Research Article

Particle matter, volatile organic compounds, and occupational allergens: correlation and sources in laboratory animal facilities

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Abstract
Exposure to airborne allergens from mice and rats can lead to laboratory animal asthma or allergy. Several biological methods can measure allergens contained in aerosols; however, they are time and cost intensive. An innovative methodology is proposed to warn laboratory animal facility workers of a possible rise in mouse and rat allergens by measuring the relationships between airborne allergens, particulate matter (PM), and volatile organic compounds (VOCs). By using a low-cost sensor (average difference respect to reference methods of 3 and 9% for $PM_{2.5}$ and VOC, respectively), Spearman's rank correlation between allergens and time-averaged PM and VOCs was found to be 0.3 and − 0.07, respectively. These numbers indicate a poor correlation between allergens and $PM_{2.5}$ and VOC; however, by considering only the spikes in PM minute-by-minute data, the relation between time-average PM and allergens increases up to 0.71. This high value indicates the applicability of PM low-cost sensors in laboratory animal centers as a warning sign of raising values of allergens. Mouse and rat allergens are present in the animals' urine, which can become aerosolized during animal activity, or task activities carried out in the laboratory facility. While previous references established a correlation between activities and mouse and rat allergens, the results are outdated and refer to a limited number of activities. For example, washing lab coats, changing uniforms, sitting in an office space, having lunch, or walking in any corridor are shown in this study to contain on average 1.77, 0.96, 0.65, 0.88, and 1.62 ng/m^3 of rat allergens, respectively. Thus, locations that do not contain any direct source of allergens are positive to the presence of mouse and rat allergens.

Keywords Rat and mouse allergies · Task-based allergens · Particulate matter · Volatile organic compound · Real-time monitoring

1 Introduction

In laboratory animal facilities (LAFs), animal allergens and ammonia are the main sources of indoor pollution [12, 44]. Rats and mice are the most common animals used in laboratory research [12]. Urine from rats and mice is the main source of allergenic proteins, rat urinary allergen, (RUA) and mouse urinary allergen (MUA), respectively. Airborne allergens are inhaled by workers in LAFs [24]. Exposure to RUA or MUA can cause workers to develop an occupational allergy. One-third of LAF workers develop an occupational allergy, and a third of these have symptomatic asthma [24].

ELISA (enzyme-linked immunosorbent assay) is commonly selected to quantitatively measure RUA and MUA

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produced by mouse and rat urines [12, 17, 24, 25, 28]. The sensitivity of the ELISA is enhanced by adding a second antibody, called "sandwich ELISA" [28, 46]. While ELISA-type assays are highly specific, with the ability to measure RUA and MUA as dilute as 0.029 ng/ml [32], this technique requires extensive sample preparation [47].

Most airborne RUA and MUA of concern are carried on small particulate matter (PM) that ranges in size from 0.8 to about 8 µm [25, 53]. Most of these particles can become "airborne bystanders" by remaining suspended in the workplace air for more than 60 min [29]. Larger carrier particles (10–100 µm) tend to sediment in the upper respiratory tract preventing allergens from entering the lungs. Consequently, airborne RUA and MUA are commonly collected along with PM smaller than 100 µm (PM_{100}) [53], which are considered the inhalable. If a known particle size is investigated, a correlation between PM size and airborne RUA and MUA mass can be defined. Nieuwenhuijsen et al. [39] found a relationship between total dust and RUA measurements without, however, using a large number of samples and plotting the data. Burstyn et al. [11] determined that judged that PM is an unjustifiably costly proxy of exposure to allergens. However, this conclusion was achieved by measuring dusts generated in bakeries. Another expected relationship is between airborne RUA and MUA and volatile organic compounds (VOCs) since urine also contains ammonia and numerous VOCs.

A low-cost methodology that correlates PM and VOCs with airborne allergens would be much easier to implement in LAFs for day-to-day monitoring versus the ELISA method. This methodology might not substitute a more accurate and traditional procedure [26], but it could allow early warning of high allergen levels. Moreover, by analyzing the PM and VOC concentrations, the sources of RUA and MUA emissions could be identified.

The past studies have investigated processes where workers are exposed to elevated levels of allergens in LAFs [15, 20, 21, 42]. Feary et al. [21] determines that modern LAF show a lower presence of airborne allergens. They verified the presence of airborne allergens respect to the room type and the ventilation system. Glueck et al. [24] collected allergens in the personal breathing zones of 7 caretakers during full workdays for one week. They found that the number of cages and animals handled proportionally increases RUA and MUA exposures. However, due to a short collection time (about 15 min), only 4 of 39 samples yielded results above the limit of detection (LOD), precluding further statistical analysis. Eggleston et al. [19] used personal air sampling devices and showed that exposure level was related to the tasks performed, with 3 to 10 times higher allergen concentrations during tasks that dealt with 'active animals' (feeding, cleaning, handling) compared with 'inactive animals' (surgery, euthanasia). Gordon et al. [25] showed that lower stocks of dirty cages and closed type cages can decrease the quantity of airborne allergens. Wald and Stave (2003) noted that personal exposures tasks, such as cage changing, surgery, and cage processing, were tenfold higher than nearby stationary samples even if the stationary samples were collected in close proximity to each task. Personal air sampling has shown that exposure intensity varies with the facility, type of job, type of tasks performed and task duration. Most studies related to airborne RUA and MUA in LAFs are older than 20 years; since over this period of time, engineering controls change, there is need for a more updated analysis [30].

Therefore, this project has two main goals: (1) to investigate an innovative methodology that can correlate with the measurements of airborne RUA and MUA, and (2) to quantitatively verify, by using the reference method, the major sources of airborne mouse and rat urinary allergens in a LAF. To achieve these goals, we used different instruments and techniques in different environments of the LAFs in order to stress, if any, the correlation between rat and mouse allergens and other common indoor pollutants, such as PM and VOC. The employment of low-cost sensors could potentially be useful as a warning sign of sudden rises in allergen levels; a warning can prevent exposures to high levels of allergens and the chance of a worker in LAFs to develop occupational allergies.

2 Materials and methods

2.1 Samples collection

Particulate matter (PM), volatile organic compound (VOC), and rat and mouse allergens are the indoor pollutants of interest in an animal care center. PM was measured mostly using the uHoo low-cost monitors. A total of six units were available which were interchanged while used in different activities; two units were used at each activity. However, in several occasions, a TSI DustTrak II 8532 Aerosol Monitor was used to record the PM at different size bins. Moreover, as a further validation of the low-cost sensors, $PM_{2.5}$ was collected on a filter; such collection allowed to derive the weight of the $PM_{2.5}$ per collection time. Previous references suggested to collect PM_{100} in order to determine the amount of mouse and rat allergens contained in inhalable dusts [27]. Thus, PM_{100} was collected using a system of a pump and a sampler.

The samples were collected by static devices placed at about 1 m of distance from the main activity. Furthermore, all the devices were placed close to each other on a stand with a height of one meter and a half. An image of collection station is reported in Fig. 1.

Fig. 1 Image of the collection station including uHoo low-cost monitors, DustTrack monitor, and a system of pump and a seven-hole sampler. The measured indoor pollutants per each device are shown in brackets

2.1.1 PM_{100} and allergen samples

PM_{100}, which is also called inhalable [36], and allergen exposures were quantified on the same filter. The samples were collected using a seven-hole sampler (Head for MDHS 14, SKC Inc.). Teflon filters of 25-mm diameter and 0.45-µm pore size were placed in the seven-hole sampler which was connected to the collection pump through a ¼ inch tube. A GilAir Plus personal sampling pump was calibrated, using a gilibrator, at 2 lpm and placed in an area for sampling. The filters were pre- and post-weighed three times by manual gravimetric analysis using a Sartorius micro balance. A total of six field blanks, two per each facility, were collected. One transportation blank filter was collected per each facility. Filters were removed from the cassette by using tweezer cleaned with ethanol and each cassette was cleaned before using.

2.1.2 $PM_{2.5}$ samples

$PM_{2.5}$, which is also called fine inhalable, exposures were measured using an Ultrasonic Personal Air Sampler. Teflon filters of 37-mm diameter and 2-µm pore size were placed in the UPAS's internal cassette. The UPAS was calibrated at 1 lpm. A total of six field blanks, two per each facility, were collected. One transportation blank was collected per each facility. The filters were pre- and post-weighed by automated gravimetric analysis.

For these samples as well as the previously described PM_{100} samples, the filters were conditioned for at least 24 h before weighing. The recorded temperature and relative humidity of the weighing room were between 20 and 23℃ and 30 and 40% RH, respectively. Static charges on filters were removed by passing them between ionizing cartridges. The weighting procedure was repeated at least three times per filter.

2.1.3 Volatile organic compound (VOC)

The total VOC were measured in real-time using both a reference method and low-cost sensors, as shown in the following sections. Sorbent tubes were not used since they commonly serve to collect one or more specific VOC.

2.2 Analytical reference methods

2.2.1 Particulate matter (PM)

Light scattering is the most common technique used in recent studies involving portable, real-time PM measurements [22, 31]. Benefits of light scattering include the low-cost, suitability for a broad range of PM sizes (0.2–700 µm), ease of calibration, and fast analysis time (about 1 min) [2, 4, 5]. The supply of low-cost, real-time sensors that measure a broad range PM sizes is constantly growing. However, they usually have lower accuracy compared to other reference methods [6, 8, 13, 54]. A DustTrak DRX 8532 Aerosol Monitor was used to record PM with a diameter lower than 1 µm (PM_1), 2.5 µm ($PM_{2.5}$), and 10 µm (PM_{10}). Gravimetric analysis is a verification of the results obtained with light scattering techniques.

2.2.2 Volatile organic compound (VOC)

OSHA has published Method ID-188 for measuring ambient ammonia concentrations in the workplace (Safety and Administration [48]. This reference method has several disadvantages, such as the use of toxic liquids, long sample preparation times, and complicated sample analysis

procedure [35, 43]. A few portable ammonia sensors are commercially available, although they provide lower accuracy than the OSHA reference method [9, 16, 49]. In LAFs, ammonia is a major volatile contaminant. Measuring VOC could be enough to estimate ammonia levels [49]. In the facilities tested, levels of ammonia were periodically tested using GX6000 from RKI Instruments.

2.2.3 RUA and MUA allergens

ELISA-sandwich assays were used to quantify airborne RUA and MUA. Mus m 1 and Rat n 1 ELISA kits were purchased from Indoor Biotechnologies. The analytical procedure has been extensively described in previous references [1, 41, 46]. A PM_{100} filter was used for the collection of airborne allergens.

2.3 Low-cost multi-channel sensor

The low-cost monitors selected were uHoo Indoor Air Quality monitors. These sensors have been used in other indoor environments [5, 7] and have been selected due to their availability and low cost. Values recorded with uHoo monitors ($PM_{2.5}$ and VOC) in one LAF were compared with reference devices. More information on some properties and the calibration of the low-cost monitors are reported in Table S.1, Figure S.1, and Figure S.2 in the Supplementary Information (SI).

2.4 Data analysis

In the literature, there is no clear definition of which levels of rat and mouse allergens is concerning for developing occupational allergens. However, to easy distinguish the cases studies, levels of allergens are considered low when below 0.5 ng/m^3 and high when above 5 ng/m^3. Concentrations of airborne PM and airborne allergens are commonly shown in a logarithm scale [10, 38]. The time-averaged values of PM are commonly shown [23]. An analysis of the spikes in real-time data of PM is contained in a few previous references [10, 40].

Methods to analyze peaks in real-time data can be multiple and complicated [45]. Therefore, the selection of different average percentiles can generate valuable and easily accessible information on peaks and spikes of real-time data. The selection of the 15th and 5th percentiles was arbitrary, though, it provided insight into the peak concentrations of indoor pollutants over the data collection period. Similar parameters have been used in previous references [5, 18, 33]. To determine these two parameters ($\mu_{15\%}$ and $\mu_{5\%}$), the 15th and the 5th percentile of the minute-by-minute data of each test were determined. Per each test, OriginPro was used to generate a normal probability distribution, from which the percentiles were extracted. Subsequently, the minute-by-minute data remained above the 15th and 5th percentile were averaged generating, thus, the variables $\mu_{15\%}$ and $\mu_{5\%}$. In order to consider the peaks of real-time data, only the positive percentiles were used. Other average percentiles, 35, 25, and 2%, have been tried and results are shown in Table S.2 in the SI. Lower percentiles would, in some case, represent one or two peaks, concerning then, data of only a few minutes. Higher percentiles poorly differed from the total average μ and minor differences are encountered. Subsequently, to correlate one pollutant, PM or VOC, and the rat or the mouse allergens, a linear regression and a Spearman's rank correlation was performed by using OriginPro [3]. A Spearman correlation was selected due to the nonlinear data representation.

2.5 Characterization parameters

2.5.1 Facilities

Three LAFs located at the University of British Columbia were tested. The facilities differed according to their operating procedures and, for simplicity, they are named 1, 2, and 3. Facilities 1 and 2 contain only rats and mice and are located underground. Each room of these facilities shows an average value of air changes per hour of about 15. Facility number 3 is located at ground level and operates many large-animal containment stalls open to the outdoors. In this facility, while every room has a similar ventilation flow than the other facilities, one room for cage changing has no ventilation while tests were performed.

2.5.2 Cage types

The cages differed in ventilation system, size, and insulation. "Opti" (Optirat and Optimice for mouse and rat, respectively) cages are considered isolated since it can be connected to a ventilation system that helps remove airborne contaminants [37]. Other types of cages include static close top ("close top"), static open top ("open top"), static half-opened ("half-open"), and large metal cages ("metal"). Static cages do not have a localized ventilation system; the close and open top cages differ in the presence and absence of a cover, respectively. In the half-open cages, a portion of the cage top is opened. The large metal cage is fully opened and is used for rats and large rodents.

2.5.3 Activities analyzed

Table 1 shows the locations and activities analyzed in the three facilities ("F") tested. The columns labeled "Person" and "Min" refer to the average number of people and the

Table 1 List of each activity analyzed along with the major species, number of people (Person), average time spent by each occupant (Min), number of cages (Cage), cage type (Type), related facility (*F*), and the use of the low-cost sensors (LC)

Group	ID	Species	Person	Min	Cage	Type	*F*	LC	Comments
Overnight	a	Rat	1	15	49	Open top	1	NA	No uncommon activities
	b	Rat	1	15	67	Opti	1	×	No uncommon activities
	c	Rat	1	15	33	Opti	2	×	No uncommon activities
	d	Rat	1	15	22	Metal	3	×	No uncommon activities
	e	Mouse	1	15	240	Opti	2	×	Reproductive activity
	f	Mouse	1	15	214	Opti	2	NA	No uncommon activities
Lunch	a	Mouse	5	90	NA	NA	1	NA	Inside the controlled area
	b	Both	6	90	NA	NA	1	NA	
	c	Both	15	120	NA	NA	3	NA	Outside the controlled area
Laundry	a	Both	2	90	NA	NA	1	×	Closed door
	b	Both	2	90	NA	NA	3	×	Opened door to dirty cage processing
Office	a	Both	1	120	NA	NA	1	×	Closed doors, access only to managers and inside controlled area.
	b	Both	4	90	NA	NA	2	×	Closed doors and inside controlled area.
	c	Both	6	240	NA	NA	3	×	Opened doors and outside controlled area.
Changing laboratory	a	Both	10	30	NA	NA	2	×	Male changing room
	a	Both	10	240	NA	NA	3	×	Classroom for veterinary students
	b	Both	15	180	NA	NA	2	×	Use of bioluminescence, isoflurane anesthetic
	c	Both	1	300	NA	NA	3	×	Imaging and analyzing samples
	d	Both	1	60	NA	NA	3	NA	Hydrogen peroxide to dissolve carcasses
Corridor	a	Both	4	30	100	Opti	1	×	High traffic for rat cages (at the corner of two hallways)
	b	Both	4	10	200	Close top	1	NA	Cages passing by before cleaning area
	c	Both	50	10	50	Opti	2	NA	Joining hallway clean and dirty areas
	d	Both	5	15	500	Opti	2	×	Hallway before dirty cage processing
	e	Both	5	15	50	Open top	3	×	Empty cages for future used
	f	Both	50	30	50	Opti	2	×	Dirty cages storage before cleaning
Surgery	a	Rat	20	300	NA	NA	2	NA	Invasive procedures, anesthetics, isoflurane, CO_2
	b	Mouse	10	300	NA	NA	2	×	
	c	Mouse	2	360	NA	NA	1	NA	Brain surgery
	d	Rat	1	360	NA	NA	1	NA	Stereo tax
Health check	a	Rat	2	45	33	Opti	2	×	Typical heath check
	b	Rat	2	45	22	Metal	3	×	Socializing with rats
	c	Rat	2	45	12	Open top	3	NA	Typical health check
	d	Mouse	1	5	33	Opti	1	NA	Nylon bagged Opti cages
	e	Mouse	3	45	25	Opti	2	NA	Typical health check
	f	Mouse	2	45	99	Opti	2	NA	Typical health check
Cage changing	a	Rat	1	150	40	Half open	1	×	Typical cage changing procedure
	b	Rat	1	150	67	Open top	1	×	Typical cage changing procedure
	c	Rat	1	150	20	Half open	1	×	Typical cage changing procedure
	d	Rat	1	210	21	Open top	1	×	Close to vacuum
	e	Rat	1	120	48	Open top	1	×	High traffic of coming and going cages
	f	Mouse	1	150	105	Opti	1	NA	Typical cage changing procedure
	g	Mouse	1	150	131	Open top	1	NA	Typical cage changing procedure
	h	Mouse	3	210	214	Opti	2	NA	Close to vacuum
	i	Mouse	4	210	248	Opti	2	×	Close to vacuum
	l	Mouse	4	210	50	Opti	2	NA	Close to vacuum
	m	Both	2	150	154	Opti	2	×	Typical cage changing procedure

Table 1 (continued)

Group	ID	Species	Person	Min	Cage	Type	*F*	LC	Comments
Clean cage processing mostly for storage	a	Both	2	240	NA	All	1	×	Sterile holding (sterilization station)
	b	Both	4	60	NA	All	2	×	
	c	Both	1	240	NA	All	1	NA	Unloading and filling cages with bedding
	d	Both	6	270	NA	All	2	NA	Receiving, clean cages, assembling
Dirty cage processing	a	Both	2	210	700	All	1	×	Near bottle station, far from tunnel washer
	b	Both	2	480	700	All	1		Dumping cages and loading on washer
	c	Both	3	480	500	All	2	×	Higher number of mice cages (> 300)
	d	Both	4	210	50	All	3	×	Typical dirty cage procedure

In addition, some comments are added where additional information on the location or the activity are shown

average minutes spent by each worker in each room per day, respectively. Cage number and type are listed for each location and activity under "Cage" and "Type," respectively. Allergens, gravimetric PM_{100}, and gravimetric $PM_{2.5}$ were tested; however, real-time PM and VOC monitoring was used in a few activities, as indicated by "×" in the column labeled "LC." The "overnight" activities covered the time gap between 4 pm and 9 am of the next day. Rats and mice are nocturnal animals, thus more active at night. Some workers might spend 15 min in each location during overnight shifts. The cage processing activities of "clean cage processing" and "dirty cage processing" identify clean cages that are mostly stored and assembled and dirty cages that are emptied of their bedding, placed in the tunnel washer, and placed on a rack to dry, respectively.

3 Results

Analyzing the rat and mouse allergen in different LAFs generated two main outcomes, generating an alternative technique to estimate the airborne allergens and determining the typical causes of allergens in a LAF. The first outcome can be achieved by comparing other indoor pollutants common in a LAF and rat and mouse allergens. The second outcome can be obtained by correlating different activities and locations with the number of airborne allergens.

3.1 Comparison between indoor pollutants and rat and mouse allergens

Two indoor pollutants are estimated to be connected with mouse and rat airborne allergens: VOC and PM. While VOC (which in the LAFs tested differs from ammonia measured levels of at most 11%) are commonly measured by using a meter or "real-time" devices, the most common technique to measure PM is the gravimetric analysis. The possibility of performing gravimetric analysis in parallel to another filter-based technique makes this technique the most common in deriving the mass of PM [52]. However, the gravimetric analysis can show a few drawbacks, such as high losses involved with transportation or manual handling of the filters, static charge of the filter media, and dependence on the environmental conditions [52]. Therefore, there is no visible trend between the PM reached using gravimetric analysis and mouse or rat allergens, Fig. 2. In addition, the poor correlation between PM_{100} and allergens could be also due to the presence of other dust PM that does not contain animal allergens (images and chemical analysis of PM are shown in Figure S.5 in the SI).

With the aim to discover an alternative methodology to estimate the levels of mouse and rat airborne allergens in a typical LAFs, PM and VOC low-cost sensors are employed. Before the intense use of any low-cost monitors, which are commonly known for their poor accuracy, a validation procedure is required, and it can be achieved by comparing its reading with common reference methods. Real-time VOC measurements have been compared with a VOC meter (MiniRAE 3000), and the average difference is 9% (Figure S.1 in the SI). In particular, the difference between VOC low-cost sensor and the reference method is lower than 2% for levels below 30 ppb but at a maximum of 16% for levels higher than 60 ppb (Figure S.1 in the SI). Real-time PM has been compared with both gravimetric analysis and a TSI DustTrak II 8532 Aerosol Monitor. While the first comparison generates an average difference of 14%, the second shows only 3% (Figure S.1 in the SI and Fig. 3 a). The higher difference with the gravimetric analysis is mostly due to bias and precision errors involved in the gravimetric analysis [34]. Else, the differences between the selected low-cost monitor and the VOC meter and the aerosol monitor can be used as a validation of the applicability of these low-cost sensors to measure real-time pollutants in a LAF.

The most common size bin of PM measured by commercially available low-cost monitors is $PM_{2.5}$ [51]. However,

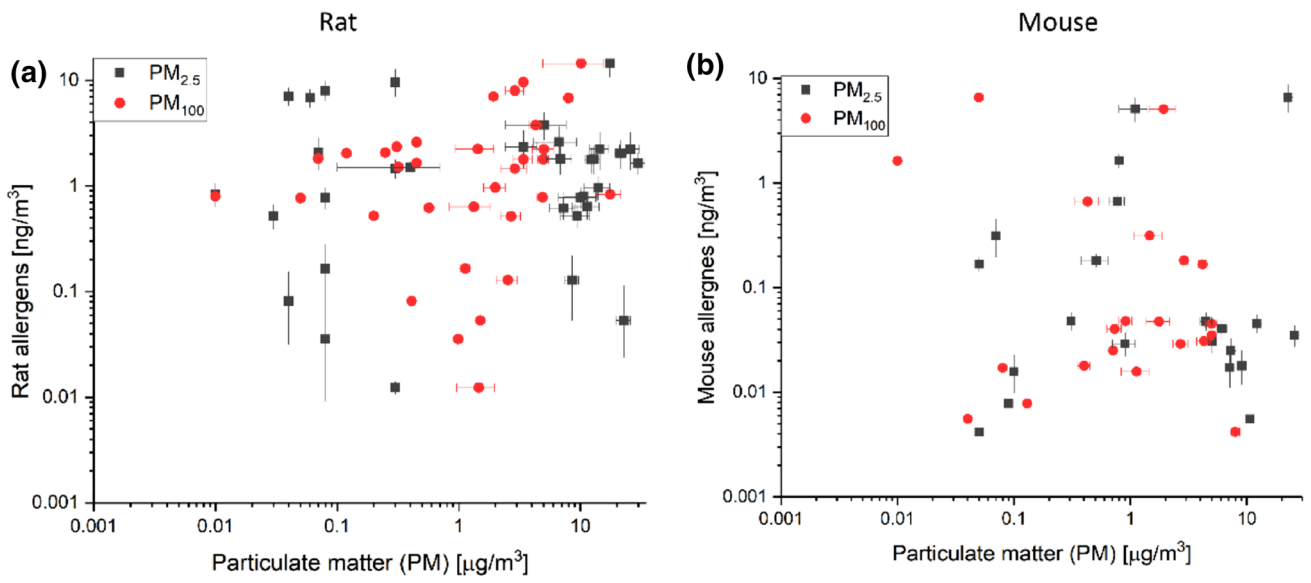

Fig. 2 Correlation between the particulate matter (PM) with a diameter lower than 2.5 ($PM_{2.5}$) and 100 µm (PM_{100}) obtained using gravimetric analysis and rat **a** and mouse **b** allergens

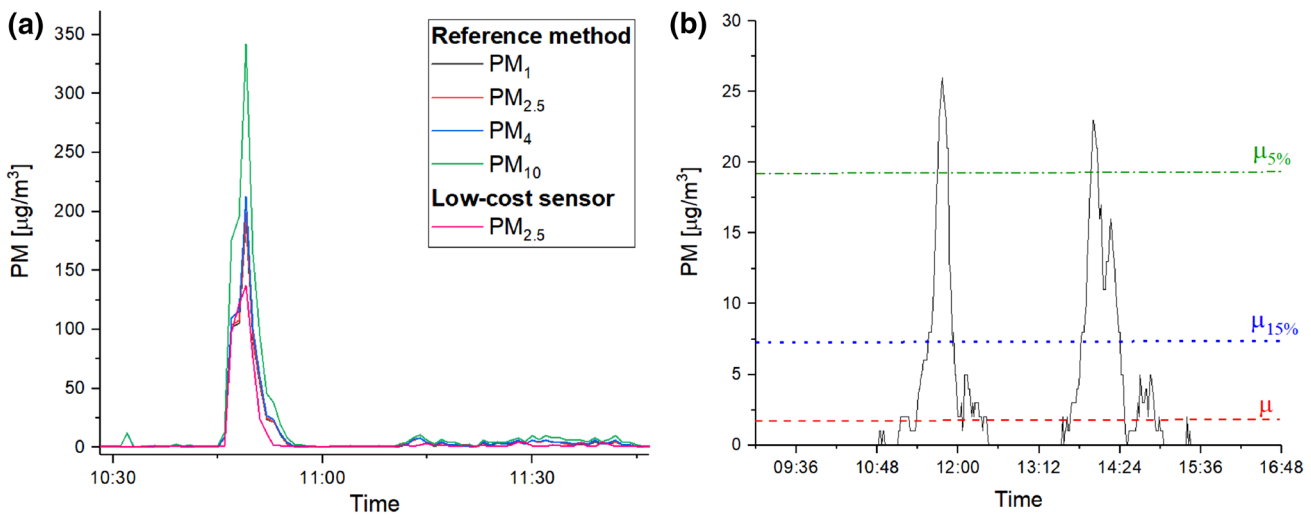

Fig. 3 **a** Measurement of various sizes of particulate matter using a real-time, low-cost sensor, and a TSI DustTrak (reference method). Measurements were collected during the representative sample of cage changing (i) (see Table 1). **b** Real-time concentration of $PM_{2.5}$ measured with the low-cost sensor during the activity of corridor (*d*) (see Table 1). In this example, the average µ of the total collection time, the average $\mu_{15\%}$ and $\mu_{5\%}$ of all the peaks contained in the 15th and 5th percentile, respectively

samples of airborne allergens are collected at the inhalable size bin of PM, which is considered to be PM_{100} [11]. Therefore, to motivate the use of a $PM_{2.5}$ low-cost monitor in estimating airborne allergens exposures, real-time variations of different size bins of PM need to be tested. DustTrak Aerosol Monitor has the ability of segregating real-time monitoring of PM according to their maximum diameter, 1 µm (PM_1), 2.5 µm ($PM_{2.5}$), 4 µm (PM_4), 10 µm (PM_{10}). As far as the authors know, PM_{10} is the highest dimension of PM that can be measured in real-time [14]. An example of real-time monitoring of four sizes of PM is shown Fig. 3 a), for the activity of cage changing (i) (see Table 1). Data obtained from a TSI DustTrak and the low-cost sensors are shown and, thus, Fig. 3a can be a visual example of the similarity in the measurements achieved by the two different methods.

The similarity of the trend of all PM size bins in time is clear in Fig. 3a. The distribution of the sizes of PM, such as

PM_{10}, PM_4, and PM_1 is very similar to the $PM_{2.5}$, which is more commonly used in commercially available low-cost sensors. By averaging all the data collected in this project, PM_{10} was about 35% greater than $PM_{2.5}$; however, PM_4 and PM_1 showed a difference of less than 5% compared to $PM_{2.5}$. This result suggests that analyzing the $PM_{2.5}$ can be a valid option for activities in a typical LAF.

An advantage of minute-by-minute data is to visualize sudden rises. In order to consider the spikes in real-time monitoring, the parameters $\mu_{15\%}$, and $\mu_{5\%}$ are introduced, as shown in Fig. 3b (and in Figure S.3 in the SI) that shows data for corridor (*d*) (see Table 1). While the parameter μ specifies the average concentration obtained using the low-cost sensor, the parameters $\mu_{15\%}$ and $\mu_{5\%}$ indicate the average concentration when only considering the 15th and 5th percentile out of the data collected. The selection of these two percentiles is arbitrary, the smaller is the percentile and the lower the number of peaks is considered. The analysis of these two percentiles was pursued to consider sudden rises in PM or VOC concentration which represented specific activities. A more detailed explanation of these two parameters is shown in a previous Ref. [5].

The averages $\mu_{15\%}$, and $\mu_{5\%}$ were used to look for a correlation between $PM_{2.5}$ and allergens Fig. 4a and b and VOC and allergens c) and d), shown in logarithmic scale. The error bars on the y and *x*-axis relate, respectively, to the systematic errors involved in the ELISA technique and to the differences between the two low-cost monitors used at each activity and location. The positive results obtained for the allergens are plotted against the averages μ, $\mu_{15\%}$, and $\mu_{5\%}$ of the data collected using the low-cost sensors. In case of $PM_{2.5}$, real-time measurements obtained with the reference method, TSI DustTrak, are not shown in Fig. 4 since a few activities were considered. The correlation between VOC, measured by low-cost monitors, and rat and mouse allergens, measured by using the ELISA technique, does not appear linear. It is evident by observing Fig. 4a

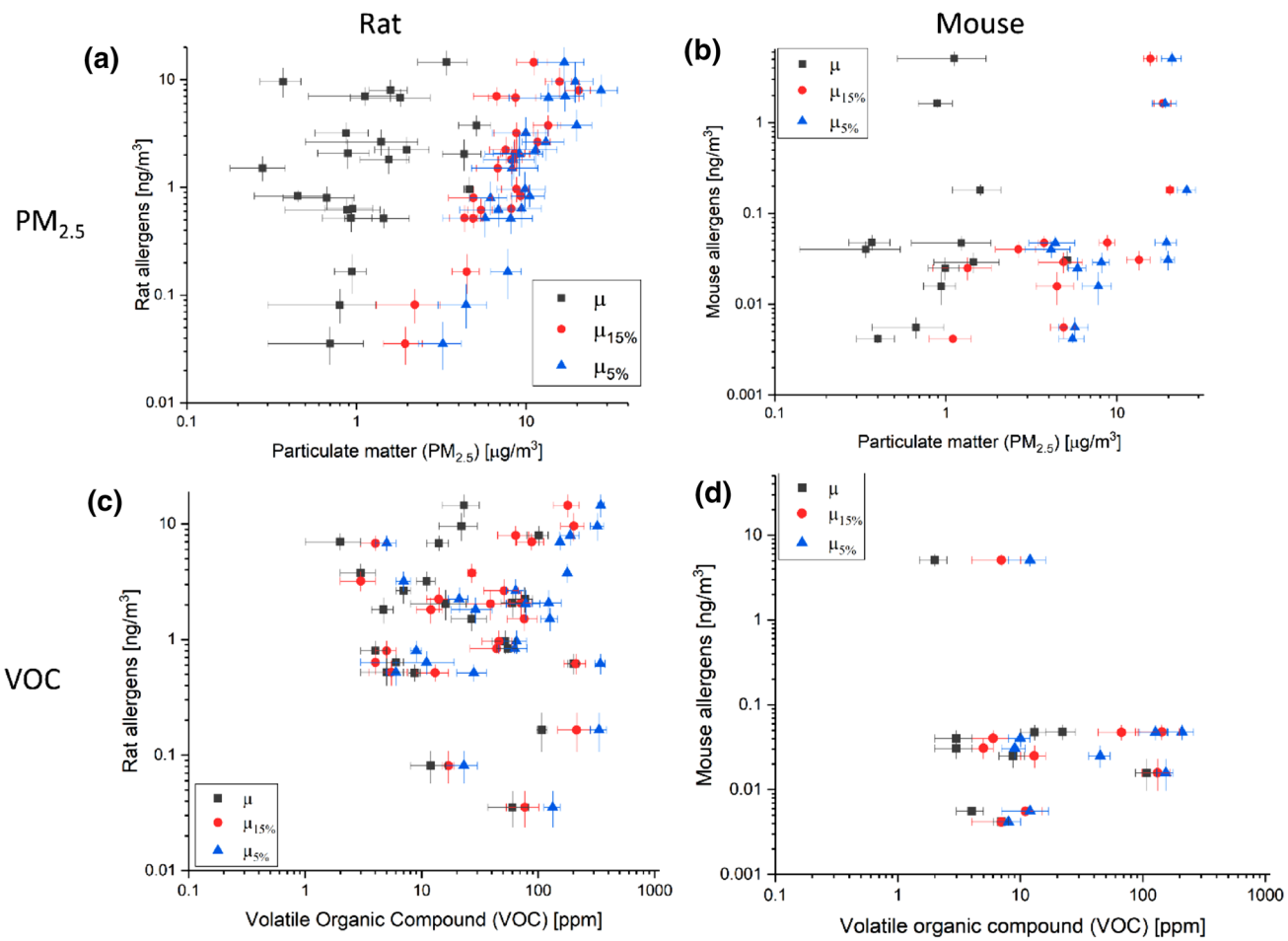

Fig. 4 Correlation between low-cost sensors measurements of $PM_{2.5}$ and rat (**a**) and mouse allergens (**b**) and between low-cost monitors measurement of VOC and rat (**c**) and mouse allergens (**d**). The average μ is calculated by averaging minute-by-minute data for the collection time. The average of the 15th and 5th percentiles are shown as $\mu_{15\%}$ and $\mu_{5\%}$, respectively

that the averages $\mu_{15\%}$, and $\mu_{5\%}$ of $PM_{2.5}$ show a more linear correlation with the rat allergens. Due to a lower number of cases with a value of mouse allergens above LOD, this correlation is less clear (Fig. 4b.

A visible and distinguishable trend between two variables is not sufficient to determine the linearity of their relation. Therefore, Table 2 shows additional parameters that help to further clarify the correlation between $PM_{2.5}$ or VOC and rat or mouse allergens. When the coefficient of determination R^2 is close to the unity, the linear relation is assumed to be optimal. The highest values of R^2 can be found in the regression between $PM_{2.5}$ and rat allergens. Rat allergens show a higher R^2 since more positive results were found; 8 and 27 out 60 samples yielded values above LOD for mouse and rat allergens, respectively. The differences between PM and allergens counts might be due to the presence, in some cases, of other sources of dust, such as rodent bedding.

The lowest values of R^2 relate to the regression between VOC and both mouse and rat allergens (0.13 and 0.2 for $\mu_{5\%}$). This suggests that VOC is probably not a valuable option to pursue in searching for an alternative technique to estimate the levels of airborne mouse and rat allergens. Such conclusion is confirmed by observing the other parameters introduced in Table 2. A possible reason of such poor linearity between VOC and airborne allergens is the systematic errors involved with VOC sensors. Since the term VOC gathers a broad range of gases, by definition, the selectivity of a VOC low-cost sensor is commonly low [50].

The selection of different percentiles of data enhances the linearity of any relationship shown in Table 2. For example, by considering the average of the 5th percentile of VOC concentrations, $\mu_{5\%}$, compared to the overall VOC average, μ, the Spearman's correlation coefficient with the rat allergens increases from −0.31 to 0.32. These values are, again, emphasizing a poor correlation between VOC and animal allergens. However, it does not surpass the high correlation number between $PM_{2.5}$ $\mu_{5\%}$ and rat allergens, 0.71. This value might appear low; nevertheless, it is considered showing a strong correlation due to the many variables involved in the differences in allergens generation between different facilities.

The good linearity of the regression between $PM_{2.5}$ $\mu_{5\%}$, and $\mu_{15\%}$ and rat allergens is confirmed also by the residual sum squares, the slope, and the intercept. The residual sum squares show the discrepancy between the data and an estimation model; thus, lower values indicate a tight fit between the two regressed variables. The lowest residual sum square can be found in the regression between $PM_{2.5}$ $\mu_{5\%}$, and rat allergens. Besides, this regression contains the lowest errors in the intercept and slope, further underlying the tight fit.

For the first time ever, a positive and strong correlation between PM and allergens is detected. Low-cost sensors can broaden their applications to environments notorious to be filled with allergens dusts, such as LAFs. Even though the correlation between real-time $PM_{2.5}$ and rat allergens is not close to unity, a low-cost sensor can be used as a warning sign of possible exposure of workers to allergens. Being real-time measurements, implementing the protection right after being warn would be beneficial to reduce the total exposure of a worker to rat allergens. For an accurate measurement, the traditional technique, ELISA, is still recommended.

Table 2 Statistical analysis of the linearity correlation between the rat allergens and the $PM_{2.5}$ and VOC measured using a low-cost monitor

Animal	Rat			Mouse		
Average	μ	$\mu_{15\%}$	$\mu_{5\%}$	μ	$\mu_{15\%}$	$\mu_{5\%}$
$PM_{2.5}$						
R^2	0.05	0.71	0.76	0.11	0.31	0.39
Slope	−0.05 ± 0.18	0.31 ± 0.04	0.29 ± 0.03	−0.08 ± 0.26	0.24 ± 0.11	0.18 ± 0.07
Intercept	0.47 ± 0.07	0.85 ± 0.02	0.98 ± 0.02	0.38 ± 0.41	1.24 ± 0.13	1.32 ± 0.11
Residual Sum Squares	215	33.4	11.7	257	189	91
Spearman's rank coefficient	−0.06	0.68	0.71	−0.09	0.31	0.49
VOC						
R^2	0.09	0.13	0.20	0.06	0.12	0.13
Slope	−0.35 ± 0.23	0.13 ± 0.10	0.28 ± 0.08	−0.16 ± 0.22	−0.12 ± 0.37	−0.07 ± 0.37
Intercept	1.46 ± 0.11	1.41 ± 0.11	2.03 ± 0.12	0.74 ± 0.41	1.02 ± 0.62	1.49 ± 0.63
Residual sum squares	854	467	333	180	116	232
Spearman's rank coefficient	−0.31	0.19	0.32	−0.25	−0.12	−0.13

3.2 Correlation between activities and rat and mouse allergens

The second goal of this project regards was to correlate a broad range of activities and levels of allergens. Figure 5 shows relative concentrations of rat allergens (a), and mouse allergens (b) at each facility, for a given activity (detailed information in Table S.2 in the SI). In this project, activities that have never been studied previously were considered; examples are "laundry," "office," "lunch," and "surgery." Samples collected during the activity of "laundry" contain similar allergens content for two of the three facilities studied. In facility 2, the laundry room has a door opened toward the dirty clean processing (about 3 meters distance between the two zones), making the average level of allergens slightly higher compared to the first facility, where the laundry room is closed (a 10 m^3 closed doors room). In the lunch rooms of the first facility (two 40 m^3 closed doors shared room located at each floor of the facility), both rats and mice allergens above LOD are found. For these cases, lunch rooms are located inside the area that requires the use of proper clothing and protection. However, about 4 workers spent 90 min in the lunch room without changing their laboratory coats and, perhaps, right after being in zones with high content of allergens. This behavior explains the increased levels of allergens that were completely unexpected for a lunch room.

The lunch room of the third facility is located outside the controlled area (about 100 m^3 shared room located at 10 m from the controlled area). Rat allergens in the lunch room of the third facility were lower than LOD, although a value right below LOD (0.17 ng/m^3) was found, Fig. 5. However, in facility 3, workers spent more than 90 min in the lunch room wearing their laboratory coats. The allergens on their coats were likely came as result of spending time in contaminated areas. A similar result was obtained for the activity of "office" in the second and third facility (both shared room of about 25 m^3 with closed doors). While for the second facility, the office (about 25 m^3) is a shared room with closed doors, the third facility has an open office space located right in the middle between zones with high allergens content (about 20 m^3 of opened space located in the hallway connected different zones). This was hypothesized to be the difference for the higher concentration of allergens in the office of the second facility and emphasize the importance of leaving shared spaces outside the controlled area. The levels of allergens contained in one facility show an average value of 0.96 ng/m^3, five times higher than LOD. The source of allergens in a changing room was assumed to be frequent entrances of workers wearing their laboratory coats impregnated with dust particles embedded with allergens. In the changing room, the workers took their dirty coats and placed them in an open container; the laboratory coats were taken to the laundry room once per day.

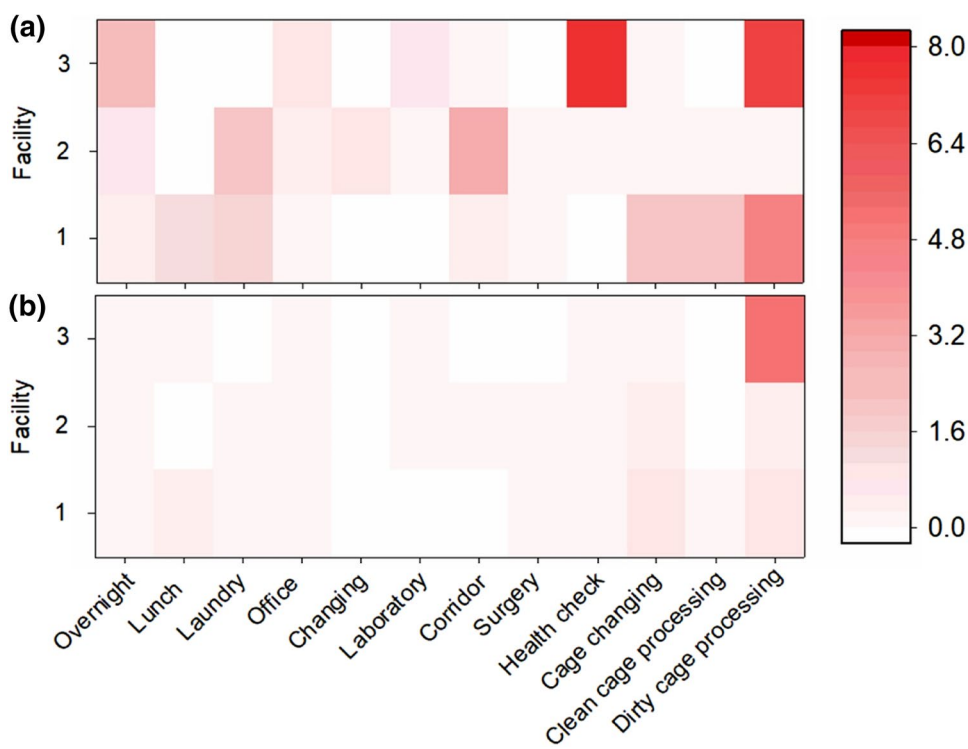

Fig. 5 Rat (**a**) and mouse (**b**) allergen concentrations in comparison with each activity. The three-facility tested are kept separated in order to show the differences between them

During surgery and in laboratory activity, a low number of animals were used and the levels of allergens were below LOD. However, the laboratory of the third facility was used once a week for a veterinary class where about 10 to 20 animals were dissected. Poor cleaning is the probable reason for the 0.6 ng/m^3 left in the room while no dissection or testing was occurring. A location that has not been tested before is the "corridor," Table 1. The corridor that had the highest concentration of rat allergens above 3 ng/m^3 is located in the second facility and is at the entrance of the dirty cage processing, an area known to be filled with allergens. Positive levels of allergens were found in corridors with high traffic or a high number of dirty cages, Fig. 5. Therefore, the highest levels of allergens have been found for the activities related to cage environments, Fig. 5.

Figure 6 shows the influence of cage number and cage type on the average concentrations of rat allergens; the correlation with mouse allergens are not reported due to a much lower number of samples with a positive number of allergens detected. More information is reported in Figure S.4 and Table S.3 in the SI. Both in the health check and cage changing activities, the highest allergen numbers were found when metal cages were treated (above 14 ng/m^3, Fig. 6) or when a high number of cages was processed (above 6 ng/m^3, Fig. 6). As suggested by previous references [19, 25, 39], clean cage and dirty cage processing were the activities with the highest levels of allergens. It was a major finding of this study that high levels of allergens (> 5 ng/m^3) were found in shared places, such as lunch room, offices, and changing rooms. In fact, by considering the amount of time spent in this area by workers, sensitization to animals' allergens over time is likely.

The presence of allergens in shared places is in contrast to the correlation between spikes in particulate matter and allergens content, shown in Figs. 5 and 6. The identification of animal allergens in communal areas outside the holding rooms increasing exposure of workers to these allergens increasing the chance of sensitization. Therefore, the use of a real-time monitoring for detecting the particulate matter could be used as a warning of allergen presence only in case of activities that involve the presence of the

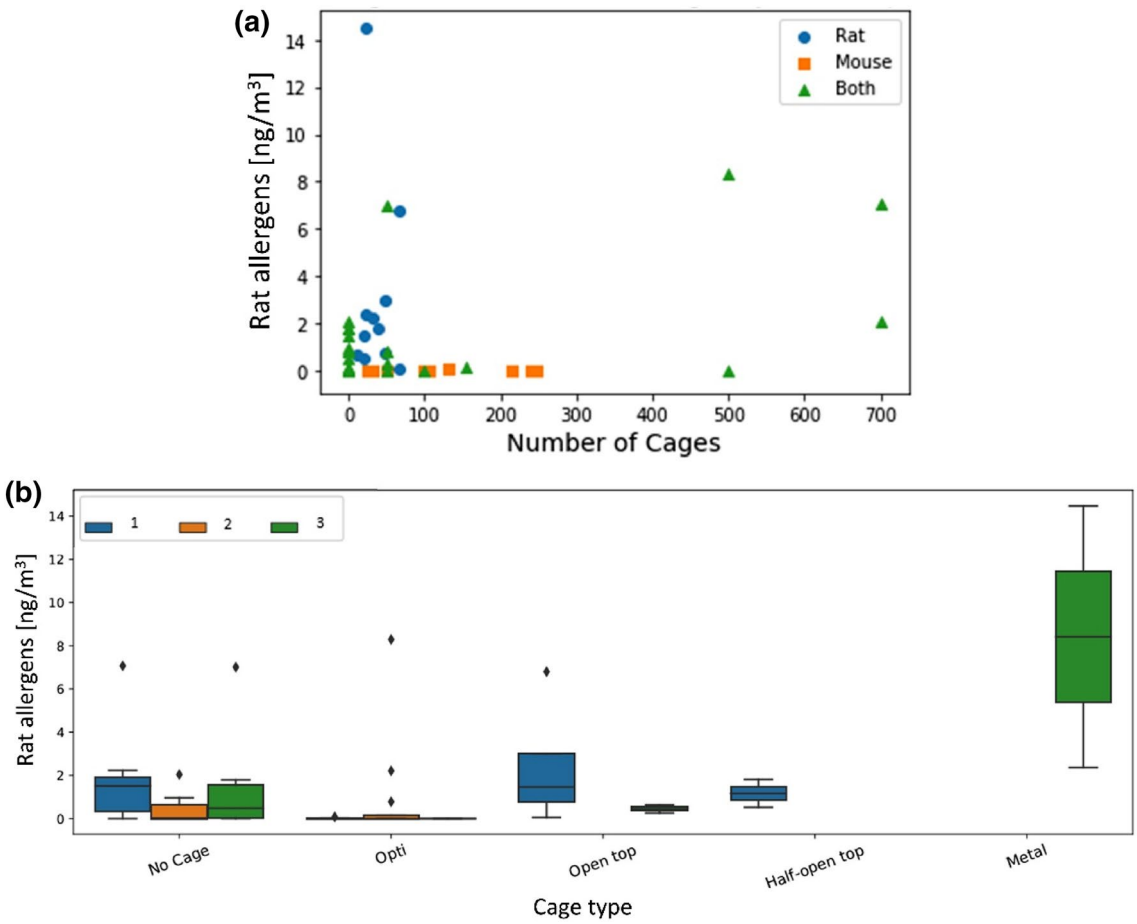

Fig. 6 Box chart of rat allergens in relation to a few activities considered. The error indicates the error bars derived by a difference in cage number, cage type, or in sub-activities a)

allergen source, as shown in Fig. 7. In case of the shared places or in locations where animals are not present, laboratory coats should be removed to possibly reduce the allergen content.

4 Conclusion

In this project, three breakthrough results were found. Firstly, it was confirmed a poor correlation between volatile organic compound (VOC) and airborne animal allergens; average VOC and animal allergens show a Spearman's coefficient of − 0.07. Secondly, a linear correlation between particulate matter with a diameter below 2.5 µm ($PM_{2.5}$) and airborne allergens was identified. The previous studies showed that $PM_{2.5}$ measured by gravimetric analysis and airborne allergens do not show any linearity. However, by employing a real-time measurement of $PM_{2.5}$ and averaging the spikes of $PM_{2.5}$, a Spearman's correlation of 0.71 was found with the rat allergens. Even though the correlation is not close to unity, this result introduces the possibility of using a low-cost sensor to warn the worker of a possible exposure to rat or mouse allergens. Whenever a large spike in $PM_{2.5}$ appears in the real-time readings, the worker could be advised to use PPE or to leave the room to reduce personal exposure. Therefore, the use of low-cost $PM_{2.5}$ sensors could be suggested in laboratory animal facilities (LAFs). In particular, in activities where the source of allergens is present, such as health check and cage changing, real-time measurements of $PM_{2.5}$ could be correlated with airborne allergens.

Secondly, high levels of rat and mouse allergens (> 5 ng/m^3) were found in unexpected location and activities, such as lunch rooms, changing rooms, offices, and laundry rooms. The presence of allergens in these rooms shows the potential for any worker in a laboratory animal facility to be exposed to rat or mouse allergen. In addition, airborne rat and mouse allergens in atypical rooms can be carried on laboratory coats of workers that had direct contact with laboratory animals. A limitation of this project is the total number of samples analyzed; 60 locations and activities in three different facilities are studied. Another limitation can be encountered in the cleaning and ventilation systems, which differ in the three facilities analyzed. The quantity of allergens collected in some activities, such as wet and dry cleaning, could be influenced by the differences in ventilation system. In addition, in some activities, such as clean or dirty cage processing, sources of $PM_{2.5}$ or VOC could be various challenging the determination of a possible correlation between these indoor pollutants and airborne animal allergens. In particular, in activities where the source of allergens is present, such as health check and cage changing, real-time measurements of $PM_{2.5}$ could be correlated with airborne allergens. However, even if allergens are present in locations where no animals are present, the use of real-time monitors as a warning of allergen presence is limited for activities or locations that involve the direct sources of allergens.

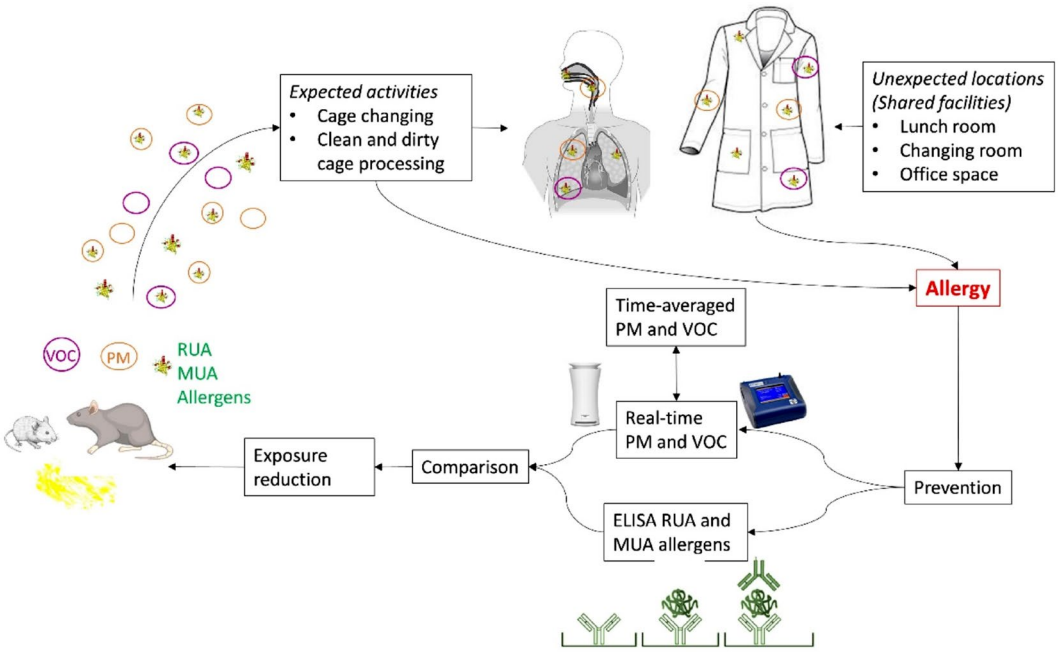

Fig. 7 Summary of the main outcomes of this project: determination of an alternative technique to use as a warning of sudden rises in airborne allergens, and detection of airborne allergens in unexpected shared facilities, such as lunch room, changing room, and office space

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supplementary information

Materials and Methods

Low-cost monitor selected

uHoo monitors passively collect a broad range of indoor pollutants. They are selected due to their availability and low cost (about 300 $). A detailed explanation of the uHoo low-cost monitor is reported in a previous reference (Baldelli et al., 2020). However, more information on the $PM_{2.5}$ and VOC sensors included in uHoo monitors are shown in Table S.1.

Table S.1 Detectable range, resolution, and estimated accuracy of the uHoo $PM_{2.5}$ and VOC low-cost sensors.

Sensor	Type	Manufacturer	Detectable range	Resolution	Estimated accuracy
$PM_{2.5}$	Optical scattering	Shinyei Kaisha	0 to 200 µg/m^3	0.1 µg/m^3	± 20 µg/m^3 or 10% of reading
TVOC	Metal oxide	AMS	0 to 1,000 ppb	1 ppb	± 10 ppb or 5% of reading, based on the types of VOC

Validation of low-cost monitors used

The uHoo low cost sensors have been previously tested in other environments (Baldelli and Loosley, 2020; Baldelli et al., 2020). The tested low-cost monitors were found to produce reliable results when tested in laboratory conditions. In an enclosed chamber, controlled amounts of PM and ethylene were used to calibrate the PM and the VOC sensors. The response of the sensors was validated against reference methods; in case of PM and ethylene were an Optical Particle Sizer (OPS) and theoretical calculations. The R-squared and the Spearman's Rank Correlation Coefficient (r_s) were above 0.8 and 0.6 for $PM_{2.5}$ and VOC sensors when compared to reference methods and in stable conditions (Baldelli and Loosley, 2020) Moreover, Even though the r_s was above 0.98, PM2.5 sensors were limited by a minimum reading of about 4.8 µg/m^3. In addition, when placed in a common household, r_s coefficients varied between 0.765 to 0.894 for $PM_{2.5}$ (the TVOC sensor was not tested). However, limitations in the process of validating the VOC sensors were found in the lack of a reference method that would show a higher accuracy than the theoretical methods used.

Even though one publication was dedicated to the validation of the performance of each sensor contained in uHoo monitors, the response of these low-cost sensors was tested against reference methods in one of the laboratory animal facility analyzed.Before performing the tests in this

project, the low-cost sensors were placed in the third facility tested (Number 3) for two days and one night. Values of $PM_{2.5}$ and VOC were compared with TSI DustTrak II 8532 Aerosol Monitor and with MiniRAE 3000 (RAE systems, Sunnyvale, California, United States). In case of the results shown in Figure S.1 a) and b), the sensors were placed in the activity "Health check" b), called as "Rat room" in Figure S.1, and in a room filled of OptiMouse cages that was not tested in the main publication. The Environmental Protection Agency (EPA) time weighted average (TWA) limit of 25 µg/m^3 for $PM_{2.5}$ and the World Health Organization (WHO) indoor short and long-term exposure limit of 100 ppb for VOCs are shown in Figure S.1 a) and b) respectively. The average difference between the readings of low-cost sensors and reference method is 5 and 4 for $PM_{2.5}$ and VOCs, respectively.

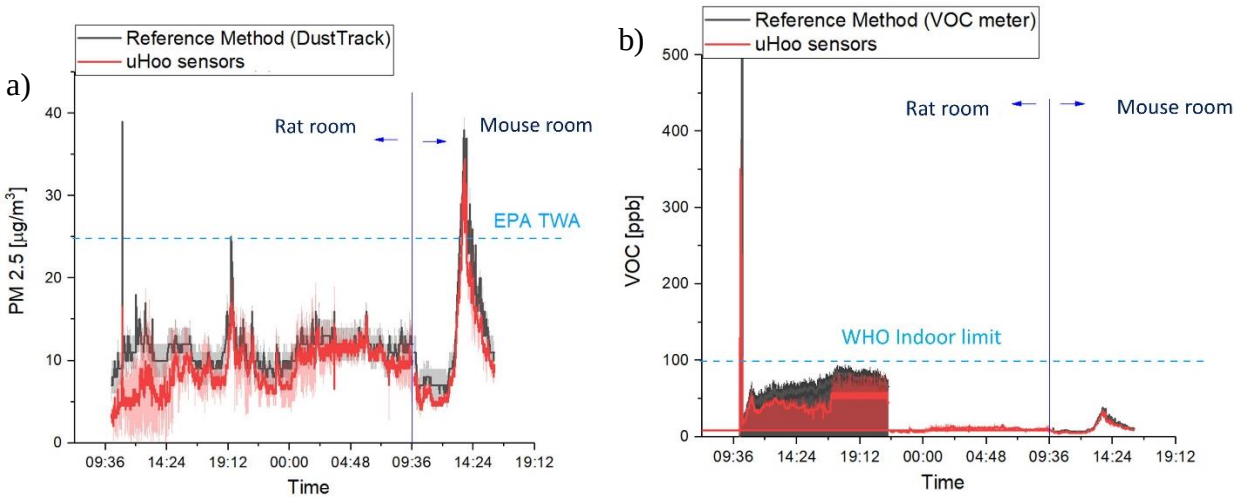

Figure S. 1 Comparison of uHoo monitors readings with reference methods for $PM_{2.5}$ (a) and VOC (b)

Microscopy

A Helios Focused Ion Beam – Scanning Electron Microscope (FIB-SEM) was used. An SEM image allowed visualization of the most common appearance of a PM collected on Teflon filters. In addition, an Energy Dispersive Xray (EDX) detector was used to identify the location of the mouse and rat allergens in dust particulates. Four samples obtained from four different activities were analyzed. Six spots on each sample were analyzed.

Samples were mounted and sputter coated with 10nm iridium (EM MED 020, Leica Microsystems Inc, Concord, ON, Canada). Samples were imaged using 2keV and 0.2nA current in BSE mode (Helios 650, Thermofisher, Hillsboro, OR, USA). The distribution of the chemical elements was obtained using 10keV and 3.2nA current with an EDAX Pegasus EDX system with

TEASM software (EDAX, Mahwah, NJ, USA). Point analysis mode for a duration of 30 sec per point.

Results and discussion

Comparison between indoor pollutants and rat and mouse allergens

In the main publication, one example of real-time data collected using the low-cost sensor and the reference method, DustTrak TSI, is shown. In Figure S. 2, the comparison of the averages μ and $\mu_{5\%}$ between all the activity where both a low-cost and the reference method, DustTrak TSI, are placed. Figure S. 2 shows a good agreement between the two methods of detecting $PM_{2.5}$. However, the DustTrak commonly shows higher averages since it consists of an active collection; DustTrak uses 1 lpm as a flow rate, while low-cost sensors are passive collectors.

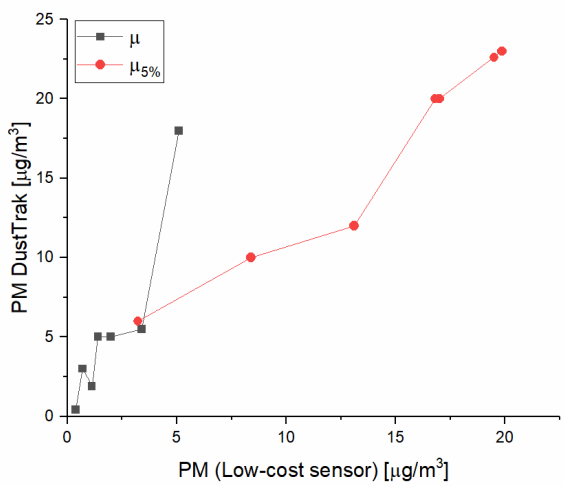

Figure S. 2 Comparison of the average of the whole data set for a single activity (μ) and of the average of the data higher than twice μ ($\mu_{5\%}$) between the low-cost sensors and the reference method, DustTrak TSI.

Definition of the variables $\mu_{5\%}$ and $\mu_{15\%}$

The variables $\mu_{5\%}$ *and* $\mu_{15\%}$ were used with the goal of looking for a possible correlation between airborne allergens and the peaks contained in the real-time readings of $PM_{2.5}$ and VOC collected using the low-cost sensors. While a deeper explanation is reported in the main manuscript, additional visual examples of these two variables are shown in Figure S.3.

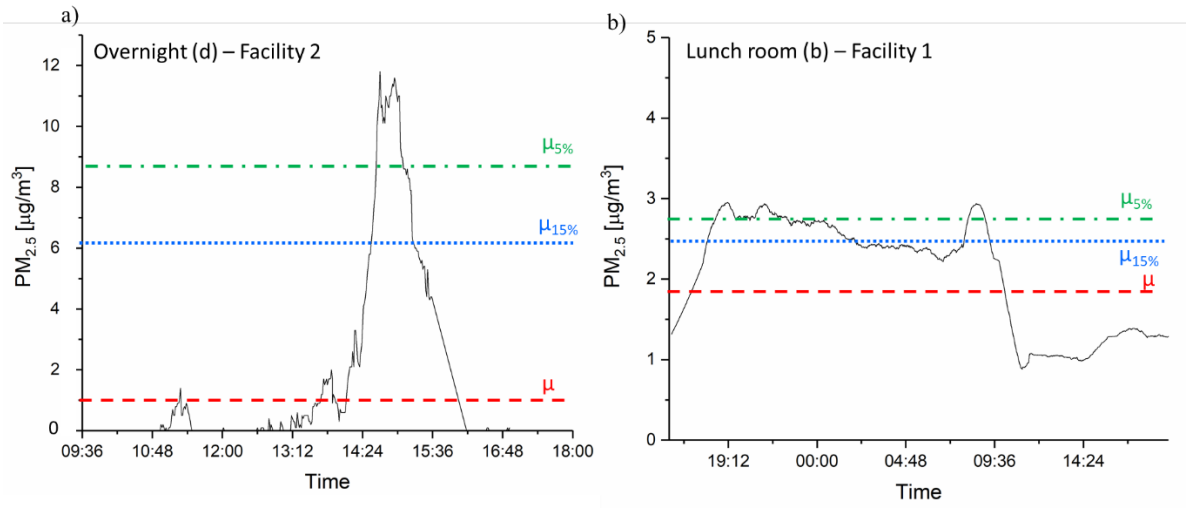

Figure S.3 Additional examples of the determination of the parameters $\mu_{5\%}$ and $\mu_{15\%}$.

The selection of the 15 and 5 percentiles of real-time data are arbitrary, as mentioned in the main manuscript. However, before doing this selection, some other percentiles have been considered. The percentiles of 35, 25, and 2 have been tried for the regression between the $PM_{2.5}$ and rat allergens, as shown in Table S.2.

Table S. 2 Statistical analysis of the linearity correlation between the rat allergens and the $PM_{2.5}$ measured using a low-cost monitor and by using different average percentiles, 35, 25, 15, 5, and 2

Average	μ	$\mu_{35\%}$	$\mu_{25\%}$	$\mu_{15\%}$	$\mu_{5\%}$	$\mu_{2\%}$
R^2	0.05	0.13	0.41	0.71	0.76	0.69
Slope	-0.05 ± 0.18	0.01 ± 0.24	0.04 ± 0.09	0.31 ± 0.04	0.29 ± 0.03	0.34 ± 0.05
Intercept	0.47 ± 0.07	0.51 ± 0.09	0.66 ± 0.06	0.85 ± 0.02	0.98 ± 0.02	0.91 ± 0.04
Residual Sum Squares	215	107	51	33.4	11.7	18
Spearman's rank coefficient	-0.06	0.41	0.57	0.76	0.83	0.65

Correlation between activities and rat and mouse allergens

The second aim of this publication is to determine the levels of allergens according to different activities involving rats and mice. In the main manuscript, all the data related to the allergens are presented for rats since more positive cases were found. However, mouse allergens were tested for every activity and location analyzed, as shown in Figure S. .

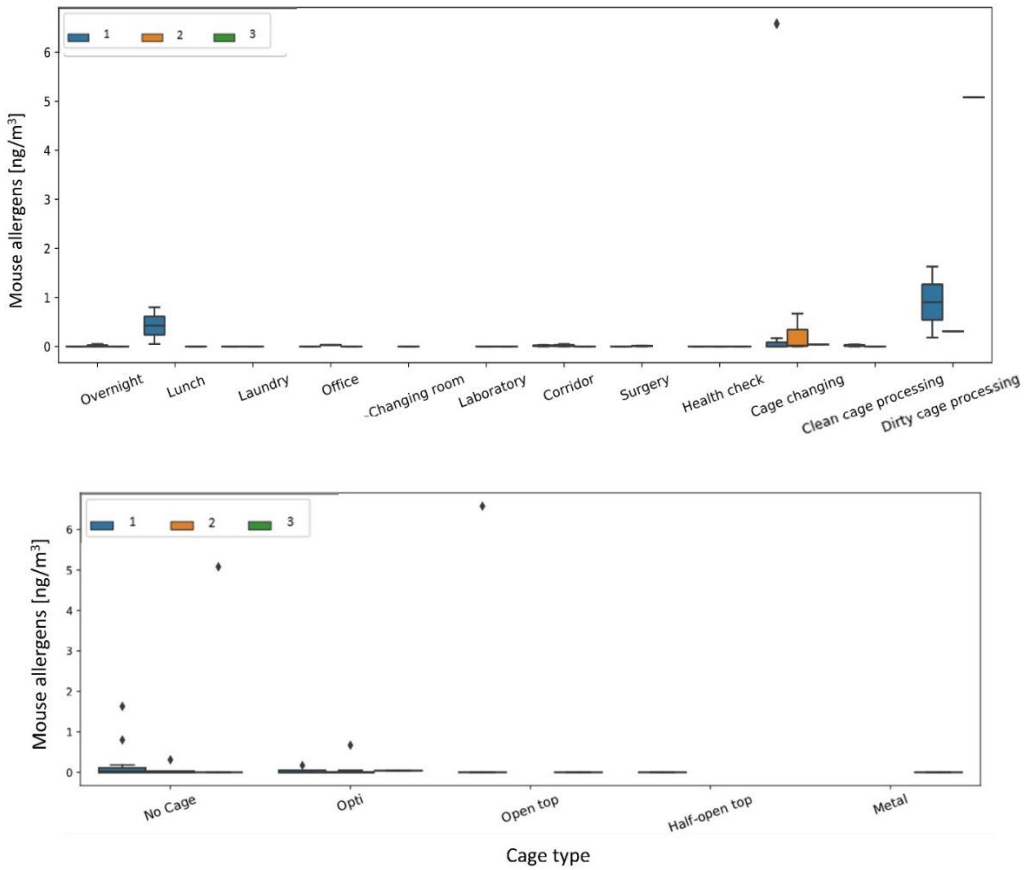

Figure S. 4 Distribution of the detected mouse allergens respect to each activity and facility tested.

Summary table

InTable S.3, the summary of the amount of mouse and rat allergens encountered in each activity and in each location is shown. In addition, the limit of detection (LOD) of ELISA for mouse and rat allergens is 0.09 and 0.2 ng/m^3. The lower amount of mouse samples is due to the higher presence of rats compared to mice in the three facilities studied.

Table S. 3 Summary of the mouse and rat allergens. The red font indicates activity that only involves rat, blue indicates only mice, and yellow both rats and mice. Bold numbers identify values above the LOD.

Activity	ID	Species	Mouse allergens [ng/m^3] LOD = 0.09 ng/m^3	Rat allergens [ng/m^3] LOD = 0.2 ng/m^3
Overnight	a	Rat	< LOD	**0.77**
	b	Rat	< LOD	0.08 (< LOD)
	c	Rat	< LOD	**2.51**
	d	Rat	< LOD	**2.36**
	e	Mouse	< LOD	< LOD
	f	Mouse	0.05 (< LOD)	< LOD
Lunch	a	Mouse	**0.24**	**0.98**
	b	Both	0.03 (< LOD)	**1.72**
	c	Both	< LOD	0.18 (< LOD)
Laundry	a	Both	< LOD	**1.51**
	b	Both	< LOD	**2.04**
Office	a	Both	< LOD	0.03 (< LOD)
	b	Both	0.04 (< LOD)	**0.52**
	c	Both	< LOD	**0.83**
Changing	a	Both	< LOD	**0.96**
Laboratory	a	Both	< LOD	**1.79**
	b	Both	< LOD	0.04 (< LOD)
	c	Both	< LOD	< LOD
	d	Both	< LOD	< LOD
Corridor	a	Both	< LOD	0.02 (< LOD)
	b	Both	0.03 (< LOD)	**0.62**
	c	Both	< LOD	**0.78**
	d	Both	0.05 (< LOD)	**8.29**
	e	Both	< LOD	**0.26**
	f	Both	0.01 (< LOD)	< LOD
Surgery	a	Rat	< LOD	< LOD
	b	Mouse	0.05 (< LOD)	< LOD
	c	Mouse	< LOD	< LOD
	d	Rat	< LOD	< LOD
Health check	a	Rat	< LOD	< LOD
	b	Rat	< LOD	**14.47**
	c	Rat	< LOD	**0.63**
	d	Mouse	< LOD	< LOD
	e	Mouse	< LOD	< LOD
	f	Mouse	< LOD	< LOD
Cage changing	a	Rat	< LOD	**1.81**
	b	Rat	< LOD	**6.81**
	c	Rat	< LOD	**0.52**
	d	Rat	< LOD	**1.46**
	e	Rat	< LOD	**3.05**
	f	Mouse	**0.17**	< LOD
	g	Mouse	**6.59**	0.05 (< LOD)
	h	Mouse	**0.67**	< LOD
	i	Mouse	< LOD	< LOD
	l	Mouse	0.04 (< LOD)	< LOD
	m	Both	0.02 (< LOD)	0.16 (< LOD)
Clean cage processing	a	Both	< LOD	**1.65**
	b	Both	< LOD	< LOD
	c	Both	0.06 (< LOD)	**3.23**
	d	Both	< LOD	< LOD
Dirty cage processing	a	Both	**1.63**	**2.07**
	b	Both	**0.18**	**7.97**
	c	Both	**0.31**	0.03 (< LOD)
	d	Both	**5.08**	**7.03**

Morphology of collected dusts

Microscopy is a common technique used to visualize and analyze PM collected on a filter (Baldelli and Vehring, 2016; Baldelli et al., 2019). Particulate matter was imaged to identify the distribution of the allergens in dust microparticles generated in LAF. An example of dust particles are shown in Figure S.5, where the activities of " "Dirty cage processing – d" a), and "Cage changing – a" b), as listed in Table 1 in the main publication, are shown.

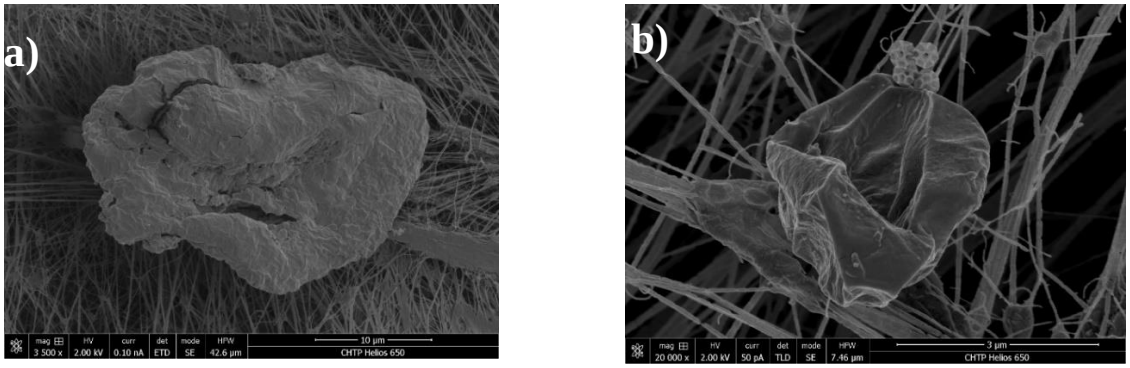

Figure S.5 Examples of particulates found in the activities of "Dirty cage processing – d" a), and "Cage changing – a" b), as listed in Table 1 in the main publication

Figure S.5 a) and b) show that by imaging dust particulates with a common SEM, airborne allergens cannot be visually recognized due to their nano ranged size. However, RUA and MUA allergens are long chain proteins mostly composed of carbon, oxygen, and nitrogen. Furthermore, rat Mups bind different chemicals, such as 2-sec-butyl-4,5-dihydrothiazole, 6-hydroxy-6-methyl-3-heptanone, 2,3 dihydro-exo-brevicomin, 1-Chlorodecane, with 2-methyl-N-phenyl-2-propenamide, hexadecane, 2,6,11-trimethyl decane, limonene-1,2-epoxide, listed from the most to the least prominent (Cavaggioni and Mucignat-Caretta, 2000). These ligands incorporate S and Cl, which can stand out in X-ray dispersion studies since most ligands only incorporate C, O, H, and N. In the sample studies in Figure S.5, the dust particulates, for the activity of "Dirty cage processing", "cage cleaning" and "corridor", are mostly made of pine bedding that is filling dirty cages cleaned during the dirty cage processing. This type of bedding consists of common paper that contains 42% oxygen, 6% hydrogen, 1% nitrogen, and 1% other elements (mainly calcium, potassium, sodium, magnesium, iron, and manganese). Since facility 1 is underground, for the activity of "Office", presence of traces of Al, Ca, and Si in indoor dust can be expected, however, in minor percentage compared to the elements produced by the indoor activities (Maskey et al., 2011). As a result, the peaks of S and Cl in Figure S. 3 might be traceable to rat or mouse antigens or other proteins, such as albumin from rat and mouse urines.

A higher number of samples would be necessary to verify and quantify the allergens contained in dust microparticles; however, this could potentially be another alternative to ELISA techniques. Furthermore, the chemical elements are analyzed for the cases of Office – a" a), "Corridor -a" b),"Dirty cage processing – d" c), and "Cage changing – a" d), as listed in Table 1 in the main publication. Interestingly, the same chemical elements are identified in each of the four activities analyzed. The standard deviation shown in Figure S.6 are obtained by the different signals obtained by the four spots analyzed per each case.

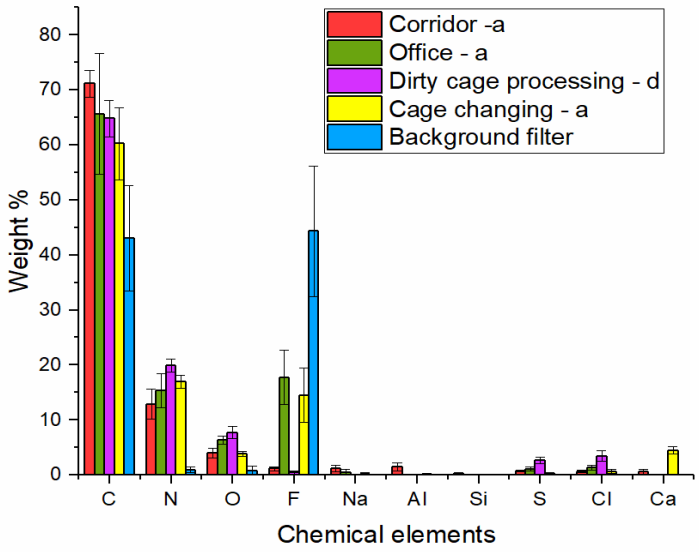

Figure S. 36 Histogram of the most common chemical elements distribution of each sample analyzed. The following activities are studied: "Office – a" a), "Corridor -a" b),"Dirty cage processing – d" c), and "Cage changing – a" d), as listed in Table 1 in the main publication. Furthermore, the background signal of the filter is shown/

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