and hydrogen peroxide were also considered based on previous *promotora* experience in beauty salons and some enrolled salon participants asking about these chemicals. Ultimately, the team decided not to sample these chemicals given the reliability of available real-time instruments and other sampling methods in an already difficult situation, not to mention nuanced results interpretation and unbudgeted material and personnel cost. However, it was clear that these contaminants were of upmost concern for stylists and future work should consider sampling for them if the study allows.

In conclusion, we were able to study full-shift VOC exposures for 47 participants in a total of 20 very small auto repair shops and beauty salons in a predominantly Spanish-speaking population using discrete methods, resulting in almost no participant or shop dropout. While this study was completed prior to the COVID-19 pandemic, which inarguably has and will continue to alter work tasks and other exposure factors (e.g., cleaning practices, number of clients, ventilation) (40), methods developed in this study are no less pertinent. Lessons learned here can assist future studies of inhalation exposures in other hard-to-reach occupational populations.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by University of Arizona Human Subjects Protection Program. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NL: Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. FS: Investigation, Methodology, Project administration, Writing – review & editing. IC: Investigation, Methodology, Project administration, Writing – review & editing. RW: Investigation, Methodology, Writing – review & editing. NL-G: Investigation, Methodology, Writing – review & editing, Validation. KP: Investigation, Methodology, Writing – review & editing. AW: Methodology, Writing – review & editing, Conceptualization, Funding acquisition, Project administration. BW: Methodology, Writing – review & editing, Data curation, Formal analysis, Software. CQ: Methodology, Writing – review & editing, Investigation. AL: Methodology, Writing – review & editing, Investigation. SG:

Methodology, Writing – review & editing. MB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Writing – review & editing. SC: Conceptualization, Funding acquisition, Methodology, Writing – review & editing. MI: Conceptualization, Funding acquisition, Methodology, Writing – review & editing. PB: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1300677/full#supplementary-material

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EDITED BY
Urs Schlüter,
Federal Institute for Occupational Safety and Health, Germany

REVIEWED BY
Carolin Dumke,
Federal Institute for Occupational Safety and Health, Germany
Rachel Kerr,
Health and Safety Laboratory (HSL), United Kingdom

*CORRESPONDENCE
Stephan Koslitz
✉ stephan.koslitz@dguv.de

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Biomonitoring of polycyclic aromatic hydrocarbons in firefighters at fire training facilities and in employees at respiratory protection and hose workshops

Stephan Koslitz[1]*, Birgit Heinrich[2], Heiko U. Käfferlein[1], Holger M. Koch[1], Tim Pelzl[3], Katrin Pitzke[2], Daniel Köster[2], Tobias Weiß[1], Volker Harth[4], Thomas Brüning[1], Thomas Behrens[1] and Dirk Taeger[1]

[1]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum (IPA), Bochum, Germany, [2]Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA), Sankt Augustin, Germany, [3]Department of Fire Services, Rescue Services, and Fire Protection of the German Social Accident Insurance, German Social Accident Insurance Institution for the Public Sector in Baden-Württemberg, Stuttgart, Germany, [4]Institute for Occupational and Maritime Medicine (ZfAM), University Medical Centre Hamburg-Eppendorf (UKE), Hamburg, Germany

Introduction: Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic to humans and are formed by incomplete combustion. PAHs are always present during firefighting operations, and fire department members can be exposed to them in the workplace.

Methods: In this study, we analyzed 1-hydroxypyrene (1-OHP) in 36 urine samples from nine firefighters, collected before and after fire training sessions, and 32 urine samples from eight employees at respiratory protection and hose workshops. To assess breakthrough PAH exposure through personal protective equipment and potential dermal uptake, some of the workshop employees wore cotton garments under their regular workwear. Cotton samples were then examined for the presence of 17 semi-volatile and low-volatility PAHs.

Results: After firefighting exercises, we observed approximately a fivefold increase in mean 1-OHP concentrations in samples from firefighters, from 0.24 μg/L to 1.17 μg/L (maximum: 5.31 μg/L). In contrast, 1-OHP levels in workshop employees were found to be low, with the majority of urine samples yielding concentrations below the limit of quantification (LOQ: 0.05 μg/L, maximum: 0.11 μg/L). Similarly, low PAH levels were found on the workshop employees' cotton undergarments, with maximum concentrations of 250 and 205 ng/g for pyrene and benzo[a]pyrene, respectively.

Discussion: In conclusion, significant increases in 1-OHP in urine were observed in firefighters after training sessions, whereas work-related exposure remained low among workshop employees.

KEYWORDS

firefighting, workplace, PAH, exposure, occupational hygiene, urine

1 Introduction

Approximately 40,000 full-time and 1.3 million volunteer firefighters in Germany may be exposed to a wide variety of hazardous chemicals during firefighting operations. The compounds formed during combustion depend, among other things, on the burned material, ventilation (oxygen supply), and temperature. Potential hazards include carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs), benzene, asbestos, cadmium, or silica (1).

In 2007, firefighting work was classified as potentially carcinogenic to humans by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) (Group 2B) (1, 2). A meta-analysis by LeMasters and colleagues (3) provided the basis for this classification. Subsequently, several epidemiological studies on the cancer risk of firefighters were published, including additional meta-analyses (4, 5). These studies reported an increase in overall cancer incidence and in mortality of certain cancers, such as melanoma of the skin, prostate cancer, and mesothelioma. However, the studies showed great heterogeneity in their results. In addition, time- and country-specific effects were also observed (5). Based on the most recent data, IARC re-classified in 2023 occupational exposure as a firefighter as "carcinogenic to humans (Group 1) based on sufficient evidence of cancer in humans" (6, 7). Exposures potentially causal for increased cancer risks, such as PAHs, asbestos, and solar UV radiation, were also mentioned. Robustness of results was observed across sensitivity analyses on mesothelioma and bladder cancer (8).

The majority of human biomonitoring studies to date have dealt with exposures during fire training situations (9–14), although data are still limited. Only one study has been conducted in Germany (14). In addition, exposure of employees who clean contaminated firefighting equipment, in particular, respirators and hoses, has not yet been investigated. Compared to firefighters, employees in workshops are less involved in active firefighting and often do not always wear any personal protective equipment (PPE) that prevents the uptake of hazardous substances. Therefore, we considered workshop employees at fire stations to be an important group of workers who could be exposed to hazardous substances such as PAHs during the cleaning of contaminated firefighting equipment.

2 Methods

2.1 Study participants and exposure scenarios

Members of the fire brigades of Berlin and Hamburg were invited to participate in the study. In addition to active firefighters, employees of the respiratory protection and hose workshops, and emergency workers at a training facility in Berlin were also included. This cross-sectional study was conducted between 2018 and 2020. In a previous publication, we reported results on firefighters who participated in real-life firefighting scenarios, such as building and car fires (15). In this study, we present data on firefighters who participated in firefighting exercises ($N = 9$) and who were employed in the respiratory protection and hose workshops ($N = 8$); these data were not part of the previous publication (Table 1). Trainee firefighters and workshop employees were informed of the aim and scope of the study on-site and gave written informed consent. The study was approved by the ethics committee of the Ruhr University Bochum, Germany (IRB 17-6071).

TABLE 1 Characteristics of the study population.

Characteristics	Fire training facilities	Workshops
Women	1	1
Men	8	7
Age (mean, min–max)	30.6 (20.4–41.2)	41.4 (26.5–53.0)
Years in fire department (mean, min–max)	6.4 (1.8–15.8)	13.9 (0.8–28.2)
Current non-smokers	4 (44.4 %)	7 (87.5 %)
Current smokers	5 (65.6 %)	1 (12.5 %)

The training scenarios studied consisted of classical flashover training in a container in an enclosed space with high smoke density. The training fire was generated by burning wood. Due to the high smoke scenario, all firefighters wore a self-contained breathing apparatus (SCBA) and standard personal protective equipment that included gloves, fire hoods, and helmets. There were two different roles during these exercise sessions: trainer and trainee. Trainers, typically skilled firefighters, stayed longer in the container than trainees. A training session for the instructors generally lasted 180 min, with ∼90 min of direct fire/smoke exposure, whereas the duration of exposure for trainees was much shorter, i.e., 60 and 30 min, respectively. Overall, the training situation of the firefighters, although not completely identical with regard to the burning material and ventilation conditions, can be best compared to that of an attack squad in a fully developed building fire inside a building.

The workshop employees mainly cleaned contaminated SCBAs and dirty hoses that were brought back by the firefighters from training exercises or from fighting real fires such as building and car fires. Usually, the contaminated equipment was first stored outside in a closed container before being brought into the room for cleaning. No protective measures were taken by workshop employees other than the voluntary use of gloves or regular work coats. Frequently, the equipment was inserted directly into the cleaning machine by hand without further pre-cleaning.

2.2 Urine collection and analysis

A urine sample was collected from each participant at an initial appointment with the fire station physician ("baseline sample"). The samples were frozen at −20°C and stored until analysis. A self-administered questionnaire was administered consisting of questions on potential co-exposures to PAHs, including, among others, smoking habits and diet. A bag with additional urine containers and an additional questionnaire to store at the workplace was handed out.

The nine firefighters who participated in the training provided three urine samples each: these were provided 2, 6, and ∼14 h after training. Together with the baseline samples, a total of 36 samples were collected. The eight workshop employees were also asked to collect three urine samples each after finishing work. Because the workshop employees were assumed to have continuous exposure during their entire work shift, they collected a urine sample 2 h after finishing work and additional urine samples before going to sleep and the following morning. However, the final number of samples was 30 (two samples, each at the third sampling time, were not provided). In general, the recommended time point for biomonitoring of work-associated PAH exposure in terms of 1-OHP is directly after the shift (16). We additionally chose "late sampling time points" (6 and 14 h and pre- and post-sleep, depending on the group) because potential dermal exposure might lead to the delayed uptake of PAHs and excretion of PAH metabolites in urine.

Urine samples were aliquoted and analyzed for 1-OHP as previously described (17). The limit of quantification (LOQ) was 0.05 µg/L of 1-OHP in urine. The coefficient of variation was <5%. External quality assurance was performed by successful participation in the German External Quality Assessment Scheme for analyses in biological materials (G-EQUAS) (18).

Creatinine was determined based on the Jaffé method (L.u.P. GmbH Labor- und Praxis Service, Bochum, Germany). Creatinine levels between 0.3 and 3.0 g/L are usually considered normal for regularly hydrated persons, whereas urine collection and biomonitoring should be repeated when creatinine levels outside this range are observed (19). However, in the case of the trainee firefighters, we observed creatinine concentrations of up to 4.0 g/L. Because sufficient hydration was difficult to achieve during firefighter training and all firefighters were well-trained individuals with a high muscle-mass-to-body-weight ratio, we chose to include all urine samples to calculate creatine-adjusted 1-OHP levels.

2.3 Interpretation of biomonitoring results

For exposure and risk assessment of urinary 1-OHP levels, the Biological Exposure Index (BEI®) of the US-American Conference of Governmental Industrial Hygienists (ACGIH) was used. The guidance value of 2.5 µg/L urine does not differentiate between smokers and non-smokers and is a health-based guidance value (20). The BEI® generally indicates a concentration below which nearly all workers should not experience adverse health effects, i.e., in case of PAH exposure and mutagenic (DNA-damaging) effects.

As a second guidance value, the biological reference value (BAR) of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) of the Deutsche Forschungsgemeinschaft (DFG) was used. This guidance level of 0.3 µg/g creatinine is valid for non-smokers only and is not health-based (21). The BAR describes the background level of 1-OHP (in terms of the 95th percentile), which is present in a reference population of people of working age who are not occupationally exposed to PAHs.

Because there is no BAR for smokers, the 95th percentile among smoking individuals from the general population of the 1998 Environmental Survey in Germany (22) was used to interpret the biomonitoring results for smokers (0.7 µg/g creatinine).

2.4 Assessing potential skin contamination with PAHs

To assess the potential for PAH contamination of the skin, workshop employees were offered the use of cotton undergarments underneath their regular workwear. For this purpose, cotton gloves (Würth, Künzelsau, Germany) and cotton shirts (HessNatur, Butzbach, Germany) were provided. The gloves and shirts were checked for the absence of PAHs prior to use. The LOQs were between 2.5 ng (for anthracene) and 50 ng (for naphthalene) (for details, see Table 3). Four out of the eight study participants wore nitrile gloves, and two of these also wore cotton gloves under their nitrile gloves. One person wore only cotton gloves. The remaining three employees wore no gloves at all. Cotton shirts were worn by three employees.

After use, the cotton shirts and gloves were dried at room temperature, packed carefully to prevent cross-contamination, and stored at −20°C. Under this approach (which was adopted for practical reasons), considerable losses of volatile (i.e., low molecular weight) PAHs could not be avoided. Therefore, only analytical results for benzo[e]pyrene and higher were considered valid.

For sample preparation, a standardized punch (diameter 35 mm, Hoffmann SE, Germany) was used. For example, an area of 9.6 cm^2 was punched out of the gloves both at predefined points and at certain hotspots that were visibly contaminated by soot (Figure 1) and analyzed for PAHs as previously described (23). In brief, the cotton pieces were first weighed to take varying fabric thicknesses and seams into account. Then, the samples were mixed with 2.5 mL of acetonitrile/methanol (60/40 v/v), treated for 60 min in an ultrasonic bath, and shaken on a laboratory shaker. The filtered extracts (PTFE) were finally analyzed using high-performance liquid chromatography coupled with diode array and fluorescence detection (HPLC/DAD-FLD). Concentrations of 16 PAHs from the US Environmental Protection Agency (EPA) list, plus benzo[e]pyrene, were determined (24). Naphthalene, acenaphthylene, and acenaphthene were detected by DAD; the other compounds were detected by FLD. The coefficient of variation was <5%. Analytical results are presented in ng/g fabric.

2.5 Interpretation of cotton results

To interpret the PAH concentrations in the punched cotton pieces (EU), Regulation 2018/1513 was used (25). This regulation describes the current EU restrictions on the manufacture, sale, and use of selected carcinogens, mutagens, and reproductive toxicants (category 1A, 1B) in clothing and related accessories, including textiles and footwear. Currently, maximum values of 1 ppm (= 1 mg/kg = 1.000 ng/g) in new clothing materials are enforced for benz[a]anthracene, chrysene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene (B[a]P), and dibenz[ah]anthracene.

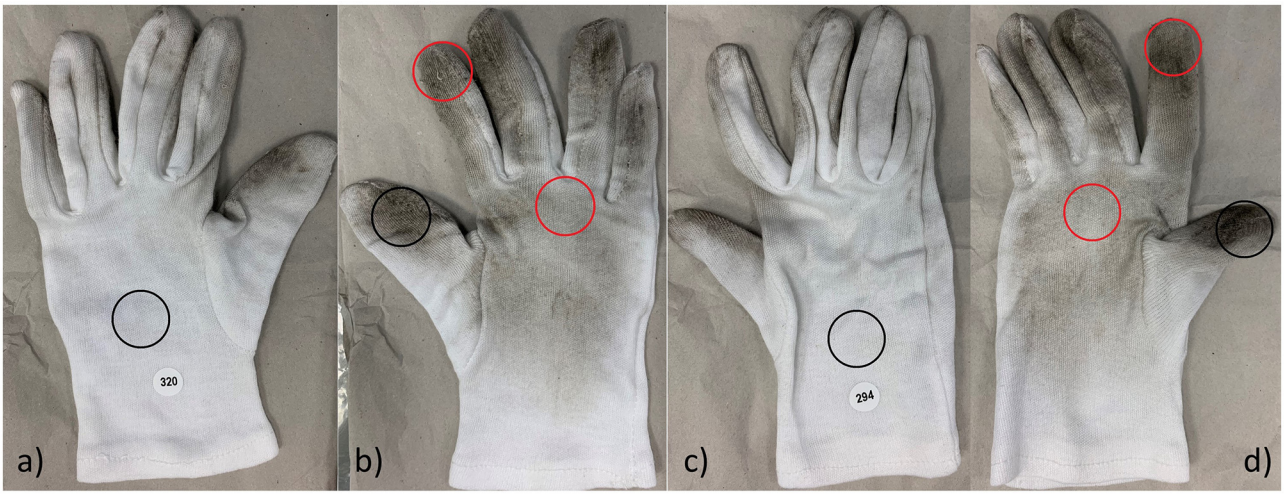

FIGURE 1
Standardized punch areas on the cotton gloves of the left **(a, b)** and right **(c, d)** hands. The black circles represent pre-selected punch sites defined prior to starting the study; the red circles represent post-selected punch sites with potential additional exposure hotspots, characterized by visible contamination with soot. Please note that the "darkness" of the stain is not a proxy for PAH contamination (see "Results").

2.6 Statistical analysis

Descriptive statistics were used to characterize 1-OHP concentrations at the four sample time points (baseline plus three post-event time points) and PAH measurements in the cotton samples. Because of the lack of normal distribution of the measurements, the median and the arithmetic mean, the minimum, and the maximum were calculated. Concentrations were plotted against time points for each participant. The non-parametric Wilcoxon matched-pairs signed-rank test was used to compare the median levels of 1-OHP occurring after the shift to those measured at baseline. The software package SAS version 9.4 (SAS Institute Inc, Cary, NC, USA), was used for analyses. For graphs, GraphPad Prism Version 9.5.0 (GraphPad Software, Boston, USA) was employed.

3 Results

3.1 Firefighters at training facilities

1-OHP concentrations in the baseline urine samples were generally low (maximum: 0.96 µg/L or 0.28 µg/g creatinine) and within the range of the BAR levels of non-smokers (Table 2). After training, 1-OHP levels were above the LOQ in the majority of cases (25 out of 27 cases). Two urine samples from two different firefighters (4.30 µg/L and 5.31 µg/L) exceeded the BEI® level for 1-OHP of 2.50 µg/L. Of note, both firefighters were non-smokers, and their respective baseline samples were below the LOQ. One of the firefighters, an instructor who used an SCBA for 120 min during the training session, showed an unusual pattern of 1-OHP excretion: 1-OHP concentration exceeded the BEI® at sampling point 1 (4.30 µg/L), then dropped considerably (0.35 µg/L), and almost reached the BEI® level again at the third sampling point (2.30 µg/L) (Figure 2A). This pattern remained after creatinine correction (1.38, 0.29 and 1.25 µg/g creatinine). Interestingly, the remaining firefighters, some of whom worked up to 180 min using an SCBA, showed no increase beyond the BEI®.

When evaluating the creatinine-corrected concentrations, in 55.6% of the firefighters (five out of nine), the respective reference level for the firefighter (smoker or non-smoker) was exceeded by up to eight-fold, thus suggesting firefighting-associated exposure to PAHs (Figure 2B), whereas the remaining four firefighters remained within the respective reference level for smokers or non-smokers.

3.2 Employees at workshops

Compared to those of the firefighters after training sessions, 1-OHP concentrations measured in the workshop employees were much lower. All 1-OHP measurements were below the BEI® and below the respective BAR for smokers or non-smokers, depending on the participant's smoking status.

Almost all samples (six out of eight) were below the LOQ at the baseline time point. Even after the employees had completed their cleaning tasks, 59% (13 out of the 22 post-shift samples) remained below the LOQ, thus leading to mean and median concentrations at or below the LOQ. The maximum observed concentration was 0.12 µg/L, which was approximately twice the LOQ. This value was observed in a baseline urine sample. Of the two workshop employees with 1-OHP values above the LOQ at baseline, one was a smoker (0.12 µg/L) and the other was a non-smoker (0.06 µg/L) who reported having eaten smoked and grilled products in the 24 h before urine sampling.

Three of the seven workshop employees provided gloves that they had worn, and in total, 24 cotton pieces were analyzed. For two of the three workshop employees, all PAH levels provided in the samples were below the respective LOQs. However, a wide range of PAH levels was quantified in the

TABLE 2 Summary of urinary 1-OHP measurements ($N = 17$).

Parameter	1-OHP (μg/L)						1-OHP (μg/g creatinine)		
	N	N (>LOQ)	Mean	Median	Range	*P*-value‡	Mean†	Median†	Range†
Fire training facilities									
Baseline	9	5	0.24	0.10	<LOQ*–0.96		0.21	0.21	0.15–0.28
1st sampling	9	8	1.16	0.52	0.12–4.30	0.0195	0.53	0.24	0.17–1.42
2nd sampling	9	8	1.17	0.61	0.29–5.31	0.0078	0.64	0.37	0.25–2.61
3rd sampling	9	9	0.73	0.50	0.27–2.30	0.0742	0.48	0.30	0.28–1.25
Workshops									
Baseline	8	2	<LOQ	<LOQ	<LOQ–0.12		-	-	-
1st sampling	8	3	<LOQ	<LOQ	<LOQ–0.11		-	-	-
2nd sampling	8	4	0.05	<LOQ	<LOQ–0.10		0.06	0.06	0.03–0.08
3rd sampling	6	2	0.05	<LOQ	<LOQ–0.10		-	-	-

* If values for volume-related levels were <LOQ (0.05 μg/L), ½ LOQ was used for statistical analysis; only volume-related levels >LOQ were corrected for creatinine. ‡ p-values for the Wilcoxon matched-pairs signed-rank test compared to baseline results. † If no values are reported, ≤3 volume-related 1-OHP values were >LOQ.

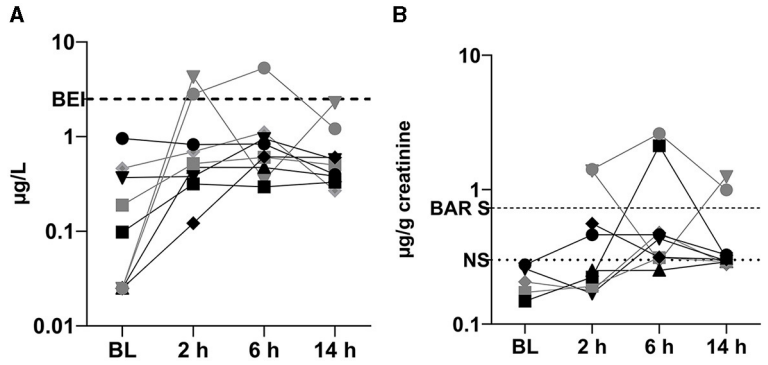

FIGURE 2
Volume-related **(A)** and creatinine-related **(B)** 1-OHP concentrations before and after firefighting training sessions, classified by smoking status (black symbols: smokers; gray symbols: non-smokers). BL, baseline measurement.

eight punch samples from the pair of cotton gloves provided by the third workshop employee (Table 3), who was wearing only cotton gloves during work (with no additional nitrile gloves over them). Therefore, the PAHs found in the cotton material would have been on the employee's hands if he had worked without gloves.

The samples included the well-accepted carcinogen benzo[a]pyrene (37–205 ng/g) and pyrene, which is not known to be carcinogenic (34–250 ng/g). The latter is the parent compound of 1-OHP in urine, which was detected in the employee at regular background levels. Generally, the punch pieces taken from the back of the hands had lower contamination levels than the samples from the thumbs, the hotspots on the palms, and especially the index fingers. The highest concentration levels were observed in the left index finger (Figure 1b). Of note, all concentrations were a factor of 4–5 below the level allowed by (EU) Regulation 2018/1513 for new textile products (25).

All three subjects who wore cotton gloves, also provided cotton shirts. The PAH levels in all 18 punch samples that were taken from the shirts were below the respective LOQ.

4 Discussion

Trainee firefighters and workshop employees showed significant differences in their exposure to PAHs. Whereas, firefighters exhibited an almost five- to sixfold increase in mean urinary 1-OHP concentration (in μg/L) after a shift compared to the baseline measurements, employees in the workshop were not occupationally exposed to PAHs. The latter exhibited baseline as well as post-work 1-OHP levels that were clearly within the respective reference values for smokers and non-smokers in the general German population.

Despite similar exposure settings due to the use of standardized training procedures, we observed a wide range of 1-OHP levels in urine after the training sessions, although the variability was less pronounced for creatinine-normalized levels (0.17–2.61 μg/g creatinine) compared to volume-related levels (0.12–5.31 μg/L). Furthermore, all firefighters wore similar personal protective equipment as they were all part of the same fire brigade and were equipped with the same PPE. There were some differences between firefighters in terms of the amount of time for which the SCBA

TABLE 3 Summary of PAH concentration for one pair of cotton gloves (see also Figure 1) from the respiratory protection and hose workshops ($N = 8$).

PAHs	LOQ [ng/punch sample]	N > LOQ	Mean [ng/g]	Range (min-max) [ng/g]
Naphthalene	50	0	-	-
Acenaphthylene	50	0	-	-
Acenaphthene	25	0	-	-
Fluorene	25	0	-	-
Phenanthrene	13	7	184	76–398
Anthracene	2.5	5	22	11–44
Fluoranthene	11	6	136	85–261
Pyrene	5.0	7	131	34–250
Benz[a]anthracene	3.8	6	101	52–170
Chrysene	3.8	6	95	42–170
Benzo[e]pyrene	25	4	153	131–182
Benzo[b]fluoranthene	5.0	6	107	34–172
Benzo[k]fluoranthene	4.5	5	71	34–100
Benzo[a]pyrene	4.3	6	133	37–205
Dibenz[ah]anthracene	7.3	0	-	-
Benzo[ghi]perylene	4.3	5	112	53–159
Indeno[1,2,3-cd]pyrene	7.5	5	119	57–159

Sixteen EPA-PAH compounds were measured; gray shading indicates selected PAH compounds that are also regulated by EU regulation 2018/1513 (maximum levels allowed in new textiles marketed in the EU: 1 ppm = 1 mg/kg = 1.000 ng/g; benzo[j]fluoranthene was not measured).

was worn, although this did not affect internal exposure levels (data not shown).

Generally, our results are in line with those of previous studies after firefighting training in various countries (9–14). In these studies, a wide range of exposure levels (mostly presented in volume-related levels, µg/L) was observed. The majority of studies observed approximately a two- to sevenfold increase in 1-OHP levels after training sessions (9, 11–14) and, in particular, after burning of chipboard in containers, which is in line with our findings. In addition, a two- to threefold increase in the levels of other hydroxylated PAH in urine samples, such as OH-naphthalenes, OH-fluoranthene, and OH-phenanthrenes, was also observed (14). Interestingly, when diesel was used to burn fires in containers as well as in barrels, no significant increase in 1-OHP could be observed (9, 13). In contrast, increases in 1-OHP levels by up to 30-fold were observed in firefighting trainers when conducting several fire training exercises in a row (i.e., three fire training exercises per day) (10), thus indicating that increased numbers of fire training exercises in a short period of time may result in increased PAH exposure levels.

Although the differences are most likely negligible, the increases in the 1-OHP levels of firefighters conducting training exercises, as reported here, and those in previous studies appear slightly higher compared to those that have been reported in firefighters after real fire missions (15, 26–28). There, only a two- to threefold increase in 1-OHP levels has been observed. These slight differences also became apparent when evaluating the frequency of BEI® exceedance. Of the nine firefighters in our study, two exceeded the BEI® (one of them at two sampling points). Comparing this to our previously published study on firefighters in real firefighting missions (15), we also observed two instances of BEI® exceedance, but this was among a total of 77 firefighters. Reasons may include slightly varying exposure circumstances, such as greater distances when extinguishing real fires or in the presence of fully deployed fires. Therefore, 1-OHP levels in firefighters conducting training sessions are more similar to those of attack teams in the field, i.e., firefighters getting close to flames and smoke in fighting fires where respiratory protection is needed.

The observed increase in 1-OHP in our study was less pronounced (about twofold) after adjustment of the levels by creatinine. However, because the majority of previous studies reported volume-related concentrations, no direct comparison was possible. We recommend that results should be presented as both volume- and creatinine-related levels to better compare results between studies. In addition, in presenting biomonitoring results for subjects with a high muscle-to-body-mass ratio (such as firefighters) creatinine correction seems reasonable.

The 1-OHP levels in the urine of firefighters (either after training or after fighting real fires) appear low relative to those of industrial workers (29). These lower exposure levels became particularly evident when comparing creatinine-normalized values. Median 1-OHP levels in our study after training exercises (0.37 µg/g creatinine) and in our previous study (15) investigating firefighters after real firefighting missions (0.12 µg/g creatinine) were ~10- to 100-fold lower compared to those in industrial workers, i.e., workers employed in the production of coke (3.8 µg/g

creatinine), refractory materials (8.4 µg/g creatinine), carbon electrodes (9.7 µg/g creatinine), and steel (13.5 µg/g creatinine). Even the maximum 1-OHP concentration observed in our study (2.61 µg/g creatinine) was lower than the median concentrations in workers at the aforementioned industrial workplaces (29). Nonetheless, despite the short time of exposure and the use of protective equipment, the slightly increased levels of 1-OHP in firefighters above normal background levels are evidence of firefighting-associated exposure to PAHs. The differences in internal exposure between firefighters and industrial workers, next to differences in external exposure levels, are most likely caused by the use of special protective equipment (including SCBA). Compared to firefighters, industrial workers usually wear, if they use any PPE at all, dust masks (FFP3), overalls, and leather gloves.

Although this interpretation is speculative because direct evidence is missing, PAH exposure in firefighters occurs most likely via dermal uptake. First, personal protective equipment, including SCBA, was frequently used; thus, inhalation exposure during firefighting can be excluded almost with certainty. Second, and in line with other studies (10, 12–14), the peaks of 1-OHP excretion in the urine of firefighters were always slightly delayed, i.e., they occurred 4–6 h after finishing the training or after real fire missions (i.e., the at second sampling point) (15). These findings are in line with delayed absorption, metabolism, and excretion of PAH after dermal uptake. Interestingly, current regulatory guidelines for assessing PAH exposure in terms of 1-OHP in urine recommend urine collection directly after the end of the work shift. However, these guidelines pertain specifically to respiratory exposure routes. In our investigation, where dermal absorption is the major route of exposure, biomonitoring directly after a shift may underestimate exposure levels.

In contrast to firefighters, PAH exposure of employees who clean contaminated firefighting equipment, in particular respirators and hoses, has not previously been investigated. We were able to demonstrate that PAHs were clearly present in the work environment in terms of contaminated equipment. For example, we detected a wide range of semi-volatile and low-volatility PAHs in the punched cotton glove samples of a worker who had worked with contaminated firefighting equipment. Interestingly, based on the REACH regulation for marketing new clothes on the European market, the gloves still could have been sold on the market (25). The observed amounts of seven selected PAH compounds that are regulated by the guidelines were, in each case, below the current EU threshold value of 1 mg/kg (=1,000 ng/g) (Table 3). Nonetheless, the general validity of this finding is certainly limited due to our measurements having been obtained in only a single pair of gloves. The extent of contamination is most likely different each day and might strongly depend on where the equipment was used during the previous firefighting operation. However, because respiratory protection and hose workshops are operated centrally for fire stations, the materials of several firefighting operations are usually cleaned in a single day. Therefore, the PAH residues found on the gloves in our study may also have been derived from contaminated equipment that has been previously used by firefighting attack teams.

Of utmost importance, four of the eight workers in the workshop wore gloves (cotton and/or nitrile gloves). Therefore, it is not surprising that no work-related internal exposure to PAHs, in the form of increased urinary 1-OHP, was observed in the workshop employees. In the majority of cases, 1-OHP levels were below the LOQ, and the maximum observed concentration (0.12 µg/L) was more than 20 times lower than the BEI®. Moreover, depending on the smoking status of the employees, no exceedance of the respective reference values for smokers or non-smokers was recorded. The results suggest that work-associated dermal uptake of PAHs present in the work environment could be almost completely avoided. Therefore, reducing internal work-related exposure can be successfully achieved by wearing gloves.

A major strength of our study is that the internal exposure to PAHs was measured in terms of 1-OHP, i.e., the amount of PAH that was actually taken up by firefighters and workshop employees was examined. Our results show that, irrespective of the presence of PAHs during fires or on contaminated firefighting equipment, protective clothing is highly efficient in minimizing the uptake of PAH. By using simple cotton gloves, we were also able to show that significant contact with PAHs can occur in employees of respiratory protection and hose workshops. Therefore, the use of such gloves is clearly recommended.

The limitations of the study include the fact that the study population was rather small and was not a random sample of firefighters at fire training facilities and workshop employees. Another limitation is that the specific working tasks of workshop employees and the actual contamination of the firefighting equipment remain unknown. There also might have been exposure by inhalation to volatile PAHs that might have been missed by measuring 1-OHP in urine.

5 Conclusions

By using a biomonitoring approach, we showed that using personal protective equipment during training sessions (such as SCBA and firefighter clothing) is highly effective in minimizing PAH exposure. The same applies to the wearing of gloves among workshop employees who are responsible for cleaning firefighters' PAH-contaminated protective gear. Overall, compared to industrial workers, exposure to PAHs in firefighters and employees in firefighting-associated jobs such as cleaning protective gear is low. However, due to the limited number of participants involved in our study and the lack of previous studies on workshop employees, the results should be confirmed in a larger study.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ruhr University Bochum, Germany. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing—original draft. BH: Investigation, Methodology, Writing—review & editing. HUK: Project administration, Supervision, Writing—review & editing. HMK: Methodology, Supervision, Writing—review & editing. TP: Conceptualization, Funding acquisition, Project administration, Writing—review & editing. KP: Investigation, Resources, Writing—review & editing. DK: Investigation, Methodology, Writing—review & editing. TW: Resources, Supervision, Writing—review & editing. VH: Investigation, Resources, Supervision, Writing—review & editing. TBr: Project administration, Resources, Supervision, Writing—review & editing. TBe: Funding acquisition, Resources, Supervision, Writing—review & editing. DT: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing—original draft.

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Conflict of interest

SK, HUK, HMK, TW, TBr, TBe, and DT [as staff of the Institute for Prevention and Occupational Medicine (IPA)], BH, DK, and KP [as staff of the Institute for Occupational Safety and Health of the German Social Accident Insurance (IFA)], and TP (as staff of the Department of Fire Services, Rescue Services, and Fire Protection of the German Social Accident Insurance) are employed by the study's main financing body, the German Social Accident Insurance. IPA is an independent research institute of the Ruhr University Bochum.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Urs Schlüter,
Federal Institute for Occupational Safety and Health, Germany

REVIEWED BY
Andrea Spinazzè,
University of Insubria, Italy
Peter E. J. Baldwin,
Health and Safety Executive, United Kingdom
Jessica Meyer,
Federal Institute for Occupational Safety and Health, Germany

*CORRESPONDENCE
Frederic Clerc
frederic.clerc@inrs.fr

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A control banding method for chemical risk assessment in occupational settings in France

Abir Aachimi[1,2,3], Florian Marc[2], Nathalie Bonvallot[3] and Frederic Clerc[1]*

[1]Department of Pollutant Metrology, Institut National de Recherche et de Sécurité (INRS), Paris, France, [2]Department of Expertise and Technical Consulting, Institut National de Recherche et de Sécurité (INRS), Paris, France, [3]Univ Rennes, EHESP, Inserm, Irset (Institut de recherche en santé, environnement et travail), Rennes, France

Background: This study describes a method whose aim is to help companies assess the chemical occupational risks related to labeled products and industrial chemical emissions. The method is intended to be used by industrial hygienists at the scale of one company. Both inhalation and cutaneous exposure routes are taken into account.

Methods: The method relies on a control-banding scheme. A work situation is described by exposure parameters such as the process or the local exhaust ventilation and by the hazard of the product. Each possible value of the parameters is associated with a "band," which is associated with an integer value. The multiplication of these values results in a score, which represents a priority for intervention. The higher the score, the more the situation warrants investigation for implementing prevention measures, such as chemical substitution and the addition of local exhaust ventilation. To simplify communication, the priority is associated with a colored priority band: red for "very high priority," orange for "high priority," and green for "moderate priority." The priority bands are computed for all work situations performed in a company.

Results: An example of the use of this method is described in a French façade insulation company.

Conclusion: A tool named Seirich was developed to implement this method and promote good practices for helping industrial hygienists in the prioritization of interventions for reducing chemical risk in France.

KEYWORDS

chemical risk assessment, control banding, chemical product, industrial hygiene, priority of intervention

1 Introduction

Occupational health and safety consists of identifying, assessing, prioritizing, and reducing health risks related to exposure to workplace hazards to ensure the safety of employees. In the specific case of chemical risk assessment, a four-step approach is commonly used: identification of the hazard, characterization of the hazard, exposure assessment, and risk characterization

(United States Environmental Protection Agency).[1] In this context, the combination of hazard and exposure data available at the workplace is used. The most accurate way to assess risk is, first, to identify all chemical products found at the workplace and estimate their potential adverse effects with dose–response relationships and, second, to measure workers' personal exposure through biomonitoring or atmospheric sampling according to Landberg et al. (1). Nevertheless, this approach is often difficult to practically implement by companies due to the lack of competencies, information, and resources. Indeed, the time and money required to conduct exposure measurements within the normative constraints (2) and the many uncertainties associated with the characterization of the products' potential hazards are not always tractable and even suitable for the size of a company using thousands of chemical products. The "control banding" method can be used as an alternative solution as it uses simplified and more accessible parameters.

Control banding is a qualitative method to assess and manage workplace risks. It consists of matching the "class" for health hazards, exposure potential, and risk mitigation measures. The result of this matching is the generation of a "risk band" that represents the level of risk, which helps the hygienist prioritize and determine prevention action plans as described in Zalk and Nelson (3) and Zalk and Heussen (4). According to Naumann et al. (5), this method was first developed in the 1980s within the pharmaceutical industry to ensure the safety of workers regarding the use of products for which little information was available. To make this method user-friendly and accessible to all companies and to determine an appropriate control strategy for occupational risks, several tools were then developed. As an example, 30 years ago, the UK Health and Safety Executive developed "COSHH Essentials" described in Brooke (6) and Garrod et al. (7) and in the Health and Safety Executive (8) guidance, which is a control-banding tool that determines, through advice and guidance, a control approach to monitor substances that may affect workers' health. More recently, in 2008, in the context of a Dutch program to reinforce the working conditions policy on hazardous substances, the web-based tool "Stoffenmanager," described by Cherrie et al. (9) and Marquart et al. (10), was developed to identify chemical hazards and control exposure in the workplace. The hazard banding scheme consists of allocating substances to particular hazard groups based on their toxicological classification and labeling under the CLP regulation, as mentioned by Garrod et al. (7). In 2010, "EMKG" (Einfaches Maßnahmenkonzept Gefahrstoffe) was developed by the German Federal Institute for Occupational Safety and Health (11). As with the other tools, EMKG offers a simple approach to evaluate occupational risks and identify management measures requiring only a minimal number of input parameters.

In 2005, the French National Research and Safety Institute for occupational risk prevention (12), in collaboration with the National Prevention and Protection Centre (CNPP), developed a simplified control banding method described by Vincent et al. (13). The method is intended to be used by anyone with minimal knowledge of chemical risks, using simple and easily accessible parameters. This method evaluates the chemical risks resulting from the potential hazard and exposure to the products used during a task. Later, in 2008, the EU CLP regulation was introduced: the method was updated to support the "H" hazard statements instead of the "R-phrases." The method is therefore always based on a qualitative assessment of chemical risks, and the output is a relative prioritization of products and industrial chemical emissions for each task performed in the company. The aim of this prioritization is to sort work situations that warrant investigation for implementing prevention measures. Concretely, a hazard band and score are assigned to each product used with regard to the "H" hazard statements. Then, an exposure band and score are assigned, based on sub-scores for each descriptive parameter influencing exposure (process, protective equipment, etc.). Finally, the hazard score and the exposure score are multiplied, and the resulting score is a relative prioritization of the chemical product (Figure 1).

In the first part of this article, the control banding method mostly used by French companies is described. In the second part of this article, a case study of a French insulation and house façade repair company is presented. The workstation chosen for the assessment was the "installation of thermal insulation," which includes numerous tasks conducted with different products used or emitted.

2 Materials and methods

The proposed control banding method has a broad domain of applicability. Since it focuses on the chemical products (a mixture of substances), it allows us to prioritize any CLP-labeled chemical product used in the company, whatever their toxicity since the starting point is the H statements. The chemical products not submitted to the CLP regulations (for example, cosmetics, food products, or waste) and the chemical industrial emissions can also be prioritized. The method consists of three main steps: (I) assignment of the hazard class and score; (II) assignment of the exposure class and score; and (III) calculation of the priority score and assignment of the colored priority band: red for "very high priority," orange for "high priority," and green for "moderate priority." This method has to be followed by the set-up of a prevention action plan to eliminate or reduce the risks threatening the health and safety of employees.

2.1 Step 1: assignment of the hazard class and score

In a preliminary task, a map of working areas, workstations, and tasks performed at the company must be prepared. Then, the chemical hazards for each task can be inventoried. For each product, the hazard may be related to a labeled product covered by the European labeling regulation (CLP; i.e., paints, inks, and solvents), a product not covered by the CLP labeling (i.e., flour, sugar, and cosmetic products) or industrial chemical emissions during a particular process without a precise description of products (i.e., wood sanding dust or welding fumes). The hazard is expressed as a hazard class and its corresponding score is expressed as an integer. The hazard class is attributed differently depending on the nature of the chemical:

- For the labeled products covered by CLP labeling, the hazard class is determined through the H and EUH statements available in the SDS or on the product label. Each H or EUH statement is associated with a hazard score according to gravity and potential for

1 https://www.epa.gov/risk/human-health-risk-assessment

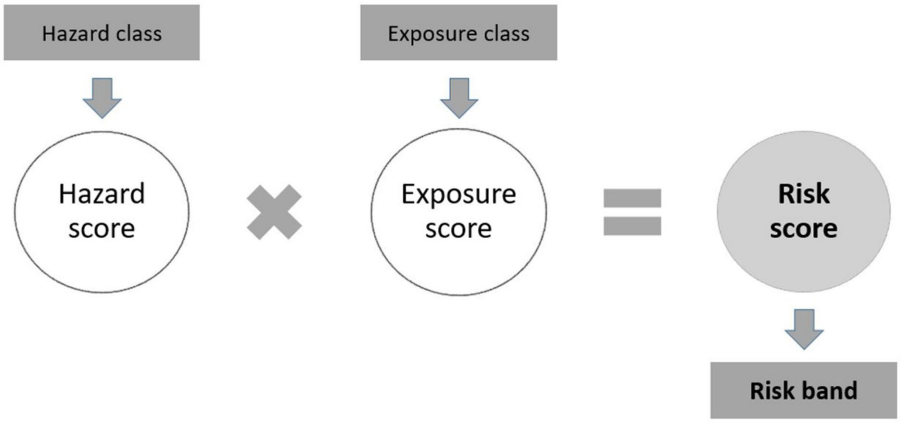

FIGURE 1
Principles of assessment for chemical risks using the control-banding method.

immediacy of effect mentioned by the statement. If a product has several hazard statements, the most severe is considered. An overview of the hazard classification for the inhalation route is presented in Table 1. The same principle is used for dermal exposure (data not shown).

• For the chemical products not covered by CLP labeling and the industrial chemical emissions, the hazard is defined by a consensus of a group of experts in the field of chemical risk prevention. The substances emitted, their toxicity and reactivity, as well as their generation are considered to determine these hazard classes.

In both cases, the assignment of hazard classes process was conducted over months by a group of +20 experts in the field of chemical risk prevention. The results are directly inspired by those from the Health and Safety Executive (HSE), 2008 (8) and in the end very similar to those proposed by (14).

TABLE 1 Overview of inhalation hazard classification according to gravity and potential for immediacy of effect.

Inhalation hazard statement according to the CLP regulation	Hazard class
No CLP classification	Very low
Products with moderate local effects, e.g., irritants	Low
Products with acute or chronic moderate toxicity, products with severe local effects, e.g., corrosive, and cutaneous sensitizer products	Medium
Products with immediate effects, products with acute or chronic severe toxicity, e.g., carcinogenic products	High
Products with lethal effects or immediate severe systemic effects, e.g., respiratory sensitizers	Very high

2.2 Step 2: assignment of the exposure class and score

For the inhalation route, five parameters are needed to evaluate the exposure score (Figure 2). The different modalities of these parameters and their relative classification are listed in Table 2.

• The physical state can be "liquid," "solid," or "gas." It is used to describe the potential of the substance to become airborne. When it is a liquid, this potential is defined by the vapor pressure, and in this case, the temperature of use and the boiling temperature can be used (EUSES, European Union System for the Evaluation of Substances, available[2]). When it is a solid, including powders, the potential is related to the dustiness: the finer the powder, the higher the potential. When it is a gas, the potential is always at the maximum level because gases are considered to generate maximum exposure.

• The type of process is used to define the level of dispersion of the product in the workplace. It can be defined by using the REACH process reference framework (PROC) defined in the European Chemicals Agency (15) guidance or by using the four modalities defined in the Technical Guidance Document on Risk Assessment (16).

• Collective protective equipment concerns the installation of ventilation controls and local exhaust ventilation, which contributes to the protection of employees' health. These measures help to reduce the levels of exposure to chemicals for employees.

• The daily amount corresponds to the amount of product used during a specific task over a day (8 h) or during a work sequence. The daily amount is only used with dispersive processes; it defines the amount of product dispersed voluntarily in the work atmosphere.

• The duration of the task performed by the employee is considered when the most severe hazard occurs after repeated exposure over time (chronic exposure, i.e., carcinogenic products). On the contrary, the duration of exposure is not considered when the most severe hazard occurs after acute exposure (i.e., highly toxic products that can cause immediate irreversible effects).

For the dermal route, which includes both the skin and eyes, four parameters are needed to assess the exposure (Figure 3). The different modalities of these parameters and their relative classification are listed in Table 3.

• The exposure scenario corresponds to the nature of the operations performed by the employee. There are four modalities

2 https://echa.europa.eu/fr/support/dossier-submission-tools/euses

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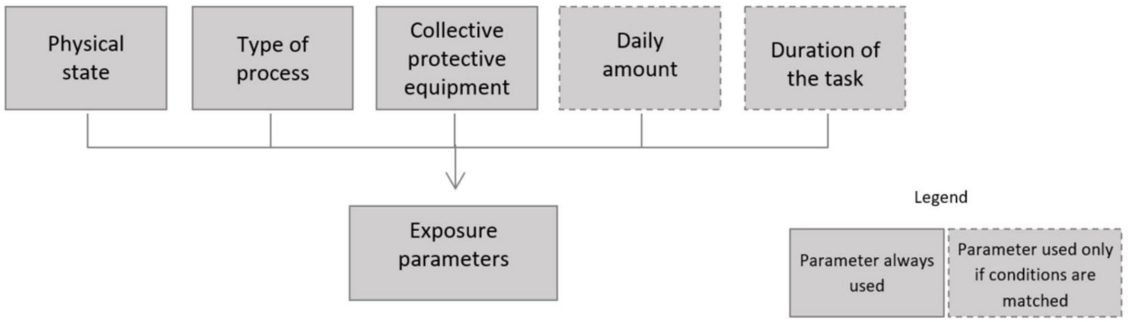

FIGURE 2
Exposure characterization parameters for the inhalation route.

TABLE 2 Modalities and classes for the inhalation exposure parameters.

Parameters	Description		Class
Physical state	Solids	Pellets, chips, and solids with little brittleness	Low
		Powder or grains (e.g., crystallized sugar)	Medium
		Fine powder, airborne dust generation during handling (e.g., powdered sugar, flour, and plaster)	High
	Liquids	Vapor pressure lower than 500 Pa	Low
		Vapor pressure between 500 and 10,000 Pa	Medium
		Vapor pressure above 10,000 Pa	High
	Gas	Usually in a pressurized bottle	High
Process	Enclosed	Any process that is completely contained	Very low
	Enclosed but regularly opened	Any process that is confined but can be opened during the filling, emptying, or control phases	Low
	Open	Any process where the material is localized without specific dispersion and without specific containment	Medium
	Dispersive	Any process, which by the energy deployed or the absence of containment generates emissions into the working atmosphere	High
Collective protective equipment	Indoors	Fume cupboard	Very low
		Other local exhaust ventilation (extractor hood, extraction slit, and extraction table)	Low
		General ventilation	Medium
		No extraction device	High
	Outdoors (natural ventilation)		Medium
Daily amount	< 10 g		Very low
	[10–100 g]		Low
	[100–1 kg]		Medium
	[1–10 kg]		High
	≥ 10 kg		Very high
Duration	< 15 min		Very low
	[15 min–1 h]		Low
	[1–4 h]		Medium
	≥ 4 h		High

The modalities are ranked in relative terms, with regard to each parameter, and ranked from "very low" to "high".

for the exposure scenario describing a part of the exposure level.

• The exposed surface corresponds to the total surface area of skin that can be exposed to the product without considering personal protective equipment.

• The daily amount is taken into account in the same way as for the inhalation route. This parameter is considered when the effects appear because of exposure through skin penetration (systemic effects). It is not used when the product produces local effects.

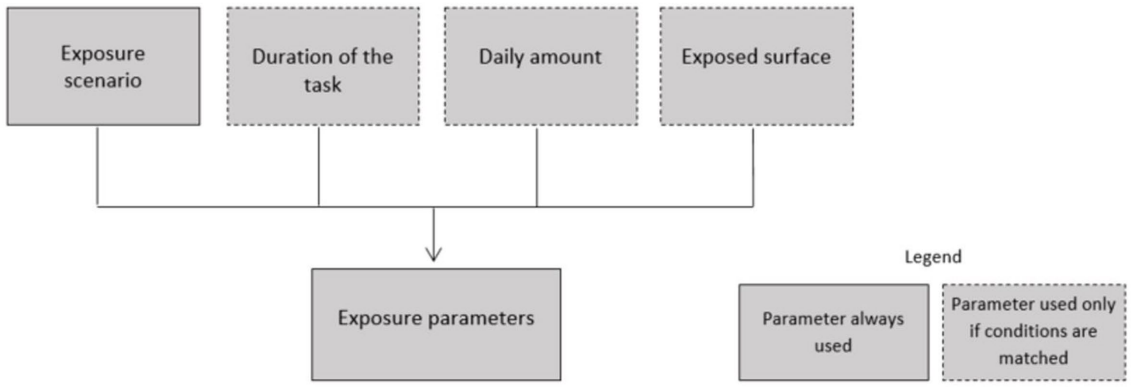

FIGURE 3
Exposure characterization parameters for dermal exposure.

• The duration is considered in the same way, with the same modalities, as for the inhalation route.

An integer value is allocated to each of the abovementioned entry parameter modalities, and the exposure score is the multiplication of these integer values.

2.3 Step 3: calculation of the priority score and assignment of the priority band

The priority score is calculated by multiplying the hazard score and the exposure score: one for the inhalation route and another for the dermal route. The value attributed to the hazard score has the most important weight compared to the exposure score. Then, the inhalation route priority band and the dermal route priority band are assigned with regard to their respective priority score: "moderate priority" (green color), "high priority" (orange color), and "very high priority" (red color). The priority bands are calculated for each work situation in the company. Then, the work situations are sorted according to their respective priority.

3 Results. Example of application for a workplace: installation of thermal insulation

In 2019, a visit to a company specialized in the insulation and repair of house façades was conducted. The company was identified following a request made by a hygienist from the French public health insurance service, explaining that the director of this company wanted to evaluate and establish an action plan to reduce the potential chemical risks within his company. The aim of the visit was to contact the director, understand his needs, and explain the usefulness of the method and its usage. To do this and to facilitate the task, the authors suggested carrying out an assessment of one of the company's workstations, from the inventory to the action plan, according to the three steps defined above.

3.1 Step 1: assignment of the hazard class and score

Different workstations using chemical products were identified in the company: scaffolding, installation of thermal insulation, repair and renovation of façades, painting, and coating. The workstation chosen for the assessment was the "installation of thermal insulation" due to the numerous tasks conducted with different products used or emitted. Information concerning the tasks and the products was collected during the company visit. Table 3 represents the eight tasks performed with the inventory of labeled products and industrial chemical emissions.

3.2 Step 2: assignment of exposure class and score

The details regarding the calculation of priority level via both inhalation and dermal routes are shown in Table 4 for all labeled products used in the workstation. For industrial chemical emissions, the determination details are shown in Table 5.

3.3 Step 3: calculation of the priority score and assignment of the priority band

Figure 4 represents the sorted list of products used during each task according to their respective inhalation and dermal priority bands.

Regarding the priorities for the inhalation route illustrated in this example, the four products used during tasks with "very high inhalation priority" were as follows: (1) the expanding foam used to fill fractional gaps, (2) the surface hardener, (3) the bonding resin used for the façade coating, (4) and the primer used as a fixative between the lattice and the plaster. Moreover, two industrial chemical emissions also showed "very high inhalation priority": the dust emitted (1) during the surface preparation and installation of the starting rails and (2) during the treatment of protruding angles. The next seven products and the plastic combustion fumes released during the cutting of polystyrene insulation boards had a high priority, as shown in orange in Figure 4. Regarding priorities related to the dermal route, six

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TABLE 3 Modalities and classes for dermal exposure parameters.

Parameters	Description	Class
Exposure scenario	No possible contact of the product with the body	Very low
	Possible contact of the product with a part of the body (e.g., handling of a cloth soaked with a product or tools contaminated by a product)	Low
	Possible generation of splashes or aerosols (e.g., projection of drops during spill operations and projection of oil mists by rotating machines)	Medium
	Possible immersion of a part of the body in the product (e.g., manual placing or removal of parts in chemical baths, during degreasing, and rinsing operations)	High
Exposed surface	One hand	Very low
	Both hands	Low
	Lower or upper limbs	Medium
	The whole body or face	High
Daily amount	< 10 g	Very low
	[10–100 g]	Low
	[100 g–1 kg]	Medium
	[1 kg–10 kg]	High
	≥10 kg	Very high
Duration	<15 min	Very low
	[15 min–1 h]	Low
	[1–4 h]	Medium
	≥ 4 h	High

The modalities are ranked in relative terms, with regards to each parameter, and ranked (from "very low" to "high").

TABLE 4 Tasks performed in the workstation with labeled products and industrial chemical emissions.

Task	Labeled product	Chemical emissions
Surface preparation and installation of the starting rails (sanding and drilling)	–	Dust emissions
Hot wire cutting of polystyrene insulation boards	–	Plastic combustion fumes
Treatment of protruding angles (reinforcing strips)	–	Dust emissions
Filling fractional gaps	Expanding foam and silicone sealant	–
Adding wefts	Epoxy bonding mortar	–
Adding fixative between lattice and plaster	Primer	–
Façade coating	Surface hardener, bonding resin, porosity regulator, and façade coat	–
Finishes and removal of residues	Hydrochloric acid; 2 waterproofing products, façade coat	–

products were used during tasks with "very high priority" as follows: (1&4) the façade coat, (2) the surface hardener, (3) the bonding resin, all used for the façade coating and the finishing; (5) the hydrochloric acid used for the finishing task; and (6) the expanding foam used to fill the fractional gaps. Moreover, one industrial chemical emission also showed "very high dermal priority": the dust released during the treatment of protruding angles.

The aim of this prioritization at a company is to guide the development and the follow-up of a preventive or corrective action plan helpful to reduce occupational risks for the most problematic situations. Therefore, to help the company determine the appropriate actions, an occupational hygienist from the French public health insurance service was asked to review the results. A precise action plan was established. In particular, the substitution of the expanding foam, the surface hardener, and the bonding resin were required because their use was considered a "very high priority" for inhalation and dermal routes. The dust emitted during the surface preparation and the treatment of protruding angles presented a very high inhalation priority. Since these tasks are performed outdoors, the use of collective protective equipment is not applicable. For this reason, the use of personal respiratory protective equipment is highly recommended to avoid the risks related to this task. In addition, given the very high priority via the dermal route for the treatment of protruding angles, the use of dermal protective equipment (goggles and gloves) is recommended during the treatment of protruding angles (Table 6).

4 Discussion

The method can be used to attribute an intervention priority to work situations involving exposure to chemical products through inhalation and dermal routes. This method's domain of applicability

TABLE 5 Hazard and exposure data and levels assigned by the method to calculate the inhalation and dermal chemical priority scores for all labeled products used in the workstation.

	Inhalation route				Dermal route			
	Task: Filling fractional gaps The residual fractional gaps in the polystyrene boards are filled with chemical products, depending on the size of the gap. This operation is done manually by the worker, either with an aerosol of expanding foam or with a silicone gun.							
	P1: Silicone sealant		**P2: Expanding foam**		**P1: Silicone sealant**		**P2: Expanding foam**	
	Data	*Level*	*Data*	*Level*	*Data*	*Level*	*Data*	*Level*
Hazard	No CLP statement	Very low	*- May cause respiratory irritation* *- May cause allergy or asthmatic symptoms or breathing difficulties if inhaled* *- Suspected of causing cancer* *- May cause harm to breast-fed children* *- May cause damage to organs through prolonged or repeated exposure*	Very high	No CLP statement	Very low	*- Causes skin irritation* *- May cause an allergic skin reaction* *- Causes serious eye irritation*	Medium
Exposure	Physical state: Paste, considered in liquid category	Low	Physical state: Foam, considered in the liquid category	Low	Exposure scenario: Possible contact of the product with a part of the body	Low	Exposure scenario: Possible contact of the product with a part of the body	Low
	Process: dispersive	High	Process: dispersive	High	Exposed surface: both hands	Low	Exposed surface: both hands	Low
	CPE: Outdoor work	Medium	CPE: outdoor work	Medium	Duration: Not required	–	Duration: Not required	–
	Duration: Not required	–	Duration: Not required	–	Daily amount: 3 L	High	Daily amount: 7 L	High
	Daily amount: 3 L	High	Daily amount: 7 L	High				
Priority	**Moderate**		**Very high**		**Moderate**		**Very high**	
	Task: Adding lattice All polystyrene boards on the whole façade are covered by a metallic lattice, which is sealed on the façade with mortar. This lattice will support the coating. This operation is done manually by the worker. He applies the mortar from a mason's through with a trowel. Then the lattice is sealed into the mortar.							
	P: Epoxy bonding mortar				**P: Epoxy bonding mortar**			
	Data		*Level*		*Data*		*Level*	
Hazard	No hazard statement		Very low		*- Causes skin irritation* *- Causes serious eye damage*		Medium	

(Continued)

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TABLE 5 (Continued)

	Inhalation route				Dermal route			
Exposure	Physical state: Fine powder		High		Exposure scenario: Possible contact of the product with a part of the body		Low	
	Process: Open		High		Exposed surface: Both hands		Low	
	CPE: Outdoor work		Medium		Duration: Not required		–	
	Duration: Not required		–		Daily amount: 279 kg		Very high	
	Daily amount: 279 kg		Very high					
Priority	**High**				**High**			
	Task: Adding fixative between lattice and plaster Once the lattice is installed and the mortar is dry, a layer of primer is applied manually with a roller. The primer is used from a bucket.							
	P: Primer				**P: Primer**			
	Data		*Level*		*Data*		*Level*	
Hazard	*-May produce an allergic reaction*		High		*-May produce an allergic reaction*		High	
Exposure	Physical state: viscous liquid, considered in liquid category		Low		Exposure scenario: Possible contact of the product with a part of the body		Low	
	Process: Open		High		Exposed surface: Both hands		Low	
	CPE: Outdoor work		Medium		Duration: Not required		–	
	Duration: Not required		–		Daily amount: 8 kg		High	
	Daily amount: 8 kg		High					
Priority	**Very high**				**High**			
	Task: Façade coating First, the worker prepares the mixture. The different products are poured manually into a bucket and mixed with a paint mixer. The mixture is then manually applied with a trowel and roller.							
	P1: Surface hardener		**P2: bonding resin**		**P1: Surface hardener**		**P2: bonding resin**	
	Data	*Level*	*Data*	*Level*	*Data*	*Level*	*Data*	*Level*
Hazard	*-May produce an allergic reaction*	High	May produce an allergic reaction.	High	*-May produce an allergic reaction*	High	-May produce an allergic reaction	High
Exposure	Physical state: liquid	Low	Physical state: liquid	Low	Exposure scenario: Possible contact of the product with a part of the body	High	Exposure scenario: Possible contact of the product with a part of the body	High
	Process: dispersive	High	Process: Dispersive	High	Exposed surface: both hands	High	Exposed surface: both hands	High
	CPE: outdoor work	Medium	CPE: outdoor work	Medium	Duration: not required	–	Duration: not require	–
	Duration: not required	–	Duration: not required	–	Daily amount: 18 kg	Very high	Daily amount: 14 kg	Very high
	Daily amount: 18 kg	Very high	Daily amount: 14 kg	Very high				

(Continued)

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TABLE 5 (Continued)

	Inhalation route				Dermal route			
Priority	**High**		**Very high**		**High**		**High**	
	P3: Porosity regulator		**P4: Façade coat**		**P3: Porosity regulator**		**P4: Façade coat**	
	Data	*Level*	*Data*	*Level*	*Data*	*Level*	*Data*	*Level*
Hazard	*-May cause respiratory irritation.*	Low	*-May produce an allergic reaction.*	High	*-Causes skin irritation. Causes serious eye damage.*	Medium	*-May produce an allergic reaction.*	High
Exposure	Physical state: fine powder	High	Physical state: paste, considered in the liquid category.	Low	Exposure scenario: Possible contact of the product with a part of the body	Low	Exposure scenario: Possible contact of the product with a part of the body	Low
	Process: dispersive	High	Process: dispersive	High	Exposed surface: the whole body or face	High	Exposed surface: the whole body or face	High
	CPE: outdoor work	Medium	CPE: outdoor work	Medium	Duration: not required	–	Duration: not required	–
	Duration: not required	–	Duration: not required	–	Daily amount: 5 kg	High	Daily amount: 270 kg	Very high
	Daily amount: 5 kg	High	Daily amount:270 kg	Very high				
Priority	**High**		**High**		**High**		**Very high**	
	Task 8: Finishes and removal of residues The façade is manually ground and sandpapered where needed. The waterproofing product is applied manually with a roller, and hydrochloric acid is used to remove the residues. More façade coat is applied manually with a smaller trowel where needed to obtain a smooth finish.							
	P1: Hydrochloric acid		**P2: Waterproofing product (1)**		**P1: Hydrochloric acid**		**P2: Waterproofing product (1)**	
	Data	*Level*	*Data*	*Level*	*Data*	*Level*	*Data*	*Level*
Hazard	*-May cause respiratory irritation*	Low	No CLP statement	Very low	*-Causes severe skin burns and eye damage*	High	*-Causes skin irritation* *-May cause an allergic skin reaction* *-Causes serious eye damage*	Medium
Exposure	Physical state: liquid	Low	Physical state: fine powder	High	Exposure scenario: Possible contact of the product with a part of the body	Low	Exposure scenario: possible contact of the product with a part of the body	Low
	Process: dispersive	High	Process: dispersive	High	Exposed surface: the whole body or face	High	Exposed surface: the whole body or face	High
	CPE: outdoor work	Medium	CPE: outdoor work	Medium	Duration: not required	–	Duration: not required	–
	Duration: not required	–	Duration: not required	–	Daily amount: 3 L	High	Daily amount: 18 kg	Very high
	Daily amount: 3 L	High	Daily amount: 18 kg	Very high				

(Continued)

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TABLE 5 (Continued)

	Inhalation route				Dermal route			
Priority	**High**		**High**		**Very high**		**High**	
	P3: Waterproofing product (2)		**P4: Façade coat**		**P3: Waterproofing product (2)**		**P4: Façade coat**	
	Data	*Level*	*Data*	*Level*	*Data*	*Level*	*Data*	*Level*
Hazard	No CLP statement	Very low	*-May produce an allergic reaction.*	High	*- Causes skin irritation* *- May cause an allergic skin reaction* *- Causes serious eye damage*	Medium	*-May produce an allergic reaction.*	High
Exposure	Physical state: fine powder	High	Physical state: paste, considered in the liquid category.	Low	Exposure scenario: possible contact of the product with a part of the body	Low	Exposure scenario: possible contact of the product with a part of the body	Low
	Process: dispersive	High	Process: dispersive	High	Exposed surface: the whole body or face	High	Exposed surface: the whole body or face	High
	CPE: outdoor work	Medium	CPE: outdoor work	Medium	Duration: not required	–	Duration: not required	–
	Duration: not required	–	Duration: not required	–	Daily amount: 18 kg	Very high	Daily amount: 270 kg	Very high
	Daily amount: 18 kg	Very high	Daily amount:270 kg	Very high				
Priority	**High**		**High**		**High**		**Very high**	

*CPE corresponds to collective protective equipment.

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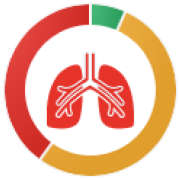

Inhalation	Skin - eye
Filling of the fractional gaps - Expanding foam	Facade coating - Facade coat
Facade coating - Surface hardener	Facade coating - Surface hardener
Facade coating - bonding resin	Facade coating - bonding resin
Adding fixative between wefts and plaster - Primer	Finishing and cleaning - Facade coat
Facade coating - Facade coat	Removal of residues - Hydrochloric acid 33%
Finishing and cleaning - Facade coat	Filling of the fractional gaps - Expanding foam
Addition of wefts - Epoxy bonding mortar	Addition of waterproofing agents - waterproofing product (1)
Addition of waterproofing agents - waterproofing product (1)	Addition of waterproofing agents - waterproofing product (2)
Addition of waterproofing agents - waterproofing Removal of residues - Hydrochloric acid 33%	Adding fixative between wefts and plaster - Primer
Facade coating - Porosity regulator	Addition of wefts - Epoxy bonding mortar
Filling of the fractional gaps - Mastic silicone	Filling of the fractional gaps - Mastic silicone

Industrial chemical emissions

Surface preparation and installation of the starting rails (sanding and drilling) - Dust emissions during sanding	Treatment of protruding angles (reinforcing strips) - Dust emissions
Treatment of protruding angles (reinforcing strips) - Dust emissions	Cutting and fixing of polystyrene insulation boards - Plastic fumes
Cutting and fixing of polystyrene insulation boards - Plastic fumes	Surface preparation and installation of the starting rails (sanding and drilling) - Dust emissions during sanding

FIGURE 4
Prioritization results according to the risk scores for inhalation and dermal (skin and eyes) exposure for labeled products and industrial chemical emissions.

extends to almost all types of products except for non-specific powders (i.e., without CLP statements). Moreover, the priorities can be attributed according to any type of working situation, whatever the task or the process involved.

To evaluate the hazard, the labeled products are associated with hazard classes based on their H and EUH statements. In addition to the major sources mentioned previously in this article, other tools such as Stoffenmanager, EMKG, and Ecetoc TRA described in Bögi et al. (17) use similar schemes. As there is no reference methodology for assigning each hazard statement to a specific band, the assignments made by each tool are different with the use of different rules. In these tools, the carcinogenic, mutagenic and reprotoxic hazards are often associated with the most severe hazard band. In the proposed methodology, the most severe band refers to lethal acute toxicity. A similarity between these tools is the classification of products capable of causing harm to unborn babies or impacting negatively on fertility, which is classified just after the classification of the most severe hazards. The qualitative identification of hazards includes subjectivity related to the use of expert judgments that are based on training and experience. As for the risks, hazard perceptions depend on many variables, such as personal and socio-demographic aspects, and the professional experience of the evaluators, as noted by Skjong et al. (18). Since different institutions and individuals develop these different tools, this may explain the differences in the hazard ranking tables. Moreover, as control banding is a relative method, the prioritization of the hazard into five classes helps to rank and prioritize products according to their level of dangerousness, but the least severe class in the hazard table does not mean that the hazard represented is not considerable.

To assess the exposure, most models cited above evaluate the concentration of substances contained in the products in the worker's breathing zone. This concentration is compared to occupational exposure limits (OELs) to assess the chemical risk, expressed as "above OEL" or "below OEL." By comparison, in this method, a risk assessment is conducted regarding the use of products and not only the substances. This is considered more convenient to field practitioners since workers are usually exposed to a mixture of substances that constitute the products and not to the substances

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TABLE 6 Hazard and exposure data and levels assigned by the method to calculate the inhalation and dermal chemical risk scores for all industrial emissions released in the workstation.

	Inhalation route		Dermal route	
	Task: Surface preparation and installation of the starting rails (sanding and drilling) The façade is ground where needed, metallic fasteners are installed in drilled holes, and metallic rails are installed horizontally and vertically on the façade. Different handheld tools can be used (driller, grinder, or perforator) and also manual hammer and chisel. The fasteners and rails are installed manually			
	Data	**Level**	**Data**	**Level**
Hazard	Dust emissions	High	Dust emissions	Very low
Exposure	Physical state: not required for emissions	–	Exposure scenario: possible generation of splashes or aerosols (e.g., projection of drops during spill operations and projection of oil mists by rotating machines).	Medium
	Process: dispersive	High	Exposed surface: the whole body or face	High
	CPE: outdoor work	Medium	Duration: 1 h–4 h	Medium
	Duration: 1 h–4 h	Medium	Daily amount: not required for emissions	–
	Daily amount: not required for emissions	–		
Priority	**Very high**		**Moderate**	
	Task: Hot wire cutting of polystyrene insulation boards The polystyrene boards are installed on the rails, and some of them need to be cut to the correct size on the ground. This operation is done with a special hot wire tool.			
	Data	**Level**	**Data**	**Level**
Hazard	Plastic combustion fumes	High	Plastic combustion fumes	Very low
Exposure	Physical state: not required for emissions	–	Exposure scenario: possible generation of splashes or aerosols (e.g., projection of drops during spill operations and projection of oil mists by rotating machines).	Medium
	Process: dispersive	High	Exposed surface: the whole body or face	High
	CPE: Outdoor work	Medium	Duration: 15 min–1 h	Low
	Duration: 15 min–1 h	Low	Daily amount: not required for emissions	–
	Daily amount: not required for emissions	–		
Priority	**High**		**Moderate**	
	Task: Treatment of protruding angles (reinforcing strips) Once the polystyrene boards and coat are applied, some strips have to be installed on the angles so there is no fragmentation of edges. The strips are cut manually, the edges are ground and sandpapered where needed.			
	Data	**Level**	**Data**	**Level**
Hazard	Dust emissions	High	Dust emissions	High
Exposure	Physical state: not required for emissions	–	Exposure scenario: Possible generation of splashes or aerosols (e.g., projection of drops during spill operations and projection of oil mists by rotating machines).	Medium
	Process: dispersive	High	Exposed surface: The whole body or face	High
	CPE: outdoor work	Medium	Duration: 1 h–4 h	Medium
	Duration: 1 h–4 h	Medium	Daily amount: not required for emissions	–
	Daily amount: not required for emissions	–		
Priority	**Very high**		**Very high**	

*CPE corresponds to collective protective equipment.

individually. However, even if this method provides a risk assessment of the products used in the company, it does not replace the regulations related to the monitoring of occupational exposure, which, in all cases, require employers to carry out exposure measurements for regulated substances that are considered to be of concern and to compare them with occupational exposure limit values.

In this method, the input parameters must be easily accessible. The parameters that are difficult to access, but which are essential for evaluation, are simplified. For example, the air change rate is represented by the type of mitigation system used and the product volatility, which is defined by the vapor pressure, and can be estimated by using the boiling point and the temperature of use if the vapor pressure is not available. Moreover, the frequency of use of products is not considered relevant because the aim is to evaluate the risk resulting from the exposure of the worker during the task (at the time he/she performs the work operation) and not at the workplace in general. The number of exposed workers in the workplace is an important parameter in risk management. However, regardless of the number of workers in the area of potential damage, the severity of this damage must be the same: this parameter does not influence the risk assessment. The volume and/or the surface area of the work zone is also not considered because it is not easily accessible to all users.

Even relying on a robust control banding methodology, chemical risk assessment remains difficult. Some specific issues related to particular substances can be improved. First, when the product evaluated does not have an SDS or is not classified according to the CLP regulation for health hazards, the chemical risk given by the method is always at the minimum level. Among these unclassified products, there are powder products with non-specific effects (i.e., calcium carbonate, amorphous silica, and alumina). This type of chemical agent can cause various respiratory system pathologies resulting from pulmonary overload or carcinogenic, allergenic, or irritant substances, as mentioned in a report by the French Agency for Food, Environmental and Occupational Health and Safety – ANSES (19). The method underestimates these effects since the products do not have a classification according to the CLP regulation. This methodology limitation was reported during its use and a solution is currently being developed to rectify it. Second, endocrine disruptors are difficult to identify and the evaluation of their effects on health is a scientific challenge and an important public health issue as noted by ANSES (20) and the ECHA (21). Despite these uncertainties, a preventive approach should be implemented to limit the workers' exposure to the lowest possible level, particularly pregnant women or women of childbearing age, as recognized in the INRS (22) report. This issue and a solution to address it will be proposed in the future. Third, the quality of the assessment depends on the quality of the information from the SDSs. Meanwhile, SDSs often do not provide complete or accurate information. For example, the physicochemical properties (vapor pressure) are sometimes missing. More importantly, the product's descriptions of health effects need more improvement within the European Chemicals Agency (23) report. This lack of data in the SDSs mainly concerns powders, especially nanometric ones. These powders are not always well identified in the SDSs and information on their composition or their potential hazards is often not available. This leads to a misjudged risk assessment for this type of product. Hodson et al. (24) evaluated the reliability and accuracy of a sample of SDS specific to engineered nanomaterials. Their evaluation showed that their information quality is not sufficient to provide adequate data on the inherent health and safety hazards of engineered nanomaterials. Thus, the use of SDSs alone to characterize the products' hazards could be considered as a limitation because even though each user is asked to verify the adequacy and SDS updates, the method is not able to confirm their accuracy and the quality of data provided on the product's effects.

This method is implemented in a software named "Seirich," which was developed by the INRS in partnership with the French Ministry of Labor, national health insurance, and French professional organizations. In addition to the control banding chemical risk assessment, Seirich software guides users in the development and follow-up of a preventive or corrective action plan to reduce risks at work. A risk assessment is provided for fire and explosion hazards. The software also offers regulation information and good practices to guide the user in the implementation of preventive actions. It is available free of charge on the web[3] (French and English languages).

5 Conclusion

For more than 20 years, and particularly since the coming into force of the EU CLP regulation in 2015 (for mixtures), a constant evolution of the presented method has been conducted, with several improved versions implemented in the Seirich software. This involves either considering regulatory updates, introducing ergonomic evolutions, or adding new features. Currently, this method is widely used for occupational chemical risk assessment in France with more than 30,000 users. The INRS is committed to promoting this tool and ensuring its continuous improvement. This tool represents a very important step in the risk prevention process by allowing the identification and evaluation of chemical risks to which employees are exposed in the workplace. This must be followed by the implementation of a specific prevention action plan based on the results obtained, with the aim of eliminating or reducing the identified risks as much as possible. Finally, to allow foreign companies to use it easily, this tool is also available in an English version but is still adapted to French regulations.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://doi.org/10.1080/15459624.2021.2023161.

Author contributions

AA: Data curation, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. FM: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Validation, Writing – review & editing. NB: Conceptualization, Project administration, Resources, Supervision, Validation, Writing – review & editing. FC: Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

3 https://www.seirich.fr/

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EDITED BY
Susana Viegas,
New University of Lisbon, Portugal

REVIEWED BY
Sasho Stoleski,
Saints Cyril and Methodius University of Skopje, North Macedonia
Peter E. J. Baldwin,
Health and Safety Executive, United Kingdom

*CORRESPONDENCE
Paloma I. Beamer
✉ pbeamer@arizona.edu

†These authors have contributed equally to this work and share first authorship

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What a mix! Volatile organic compounds and worker exposure in small business beauty salons in Tucson, Arizona

Denise Moreno Ramírez[1†], Shannon Gutenkunst[2†], Nathan Lothrop[1], Carolina Quijada[1], Marvin Chaires[1], Imelda Cortez[3], Flor Sandoval[3], Fernanda J. Camargo[1], Emma V. Gallardo[1], Elmira Torabzadeh[2], Rietta Wagoner[1], Nicolas Lopez-Galvez[4], Maia Ingram[1], Dean Billheimer[1,2], Ann Marie Wolf[3] and Paloma I. Beamer[1,2*]

[1]Mel and Enid Zuckerman College of Public Health, The University of Arizona, Tucson, AZ, United States, [2]BIO5 Institute, The University of Arizona, Tucson, AZ, United States, [3]Sonora Environmental Research Institute, Inc., Tucson, AZ, United States, [4]College of Health and Human Services School of Public Health, San Diego State University, San Diego, CA, United States

Introduction: Small business beauty salons have volatile organic compounds (VOCs) in their workplace air. VOCs are present as ingredients in beauty or hair products. They may also form because of chemical reactions, where thermal-styling elements accelerate the volatilization of these compounds. Uncertainties remain about the relationship between air pollutant concentrations and the variety of beauty salon activities in a work shift. Investigating these associations can help determine high-risk services, associated products, and at-risk workers.

Methods: In this exploratory study, female community health workers recruited beauty salons from target zip codes in predominately Latino neighborhoods, including primarily Spanish-speaking small businesses. We collected salon chemical inventories, business characteristics, and participant activity logs to understand how chemicals and activities influence the total and specific VOC concentrations. We sampled personal total VOCs and specific VOCs from the same shop during the participant work shift. We also measured personal total VOCs for four work shifts per shop.

Results: A linear mixed effects model of log VOCs on the fixed effect of activity and the random effects of salon and shift within the salon showed that the variance between salons explains over half (55%) of the total variance and is 4.1 times bigger than for shifts within salons. Summa canisters detected 31 specific VOCs, and hazard scores ranged between 0 and 4.3. 2-Propanol (isopropyl alcohol) was the only VOC detected in all shifts of all salons.

Discussion: In this study, differences in VOC measurements were primarily between salons. These differences may result from differences in ventilation, services rendered, and product lines applied.

KEYWORDS

VOC exposure, beauty salons, hierarchy of controls, salon products, worker health, Spanish-speaking small businesses, heat-styling, beauty justice

1 Introduction

Beauty salons are ubiquitous. This industry is estimated to be valued at $230.64 billion worldwide, with profits increasing to $383.88 billion by 2030 (1). In the United States (U.S.), estimates suggest that in 2023, individuals will spend about $91.23 billion on hair care products alone (2). Currently, in the U.S., the beauty industry is expanding at a high rate, spurred by a general trend toward wellness (3). The beauty market has also demonstrated resilience in turbulent macroeconomic times because of the coronavirus pandemic, making it a lucrative investment for many entrepreneurs, celebrities, and influencers (4, 5). Yet, since the early twentieth century, the production of cosmetics has been dominated by a handful of multinational corporations with significant influence over the type and content of products that workers and consumers may be exposed to in beauty salons (6).

Air in beauty salons contains volatile organic compounds (VOCs) that may harm human health. These compounds are introduced through ingredients in beauty and hair products used to provide clients with the desired style. They may also form because of chemical reactions during the styling process. Often, volatilization of these compounds is accelerated by thermal hair-styling tools (e.g., hairdryers, flatirons, hot combs, and hair processors) used in hair-styling, processing, and cutting activities completed daily in salons. Thermal hair-styling tools can use different technologies ranging from ceramic to ionic and infrared to tourmaline. Typically, a hair dryer heats the air surrounding wet strands of hair, and as the capacity of the surrounding air to hold moisture increases, the water from the hair evaporates. In the case of ionic hairdryers, they contain a negative ion-generating device that helps smooth frizz. Other VOC sources in a beauty salon may include disinfection products and other environmental sources (e.g., traffic-related air pollution). Consequently, beauty salon workers and their clients are exposed to salon VOCs through their products and activities. Yet, salon workers experience more frequent and prolonged exposure to these compounds.

Beauty salons are a significant employer of women in the U.S. Most small businesses (less than 100 employees) fall under the category of the professional services sector (7). Estimates suggest approximately 80,000 beauty salons exist in the U.S. (8). Businesses with less than 20 workers employ 21 million workers, and over 20 million are employed by firms with 20–99 workers, representing about 17% of the worker population (9). Small business-sized beauty salons mostly employ racial and ethnic minority workers who often have gaps in health insurance coverage and suffer disproportionate health impacts (10).

Previous investigations on VOCs focus primarily on nail salons, yet beauty salons provide more services and are understudied. Exposures in beauty salon settings are varied and potentially even higher. Also, more adverse health outcomes are associated with performing hair processes than nail care tasks (11). Current research on beauty salons has focused on beautician exposure via biomonitoring, identifying compounds in workplace air, and how compounds interact under controlled experimental conditions (12–16). This existing research concludes by raising health concerns for beauty salon workers. One study found that workers serving primarily clientele of color have VOC concentrations in their bodies approximately four times higher than those of general women in the U.S. (16).

Understanding the relationship between air pollutant concentrations and the variety of cosmetic practices that occur throughout the work shift of a beauty salon worker is vital to developing strategies to protect worker health. Investigating these associations can help determine high-risk services, associated products, and at-risk workers. The application of controls has also been understudied in the beauty salon setting. From occupational health studies, we know the hierarchy of controls (HoC) can assist in selecting safeguards to protect worker health. More information is needed on the feasibility of safety protections and how to facilitate their implementation in an overburdened setting with esthetics as a priority. Identifying controls is especially important because many of the chemicals found in beauty salons have been shown to impact workers' reproductive systems, lung functions, cardiovascular system, cognition, and skin health (11, 17, 18). Additionally, the health impact on these workers may only be diagnosed decades after the exposure, and associations are underrecognized and underreported in occupational surveillance data (19–21). Even with the potential risk to workers and surrounding communities, few studies have quantified VOC exposures in small businesses, and most are out of date, limited in scope, or conducted outside the U.S.

The current project evolved from grassroots pollution prevention work established by Mexican community health workers (CHWs) employed by the Sonoran Environmental Research Institute, Inc. (22). CHWs are frontline public health workers who identify strongly with their communities, and they have a long history of addressing environmental health concerns (23). In this exploratory study, we sought to determine how different beauty salon activities influence workplace air compounds and whether these compounds vary more between salons than within a salon. We measured total VOCs as our primary outcome because methods that measure specific VOCs (our secondary outcome) cannot account for all the VOCs. Also, elucidating a whole class of chemicals that can be targeted with a system-wide intervention rather than one chemical at a time is more efficient. The information gained from this study will be utilized to reduce beauty salon workplace exposures to VOCs. A follow-up study aims to understand if an industrial-hygiene-enhanced CHW intervention can minimize exposure to VOCs in the workplace of beauty salons and auto shop small businesses. We concentrate our efforts on small beauty salon businesses in southern metropolitan Tucson, Arizona, with a high population identifying as Mexican and Mexican American (Latino).

2 Materials and methods

2.1 Study population and recruitment

The Solutions for a Changing World Project brings together the Sonora Environmental Research Institute, Inc. (SERI), El Rio Community Health Center (federally qualified health center), and the University of Arizona's (UA) Mel and Enid Zuckerman College of Public Health (MEZCOPH). We implemented an exploratory study to characterize the compounds in workplace air in 10 small business beauty salons in 2018. This study defines small businesses as those with 25 employees or fewer.

During the study period, we primarily focused on developing partnerships with small businesses and assessing salon exposures and

activities in four work shifts. We collected total and specific VOC samples, business characteristics (e.g., ventilation conditions and number of rooms), participant demographics, participant activity logs (e.g., salon activities, activity start and end times), and salon chemical inventories. The data collection period was from June until November 2018.

The City of Tucson is situated in the semi-arid Sonoran Desert in southern Arizona and has one of the country's highest poverty rates (24). CHW recruited beauty salons from six target ZIP codes. We focus on these ZIP codes because those who live in them have higher poverty rates, increased urban stress, and lower educational attainment. One of the nation's oldest Superfund sites is in this area (Tucson International Airport Area). The ZIP codes also contain Tucson's Latino neighborhoods and predominately Spanish-speaking small businesses. The beauty salons located here also primarily serve Latino clients.

CHW from SERI recruited participants from small businesses beauty salons in person or via phone. Some businesses were approached because of their previous interaction with SERI in a pollution prevention program. The remaining businesses were approached because they existed in a SERI database. When business owners agreed to participate in the study, the CHW obtained written permission and consent from individual workers at the salon participating in the study. Workers had to be consented separately. The business owner had to consent for the business to participate in the study, but not all workers (including the owner) at the business were required to participate in personal total VOC monitoring. Demographic information was acquired from participants.

Inclusion criteria for this study comprise being a small business beauty salon in the selected ZIP codes (25 employees or less), an owner, manager, or employee who is at least 18 years old, able to speak Spanish or English, and expected to be employed at the business for the next 3 months. Exclusion criteria include nail salons and chain beauty salon businesses, and businesses outside the targeted ZIP codes. Study subjects were not compensated for participation but would receive results from the air monitoring events and received consultation with a health insurance navigator. The UA Human Subjects Protection Program approved all human-subjects materials related to the study (#1709821542).

Salon chemical inventories, business characteristics, and participant activity logs were recorded throughout the work shifts to understand how chemicals and activities influence the total and specific VOC concentrations. Public health researchers from the U.A. were embedded in the field, observing salon activities and business characteristics (e.g., ventilation) while monitoring a work shift. Notes were taken in a form designed for field observations. The categories developed for beautician activities were: (1) administration; (2) clean-up/housekeeping; (3) hair processing; (4) hair-styling/cutting; (5) skin care; (6) taking a break; and (7) unknown. These categories were identified as the most general activities that could occur in a salon. The participant activity log also included ventilation categories: (1) central air conditioning; (2) swamp cooler; (3) mini-split/wall air conditioning unit; (4) desk/floor fan; (5) ceiling fan; (6) open door/window; and (7) local exhaust fan, as well as other business conditions such as the number of rooms present at the business. The activity log also captured the specific products applied and issues with monitoring equipment.

2.2 VOC measurements – total and specific VOCs

Total VOC measurements were collected during four work shifts per salon. Total VOCs were measured using real-time photoionization detectors (PIDs) ppbRAE 3000 (RAE Systems, Inc. San Jose, CA). The PID monitor was placed on the individual in a specific bag slung on their shoulder, belt, or near them as they performed the salon activity. A Versilon SE-200 fluorinated ethylene-propylene lined tubing (Saint-Gobain, Courbevoie, France), with one end connected to the monitor and the other end placed near the participant's face, was used to measure total VOCs measured closest to the participant's breathing zone. Each monitor was set to record total VOCs every 20 s. Public health researchers addressed issues resulting from participants' monitoring equipment during their work shifts by viewing a handheld EchoView Host (RAE Systems, Inc., San Jose, CA). Detailed methodological steps are reported by Lothrop and colleagues (25).

Specific VOC samples were collected during one or two salon visits, with half of the salons having Summa canister (Restek™ SilcoCan Air Canisters with RAVE Valve) measurements on two separate days and one salon having a true duplicate on the same day. Summa canisters were put in the room with the most expected activity on the floor in a location that would not interrupt workflow. Sample preparation, analyte determination, and measurement methods for specific VOCs followed the U.S. Environmental Protection Agency (U.S. EPA) Air Method Toxic Organics-15, which tests for 70+ VOCs (26). Test America Laboratories, Inc. completed laboratory analyses and tentatively identified additional VOCs beyond the standard set for TO-15.

2.3 Data analysis – total VOCs

Values of total VOCs below the limit of detection (LOD) and recorded as 0 parts per billion (ppb) by the PID were replaced with $\mathrm{LOD}/\sqrt{2} = 1\,\mathrm{ppb}/\sqrt{2} \approx 0.707\,\mathrm{ppb}$ before proceeding with the statistical analysis. Additionally, observations where the ppbRAE was left running in the salon but the participant left the salon were identified by notes in the activity logs and removed. Likewise, before proceeding with further analyses, observations where the ppbRAE was malfunctioning were identified in the notes as a flow fault or ppbRAE alarm in the activity logs, and then suspicious data were removed by looking at the data (mainly VOC concentrations below the LOD, but at least below the baseline level for the shop). A flow fault could occur from the tubing leading from the monitor to near the participant's face becoming crimped. When a U.A. public health researcher noticed such a flow fault, they would fix it (allowing sampling to resume) and note the time of the fix in the activity log.

Because the total VOC data were correlated in time, we used the aggregated data over each activity-ventilation span (whenever the activity or ventilation changed): each observation was the average (arithmetic mean) for each sequential activity-ventilation span during each shift. Because all total VOC measurement intervals were equal lengths (20 s), this average could be considered a time-weighted average. Because the distribution of this aggregated total VOC data was skewed, the data were log-transformed before statistical analysis was completed.

2.4 Data analysis – specific VOCs

Summa canister results for the 70+ chemicals attempted to be measured in all the small business beauty salons (following U.S. EPA Air Method Toxic Organics – 15) and detected in at least one salon were plotted and used in this analysis. Given the complexity of the mixtures in each of the salons and to allow comparisons between salons we estimated a hazard score for the mixture. The hazard score was determined by calculating the measured VOC concentration in ppb divided by that VOC's reference concentration in ppb and summing that quantity for all measured VOCs in that Summa canister in a salon on a given date. U.S. EPA's inhalation reference concentration (RfC) values were used as the reference concentration because they were available for more chemicals in this study than ACGIH TLV (see Supplementary material).

If a chemical from the Summa canister result did not have a given U.S. EPA Inhalation RfC value (in units of micrograms per cubic meter or μg/m^3), then an estimated inhalation reference value was calculated using the inhalation cancer unit risk factor (IUR) of the chemical. The IUR was converted to the desired μg/m^3 units using the following conversion:

$$RfC_{fromIUR} = \left(10^{-4}\right) / \left(IUR\right)$$

The 10^{-4} value is used to be consistent with Hazardous Air Pollutants (HAP) guidelines for cancer risk assessment. If the chemical from the Summa canister did not have a U.S. EPA inhalation RfC value nor an IUR value, an assumed inhalation reference value was produced using the reference oral dose (RfD in mg/(kg * day)) of the chemicals. This assumed inhalation value was converted to inhalation units in μg/m^3 with the following equation:

$$RfC_{fromRfD}\left(\mu g / m^3\right) = RfD\left(mg / \left(kg * day\right)\right) x\left(70\ kg / 20\ m^3 / day\right) x 1000\ \mu g / mg$$

The assumptions for the conversion were 70 kilograms as the average adult body weight and the average daily adult inhalation rate of 20 m^3 per day (both values were derived from the U.S. EPA Exposure Factors Handbook). For benzyl chloride, the U.S. EPA cancer oral slope factor (in units of risk per mg/(kg * day)) to μg/m^3 was used to produce an assumed inhalation reference value.

$$RfC_{benzylchloride}\left(\mu g / m^3\right) = [\left(10^{-4}\right) / \left(\begin{matrix} cancer\ oral\ slope\ factor\ in\ risk\ per\ mg \\ /kg - day \end{matrix}\right)] * 1000\ \mu g / mg$$

After all these assumptions were made, we ended up with 56 number of RfCs which were used in the calculation of the hazard scores (see table at start of Supplementary material for the reference value and its source for each chemical).

2.5 Statistical analysis

Data cleaning and statistical analyses were performed using R version 4.3.1, the *tidyverse* package for data manipulation, the *lme4* package for linear mixed effects models, and other packages listed in the Supplementary material (27–29). A value of $p < 0.05$ was assumed to be statistically significant. Descriptive statistics were calculated to characterize the data.

To examine the relationship between total VOCs and beautician activities, salons, and shifts within salons, we fit a linear mixed effects model of log VOCs on the fixed effect of activity and the random effects of salon and shift within salon. Each salon and shift within a salon were allowed to have a different intercept, and shifts were placed within salons in the model to account for their nested structure. Note that a frequency table of observations for salon and activity showed that skincare had limited observations, so this beautician activity was dropped from the mixed effects model.

3 Results

3.1 Participants

CHWs visited 15 small business beauty salons to recruit the 10 salons participating in this study. From these 10 salons, nine out of 10 (90%) eligible owners and 14 out of 25 (56%) employees consented to personal total VOC sampling. All shops allowed for the monitoring of specific VOC sampling.

Demographic information is provided in Table 1. The 23 beauty shop workers who agreed to wear PIDs were Latino between the ages of 30 and 65, primarily female (21/23 = 91.3%), with a mix of employees (14/23 = 60.9%) and owners (9/23 = 39.1%). Most participants also consented in Spanish (22/23 = 95.7%) or were Spanish speaking. No managers participated in this study.

TABLE 1 Participant demographics for the 23 participants from salons 1–10 with total VOCs data.

	Overall (*N* = 23)
Age	
Missing	3
Mean (SD)	46.5 (9.3)
Range	30.0–65.0
Gender	
Female	21 (91.3%)
Male	2 (8.7%)
Worker type	
Employee	14 (60.9%)
Owner	9 (39.1%)
Ethnicity	
Latino	23 (100.0%)
Consent language	
English	1 (4.3%)
Spanish	22 (95.7%)

3.2 Total VOCs

Total VOC samples were collected from 23 participants throughout 40 shifts at 10 beauty salons from the recruitment area using PIDs. Before data cleaning of times when the monitor was left running in the salon, but the participant left the salon, and of times when the monitor malfunctioned, there were 8,903/49,624 = 17.9% of the observations below the LOD; after there were 6,472/45,471 = 14.2% below the LOD. Once the data were aggregated over the activity-ventilation span as described above, 100/1,200 = 8.3% of the observations were below the LOD.

Because the total VOC data were correlated in time, we used aggregated data over the activity-ventilation span. The number of data points during each work shift (1–4) and salon (B001-B010) after aggregation is shown in Table 2.

After aggregation, the total VOCs ranged from less than the LOD of 1 ppb to a maximum of 76,892 ppb with a geometric mean of 792 ppb and median of 2,169 ppb.

3.3 Work activity and total VOCs

To examine the relationship between work activity and total VOCs, Figure 1 shows the distribution of total VOC exposure aggregated over each activity-ventilation span for each salon, as grouped by activity. Datapoints from different salons create the two-peaked structure, with certain salons (B001, B004, B005, B009, B010) dominating contributions to the peak at higher VOCs (between 1,000 and 10,000) and others (B006, B007, B008) dominating contributions to the peak below (between 100 and 1,000 ppb), regardless of activity. Salon B002 and B003 contribute to both peaks and the lower peak, respectively. Thus, this figure shows evidence of the association between salon and VOC exposure. It also shows how small the effect size of activity is compared to that of the salon because it shows only slight (almost unnoticeable) differences in VOC levels between activities.

Real-time total VOC concentrations are highly variable over the work shift. In the following example, showing real-time toral VOC data from one work shift (Figure 2), peak exposures occurred when hair oil was applied before thermal application during the hair-styling/cutting activity. Furthermore, peak exposures occurred frequently in other shifts when thermal styling occurs after applying hair oil product.

3.4 Mixed model

We fit a linear mixed effects model of log total VOC concentration on the fixed effect of activity and the random effects of salon and shift within the salon. The variance between salons accounts for over half (55%) of the total variance in log total VOC concentration and is 4.1 times bigger than that for shifts within salons (Table 3). This indicates that differences between salons like ventilation or beauty product lines contribute more to VOC exposures than specific worker behaviors or activities.

Analysis of variance shows that activity is significantly associated with the log of the total VOC concentrations ($p = 0.001 < 0.05$; Table 4). However, as noted previously, activity has a small effect size: it does not explain much of the variance in log VOCs.

3.5 Specific VOCs

The Summa canisters detected 31 specific VOCs, and hazard scores ranged between 0 and 4.3 (Figure 3). Hazard scores were similar within a salon, even though different chemicals were detected on different days or even the same day for the duplicate. 2-Propanol (isopropyl alcohol) was the only VOC detected in all Summa canisters in all salons. The other most common chemicals detected were acetone (found in 13/15 canisters and 9/10 shops), toluene (found in 11/15 canisters and 8/10 shops), ethyl acetate (found in 10/15 canisters and 7/10 shops), propene (found in 9/15 canisters and 7/10 shops) and MEK (found in 8/15 canisters and 7/10 shops). The most hazardous chemicals that were found in the salons (based on having low reference values) were naphthalene, chloroform, tetrachloroethene, benzene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, o-xylene, and m,p-xylenes. The specific chemicals driving large (> 1) hazard scores were 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene, naphthalene, acetone, chloroform, benzene, m,p-xylenes, n-hexane, and o-xylene.

4 Discussion

In this study, we measured the indoor concentrations of VOCs in small business beauty salons serving primarily Latino and Spanish-speaking clientele in a low-income community in Tucson, Arizona. We demonstrated that real-time total VOC concentrations can vary over the work shift, while applying hair oil followed by thermal styling leads to peak exposures. However, the most significant variance in the mixed model was between shops. The most common specific VOC found in our study is 2-Propanol, often used in personal care products and other products used in salons. Specific VOC concentrations that were the most common include acetone, toluene, ethyl acetate, propene, and MEK; specific VOCs that were the most hazardous were naphthalene, chloroform, tetrachloroethene, benzene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, o-xylene, and

TABLE 2 The total number of data points after aggregating the data over each activity-ventilation span, for each shift (1–4) in each beauty salon (B001-B010).

Shift/ Salon	B001	B002	B003	B004	B005	B006	B007	B008	B009	B010
1	47	8	42	24	29	34	35	21	22	16
2	46	28	46	27	14	9	49	14	19	42
3	39	22	8	42	35	21	23	29	52	35
4	83	27	36	26	15	14	30	25	28	38

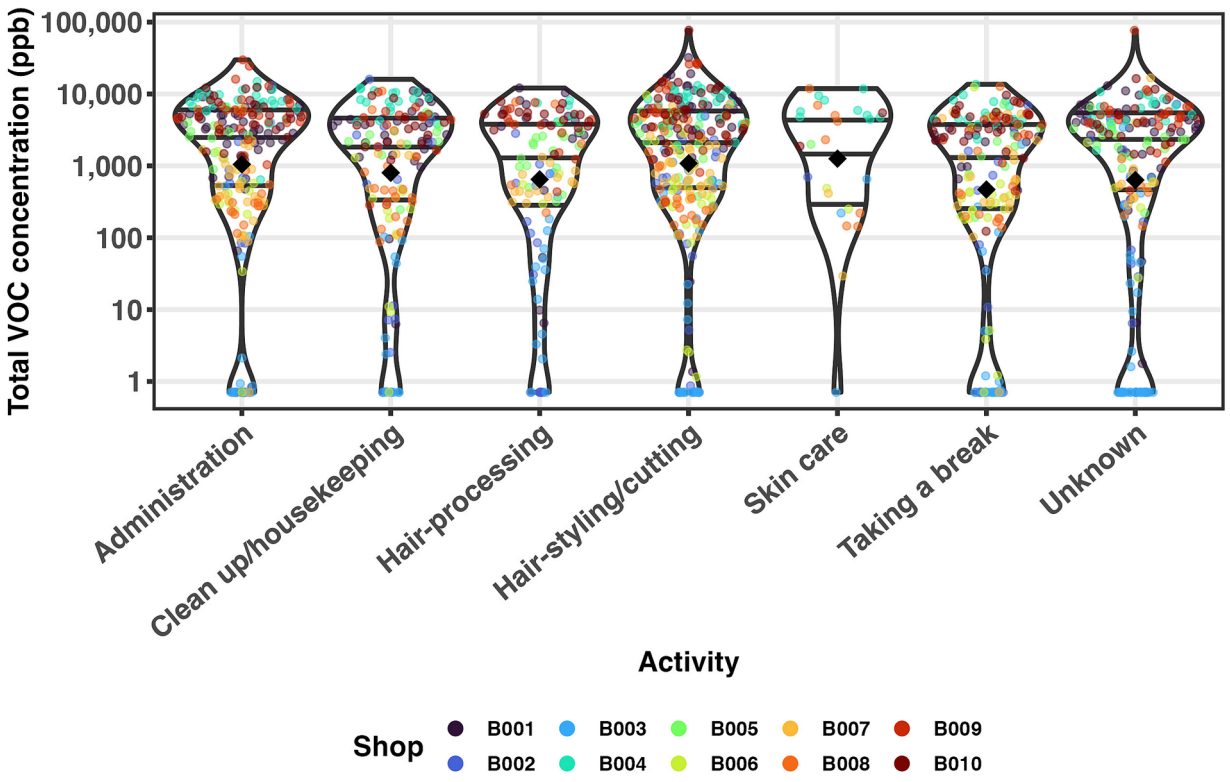

FIGURE 1
Total VOC concentration grouped by activity and colored by salon. The first quartile (Q1), median, and third quartile (Q3) are shown by black horizontal lines; a black diamond shows the geometric mean. Because of the temporal autocorrelation of the real-time VOC data, each data point represents a time-weighted average for each activity-ventilation span. Due to the skewed distribution of the total VOC concentration, the plot is on a log scale.

m,p-xylenes. Other studies investigating the air in beauty salon settings have found a combination of aromatics, esters, ketones, and terpenes (30). More data about workplace exposures in this setting are needed to develop a clearer picture of the fate and transport of these compounds. Additionally, robust workplace policies are needed that protect worker health instead of placing responsibility on them (31).

In this study, total VOCs aggregated over the activity-ventilation span ranged from less than the LOD of 1 ppb to a maximum of 76,892 ppb. Previous studies measuring total VOC concentration in beauty salons report values between 28 ppb and 5,248 ppb (12, 32). The maximum result from our studies is more elevated than those of these previous studies. In nail salon studies, higher total VOC concentrations (54,880 ppb) have been measured, like our maximum range (33). Further studies are needed to understand what activities and beauty products drive maximum values.

A key finding of this study was that there is more variation in VOC concentration between salons than within work shifts within salons. Most of the salons used central air conditioning for ventilation. We did not measure air exchange rates; therefore, differences in ventilation may be one of the drivers between these differences in salons. It needs to be further explored. Another key difference may be in the beauty product lines each salon utilizes. Each beauty product can have multiple variants within product lines with different chemical formulas. Small businesses in our study depend on various products and brands, unlike larger salons or salon spas that carry exclusive product lines. Choice of product(s) is primarily related to preference (client or beautician) and economics. In combination, varying products used in these small businesses tied to services can contribute to the differences in VOC concentrations between salons.

In some cases, individual salon owners and workers use different products and brands because workers often rent booths or chairs within the same salon. These salon workers are considered independent small businesses. Each small business can introduce different products and associated activities in this case. Also, beauty products generally have multiple variants within the product line with different chemical formulas. Sometimes, missing ingredient information can also be the case. Specialized products, such as Brazilian Blowout®, are tied to an exclusive service that requires specific beautician training. Yet, it can be the case that sometimes training protocols are not followed, and uncertified beauticians still use the product. These services' uniqueness and associated products contribute to VOC variation between beauty salons. Therefore, choices made by each salon worker can affect the exposure of their colleagues to VOCs in the workplace, underscoring the importance of awareness in this context.

Styling products applied to the client's hair in combination with thermal-styling can further explain the variation in total VOCs and the number of specific VOCs generated between salons. Heat introduced in the hair styling activity can accelerate the creation and volatilization of these compounds from the hair cuticle and scalp where they are applied initially. Thermal heat styling is accomplished by flat irons, hair dryers, hair processors, and styling combs, with potentially different heat elements. If a salon setting does not have adequate ventilation or air mixing, heat may stay in the indoor

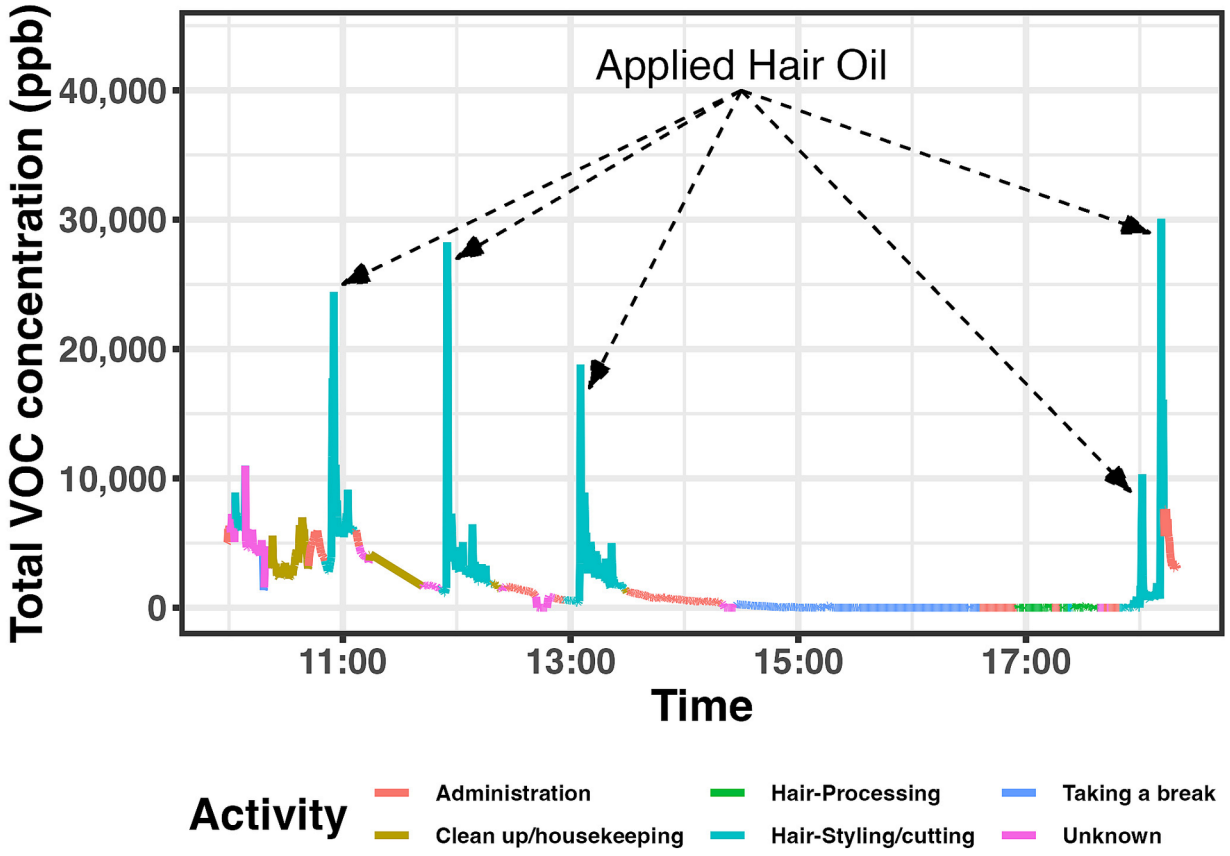

FIGURE 2
Real-time total VOC data from one shift (B001W01–2018-07-03) with labeled peaks.

TABLE 3 Variances and standard deviations from the model of log VOCs on the fixed effect of activity and the random effects of salon and shift within salon.

Groups	Variance	Standard deviation	Percent total variance
Shift within salon	1.1	1.0	13
Salon	4.5	2.1	55
Residual	2.6	1.6	32

TABLE 4 Analysis of variance for the model of log VOCs on the fixed effect of activity and the random effects of salon and shift within salon with the Kenward-Roger degrees of freedom method.

	SS	MS	Num df	Den df	*F* value	*p*-value
Activity	53.7	10.7	5	1,136	4.052	0.001

These are Type III sums of squares, which is the sum of squares given all other effects in the model. SS, sum of squares; MS, mean squares; Num df, numerator degrees of freedom; Den df, denominator degrees of freedom.

environment, potentially causing VOCs to combine further. For example, we did not see the VOC peaks from the hair oil (Figure 2) until after the beautician applied thermal styling. The safety data sheet for this specific product stated not to apply heat.

Tsigonia and colleagues (30) determined that the most significant variation in total VOC concentration depends on the use of products and associated characteristics, the number of services rendered, and the ventilation type in the salon space. In this and other studies, the total VOCs measured in beauty salons during a workday also showed significant variation (30, 34–36). Indoor air in beauty salon environments is a complex mixture of chemical ingredients, byproducts, vapors, and aerosols. Heat presented in this environment may increase the speed of chemical reactions. More controlled studies are needed to determine the interaction of the compounds and thermal styling in this environment.

Although there were no exceedances of occupational exposure guidelines, there were some exceedances of the U.S. EPA reference values. Some beauty salon business measurements resulted in hazard scores over the value of one. Settings with specific VOC concentrations exceeding the reference values or settings with hazard scores greater than one may be exposed to levels that may impact health. Exposure to high VOCs in beauty salons is expected to impact customers' health less than workers because VOCs' health effects are cumulative over time, and customers spend much less time in this high-VOC environment. Additionally, beauty salon workers handle diverse beauty products and have additional routes of exposure. In other studies, common VOCs detected include toluene, ethyl acetate, benzene, and acetone (30, 37, 38). The level of biomarkers in urine representing toluene exposure (N-acetyl-S-(benzyl)-L-cysteine) was

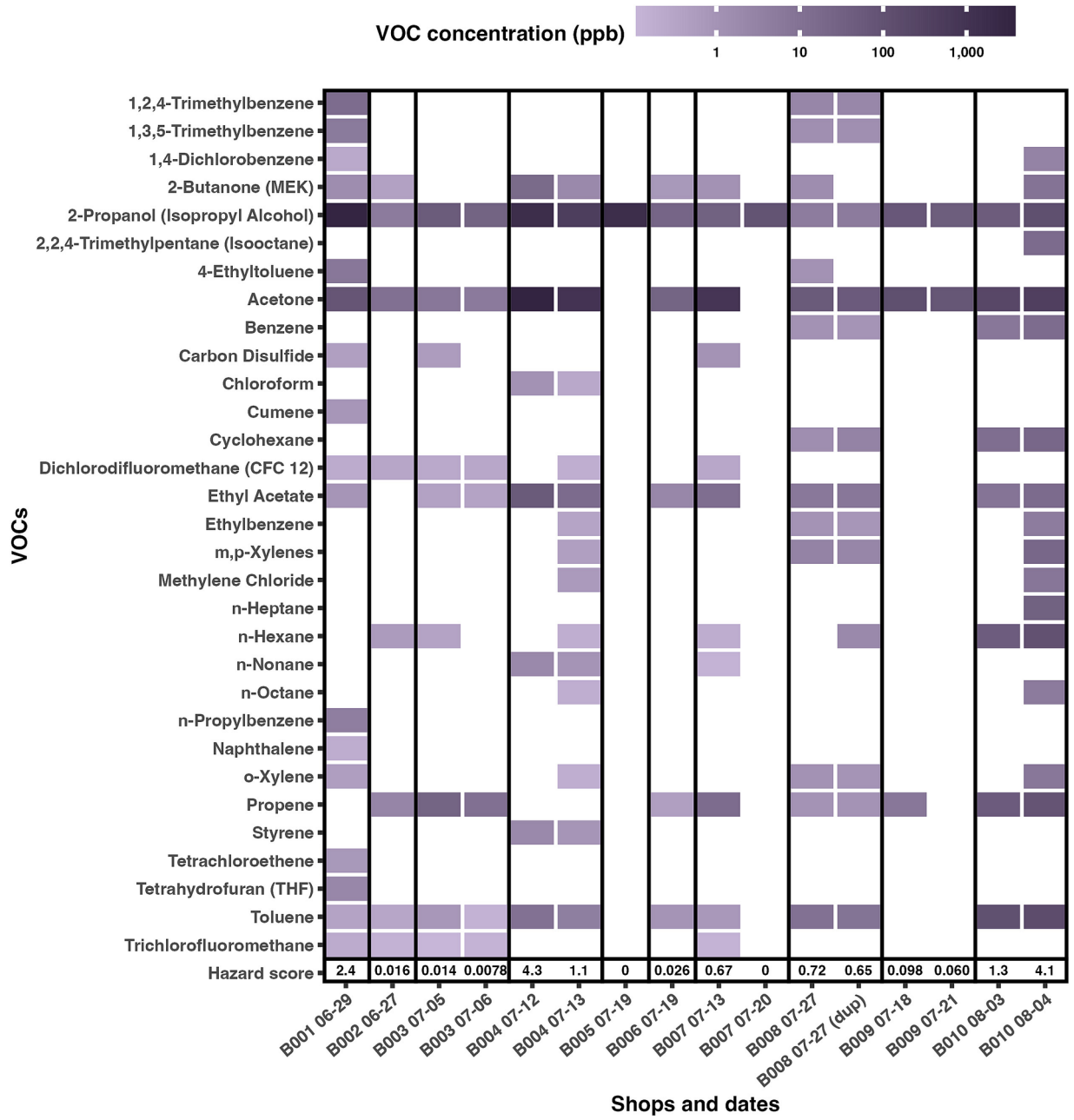

FIGURE 3
VOC concentration (ppb) of specific VOCs measured by the Summa canisters in 10 beauty salons, for VOCs measured according to the US EPA TO-15 method detected in at least one salon. The hazard score was determined by calculating the measured VOC concentration in ppb divided by that VOC's reference value in ppb and summing that quantity for all measured VOCs in a Summa canister in a salon on a given date, and it is presented in the last row of the figure.

reported by beauticians who used semi-permanent hair coloring formulations (39). Lamplugh and colleagues (40) found similar VOC compounds detected in our study as those in nail salons. The specific chemicals driving the hazard scores are reported in controlled studies focused on heating flame-retardant synthetic hair (37).

Other VOC sources, not associated with beauty or hair products, may also be present in indoor workplace air, contributing to the total VOCs present. A building and indoor space contains many natural and synthetic VOC sources that can add to this already burdened workplace environment (39). These additional sources, such as off-gassing paint or the use of cleaning products, add to the problem. These other VOC sources were not the focus of this study, so additional longitudinal measurements should be conducted to tease out the sources.

The percentage of specific VOCs detected by the Summa canister was low compared to the total VOCs detected by the PID. As there are likely 1000s of compounds present in the workplace, as expected, Summa canisters do not measure all the specific VOCs in the indoor air, and it is limited to only the 70 compounds that samples were analyzed for. The beauty salon setting is not a closed system. Chemical compounds generated in indoor air from different sources can impact general workers' exposure, adding to their total chemical burden.

Results from the Summa canister data analysis also determined the presence of other VOCs not in the U.S. EPA Air Method Toxic Organics −15, such as 2-methyl-1-butene. Compounds may mix and interact in the air in beauty salon environments, generating additional compounds. Also, thousands of chemicals are used in styling products, with many that still need reference values or analytical methods, so we cannot measure all of them. Therefore, the advantage of using the PID is to get an aggregate estimate of the total VOC exposures in the salon environment. Summa canisters cannot measure all the VOCs, but the data obtained is still helpful for future studies to identify ingredient generation rates and transport mechanisms.

Exposure scientists have also shown an increased chemical burden in beauty salons catering to women of color clientele (39–41). The literature outlines sociocultural and economic explanations that overtly drive high beauty product sales and associated activities. Racialized marketing and beauty standards perpetuate the purchase and use of products and services that contain more harmful chemicals than those targeted to their white counterparts (42, 43). Thus, participating beauticians, like in our study in salons mainly catering to clientele of color, may have a higher chemical burden in these salons (39, 40).

Previous studies have pointed to ventilation significantly influencing beauty salon air quality (35). In this study, ventilation was air-conditioning primarily, and we did not record air exchange rates, so we cannot formally assess the relationship between ventilation and total VOC concentrations. Another limitation is that we used the U.S. EPA reference values compared to occupation standards meant to protect the worker. Occupational standards are higher than environmental standards. Yet, new Occupational Safety and Health Administration (OSHA) standards, which are based on the feasibility of achieving a level in the worst segment(s) of industry, may not be sufficiently protective in sectors where exposures can be better controlled (44). None of the compounds exceeded the values of the OSHA or American Conference of Governmental Industrial Hygienists. Meanwhile, a key strength of the project is using the PID to understand the total VOCs in the air and data on beautician activity, business ventilation, and chemical inventory. Detailed data about a workplace setting can strengthen future studies regarding interventions.

The beauty salon is a complex and understudied occupational setting. Our findings confirm that applying styling products to the client's hair and subsequent thermal-styling may explain part of the VOC variation between salons. While ventilation likely accounts for the main differences, beauty and styling products used in salons may also contribute to the VOC variation. The workplace environment (including indoor and outdoor areas surrounding the salon) may also add to the VOC variability between salons. The specific VOCs detected by the Summa canister are only a proportion of those that may exist in workplace air. The results of this study add to the evidence suggesting that salon workers can be exposed to steadily high concentrations of VOCs with periodic very high spikes. Following both the socioecological model of health and the hierarchy of controls, regulation, and inspection of industrial facilities that produce these products and precautionary development of product lines will significantly impact beauticians' exposures to VOCs more than controls implemented on a shop-by-shop basis (31, 45). The results demonstrate that salon settings are incredibly diverse and poorly understood. To our knowledge, only a few studies have set out to assess workplace concentrations of VOCs in beauty salons with Latino workers focused on predominantly Spanish-speaking clients. Because of the unknown interaction of VOCs and the entirety of the variables involved, an expanded study design to capture more beauty salon spatial variability of VOCs during real-time would provide a more holistic perspective of what is happening.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Human Subjects Protection Program, University of Arizona. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

DMR: Data interpretation, Writing – original draft, Writing – review & editing. SG: Formal analysis, Writing – original draft, Writing – review & editing. NL: Methodology, Writing – review & editing. CQ: Investigation, Writing – review & editing. MC: Investigation, Writing – review & editing. IC: Investigation, Writing – review & editing. FS: Investigation, Writing – review & editing. FC: Investigation, Writing – review & editing. EG: Investigation, Writing – review & editing. ET: Formal analysis, Writing – review & editing. RW: Investigation, Writing – review & editing. NL-G: Investigation, Writing – review & editing. MI: Investigation, Writing – review & editing. DB: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. AW: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. PB: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1300291/full#supplementary-material

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EDITED BY
Luigi Vimercati,
University of Bari Aldo Moro, Italy

REVIEWED BY
Anna Kozajda,
Nofer Institute of Occupational Medicine, Poland
Somayeh Dolatabadi,
Westerdijk Fungal Biodiversity Institute, Netherlands

*CORRESPONDENCE
Carla Viegas
✉ carla.viegas@estesl.ipl.pt

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Comprehensive assessment of occupational exposure to microbial contamination in waste sorting facilities from Norway

Carla Viegas[1,2]*, Elke Eriksen[3], Bianca Gomes[1,4], Marta Dias[1,2], Renata Cervantes[1,2], Pedro Pena[1,2], Elisabete Carolino[1], Magdalena Twarużek[5], Liliana Aranha Caetano[1,6], Susana Viegas[1,2], Pål Graff[3], Anani Komlavi Afanou[3] and Anne Straumfors[3]

[1]H&TRC – Health & Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, Lisbon, Portugal, [2]NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, NOVA University Lisbon, Lisbon, Portugal, [3]National Institute of Occupational Health (STAMI), Oslo, Norway, [4]CE3C – Center for Ecology, Evolution and Environmental Change, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal, [5]Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza, Bydgoszcz, Poland, [6]Research Institute for Medicines (iMed.uLisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Introduction: It is of upmost importance to contribute to fill the knowledge gap concerning the characterization of the occupational exposure to microbial agents in the waste sorting setting (automated and manual sorting).

Methods: This study intends to apply a comprehensive field sampling and laboratory protocol (culture based-methods and molecular tools), assess fungal azole resistance, as well as to elucidate on potential exposure related health effects (cytotoxicity analyses). Skin-biota samples (eSwabs) were performed on workers and controls to identify other exposure routes.

Results: In personal filter samples the guidelines in one automated industry surpassed the guidelines for fungi. Seasonal influence on viable microbial contamination including fungi with reduced susceptibility to the tested azoles was observed, besides the observed reduced susceptibility of pathogens of critical priority (Mucorales and *Fusarium* sp.). *Aspergillus* sections with potential toxigenic effect and with clinical relevance were also detected in all the sampling methods.

Discussion: The results regarding skin-biota in both controls´ and workers´ hands claim attention for the possible exposure due to hand to face/mouth contact. This study allowed concluding that working in automated and manual waste sorting plants imply high exposure to microbial agents.

KEYWORDS

occupational exposure assessment, microbial agents, manual and automated waste sorting, azole resistance screening, *Aspergillus* spp.

1 Introduction

There is still much to investigate to fill the knowledge gap regarding the most suitable protocols (from the field to the lab) to assess occupational exposure to microbiological agents and to conclude about the potential health risks in each occupational environment (1, 2). One of the most challenging occupational environment is the waste sorting industry (3, 4) due to

several reasons: (a) the waste materials can serve as substrate and provide the needed nutrients for the microorganisms' proliferation (5); (b) the dust generated in all the workplaces can be a perfect vehicle for the microbial contamination dissemination and reach the workers respiratory tract (3, 6, 7); (c) the waste handling (domestic triage, transport duration, …) before reaching a sorting unit can vary greatly among city, region and country and this will affect the microbial contaminants in the waste and, consequently, the workers' exposure (8). These variables, among others, remain to have influence on workers' exposure to microbial contaminants even in modern automated waste sorting plants (9); (d) and the fact that this occupational environment has been reported as a hot spot for two emergent occupational risks needed to be fully addressed: mycotoxins (3, 7) and fungal azole resistance (2, 3, 10).

Waste management industries, and more specifically the ones dedicated to sorting waste, are critical to achieve the Sustainable Development Goals (SDGs) proposed by World Health Organization. Ever since the European Union's (EU) approval of the Circular Economy (CE) action plans in 2015, expectations toward the waste sorting industries to meet the CE principles have been high (11). To accomplish this endeavor the number of waste sorting facilities and respective workforce is expected to increase in all the EU countries and partners. As the European Economic Area agreement grants Norway access to the EU's single market the need to achieve these principles will be of upmost importance also for this country.

Norwegian employers are subjected to national regulation that implies the assessment and prevention of exposure to occupational risks (12) and specifically to biologic agents (12). Although there is scientific evidence that associates occupational exposure to microbial agents (bacteria and fungi) to health outcomes (1, 13–15), the health risks due to occupational exposure to microorganisms are frequently less recognized and underreported when compared to chemical exposures (2). In addition, exposure-related health effects on the respiratory tract have been reported in waste workers (16, 17). Indeed, previous studies already concluded that the waste management setting implies high exposure to microorganisms (2, 8, 9, 18). However, exposure determinants, characteristics of the determinants and the possible health effects related still need to be fully unveiled. In this study we intend to complement the findings obtained in previous studies (9, 19–21) and to contribute to fill the knowledge gap regarding occupational exposure to microbial agents in the waste sorting setting (performed automatically and manually) applying a novel and comprehensive field (active and passive sampling methods) and laboratory protocol (culture based-methods and molecular tools). This will allow to understand if the type of sorting influence the microbiological contamination and main features. Furthermore, this study aimed to assess fungal azole resistance, as well as to elucidate potential exposure related health effects (through cytotoxicity analyses). Skin-biota samples were also performed on workers and controls to identify other exposure routes besides inhalation.

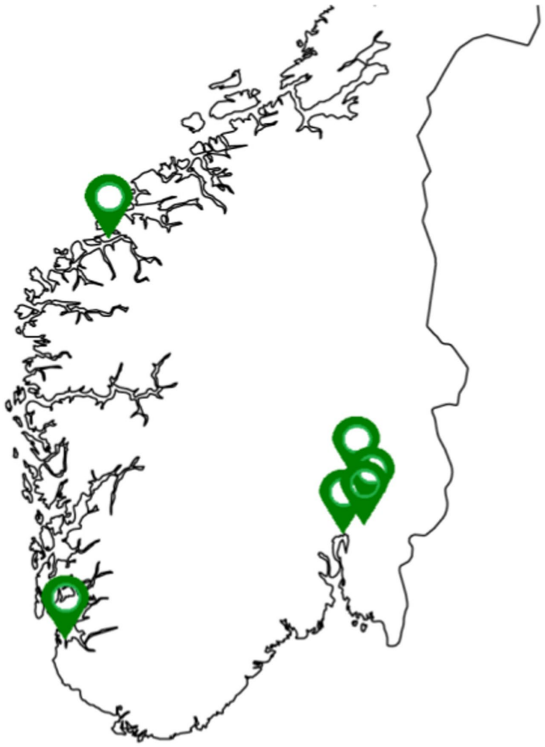

FIGURE 1
Geographical distribution of the waste sorting plants considered.

2 Materials and methods

2.1 Waste sorting plants characterization

The sampling campaign of waste sorting plants occurred between June 2020 and November 2021. Three manual (private companies) and three automated (inter-municipal) waste sorting plants enrolled in the study were assessed, in western and eastern Norway (Figure 1).

Waste sorting differed among plants. In manual plants, primarily pre-sorted waste from housing collectives and local businesses was treated. Plastic and paper/cardboard waste was sorted by hand (with valuable material being returned to the value chain), whereas residual waste was sorted by excavators, shredded and transported to incineration plants. Regarding the work tasks performed, manual plant workers performed the same task throughout the workday, every working day. Investigated work operations included manual sorting of plastics and paper/cardboard, controlling incoming waste and driving excavators. In automated waste sorting plants, unsorted residual waste from domestic homes was received, and sorting was achieved by modern, fully automated waste sorting lines that used ballistic separation, air-pressure, and infrared technologies to fractionize the incoming waste. Investigated work operations included control of incoming waste, cleaning and maintenance of sorting machines, supervision of sorting lines from a secluded control room, as well as driving excavators/trucks in waste reception and storage. All plants were visited at least once, one plant was visited twice, and one plant was visited three times.

2.2 Workers population involved in skin-biota evaluation

Workers of all the waste sorting facilities enrolled in the study were invited to voluntarily participate in the project. From these companies, a total of 98 participants (73 waste workers – exposed

group, 25 offices personal – control group) were enrolled in the study. All controls were office personal from the respective waste sorting plant that at times visited the waste sorting hall. Sample collection happened in the clean zone of each respective sorting plant. Thus, both exposed workers and controls likely had sanitized their hands immediately prior to entering the office area.

Skin-biota samples of the dorsal side of the left hand were collected on day 3 of the sampling campaign (Wednesday) of both exposed workers and the control group. Workers' hands were "swabbed" right before the lunch break, as they came from the sorting hall.

This study complied with the Helsinki Declaration and Oviedo Convention and all data were stored and analyzed in accordance with the Portuguese General Data Protection Regulation (GDPR) law n° 58/2019. The study was approved by the Regional Committees for Medical research Ethics South East Norway, REK South East (ref. no. 34312). Workers were invited to voluntarily participate in the project and before their enrolment all volunteers filled a written informed consent.

2.3 Workplace sampling campaign performed and samples extraction

Personal air-filter samples, impingement samples (Coriolis air sampler), electrostatic dust collectors (EDC), and settled dust were collected throughout nine sampling campaigns over a period of 18 months. The workplaces assessed and the sampling methods used are described in Table 1.

Air-filter samples were collected on 25 mm glass fiber filters (pore size 1 µm, GF/A, Whatman, UK) that were mounted in PAS-6 filter cassettes (22). Filter cassettes were attached to air-pumps (GS5200, GSA Messgerätebau GmbH, Germany) and operated at an average air flow of 2 L/min (±10%). The airflow was measured using a Defender 510 (TPF Control B.V., The Netherlands) prior to and after exposure. Filters were extracted for 30 min in sterile conditions with 5 mL NaCl 0.9% + Tween 80 0.05% (250 rpm, room temperature), and stored at −80°C until shipment/analysis (2.5 mL glycerol was added for conservation).

Workplace air samples were collected using a Coriolis µ (Bertin technologies, France). Sterile autoclaved cones were filled with 15 mL sterile filtrated PBS and operated at an air flow of 200 L/min for 10 min. Samples were stored on ice during transport and stored at −80°C until shipment/analysis (2.5 mL glycerol was added for conservation) and used for further molecular detection.

Settled dust was collected using a sterile spatula and stored at −80° C until shipment/analysis. Dust samples were suspended in 0.1% Tween 80 saline (0.9% NaCl) solution (250 rpm, 30 min), using 9.1 mL solution for 1 g of settled dust sample (23).

Electrostatic dust cloths (EDC) were packed under sterile conditions and exposed for 14 days in the workstations. After exposure, the EDCs were returned to The National Institute of Occupational Health in Norway (STAMI) by mail. Upon arrival, EDCs were extracted for 60 min in 20 mL sterile MilliQ water added 0.05% Tween 20 by orbital shaking at 300 rpm at room temperature. Subsequently, eluates were aliquoted, and stored (after glycerol addition) at −80°C until shipment/analysis.

Skin biota samples were collected by swabbing an area of approximately 5 cm^2 on the dorsal side of the workers hand with circulating motions (for about 10 s). The samples were collected with sterile Copan eSwab 480C regular flocked swab with 1 mL Liquid Amies Medium in Skirted Tube with Plastic White Capture Cap (Copan, Italy). The sampling of skin biota was conducted during work hours. Hand sanitation was performed before samples collection due to strict hygienic measures due to the pandemic. Samples were collected in both the exposed and control group, using the same protocol. The samples were kept refrigerated (0 to 4°C) until arrival at the laboratory, and then frozen at −80°C until further analysis.

2.4 Prevalence of cultivable fungi and bacteria

In order to assess the viable microbiota, 150 µL of the prepared sample extracts were inoculated in selective media, as follows: malt extract agar (MEA) supplemented with chloramphenicol (0.05%), and dichloran-glycerol agar (DG18) for fungi (27°C, 5–7 days); tryptic soy agar (TSA) supplemented with nystatin (0.2%) (30°C, 7 days), and Violet Red bile agar (VRBA) (35°C, 7 days) for mesophilic and Gram-negative bacteria, respectively. Microbial quantification was determined as colony-forming units (CFU) and CFU concentration ($CFU.m^{-3}/m^{-2}/m^{-2}.day^{-1}/g^{-1}$) depending on the used sampling method. Additionally, fungal species/genera were identified by a trained mycologist through notation of macro and microscopic characteristics (24).

TABLE 1 Workplaces assessed, and sampling methods applied.

Plants	Waste tons sorted/ year	Number of exposed workers	Number of contsrols	Workplaces assessed	Samples type and number			
					Air filter samples	Settled dust	Coriolis air sampler	eSwab
Plant A	50,000	29	8	Automated WSP*	24	11	7	22
Plant B	75,000	17	7	Automated WSP	8	8	3	21
Plant C	22,000	7	5	Automated WSP	5	4	0	7
Plant D	140,000	9	2	Manual WSP	6	3	0	0
Plant E	46,000	4	0	Manual WSP	3	3	0	0
Plant F	347,000	7	3	Manual WSP	6	0	0	6

*WSP, Waste sorting plant.

2.5 Screening of azole-resistance

In order to address the growing urgency of fungal resistance (25), a preliminary screening of azole-resistance was conducted, as previously reported (26). Briefly, 150 µL of EDC, filter and settled dust samples' extracts were seeded on azole-supplemented Sabouraud dextrose agar (SDA) media (Frilabo, Maia, Portugal) with final concentrations of 4 mg/L itraconazole (ICZ), 2 mg/L voriconazole (VCZ), and 0.5 mg/L posaconazole (PCZ) [adapted from: Arendrup et al. (27); The European Committee on Antimicrobial Susceptibility Testing (28)]. *A. fumigatus* reference strain (ATCC 204305) and pan-azole-resistant *A. fumigatus* strain (both provided by the National Health Institute Doctor Ricardo Jorge, IP) were used as negative and positive control, respectively. Fungal species/genera were identified after 48 to 72 h' incubation at 27°C, as described elsewhere (23).

2.6 Molecular detection of *Aspergillus* sections

Six important *Aspergillus* sections were targeted in air samples, filter and settled dust samples' extracts by quantitative PCR (qPCR) using the CFX-Connect PCR System (Bio-Rad), according to a previously reported method (23) and to complement the results already obtained in previous studies (19, 21). For fungal DNA isolation, the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, USA) was used. Reactions were performed in a 20 µL final volume containing 1 × iQ Supermix (Bio-Rad, Portugal), 0.5 µM of each primer, and 0.375 µM of TaqMan probe. qPCR conditions included a three-step reaction consisting of 40 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s.

The used controls were water (negative control) and a reference strain DNA (positive control). The reference strains were kindly provided by the reference Unit for Parasitic and Fungal Infections from the Department of Infectious Diseases, National Health Institute Doctor Ricardo Jorge, IP. All reference strains were sequenced for ITS, B-tubulin, and Calmodulin.

2.7 Screening for cytotoxicity

In order to assess the toxicological effects of samples collected in the waste sorting plants, human alveolar epithelial (A549) cells and human liver carcinoma (HepG2) cells were exposed to filter ($N = 18$) and settled dust ($N = 11$) samples' extracts and screened for cytotoxicity.

Firstly, cells were maintained in Eagle's Minimum Essential Medium (MEM) supplemented with 10,000 units penicillin and 10 mg/mL streptomycin in 0.9% NaCl and fetal bovine serum (Sigma-Aldrich, USA). Then, cells were detached with 0.25% (w/v) Trypsin 0.53 mM EDTA. Cell suspensions (100 µL) with 2.0×10^5 HepG2 cells/ml and 4.7×10^5 A549 cells/ml densities (Scepter™ 2.0 Cell Counter, Merck) were transferred to a 96-well plate and incubated with series of five sample dilutions (D1:2, first dilution as half the equivalent of 1 mL of the sample) for 48 h at 5% CO2, 37°C, and humid atmosphere.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine cell viability, measured at 510 nm (ELISA LEDETECT 96, biomed Dr. Wieser GmbH; MikroWin 2013SC software), as previously described (29). The lowest concentration dropping absorption to <50% of cell metabolic activity (IC50) was defined as threshold toxicity level.

2.8 Statistical analysis

Data were analyzed in SPSS statistical software, version 27.0 for windows. The results were considered significant at the 5% significance level. To test the normality of the data, the Kolmogorov–Smirnov test or the Shapiro–Wilk test were used, according to the sample size. To characterize the sample, frequency analysis was used (n, %) for qualitative data and for quantitative data, the logarithm of bacterial and fungal counts and resistance to azoles was used. To compare bacterial and fungal contamination and resistance to azoles between two independent groups, the Mann–Whitney test was used (evaluate season effect, to compare industries in the summer, to compare the type of workplaces assessed), and between $k > 2$ independent groups (to compare industries in the autumn), the Kruskal-Wallis test was used, since the normality assumption was not verified. When statistically significant differences were detected, the Kruskal-Wallis, multiple comparison tests were used. To compare the culture media, the Wilcoxon Signed Ranks (comparison of two media) and Friedman (comparison of $k > 2$ media) tests were used, since the assumption of normality was not verified. To study the relationship between bacterial, fungal and resistance to azoles by sampling method, Spearman correlation coefficient was used. To assess species diversity, Simpson and Shannon indices, given by $\text{Shannon Index}\,(\mathrm{H}) = -\sum_{i=1}^{s} p_i \ln(p_i)$ and $\text{Simpson Index}\,(\mathrm{D}) = \frac{1}{\sum_{i=1}^{s} p_i^2}$, were used, where pi is the proportion (ni/n) of individuals of one particular species found (ni) divided by the total number of individuals found (n).

3 Results

3.1 Viable bacterial contamination

Personal filter samples had the highest counts on total bacterial contamination (Manual: 8.15×10^1 CFU.m^{-3}; Automated: 2.67×10^5 CFU.m^{-3}), compared to the counts of Gram-negative bacteria (Manual: 2.29×10^1 CFU.m^{-3}; Automated: 2.18×10^2 CFU.m^{-3}) (Figure 2).

EDC total bacterial counts ranged between 1.21×10^2 CFU.m^{-2}.day^{-1} in automated industries and 1.21×10^2 CFU.m^{-2}.day^{-1} in manual industries. As for Gram-negative counts, automated industries presented 1.21×10^2 CFU.m^{-2}.day^{-1}, while on manual industries presented 6.07×10^1 CFU.m^{-2}.day^{-1}. Total bacteria counts in settled dust ranged from 2.92×10^3 CFU.g^{-1} in automated industries to 1.57×10^2 CFU.g^{-1} in manual industries, whereas Gram-negative counts ranged from 1.81×10^3 to 8.87×10^1 CFU.g^{-1}, respectively on automated and manual industries (Figure 3).

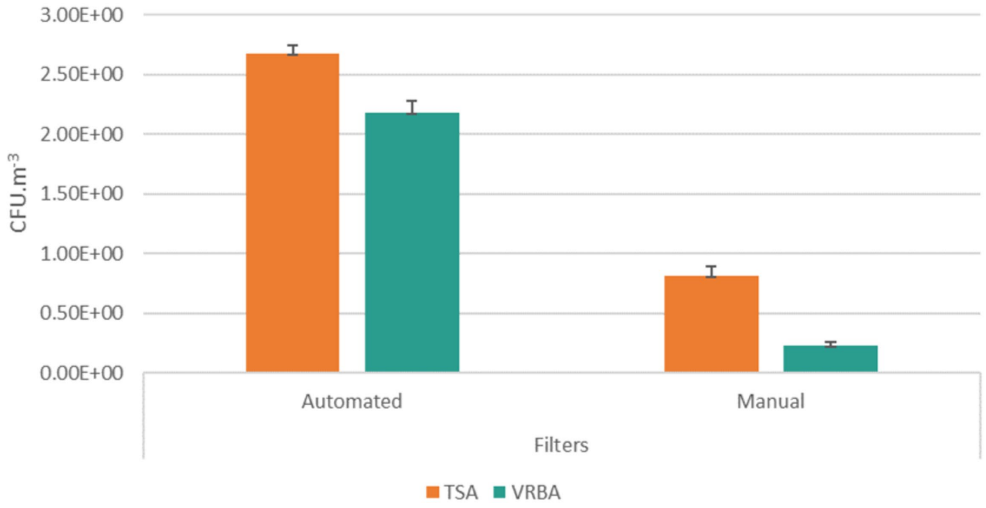

FIGURE 2
Bacterial distribution (total bacteria, TSA; Gram negative bacteria, VRBA) in filter samples from automated and manual industries (CFU.m – 3) and the standard error for each case.

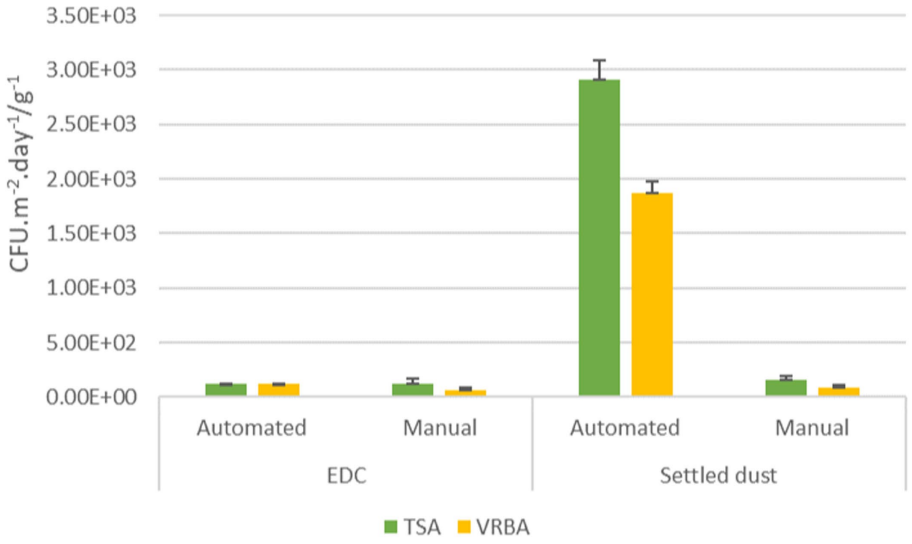

FIGURE 3
Bacterial distribution (TSA; VRBA) in automated and manual industries among the passive sampling matrices (EDC: CFU.m^{-2}.day^{-1}; Settled dust: CFU.g^{-1}) and the standard error for each case.

In swabs from workers' hands, the contamination of total bacteria in control workers was 3.94×10^6 CFU.m^{-2} while on exposed workers was 3.40×10^6 CFU.m^{-2}. Considering Gram-negative bacteria, no contamination was detected on control workers, while on exposed workers contamination reached 1.26×10^6 CFU.m^{-2} (Figure 4).

3.2 Viable fungal contamination

Personal filter samples had higher counts in company A (MEA 2.85×10^2 CFU.m^{-3}; DG18 1.39×10^3 CFU.m^{-3}) and C (MEA 8.0×10^2 CFU.m^{-3}; DG18 1.43×10^2 CFU.m^{-3}) among the automated industries. On the manual industries, industry E (MEA 4.7×10^2 CFU.m^{-3}; DG18 3.40×10^2 CFU.m^{-3}) and F (MEA 4.17×10^2 CFU.m^{-3}; DG18 3.70×10^2 CFU.m^{-3}) had the highest fungal counts (Figure 5).

EDC had the highest counts in industry A (DG18 7.58×10^0 CFU.m^{-2}.day^{-1}) and D (MEA 7.58×10^1 CFU.m^{-2}.day-1; DG18 1.21×10^2 CFU.m^{-2}.day^{-1}), while the settled dust samples, the counts ranged from 7.90×10^1 CFU.g^{-1} in industry C to 3.59×10^2 CFU.g^{-1} in industry A on DG18, and from1.84×10^2 CFU.g^{-1} in industry A to 2.75×10^2 CFU.g^{-1} in industry D on MEA (Supplementary Figures S1A,B).

Higher counts were observed in eSwabs from exposed workers (MEA 5.40×10^4 CFU.m^{-2}; DG18 2.00×10^4 CFU.m^{-2}) than in control workers (MEA 6.00×10^3 CFU.m^{-2}; DG18 6.00×10^3 CFU.m^{-2}) (Figure 6).

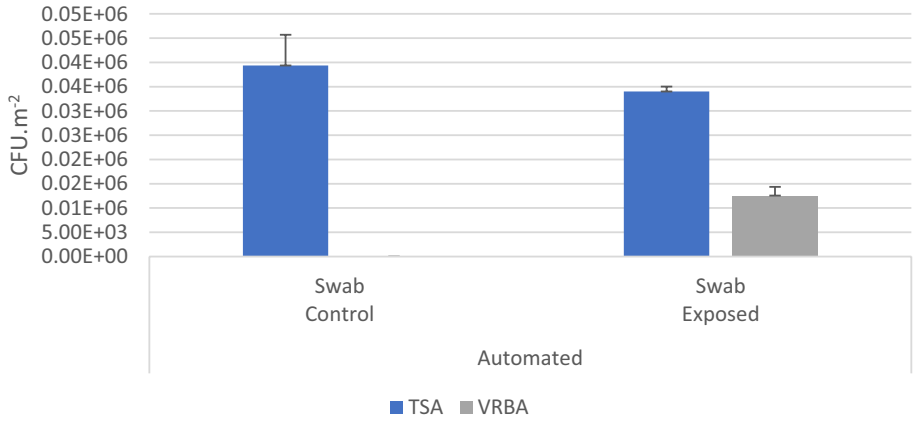

FIGURE 4
Bacterial (TSA; VRBA) distribution in automated industries in swabs from the workers' hands (CFU.m^{-2}) and the standard error for each case.

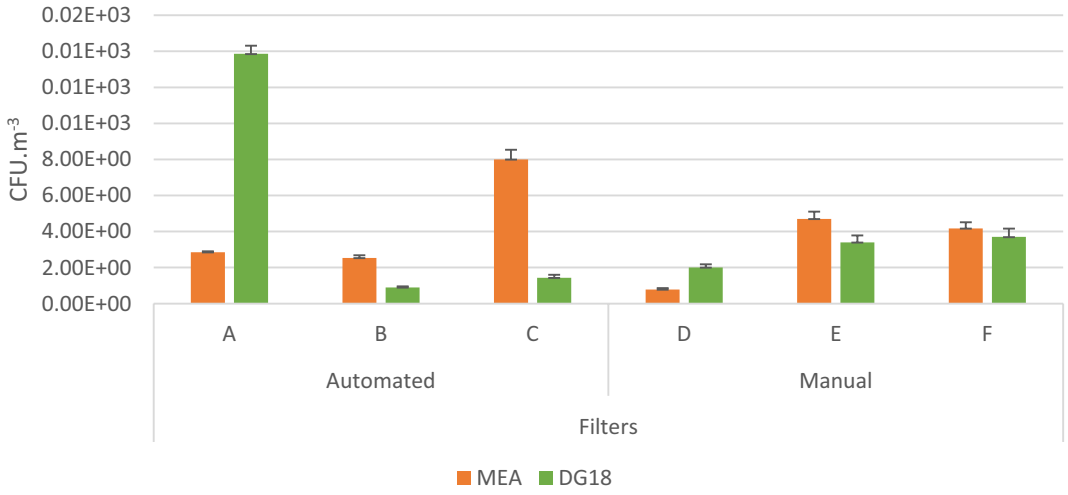

FIGURE 5
Fungal distribution (MEA; DG18) in automated and manual industries among filter samples (CFU.m^{-3}) and the standard error for each case.

Penicillium sp. was the most prevalent fungal genus in personal airborne filter samples from both automated and manual industries (Table 2).

Regarding automated plants, *Penicillium* sp. was the most prevent genus in industries A (EDC: 100% DG18; SD: 64.1% MEA, 77.7% DG18), B (SD: 71.3% MEA, 83.5% DG18) and C (SD: 83.1% MEA, 65.8% DG18). In the manual industries, *Penicillium* sp. was the most prevalent genus in industry D (EDC: 60% MEA, 100% DG18; SD: 82.5% MEA, 82.5% DG18) (Supplementary Table S1). *Penicillium* sp. was also the most prevalent genus in eSwab samples at automated plants, except unexposed controls from industry A where the most prevalent genus was *Cladosporium* sp. (66.7% MEA) (Supplementary Table S2).

Among *Aspergillus* sections present in MEA, *Nigri* was the most prevalent section in personal filter samples from workers at industries A, B, C, D and E (100%). *Fumigati* section also showed to be prevalent in filter samples (Industry F 67.8%).

The most prevalent *Aspergillus* section in filters on DG18 were *Circumdati* (Industries A 73.7%; B 100%; C 36%; E 16.7%; F 67%), *Nidulantes* and *Aspergilli* (industry D 100%; E 83.3%, respectively). The most prevalent *Aspergillus* section in EDC samples cultivated on MEA was *Nidulantes* (100%), while in settled dust samples, *Nigri* was the most prevalent section in industries A (100%), B (75%), C (96.7%), and D (100%). The second most prevalent section in MEA was *Nidulantes* in settled dust (Industry B 25%; D 3.33%). When cultivating on DG18, the most prevalent section was *Flavi* (A: 1.3%; B: 72%; C: 3.7%; and D: 23.1%). The second most prevalent sections were *Nigri* (A: 16.3%; B: 28%; C: 33.3%; and D 38.5%) and *Circumdati* (A: 17.5%; C: 59.3%; D: 7.7%) (Figure 7).

In eSwabs from exposed workers' hands, either in MEA and DG18, the sections *Nigri* and *Fumigati* were the only *Aspergillus* sections identified (Plant B 100%).

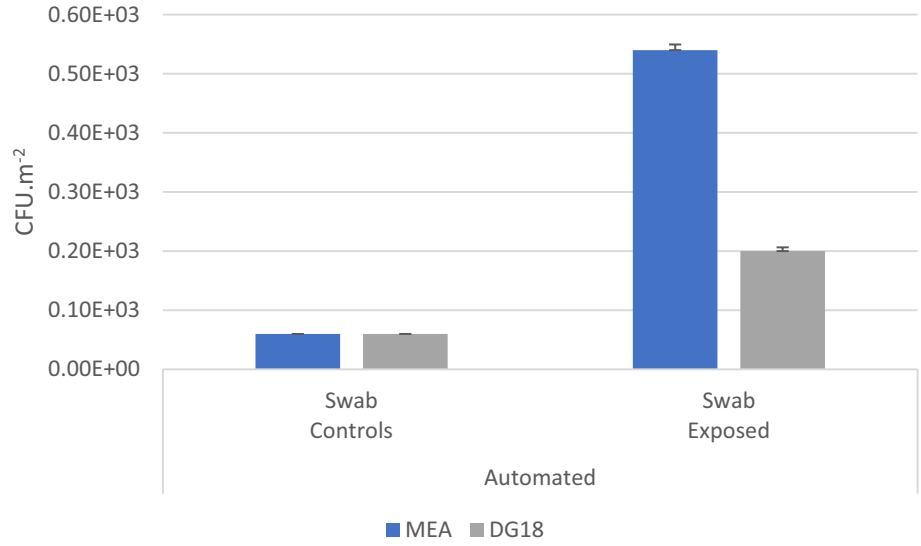

FIGURE 6
Fungal (MEA; DG18) counts in swabs from the hands of workers at the automated industries (CFU.m^{-2}) and the standard error for each case.

TABLE 2 Fungal distribution in industries A to F on filters (log [CFU.m^{-3}]).

Matrix	Workplaces assessed plant	Industry	MEA			DG18		
			ID	CFU.m^{-3}	%	ID	CFU.m^{-3}	%
Filters	Automated	A	*Aspergillus* sp. *Penicillium* sp.	2.15×10^1 2.64×10^2	7.6 92.4	*Aspergillus* sp. *Penicillium* sp.	2.17×10^1 1.36×10^3	1.6 98.4
		B	*Cladosporium* sp. *Aspergillus* sp. *Penicillium* sp. *Rhizopus* sp.	1.37×10^0 4.00×10^1 1.36×10^2 7.59×10^1	0.5 15.8 53.7 30.0	*Aspergillus* sp. *Penicillium* sp.	2.54×10^0 8.70×10^1	2.8 97.2
		C	*Aspergillus* sp. *Penicillium* sp. *Rhizopus* sp.	6.91×10^1 5.47×10^2 1.84×10^2	8.6 68.3 23.0	*Aspergillus* sp. *Cladosporium* sp. *Penicillium* sp.	3.85×10^1 1.14×10^0 1.4×10^2	26.9 0.8 72.3
	Manual	D	*Cladosporium* sp. *Aspergillus* sp. *Penicillium* sp. *Rhizopus* sp.	2.11×10^0 3.17×10^0 6.97×10^1 3.16×10^0	2.7 4.1 89.2 4.0	*Mucor* sp. *Aspergillus* sp. *Penicillium* sp.	5.29×10^0 1.08×10^0 1.94×10^2	2.6 0.5 96.8
		E	*Aspergillus* sp. *Penicillium* sp. *Rhizopus* sp.	4.44×10^0 4.48×10^2 1.78×10^1	0.9 95.3 3.8	*Aspergillus* sp. *Penicillium* sp. *Syncephalastrum racemosum*	6.67×10^0 3.31×10^2 2.22×10^0	2.0 97.4 0.7
		F	*Aspergillus* sp. *Paecilomyces* sp. *Penicillium* sp.	2.79×10^1 1.13×10^0 3.88×10^2	6.7 0.3 93.0	*Aspergillus* sp. *Penicillium* sp.	1.23×10^1 3.58×10^2	3.3 96.7

3.3 Fungal distribution in azole-supplemented media

The burden of fungal resistance is depicted in Figure 8 per industry type (automated vs. manual). Higher fungal counts with reduced azole susceptibility were observed in the automated industry by filter and settled dust sampling.

Regarding fungal diversity in azole-supplemented media (Table 3), *Penicillium* sp. was the most prevalent (ICZ, VCZ and PCZ) by filter sampling, followed by *Mucorales* order (*Mucor* sp., *Rhizopus* sp.) (PCZ and ICZ). *Aspergillus* sp. was also present in filters from manual (ICZ and PCZ) and automated (VCZ and PCZ) plants. Five *Aspergillus* sections were identified, including *Circumdati* (VCZ and ICZ) and *Nidulantes* (PCZ) with reduced susceptibility to azoles.

Settled dust and EDC sampling also enabled the identification of *Mucor* sp. and *Rhizopus* sp. in azole-supplemented media, but not *Aspergillus* sp. (Supplementary Table S3).

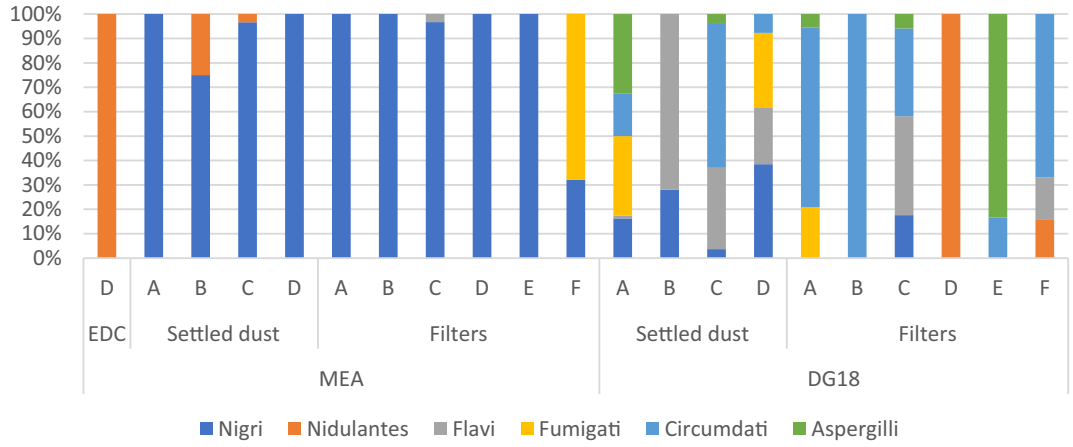

FIGURE 7
Aspergillus sections distribution in DG18 and MEA in industries A to F (Filters: CFU.m^{-3}; EDC: CFU.m^{-2}.day^{-1}; Settled dust: CFU.g^{-1}).

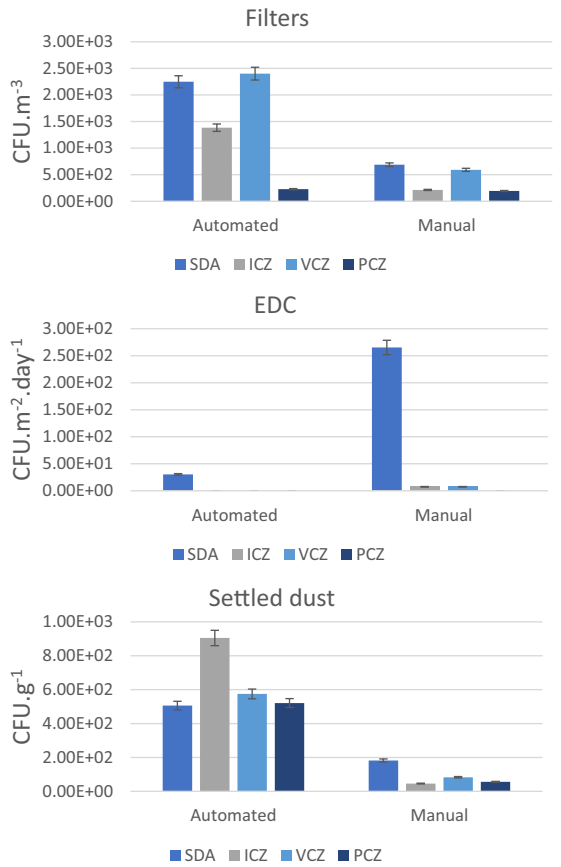

FIGURE 8
Fungal counts in azole screening in automated and manual industries, per sampling method: Filters (CFU.m^{-3}); EDC (CFU.m^{-2}.day^{-1}); Settled dust (CFU.g^{-1}) and the standard error for each case.

3.4 Detection of the targeted fungal sections

Regarding the four *Aspergillus* sections investigated by PCR, two of them were detected. *Aspergillus* section *Fumigati*, was detected in settled dust samples (4 out of 29, 13.79%) and in filter samples (1 out of 58, 1.72%). *Aspergillus* section *Circumdati* was detected in settled dust samples (1 out of 29, 3.45%), and also in filter samples (2 out of 58, 3.45%) (Supplementary Table S4).

3.5 Cytotoxicity results

Based on the ability to decrease cell metabolic activity (IC50), cytotoxicity levels were determined in extracts of personal filters and settled dust samples as depicted in Table 4. Six percent of the filter sample extracts were highly cytotoxic for A549 cells, while most filters were low cytotoxic for both cell lines. Regarding settled dust, 18% were highly cytotoxic for A549 cells, and 45% were highly cytotoxic for HepG2 cells.

3.6 Comparisons and correlation analysis

Between summer and autumn, statistically significant differences were detected among filter samples regarding: (i) bacterial counts in TSA ($p = 0.006$) and VRBA ($p < 0.0001$) with statistically highest bacterial counts during summer; (ii) fungal counts in MEA ($p = 0.001$), with statistically highest fungal counts during autumn. Statistically significant differences were also detected in azole screening in ICZ, VCZ and PCZ (p's < 0.05) with highest values during summer. In settled dust samples, statistically significant differences were detected between summer and autumn regarding to: (i) bacterial counts in TSA ($p < 0.05$) and VRBA ($p < 0.05$) with statistically highest bacterial counts during summer; (ii) azole screening in ICZ, VCZ and PCZ (p's < 0.05) with highest values during summer. In the eSwabs samples, statistically significant differences were detected in relation to bacterial counts in TSA ($p = 0.001$), with, once again, highest bacterial counts during summer (Supplementary Table S5).

As statistically significant seasonal variation was identified, the following analyzes were carried out separately by season. During summer in the filters, statistically significant differences were detected between the industries A and B, concerning bacterial counts in TSA ($p = 0.005$) and VRBA ($p = 0.035$), with industry B revealing higher counts and relatively to azole screening in ICZ ($p = 0.029$), with industry A revealing higher counts. In settled dust and swabs, no statistically significant differences were detected between industries A and B (p's > 0.05) (Supplementary Table S6).

TABLE 3 Fungal diversity in azole screening per industry type.

			SDA	4 mg/L ICZ	2 mg/L VCZ	0.5 mg/L PCZ
Matrix	Plant type	ID	CFU.m^{-3}	CFU.m^{-3}	CFU.m^{-3}	CFU.m^{-3}
Filters	Manual	*Alternaria* sp.		6.41E+00		
		Aspergillus sp.	4.29E+02	4.90E+00	1.19E+00	1.21E+01
		Chrysosporium sp.		4.75E+00	2.05E+01	1.28E+01
		Cladosporium sp.		7.61E+00	1.85E+02	5.10E+01
		Fusarium verticilloides				1.44E+00
		Fusarium solani				1.19E+00
		Lichtheimia sp.			2.24E+00	
		Mucor sp.	5.80E+01	2.80E+01	9.45E+01	1.34E+01
		Paecilomyces sp.			2.22E+01	1.30E+00
		Penicillium sp.	2.38E+03	1.48E+03	2.59E+03	4.48E+02
		Rhizopus sp.	3.69E+01	6.06E+01	7.92E+01	3.71E+00
		S. racemosum	3.21E+01	2.22E+00	7.07E+00	
	Automated	*Alternaria* sp.		6.41E+00		
		Aspergillus sp.	4.14E+02		1.19E+00	1.21E+01
		Chrysosporium sp.			2.05E+01	1.28E+01
		Cladosporium sp.		2.33E+00	1.85E+02	5.10E+01
		F. verticilloides				1.44E+00
		Fusarium solani				1.19E+00
		Lichtheimia sp.			2.24E+00	
		Mucor sp.	5.69E+01	2.69E+01	9.45E+01	1.34E+01
		Paecilomyces sp.			2.22E+01	1.30E+00
		Penicillium sp.	1.74E+03	1.30E+03	2.59E+03	4.48E+02
		Rhizopus sp.	3.47E+01	5.19E+01	7.92E+01	3.71E+00
		S. racemosum			7.07E+00	

Filters: [CFU.m^{-3}].

TABLE 4 Cytotoxicity levels of filters ($N = 18$) and settled dust ($N = 11$) diluted samples in A549 and HepG2 cellular lines.

	A549 cells				HepG2 cells			
Cytotoxicity level	High	Moderate	Low	n.d.	High	Moderate	Low	n.d.
Filters (N)	1	0	14	3	0	4	12	2
Settled dust (N)	2	4	3	2	5	2	1	3

Cytotoxicity level: High, IC50 at third or more dilutions; Moderate, IC50 at second dilution; Low, IC50 at first dilution; n.d., no cytotoxicity detected.

Statistically significant differences were detected in autumn measurements among industries in the filters, regarding to: (i) bacterial counts in TSA ($p = 0.025$), with industry B having the highest bacterial counts and industries A and D with lower counts, and in VRBA ($p = 0.002$), with industry D having the highest bacterial counts, while industries B, C, E and F with lower counts; (ii) fungal counts in MEA ($p = 0.002$), with industries C and E showing the highest fungal counts; (iii) fungal counts in SDA ($p = 0.001$), with industry E showing higher counts followed by industry C, in VCZ ($p = 0.002$), with industry D showing higher counts followed by industry B and in PCZ ($p < 0.0001$), with industry D revealing the highest values (Supplementary Table S7). As for *Aspergillus* sp., no statistically significant differences were detected ($p > 0.05$). Concerning settled dust sample method, statistically significant differences were detected regarding to: (i) bacterial counts in TSA ($p = 0.011$) and in VRBA ($p = 0.024$), with industry A revealing the highest counts; (ii) fungal counts in SDA ($p = 0.008$), with industry C showing higher values, followed by industry D, and in PCZ ($p = 0.018$), with industry A revealing the highest values, followed by industry D (Supplementary Table S7). With respect to eSwabs, no statistically significant differences were detected between industries (p's > 0.05).

The comparison of bacterial counts (TSA and VRBA), fungal counts (MEA and DG18) and azole screening (SDA, ICZ, VCZ and PCZ) in the two types of industries assessed (automated/manual) was only possible during autumn, as only automated industries were assessed during summer. In filters, statistically significant differences between manual and automated industries were only detected for fungal counts in PCZ ($p = 0.047$), with manual industries having

higher values. In settled dust, no statistically significant differences were detected (Supplementary Table S8). This analysis could not be performed on eSwabs, as data were not collected for the manual workplaces. It was also not possible to perform for the EDC, since there were only two observations. Considering *Aspergillus* sp., no statistically significant differences were detected between the types of industry ($p > 0.05$) (Supplementary Table S1).

Statistically significant differences in bacterial counts were detected between TSA and VRBA in filter samples ($p = 0.020$), with the VRBA medium presenting lower counts. Regarding fungal counts, no statistically significant differences were detected between MEA and DG18 ($p = 0.943$). Regarding azole screening, statistically significant differences were detected between culture medium ($p < 0.0001$). In Friedman's paired multiple comparisons the differences were between the PCZ and the other media (p's < 0.05) with PCZ having the lowest values (Supplementary Table S9).

In settled dust, bacterial counts in VRBA were also statistically significant lower than counts in TSA ($p < 0.0001$). No statistically significant differences were detected among fungal counts in MEA and DG18 ($p = 0.873$) nor among azole-supplemented media (Supplementary Table S9).

In eSwabs from the exposed workers, bacterial counts in VRBA were statistically significant lower than bacterial counts in TSA ($p < 0.0001$), whereas fungal counts in DG18 were statistically significant lower than fungal counts in MEA ($p = 0.013$) (Supplementary Table S9).

The azole screening and the EDC sampling method were excluded from this analysis, as the observations number was very small.

Concerning the sampling method (particularly, filters in TSA), a relation between higher bacterial counts in filters in TSA and higher values in SDA was determined. Higher bacterial counts in VRBA was related to lower fungal counts in MEA and higher fungal counts in VCZ and PCZ. Higher fungal counts in MEA was related to higher counts in DG18 and SDA and lower values in VCZ and PCZ. Higher fungal counts in DG18 was related to higher values in SDA. In azole screening, higher fungal counts in a given culture medium were related to higher values in another (Supplementary material Text S1; Table 5).

In settled dust, higher bacterial counts in TSA were related with higher counts in VRBA, lower counts on MEA and higher counts in ICZ, VCZ and PCZ. Higher bacterial counts in VRBA were related with lower fungal counts in MEA and higher values in ICZ, VCZ and PCZ. Higher fungal counts in MEA were related with lower values in ICZ, VCZ and PCZ. Higher values in ICZ were related with higher

TABLE 5 Study of the relationship between bacterial, fungal and resistance to azoles counts by sampling method.

	Culture media	Filter						
		Bacteria	Fungi		Azole screening			
		VRBA	MEA	DG18	SDA	ICZ	VCZ	PSZ
Bacteria	TSA	0.172	0.040	−0.150	0.374*	−0.007	0.149	0.034
	VRBA		−0.563**	−0.126	−0.174	0.233	0.448**	0.528**
Fungi	MEA			0.373**	0.582**	−0.116	−0.304*	−0.483**
	DG18				0.382**	0.113*	−0.091	−0.159
Azole screening	SDA					0.088	0.119	−0.227
	ICZ						0.745**	0.648**
	VCZ							0.726**
Settled dust								
Bacteria	TSA	0.979**	−0.470*	0.310	0.295	0.918**	0.791**	0.807**
	VRBA		−0.487**	0.289	0.306	0.932**	0.764**	0.799**
Fungi	MEA			0.281	0.193	−0.404*	−0.431*	−0.625**
	DG18				0.304	0.299	0.132	0.174
Azole screening	SDA					0.172	0.053	0.071
	ICZ						0.793**	0.784**
	VCZ							0.658**
Swabs								
Bacteria	TSA	0.332*	0.152	0.094				
	VRBA		0.032	−0.131				
Fungi	MEA			0.171				
	DG18							
Azole screening	SDA							
	ICZ							
	VCZ							

Spearman correlation results.

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

values in VCZ and PCZ, and higher values in VCZ were related with higher values in PCZ (Supplementary material Text S1; Table 5).

Considering the surfaces eSwabs sampling method, a significant correlation with weak intensity suggested that higher bacterial counts in TSA is related with higher counts in VRBA (Supplementary material Text S1; Table 5).

The highest fungal diversity was found in DG18 inoculated with the eSwabs of the exposed workers from automated industry B (Shannon Index (H) = 1.34 and Simpson Index (D) = 2.95), followed by filters also from industry B inoculated in MEA (Shannon Index (H) = 1.01 and Simpson Index (D) = 2.48) (Supplementary Table S10).

4 Discussion

Occupational exposure to microorganisms during waste handling is a known health hazard for exposed workers (9, 30–31). Although microbial composition of bioaerosols in traditional waste sorting has been described previously (18, 32, 33) the work environment microbiome is rarely described at automated waste sorting plants (19, 21).

The present study compares (by personal air sampling and passive methods) workers' exposure to microbial agents in waste sorting in modern automated facilities with exposure in traditional facilities, addressing selected pathogens and fungal resistance. The use of complementary sampling methods (personal, environment) and laboratorial assays (culture-based identification, molecular detection, *in vitro* cytotoxicity) allow to identify a wider spectrum of the microbiota, and screen for potential health risks in this occupational setting (18).

Despite the restricted number of assessed plants, this study confirmed a high exposure to microbial agents. The use of six selected fungal molecular targets in this study allowed comparison with previous molecular results (19, 21). The selection of these molecular targets, specific to the environment under study, was based on results from previous studies that described fungal contaminants with clinical and toxicological relevance (2, 18). The toxicological assessment of microbes is frequently done by *in vitro* assays. Previous studies in these environments indicated that dust samples and personal air samples contained ligands capable of stimulating TLR2 and TLR4 receptors, with the potential to evoke an inflammatory response in exposed workers (9, 20). In this study, the MTT assay was used to assess cell viability of A549 and HepG2 cells after exposure to dust and personal air samples.

4.1 Compliance assessment

In personal filter samples the guidelines for total bacteria (10,000 CFU.m^{-3}) were not overpassed in either automated or manual industries (34, 35), as well as in the case of gram-negative bacteria (1,000 CFU.m^{-3}) (34). Concerning fungi, one automated industry (A) surpassed the guidelines (1,000 CFU.m^{-3}) (34, 36) and, although with lower counts than other studies performed in the same setting (18, 37, 38), this fact claim attention for the need of intervention in the scope of microbial agents' risk management, even in automated industries, with less workers engaged in the different tasks. Thus, probably other variables that were not studied influence the contamination and not the type of process (manual or automatic).

4.2 Sampling and analyses approaches

For a better estimation of workers' health risks in waste sorting industries, a comprehensive sampling strategy using complementary sampling methods is of the upmost importance. An important feature of this study is the evaluation of the viable microbiota, due to the critical implication of microorganisms' viability in the health effects that can be observed, thus, being a more useful resource for accurate risk assessments (39). The use of previously described methods also enables the generation of comparable data among different studies (2, 18). Besides, we should be aware of the drawbacks to apply only molecular tools when assessing occupational exposure to microbial contamination. In fact, despite cultivation of microorganisms induce a bias in their representation (40–42) we cannot neglect the fact that the isolation of fungal isolates is vital to understand and study specific isolates (such as the ones presenting azole resistance) and to better characterize the biodiversity present in a specific occupational environment (41). Nevertheless, in automated plants EDC for sampling were used in the control room/office area of the respective plants (the expected "cleaner" areas from the facilities) and no contamination was observed, corroborating the suitability of the sampling approach.

The surveillance of antifungal resistance is considered to be critical in hot spot environments such as waste management, due to the foreseen increased prevalence of resistant fungi as an indirect consequence of climate change (2, 3, 18, 43). Indeed, previous detection of azole-resistant *Aspergillus fumigatus* harboring the TR34/L98H Mutation in a waste management facility justifies this (10). Thus, the application of multiple culture conditions (combining different culture media and incubation temperatures), used in parallel with more refined molecular methods, will provide complementary information regarding microbial diversity and, in particular, fungal diversity (41, 44). All these datasets will provide information to characterize in detail exposure and estimate all the possible impacts on workers' health (2, 41).

4.3 Fungal contamination and azole resistance screening

The seasonal influence on viable microbial contamination observed in this study, including on fungi with reduced susceptibility to the tested azoles, raises concern on the impact of climate change on the development of antimicrobial resistance (AMR). It is well described that the continuing disturbance of the environment, with extreme weather events and higher global temperatures, impacts the emergence and spread of antimicrobial resistance phenotypes in the environment (43, 45, 46). In this context, specific fungal species are expected to thrive through climate change, boosting crops' contamination by toxigenic fungal species with consequent increase of the use of fungicides. Thus, not only environmental pressures may result in new fungal diseases (47), they can also increase human exposure to mycotoxins, and prompt the development of acquired azole resistance that hampers the management of life-threatening fungal invasive infections (43).

Driven by this real menace, the World Health Organization (WHO) recently published the first fungal priority pathogens list, identifying 19 groups of human fungal pathogens associated with a higher risk of mortality or morbidity (25). However, the concern regarding the toxigenic potential of specific fungal species, sections and strains was

overlooked in the published WHO list, hindering a more accurate intervention concerning risk management measures. In fact, *Aspergillus* section *Flavi*, found in settled dust and filters samples, was not listed by WHO, although in previous studies performed in the waste sector the presence of this section resulted in occupational exposure to aflatoxin B1, a carcinogenic mycotoxin (7, 48). Furthermore, the section *Circumdati* (observed and detected by molecular tools in the same matrices), was also neglected in WHO list, although species from this section produce large amounts of ochratoxin A (OTA) (49). Several studies have linked OTA exposure with different human diseases, such as Balkan endemic nephropathy (BEN) and chronic interstitial nephropathy (CIN), as well as other renal diseases (50).

In this study, *Aspergillus* section *Fumigati,* that was listed as of critical priority by WHO and suggested as indicator of harmful fungal contamination in waste management industry (2, 18) was observed in filters and settled dust samples and detected by molecular tools in different settled dust samples, proving the widespread of this section in the assessed plants. *Fusarium* species (*F. solani* and *F. verticilloides*) and Mucorales (*Mucor*, *Rhizopus*, *Syncephalastrum* and *Lichtheimia* genera) (listed as of high priority by WHO) were also identified. In addition all the *Aspergillus* sections identified have toxigenic potential and this should be also considered when performing risk characterization.

The statistically significant lower fungal prevalence in posaconazole is in accordance with the reported superior activity of this azole (compared to itraconazole or voriconazole) against *Aspergillus* and *Mucormycetes* isolates (51). Nevertheless, the observed reduced susceptibility of pathogens of critical priority (Mucorales and *Fusarium* sp.) to posaconazole supports the need to intervene in this occupational environment. In filters, *Mucor* sp. and *Fusarium* sp. were observed in all azoles and in posaconazole only, respectively, with no differences between manual and automated industries; in settled dust, *Mucor* sp. prevalence in azoles was about 1.6-fold higher in the automated industries. Although no conclusions can be drawn regarding azole resistance as the tested azole concentrations are cut off values defined only for *Aspergillus* section *Fumigati* (not *Fusarium* sp. or Mucorales), these preliminary results raise awareness for the need of implementing surveillance programs dedicated to the fungal prioritized species in the environment.

4.4 Skin-biota samples

Strict hygiene regimes were in place, due to the ongoing pandemic, and many workers had sanitized their hands before eSwabs samples could be collected. However, the results report microbial contamination in both controls and exposed hands claiming attention for the possible exposure by hand to face/mouth contact even when strict hygienic measures are in place. The findings corroborate previous results concerning the prevalence of gastrointestinal symptoms, besides respiratory disorders, among the workers from the same units (19, 20).

4.5 Cytotoxic analyses

Cytotoxicity is one of the most important and preliminary indicators in biological risk assessment and *in vitro* toxicology (52). While chemical pollutants have been more studied through these type of resources, we propose an increment on the use of *in vitro* testing when performing environmental assessments to estimate biological effects resulting from exposure to biological agents. In this study, lower cell viability was observed for A549 and HepG2 cells exposed to settled dust, compared to cells exposed to filters. The analysis of the microbial counts in automated industries of filters and settled dust revealed a higher bacteria contamination in settled dust (2.92×10^3 CFU.g^{-1} TSA and 1.87×10^3 CFU.g^{-1} VRBA), and higher fungal counts in filters (8×10^2 CFU.m^{-3} MEA and 1.39×10^3 CFU.m^{-3} DG18). The lower cell viability observed with settled dust might be partially explained by their relatively high bacterial contamination or the prevalence of specific fungal species, besides other contaminants, such as mycotoxins, particles, or chemicals (not assessed in this study). Some phenomena well described are the cellular toxicity of toxigenic *Fusarium* sp. and its mycotoxins fumonisins (53), and *Aspergillus* section *Nidulantes* (series *Versicolores*) due to the production of sterigmatocystin with renal and hepatic toxicity (54). Not only these two fungal genus/species are potentially toxigenic and related to cytotoxicity *in vitro*, they were also found in filters with reduced susceptibility to posaconazole in this study. These findings also reinforce the need of surveillance of antifungal resistance in the environment for fungal priority species, as a contribution to proper antifungal stewardship from the environment to the bench.

5 Conclusion

This study allowed to conclude once again that working in manual and automated waste sorting plants imply high exposure to microbial agents. The approach followed, that comprehends several sampling methods and assays employed, is increasingly applied and industrial hygienists/exposure assessors should rely on this new trend to achieve a precise assessment of microbial risk.

It was possible to conclude that the fact of being automated does not result in a reduction in workers exposure to fungal pathogens associated with a high risk of mortality or morbidity. Moreover, the seasonal influence on viable microbial contamination observed claims attention for the potential impact of climate change in the occupational environment contamination and workers exposure pattern and, consequently, in the resulting health effects. Some findings should be highlighted: (a) one automated industry surpassed the guidelines for fungi (b) the presence of indicators of harmful fungal contamination (*Aspergillus* section *Fumigati*); (c) the identification of *Aspergillus* sections with toxigenic potential; (d) microbial contamination in both controls and exposed workers' hands potentiating the exposure by hand to face/mouth contact; (e) the observed reduced azole susceptibility of pathogens of critical priority (Mucorales and *Fusarium* sp.).

In vitro tools are important tools to estimate the health effects related to the overall contamination present in the workplace environment. However, more efforts in science and engineering need to be developed to design and implement risk management measures more effective in controlling workers exposure in this occupational setting. This is of particular relevance due to the boost expected and already happening in the number of waste management plants across the European Union promoted by the needed circular economy goals.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Regional Committees for Medical research Ethics South East Norway, REK South East (ref. no. 34312). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Ethical approval was not required for the studies on animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

CV: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. EE: Formal analysis, Methodology, Validation, Writing – original draft. BG: Formal analysis, Writing – original draft. MD: Formal analysis, Writing – original draft. RC: Formal analysis, Writing – original draft. PP: Formal analysis, Writing – original draft. EC: Formal analysis, Writing – original draft. MT: Formal analysis, Funding acquisition, Validation, Writing – original draft. LC: Formal analysis, Writing – original draft. SV: Methodology, Writing – review & editing. PG: Funding acquisition, Writing – review & editing. AA: Writing – review & editing. AS: Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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EDITED BY
Susana Viegas,
New University of Lisbon, Portugal

REVIEWED BY
Ana I Proykova,
Sofia University, Bulgaria
Jinglin Jiang,
Purdue University, United States
Luca Cappellin,
University of Padua, Italy

*CORRESPONDENCE
Srdjan Sabic
✉ srdjan.sabic@usys.ethz.ch
Claudia Marcolli
✉ claudia.marcolli@env.ethz.ch

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Exposure assessment during paint spraying and drying using PTR-ToF-MS

Srdjan Sabic[1]*, David Bell[2], Bojan Gasic[3], Kaspar Schmid[3], Thomas Peter[1] and Claudia Marcolli[1]*

[1]Institute for Atmospheric and Climate Science, ETH Zurich, Zurich, Switzerland, [2]Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, Villigen, Switzerland, [3]Swiss State Secretariat for Economic Affairs (SECO), Chemicals and Occupational Health, Bern, Switzerland

Spraying is a common way to distribute occupational products, but it puts worker's health at risk by exposing them to potentially harmful particles and gases. The objective of this study is to use time-resolved measurements to gain an understanding of spray applications at the process level and to compare them to predictions of exposure models. We used proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS) at 1-s time resolution to monitor the gas phase concentration of the solvents acetone, ethanol, butyl acetate, xylene and 1-methoxy-2-propy acetate during outdoor spraying and indoor drying of metal plate under various conditions of outdoor air supply. We found that during spraying, gas-phase exposure was dominated by the more volatile solvents acetone and ethanol, which exhibited strong concentration variations due to the outdoor winds. During drying, exposure strongly depended on the strength of ventilation. Under conditions with high supply of outdoor air, our measurements show a near-exponential decay of the solvent concentrations during drying. Conversely, under conditions without outdoor air supply, the drying process required hours, during which the less volatile solvents passed through a concentration maximum in the gas phase, so that the exposure during drying exceeded the exposure during spraying. The concentrations measured during spraying were then compared for each of the substances individually with the predictions of the exposure models ECETOC TRA, Stoffenmanager, and ART using TREXMO. For these conditions, ECETOC TRA and Stoffenmanager predicted exposures in the measured concentration range, albeit not conservative for all solvents and each application. In contrast, ART largely overestimated the exposure for the more volatile solvents acetone and ethanol and slightly underestimated exposure to 1M2PA for one spraying. ECETOC TRA and ART do not have options to predict exposure during drying. Stoffenmanager has the option to predict drying together with spraying, but not to predict the drying phase independently. Our study demonstrates the importance of considering both the spray cloud and solvent evaporation during the drying process. To improve workplace safety, there is a critical need for enhanced exposure models and comprehensive datasets for calibration and validation covering a broader range of exposure situations.

KEYWORDS

spraying applications, workplace exposure, proton transfer reaction time-of-flight mass spectrometry, volatile organic compounds, exposure models

1 Introduction

Spraying is a widespread application to disperse consumer and occupational products uniformly in air or on surfaces. Typical occupational uses include spraying of lacquers or paints, pesticides, wood preservatives, detergents, or disinfectants (1). Health hazards may arise from dermal exposure or inhalation of particles and gases during spraying. To ensure uniform distribution by spraying, the products are dissolved or suspended in a solvent or a solvent mixture. During application, the solvents evaporate from the sprayed surfaces, resulting in additional exposure to the vapors if workers remain in the area during the drying phase. Therefore, in spray applications, the primary exposure to the spray cloud is followed by a secondary exposure to the vapors emitted by droplets or by treated surfaces. Solvent evaporation from surfaces is also part of many wiping, brushing, rolling, or mopping applications as required in painting, lacquering, polishing, or cleaning of surfaces.

The level of exposure reached during drying of sprayed surfaces depends on factors related to the product's composition and on workplace conditions. Product-related properties are the vapor pressure of the solvents, their concentration in the product, and their miscibility with the other mixture components. The most relevant workplace properties are room size, ventilation or air exchange rate, position of the workers with respect to the emission source, and the protection measures taken, for instance with respect to duration of the occupational task.

Under the European Chemicals Act Registration, Evaluation, Authorization and restriction of Chemicals (REACH), companies are obliged to register all substances they intend to sell on the European market (2–4). Since the inception of REACH in 2007, the European Chemical Agency (ECHA) has provided safety data for a wide array of individual substances, most of which are freely accessible. In Switzerland, the safety data sheets provided to the customers together with the products include maximum allowable concentrations (MAK—"Maximale Arbeitsplatzkonzentration") for short term (15 min) and day shift (8 h) exposures (see www.suva.ch). Another parameter is Derived No-Effect Level (DNEL) that constitutes an essential toxicological exposure threshold necessitated for the assessment of chemicals seeking market entry within both the Swiss and EU regulatory frameworks, and both parameters (MAK and DNEL) are covered under the umbrella term Occupation Exposure Limits ("OEL").

To estimate whether workplace exposures exceed DNEL values, ECHA recommends the use of exposure models in a tiered approach (3, 5). Tier 1 models should provide a conservative exposure estimate requiring only a few input parameters. The most widely used Tier 1 model in Europe is European Centre for Ecotoxicology and Toxicology of Chemicals Targer Risk Assessment (ECETOC TRA) (6, 7). The higher tier models Stoffenmanager [Tier 1.5; (8, 9)] and Advanced REACH Tool (Tier 2;ART) (10) are recommended when safe use of the substance cannot be demonstrated based on the initial Tier 1 assessment (3). Yet, intercomparison of these models in different exposure situations revealed significantly different exposure estimates, which would entail disparate safety measures (4). Especially Tier 1 models did not always prove to be the most conservative, an outcome that questions the tiered workflow and rather suggests the use of multiple models to avoid exposure scenarios where safety measures are not sufficient to adequately control the risks. Therefore, to facilitate and unify the simultaneous use of different exposure models, the Translation of Exposure Models (TREXMO) tool has been developed, which includes among others ECETOC TRA, Stoffenmanager, and ART (11–13).

The different exposure models have been summarized and compared in different validation studies [e.g. (4, 14–17), which have revealed systematic under- or overprediction of exposure levels for specific models depending on exposure situations. There is consensus that further validation with more comprehensive datasets covering a broader range of exposure situations is required. Specifically, spraying applications are poorly represented. In a recent review, Hahn et al. (1) identified the need to extend mechanistic model approaches to cover combined exposure to the spray cloud and to solvent evaporation during the drying process. Yet, exposure measurements suited to improve exposure models are scarce.

Input data for model development (e.g., 8) and validation are mostly task- or shift-based exposures at workplaces [e.g., (14, 18, 19). For volatile substances, sorbent-based air sampling is used followed by isolation and identification by gas chromatography coupled with mass spectrometry (GC-MS) (20, 21). This method provides integrated exposure over the entire sampling period. Therefore, no mechanistic understanding of exposure arising from spraying and drying can be derived from such data. Time-resolved measurements are required to gain an understanding at the process level.

A method for online monitoring of volatile organic compounds (VOC) in real-time is proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS) (22, 23). This method has become popular in different research fields, e.g., in atmospheric sciences for indoor and outdoor air-quality monitoring and emission studies (24–26), in food and flavor sciences (27, 28), and in medical sciences for real-time breath analysis (29, 30). It has also been successfully applied to workplace exposure for α-diketones in coffee roasteries and breweries (31) and for VOC measurements related to building disinfection during COVID-19 (32).

Under ideal conditions, PTR-ToF-MS uses proton-transfer reaction with H_3O^+ for soft ionization to minimize molecule fragmentation, such that the molecular ion at m/z = MW (molecular weight) + 1 can be used as molecular identifier for VOCs. Due to the high mass resolution of the time-of-flight analyzer, peaks of the same mass but with different elemental composition can be discriminated (26). As PTR-ToF-MS enables continuous monitoring of VOCs at a time resolution of 1 Hz, the evolution of mass peaks in mass spectra can be assigned to specific activities. Nevertheless, because mass peaks are not unique for a specific compound, reliable identification of substances requires additional compositional information e.g., from the safety data sheet of the product. Moreover, calibration of each compound is required for quantitative evaluation of the mass spectra when the proton transfer reaction rate is not known.

In this study, we applied PTR-ToF-MS to investigate workplace exposure to a spray paint/lacquer containing five solvents in real-time. We sprayed a black paint onto a metal plate to monitor the spray cloud and the subsequent evaporation from the plate. To

simulate near-field conditions, we placed the inlet of the PTR-ToF-MS at a distance to the metal plate that corresponds to the breathing zone of a worker (< 1 m). We monitored the concentration of all five solvents in the spray, namely acetone, ethanol, butyl acetate, xylene, and 1-methoxy-2-propyl acetate (1M2PA) and compared the measured exposures with the values predicted by the exposure models ECETOC TRA (v3), Stoffenmanager (v4.0), and ART (v1.5).

2 Materials and method

2.1 Spray paint experiments

The paint used for our experiments was "Lackspray schwarz matt RAL 9005" (Albert Berner Deutschland, GmbH). The composition of the paint in terms of weight percentage according to the safety data sheet (SDS) version 07.03.2017/0013 is summarized in Table 1, including the calculated mole fractions. The listed mole fractions exclude the propellants (butane, propane, and dimethyl ether), so that the solvents ethanol, acetone, xylene, butyl acetate (BA), and 1-methoxy-2-propyl acetate add up to the entire composition. Two sets of conversions were done, one considering the lower limit (mole fraction min) and one with the upper limit (mole fraction max) of the composition range to cover the uncertainty in composition.

A metal sheet (64 cm x 64 cm) was sprayed with the spray can for 1–2 min until the surface was evenly covered using the recommended pulse spraying method, which involved dispensing short bursts of paint (see Figure 1A). The weight of the spray can was measured before and after each spraying to derive the amount of sprayed paint. Spraying was conducted outdoors, and the painted metal plate was subsequently moved indoors. During both the spraying and drying process, the PTR-ToF-MS (PTR-ToF-MS-8000, Ionicon Analytik, Austria) inlet was positioned at 30 cm (± 5 cm) from the plate to align with the workplace terminology's definition of a breathing zone [Comité Européen de Normalisation (CEN) (1998) EN1540 Workplace Atmospheres – Terminology] (see Figures 1A, C for illustration). Figure 1B, shows an image of the experimental setup employed for the spraying application. We conducted three independent spraying experiments, each with different strengths of outdoor air supply. The sprayed mass was 90 g for the first, 66 g for the second, and 85 g for the third spraying (as demanded by establishing a uniform layer of paint by spraying under outdoor conditions). The drying took place in a container with a volume of 26 m^3 (a description of the container is provided in Supplementary material), which was kept at a constant temperature of 25°C using three air conditioning units (model AK 7540, Suter Technik AG, Switzerland). Note that the installed air conditioning just regulated indoor temperature and led to internal ventilation but did not provide exchange with outdoor air. The first drying experiment was with door fully open (90 cm in width and 200 cm in height), resulting in significant exchange with outdoor air. The second drying experiment had a partially open door (with a slit of 4 cm) to limit the exchange of air. Finally, the third drying experiment was with closed door, ensuring negligible exchange with outdoor air. During the drying nobody was inside the container.

2.2 Real-Time VOC gas composition measurements with PTR-ToF-MS

We used a high-resolution PTR-ToF-MS to measure gaseous emissions during spraying with the spray paint and during drying of the sprayed surface. The operational details of the instrument have been previously published (22, 23, 33). The ion drift tube was set to standard conditions with a total voltage ranging from 550 to 600 V and a pressure of 2.4 mbar. To maintain a consistent ratio of electric field (E) to number density (N) of buffer gas molecules in the drift tube (E/N), we kept values within the range of 119–120 Td during spraying measurements and 111–112 Td during calibration measurements. These variations in E/N were not on purpose, yet the differences are relatively small (6 %) and within the overall uncertainty of the experiment. The Townsend, symbol Td, is a physical unit of E/N. This ratio is important, because it determines the mean energy of electrons, and hence the degree of ionization. It means that increasing the electric field (units V/m) by some factor has the same consequences as lowering the gas density (units cm^{-3}) by the same factor. The Townsend is defined as 1 Td = 10^{-17} V cm^2. These settings ensure that the ion drift is predominantly influenced by the H_3O^+ cluster rather than higher mass water clusters.

The proton transfer reaction can be written as:

$$H_3O^+ + R \rightarrow RH^+ + H_2O, \quad (1)$$

Here, R denotes the VOC being measured, while RH^+ represents the protonated molecule detected by the TOF-MS (Equation 1).

2.3 Calibration measurements with saturated airflows of the pure solvents

To quantify the gas-phase emissions of the spray can paint during spraying and drying, we performed reference measurements with airflows saturated with the five pure solvents obtained from Sigma-Aldrich. We have purchased acetone (ACS reagent with purity of ≥ 99.5 %), ethanol (for molecular biology), xylene (xylenes, isomers plus ethylbenzene, reagent grade), butyl acetate (purity of 99.5%), and 1-methoxy-2 propyl acetate (purity of ≥ 99.5 %). The measurement setup is outlined in Figure 2, setup A. We equilibrated each solvent in a 0.5 L Schott bottle for up to 30 min with closed inlet and outlet lines. Once equilibrium between the gas and the condensed phase was established, air with a flow rate of 0.03–0.05 L/min was passed through the bottle. Due to the high vapor pressures of the pure solvents, we introduced two dilution stages to keep the solvent signals within the linear PTR-ToF-MS measurement range, and two mixing regions (widened part of the metal tubing) to ensure better mixing. Dilution factors (DF) were calculated as given in Equation (2).

$$DF_X = \frac{f_X^{sat} + f_1^{zero}}{f_X^{sat}} \times \frac{f_X^{sat} + f_1^{zero} + f_2^{zero} - f_1^{exh}}{f_X^{sat} + f_1^{zero} - f_1^{exh}}, \quad (2)$$

where f_X^{sat} is the air flow saturated with species X from the bottle, $f_1^{zero} + f_2^{zero}$ are the flows of zero air entering the main flow line

TABLE 1 Composition of the spray can paint in wt% and its conversion to mole fraction neglecting propellants and substances present only in traces (<1 %).

Composition	Weight percentage (wt %) (Min)–(Max)	Mole fraction min (Min)	Mole fraction max (Max)	Molecular weight
Acetone	20–40	0.87	0.68	58.08
Ethanol	1–< 5	0.05	0.11	46.07
Butyl acetate	1–< 10	0.03	0.08	116.16
Xylene	1–< 10	0.02	0.09	106.16
1-Mehoxy-2-propyl acetate (1M2PA)	1–< 5	0.03	0.04	132.16
Butyl glycollate	0.01–< 1	-	-	132.16
Oleic acid, compound with (Z)-N-octadec-9-enylpropane-1,3-diamine (2:1)	0.001–< 0.1	-	-	-
Butane	10–20	-	-	58.12
Propane	5–15	-	-	44.09
Dimethyl ether	10–< 20	-	-	46.07

FIGURE 1
Images illustrating the spraying experiment: **(A)** Outdoor spraying of the product; **(B)** Painted metal plate (outdoors); **(C)** Drying of the paint (indoors).

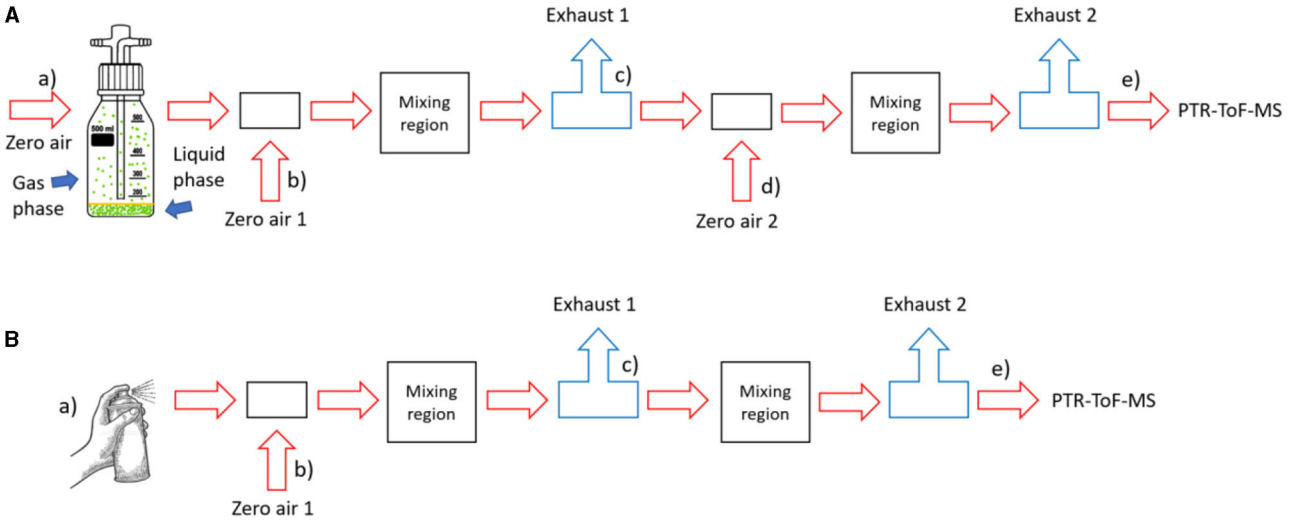

FIGURE 2
Measurement setup: **(A)** for reference measurements with pure solvents; **(B)** for spraying experiments. More details are given in SM. The lowercase alphabet letters, enclosed in brackets, denote the position of the mass flow controllers, with the sole exception being the notation "a)" within the B section of the illustration: in this position there was no mass flow controller.

TABLE 2 Ion peaks used for evaluation, dilution factors (*DF*) used for calibration and spray paint measurements, calibration factors (*CF*) derived for the selected ion peaks, and saturation vapor pressures (ECHA webpage: https://echa.europa.eu/home).

Substance	Ion peaks m/z	Dilution factor (*DF*)	Calibration factor (*CF*)	Vapor pressures at 25°C [Pa]
Acetone	60.05	30,401	1.02	32,130
Ethanol	45.03	30,401	5.9	16,926
Butyl acetate	117.05	7,525	1,898	1,200
Xylene	107.08	30,401	4.0	1,048
1M2PA	133.08	602	884	517
Spray can paint	-	168	-	

at positions 1 and 2, respectively, and f_1^{exh} is the flow through the exhaust at position 1. Input parameters are presented in Supplementary Table S1 and the resulting DF_X are listed in Table 2.

Additionally, for each solvent, we have measured a lower concentration in a separate setup (injecting a defined amount into a chamber). This process confirmed the obtained calibration factor and helped minimize uncertainties (description of the chamber experiments and the calibration factors derived from them are presented in the Supplementary material). For the spraying experiments, we use the calibration factors from the bottle experiments as they give a lower limit of the concentrations. Moreover, we have corrected for the transmission efficiency (the corresponding curve and equation is presented in the Supplementary Figure S1).

2.4 Application to the measurements of complex mixtures

Because of the complexity of the mass spectra of the spray paint with overlapping ion signals from the five solvents, we rely on just one ion peak for each substance in our evaluation. For xylene and 1M2PA we chose the peaks of the parent ions, which are $C_8H_{11}^+$ (m/z = 107.08) and $C_6H_{13}O_3^+$ (m/z = 133.08), respectively. As the high vapor pressure of acetone leads to a very strong signal of the parent ion peak, which was outside the linear range of the instrument despite dilution, the isotope peak of the parent ion at m/z = 60.05 (^{13}C-$C_2H_7O^+$) was used to ensure linearity of the PTR-MS signal because we observed that the signal of the acetone parent ion at m/z = 59 was above the linearity range of the instrument recommended by the manufacturer as it exceeded 3 ppm even after dilution, which is just above linearity range of the instrument. During spraying, we observed a decrease of the H_3O^+ intensity by 5–10 % associated with the peaks that exceeded the linearity range of the instrument even without saturating the detector. As the measured parent ion peak intensity $I_{117.05}$ of butyl acetate at m/z = 117.05 also contained shares of a major fragment of 1M2PA, we subtracted the contribution of the 1M2PA fragment, equaling 0.672 of the measured intensity of the parent 1M2PA ion peak ($0.672 \times I_{133.08}$). This yields a net butyl acetate signal with intensity of $I_{117.05} - 0.672 \times I_{133.08}$.

Ethanol was the most difficult substance to quantify during spraying as its parent ion peak and all its fragments overlap with the propellant dimethyl ether of the spray can paint. We chose the mass peak at m/z = 45.03 ($C_2H_5O^+$), which proved to be the highest signal in the calibration measurements with pure ethanol and at the same time specific for ethanol in the solvent mixture. However, we needed to exercise caution due to the interference caused by dimethyl ether. This interference could potentially lead to an overestimation of the concentration measured during spraying, owing to the presence of dimethyl ether and fragments from other components in the spray paint. Therefore, we chose to represent m/z = 45.03 as an upper limit for ethanol. The evaluation of the ethanol concentration during drying, on the other hand, should not have been affected by interference from dimethyl ether because we transferred the plate inside the container for spraying while the overspray cloud and propellants remained outside. The resulting dilution factors (*DF*) and the calibration factors (*CF*) obtained by comparing the partial pressures derived from the intensity of the selected peaks with compiled vapor pressures are presented in Table 2.

2.5 Exposure assessment with occupational exposure models

We compared our measurements with predictions from Tier 1–2 exposure models available in TREXMO 2.0, specifically ART (version 1.5), Stoffenmanager (version 4.0), and ECETOC TRA (version 3). We used the option to run them all individually within TREXMO (without translation tool), thus avoiding any ambiguity through automatically translating between models. The relevant information for the source term, activity term, and control term are listed in Table 3. Note that in ECETOC TRA the concentrations cannot be inserted exactly but are just selected as >25 %, 5–25 %, 1–5 %, or <1 %.

AIOMFAC was used to determine activity coefficients for exposure assessment with ART. We considered the lower limit (mole fraction min) and upper limit (mole fraction max) of the composition range as input for TREXMO and to calculate activity coefficients with AIOMFAC.

To estimate the combined exposure to the solvent mixture, we calculate the sum index (*SI*) from the individual MAK values using the following formula:

$$SI = \frac{C1}{MAK1} + \frac{C2}{MAK2} + \frac{C3}{MAK3} + \frac{C4}{MAK4} + \frac{C5}{MAK5}, \quad (3)$$

TABLE 3 Exposure model parameters set for the exposure assessment of the spraying application.

Source term (variables)	Input for models	Model
State of the substance	Liquid	All models
Vapor pressure	Substance specific (see Table 2)	All models
Concentration present in the product	Substance specific	All models
Mole fraction	Substance specific (see Table 1)	ART
Activity coefficient	Substance specific [a]	ART
Molecular weight	Substance specific (see Table 1)	ECETOC TRA, ART
Distance from the source	Less than 1 m (near-field zone)	All models
Workshop cleaning and maintenance/Surface contamination	No daily cleaning of workshop	All models
Activity term	**Input for models**	**Model**
Number of employees carrying out the same task simultaneously	1	Stoffenmanager
Task followed by evaporation	Yes (far-field exposure possible)	Stoffenmanager
Type of handling/Select process category (PROC)/Activity class	Handling of liquids using low pressure low speed and on medium sized surfaces/PROC 11: non-industrial spraying/Spray application of liquids	All models
Task duration	480 min/> 4 h	All models
Type of setting	Professional	ECETOC TRA
Activity sub-class	Surface spraying of liquids	ART
Situation which best represents activity	Moderate application rate (0.3–3 L/min)	ART
Direction of spraying	Only horizontal and downward spraying	ART
Spray technique	Spraying with no or low compressed air use	ART
Control term	**Input for models**	**Model**
Select the volume of working room/Ventilation/Exposure site	Outdoors	All models
Select available control measures/localized controls	No control measures at source	ART and Stoffenmanager
Select personal protective equipment	No protection	Stoffenmanager and ECETOC TRA
Distance of exposure source from the building	Close to building	ART

[a]To calculate activity coefficients, we used AIOMFAC, an online tool readily available online (www.aiomfac.caltech.edu).

where *C1–C5* are the concentrations of the five solvents and *MAK1–MAK5* their MAK values (Equation 3).

3 Results

Figures 3–5 show the time-resolved concentrations of the five solvents in the spray paint evaluated based on the ion peaks listed in Table 2. The measurements are divided into the spraying phase (left columns) performed outdoors in front of the container, followed by the drying phase (right columns), which took place within the container. Note that the spraying is shown with the instrument time resolution of 1 second, while for the drying, the data was smoothed by taking 10 seconds averages. The green sections after the spraying period mark the transfer of the plate into the container and the re-installation of the inlet in front of the plate at a distance of 30 cm. The drying period shown on the right-hand panels starts after positioning the inlet. Table 4 lists the mean gas phase concentrations of each solvent for the spraying period and the highest concentrations reached during evaporation (blue sections), the maximum concentration reached by a spike and the concentration before the measurement was stopped.

3.1 Time-resolved concentrations measured during spray paint application

As spray painting was always performed in front of the container in the same manner, we are able to compare the three results to evaluate the reproducibility of the spraying process.

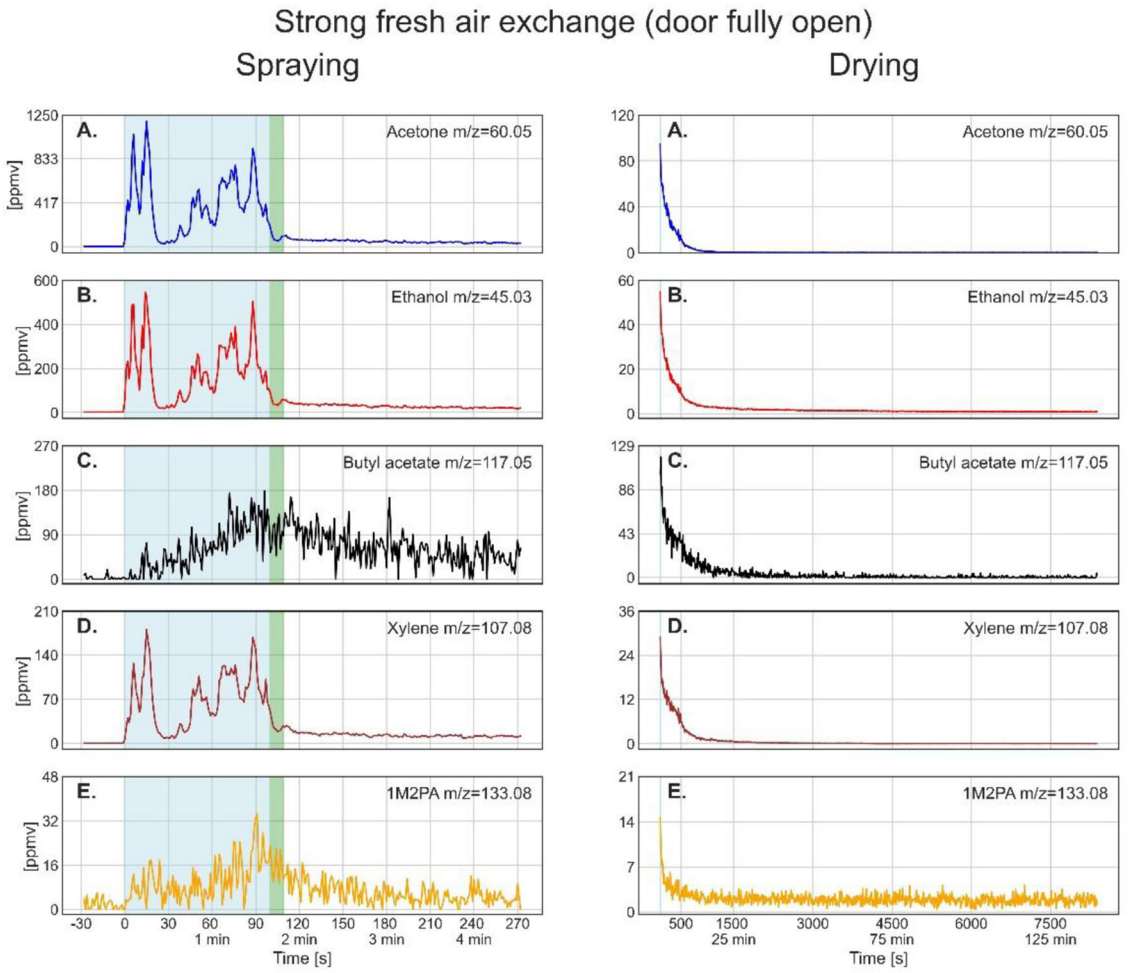

FIGURE 3
Time series of the monitored solvents in the order of their vapor pressures (from high to low) for the spray paint application with open door during drying. In the left column, measurements during the spraying phase are shown. The drying phase, in the right column, started at 108 s. The light blue shaded regions in the left column mark the effective spraying period. The green shaded area represents the transfer of the metal plate and PTR-ToF-MS inlet from outdoors into the walk-in container. The thin light blue segments in the right column denote the maximum of solvent concentration during drying, for which the average concentrations are given in Table 4.

For acetone, ethanol, and xylene, the left columns of Figures 3–5 show strongly varying concentrations within one application and between the three applications with almost the same pattern of peaks for all three solvents. Butyl acetate and 1M2PA, on the other hand, exhibit a much weaker and noisy gas-phase signal during spraying. Assuming that the gas phase concentration during spraying is dominated by the evaporation of overspray droplets with only minor contributions from evaporation from the plate, the strong variations in gas-phase concentrations of acetone, ethanol, and xylene can be explained by the applied line-by-line pulsed spraying method together with air movements and wind, which blew the overspray away from the inlet in an irregular pattern. This pattern is much weaker or even absent for butyl acetate and 1M2PA, which shows that these solvents hardly evaporated during spraying and confirms that PTR-ToF-MS measured exclusively the gas phase with no droplets entering the inlet. The large variability in the measured concentrations of acetone, ethanol, and xylene explains the large standard deviation in Table 4 for these solvents during spraying. In comparison, the standard deviations of butyl acetate and 1M2PA are smaller due to the noisiness of their weak signal, which is owed to the lower sensitivity of PTR-ToF-MS to esters. Considering all this, the mean concentrations of the solvents during spraying show reasonable agreement with each other. Nevertheless, the differences in average exposure vary considerably. The concentration of acetone, ethanol, and xylene vary all by a factor of about 1.5, butyl acetate by a factor of 3, and 1M2PA even by 6.7, when we compare the three sprayings.

3.2 Drying dynamics and ventilation conditions

The drying process varied significantly depending on the ventilation conditions. For the open-door experiment (Figure 3), the gas phase concentrations show a near-exponential decay for

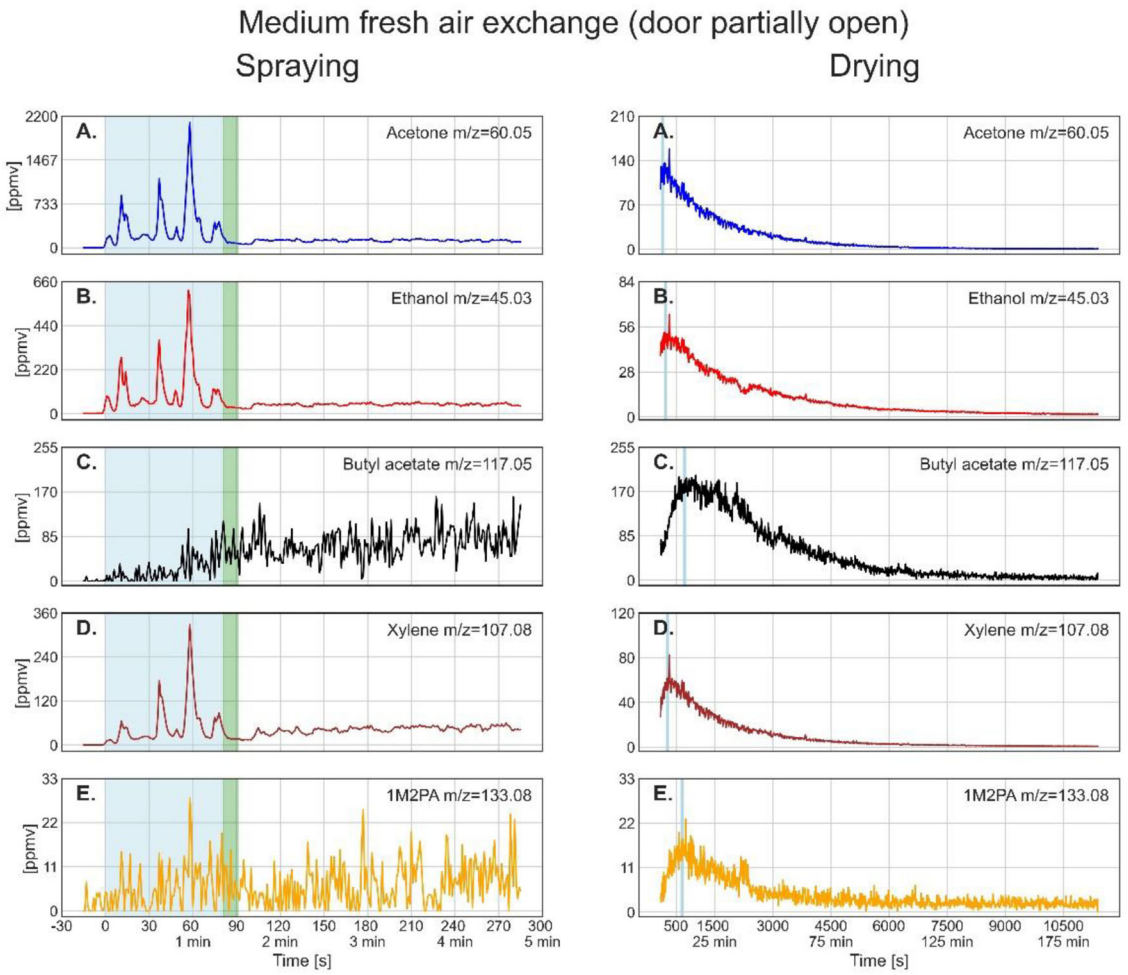

FIGURE 4
Time series of the monitored solvents in the order of their vapor pressures (from high to low) for the spray paint application with partially open (4 cm) door. In the left column, measurements during the spraying phase are shown. The drying phase, in the right column, started at 98 s. The light blue shaded regions in the left column mark the effective spraying period. The green shaded area represents the transfer of the metal plate and PTR-ToF-MS inlet from outdoors into the walk-in container. The thin light blue segments in the right column denote the maximum of solvent concentration during drying, for which the average concentrations are given in Table 4.

all solvents and reach constant values within the measurement uncertainties after 1,000 s (around 16 min). Comparing the end concentrations with the average outdoor signal before the measurement started (acetone: 0.2 ± 0.2 ppmv; ethanol: 1.2 ± 0.4 ppmv; butyl acetate: 0.6 ± 6.3 ppmv; xylene: 0.04 ± 0.02 ppmv; 1M2PA: 1.9 ± 2 ppmv) shows that they correspond to background values. The rather high background signal and uncertainties can be explained by the dilution step that was applied to measure the high concentrations during spraying, because converting the values back to the real concentrations increased the noise level. Comparison of spraying and drying signal intensities shows that the main exposure to acetone, ethanol, and xylene occurred during spraying. For butyl acetate and 1M2PA, the maximum measured signals during the drying phase were above the average signal during spraying, as these substances build up only slowly during spraying.

For partially-open door during drying (4 cm) (Figure 4), acetone shows again a near-exponential decay in the gas phase concentration, while the concentrations of ethanol, butyl acetate, xylene, and 1M2PA first exhibit an increase followed by a near-exponential decay, which is clearly slower than the one for the open-door situation. Thus, the maximum concentration during drying was reached later and persisted longer. For butyl acetate and 1M2PA, it took around 10 min to reach the maximum concentration, which by then clearly topped the concentration reached during spraying (see Table 4, maximum of drying). Butyl acetate levels remained above the concentration reached during spraying for over 30 min. The maximum in gas-phase concentrations observed for butyl acetate, xylene, and 1M2PA can be explained by their relative increase in terms of mole fraction within the paint layer due to evaporation of the more volatile solvents acetone and ethanol, leading to an increase of partial vapor pressures. The gas phase concentrations of the solvents at the end of the measurement after about 11,300 s (about 188 min) are slightly higher than the values measured for the open-door experiment, maybe because of ongoing evaporation or slow diffusion out of the container.

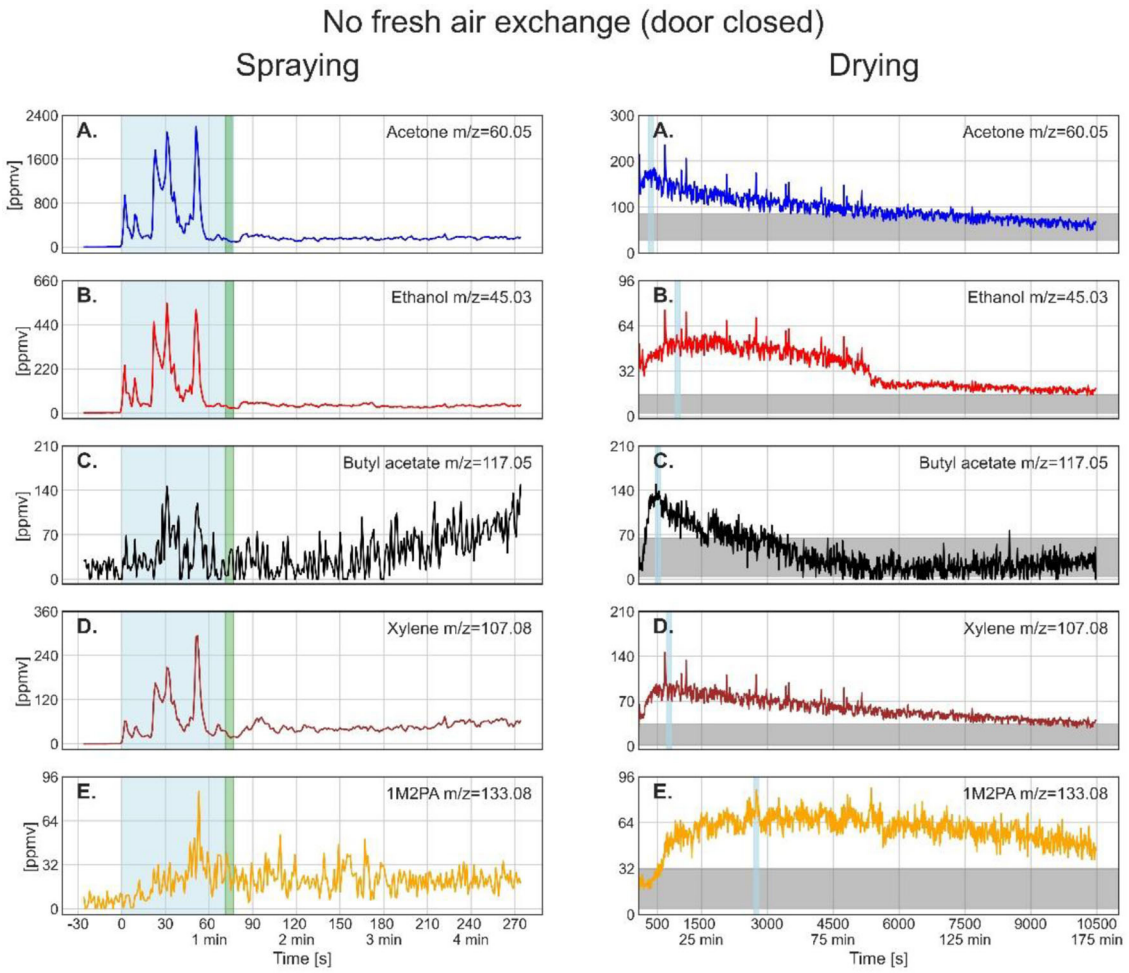

FIGURE 5
Time series of the monitored solvents in the order of their vapor pressures (from high to low) for the spray paint application with closed door during drying. In the left column, measurements during the spraying phase are shown. The drying phase, in the right column, started at 77s. The light blue shaded regions in the left column mark the effective spraying period. The green shaded area represents the transfer of the metal plate and PTR-ToF-MS inlet from outdoors into the walk-in container. The thin light blue segments in the right column denote the maximum of solvent concentration during drying for which the average concentrations are given in Table 4. The horizontal gray bar shows the solvent concentration calculated for homogeneous distribution within the container after full evaporation from the plate assuming an airtight room and no wall loss.

When the door was closed during drying, all solvents showed first an increase before their concentrations started to decrease. Therefore, an increase in partial vapor pressure of some components at the expense of the others cannot fully explain this behavior. Rather, slow gas-phase diffusion seems to be relevant, having led to a time delay between evaporation from the plate and reaching the inlet of the instrument. Gas-phase diffusion limitations are confirmed by the spikes that appeared for all solvents simultaneously in the mass spectra. We ascribe these to eddy diffusion, causing direct motion of air from the plate to the instrument inlet. These air flows therefore reflect the higher solvent concentration in the vicinity of the plate surface compared with the lower average concentration close to the inlet.

After having reached the maximum, the solvent concentrations did not show an exponential decrease, but rather a linear or irregular one. Moreover, all solvents except butyl acetate were still decreasing in concentration at the end of the measurement time after about 10,400 s (173 min). As the air conditioning system was not connected to outdoors, air was just recirculated within the room thus stimulating eddy diffusion. The horizontal gray bars in Figure 5 show the estimated level of the solvents assuming an airtight room. Their width reflects the uncertainties in the composition of the paint as disclosed in the safety data sheet, and the estimated loss of paint to overspray during outdoor spraying, which we assumed to be 40–60 % for an airless spray (34). The concentrations of acetone and butyl acetate are well within this uncertainty range in accordance with a homogeneous distribution in the container, while concentrations of ethanol and xylene are just approaching the gray bar, and 1M2PA is even above it, pointing to continuing evaporation of these solvents from the plate after the measurement was stopped.

Another observation during the closed-door drying phase was a sudden decrease in ethanol intensity after about 5500 s (around 92 min), where also the occurrence of spikes ended. We had to

TABLE 4 Mean solvent concentrations in ppmv with standard deviations averaged over the PTR-ToF-MS signal with 1 s time resolution (blue sections marked in Figures 3–5), the time in seconds when the maximum of drying was reached, and the end concentration of the measurements.

Experiment	Task (Time interval)	Acetone m/z = 60	Ethanol m/z = 45	Butyl Acetate m/z = 117	Xylene m/z = 107	1M2PA m/z = 133
Open door experiment	Spraying (100 s)	400 ± 272	189 ± 129	60 ± 48	70 ± 44	10 ± 7.4
	Maximum of drying (10 s)	85 ± 15	49 ± 7.2	121 ± 25	24 ± 3.7	12 ± 3.9
	(Start time of maximum)	(117 s)	(117 s)	(117 s)	(117 s)	(117 s)
	End of drying (120 s)	0.2 ± 0.2	0.8 ± 0.2	0 ± 4.3	0.1 ± 0.03	1.9 ± 2
Partially open-door (4 cm) experiment	Spraying (79 s)	369 ± 375	119 ± 118	20 ± 22	53 ± 60	5.8 ± 5.6
	Maximum of drying excluding spikes (60 s)	124 ± 15	48 ± 5.4	184 ± 54	49 ± 5.7	11 ± 7.8
	(Start time of maximum)	(123 s)	(206 s)	(620 s)	(266 s)	(698 s)
	Maximum of drying including spikes (7 s)	69 ± 5.5	172 ± 11	-	79 ± 4.6	-
	(Start time of the spike)	(350 s)	(350 s)	-	(350 s)	-
	End of drying (120 s)	0.7 ± 0.3	1.7 ± 0.4	3.3 ± 8	0.6 ± 0.1	2.3 ± 2.6
Closed door experiment	Spraying (71 s)	615 ± 554	178 ± 162	21 ± 15	74 ± 65	39 ± 36
	Maximum of drying excluding spikes (120 s)	170 ± 23	51 ± 4.4	132 ± 38	77 ± 9.8	75 ± 15
	(Start time of maximum)	(301 s)	(907 s)	(450 s)	(718 s)	(2,696 s)
	Maximum of drying including spikes (14 s)	233 ± 21	74 ± 5.3	-	127 ± 10	-
	(Start time of the spike)	(682 s)	(682 s)	-	(682 s)	-
	End of drying (120 s)	59 ± 10	17 ± 2.7	32 ± 46	30 ± 4.1	45 ± 17

perform the closed-door experiment twice because the first time, the spray can turned empty in the middle of spraying, requiring switching to a new one, which was not shaken before spraying. Nevertheless, we share these results in SM to show that in this experiment, all solvents showed a clear decrease in evaporation rate also after 5,500 s (around 92 min), evidencing that this feature does not seem to be accidental but might be due to an abrupt decrease of diffusion within the paint layer, potentially due to a discontinuity in the drying process, e.g., through film formation on top of the paint layer. Note that the slight increase in butyl acetate concentration after 5,500 s (around 92 min) might be an artifact because the concentration of this ester could only be evaluated after the 1M2PA concentration was subtracted from the butyl acetate parent peak, constituting a source of increased uncertainty and bias.

In a next step, we compared the measured solvent concentrations during spraying with predictions from the exposure models ECETOC TRA (v3), Stoffenmanager (v4), and ART (1.5). We took the models activity-based estimate exposures (480 min) at different percentile levels. To compare with our measurements, we selected daylong spraying (>4 h) for ECETOC TRA. The TRA exposure results represent the 75th percentile of the calculated exposure (7). For ART and Stoffenmanager, we selected 50th and 90th percentiles, the latter one being the recommended percentile under REACH for risk characterization (3). For comparison with the model predictions, we assumed spraying for a dayshift with the mean concentration measured during the 70–100 s actual spraying time. All input parameters for TREXMO are listed in Table 3 and the comparison between measurements and model predictions are shown in Table 5. Note that we converted the exposures given in mg/m^3 by the models to ppmv for easier comparison with measurements.

ECETOC TRA, which should, as a Tier 1 model, provide a conservative estimate of exposure, does not fully reach this goal for all solvents as also reported by Savic et al. (4). Specifically, the predicted exposure to ethanol is slightly underestimated for two sprayings. Nevertheless, ECETOC TRA predicts all solvents in the right concentration range. Note that for this model, the paint composition cannot be entered exactly but just in terms of >25 %, 5–25 %, 1–5 %, and <1 %.

Stoffenmanager, the Tier 1.5 model, shows a difference of around one order of magnitude between exposure estimates for the 50th percentile compared with the 90th, with the predictions at the 50th percentile being clearly too low when compared to the measured values. For the 90th percentile, Stoffenmanager predicts all solvents in the right concentration range, albeit the less volatile ones (butyl acetate, xylene, 1M2PA) too low. One obstacle for accurate predictions is the wide concentration range given in the safety data sheet for the paint composition, leading to differences in prediction of more than a factor of 3 for butyl acetate and xylene

TABLE 5 Comparison between measured mean solvent concentration levels in ppmv during spraying and exposures predicted by the models ECETOC TRA, Stoffenmanager, and ART.

Experiments/ substances	Acetone m/z = 60	Ethanol m/z = 45	Butyl Acetate m/z = 117	Xylene m/z = 107	1M2PA m/z = 133
Spraying 1	400	189	60	70	10
Spraying 2	369	119	20	53	5.8
Spraying 3	615	172	21	74	39
ECETOC TRA (v3) 75 %-ile (Min, Max)	420, 700	140, 140	70, 210	70, 210	70, 70
Stoffenmanager (v4.0) 50 %-ile (Min, Max)	39, 56	7.4, 17	0.7, 2.5	0.7, 2.5	0.4, 1.0
Stoffenmanager (v4.0) 90 %-ile (Min, Max)	352, 509	67, 157	6.5, 22	6.7, 23	3.7, 8.7
ART (v1.5) 50 %-ile (Min, Max)	4,210*, 4,210*	1,274, 2,760	10, 27	16, 60	3.7, 4.8
ART (v1.5) 90 %-ile (Min, Max)	4,210*, 4,210*	5,307*, 5,307*	72, 183	106, 391	24, 31
Swiss MAK values**	500	500	100	100	50
Derived No-Effect Level (DNEL)***- short	1,019	1,008	126	67	51
Derived No-Effect Level (DNEL)*** - long	509	504	63	18	51

(Min, Max)-values refer to composition uncertainty (see Table 1).
*Maximum concentration output from ART (35).
**Limit values in the workplace: Current MAK and BAT values (suva.ch) values of 2023.
***0013 (Regulation (EC) No. 1907/2006, Annex 2).

(Table 4, [Min, Max] values). Thus, the advantage of entering the composition exactly is counterbalanced by the imprecise composition disclosed in the safety data sheets. Note that we used in this study Stoffenmanager (v4), which version incorporated in TREXMO. We repeated these calculations with the latest version of Stoffenmanager available online (v8, gestis.stoffenmanager.com) and found that the output is the same.

Finally, the Tier 2 model ART provides the upper ceiling values for the more volatile solvents acetone and ethanol for both the 50th and 90th percentiles, while for the less volatile substances butyl acetate, xylene, and 1M2PA the predictions are low compared with the measured values for the 50th and rather higher than measurements for the 90th percentile. ART is conservative for all solvents except for 1M2PA, for which the third spraying exceeds the upper estimate.

All solvents remained below the OEL during spraying except for acetone, which exceeded the short-term OEL during Spraying 3. Xylene exceeded the short-term DNEL value during two sprayings and the long-term DNEL value during all sprayings. Yet, the measured exposures would only be realized when spraying lasted for 8 h. Yet, the sum indices (SI) of the combined exposure to all solvents, clearly exceeded the allowable concentration ($SI < 1$) for all three sprayings, reaching values of 2.7 (spraying 1), 1.8 (spraying 2), and even 3.3 (spraying 3).

4 Discussion

This study presents a novel approach to assess workplace exposure during spray applications, using PTR-ToF-MS to monitor solvent concentrations in real time (22, 23). Our results show that this technique provides a comprehensive picture of exposure dynamics, covering both the spraying and the drying. With this approach, two goals can be reached, (i) giving process level-insights in spray applications that cannot be reached if only average concentrations during an arbitrary time period are measured; and (ii) providing reliable datasets for exposure model validation and development.

4.1 Relevance of spraying and drying for exposure to vapors

This study shows that online monitoring in spray applications can provide process-level insights that cannot be obtained by offline analysis. Owing to the small air volume of the container, we performed the spraying outdoors and then moved the freshly sprayed plate and the PTR-ToF-MS inlet indoors to monitor the drying process. Like this, the drying was not influenced by the dispersing overspray cloud. This procedure allowed us on one hand to obtain the spraying in replicate for comparison between each other, and, on the other hand, to investigate the role of ventilation by varying strengths of fresh air supply.

Online monitoring of the spraying process revealed that the gas-phase exposure during spraying strongly depends on the vapor pressure of the substances with the more volatile ones strongly dominating the total exposure. Conversely, exposure during drying is strongly influenced by the ventilation conditions. If drying takes place in a small room with no or limited outdoor air-exchange, gas-phase concentrations of the less volatile solvents build up

and, as drying proceeds, start to dominate the total exposure to solvent vapors. These findings are supported by a recent study from Ding et al. (32), who measured real-time worker's exposure during Covid-19 disinfection activities (spraying, wiping, drying off). In 30, the PTR-ToF-MS inlet was connected within the breathing zone (about 10 cm from the nose) on the researcher's working suit while he moved within the room to disinfect different indoor spaces in a small bathroom by spraying a thymol- and plant-based disinfectant for a total of 10 min, followed by wiping each location dry for additional 10 min and an additional 60 min measurement period to register the decay. Like our results, this study found highly varying concentrations of the more volatile terpene components during spraying, while the concentration of the less volatile thymol peaked at the end of the wiping period. Both substances then show near-exponential decay. Like in our study with five solvents, gas-phase exposure to the less volatile substances became more relevant during drying than it was during spraying. Such detailed and time-resolved measurements offer a database to improve the understanding of spraying applications on a process level.

4.2 Implications for exposure models

Comparison of spraying measurements with predictions from exposure models showed that ECETOC TRA (v3) predicts concentrations in the measured range for all solvents, albeit not conservative for all three sprayings and all solvents (acetone, ethanol, xylene, butyl acetate, and 1M2PA). In previous studies, ECETOC TRA has been criticized for not being sufficiently conservative for industrial spraying applications (1). Landberg et al. (36) reported the underprediction of exposure by ECETOC TRA (v3) for chassis spray painting. Interestingly, the substance that was underpredicted was xylene, which was predicted in the right concentration range in our spray-painting application. In a broader study covering occupational exposure situations including spraying, Spinazzè et al. (37) found the performance of ECETOC TRA (v3.1) to be not acceptable in terms of accuracy for exposures to organic solvents and pesticides, as it led to too high as well as too low exposure estimates. However, we found neither large over- nor large underestimates in our spray application for the substances analyzed.

When Stoffenmanager, the Tier 1.5 model, was evaluated at the 90th percentile, it predicted the more volatile solvents in the measured concentration range, yet it rather underestimated exposure especially for the less volatile solvents butyl acetate, xylene, and 1M2PA. For the 50th percentile, predictions were clearly too low. Previously, Landberg et al. (36) also tested Stoffenmanager for a chassis spray painting application and found xylene to be underpredicted, yet in the right concentration range when the 90th percentile was used. For the 50th percentile, the concentration was clearly underpredicted (19), which is in agreement with our findings.

ART, the Tier 2 model, overpredicted the more volatile solvents acetone and ethanol considerably while the less volatile ones were in the right concentration range. Overall, ART was the most conservative model for all solvents but 1M2PA, for which ECETOC TRA was more conservative. This outcome agrees with Landberg et al. (36), who concluded that ART was the most conservative model when compared with ECETOC TRA and Stoffenmanager. This is in contrast to the expectation that ART should be the least generic and most accurate model, as was also found by Savic et al. (4). Instead, ECETOC TRA, which is supposed to be conservative, was the least.

We did not compare the drying phase with model predictions because drying is not covered by ECETOC TRA and ART. Only Stoffenmanager offers the option to model drying, but only in conjunction with spraying and under the same ventilation conditions. Thus, our experimental setting of outdoor spraying and indoor drying is not covered. However, this study shows that a comprehensive description of spraying, including drying, is urgently needed in exposure models to capture high exposures to less volatile solvents during the drying phase, especially when ventilation conditions are not ideal.

5 Conclusion

In this study we used a PTR-ToF-MS system to monitor the gas-phase concentration during spraying and drying of a spray can paint. We established a dataset that consists of the time-resolved gas-phase concentration of acetone, ethanol, xylene, butyl acetate, and 1M2PA during spraying performed three times outdoors, always in the same manner, and the evolution of the concentration of the same solvents while the paint was drying indoors in a container. For the drying phase, we varied the ventilation conditions each time: spraying 1 was performed with open door, spraying 2 with partially open door, and spraying 3 with closed door.

Owing to the high time resolution of PTR-ToF-MS, the measurements revealed strongly fluctuating overspray concentrations during spraying, leading to an average exposure that varied by a factor of 1.5 for acetone, ethanol, and xylene, and even by a factor of more than 6 for 1M2PA when comparing the three sprayings. For this reason, measuring and modeling must first be compared neutrally: measurements may be very accurate at a particular location and time, yet, they might not be very representative. Conversely, modeling at the given place and time may not by perfect, but may be more representative.

For the drying phase, we observed a strong influence of the ventilation conditions: for acetone and ethanol, the average gas phase concentration during spraying was higher than the maximum concentration during drying for all ventilation conditions; for xylene, butyl acetate, and 1M2PA, the maximum concentration during drying was equal to the average concentration during spraying with the door partially open, and for butyl acetate and 1M2PA, the concentration during drying clearly exceeded the exposure during spraying with the door closed. This highlights the relevance of drying for estimating total exposure to spray paints when ventilation conditions are not ideal. Hence, we recommend that drying should be integrated into the model predictions.

Comparison of the spraying phase with exposure model predictions showed that ECETOC TRA (v3) and Stoffenmanager (v4) predicted exposure in the measured concentration range, albeit not conservative for all solvents and all sprayings. On

the contrary, ART (v1.5) strongly overestimated the exposure for the more volatile solvents acetone and ethanol and slightly underestimated exposure to 1M2PA for one spraying. Again, the large variability of overspray vapor concentrations due to random air flows during the outdoor spraying highlights the difficulty in acquiring a representative database as input for exposure models when measurement conditions are very random (e.g. due to wind or variable air circulation).

As an important result of this work, it became clear that more attention should be paid to the drying phase, especially when the less volatile solvents are the more hazardous ones and when ventilation conditions are not ideal. It should be noted that evaporation of less volatile solvents during product drying is not limited to spraying applications but also occurs during wiping, brushing, rolling, or mopping. Some of these activities may well be performed in small spaces with limited ventilation. Therefore, the inclusion of the drying phase in exposure model predictions is strongly warranted. Also here, online-monitoring techniques such as PTR-ToF-MS should be considered as the methods of choice for model development and improvement.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SS: Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing—original draft, Writing—review & editing. DB: Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing—review & editing. BG: Conceptualization, Funding acquisition, Writing—review & editing. KS: Conceptualization, Funding acquisition, Writing—review & editing. TP: Funding acquisition, Supervision, Writing—review & editing. CM: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing—original draft, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1327187/full#supplementary-material

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EDITED BY
Susana Viegas,
New University of Lisbon, Portugal

REVIEWED BY
Ilse Bente Ottenbros,
Netherlands Organisation for Applied Scientific Research (TNO), Netherlands
Seeta Dewali,
Kumaun University, India
Craig Sams,
Health and Safety Executive, United Kingdom

*CORRESPONDENCE
Heiko U. Käfferlein
✉ heiko.kaefferlein@dguv.de
Sonja A. Wrobel
✉ sonja.wrobel@dguv.de

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Human biomonitoring of neonicotinoid exposures: case studies after the use of a spray-agent to ornamental plants and a topical medication to pets

Sonja A. Wrobel[1]*, Stephan Koslitz[1], Daniel Bury[1], Heiko Hayen[2], Holger M. Koch[1], Thomas Brüning[1] and Heiko U. Käfferlein[1]*

[1]Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Ruhr University Bochum (IPA), Bochum, Germany, [2]Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany

Acetamiprid (ACE) and imidacloprid (IMI) are insecticides of global importance and are used as spray and watering agents for ornamental plants to control biting and sucking insects or as topical medications on pets to remove and control fleas. Human biomonitoring data on ACE and IMI exposures when applying these products are limited. We investigated exposures to ACE and IMI in male volunteers after the domestic application of either an ACE-containing agent or an IMI-containing spot-on medication. Complete and consecutive urine samples were collected for up to 56 h after application. Urine samples were analyzed for ACE, IMI, and their respective metabolites (*N*-desmethyl-ACE, IMI-olefin, and sum of 4–/5-hydroxy-IMI) by liquid chromatography–tandem mass spectrometry. Fairly uniform concentrations of *N*-desmethyl-ACE could be observed before and after orchid treatment, so that an ACE exposure associated with orchid treatment can most likely be excluded. In contrast, after the application of the IMI-containing medication, elevated concentrations of IMI, 4–/5-hydroxy-IMI, and IMI-olefin were quantified in urine samples post-20 h with maximum concentrations of 3.1, 14.9, and 8.0 µg/g creatinine, respectively, well above general background levels. Nevertheless, the IMI intake (10.6 µg/kg bw), calculated from the excreted amounts, was around five times below the current European acceptable daily intake. Based on the case results here, household exposures to ACE and IMI after spray treatment of ornamental plants and anti-flea treatment of dogs can be regarded as low and safe. However, people regularly applying neonicotinoid-containing formulations, such as professional gardeners and employees in animal shelters, should be studied in more detail.

KEYWORDS

risk assessment, daily intake, absorbed dose, insecticide, urine, LC–MS/MS, pet, ornamental plants

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1 Introduction

The domestic use of insecticides is common, e.g., to control pests in ornamental plants and pets (1–3). Therefore, human exposure to insecticides is plausible and can occur in non-occupational settings as well. Nevertheless, actual information on exposure levels in humans after domestic use of insecticides is limited (2, 3). Additionally, there is continued discussion and rising concern about the environmental impact of veterinary medication (4, 5).

Acetamiprid (ACE) and imidacloprid (IMI) are two neonicotinoid insecticides (NNIs), which are often used as active ingredients in spray and watering agents for ornamental plants and veterinary medication to remove and control fleas in Germany (6, 7). In humans, prior to urinary excretion, ACE is mainly metabolized to *N*-desmethyl (dme)-ACE (Figure 1A) and IMI to 4–/and 5-hydroxy (OH)-IMI and IMI-olefin (Figure 1B) (8).

While human biomonitoring data on the exposure to NNIs of the general population became available in recent years (9–11), exposure data related to sources, especially domestic use of these insecticides, are limited. Therefore, the main aim of this research was to investigate if exposure to ACE and IMI occurs in two typical household scenarios, i.e., using an ACE-containing spray-treating agent on orchids or an IMI-containing spot-on medication on a dog. For this purpose, the urinary excretions of ACE and IMI, as well as their metabolites, were followed by a single male volunteer for each of the aforementioned applications.

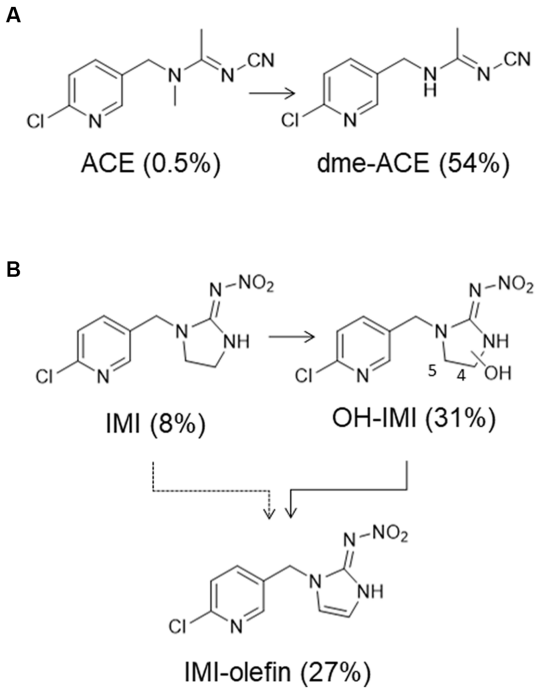

FIGURE 1
Human metabolism of (**A**) ACE and (**B**) IMI. Numbers in parentheses represent the urinary excretion fractions (F_{ue}s) relative to an oral dose, as previously published (8). For IMI, the summed biomarkers excreted in urine account for 66% of the oral dose.

2 Materials and method

2.1 Applied products

An ACE-containing spray-agent for ornamental plants ('Substral Celaflor – Schädlingsfrei CAREO Konzentrat', Evergreen Garden Care Deutschland GmbH, Mainz, Germany, Supplementary Figure 1) and an IMI-containing spot-on solution product for dogs ('Advantix', KVP Pharma + Veterinär Produkte GmbH, Kiel, Germany, Supplementary Figure 2) were purchased by the volunteers for the intended use at home (´over-the-counter´ products).

2.2 Study design

For applying a spray agent on ornamental plants ('ACE case study'), 5 mL of the ACE-containing spray agent (5 g/L; 0.5wt.–%) were diluted in 500 mL of tap water (final concentration of the active ingredient: approximately 50 mg/L) according to the manufacturer's instructions. Ornamental plants (orchids) were then sprayed directly on the leaves and roots from close range to treat for small infestations of leaf scale aphids and mealybugs (Supplementary Figure 3). Both spraying and watering of the ACE formulation were recommended by the manufacturer, depending on the crops and application site. Although watering was recommended for ornamental plants in pots indoors, we opted for spraying to possibly create a scenario with increased exposure. For topically applying the medication on pets ('IMI case study'), the ready-to-use IMI-containing spot-on solution (2.5 mL containing 1 g/L of IMI) was applied at three spots to the dog's back directly on the skin by manually splitting the hair according to the manufacturer's instructions (Supplementary Figure 4). No gloves were worn in either case study (ACE or IMI application), as this was not explicitly recommended by the manufacturers.

Orchids were simply air-dried after product application and no direct contact occurred later on. The first dog contact in terms of petting and cuddling after treatment was reported at 8.5 h post-application.

Urine samples were collected directly before the application of the NNI-containing agents (t_0) and consecutively and completely during the following 48 (ACE case study) or 56 h (IMI case study). The time periods were set to a minimum of 48 h to stay in line with our previously performed studies in volunteers after the oral dosage of neonicotinoids (8, 9). Urine samples were stored frozen (−20°C) in 250 mL polyethylene (PE) containers until analysis. The study was carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki, written informed consent (IRB Reg. No.: 18-6680-BR)).

2.3 Urine analyses

Quantification of ACE, IMI, and their specific metabolites dme-ACE, OH-IMI, and IMI-olefin was performed by stable isotope dilution analysis using online-solid phase extraction (SPE)-LC–MS/MS as previously published (10). In brief, stable isotope-labeled internal standards, buffer, and pure β-glucuronidase from *E. coli* K12 were added to urine samples, and the samples were then incubated in a water bath at 37°C for 1 h for the hydrolysis of glucuronic acid

conjugates. After incubation, samples were frozen overnight, thawed, equilibrated to room temperature, and centrifuged (1900 *g*, 10 min). A measure of 50 µL of the supernatant was injected into the LC–MS system. Limits of quantification (LOQ) were 0.06 µg/L (ACE), 0.15 µg/L (dme-ACE), 0.19 µg/L (IMI), 1.00 µg/L (OH-IMI), and 2.10 µg/L (IMI-olefin). The creatinine concentration of the urine samples was determined by the Jaffé method (L.u.P. GmbH Labor und Praxisservice, Bochum, Germany).

2.4 Estimation of NNI intakes

To back-calculate the NNI intakes (in µg/kg body weight) from urinary biomarker excretion, previously published quantitative toxicokinetic data on ACE and IMI derived in humans, including urinary excretion fractions (F_{ue}s), were used (8). NNI intakes were calculated over the complete study time (up to 48 or 56 h after application) using Equation 1.

$$NNI\ intake = \frac{\sum(c_{i-ne} \times V_i)}{F_{ue-ne} \times bw} \times M_n \quad (1)$$

with $c_{i\text{-}ne}$ being either the dme-ACE concentration or sum of the excreted IMI biomarker concentrations at time point i in mol/L, V_i is the volume of the corresponding urine sample in L at time point *i*, $F_{ue\text{-}ne}$ is the urinary excretion fractions of dme-ACE or the sum of the individual F_{ue}s of the IMI biomarkers excreted via urine within 48 h after oral application relative to the incorporated NNI dose (see Figure 1), bw is the body weight of the volunteer in kg (see Supplementary Table 1), and M_n is the molar masses of either ACE or IMI. Molar masses of ACE, dme-ACE, IMI, OH-IMI, and IMI-olefin were 223, 209, 256, 272, and 254 g/mol, respectively.

3 Results

3.1 ACE case study

In the ACE case study, ACE itself was not quantifiable above the LOQ in any of the samples. In contrast, its metabolite dme-ACE was already quantifiable in the t_0 sample and continuously until 44 h after spraying (Figure 2). In the last two samples, dme-ACE was below the LOQ. The maximum measured concentration (c_{max}) was 0.32 µg/g creatinine.

Fairly uniform concentrations of dme-ACE were observed over the whole study period without an identifiable excretion pattern for both volume- and creatinine-adjusted concentrations. The total excretion of dme-ACE over the observation time was 0.80 µg, which corresponded to an ACE intake of 0.01 µg/kg body weight.

3.2 IMI case study

In the IMI case study, the concentrations of IMI, OH-IMI, and IMI-olefin were below the LOQ at t_0. OH-IMI was the first metabolite to emerge with concentrations above the LOQ in two samples at 13 and 15 h after dog treatment (or 4.5 and 6.5 h after petting and cuddling with the dog). All three analytes were then quantifiable in all urine samples 20 h post-application (or 11.5 h after the first post-application contact with the dog); see Figure 3.

A clear treatment-associated time-concentration curve was observed for all three urinary IMI exposure biomarkers starting 20 h after the application (or 11.5 h after the first post-application contact with the dog). Creatinine adjustment (for differing urinary dilutions) leads to a considerable smoothing of the curve compared to unadjusted µg/L levels. Creatinine-corrected urinary concentrations rather constantly increased to reach their maximum 38 h after the application, with corresponding c_{max} of 3.1, 14.9, and 8.0 µg/g creatinine for IMI, OH-IMI, and IMI-olefin, respectively. Thereafter, the levels slowly decreased but remained well above the LOQ 56 h after application. The total excretion of IMI, OH-IMI, and IMI-olefin (until the last sampling point 56 h post-application) was 73.5, 439.6, and 212.0 µg, respectively, corresponding to an oral dose equivalent IMI intake of 10.6 µg/kg body weight based on the summed three urinary biomarkers.

3.3 Discussion

In the ACE case study, no unchanged ACE was found in any of the urine samples at concentrations above the LOQ (0.06 µg/L). This is not surprising, as ACE is known to be rapidly metabolized into dme-ACE

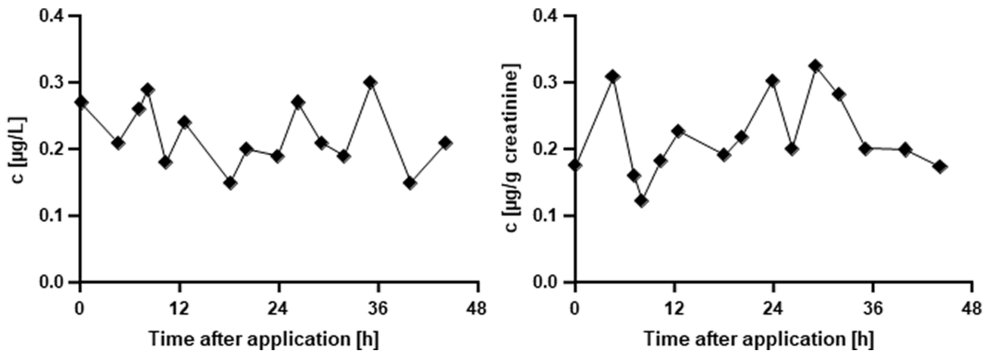

FIGURE 2
Urinary concentration of dme-ACE after in-house use of an ACE-containing spray agent; left, absolute concentrations in µg/L; right, creatinine adjusted concentrations in µg/g creatinine. Values below LOQ are not shown.

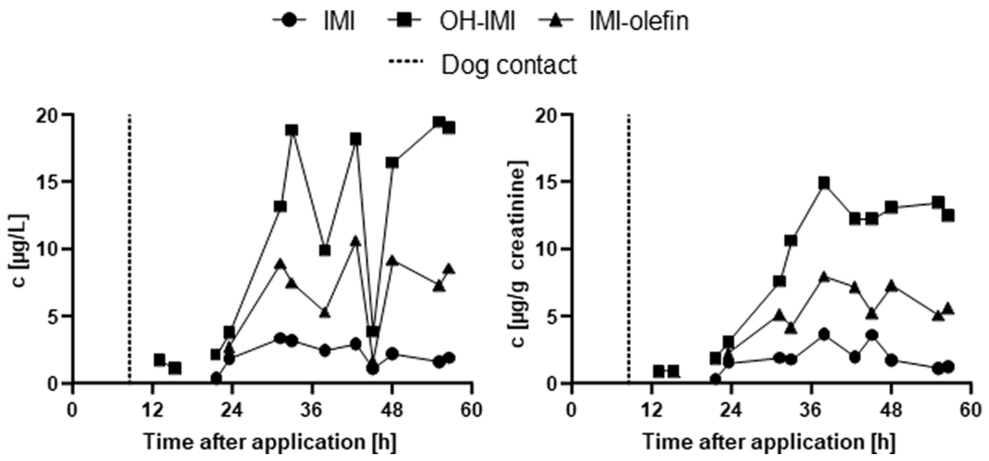

FIGURE 3
Urinary excretion of IMI, OH-IMI, and IMI-olefin after the application of an IMI-containing spot-on solution on a dog; left, absolute concentrations in µg/L; right, creatinine adjusted concentrations in µg/g creatinine. The dashed line represents the time point (8.5 h) indicating the first skin contact with the dog after treatment. Values below LOQ are not shown.

(8, 12), and the F_{ue} of the unchanged ACE is very low (0.5%, see Figure 1). Contrary to that, dme-ACE was found in almost all urine samples, including the one at t_0 before the spray application (Figure 2), thus suggesting background exposures to ACE (possibly via diet) in the volunteer and already prior to spraying the orchids. All measured concentrations were rather close to the LOQ of 0.15 µg/L or even below the LOQ ($n = 3$), which also explains the absence of unchanged ACE. All dme-ACE concentrations above the LOQ ($n = 13$) (median 0.21 µg/L, 95th percentile 0.29 µg/L) were well in the background range of ACE exposures previously reported in individuals from the German general population (median 0.38 µg/L, 95th percentile of 0.83 µg/L) (10). Overall, the lack of a classical excretion pattern and the rather uniform concentrations of dme-ACE suggest constant environmental ACE exposures in our volunteers that were not related to the spray-treating of the orchids but, most likely, to diet. The estimated ACE intake for our volunteer based on the excretion of dme-ACE (0.01 µg/kg body weight) was comparable to the intakes previously estimated for the German general population (median DI: 0.03 µg/kg body weight/day) (8) and thus well below the current acceptable daily intakes of the European Union of 25 µg/kg body weight/day for ACE (13).

In the IMI case study, we observed classical post-exposure excretion patterns in terms of, first, increasing concentrations followed by decreasing levels for all analytes (Figure 3). Neither IMI nor its metabolites, OH-IMI and IMI-olefin, were quantifiable in the urine sample before the dog was treated (t_0). This result is in line with previous findings in Germany, where most investigated urine samples did not show any IMI exposure biomarkers. In contrast to ACE, the use of IMI has been restricted to greenhouse uses in the European Union since 2013 (14) due to its toxicity in pollinators, thus limiting the presence of IMI in crops and, consequently, background exposures of the general population of Germany/Europe via diet. The excretion pattern of IMI and its metabolites after topical application of the spot-on solution to a dog is therefore clearly associated with the aforementioned treatment. Interestingly, IMI biomarkers started to be detected in urine only after petting the dog at 8.5 h post-treatment rather than directly after the topical application. We therefore assume that the petting of the treated dog is the cause of exposure rather than the original application of the spot-on agent (Figure 3). The IMI intake was calculated based on the sum of the urinary IMI, OH-IMI, and IMI-olefin in urine and their known urinary excretion fraction (15). However, as visible from the excretion kinetics (Figure 3), the urinary excretion of IMI has not been completed within the sample collection period. There are several reasons for this: From our oral dosing study, we know that the elimination half-times of IMI and its metabolites after oral dosage are rather long (12–23 h). Given the delayed uptake in our study, a total collection time of 56 h might not have been sufficient. Dermal uptake must be considered the major route of exposure in our study, similar to other studies that previously investigated exposures to active compounds in flea-controlling veterinary products (3). Compared to oral uptake, dermal uptake is slower and results in a delayed urinary excretion of IMI and its metabolites. However, human toxicokinetics after the dermal uptake of IMI have not yet been investigated in detail. Moreover, cuddling with dogs occurs infrequently and therefore cannot be considered a single exposure event (such as the spot application itself or the "first" cuddling of the dog). Because several succeeding exposure events occur at infrequent intervals, we must assume that more than 10.6 µg/kg body weight of IMI will be taken up (although the total uptake is distributed across several days post-application). Nevertheless, in our single treatment study, we could evidence the uptake of IMI in a dog owner after topically applying an ´over-the-counter´ product for controlling fleas. Overall, this single treatment did not result in an exceedance and was about a factor of 5 below the current acceptable daily intake of the European Union of 60 µg/kg body weight/day for IMI (13).

All data presented here are based on a single volunteer for each substance only and should be regarded qualitatively. Further studies including more volunteers and under varying exposure situations would be needed to assess the range of exposure quantitatively and the toxicological significance of these exposures.

4 Conclusion

Human biomonitoring has the advantage of reliably quantifying the total body burden that can occur during the use of NNI products in occupational or private environments, irrespective of the complexity of potential exposure routes (dermal, oral, and inhalation), capturing all routes and sources of exposure. Our data give first insights into ACE and IMI exposures after two different, even though specific household applications. The applications were carried out in such a way that was more likely to result in increased exposure, i.e., preparing and applying all solutions without dermal protection (no use of gloves) and, in the case of the ACE case study, spraying the plants rather than watering them. For the use of the ACE-containing plant protection product, we found no additional treatment-related exposure on top of the general background exposure to ACE. For the dog treatment with IMI, we clearly found exposures were almost exclusively related to the cuddling of the dog rather than the direct topical application of the flea-control product itself. Furthermore, we have to assume that multiple exposure routes (inhalation of dog dander and/or fine hair and dermal penetration) contributed to the total exposure, which would have been difficult to capture with exposure assessment techniques other than human biomonitoring. Therefore, although these two case studies certainly cannot be generalized with regard to every single domestic exposure situation, our study reveals that the use of spot-on medications must be considered more relevant than spray-treating ornamental plants indoors. However, the back-calculation of oral intake equivalents by reverse dosimetry from urinary biomarkers did not indicate critical IMI intake levels yet for the studied dog owner after a single application.

Generally, future studies should investigate the dermal absorption of neonicotinoids. In addition, settings presumably associated with increased exposures, such as occupational exposures of farmers, gardeners, and employees in animal shelters and veterinary practices, should be studied in more detail, and a higher number of study subjects should be used as close and continued contact with treated pets or contact with multiple treated pets might lead to cumulated exposures approaching or exceeding the ADI for IMI.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethik-Kommission der Medizinischen Fakultät der Ruhr-Universität Bochum. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. SK: Conceptualization, Visualization, Writing – review & editing. DB: Supervision, Visualization, Writing – review & editing. HH: Supervision, Writing – review & editing. HKo: Resources, Supervision, Writing – review & editing. TB: Resources, Writing – review & editing. HKä: Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1321138/full#supplementary-material

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EDITED BY
Brian Curwin,
National Institute for Occupational Safety and Health, United States

REVIEWED BY
Abas Shkembi,
University of Michigan, United States
Raghawendra Kumar,
Central Salt & Marine Chemicals Research Institute (CSIR), India

*CORRESPONDENCE
Stefan Hahn
✉ stefan.hahn@item.fraunhofer.de

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A generic approach to estimate airborne concentrations of substances released by indoor spray processes using a deterministic 2-box model

Stefan Hahn[1]*, Katharina Schwarz[1], Norman Nowak[1], Janine Schwarz[2], Jessica Meyer[2] and Wolfgang Koch[1]

[1]Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany, [2]Unit 4.I.4 Exposure Assessment, Exposure Science, Division 4 Hazardous Substances and Biological Agents, Federal Institute for Occupational Safety and Health (BAuA), Dortmund, Germany

Sprays are used both in workplace and consumer settings. Although spraying has advantages, such as uniform distribution of substances on surfaces in a highly efficient manner, it is often associated with a high inhalation burden. For an adequate risk assessment, this exposure has to be reliably quantified. Exposure models of varying complexity are available, which are applicable to spray applications. However, a need for improvement has been identified. In this contribution, a simple 2-box approach is suggested for the assessment of the time-weighted averaged exposure concentration (TWA) using a minimum of input data. At the moment, the model is restricted to binary spray liquids composed of a non-volatile fraction and volatile solvents. The model output can be refined by introducing correction factors based on the classification and categorization of two key parameters, the droplet size class and the vapor pressure class of the solvent, or by using a data set of experimentally determined airborne release fractions related to the used spray equipment. A comparison of model results with measured data collected at real workplaces showed that this simple model based on readily available input parameters is very useful for screening purposes. The generic 2-box spray model without refinement overestimates the measurements of the considered scenarios in approximately 50% of the cases by more than a factor of 100. The generic 2-box model performs better for room spraying than for surface spraying, as the airborne fraction in the latter case is clearly overestimated. This conservatism of the prediction was significantly reduced when correction factors or experimentally determined airborne release fractions were used in addition to the generic input parameters. The resulting predictions still overestimate the exposure (ratio tool estimate to measured TWA > 10) or they are accurate (ratio 0.5–10). If the available information on boundary conditions (application type, equipment) does not justify the usage of airborne release fraction, room spraying should be used resulting in the highest exposure estimate. The model scope may be extended to (semi)volatile substances. However, acceptance may be compromised by the limited availability of measured data for this group of substances and thus may have limited potency to evaluate the model prediction.

KEYWORDS

aerosol, occupational exposure, spraying application, modeling, screening

1 Introduction

For manufacturing and marketing chemicals or biocidal products in the European Union, enterprises must fulfill legal requirements. According to European chemicals legislation, a risk assessment is usually necessary, which also includes the assessment of occupational exposure that can be model-based if relevant monitoring data are not available. This model-based exposure assessment often follows a tiered approach, where it is expected that the degree of conservatism for the prediction decreases with increasing levels of detail and accuracy of the prediction. This comprises a series of models with increasing complexity and degree of detail. This means that the models should span a range from simple generic models, which need basic and easily obtained input parameters, to sophisticated (e.g., deterministic or probabilistic) models for which comprehensive information on the processes is required. Depending on the availability of input parameters, the suitable tier level can be selected for the exposure assessment. Due to these different demands and needs, there is a large number of models available for the exposure assessment (1). A review of available models and the status and further needs for modeling spraying activities are given in Hahn, Meyer (2). Spray applications are activities used to atomize liquids into droplets for dispersion of, e.g., pesticides, biocides, and paints (3). Thereby, spraying has several advantages such as uniform distribution of substances on surfaces in a highly efficient manner. However, non-volatile substances will become airborne as aerosols and thus inhalable by these activities. Moreover, the surface area of the products will increase so that volatile substances can more easily evaporate, resulting in potentially higher air concentrations. Tasks such as spraying solvents or pesticides can produce very high exposure levels (3), which are linked to several chronic health impacts such as cancer, neurotoxic effects, or reproductive toxicity. Respiratory effects such as temporary irritation and asthma during spray cleaning and by disinfection products have been discussed by Clausen, Frederiksen (4). The authors found that especially corrosive chemicals are chemicals of concern regarding respiratory effects (e.g., asthma). Furthermore, they concluded that the assessed epidemiological studies provide some evidence of increased asthma risk or worsening of asthma symptoms while using spray cleaning products in a professional or private context. Overall, occupational exposures continue to cause an important health burden worldwide, justifying the need for ongoing prevention and control initiatives (5). Spraying activities are often associated with high inhalation burden, and spray products require additional considerations to assess potential inhalation exposure.

The exposure assessment by modeling of chemicals applied by spray processes is challenging because of the high number of parameters and the variance of their values having influence on the airborne concentration. Especially the higher tier models require full details of the spraying process and the dispersion mechanisms, which are often not available. For this reason, simple model approaches and the improvement of existing spraying models are a valuable addition (6, 7).

The inhalation exposure during spraying is determined by the rate at which the spray is released into the air, the dispersion and the maturation of the released droplets by deposition (mainly settling on horizontal surfaces), and their evaporation. There are two modes of spray application: surface spraying and room spraying. While for room spraying, the entire mass released by the spraying system becomes airborne, only part of it – the overspray or airborne fraction – is available for airborne transport and exposure during surface spraying. The relevance of the exposure-determining mechanisms depends on the spray technology (such as airless versus air assisted, propellant sprays) and the associated parameters such as spray nozzle parameters, spray angle, distance to wall, and droplet size distribution. Further parameters related to the formulation are its chemical composition, the mass fraction, and partial vapor pressures of the relevant compounds (e.g., active substance or pigments and solvents).

In this article, we present a simple tier 1 approach for the assessment of the time averaged exposure concentration using only a minimum of input information and discuss possible refinements based on the classification and categorization of two key parameters: the droplet spectrum and the solvent vapor pressure. The model results are compared with measurements carried out at real workplaces. The degree of conservatism is assessed and discussed. Currently the model is restricted to binary spray liquids composed of a non-volatile fraction and volatile solvents.

2 Materials and methods

2.1 Modeling approaches

There are numerous approaches available for indoor occupational exposure modeling (1). Only very few of them focus on spray processes (2). A common way to assess indoor exposure concentration by deterministic modeling is to balance the mass flows of sources and sinks inside a closed system.

2.1.1 Generic 2-box spray model

On the lowest level a mass balance model requires knowledge of only a few generic parameters: the source strength of the spray process and the removal rate by air exchange together with the room volume, and spraying and post-spraying duration. Further mechanisms which also determine the air concentration, such as spray maturation by droplet evaporation and mass losses due to droplet settling onto the floor and other surfaces for example, are neglected in this modeling approach.

The mass balance model suggested here as a tier 1 screening model is based on a well mixed 2-box approach as shown in Figure 1, characterized by a personal volume, V_p, and a room volume, V_r. We consider a single spraying event of duration, T, composed of a spraying period, T_s, and a post spraying time, $T - T_s$. The spray liquid is usually a system composed of N (non-volatile) substances with mass fraction, ϕ_i, (total mass fraction, $\phi = \sum_1^N \phi_i \ll 1$), and solvents with the complementary mass faction, $1-\phi$. The room air is constantly exchanged with exchange rate, Γ. The spray is released at a constant mass flow rate, $\dot{M}$.

In the 2-box approach, the near field is defined by a personal volume, V_p, which is fed by the constant mass flow rate of the spray, $\dot{M}$. Due to the movement of the spray operator, for example during wall spraying and/or the entrained airflow related to the spray process, the personal volume is exchanged by a constant airflow rate, $Q_p = V_p / T_p$. Thereby T_p represents the residence time of the spray mass in the personal volume. Subsequently the mass will pass into the far-field room volume, V_R, where it is assumed to be instantaneously

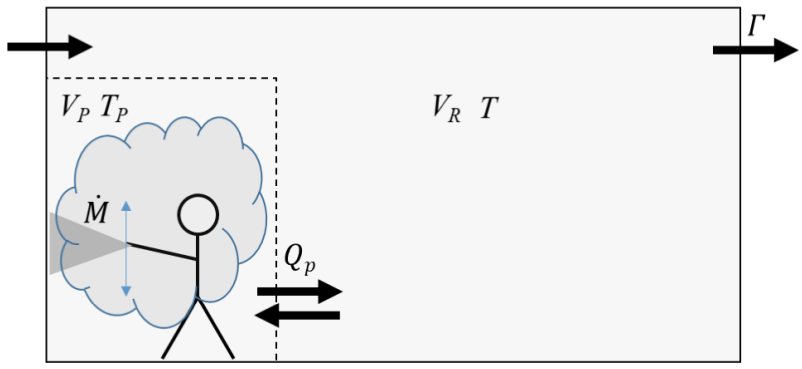

FIGURE 1
Schematics of the proposed 2-box approach. Assumptions: the personal volume (V_P) is small compared to the room volume (V_R), and the residence time (T_p) of the spray inside the personal volume is small compared to the exposure time T (spraying and post spraying). Q_p = 100 m³/min, which, for example, corresponds to T_p of 0.1 min for V_P of 10 m³.

homogenized inside the entire volume. This causes a constant (near field) concentration of the sprayed substances (and solvents) inside the personal volume during the spraying time, T_s.

Accordingly, for the (non-volatile) substance the concentration in the near field (Eq. 1) can be expressed as

$$C_{\phi_i,\mathrm{nf}} = \frac{\dot{M}\phi_i}{Q_p} \tag{1}$$

and the concentration pattern in the far field (Eq. 2) taking into account the air exchange rate, Γ, is

$$C_{\phi_i,ff}(t) = \frac{\dot{M}\phi_i}{V_r\Gamma}\begin{cases} \left(1 - e^{-\Gamma t}\right) & for\ t < T_s \\ \left(1 - e^{-\Gamma T_s}\right)e^{-\Gamma(t-T_s)} & for\ T_s \le t < T \end{cases} \tag{2}$$

inside the entire room volume, V_R, during the spraying time and post spraying time if the material is instantaneously mixed. Time integration yields the contributions to the time weighted average (TWA) mass concentration of the sprayed formulation in the personal and the room volume:

$$\bar{C}_{\phi_i,nf} = \frac{\dot{M}\phi_i}{Q_p}\cdot\frac{T_s}{T} \tag{3}$$

$$\bar{C}_{\phi_i,ff} = \dot{M}\phi_i\cdot\frac{T_s}{V_R}\cdot\frac{1}{\Gamma T}\left[1 - \frac{1}{\Gamma T_s}\left(1 - e^{-\Gamma T_s}\right) + \frac{1}{\Gamma T_s}\left\{1 - e^{-\Gamma(T-T_s)}\right\}\left\{1 - e^{-\Gamma T_s}\right\}\right] \tag{4}$$

The TWA of the exposure concentration is approximated by the sum of the two terms which is a good approximation for $T_p \ll T$:

$$\bar{C}\phi_i = \bar{C}_{\phi_i,nf} + \bar{C}_{\phi_i,ff} \tag{5}$$

In summary, the mandatory input parameters for the simple generic 2-box model used here are: the room volume, the air exchange rate, the spraying and exposure time, the mass flow rate of the sprayed liquid, and the mass fraction of the substance under consideration in the sprayed liquid. For the volumetric exchange flow rate, Q_p, of the personal volume, a value of 100 m³/min is suggested as a fixed value. This value is larger than values found in the literature for stationary sources ranging up to 30 m³/min (8). The higher value has been chosen due to the movement of the sprayer and the forced airflow by air entrainment into the spray. For a value of 10 m³ for the personal volume V_p, the volume flow rate of 100 m³/min corresponds to a residence time, T_p, of the spray in the personal volume of 0.1 min.

2.1.2 Refined generic 2-box spray model

The generic 2-box model (Eqs. 3–5) assumes that all sprayed amounts end up in the air with the source term quantified by the release rate of the substance. For volatile substances, this approach assuming (instantaneous) complete evaporation is sufficient taking into account air exchange. For a non-volatile substance, the approach is expected to be over-conservative since droplet evaporation and settling is not taken into account as well as the reduced airborne fraction F_A for surface spraying which is due to wall deposition.

For room spraying, a value of 1 is suggested for F_A considering that all sprayed mass is released to air. For surface spraying, the value is usually <1 and strongly depends on the details of the spray nozzle such as cross-sectional surface area, spray angle, and exit velocity on the spray as well as on the droplet spectrum generated in the spray process. These parameters may vary significantly for different spraying systems, and usually the operational parameters of the spraying system are not known in detail. A default value of 30% for the airborne fraction seems to be a reasonable worst-case for surface spray applications since spraying systems leading to higher overspray formation are unlikely to be used for this type of application considering that the intention is that the substance is on the surface and not off the target. Measurements by Schwarz, Koch (9) and estimations derived from a detailed wall impaction model presented in Hahn, Schwarz (10) support this assumption.

Droplet evaporation is determined by the solvent vapor pressure and settling depends on the (resulting) droplet size distribution. For exposure situations for which at least some generic information or

assumptions on solvent vapor pressure and droplet size distribution are available, a refined version of the generic 2-box model was developed. This refined 2-box model accounts for droplet maturation and settling by correction factors ξ and κ applied to the far field and near field contribution of the TWA concentration "Applying these correction factors to Eq. 5, results in the following Eq. 6.

$$\bar{C}_{\phi_i,corr} = \left(\bar{C}_{\phi_i,nf}\cdot\xi + \bar{C}_{\phi_i,ff}\cdot\kappa\right)\cdot F_A \quad (6)$$

The correction factors were calculated using a more detailed analytical well stirred one compartment model applied to the personal volume and the room volume that takes into account the aerosol dynamics of droplet evaporation and droplet settling. The details of this analytical spray model are presented in the Supplementary material.

The correction factors to the simple generic 2-box model related to droplet evaporation and settling are given by

$$\xi = \bar{\tilde{C}}_{\phi_i,nf} / \bar{C}_{\phi_i,nf} \text{ and } \kappa = \bar{\tilde{C}}_{\phi_i,ff} / \bar{C}_{\phi_i,ff} \quad (7)$$

where $\bar{\tilde{C}}_{\phi_i,nf}$ and $\bar{\tilde{C}}_{\phi_i,ff}$ are the TWA concentrations calculated by the extended analytical model. In a comparison of Eq. 7 with Eqs 3, 4, it is obvious that the correction factors (ratios of two concentration values) are independent of the room volume and the mass flow rate of the spray liquid because both calculated concentrations depend in the same way on these parameters. Parameters primarily influencing the values of the correction factors are the droplet distribution, which can be described by a lognormal distribution with variable mass median droplet diameter (MMD) and constant geometric standard deviation, $\sigma_g = 1.8$ (11), and the vapor pressure of the solvent as well as the spraying and exposure times, T_s and T. Further parameters are the air exchange rate, Γ, the settling height, H_s, and the volume fraction of the non-volatile substances, ϕ.

For the model runs to derive the correction factors, the following model input data were used: The solvent vapor pressures values were chosen in steps of 0.01, 0.1, 1, 10, 100, 10,000, and 10,000 Pa. The mass median droplet diameters of the spray droplet spectrum were chosen in steps of 10, 20, 40, 80, 160, 320, and 640 µm. The geometric standard deviation was set to a constant value of 1.8. The overall effect of settling losses that determine the correction factors for the far-field contribution depends on the time scales and on the air exchange rate. With large values for the air exchange rate, for example, mass losses by settling become less important compared to mass losses by ventilation. Therefore, the spraying time, T_s, the exposure time, T and the air exchange rate, Γ, were chosen according to Table 1. Table 1 represents typical values for real life application of spray: short, medium and long spraying times as well as short and long post exposure times (per treated location). Far-field correction factors were calculated for 0, 10, and 20 h^{-1} for the exposure durations of 6 and 15 min.

TABLE 1 Values of spraying and exposure time used for calculation of the correction factors.

T_S [min]	T [min]	T / T_S	Γ [1/h]
1	6	6	0, 10, 20
10	15	1.5	0, 10, 20
10	60	6	0, 5, 10, 20
60	65	1.08	0, 5, 10, 20

The value of the settling height was set to 3 m as a typical room height. The effect of settling on the TWA after evaporation of the solvent is influenced by the volume fraction of all non-volatile compounds in the application solution. Here a value of 0.01 was chosen to be conservative. Larger values of the volume fraction would lead to smaller values of the correction factor, i.e., lower concentration because the size (MMD) of the matured aerosol (after solvent evaporation) is larger resulting in higher settling losses. Values of the volume fraction of non-volatiles smaller than 0.01 seemed unlikely in practice, as other impurities add to the background concentration of non-volatiles in the final spray solution. For example, the formulation does not often contain only one non-volatile substance. In addition, concentrated solutions of the formulation containing the non-volatile compound [for example quaternary ammonium compound (QACs)] are typically diluted with tab water, and tab water usually contains other non-volatile compounds such as salts, e.g., a medium water hardness of 14° is equivalent to 0.25 g/L calcium carbonate. A value of 1,000 kg/m^3 was assumed as default for the solvent and substance densities.

The vapor pressure of the solvent (which can be a mixture of different compounds) and the droplet spectrum may cover a range of values and are not always readily available. Therefore, a categorization of the parameters was carried out. Three vapor pressure classes, 1–3 (class 1: ≤ 10 Pa, class 2: 10–1,000 Pa, class 3: ≥ 1,000 Pa), and two size classes (fine spray: MMD 10, 20, 40, 79 µm and coarse spray: MMD 80, 160, 320, 640 µm) were chosen. The fine spray is representative for room spraying using propellant aerosol cans or fogging systems as particles are intended to have a long residence time in the air. High pressure spraying typically generates fine droplets whereas coarse sprays result from spray nozzles operated at low pressure (< 6 bar). In order to adjust the generic 2-box model using the restricted information on vapor pressure and droplet size, the mean value, $\bar{\kappa}$, of the κ-values belonging to the parameters that determine the vapor pressure and droplet size class was calculated. This was done for all scenario parameters listed in Table 1, resulting in $3 \times 2 \times 14 = 84$ values for $\bar{\kappa}$ for the far-field correction. The calculated values are listed in Table 2.

The near field correction factors, ξ, were calculated for one scenario only. It was characterized by a droplet residence time inside the personal volume of 0.1 min. The residence time was obtained from the air exchange flow rate of 100 m^3/min and an assumed personal volume of 10 m^3. A total of $3 \times 2 \times 1 = 6$ mean values, $\bar{\xi}$, of the mean near field correction factor was calculated (Table 2).

2.1.3 Generic 2-box spray model using release fraction

The default value for the airborne release fraction during surface spraying of 30% is probably too conservative when treating flat surfaces such as the walls and the floor of the room. It may be justified that the spray partly passes the surfaces to be treated such as spraying on industrial appliances with structured surfaces (tubes, grids) or carrying out disinfection tasks in stables.

An alternative to select this value is to use measured values of the airborne release fraction of (non-volatile) compounds obtained in control chamber experiments (9, 12–14). The airborne release fraction

TABLE 2 Mean values of the near- and far-field correction factors $\bar{\xi}$ and $\bar{\kappa}$.

T_S [min]	T [min]	Γ [1/h]	1 fine	1 coarse	2 fine	2 coarse	3 fine	3 coarse
			Mean far-field correction factor, $\bar{\kappa}$					
1	6	0	0.38	0.03	0.61	0.09	0.69	0.16
		10	0.40	0.03	0.61	0.10	0.70	0.17
		20	0.41	0.04	0.62	0.10	0.71	0.18
10	15	0	0.35	0.02	0.59	0.08	0.66	0.13
		10	0.38	0.03	0.60	0.09	0.68	0.15
		20	0.40	0.04	0.62	0.10	0.70	0.17
10	60	0	0.27	0.01	0.45	0.04	0.48	0.05
		5	0.32	0.02	0.54	0.06	0.59	0.10
		10	0.36	0.03	0.58	0,08	0.65	0.13
		20	0.40	0.04	0.61	0.10	0.70	0.17
60	65	0	0.29	0.01	0.49	0.04	0.52	0.06
		5	0.33	0.02	0.55	0.07	0.61	0.10
		10	0.36	0.03	0.59	0.08	0.66	0.13
		20	0.40	0.04	0.62	0.10	0.70	0.17
			Mean near-field correction factor, $\bar{\xi}$					
0.1	0.1	0	0.65	0.34	0.73	0.36	0.84	0.43

The numbers 1, 2, and 3 denote the vapor pressure classes; the expressions "fine" and "coarse" characterize the droplet size distribution.

takes into account overspray formation and settling losses in the immediate vicinity of the spray nozzle. In the study by Schwarz, Koch (9), the airborne release fraction of non-volatile substances in the inhalable aerosol was roughly classified into $F_A = 0.01$ for flat fan and hollow cone spray nozzles operated at low (1–3 bar) and high (<10 bar) pressures and $F_A = 0.1$ for handheld pump sprays. It was measured under conditions of realistic application for the spray technology.

2.2 Workplace measurement data

The model and its two refinement options were evaluated by comparing model calculations with available experimental measurement data for typical workplaces. Different sources of suitable workplace measurements data have been identified. Measurement data are available from BAuA reports [F1702 (15); F2137 (16), F2366 (9)]. Data from F2137 (16) were already used for evaluation of the SprayExpo model. In addition, suitable data were available from the Biocides Human Health Exposure Methodology (17): Spraying Model 2 contains data from HSE (18) and Spraying Model 10 contains data from TNO report V3806 (19). Further data were published for insect sprays by Berger-Preiss, Koch (20), which were also used for evaluation of the performance of ConsExpoWeb on modeling consumer exposure to spray products (21). For the modeling exercise, the experimental conditions described in the identified studies were coded for the input parameters required and simulated using the described models (see Supplementary material).

In total for evaluation of the screening models, 34 scenarios were used from the BAuA reports [F1702 (15), F2137 (16), F2366 (9)] representing different sprayed masses, room volumes, and spraying times as well as different application types (surface or room spraying) and mass median diameters (MMD) of the sprayed aerosols. The use areas covered antifouling, pest control, wood protection, stored product protection as well as disinfection of tables, walls, or pool sides or treatment of animal housings. Room volumes ranged from relatively small rooms with 13.3 m^3 to very large with 10,700 m^3. For the ventilation rates, specific values were available for three scenarios. In the cases where information was missing, a rough estimation was made: for antifouling scenarios, an air exchange rate of 10 h^{-1} has been assumed, for animal housings a rate of 1 h^{-1} due to open doors, and for all other scenarios a rate of 0.6 h^{-1}. Spraying time was usually equal to exposure time (sampling time) and ranged from 4 to 103 min. Exceptional cases are the table disinfection at which spraying time of 1 and 1.28 min were shorter than the exposure time of 4.5 and 4.45 min, respectively.

Data from HSE (18) are available including 13 scenarios with measured data for inhaled exposure during spray application (in total 20 scenarios considering dermal exposure data). Spraying indoors ranged from small-scale domestic to large-scale applications. The room volume spanned a high variability from living rooms to church halls, but specific values on room volumes and ventilation rates were not given. Spraying was done onto hard surfaces, and the direction was usually "around," partly overhead and for some scenarios downwards. Besides spraying, half of the scenarios also included irrigation (injection into holes), but information on the fraction of irrigation on the whole process was not given. Spray pressures ranged from 320 to 1,050 kPa and spraying activity from 6 to 95 min. For the simulation, a worst-case assumption was used to cover uncertainties in the boundary conditions: all mass is sprayed not irrigated, particle size class is fine, and ventilation is low (0.6 h^{-1}).

In the TNO report V3806 (19), in total 16 scenarios were given on surface spraying for pest control in different areas such as private

home, chicken stable, transit store, and restaurant. The room volumes varied from 192 to 40,728 m^3 and the treated surface (no input parameter) from 64 to 5,556 m^2. Ventilation was given qualitatively with no ventilation, natural ventilation, or mechanical ventilation, so that for modeling 0, 0.6, and 10 h^{-1} were selected, respectively. Generally, equipment with a shoulder strap was used with <3 bar. However, one scenario comprised equipment with >3 bar and another scenario with equipment for fogging. Different temperatures were given for each scenario which had no impact on the modeled results using the described models, as the temperature range of 7.5 to 20.1°C had no influence on the assignments on vapor pressure classes of the solvent or non-volatile substance. The amount sprayed and the measured concentration of the substance of interest in the spray tank were specified for each scenario. Sampling time was used for spraying time and exposure time, as directly linked to the measured TWA given. Overall, although some minor uncertainties regarding ventilation or particle sizes existed, sufficient information on the boundary conditions were available for these measurements.

Measured data for application of 5 different insect sprays were presented in Berger-Preiss, Koch (20), and each product consisted of 2–3 non-volatile substances. The insect sprays were applied all for room spraying with a fine aerosol (MMD < 40 µm), and each in three different time scales [variation in spraying (10 s to 2 min) and exposure time (2.2 –to 60 min)] as well as in sprayed amount (9.5 to 189.2 g) resulted in 15 different scenarios. Only the substance with the highest content was selected for the modeling exercise for each scenario and was compared to the measured value of this compound. Room volume was relatively small (about 40 m^3), and a low ventilation rate of 0.6 h^{-1} was presumed.

Finally, the modeled time-weighted average (TWA) air concentrations using the described models were compared with the measured air concentrations to evaluate the performance of the described models.

2.3 Statistical methodology

In recent publications, statistical parameters have been proposed and discussed to evaluate performance and accuracy of models (8, 22–24). However, no agreed standards exist (25). In the following, the ratio modeled/measured concentrations have been calculated for each workplace scenario. Based on these ratios, the percentage of the number of scenarios with ratio < 0.5, 0.5–10, 10–100, and > 100 has been derived.

3 Results

3.1 Correction factors

For practical application of the generic 2-box model and the refinement using correction factors, the latter should be calculated in advance. For this purpose, a series of model runs was performed with the analytical model covering the range of expected exposure scenarios (see section 2.1.2).

Figure 2 shows results calculated for the parameters of the second and third row of Table 1 representing spraying times of 10 min, which are typical for disinfection of surfaces inside a room. Parameters of the calculations varied with respect to the post exposure time (15 versus 60 min and the air exchange rate (0 versus 20 h^{-1}). No correction to the far-field contribution of the generic 2-box model (Eq. 4) results in $\kappa = 1$. The smaller the κ-value, the larger the deviation of the concentration calculated with the analytical model related to mass losses from droplet settling compared to the generic 2-box model without refinements. The main parameter of influence on the κ-value is the *MMD* of the droplet spectrum. The droplet size dependence is reduced to high values of the air exchange rate. This is because the residence time of the substance is smaller and, therefore, also the time that the settling mechanism is effective. Please note, the main influence of the air exchange rate on the TWA concentration is already accounted for in the generic 2-box model.

The dots show exemplary results for a MMD of 320 µm and a solvent vapor pressure of 1,000 Pa. For the 60 min exposure time (a, b) a reduction of the correction factor can be observed from $\kappa = 0.0428$ for 20 h^{-1} to $\kappa = 0.0064$ for zero air exchange. Obviously this difference is reduced for the shorter exposure time of 15 min (c, d): $\kappa = 0.0402$ for 20 h^{-1} and $\kappa = 0.0245$ for 0 h^{-1}. For the large Γ -value of 20 h^{-1} the influence of exposure time on κ is small ($\kappa = 0.0402$ for T = 15 min and $\kappa = 0.0428$ for T = 60 min) since the mean residence time where settling is effective is 3 min for both scenarios.

Figure 2 also shows that, compared to the droplet spectrum, the dependence of κ on vapor pressure is smaller. This is because for values above 1,000 Pa, the regime for most of the solvents, κ is nearly independent of vapor pressure because solvent evaporation from the spray droplets is fast and the mass losses are determined by the residual dry aerosol. At the low end of the vapor pressure scale (<1 Pa), there is virtually no droplet evaporation, and mass losses are determined by the size of the spray droplets.

Generally the far-field correction factors are smaller for coarse sprays than for fine sprays. For water which is classified as vapor pressure class 3, the correction factors for the fine droplet spectrum vary between 0.48 and 0.70 and for the coarse droplet spectrum between 0.05 and 0.17. For short exposure times, the influence of the air exchange rate on the mean far-field correction factors is small. For 20-fold air exchange rate per hour, for example, the correction factors are independent of the spraying and exposure times since settling is active only during the residence time $1/\Gamma = 3$ min which is smaller than all the exposure time scale considered here. For the near field, the fine mode correction factor is close to 1 for the fine spectrum and about 0.4 for the coarse mode spectrum.

3.2 Comparison with workplace measurements

The model and its refinements were compared with monitoring results obtained at workplaces. In total, 78 measurements from different sources were used for comparison. The measured substances in the spray formulations were all non-volatile. Most of the solvents (mainly water) belonged to vapor pressure class 3, which was important for the maturation of the droplets and thus the correction factor used for the refined generic 2-box model. However, measurement data for the solvents were usually not available, so only the concentrations of the non-volatile substances were available for comparison.

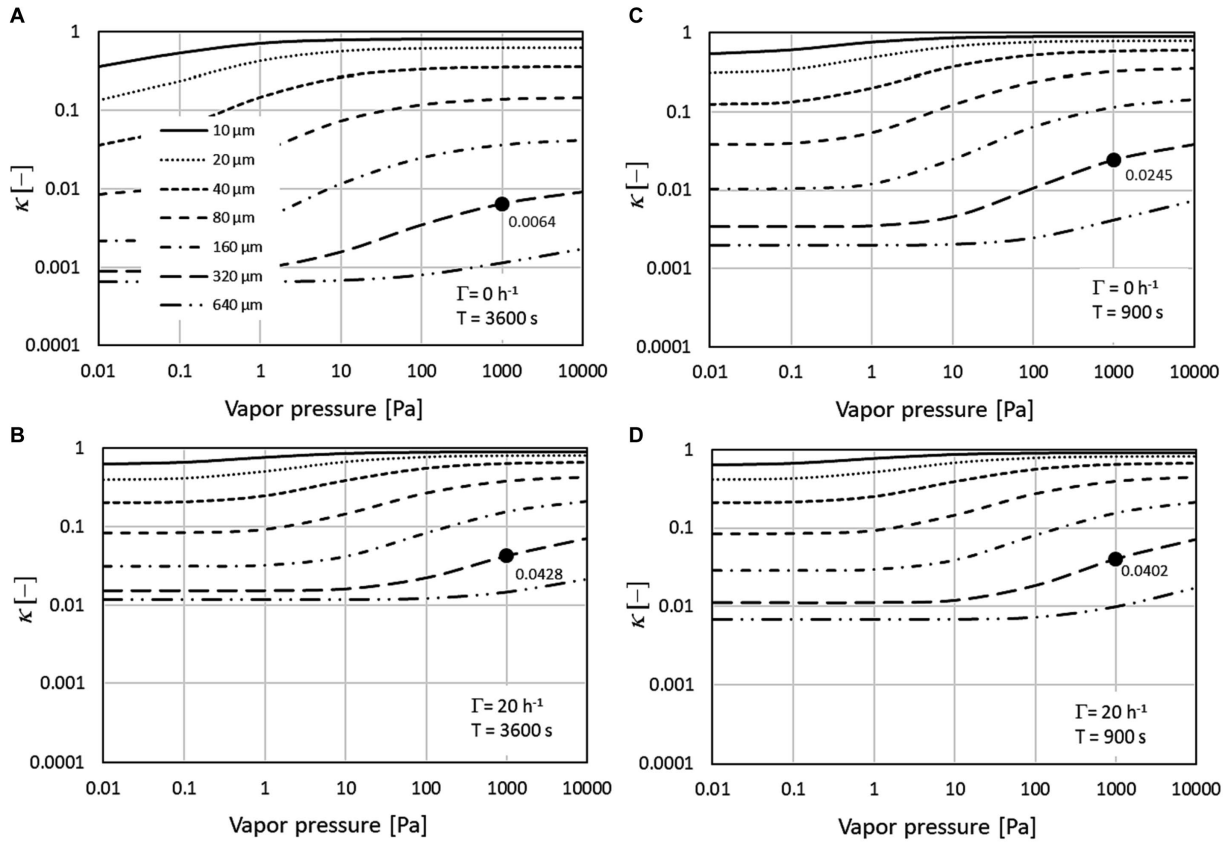

FIGURE 2
Correction factors for 10 min spraying duration (Ts) and overall exposure time (T) of 60 min **(A,B)** or 15 min **(C,D)**. Comparison between scenarios without air exchange **(A,C)** and an exchange rate of 20 h^{-1} **(B,D)**. The dots are the exemplary values for a solvent vapor pressure of 1,000 Pa and a droplet spectrum with MMD of 320 µm.

3.2.1 Data from BAuA reports

The comparison of the modeled TWA with workplace data from the BAuA reports is shown in Figure 3 (15, 16, 9). For surface spraying, the generic 2-box spray model (Figure 3A) usually overestimates the measured TWA values by a factor of 100 and larger. Using the refined generic 2-box spray model (Figure 3B) reduces the conservatism of the model due to applying the correction factors $\bar{\kappa}$ and the default airborne fraction of 30%. However, the modeled TWA values for the surface spraying scenarios are mostly still at least a factor of 10 above the measured TWA values. This changes when the data-based classification of the airborne release fractions associated with the spray technology is used for $F_A$in the generic 2-box spray model. The predictions for surface spraying become significantly less conservative with many of the scenarios falling within the range between the measured TWA and 10-fold above the measured TWA (Figure 3C).

For room spraying the situation is quite different. The modeled TWA values are above the measured TWA using the generic 2-box spray model (Figure 3A), but only a few scenarios are highly overestimated (> factor 100). Considering the settling of particles using the correction factors $\bar{\kappa}$ significantly reduces the conservatism of the model if coarse particles are present (refined generic 2-box spray model, Figure 3B). Using the airborne release fraction approach (Figure 3C) will not change the estimate in comparison to the generic 2-box spray model (Figure 3A), as for room spraying all sprayed liquid becomes airborne (airborne release fraction $F_A = 1$).

The data points above the 1:100 line in Figures 3A,C represent stable disinfection and wood protection scenarios. In these scenarios not only flat surfaces are treated but also beams and grids, and thus parts of the spray pass the surfaces to be treated. The actual application type is consequently a mixture of room and surface spraying. Room disinfection with $F_A = 1$ has been selected for these scenarios as a worst-case assumption. However, a significant part of the spray is expected to be on the surface, so that F_A is actually <1. Using for these scenarios surface spraying (Figure 4) would shift the model closer to the measured data if the airborne release fraction approach is used with an $F_A = 0.01$. The TWA values calculated by the refined generic 2-box spray model are less conservative than the generic 2-box spray model but are still apparently higher than the release fraction approach. For these specific scenarios and measurements, the selection of surface spraying instead of room spraying is a refinement option, is still conservative, and seems to be more appropriate than room spraying.

3.2.2 Data from model 2 of biocides human health exposure methodology

For the workplace data from Garrod, Rimmer (18), all scenarios were coded as surface spraying with a particle size "fine." In the

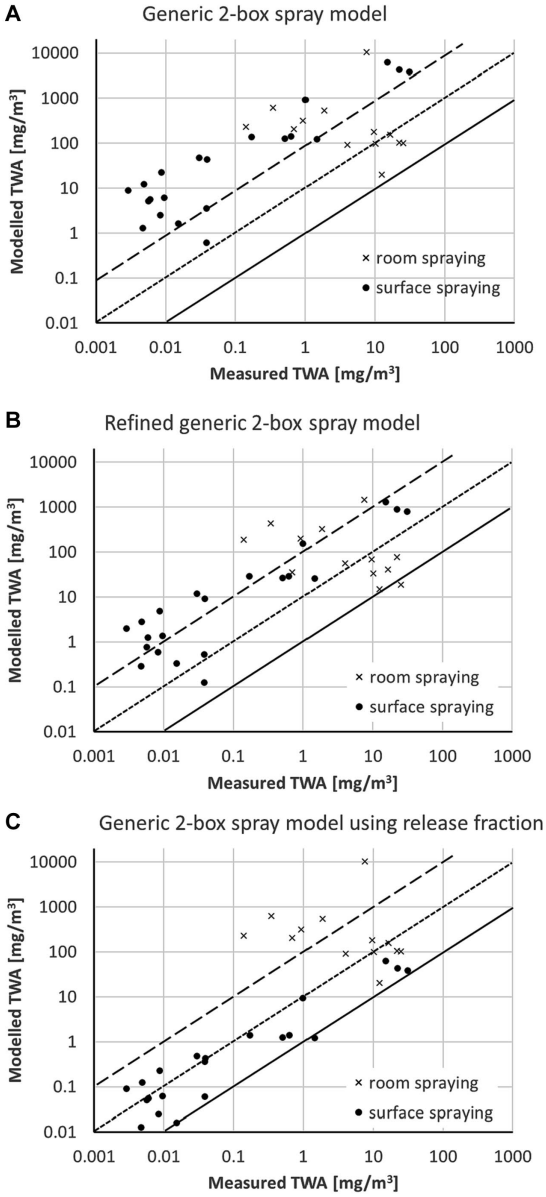

FIGURE 3
Comparison of modeled TWA values with measured data from the BAuA reports (15, 16, 9) using **(A)** the generic 2-box spray model, **(B)** the refined generic 2-box spray model, and **(C)** the generic 2-box spray model using release fraction; solid line represents 1:1-line, the dotted line a 10-fold, and the dashed line a 100-fold overestimation of the model.

publication of Garrod, the room volume of each workplace was not specified. However, the uncertainty of the room volume was not expected to have much impact. Figure 5 shows the dependency of the modeled TWA from the input parameter room volume. With increasing room volume, the TWA converges to a value which is determined by the concentration in the near field (personal volume). This clearly shows the strength of the 2-box model. Subsequently, simulations were performed for all scenarios using two different room volumes (1,000 m^3 and 10,000 m^3), which are probably too high, for example, for the sampled scenarios in living rooms but too small for example for the sampled scenarios in a chapel. The comparison of modeled TWA with the measured workplace data is shown in Figure 6. For both assumed room volumes, the modeled TWA are often at least by a factor of 100 higher than the measured TWA values using the generic 2-box spray model (Figure 6A). The TWA is more realistic but in most cases still higher than the measured value using the refined generic 2-box spray model (Figure 6B). The third model using the release fraction of 0.01 with the generic 2-box spray model results in TWA values significantly below the measured values for some scenarios (Figure 6C). This indicates that the airborne release fraction of 0.01 may not be appropriate for these situations. However, information on some relevant boundary conditions are not available, resulting in a high uncertainty of the input parameters and thus also in an uncertainty of the modeled TWA.

3.2.3 Data from model 10 of biocides human health exposure methodology

The TNO data were mostly coded as surface spraying as well (19). One scenario is for fogging application, and thus it cannot be assumed that deposition on surface during application is relevant. This scenario was assigned as a worst-case approach to application type room spraying. The comparison of modeled TWA with the workplace data is shown in Figure 7. Again the generic 2-box spray model (Figure 7A) usually highly overestimates the measured TWA by a factor of >100 due to neglecting deposition on the surfaces. Applying the correction factor (Figure 7B) will reduce the modeled TWA but is still conservative. Using the airborne release fraction approach in the generic 2-box spray model results in an estimation nearest to the 1:1 line.

3.2.4 Data for insect sprays

The measurements of Berger-Preiss, Koch (20) were solely simulated as room spraying with a particle size class of "fine." The comparison of modeled TWA with measured workplace data is shown in Figure 8. For room spraying all sprayed liquid becomes airborne (F_A = 1), and thus there is no difference between Figures 8A,C. The modeled data using the generic 2-box spray model are usually up to a factor of 10 above the 1:1 line. The refined generic 2-box spray model (Figure 8B) results in slightly reduced modeled TWA values, which is due to the consideration of the settling and which is only marginal for fine particles.

4 Discussion

In this article, a generic 2-box spray model is presented for screening purposes in order to estimate the exposure during spraying activities. In addition, approaches are suggested to refine the model outcome without using higher tier tools. In recent publications, criteria has been proposed and discussed to evaluate performance and accuracy of models which are based on the ratio modeled/measured concentrations (8, 22–24). However, these criteria seem to be too ambitious for screening models, as such screening models (tier 1 models) should represent the best possible compromise between accuracy and simplicity, and, therefore, often the modeled estimates are significantly higher than the actual exposure. For this reason an underestimation was assigned to a ratio < 0.5, an accurate estimation for ratio 0.5–10, an overestimation to ratio 10–100, and a high

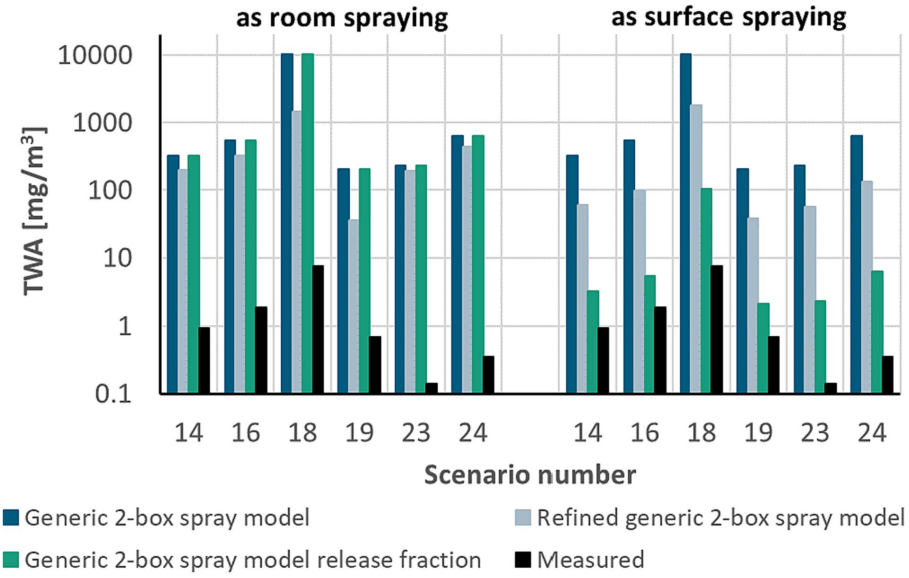

FIGURE 4
Comparison of modeled and measured TWA values for scenarios calculated for both room and surface spraying, as application type is unclear. For scenarios numbers and specific information, see Supplementary material.

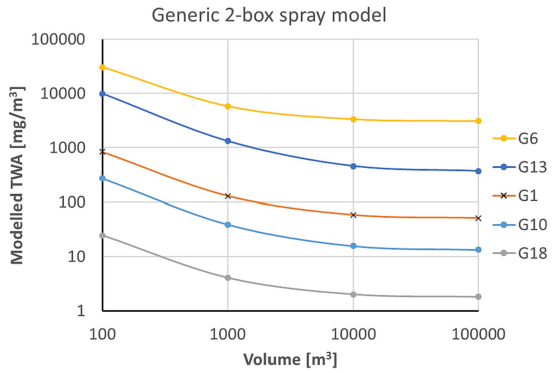

FIGURE 5
Dependence of modeled TWA values from room volume used in the generic 2-box spray model for exemplary scenarios from HSE (18).

overestimation to ratio > 100. An overview of the statistical data evaluation is given in Table 3.

All three models give conservative results, as almost all modeled values are higher than the measured values. The prediction of the generic 2-box spray model highly overestimates the measurements for the considered scenarios in approximately 60% of the cases by more than a factor of 100. However, performance of the generic 2-box spray model is excellent for room spraying, and the modeled value is usually maximum a factor 10 higher than the measured value. Exemptions are the scenarios for which application type is not an unambiguous assignment, and thus room spraying is used as a worst-case assumption. If detailed information on the application type and equipment are available, the selection of surface spraying and measurement or defining the airborne release fraction may be an appropriate refinement option. However, if only limited information on the scenario and equipment is available, the default of 30% airborne fraction or even room spraying should be selected as a worst-case approach to avoid underestimation.

Underestimation is generally not observed for the generic 2-box spray model which is not surprising as the model assumes that all sprayed amount is airborne regardless of surface or room spraying. In addition, TWA values during spraying are determined by the concentration in the near field (personal volume) so that by using the 2-box model approach the influence of the dilution within the room volume has minor impact and an underestimation is not expected.

For surface spraying the overestimation in the generic 2-box spray model is primarily based on neglecting intended deposition of spray on the treated surface. Taking settling into account, which is implemented in the refined generic 2-box spray model, reduces the conservatism. Although settling is dependent on the particle size, it is not necessary to know the particle size distribution in detail but it is sufficient to rather have a rough classification into fine or coarse spray. Further refinement option addresses the airborne fraction which is important for surface spraying only. An airborne fraction of 30% is suggested as a default in the refined generic 2-box model and at the same time only fine particles as airborne are considered. Using the experimentally determined airborne release fractions, which indirectly reflect the complex characteristics of the spraying equipment and which are much smaller than the suggested default value, results in the least conservative and thus most accurate prediction of measured concentrations. In more than 60% of the cases, the modeled value for the spraying scenarios is then below a factor of 10 above the measured values. It is worth mentioning that for the generic 2-box model based on airborne release fractions no information on the droplet size distribution is required.

Underestimation has been observed for the HSE data (18) when using the airborne release fraction approach on the generic 2-box model. It seems that the used airborne release fraction is not appropriate to all these scenarios and some are rather similar to room spraying. However, in this case, the coding of the scenarios has a high

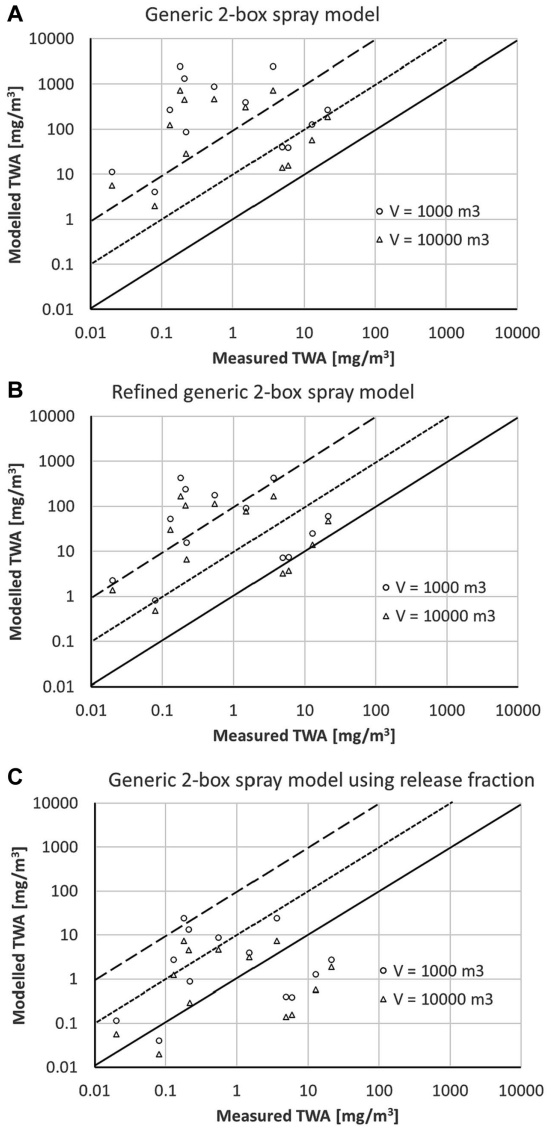

FIGURE 6
Comparison of modeled TWA values with measured data from HSE (18) using **(A)** the generic 2-box spray model, **(B)** the refined generic 2 box spray model, and **(C)** the generic 2-box spray model using release fraction; solid line represents 1:1-line, the dotted line a 10-fold, and the dashed line a 100-fold overestimation of the model.

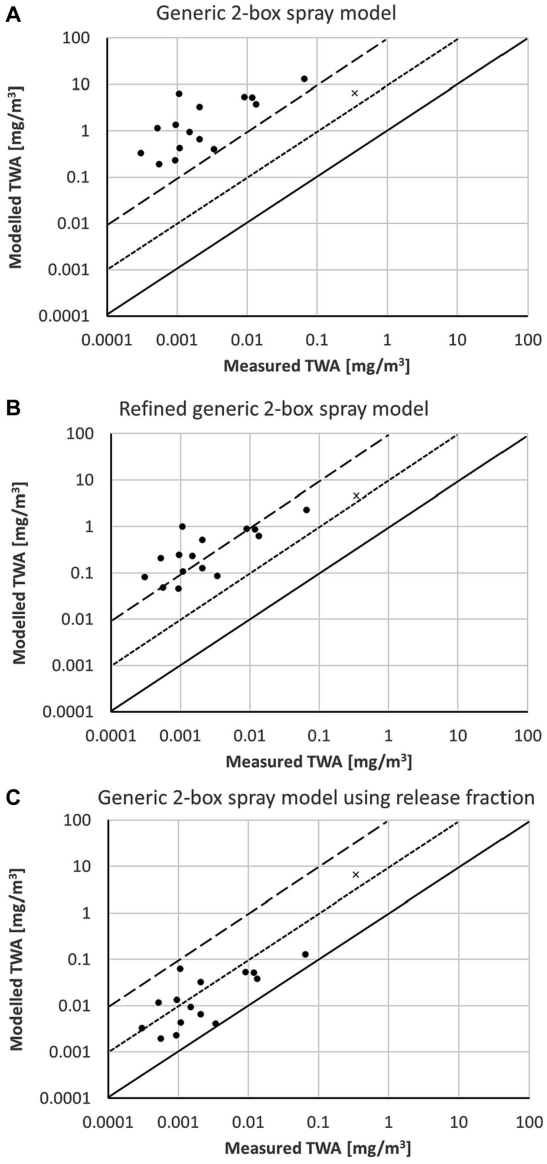

FIGURE 7
Comparison of modeled TWA values with measured data from TNO (19) using **(A)** the generic 2-box spray model, **(B)** the refined generic 2 box spray model, and **(C)** the generic 2-box spray model using release fraction; solid line represents 1:1-line, the dotted line a 10-fold, and the dashed line a 100-fold overestimation of the model; all scenarios surface spraying with one exemption for fogging.

uncertainty due to missing information on room volume, ventilation, application, and equipment. As a worst-case approach, room spraying should be selected if sufficient information is not available. However, for the BAuA data, it has been demonstrated that room spraying could also result in high overestimation and surface spraying considering the airborne release fraction is the better choice. In case of doubt, the airborne release fraction should be measured experimentally.

The evaluation of the performance of the presented screening models has only taken the measurement of non-volatile substances into consideration so far. However, the generic 2-box spray model approach, at which all sprayed liquid becomes airborne, is principally also applicable to volatile substances. Even if volatile substances deposit on the surface, they will become airborne by evaporation. For this reason, assuming that all substances are after spraying, airborne is a reasonable worst-case assumption. As only limited data are available for volatiles during spraying, this model domain can hardly be evaluated quantitatively. In Hahn, Schwarz (10) some information on volatiles are presented that supports the expectation that the presented screening approach may be conservative for volatile substances. A potential refinement for volatiles substances may only be possible using higher tier tools such as SprayEva (26). In addition, the presented model is evaluated so far mostly for indoor application only. As mentioned earlier, often spraying activities are applied outdoors (e.g., pesticides). For outdoor environments, the far field volume will be large, and additional distribution processes have to considered such as wind speed and direction. Wind will have an influence on the mass flow out of the personal volume but could also be directed into the personal volume which makes a prediction of the

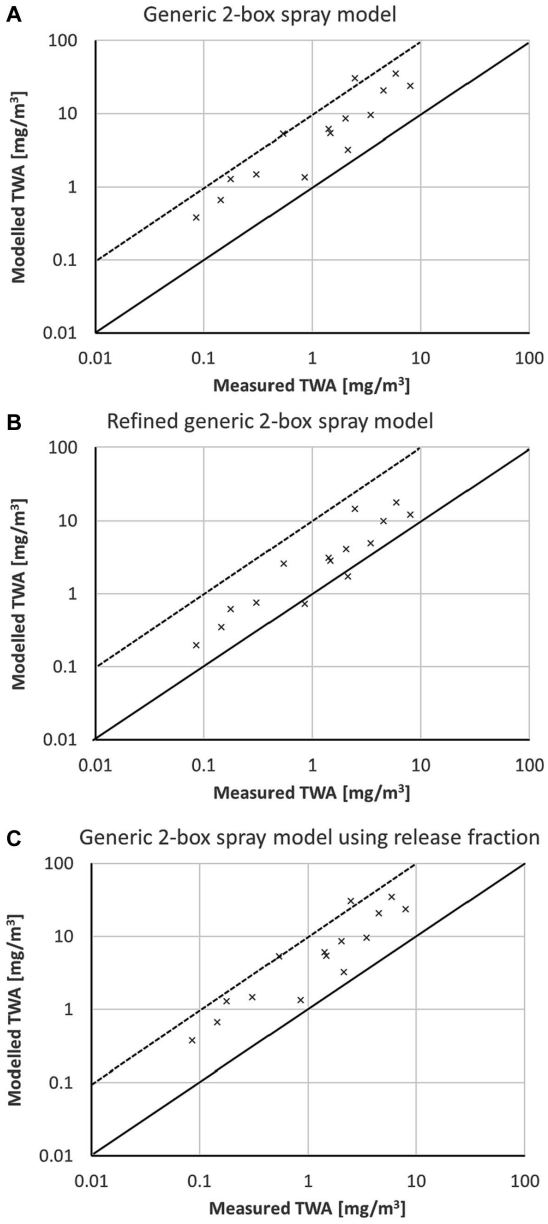

FIGURE 8
Comparison of modeled TWA values with measured data for insect sprays (20) using **(A)** the generic 2-box spray model, **(B)** the refined generic 2 box spray model, and **(C)** the generic 2-box spray model using release fraction; solid line represents 1:1-line, the dotted line a 10-fold overestimation of the model; all scenarios room spraying.

inhalable exposure more complex. Adaptation of the model and maybe different default input parameters may be required to be applicable to outdoor processes without limitations.

As mentioned above, a review of available models suitable to assess exposure during spraying activities is given in Hahn, Meyer (2). For example ECETOC TRA, ART or Stoffenmanager® provide approaches to predict exposure during spraying activities. The majority of the available models are based on empirical data such as the TNsG spraying models [Biocides Human Health Exposure Methodology (17)]. Two datasets (HSE, TNO) provided by the TNsG spraying models have been used for evaluation of the presented screening models. The available mass-balance models (SprayExpo and ConsExpo) are regarded as higher tier models, as they need detailed information on the exposure situations such as information on particle size distribution. A comparison between the different models is beyond the scope of this publication, but there is a need for simple model approaches (6, 7).

ConsExpoWeb contains two model approaches: an instantaneous release model as screening and the more sophisticated spraying model mentioned above. The first one is similar to the presented generic 2-box spray model and considers the released mass, the weight fraction of the compound, the room volume, the exposure duration, and the ventilation. However, all the released amount is instantaneously released and homogenized in the air and does not consider the spraying time. In addition, it is based on a well-mixed room concept which is sufficient for small rooms usually presenting consumer exposure. However, for workplaces, often larger rooms are more typical, and thus a 2-box model concept seems to be more appropriate.

Several authors presented 2-box models. i.e. based on near field and far field (NF/FF) approaches (24, 27–34). Most of these approaches are applied to volatile substances which are evaporating from a source within the near field. The concept has been applied to spraying as well (29, 30), whereas Hofstetter, Spencer (29) concentrated on volatile compounds only. Critical parameters for the NF/FF model are the size of the near field (24) or the mass flow between near field and far field. Mass flow rates between near field and far field have been reported for several indoor environments in the range between 0.24 and 30 m^3/min (8). The higher value of 100 m^3/min has been proposed (see section 2.1.1) due to the movement of the sprayer and the forced airflow by air entrainment into the spray. Usually, near-field volumes of less than 1 to 25 m^3 are suggested in literature (often 2x2x2 = 8 m^3). A medium volume for the near field of 10 m^3 corresponds for the proposed mass flow of 100 m^3/min to a residence time of 0.1 min in the personal volume.

A 2-box model is also available in the IHMOD™ Tool published by AIHA. However, it is not developed specifically for spraying activities. For this reason, it considers the mass generation but does not consider the sink by settling or the deposition on the treated surface. This is also not considered in the presented generic 2-box spray model, but it is considered by the correction factors used for the refined generic 2-box spray model. In addition, the airborne release fraction approach considers the fraction which will adhere on the treated surface in the case of surface spraying as well as settling losses in the immediate vicinity of the treated area.

For the screening models (tier 1 approach) which are presented here, only easily obtainable input information is required. These are the room volume, the air exchange rate, the spraying and exposure time, the mass flow rate of the sprayed liquid, and the mass fraction of the substance under consideration in the sprayed liquid. For the refinements only information is required about the application type, i.e., surface or room spraying, and the vapor pressure class of the solvent. Additional refinement is possible if measured airborne release fraction is available or at least information about equipment which justifies the selection. If more information is available such as, for example, a detailed characterization of the spray droplet spectrum, higher tier models can be used as, for example, the analytical approach presented in the Supplementary material, SprayExpo (16, 35), and SprayEva (26).

The presented screening models can be regarded as a stage of extension for the ConsExpoWeb instantaneous release model considering the spraying time and the two-box model concept or for

TABLE 3 Statistical data using the 2-box spray models.

		Generic	Refined generic	Generic release fraction
BAuA data (15, 16, 9); number of entities 34; different application types (surface and room spraying; different particle size classes)	Underestimation (T/M < 0.5)	0.0%	0.0%	0.0%
	Accurate (0.5 < T/M < 10)	14.7%	20.6%	61.8%
	Overestimation (10 < T/M < 100)	14.7%	35.3%	20.6%
	High overestimation (T/M > 100)	70.6%	44.1% [a]	17.6% [a]
HSE data (18); number of entities 13; surface spraying, uncertainty regarding room volume (V = 1,000 m^3), and particle size class (fine)	Underestimation (T/M < 0.5)	0.0%	0.0%	30.8%
	Accurate (0.5 < T/M < 10)	15.4%	30.8%	38.5%
	Overestimation (10 < T/M < 100)	23.1%	23.1%	23.1%
	High overestimation (T/M > 100)	61.5%	46.2%	7.7%
TNO data (19); number of entities 16; surface spraying (and one room spraying/fogging); uncertainty regarding particle size classes (mostly coarse used)	Underestimation (T/M < 0.5)	0.0%	0.0%	0.0%
	Accurate (0.5 < T/M < 10)	0.0%	0.0%	62.5%
	Overestimation (10 < T/M < 100)	6.3%	62.5%	37.5%
	High overestimation (T/M > 100)	93.8%	37.5%	0.0%
Insect sprays (20); number of entities 15; room spraying, particle size class fine	Underestimation (T/M < 0.5)	0.0%	0.0%	0.0%
	Accurate (0.5 < T/M < 10)	93.3%	100%	93.3%
	Overestimation (10 < T/M < 100)	6.7%	0.0%	6.7%
	High overestimation (T/M > 100)	0.0%	0.0%	0.0%

T / M = ratio tool estimate to measured TWA; a = using surface spraying will reduce high overestimation.

the AIHA model considering spraying activities and processes. Ultimately, the presented screening models expands the possibilities to use modeled data in regulatory authorization processes.

Although spraying has several advantages, the sprayed substances will become airborne and inhalable. As a result, several diseases are induced by these (workplace) activities which has been discussed, for example, by Clausen, Frederiksen (4). To prevent, control, and avoid these diseases, occupational health practitioners and exposure and risk assessors can make use of the presented generic 2-box model as a possible addition to workplace measurements. The model can be used for a first estimate to determine where at the workplaces concern about human health is expected, where more information is necessary (higher tier modeling and measurements), or where risk mitigation measures are needed. In comparison to the higher tier models, only easily obtainable input information is required. The generic 2-box model usually produces conservative exposure estimates. Thus, if the results of a risk analysis indicate that adverse health impacts are likely, the refinement options based on correction factors and measurement of release fractions provide an alternative to considering burdensome risk mitigation measures. The model can also be used to evaluate the impact of varying mass fraction, MMD, etc., in order to make recommendations for safe and sustainable by design (36, 37) products and systems, for example by altering the design of a spraying device and scenario. In consequence, the model will help to realize and control adverse human health effects during spraying of (corrosive) chemicals, which are often associated with a high inhalation burden.

5 Conclusion

The presented screening model is intended to be a simple introduction to exposure modeling of spraying activities, which also allows more refined estimates with slight adjustments. The model approaches using generic input parameters allow a conservative prediction of exposure concentrations for spray applications. However, the over-prediction of measured concentrations is quite large in particular for surface spraying due to significant overestimation of the airborne fraction. This can be reduced by using correction factors or the concept of airborne release fractions in which overspray formation and early spray aging is determined experimentally and categorized in view of the spray technology used. It would be worthwhile to enlarge the database of airborne release fractions and refine the categories in view of the specific scenarios and spray technologies. Combining this data set with the generic 2-box spray model could be a practical tool for conservative exposure prediction.

Overall, these screening models will complement the available models to assess spraying activities at workplaces. We have shown a way to replace necessary detailed technical information about the spray equipment (e.g., particle size distribution, nozzle information) with simple measurements or extraction of results from more complex modeling. Depending on the methodology used, different accuracies can be achieved.

Data availability statement

All contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SH: Conceptualization, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft. KS: Formal analysis, Methodology, Writing – review & editing. NN: Formal analysis, Software, Writing – review & editing. JS: Conceptualization, Project

administration, Writing – review & editing. JM: Conceptualization, Supervision, Writing – review & editing. WK: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1329096/full#supplementary-material

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Cathryn Lee,
The University of Chicago, United States

REVIEWED BY
Elliott Horner,
UL Solutions, United States
Malcolm D. Richardson,
The University of Manchester,
United Kingdom

*CORRESPONDENCE
Carla Viegas
✉ carla.viegas@estesl.ipl.pt
Liliana Aranha Caetano
✉ liliana.caetano@estesl.ipl.pt

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Portuguese cork industry: filling the knowledge gap regarding occupational exposure to fungi and related health effects

Carla Viegas[1,2]*, Marta Dias[1,2], Cátia Pacífico[1], Tiago Faria[3], Anália Clérigo[1], Hermínia Brites[1], Liliana Aranha Caetano[1,4]*, Elisabete Carolino[1], Anita Quintal Gomes[1,5] and Susana Viegas[1,2,4]

[1]H&TRC – Health and Technology Research Center, ESTeSL – Escola Superior de Tecnologia e Saúde, Instituto Politécnico de Lisboa, Lisbon, Portugal, [2]NOVA National School of Public Health, Public Health Research Centre, Comprehensive Health Research Center, CHRC, REAL, CCAL, NOVA University Lisbon, Lisbon, Portugal, [3]Centro de Ciências e Tecnologias Nucleares (C2TN), Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal, [4]Research Institute for Medicines (iMed. ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal, [5]Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisbon, Portugal

Introduction: The presence of the *Penicillium* section *Aspergilloides* (formerly known as *Penicillium glabrum*) in the cork industry involves the risk of respiratory diseases such as suberosis.

Methods: The aim of this study was to corroborate the predominant fungi present in this occupational environment by performing a mycological analysis of 360 workers' nasal exudates collected by nasal swabs. Additionally, evaluation of respiratory disorders among the cork workers was also performed by spirometry.

Results: *Penicillium* section *Aspergilloides* was detected by qPCR in 37 out of the 360 nasal swabs collected from workers' samples. From those, 25 remained negative for *Penicillium* sp. when using culture-based methods. A significant association was found between ventilatory defects and years of work in the cork industry, with those people working for 10 or more years in this industry having an approximately two-fold increased risk of having ventilatory defects compared to those working less time in this setting. Among the workers who detected the presence of *Penicillium* section *Aspergilloides*, those with symptoms presented slightly higher average values of CFU.

Discussion: Overall, the results obtained in this study show that working in the cork industry may have adverse effects on worker's respiratory health. Nevertheless, more studies are needed (e.g., using serological assays) to clarify the impact of each risk factor (fungi and dust) on disease etiology.

KEYWORDS

***Penicillium glabrum* complex, *Aspergillus* sp., spirometry, health effects, suberosis**

1 Introduction

Portugal produced 49.6% of all worldwide cork in 2019, with 640 companies working in this production sector with 8,343 direct workers and an overall profit of 718 M euros each year (1). Additionally, two-thirds of worldwide cork exportation originates in Portugal, 77.4% from semi-processed products, 82.3% from processed products from natural cork, and 68% from agglomerate products.

The presence of the *Penicillium* section *Aspergilloides* (formerly known as *Penicillium glabrum*) in this industry involves the risk of respiratory diseases such as suberosis, a type of hypersensitivity pneumonitis that is one of the most prevalent diseases among cork workers (2–9). Epidemiologic studies have already reported an estimated prevalence between 9 and 19% of suberosis among Portuguese cork workers (3).

Penicillium section *Aspergilloides* and *Chrysonilia sitophila* were both reported as the dominant fungal species in all stages of cork production (10–12), corroborating their role in respiratory disorders in this setting (10, 12, 13). In addition, despite not being fully understood, an altered immune response to inhalation of antigens produced by these species can also trigger in susceptible individuals an inflammatory cascade that can progress to lung fibrosis (14).

Aspergillus section *Fumigati*, one of the most ubiquitous saprophytic fungi (15), has also been observed in cork industries (12). It is suggested as an indicator of harmful fungal contamination in different occupational environments (16–18), with several fungal species from the *Fumigati* section implicated in the development of suberosis (9, 19). Thus, an additional health risk should be considered for exposed workers (17, 18, 20).

Aspergillus section *Fumigati* is also ranked as a fungal species of critical priority, as it is considered one of the potential pathogenic species with higher clinical relevance, partly due to the prevalence of azole-resistant phenotypes both in clinical and environmental isolates (21).

A pilot study has previously shown that exposure to particles is also a concern particularly associated with the respirable fraction that occurs during manual intervention in the task of agglomerating cork (12).

The nose cavity is the primary entry point for inhaled air and, consequently, the first region of the respiratory tract in contact with airborne fungi, among other occupational risk factors (22–27). In this context, the use of the nasal swab procedure for sampling is of utmost importance since it allows fungal detection in the nasal cavity, being an easy and painless collection method that can be performed everywhere with no need for additional equipment (22, 25).

The aim of this study was to corroborate the predominant fungi present in this occupational environment by performing a mycological analysis of 360 workers' nasal exudates collected by nasal swabs. Additionally, evaluation of respiratory impairment among the cork workers was also performed through spirometry.

2 Materials and methods

2.1 Previous environmental monitoring

Three cork plants were included in the study developed between January and February 2014. Plant A was located in the Évora district, while plants B and C were located in the Santarém district. Plant A employed 41 workers and produced cork boards for further processing by other industries. Plant B employed 165 workers and mainly produced natural bottle corks. Plant C employed 154 workers and specialized in several cork-derived articles such as cork tiles, papers, and textiles (Figure 1).

All three plants provide respiratory protection equipment (RPE) to their workers, but workers do not use this equipment in a consistent manner. All the plants work 5 days a week in two 8-h shifts. To assess occupational exposure to fungal contamination, air samples of 50–100 L were collected through an impaction method with a flow rate of 140 L min-1 onto malt extract agar (MEA) supplemented with chloramphenicol (0.05%) (Frilabo, Portugal) using the Millipore air Tester (Millipore). Surface samples were collected by swabbing the surfaces of the same indoor sites, using a 10-by-10 cm square stencil disinfected with 70% alcohol solution between samples according to the International Standard ISO 18593 (2004). The obtained swabs were then plated onto MEA. Air samples of 250 L were collected using the impinger Coriolis μ air sampler (Bertin Technologies) at 300 L min-1 airflow rate. Samples were collected onto 10 mL sterile phosphate-buffered saline with 0.05% Triton X-100, and the collection liquid was subsequently used for DNA extraction. Samples collected were analyzed using culture-based (air samples collected by impaction and surface samples) and molecular methods (air samples collected by impinger) following the procedures applied in previous research work (12).

In the previous study (12), Plant C showed an increased air fungal diversity compared with the other two plants, among which the most prevalent was *Penicillium* sp. (76.5%). The distribution of fungal species in the surface samples of Plants A and B was similar, with isolates from the *Aspergillus* section *Fumigati* being the only ones found besides *C. sitophila*. In Plant C, the most prevalent genera were *Trichoderma* sp. and *Penicillium* sp. (52.9%; 29.4%). All three plants had higher fungal loads indoors than outdoors. Real-time PCR identified the *Penicillium* section *Aspergilloides* in 10 out of the 12 air samples, that is, in six more sampling sites than the culture-based methods (12).

2.2 Study population

In total, 360 workers from the 3 companies were enrolled in the study (plant A—41 workers, plant B—165 workers, and plant C—154 workers). A control group (38) with administrative tasks outside these companies was also engaged in the study. The 360 workers from the three cork plants participated in both the nasal swab assay and spirometric study.

All workers and control group subjects gave written informed consent to participate in the study. This study complied with the Helsinki Declaration and Oviedo Convention, and all data were stored and analyzed in accordance with the Portuguese General Data Protection Regulation (GDPR) law n° 58/2019.

2.3 Nasal swab assay

Two consecutive swab samples, with sterilized cotton swabs, were taken from one nostril at the end of the work shift. The swabs were rotated against the internal anterior walls of the nostril and then placed in the provided transport tube. One of the swab samples of each worker was plated onto malt extract agar (MEA) supplemented

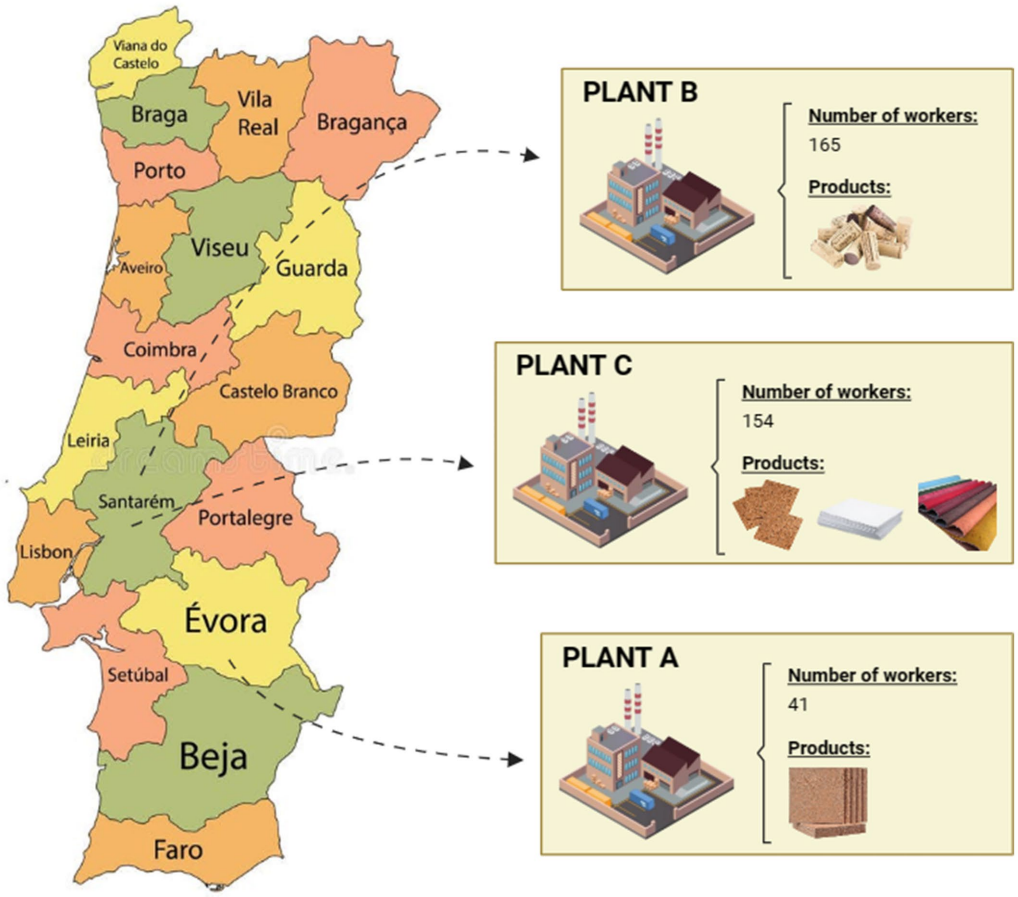

FIGURE 1
Geographical distribution of the cork plants assessed.

with chloramphenicol (0.05%) (Frilabo, Portugal). The samples collected this way were subsequently incubated at 27°C for 5 to 7 days. The fungal species were quantified (CFU per worker) and identified microscopically through macro and microscopic characteristics according to De Hoog et al. (28).

The other swab sample was eluted into 1 mL of PBS, centrifuged at 250 rpm (5 g) for 30 s, and then frozen at −80°C until DNA extraction. This sample was subsequently centrifuged for 30 min at 3500 rpm. The supernatant was discarded, and the pellet was re-suspended in 200 µL of distilled water. DNA was then extracted using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, United States) according to the recommendations of the manufacturer. Molecular identification of *Penicillium* section *Aspergilloides* (*P. glabrum* complex) and *Aspergillus* section *Fumigati* (Table 1) was achieved by real-time PCR (RT-PCR) using the Rotor-Gene 6,000 qPCR Detection System (Corbett-Quiagen, Germany). Primers and probes for *Penicillium* section *Aspergelloides* were designed with Primer Express software for the Calmodulin (CaM) gene of *Penicillium* section *Aspergilloides* strain AS3.15335. Primers for *Aspergillus* section *Fumigati* were described by Cruz-Perez et al. (29). Reactions included 1× iQ Supermix (Bio-Rad, Portugal), 0.5 µM of each primer (Table 1), and 0.375 µM of TaqMan probe in a total volume of 20 µL. Amplification followed a three-step PCR: 40 cycles with denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s. For each gene amplified, a non-template control and a positive control were included. The positive control consisted of DNA obtained from a reference strain belonging to the culture collection of the Reference Unit for Parasitic and Fungal Infections, Department of Infectious Diseases of the National

TABLE 1 Sequence of primers and TaqMan probes used for real-time PCR.

Fungal species targeted	Sequences	Reference
Penicillium section *Aspergilloides*		
Primer forward	5'-TGCCTGGACCGGAACCTA-3′	
Primer reverse	5'-CACCATCGCCATCCTTGTC-3'	Designed for this study
Probe	5'-TGAATGCTTTCCCGTAATA-3′	(information above)
Aspergillus* section *Fumigati		
Primer Forward	5'-CGCGTCCGGTCCTCG-3'	
Primer Reverse	5'-TTAGAAAAATAAAGTTGGGTGTCGG −3′	(29)
Probe	5'-TGTCACCTGCTCTGTAGGCCCG −3′	

Institute of Health Dr. Ricardo Jorge was included. These strains have been sequenced for ITS, B-tubulin, and calmodulin.

2.4 Spirometry

An individual questionnaire was applied to obtain data on (1) smoking habits, (2) history of known lung disease, (3) presence of respiratory symptoms, and (4) exposure history.

Spirometries were performed using an MK8 Microlab spirometer. The spirometer was always calibrated before data collection, with a 3-L syringe to a total of 12 L. Values from calibration were accepted if results were within a ± 3% range. The spirometer used met the international standards with respect to flow rate and duration of the test. A minimum of three acceptable flow-volume curves were obtained, and repeatability was verified on the two tests with the largest forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1), according to ATS/ERS 2005 guidelines (30). The following respiratory function parameters were evaluated: FVC, FEV1, and FEV1/FVC%.

A control group was not considered since the aim of our study was to identify the prevalence rate of ventilatory defects in exposed workers through comparison with reference values from the European Community for Coal and Steel (ECCS) (31). Taking this into consideration, the methodology normally used in lung function laboratories was considered suitable for this study. For interpretation purposes, the fixed cutoff of 80% of the predicted value was used. Ventilatory defects were classified as follows: (1) obstructive—FEV1/FVC% below 80%; (2) restrictive—FEV1 and FVC below 80% with a FEV1/FVC% equal or above 80%; and (3) non-specific—FEV1, FVC, and FEV1/FVC% below 80%.

2.5 Statistical analyses

Statistical analysis of all data was performed using the Statistical Package for Social Sciences (SPSS) version 24.0 for Windows. To characterize the workers' samples quantitatively, frequency analysis (n, %) for qualitative data and calculation of minimum, maximum, mean, and standard deviation were used. The criterion for significance was set at $p < 0.05$. The Shapiro–Wilk test was used to test the normality of the quantitative data. To study the association between two qualitative variables, the Chi-Square Test was used to determine whether the applicability assumptions were verified or Fisher's Exact Test otherwise. Binary logistic regression was used to identify risk factors for the presence of respiratory symptoms. Once the assumption of normality was verified, the t-test was used to compare the presence of *Penicillium* section *Aspergilloides* between those workers who have and those who do not have respiratory symptoms.

3 Results

3.1 Nasal swab assay

3.1.1 Culture-based methods

Among the 360 workers subject to nasal swab assay, 310 (86.1%) presented fungal contamination. In 119 workers, overgrowth of *Chrysonilia sitophila* was observed, which rendered impossible the quantification of the number of isolates on the plate, being considered in these cases 500 isolates per nostril, following previous procedures regarding environmental samples´ fungal quantification (12). Around 36.6% of the workers' nasal swabs presented *Penicillium* genus, 9.9% *Aspergillus* sp., and 29.1% observed more than one fungal genera (Figure 2). Within the 38 samples from the control group, 16 (42.1%) did not show any fungal growth, 44.7% presented *Penicillium* sp., and 18.4% *Cladosporium* sp. The sample from one subject presented *Mucor* sp. and other *Geotrichum* sp.

Considering the 500 isolates per nostril on the plates where overgrowth was observed, *C. sytophila* (92.3%) was the most common fungi found in the workers' noses, followed by *Penicillium* sp. (4.9%), *Rhizopus* sp. (1.5%), and *Mucor* sp. (0.7%). *Cladosporium* sp., *Alternaria* sp., *Acremonium* sp., and *Aspergillus* sp. were present in

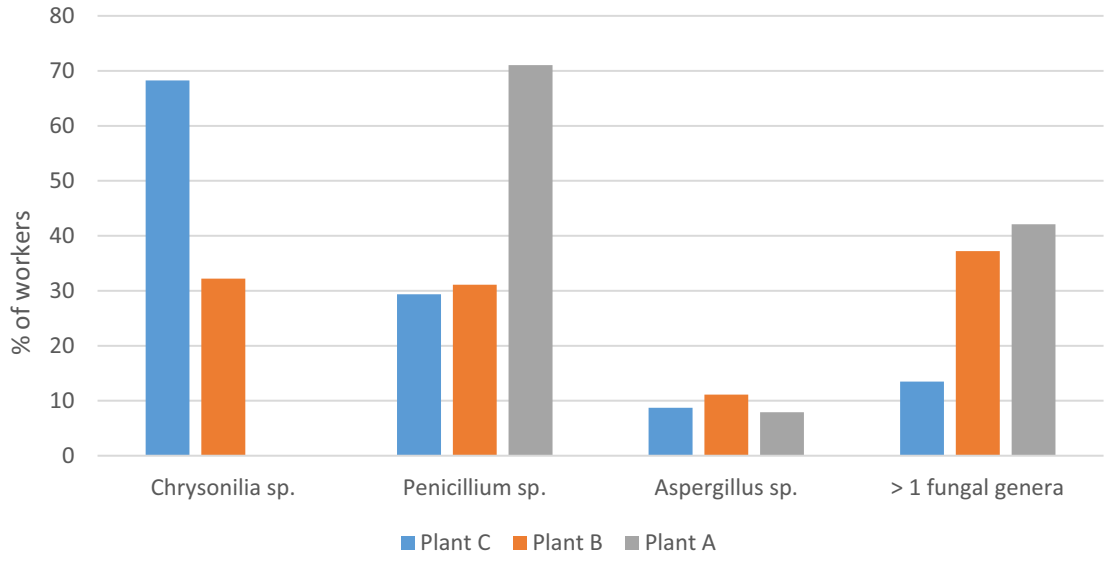

FIGURE 2
Fungal contamination distribution in the workers' nasal swabs.

lower counts, accounting for the majority of the remaining percentage. When considering workers from each cork plant, *C. sitophila* was the most common fungus isolated in workers from both plants B and C, accounting for more than 90% of the fungal diversity, while plant A presented a slightly different fungal distribution. *Penicillium* sp. represented 95.0% of the fungal species identified, followed by *Cladosporium* sp. (2.1%), *Aspergillus* sp. (1.4%), and *Acremonium* sp. (1.1%). *Alternaria* sp., *Paecilomyces* sp., and *Chrysosporium* sp. accounted for 0.1% each (Figure 3).

Fungal diversity is described in Table 2 according to the isolates number obtained in the workers' noses from the 3 plants.

3.1.2 Molecular tools

We next subjected the nasal swab samples from the 360 workers of the three different plants (Plant A—41; Plant B—165; Plant C—154) to qPCR analysis and observed successful amplification of DNA from *Penicillium* section *Aspergilloides* in 37 of the analyzed samples. From those, 25 remained negative for *Penicillium* sp. when using culture-based methods. Furthermore, in one worker, *Aspergillus* section *Fumigati* was co-amplified with *Penicillium section Aspergilloides,* and in another worker, that section was detected singularly. As expected, in the 38 controls used, none were positive for the *Penicillium section Aspergilloides* nor for the *Aspergillus* section *Fumigati*. Of note,

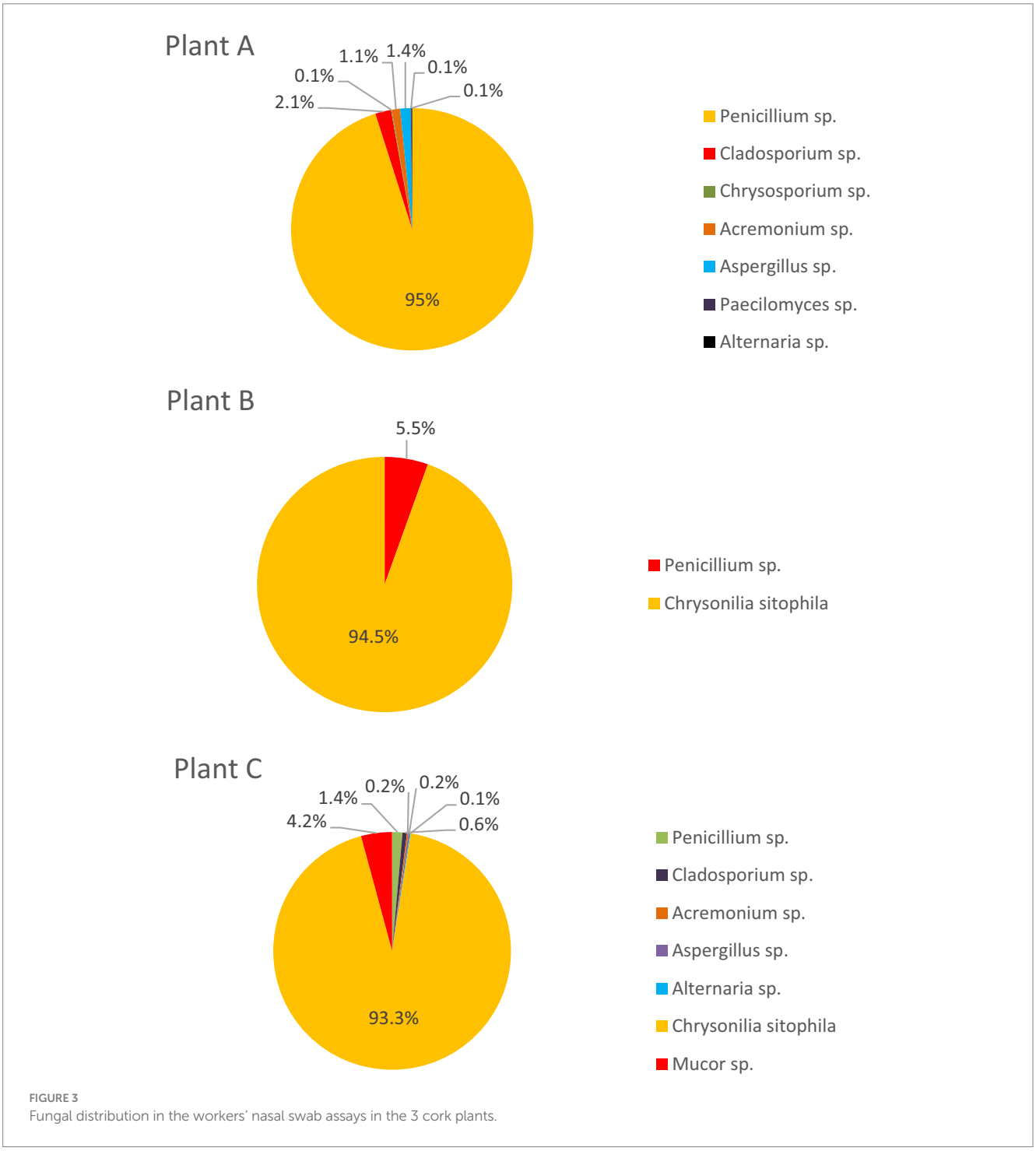

FIGURE 3
Fungal distribution in the workers' nasal swab assays in the 3 cork plants.

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samples with lower cycle threshold (CT) values very likely exhibited higher levels of the detected fungi (Figure 4).

3.2 Spirometry

Three hundred sixty workers completed the symptom questionnaire and performed spirometry. Since 40 workers had previous pulmonary pathology, only 320 were considered in the analysis. The average age of participants was 41.24 ± 10.56, and 66.9% ($n = 214$) were men. A considerable percentage ($n = 118$; 36.9%) of participants were smokers (Table 3).

TABLE 2 Fungal isolates distribution in workers' noses from the 3 cork plants.

Isolates/worker nose.	Genera/Species
0–100	*Chrysosporium* sp.
	Acremonium sp.
	Alternaria sp.
	Scopulariopsis sp.
	Fusarium verticilloides
	Aureobasidium sp.
	Neoscytalidium hialinum
	Neoscytalidium dimiatum
	Geomyces sp.
	Geotrichum sp.
	Fusarium poae
	Fusarium oxysporum
	Cladophialophora sp.
	Aspergillus sp.
100–500	*Cladosporium* sp.
500–1,000	*Mucor* sp.
1,000–2,500	*Rhizopus* sp.
> 2,500	*Chrysonilia sitophila*
	Penicillium sp.

The average number of years of work in the cork industry was 11.02 ± 8.86. The majority ($n = 168$, 52.5%) worked in this industry for 10 or more years and did not smoke ($n = 177$, 55.3%). Regarding the respiratory symptoms, the majority ($n = 193$, 61.3%) did not have symptoms (Table 4).

Concerning the ventilatory defects, 36.5% of spirometries ($n = 115$) were classified as obstructive, 0.6% ($n = 2$) as restrictive, and 1.6% ($n = 5$) as non-specific. A significant association was found between smoking habits and age with ventilatory defects ($\chi^2_1 = 5.376$, $p = 0.020$ and $\chi^2_1 = 31.565$, $p < 0.001$, respectively). For each additional year of life, the risk of the presence of ventilatory defects increased [Odds Ratio = 1.788, Confidence Interval$_{95\%}$ = (1.420, 2.252)], and in the smokers or ex-smokers, the risk of ventilatory defects was approximately 2 times higher [Odds Ratio = 1.734, Confidence Interval$_{95\%}$ = (1.087, 2.768)] (Tables 5, 6).

A significant association was found between ventilatory defects and years of work in the cork industry ($\chi^2_1 = 5.058$, $p = 0.025$), and it was found that those who worked for 10 or more years in this industry had an approximately two-fold increased risk of having ventilatory defects [Odds Ratio = 1.692, Confidence Interval$_{95\%}$ = (1.068, 2.681)], in relation to those who have worked for less than 10 years. Regarding respiratory symptoms, namely regular cough, expectoration, wheezing, and dyspnea, no significant association was detected with the number of years of work in this industry (Table 7).

The same analysis was performed separately in smokers and non-smokers. In non-smoking workers, a significant association was found between ventilatory defects and years of exposure ($\chi^2_1 = 5.762$, $p = 0.016$). It was found that those people who worked for 10 years or more in the cork industry had a two-fold increased risk of developing respiratory defects [Odds Ratio = 2.002, Confidence Interval$_{95\%}$ = (1.131, 3.543)], in relation to those who have worked for less than 10 years. In smokers, no significant association was

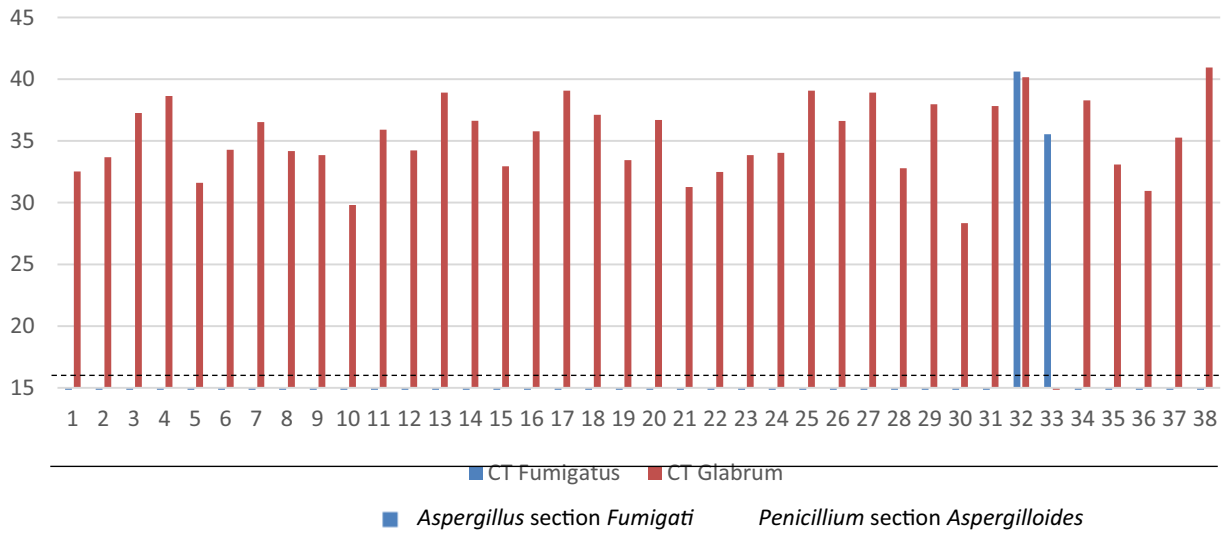

FIGURE 4
CT values of each sample for *Penicillium* section *Aspergilloides* and *Aspergillus* section *Fumigati*. The dashed line represents the CT of the positive control for *Penicillium* section *Aspergilloides* while the filled line represents the CT of the positive control for *Aspergillus* section *Fumigati*.

TABLE 3 General characteristics of the workers' samples.

Characteristics		*n* (%)	Minimum – Maximum	Mean ± Std. deviation
Age (years)			18–65	41.24 ± 10.56
Gender	Male	214 (66.9%)		
	Female	106 (33.1%)		
Height (cm)			148–198	169.97 ± 8.81
Weight (kg)			38–136	73.42 ± 13.66
Number of years in cork industry			0–51	11.02 ± 8.86
	<10 years	152 (47.5%)		
	≥10 years	168 (52.5%)		
Smoking habits	No	177 (55.3%)		
	Yes	118 (36.9%)		
	Ex-smoker[a]	25 (7.8%)		

[a]An adult who has smoked at least 100 cigarettes in his or her lifetime but who had quit smoking at the time of the interview.

TABLE 4 Spirometry data of cork workers according to smoking habits and exposure.

Smoker	Spirometry	Number of years in cork industry (exposure)									
		<10					≥10				
		n	Min	Max	Mean	SD	*N*	Min	Max	Mean	SD
No	FEV_1% Predictive	68	77.00	137.00	100.01	11.05	109	72.00	141.00	100.82	12.89
	FVC % Predictive	68	79.00	136.00	101.90	11.00	109	72.00	231.00	106.61	17.43
	FEV_1/FVC % baseline	68	58.00	96.00	82.56	6.76	109	62.00	97.00	80.92	5.88
Yes	FEV_1% Predictive	73	74.00	118.00	97.41	10.89	45	63.00	142.00	96.96	16.58
	FVC % Predictive	73	79.00	126.00	101.22	11.73	45	66.00	138.00	100.96	15.21
	FEV_1/FVC % baseline	73	60.00	96.00	80.66	6.45	45	65.00	92.00	78.82	6.61
Ex-smoker	FEV_1% Predictive	11	67.00	114.00	94.09	15.57	14	80.00	116.00	95.07	11.95
	FVC % Predictive	11	73.00	115.00	94.82	14.48	14	77.00	114.00	95.93	10.21
	FEV_1/FVC % baseline	11	74.00	87.00	82.27	3.88	14	68.00	88.00	80.43	6.28

Min, Minimum; Max, Maximum; SD, Standard Deviation.

TABLE 5 Ventilatory defects versus smoking habits.

		Smoker		Chi-Square test		
		No smoker	Smoker	Test statistic	df	*p*-vlaue
Ventilatory defects	Absence	131/198 (66.2%)	62/117 (53.0%)	5,376a	1	,020*
	Presence[a]	67/198 (33.8%)	55/117 (47.0%)			

[a]Presence corresponds to Obstructive or Restrictiveor Mixed; *Significant association at a 5% significance level.

TABLE 6 Ventilatory defects versus age.

		Age					Chi-Square test		
		<25	[25; 35]	[35; 45]	[45; 55]	≥ 55	Test statistic	df	*p*-vlaue
Ventilatory defects	Absence	14/17 (82.4%)	58/81 (71.6%)	64/87 (73.6%)	46/96 (47.9%)	11/34 (32.4%)	31,565[a]	4	,000
	Presence[a]	3/17 (17.6%)	23/81 (28.4%)	23/87 (26.4%)	50/96 (52.1%)	23/34 (67.6%)			

[a]Presence corresponds to Obstructive + Restrictive + Mixed; *Significant association at a 5% significance level.

found between the number of years of exposure and ventilatory defects ($\chi_1^2 = 0.586$, $p = 0.444$). However, although not significant, a 1.4-time higher risk of developing ventilatory defects [Odds Ratio = 1.411, Confidence $Interval_{95\%} = (0.582, 3.419)$] was found in smokers who worked for 10 or more years in the cork industry (Table 8).

In smokers who did not use RPE devices, a significant association was detected between respiratory defects and the number of years of

TABLE 7 Ventilatory defects and respiratory symptoms among exposed workers.

		Number of years in cork industry (exposure)		Qui-square test		
		<10 Count/ column total (%)	≥10 Count/ column total (%)	Test statistic	df	*p*-value
Ventilatory defects	Absence	101/149 (67.8%)	92/166 (55.4%)	5.058	1	0.025*
	Presence	48/149 (32.2%)	74/166 (44.6%)			
Cough regularly?	No	132/152 (86.8%)	138/168 (82.1%)	1.337	1	0.248
	Yes	20/152 (13.2%)	30/168 (17.9%)			
Do you have expectoration, regularly?	No	138/152 (90.8%)	149/168 (88.7%)	0.380	1	0.538
	Yes	14/152 (9.2%)	19/168 (11.3%)			
Do you have wheezing regularly?	No	152/152 (100%)	166/168 (98.8%)			0.500[b]
	Yes	0/152 (0%)	2/168 (1.2%)			
Do you have dyspnea regularly?	No	152/152 (100%)	168/168 (100%)			
	Yes	0/152 (0%)	0/168 (0%)			

[a]Changed corresponds to Obstructive + Restrictive + Mixed; [b] Fisher Exact Test; *Significant association at a 5% significance level.

TABLE 8 Ventilatory defects and respiratory symptoms among exposed workers in non-smokers and smokers.

			Number of years in cork industry (exposure)		Chi-square test		
			<10 Count/ column total (%)	> = 10 Count/ column total (%)	Test Statistic	df	*p*-value
No Smoker	Ventilatory defects	Absence	64/89 (71.9%)	78/139 (56.1%)	5.762	1	0.016*
		Presence[a]	25/89 (28.1%)	61/139 (43.9%)			
	Do you have a persistent cough?	No	83/91 (91.2%)	125/142 (88.0%)	0.586	1	0.444
		Yes	8/91 (8.8%)	17/142 (12.0%)			
	Do you have expetoration?	No	83/91 (91.2%)	132/142 (93.0%)	0.238	1	0.626
		Yes	8/91 (8.8%)	10/142 (7.0%)			
	Do you have wheezing regularly?	No	90/91 (98.9%)	137/142 (96.5%)			0.408[b]
		Yes	1/91 (1.1%)	5/142 (3.5%)			
	Do you have dyspnea?	No	91/91 (100%)	139/142 (97.9%)			0.283[b]
		Yes	0/91 (0%)	3/142 (1.3%)			
Smoker	Ventilatory defects	Absence	44/78 (56.4%)	19/48 (39.6%)	3.365	1	0.067
		Presence[a]	34/78 (43.6%)	29/48 (60.4%)			
	Do you have a persistent cough?	No	73/79 (92.4%)	41/48 (85.4%)			0.237[b]
		Yes	6/79 (7.6%)	7/48 (14.6%)			
	Do you have expetoration?	No	69/79 (87.3%)	38/48 (79.2%)	1.504	1	0.220
		Yes	10/79 (12.7%)	10/48 (2.8%)			
	Do you have wheezing regularly?	No	78/79 (98.7%)	48/48 (100%)			1.000[b]
		Yes	1/79 (1.3%)	0/48 (0%)			
	Do you have dyspnea?	No	78/79 (98.7%)	47/48 (97.9%)			1.000[b]
		Yes	1/79 (1.3%)	1/48 (2.1%)			

[a]Presence corresponds to Obstructive + Restrictive + Mixed; [b]Fisher Exact Test; *Significant association at a 5% significance level.

exposure (χ_1^2 = 5.399, p = 0.020), and it was found that workers who were in for 10 or more years in the cork industry presented a two-fold higher risk of developing respiratory defects [Odds Ratio = 2.190, Confidence Interval$_{95\%}$ = (1.124, 4.270)]. In non-smokers who used RPE, no significant association was detected (χ_1^2 = 0.213, p = 0.644). Regarding smokers who did not use an individual respiratory

protection device, a significant association between the number of years of exposure and ventilatory defects was found (χ_1^2 = 4.356, p = 0.037). Those who have been working for 10 or more years in the cork industry had a two-fold increased risk of developing respiratory defects [Odds Ratio = 2.269, Confidence Interval$_{95\%}$ = (1.045, 4.928)]. Finally, for workers who smoked in the past and who used RPE, no significant association between respiratory defects and years of exposure was found (χ_1^2 = 0.034, p = 0.853) (Table 9).

No significant association was found between the presence of *Penicillium* section *Aspergilloides* and *Aspergillus* section *Fumigati* in nasal swabs with respiratory symptoms (p = 1.000 and χ_1^2 = 0.007, p = 0.934, respectively, Table 10). However, it was observed that in the absence of both fungal species, the majority of workers did not have any respiratory symptoms (99.5 and 89.6%, respectively) (Table 10).

Considering only the cases in which the presence of *Penicillium* section *Aspergilloides* was detected, no statistically significant differences were observed between those workers who did not have respiratory symptoms and those who had (t_{30} = −0.791, p = 0.435). However, it was verified that those workers who had symptoms presented slightly higher average values of CFU ($Mean_{No\ symptoms}$ = 34.75 ± 2.87, $Mean_{With\ symptoms}$ = 35.61 ± 3.23).

4 Discussion

4.1 Main findings

This study found a significant association between ventilatory defects and years of work in the cork industry. Indeed, the risk of having ventilatory defects was approximately two-fold higher in workers who had worked for 10 or more years compared to those who had worked for less than 10 years in this industry. The same trend was observed in smokers who did not use respiratory protective equipment and worked for 10 years or more. Although *Penicillium* section *Aspergilloides* was detected in workers´ noses, the study did not find any association between respiratory effects and fungal contamination.

4.2 Nasal fungal contamination

Chrysonilia sitophila was the most prevalent fungi on workers' noses, according to previous environmental monitoring at the same plants (12). Higher fungal diversity was observed by workers' nose sampling [compared to environmental sampling obtained in the previous study (12)] and in Plant A (compared to the other two plants). In workers from plant A, *Penicillium* sp. was the most prevalent fungal genus, whereas, in workers from the other two plants, *C. sitophila* was dominant. The number of workers and type of activities inside the facilities appears to influence fungal contamination (32–34). In fact, Plant A had fewer workers and produced only cork boards, while Plants B and C produced more cork-derived articles [natural bottle corks, cork tiles, papers, and textiles (12)]. The organic dust contamination present in the cork industry is critical for workers' exposure since particles act as carriers of fungi to the upper airways (34–36). Fungal geographic distribution and dominance also vary with climate-driven patterns (37, 38), which explains the differences observed in Plant A, located in south Portugal, with warmer average temperatures.

TABLE 9 Smoking habits versus always use RPE versus number of years of exposure.

			Number of years in cork industry (exposure)		Chi-square test		
			<10	≥10	Test Statistic	df	*p*-value
Non-smoker and does not always use individual protection	Ventilatory defects	Absence	48/66 (72.7%)	56/102 (54.9%)	5.399[b]	1	0.020*
		Presence[a]	18/66 (27.3%)	46/102 (45.1%)			
Non-smoker and always uses individual protection	Ventilatory defects	Absence	12/18 (66.7%)	18/30 (60.0%)	.213[b]	1	0.644
		Presence[a]	6/18 (33.3%)	12/30 (40.0%)			
Smoker and does not always use individual protection	Ventilatory defects	Absence	40/68 (58.8%)	17/44 (38.6%)	4.356[b]	1	0.037*
		Presence[a]	28/48 (41.2%)	27/44 (61.4%)			
Smoker and always uses individual protection	Ventilatory defects	Absence	4/9 (44.4%)	2/4 (50.0%)			1.000[b]
		Presence[a]	5/9 (55.6%)	2/4 (50.0%)			

[a]Presence corresponds to Obstructive or Restrictive or Mixed; [b]Fisher Exact Test; *Significant association at a 5% significance level.

TABLE 10 Prevalence of respiratory symptoms according to the presence of *Penicillium* section *Aspergilloides* and *Aspergillus* section *Fumigati* (qPCR results).

			Ventilatory defects		Qui-Square test		
			Absence	Presence	Test Statistic	df	*p*-value
Aspergillus section *Fumigati*	Absence	count/row total (%)	192/193 (99.5%)	121/122 (99.2%)			1.000[a]
	Presence	count/row total (%)	1/193 (0.5%)	1/122 (0.8%)			
Penicillium section *Aspergilloides*	Absence	count/row total (%)	173/193 (89.6%)	109/122 (89.3%)	0.007	1	0.934
	Presence	count/row total (%)	20/193 (10.4%)	13/122 (10.7%)			

[a]Fisher Exact Test.

In this study, qPCR enabled the detection of *Penicillium* section *Aspergilloides* in 25 samples where *Penicillium* sp. had not been identified by culture. On the other hand, in highly contaminated environments, fast-growing species such as *C. sitophila* can inhibit the growth of *Penicillium* and *Aspergillus* sp. in culture (33, 34, 39, 40). Furthermore, molecular detection can be underestimated due to PCR inhibitors such as environmental contaminants (e.g., dust) (41, 42). Importantly, for occupational exposure assessments, it is crucial to determine the viability of microorganisms as it relates to inflammatory and cytotoxic effects (34, 40, 43–46). Altogether, this evidence highlights the relevance of combining culture-based methods with molecular detection (30, 47, 48).

Although workers in whom *Penicillium* section *Aspergilloides* was detected showed slightly higher values of symptoms, the association between this contaminant and respiratory disorders (10, 13) was not significant in this study. *Aspergillus* section *Fumigati*, on the other hand, was detected in two workers and can explain their reported symptoms. *Aspergillus* section *Fumigati* is commonly related to respiratory symptoms due to the small size of the conidia and to other virulence factors. Allergic bronchopulmonary aspergillosis (ABPA), rhinitis, rhinosinusitis, and severe asthma with fungal sensitization (SAFS) are some of the diseases more often associated with occupational exposure to *Aspergillus* genera (49, 50).

Whereas *Aspergillus* section *Fumigati* is critical for its public health burden and urgent need for surveillance, Mucorales and *Fusarium* spp. are also prioritized due to limited therapeutic options and fungal cross-resistance to azoles used in agriculture (21). The agricultural use of azole fungicides has been linked to the emergence of antifungal resistance in clinical practice (51). To prevent fungal infections of cork oaks, azole fungicides such as tebuconazole and benzimidazole have been used (52, 53), making the cork production sector a hotspot for the development of azole resistance. To prevent antifungal resistance, it is crucial to raise awareness and adopt interlinked, integrated, and innovative multisectoral approaches to surveillance in occupational exposure assessments.

4.3 Spirometry

Considering lung function evaluation, both smokers and non-smokers with longer exposure showed a higher prevalence rate of ventilatory defects.

We observed a significant association between ventilatory defects and years of work in the cork industry. In fact, those people who worked for 10 or more years in this industry had an approximately two-fold increased risk of having ventilatory defects. This is of particular relevance to demonstrate causality between working in the cork industry (prone to organic dust and fungi) and ventilatory defects and agrees with results previously published (6, 7, 54, 55). The average number of years of workers in the cork industry analyzed in this study was relatively small (11.02 ± 8.86 years). As such, a further increase in the years of exposure (only 52% had more than 10 years of exposure) might have an important effect on worker's health. However, the "healthy worker effect" (HWE) needs to be considered, given that severely ill and chronically disabled are commonly excluded from employment (56), leading to lower overall death rates or morbidity when compared with the general population. Other occupational epidemiologists simply describe HWE as the reduction of mortality or morbidity of occupational cohorts when compared with the general population (57). It is a special form of selection bias common to occupational cohort studies previously noted in populations occupationally exposed to different risk factors (36, 58). This might imply that more workers have health effects but already left the company at the moment of the study, resulting in the employed workforce having fewer sick people than expected. Moreover, the ventilatory defects observed in cork industry workers engaged in this study can be due to the combination of different risk factors present in the cork industry, such as cork dust and fungal contamination (54). Previous studies noted that occupational exposure could present higher health impacts among workers than smoking and that both exposures resulted in worse outcomes (59). Furthermore, long-term exposure in susceptible individuals may lead to lung fibrosis and, therefore, a restrictive ventilatory defect (60). Thus, smokers who have already had an airway disease and the related obstructive ventilatory defect are also expected to have a restrictive defect.

5 Conclusion

Our study showed that working in the cork industry may have adverse effects on worker's respiratory health. Even using a relatively low exposure-time window, it was possible to detect health effects in workers, evidencing the need to invest in risk management measures that can eliminate or reduce exposure to fungi and dust in this setting. Cork industry workplaces normally have high contamination of both fungi and dust. Thus, preventing exposure to organic dust also prevents exposure to fungi. Therefore, process containment and enclosure and, if not achievable, adequate ventilation systems (general mechanical ventilation and proper local exhaust ventilation) should be implemented. If these options are not possible to implement, then respiratory protection devices must be chosen and available as the only protection measures, particularly in tasks that involve manual handling of cork. Nevertheless, more studies are needed (e.g., using serological assays) to clarify the impact of each risk factor (fungi and dust) on disease etiology.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CV: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. MD: Formal analysis, Writing – original draft. CP: Formal analysis, Writing – original draft. TF: Formal analysis, Writing – original draft. AC: Formal analysis, Resources, Writing – original draft. HB: Formal analysis, Resources, Writing – original draft. LC: Formal analysis, Writing – original draft. EC: Formal analysis, Writing – original draft. AG: Formal analysis, Writing – original draft. SV: Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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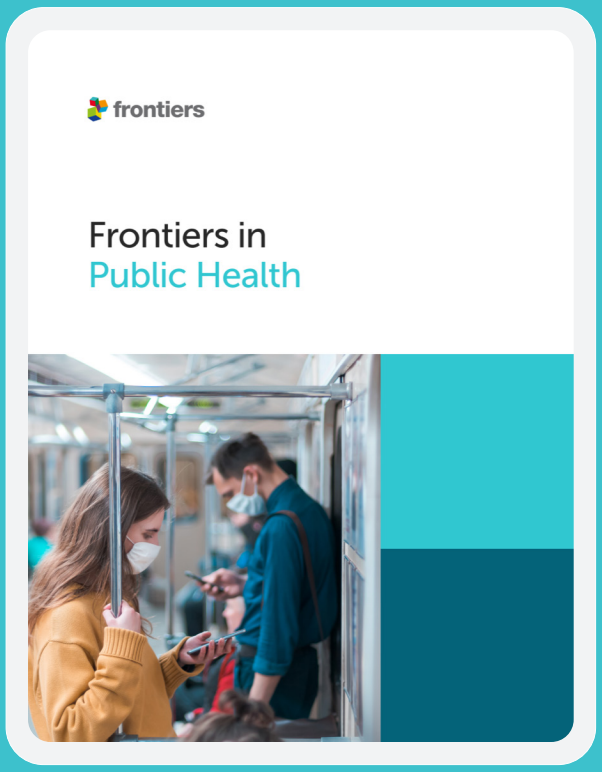

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