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*Research article*

# Airborne exposure to laboratory animal allergens: 2005–2022

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**Abstract:** Exposure to laboratory animal allergens has been a significant cause of IgE-mediated allergic symptoms and occupational asthma (OA). Mice are the predominant species used in medical and scientific facilities. We have previously published a review of our air monitoring data of mouse and rat major allergens (mus m 1 and rat n 1) expressible in ng $m^{-3}$ from 2005–2016. We have now reassessed all this largely United Kingdom (UK) air monitoring data from 2005–2022, which include 77% and 47% additional mouse and rat results, respectively. Where possible, we have categorized the results to specific tasks and areas to identify those associated with higher exposures and explored temporal trends in exposure together with an estimation of the annual incidence of OA in 2005 and 2018. A downward shift in mouse results for personal samples was apparent from 2014, with evidence of a decrease in the 90$^{th}$ percentile, but both personal and static rat samples confoundingly showed an apparent increase from 2017. Activities of "cage changing", "cage cleaning", and "cage washing, dirty side" suggested substantial personal exposures in some facilities, while "bedding dumping" and "cage scraping" suggested generally high exposures, albeit modified by any respiratory protective equipment worn. Individually ventilated cages reduce exposure, but filter changing/cleaning can still lead to high exposures. Exposure from experimental and husbandry duties were generally lower, but in some facilities activities where animals are handled outside of cages can cause significant exposures. The reduction in personal exposure to the predominant species is consistent with an estimated 55% decrease in the UK annual incidence rate of OA in 2018 from 2005. The data may help facilities to improve exposure control by identifying higher risk activities, benchmarking their own monitoring data and help further reduce the risk of sensitization and subsequent allergic respiratory ill-health.

**Keywords:** allergens; rodents; mus m 1; rat n 1; exposure monitoring; occupational asthma

**Abbreviations:** HSE: health and safety executive; IVC: individually ventilated cages; LAA: laboratory animal allergens; LOD: limit of detection; OA: occupational asthma; RPE: respiratory protective equipment

## 1. Introduction

The use of animals remains pivotal in many scientific and medical research studies undertaken in the pharmaceutical sector, contract toxicology laboratories, research institutes, and universities. This is despite considerable activity to reduce their use and replace with alternative *in vitro*, experimental models [1]. In Great Britain, some 3.3 million animals were used for scientific research in 2019 [2]. Roughly half the animals were used for experimental procedures and half for the creation or breeding of genetically modified (transgenic) animals. Mice and rats are the two major animals utilized; 61% of all animals used for experimental procedures were mice, while for genetically altered animals the predominance of mice was greater at 87%. Data from the UK Home Office suggest that during 2020 there was a 15% drop in Great Britain in the use of animals for experimental activity, which was presumed largely to have been caused by COVID-19 [3].

Airborne exposure to laboratory animal allergens (LAA), especially from mice and rats, is well recognized as causing IgE-mediated sensitization with subsequent ocular, nasal, upper, and lower respiratory symptoms [4–6]. While life threatening anaphylaxis has been uncommon and mostly associated with rodent bites [7], other symptoms can be debilitating enough to prevent further work by experienced staff with putative species. LAA is a proven and major cause of occupational asthma (OA) and rhinitis. A review of published papers covering 1980–2006 had shown a decrease in OA in those exposed to laboratory animals and suggested that this was due to the reduction in exposure [8]. In 1999/2000, Draper estimated the annual UK incidence rates of OA and rhinitis in those occupationally handling small animals as 1.56/1000 workers and 2.54/1000, respectively, although acknowledging these are likely underestimates, depending on cases being reported by specialist chest physicians to the Surveillance of Work & Occupational Related Diseases scheme (SWORD) [9].

The exact nature of the relationship between exposure to LAA and symptoms remains unclear [10–13], but it is accepted that controlling exposure is paramount. Allergic symptoms mostly occur within around the first 3 years of exposure [14] and both atopy and smoking have been recognized as risk factors [11,14]. The role of regular medical surveillance in controlling the risk of poor health outcomes has also been highlighted [12].

The potent major allergens, mus m 1 in mice and rat n 1, have been characterized, and a number of immunochemical assays have been developed over the years to monitor airborne levels of these allergens [15,16]. The sensitizing agents in mice and rats are proteins found in the urine and saliva of the animals, which contaminates fur and bedding and is readily aerosolized by activities of the animals, by staff looking after the animals, or in carrying out procedures on the rodents. The HSE (UK) produced guidance on the control of laboratory animal allergy in 2011 [17]. Control of exposure is paramount; engineering and ventilation control measures of varying complexity and cost are available. These include the use of individually ventilated cages (IVCs) for rodents [18], where potentially contaminated air is drawn within a central plenum rather than "open-topped" cages, semi-automated, or fully robotic systems for washing cages; automated vacuum operated dumping stations for soiled bedding, and ventilated work stations for procedures such as animal weighing to organ harvesting [19]. The benefit of respiratory protective equipment has also been highlighted [18,20].

Since 2005, HSE's Science and Research Centre has undertaken LAA measurements on atmospheric and surface wipe samples for occupational hygienists, and health and safety professionals

in animal facilities. This activity has not been mandated by the UK regulatory authority (HSE), but undertaken on the initiative and expense of a significant number of duty holders. In 2017, we published an initial analysis of airborne mouse and rat results for the period 2005–2016 [21]; this was based on available paper records of results. Since late 2014, we have used a standardized electronic database (EMDB) for result storage and reporting, including sample descriptors and site details. Given the potential for laboratory animals to cause sensitization, allergic symptoms, and occupational asthma, we have recovered and re-collated all previous paper-based records (2005–2014) and formulated all results from 2005 to the end of 2022 according to the standardized fields within the EMDB. There are 77% and 47% more mouse and rat results than reported earlier [21].

Our major aims of this paper are to update the previous report: Identify the levels and any temporal changes in mus m 1 and rat n 1 levels found across the overall sector using laboratory animals over the period 2005–2022, establish appropriate P90 values, and describe the range of allergen levels found in various tasks, activities and locations in order to identify those associated with higher potential exposures. In parallel, we have also estimated the UK annual incidence rate of OA in 2005 and 2018 based on a method suggested by Draper [9].

## 2. Materials and methods

Samples are collected from facilities using laboratory animals by in-house or external occupational hygiene consultants and sent to the laboratory by courier or first-class post for analysis. The UK sector using laboratory animal allergens can be subdivided into (a) fully commercial facilities, such as pharmaceutical companies (Pharma), which may operate a number of facilities, and contract toxicology laboratories; (b) research institutes, which are generally charity or government funded, and while possibly associated with a university, generally operate independently with their own health and safety departments; and (c) facilities within universities utilizing laboratory animals.

We have always recommended a standardized method for collecting air samples for allergen analyses. This involves the use of 25 mm FALP2500 PTFE filters of 1 uM pore size (Millipore, UK) mounted in IOM samplers and cassettes, operating at an air flow rate of 2 L $min^{-1}$ and collecting the inhalable bioaerosol fraction. Where static (background or area) samples are collected in a facility is the choice of consultants undertaking the sampling, personal sampling is undertaken with the pumps attached to a belt and the samplers attached at the lapel (breathing zone) of specific staff, identified by the consultant. Personal samples reflect what a worker may inhale, albeit mitigated by any respiratory protective equipment (RPE) being worn, and therefore are probably a more direct, comparable indicator of risk to respiratory health.

Although not obligatory, many submitters specifically identify the site being monitored, the volume of air sampled, as well as area/task/activity descriptors for individual samples. For results to be included in the full analysis within this paper, the minimum necessary data was the type of sample (static or personal) and the air volume sampled so that results could be expressed in ng $m^{-3}$, and samples also had to have been collected using PTFE filters and employing air flow rates around 2 L $min^{-1}$.

The extraction procedures and automated methods for mus m 1 and rat n 1 measurements have not essentially changed from 2005 and have been described previously [21]. Long term quality assurance material has been run with every batch of samples to ensure comparability of results over time. This material consists of 1% bovine serum albumin with 0.1% PRoclin 300 (Sigma-Aldrich, UK) spiked with purified allergens, aliquoted and stored at −80 °C. The analytical LOD for the mus m 1 and rat n 1 assays are 0.01 ng $ml^{-1}$, 0.025 ng $ml^{-1}$ respectively; these equate to absolute amounts of

allergen collected on the PTFE filters of 0.04 ng and 0.1 ng, respectively. This allows the calculation for each sample of a LOD in terms of ng $m^{-3}$ based on air volumes sampled. For those results less than the LOD, ((LOD)/SQRT2) was substituted and used in calculating the likely airborne exposure level in terms of ng $m^{-3}$ (around 7% of results needed this adjustment).

Given the nature of the samples analysed, largely descriptive numerical analysis employing medians and percentiles have been used, which are readily interpretable by health and safety practitioners. The P90 value is defined as the $90^{th}$ percentile of representative, results for a specific analyte accumulated up to any time point, allowing the identification of specific results that could be considered high against the general distribution of results. Where appropriate, non-parametric statistical analysis (Kruskal-Wallis, Chi-squared or Fishers exact tests for comparison between groups) or parametric analysis after log transformation such as linear regression have been used (MedCalc, Belgium, version 13.2.2). In order to look for temporal shifts, the exposure data was brigaded into three yearly groups and analyzed using the Kruskal-Wallis test.

The measurements of mouse exposure, both static and personal, were broken down by location of monitoring and tasks being undertaken. The categories were based on commonly used descriptors by those professionals submitting samples to the laboratory. However, there may be some overlap or ambiguity between these descriptors, for example “cage cleaning”, “cage changing”, and “clean out”.

Estimates of the UK annual incidence rate of OA in the sector using laboratory animals at the approximately the beginning and end of exposure monitoring period was carried out as suggested by Draper [9] using cases of OA recorded by SWORD and reported by HSE [22]. Estimates of the exposed population based on the number of personal license holders recorded by the UK Home Office and information from the Institute of Animal Technology (IAT) was used (personal communication). The OA cases data is based on annual average for 2004–2006 and 2017–2019 from the published SWORD data, as it has been acknowledged that COVID-19 in 2020–2022 impinged on the reporting of asthma and other respiratory diseases [22,23].

## 3. Results

### *3.1. Overview*

Filters from air sampling were received for analysis from 429 site visits to 89 different laboratory animal facilities in the UK. Annual animal statistics reports published for Great Britain and Northern Ireland suggest the number of designated places for animal work in the UK has been between 153–227 for the period of our monitoring. Almost 50% of the site visits were undertaken by four independent, commercial occupational hygienists, or health and safety professionals with experience of such facilities, and approximately 10% of the sites undertake annual monitoring.

Of the total number of filters submitted for mouse or rat allergen analysis, 6234 air filters have been analyzed and reported from 2005 to the end of 2022, which included details of the air volume sampled. Thus, 5441 mus m 1 and 2042 rat n 1 results were expressed in ng $m^{-3}$. Ninety-two percent of all results could be assigned as either personal air monitoring samples or static/background air samples. Sixty-seven percent were identified as static/background monitoring samples, with 33% being personal air samples. Table 1 shows the characteristics of these samples by species and type of monitoring.

**Table 1.** Characteristics of the data for the mouse (mus m 1) and rat (rat n 1) allergens expressed as ng $m^{-3}$. Geometric means (GM), medians and 90$^{th}$ percentiles (P90) shown with their respective 95$^{th}$ percentile confidence intervals.

| | All samples | | Personal | | Static | |
|---|---|---|---|---|---|---|
| | Mus m 1 | Rat n 1 | Mus m 1 | Rat n 1 | Mus m 1 | Rat n 1 |
| GM | 0.43 (0.40–0.46) | 0.36 (0.33–0.39) | 2.54 (2.27–2.85) | 0.91 (0.79–1.06) | 0.21 (0.19–0.22) | 0.16 (0.14–0.18) |
| Median | 0.33 (0.30–0.37) | 0.23 (0.20–0.27) | 2.17 (1.89–2.47) | 0.83 (0.63–0.99) | 0.15 (0.14–0.16) | 0.09 (0.08–0.10) |
| P90 | 11.38 (10.2–13.5) | 6.41 (5.48–7.91) | 53.90 (43.0–67.0) | 16.59 (12.4–19.6) | 3.04 (2.68–3.50) | 1.98 (1.49–2.89) |
| n | 5441 | 2042 | 1478 | 683 | 3340 | 1045 |

The results for the allergens were highly positively skewed. Table 1 confirms differences in results between personal and static samples; geometric means for personal compared to static samples were 14-fold higher for mus m 1 and 9-fold higher for rat n 1, thus indicating that results from these two sample types should be considered independently in terms of their interpretation. Thirty-seven percent of submitted samples could be identified as derived from commercial pharmaceutical and contract toxicology laboratories, 30% from research institutes and 33% from universities (Table 2). Interestingly, while roughly equal numbers of mice and rat samples were submitted from pharmaceutical companies/contract toxicology laboratories, only 20% and 8% of samples from universities and research institutes respectively related to exposure to rats. This is perhaps in the last category reflecting the predominance of mouse-based genetically modified (transgenic) models in current medical research. Personal and static samples from research institutes for mus m 1 tended to be lower than in both universities and Pharma/contract toxicology laboratories.

**Table 2.** Characteristics of the data for the mouse (mus m 1) and rat (rat n 1) allergens expressed as ng $m^{-3}$ by categorized source of samples. Medians (95$^{th}$ percentile confidence intervals) are shown.

| | Personal | | Static | |
|---|---|---|---|---|
| | Mus m 1 | Rat n 1 | Mus m 1 | Rat n 1 |
| Pharma & contract toxicology laboratories | 2.2 (1.27–4.84) | 0.82 (0.57–1.18) | 0.16 (0.10–0.21) | 0.10 (0.08–0.12) |
| Research Institutes | 1.22 (0.80–1.65) | 0.21 (0.09–1.01) | 0.07 (0.06–0.08) | 0.08 (0.06–0.10) |
| Universities | 2.61 (2.20–3.87) | 0.59 (0.20–1.70) | 0.23 (0.16–0.31) | 0.24 (0.14–0.36) |

### *3.2. Temporal changes*

In another analysis from this unselected sampling (i.e., not a defined research population), any evidence of changes in levels of allergens over time for mus m 1 and rat n 1 was explored. Results were brigaded into three-year periods from 2005 (Table 3). Table 3 suggests a substantial downward shift in the median values for personal mus m 1 results from 2014 compared to earlier time frames. The three time bands from 2014–2022 were statistically lower than the earlier the three time bands covering 2005–2013 ($p < 0.01$). For static air samples, results for 2011–2013 and 2014–2016 were statistically higher to both earlier and later time bands ($p < 0.01$). The rat n 1 results from personal samples show significant differences between the time frames with the last two time periods (2017–2019 and 2020–2022) being significantly higher than each of the first three time bands (2005–2013) ($p < 0.01$). A similar pattern was shown for the rat static samples with the last two time periods (2017–2019 and 2020–2022, each being significantly higher ($p < 0.01$) than all the first four time periods.

Therefore, an initial analysis of the temporal changes in the two major rodent allergen results appear to show a divergence. Personal atmospheric monitoring for mus m 1 shows a significant and sustained decrease in airborne levels since 2014, while the results for rat n 1, either personal or static, show an increase in monitoring results since 2017.

### *3.3. 90th percentiles (P90)*

The cumulating 90th percentiles (P90) over time for mus m 1 and rat n 1 for personal air samples are shown in Figure 1. By the end of 2022 the P90s for mus m 1 was 54 (CI 43-67) and 3.0 (CI 2.7-3.5) ng $m^{-3}$ for personal and static samples respectively; for rat n 1 the equivalent values for the P90 were 16.6 (CI 12.4-19.6) and 2.0 (CI 1.5-2.9) ng $m^{-3}$, respectively. As expected, the increasing numbers of results in the cumulative P90 values narrow the confidence intervals over time.

Linear regression analysis was performed for the four cumulating P90s by year from the year when more than 100 results accumulated. Personal samples for mus m 1 show a significant downward trend from 2008 ($p < 0.001$). This suggests that prevalence of higher values for this type of sample has been declining over time and is compatible with the outcomes of the earlier Kruskall-Wallis analysis. Regression analysis for mus m 1 static samples also suggests some decline from 2009 ($p = 0.04$). For rat n 1 personal and static samples, there was no evidence of any statistical significant decline from 2008 and 2009, respectively. Visual inspection of the rat data suggests that there may be some increase in the P90 values from 2011 (Figure 1).

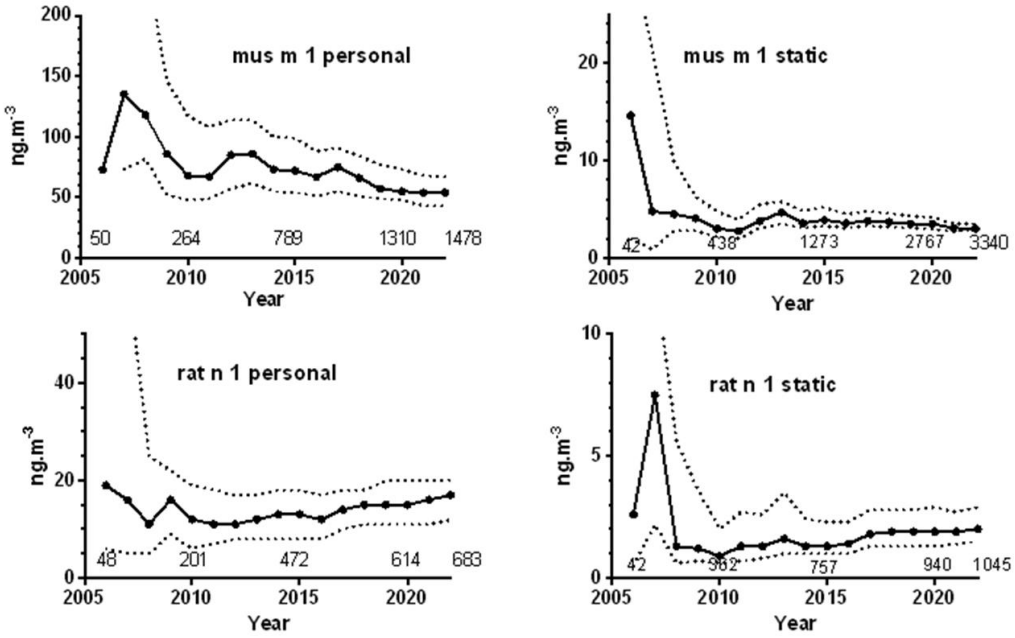


**Figure 1.** Cumulative P90s and their confidence intervals. Cumulating number of analyses in 2005, 2010, 2015, 2020, and 2022 are shown above the x axes.

### *3.4. Exposure by location of monitoring or tasks being undertaken*

Table 4 shows the median and interquartile ranges (IQR: 25th percentile to 75th percentile) brigaded by locations and work tasks. This analysis was carried out on mouse results because of the greater number of these results compared to rat.

**Table 3.** Data for mus m 1 and rat n 1 brigaded into three-year periods. Medians, interquartile range (IQR) and the 90$^{th}$ percentile (P90) together with its 95$^{th}$ percentile confidence intervals in brackets.

| Years | Mus m 1 | | Rat n 1 | |
|---|---|---|---|---|
| | Personal samples | Static samples | Personal samples | Static samples |
| 2005–2007 | Median 4.01 | Median 0.05 | Median 0.38 | Median 0.09 |
| | IQR 0.40–33.27 | IQR 0.03–0.20 | IQR 0.08–3.53 | IQR 0.06–0.36 |
| | P90 134.55 (74.43–219.97) | P90 4.78 (0.88–21.39) | P90 16.05 (5.10–76.30) | P90 7.50 (2.17–11.97) |
| | N = 74 | N = 88 | N = 72 | N = 130 |
| 2008–2010 | Median 4.257 | Median 0.04 | Median 0.20 | Median 0.03 |
| | IQR 1.12–12.86 | IQR 0.02–0.85 | IQR 0.09–0.67 | IQR 0.02–0.06 |
| | P90 48.86 (30.82–89.80) | P90 2.94(2.09–3.96) | P90 9.96 (3.75–18.41) | P90 0.53 (0.23–0.93) |
| | N = 190 | N = 350 | N = 129 | N = 232 |
| 2011–2013 | Medan 4.89 | Median 0.24 | Median 0.95 | Median 0.08 |
| | IQR 0.91–25.78 | IQR 0.04–1.11 | IQR 0.12–4.12 | IQR 0.06–0.52 |
| | P90 93.19 (66.96–139.39) | P90 5.59 (4.04–8.29) | P90 12.05 (8.18–19.87) | P90 3.95 (1.40–6.75) |
| | N = 329 | N =862 | N = 187 | N = 241 |
| 2014–2016 | Median 1.285 | Median 0.19 | Median 0.67 | Median 0.08 |
| | IQR 0.26–6.49 | IQR 0.05–0.91 | IQR 0.15–2.64 | IQR 0.05–0.21 |
| | P90 28.14 (19.88–50.26) | P90 2.98 (2.43–3.33) | P90 15.77 (6.16–25.51) | P90 0.98 (0.47–2.00) |
| | N = 318 | N =763 | N = 100 | N = 167 |
| 2017–2019 | Median 1.98 | Median 0.11 | Median 1.77 | Median 0.36 |
| | IQR 0.68–11.02 | IQR 0.05–0.57 | IQR 0.85–7.13 | IQR 0.12–1.12 |
| | P90 41.27 (32.13–72.06) | P90 2.65 (2.07–4.58) | P90 27.00 (15.96–34.62) | P90 3.21 (1.81–5.37) |
| | N = 326 | N = 605 | N = 111 | N = 139 |
| 2020–2022 | Median 1.04 | Median 0.12 | Median 1.45 | Median 0.23 |
| | IQR 0.41–5.29 | IQR 0.05–0.37 | IQR 0.25–6.43 | IQR 0.12–0.99 |
| | P90 28.80 (16.79–51.94) | P90 1.34 (0.95–1.91) | P90 23.73 (13.97–43.73) | P90 2.57 (1.72–5.55) |
| | N = 241 | N = 672 | N = 84 | N = 136 |

**Table 4.** Breakdown of mouse results in ng $m^{-3}$ by location/task.

| Location or task | Static samples | | | | Personal samples | | | |
|---|---|---|---|---|---|---|---|---|
| | N | 25th% | Median | 75th% | N | 25th% | Median | 75th% |
| Bedding dump | 51 | 0.53 | 2.60 | 23.98 | 37 | 8.52 | 40.12 | 74.94 |
| Cage changing | 53 | 0.16 | 0.38 | 0.85 | 86 | 0.73 | 4.17 | 34.86 |
| Clean out | 64 | 0.14 | 0.75 | 2.69 | 97 | 1.61 | 2.86 | 12.72 |
| Cage cleaning | 13 | 0.07 | 0.92 | 12.68 | 36 | 1.01 | 4.04 | 32.83 |
| Cage scraping | 18 | 0.28 | 6.25 | 28.51 | 26 | 11.24 | 36.57 | 145.02 |
| Cage wash | 250 | 0.10 | 0.36 | 1.90 | 115 | 1.14 | 5.47 | 18.35 |
| *Cage wash--"clean side"* | 41 | 0.03 | 0.09 | 0.19 | 10 | 0.22 | 0.57 | 2.05 |
| *Cage wash--"dirty side"* | 64 | 0.22 | 0.58 | 2.23 | 15 | 5.28 | 10.07 | 18.59 |
| Changing rooms | 165 | 0.040 | 0.08 | 0.20 | | | | |
| Corridors | 397 | 0.03 | 0.07 | 0.26 | | | | |
| *Corridors--"clean"* | 43 | 0.03 | 0.05 | 0.15 | | | | |
| *Corridors--"dirty"* | 53 | 0.05 | 0.13 | 0.40 | | | | |
| Holding rooms | 350 | 0.07 | 0.30 | 0.92 | 46 | 0.94 | 1.97 | 3.16 |
| *Holding rooms--"IVC cages"* | 36 | 0.14 | 0.50 | 0.75 | | | | |
| *Holding rooms--"open cages"* | 13 | 0.12 | 5.93 | 11.41 | | | | |
| *Holding rooms--"transgenic"* | 34 | 0.41 | 0.56 | 0.81 | | | | |
| Transgenic wards | 18 | 0.29 | 0.38 | 0.79 | 29 | 0.80 | 1.83 | 3.09 |
| Quarantine rooms | 19 | 0.05 | 0.08 | 0.23 | | | | |
| Experimental rooms | 332 | 0.04 | 0.10 | 0.30 | 276 | 0.18 | 0.69 | 2.07 |
| Husbandry | | | | | 146 | 0.53 | 1.28 | 6.46 |
| General duties | | | | | 64 | 0.19 | 0.99 | 3.91 |
| General cleaning | | | | | 29 | 0.40 | 0.92 | 8.08 |
| Filter change | 18 | 0.50 | 0.55 | 0.96 | 33 | 0.50 | 6.49 | 20.58 |
| Filter clean | | | | | 14 | 13.00 | 60.35 | 199.32 |
| Laundry | 46 | 0.03 | 0.06 | 0.30 | | | | |
| Offices | 152 | 0.04 | 0.06 | 0.12 | | | | |
| Tea/rest rooms | 75 | 0.03 | 0.05 | 0.12 | | | | |
| Plant rooms | 49 | 0.02 | 0.04 | 0.07 | | | | |

As expected, background exposures to mus m 1 in corridors, offices, canteens/tearooms, plant rooms, laundry, and changing rooms were low and significantly lower than measurements in holding rooms, where invariably rodents are housed and looked after ($p < 0.01$). Background exposures were statistically higher ($p < 0.01$) than holding rooms in areas where active jobs such as "bedding dump", "clean out", and "cage scraping" were undertaken, but not for "cage wash", "cage changing", and "cage cleaning". A small number of static samples from "holding rooms" clearly identified whether IVC or open top cages were in use. Median levels of mus m 1 of 5.93 ng $m^{-3}$ were recorded where open cages were in use compared with median values of 0.50 ng $m^{-3}$ for IVC cages, which supports previously reported findings that the use of IVC reduces but does not completely eliminate allergen exposure in such rooms [18,24].

The mus m 1 personal monitoring data clearly identifies that the activities of "bedding dump" and "cage scraping" carry a very significant risk of high exposures; while the 75$^{th}$ percentiles for "cage changing", "clean out", "cage cleaning", and "cage wash" suggest that in certain units these activities are associated with high potential allergen exposures. Higher ratios between the upper and lower boundaries of the interquartile range (IQR) expressed as percentages of the median value for "cage wash", "cage cleaning", and "cage changing" (685%, 926% and 625% respectively) may indicate significant differences between different units in how they undertake these processes and/or the exposure control measures utilized. Identification of work undertaken on the dirty side of cage washing clearly shows increased exposure in comparison to "clean" side operations of cage washing.

Generally, exposures from both static and personal monitoring in experimental rooms suggested that exposures were low (Tables 4 and 5). Although the median for the activity of "dosing" indicates generally higher exposure for this particular experimental activity, the increased upper boundary of the IQR (75$^{th}$ percentile) for "bleeding" (Table 4) may suggest that in a sizeable minority of circumstances, personal exposure for this activity may be higher. Personal samples taken from staff looking after animals ("husbandry") were generally low, but again, the elevated 75$^{th}$ percentile related to "animal receipt" suggests higher exposure values in some cases within this category (Table 5).

**Table 5.** Breakdown of personal atmospheric mouse results in ng $m^{-3}$ for experimental tasks & husbandry.

| | N | 25$^{th}$ percentile | Median | 75$^{th}$ percentile |
|---|---|---|---|---|
| **Experimental tasks** | 276 | 0.18 | 0.69 | 2.07 |
| culling | 18 | 0.28 | 0.98 | 2.99 |
| surgery | 35 | 0.12 | 0.25 | 0.91 |
| dosing | 29 | 0.31 | 1.76 | 13.49 |
| bleeding | 18 | 0.15 | 0.82 | 8.96 |
| biopsies | 12 | 0.42 | 0.53 | 0.78 |
| behavioural | 16 | 0.59 | 1.36 | 2.42 |
| **Husbandry** | 146 | 0.53 | 1.28 | 6.46 |
| animals housed in IVCs | 13 | 0.93 | 1.30 | 5.70 |
| animals housed in "open" cages | 10 | 6.60 | 46.8 | 84.64 |
| animal receipt | 13 | 1.06 | 1.52 | 16.56 |
| health checks | 36 | 0.45 | 0.61 | 1.73 |
| weighing | 10 | 0.30 | 0.64 | 0.82 |

Thus for "dosing", "bleeding", and "animal receipt" elevations in median or the 75$^{th}$ percentiles may reflect the necessary handling of live animals close to the staff's breathing zone during these tasks, although RPE may mitigate the inhaled dose. The comparison, albeit of small numbers between personal samples for husbandry activities where IVCs and "open cages" are in use is striking (Table 5), shows the level of exposure reduction IVCs can confer for this particular activity.

The mus m 1 personal monitoring data were examined to see if any activity was over or under represented in breaching the P90 level (Chi-squared or Fishers exact test). The relative risks of breaching the P90 from tasks of "bedding dump", "cage cleaning", "cage scraping" and "filter cleaning" activities" were significantly higher ($p < 0.001$); while "experimental activities" had a significantly lower risk ($p < 0.001$) of breaching the relevant P90. For personal samples related to "general duties", "holding rooms", and "transgenic wards", no sample breached the P90.

Personal exposures taken during a relatively small number of "filter changing" or "filter cleaning" activities showed that exposure could be considerable, especially for "filter cleaning" (Table 4). Whether these filters are associated with plenums, filter lids on IVC cages or room filtration systems is unclear.

### *3.5. Is the downward decrease in mus m 1 monitoring data over time influenced by the nature of the sites/tasks sampled?*

Previous analysis (Table 3) had shown a decrease in mus m 1 levels in personal samples from 2014. There is the possibility that changes in the proportion of lower risk activities submitted overtime could influence this trend. *A priori* it might be thought that those collecting and submitting samples would over time with increasing knowledge, focus on monitoring higher risk activities. Identified activities of "general duties", "husbandry", and "experimental" were considered as lower exposure activities from Table 4 and the percentage of these pooled activities of the total monitoring by year bands (Table 3) was examined. Interestingly, the mean percentage of these "low exposure" activities was 27% from 2005–2013 and increased to 38% during 2014–2022.

A two–way ANOVA on log-transformed mus m 1 data for personal samples was undertaken using both year groups and samples categorized as low exposure activity or not, as explanatory variables. While as expected the exposure category significantly influenced the mus m 1 results ($p < 0.001$), the year group also remained a significant influence on mus m1 levels ($p < 0.001$), suggesting that the decrease in levels over time is real. Examination of the estimated marginal means of mus m 1 for samples identified as either high or low exposure showed a clear decrease in the time bands from 2014, confirming the earlier analysis.

A similar analysis was performed for rat n 1 personal air samples. The year grouping was a significant explanatory variable ($p < 0.001$), but the exposure variable and the interactive term were both non-significant ($p > 0.05$). The estimated marginal means for both the high and low exposure category suggest that results were higher in 2017–2019 and 2020–2022 than earlier time bands.

### *3.6. Changes in the incidence rate of OA over time*

Annual estimates of OA from SWORD are averaged and tabulated in three-year periods (THORR06) [23]. Unfortunately, the three-year reporting periods for SWORD are one year displaced from our three yearly exposure periods shown in Table 3, so we have taken the closest SWORD period of 2004–2006 compared to our initial three yearly exposure period of 2005–2007. The average annual estimated number of OA diagnoses from SWORD for 2017–2019, the last time period for reliable reporting due to covid, have halved since 2004–2006. Estimates of the number of UK staff in the sector

based on the number of personal project licenses have marginally increased by about 13% over this period, while lower estimate of staff numbers are derived from IAT supplied information. Thus, the estimates of annual incidence rate of new cases of OA, using the two sources of workforce numbers as denominators, are calculated as between 0.68–1.67 per 1,000 staff for 2004–2006 reducing to between 0.30–0.81 per 1000 staff for 2017–2019. Therefore, over the period of the monitoring in this report, the UK annual OA incidence has decreased by around 55% between the two time periods. In 1999/2000, Draper had estimated the annual UK incidence of asthma in those occupationally handling small animals as 1.56 per 1000 workers [9].

## 4. Discussion

This paper is a retrospective analysis of data derived from commercial LAA monitoring over a 17-year period, not a research study utilizing a defined population, such as reported by Feary [18], Di Renzi [25] and Marcelloni [26]. However, it does represent a large body of LAA results from this sector in the UK. We have not mandated which facilities were monitored and where in facilities sampling was undertaken. There may possibly be some bias in the results towards facilities/companies who are more pro-actively engaged in controlling exposure and risk, and thus are willing to be bear the cost of monitoring. We are also reliant on task descriptors supplied by those submitting the samples in brigading to the task categories, so there may be some ambiguity or overlap in some descriptors, depending on the nature, size, and organization of a facility. For example, “cage changing”, “clean out”, and “cage cleaning” may have some ambiguity. Whereas, “cage scraping”, if necessary, is distinct and is the initial stage of the overall cage washing process where cages may be handled initially on the “dirty side” and again handled post-washing on the “clean side”.

The monitoring data was not undertaken as mandated by the regulatory body but by duty holders at their expense to help them control exposure in their animal units. We have therefore refrained from an over-statistical analysis of the data, but taken a more qualitative analysis of the results. The data presented may help those undertaking current LAA monitoring in terms of benchmarking their own results against the wider sector, and identify those activities and locations for closer scrutiny. The concept of the 90$^{th}$ percentile (P90) had been introduced, which has proven successful in biological monitoring as a means of driving exposures down long term [27,28]. We attempt to identify those activities where higher exposures can be found and thus direct exposure control measures to such activities. The data covers some 77% and 47% more mice and rat results, respectively, than we reported previously and covers the longer time-frame of 2005–2022 [21].

While background or static samples are useful, personal samples collected in the breathing zone of a worker reflect the likely inhaled dose of allergen by staff and risk to health, albeit mitigated by whether RPE is worn and the nature of the RPE. We do not have accurate information concerning RPE in relation to personal atmospheric samples, although this is a sector where the wearing of RPE is commonplace. The data therefore reflects potential inhalation exposures. In contrast static samples lack clarity between different sites in the spatial relationship between where the air sampler is located and the source of the allergen exposure or staff. Therefore, in terms of identifying temporal shifts in risk to workers across a number of sites, personal samples are more useful.

The data show that while the overall levels of mus m 1 in personal samples have declined since 2014 with a significant downward trend in the cumulative P90 from 2008; intriguingly, rat n 1 levels seem to have increased somewhat in the last 6 years. This divergence between trends in mouse and rat results is puzzling. The engineering control measures available to employ for mice and rats can be the same. Some of the institutes sending samples to us only use mice, while other units use either species

depending on need. Further work is needed to understand why the rat exposure data suggests a different temporal exposure pattern to mice. However, by a large majority, mice are most utilized both in experimental and transgenic work, so the reduction in exposure found in mice allergen levels is in accordance with the reduction with the incidence of UK asthma cases from LAA reported via SWORD [22,23] and our estimation of a 55% decrease in annual incidence rate of new cases of OA in the time frame of our analyses. It should be noted that the data from SWORD under-estimates the true incidence of asthma as diagnosis needs to be made by an appropriate chest physician [9,29,30]. However, it is invaluable in comparing UK annual incident rates of OA between different workplace sectors and studying temporal shifts. Smoking has been identified as a risk factor for sensitization and presentation of symptoms is those working with laboratory animals [11], and it is unclear if the reduction of smoking in Great Britain, which continues to decline [31], has had any influence on the OA statistics for LAA or other OA where smoking has been noted as a risk factor.

Under the UK Control of Substances Hazardous to Health (COSHH) Regulations, exposure to allergens should be controlled to as low as reasonably practicable. However, as with biological monitoring, identified breaches of a defined P90 can help identify areas or activities for priority investigation and action. The data also helps identify those areas and tasks where there are higher risks of exposure to animal allergens, and the breakdown of measured exposures by tasks or activities allows a unit to compare their exposure levels against similar activities in peer units and thus identify areas for a particular unit to target for further exposure control measures. The establishment of databases for both biological and environmental monitoring data, allows for the tracking of exposures, riskier activities and P90 values over time. Other laboratories are known to undertake such monitoring, and there could be some bias in the results presented in this paper. However, we include sampling visits to around 50% of UK facilities holding establishment licenses, many of them more than once and around 10% of these units have undertaken monitoring on a regular basis (annually) over the time period, with four occupational hygienists/safety professionals having undertaken 50% of the presented sampling data. We therefore consider that the results are probably representative of the situation in the UK.

Figure 1 suggests that the cumulating P90 levels for both allergens and static and personal samples tended to stabilize with 200–300 samples, being unlikely to show large temporal shifts, and the uncertainty (CI) associated with the P90 reducing considerably. Obviously, further additions to the cumulating P90 reduces the uncertainty in the P90. The value of this pragmatic, non-health-based tool lies in identifying high values for priority investigation that its recalculation over time should have a general ratcheting down effect on exposure [32,33]. The data for the P90 for mus m 1 personal samples does show a significant downward trend post 2008.

Tables 4 and 5 show that experimental tasks with mice generally reflect lower personal exposures as with animal husbandry tasks, outwith the use of "open cages". As expected, static atmospheric levels in offices, rest rooms, and corridors are generally low, but not necessarily undetectable. Higher exposure are associated with cleaning animals out, getting rid of soiled bedding, changing or cleaning filters, and elements of the case washing process. These exposure results are compatible with Di Renzi's study based on categorization of staff into three task-based work categories [25] and our earlier publication [21].

The increasing use of IVCs over "open cages" has led to a reported reduction, not elimination, of rodent allergens into these work environments [18,21,24,34], which the data in this paper confirms, suggesting around a 10-fold, or greater, reduction in atmospheric allergen levels (Tables 4 and 5). However, even where IVCs are employed to house animals, they will need to be taken out for experimental (e.g. dosing, surgery) or some husbandry activities, such as weighing of animals. There

are activities where allergen contaminated material from cages needs to be disposed of (bedding dump, clean out), cages cleaned (cage scraping, cage washing), or changed, and are all activities that can lead to potential high exposures. There are engineering solutions that have been identified [35] and can be employed to reduce exposure, such as ventilated cage changing stations, vacuum dump stations, and ventilated tables for animal husbandry etc. Fully automated robotic cage washing stations are available and our data suggests that the use of such systems can almost eliminate any allergen exposure to staff in the vicinity (data not shown). However, such systems are very expensive and generally only found within the pharmaceutical industry. Smaller facilities have to employ less automated exposure control measures for cage washing and our data shows that activities on the "dirty" side of cage washing need particular care to reduce exposure.

Maintenance on filter systems as a cause of exposure is well known across many industries and the limited data presented here suggest that attention to exposure control measures and appropriate RPE are warranted where filters are changed or cleaned. Currently the data in this paper does not distinguish between handling filters on large, central air handling systems, the plenum systems for IVCs, or any filter units on each IVC cage. Such activities on the former are unlikely to be as frequent as some other activities, but the potential exposure can be considerable. Other activities where animals are outside of their housing (animal receipt, health checks, and dosing in experimental studies) are potential sources of higher exposures. The hierarchy of control would suggest the implementation of local exhaust ventilation (LEV) should be considered rather than simple reliance on RPE, e.g., weighing of animals on a ventilated bench system. Nevertheless, this is a sector where RPE is widely used.

The data highlights the numerical differences found between static and personal atmospheric monitoring data of the same activity or location. Static samples significantly underestimate the exposures in the breathing zone of staff, yet static samples have become the predominant samples collected in this dataset. Therefore, in interpreting collected atmospheric monitoring data care must be taken that comparisons are made against data from the appropriate type of sample.

The data in this paper suggest that the median high personal exposures during activities of "bedding dumping", "cage scraping", and "filter cleaning" are high priority tasks for examination of current exposure control measures. The wide relative ranges in the IQR for personal samples from "cage changing" and "cage cleaning" could suggest either mis-classification of descriptors or that while high exposures can occur in some units for these activities, there may be available ways for controlling to lower exposure being used by other units. Tasks of "bedding dump", "cage cleaning", "cage scraping", and "filter cleaning" having a high risk of exceeding the appropriate P90 supports this analysis. Experimental and husbandry activities generally suggest lower exposures. Activities of "dosing", "bleeding", and "animal receipt", which involve the animal being closer to staff's breathing zone, may potentially cause higher exposures, depending on the nature of exposure control measures in place .

## 5. Conclusions

The data show a welcome decline in both mouse allergen levels in air samples measured in workers' breathing zones, reflecting the predominant laboratory animal species and the measurement most related to health risk, and the concomitant UK reduction in occupational asthma related to laboratory animals. However, there remains a significant incidence rate of occupational asthma in those working with laboratory animal allergens that needs to be addressed, as inherently does the apparent increase in exposure to rat allergens.

While we do not claim causality between the drop-in incidence rate of OA cases over time and exposure monitoring, we believe that the level of unmandated monitoring activity at a significant number of duty-holders' expense in this sector indicates a desire to understand and take action to control exposure, and thus reduce ill-health.

## Use of AI tools declaration

The authors declare that they have not used artificial intelligence (AI) tools in the creation of this article.

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## Conflict of interest

The authors declare no conflicts of interest in this paper.

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