



# Laboratory animal allergy: a new world

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## Purpose of review

In recent years there has been a dramatic shift in the world of animal research whereby genetically modified mice have largely supplanted rats, and individually ventilated cages have been introduced to house delicate experimental animals in place of traditional open cages. Although laboratory animal allergy remains an important cause of occupational asthma, the risks associated with contemporary practice and consequently the opportunities for primary and secondary prevention are largely unknown.

## Recent findings

Although there is clear confirmation of a widespread increase in animal experiments using mice, the evidence-base on the associated risks has lagged. Individually ventilated cages reduce ambient levels of mouse urinary protein in air but task-based exposures are unquantified. Immunological techniques to identify sensitization to mouse proteins are poorly standardized. The available evidence suggests that modern practices are, in most cases, associated with a reduced incidence of animal sensitization.

## Summary

There is a paucity of data to inform evidence-based practice in methods to control the incidence of laboratory animal allergy under the prevailing research environment; a better understanding of the relationship between exposures and outcome is urgently needed. As exposures decline, the relative importance of individual susceptibility will become prominent.

## Keywords

mouse, occupational asthma, rat

## INTRODUCTION

One of the more stimulating challenges faced by the physician with an interest in occupational respiratory disease is the requirement to keep abreast of developments in industry and other workplaces. In practice this can be achieved by maintaining communication with occupational health and safety personnel, by listening to the experiences related by our patients and by visiting their workplaces, by browsing various media and of course through the sharing of professional knowledge as exemplified by this 'update' series.

The relevant developments often concern the introduction of hazardous materials into familiar or new settings; a clear example is the increasing use of a very wide range of biological enzymes in a bewildering variety of manufacturing processes. Alternatively, technologies may be transformed so that they no longer involve the use of hazardous agents, although such developments are seldom undertaken primarily to protect workers but more usually for technological, economic or environmental ends; an example is the phasing out of diisocyanates from many industrial applications in Europe as a direct result of legislation passed to prevent chemical

contamination of ground water sources [1]. Occasionally, very major changes are made to what was a well established way of working in which the attendant risks to health were familiar to employees and to workplace health and safety professionals. Such shifts may require a radical reexamination of what we all thought we knew.

One contemporary example is in the use of laboratory animals for medical and related research. The recent, dramatic increase in genome-based research has led directly to a sharp shift in experimental models so that in most countries the dominant species is now the mouse (rather than the rat or other small mammals) because it lends itself readily to genetic modification. In Great Britain, for instance, between 2011 and 2012 there was a 22%

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## KEY POINTS

- Significant changes in animal research have occurred in recent years, with a sharp increase in the use of genetically modified mice and the introduction of individually ventilated cages (IVCs) in place of open cages.
- IVCs reduce ambient levels of mouse aeroallergen and while the incidence of sensitization to laboratory animal allergens appears to have fallen, it has not been eliminated even in those individuals working in IVC-only facilities.
- The relationship between exposure and outcome in laboratory animal allergy is incompletely understood.
- There is a poor evidence-base to inform practices to control the contemporary incidence of laboratory animal allergy; the risks associated with modern animal research need further evaluation.

increase in the use of genetically modified animals and for the first time the number of procedures involving genetically modified animals (1.91 million) was greater than the number performed on normal animals (1.68 million) [2]. Experiments using genetically modified mice (and even the animals themselves) are often exquisitely sensitive to microbial contamination and as a consequence a great deal of effort and money has been put into their safe confinement. As an unintended consequence the scientists and technicians who work with laboratory animals have undergone significant changes in both the nature and intensities of their exposures to animal proteins and thus, in their risks of laboratory animal allergy (LAA).

Almost all the available, published and familiar evidence on the prevalence, incidence and determinants of LAA has been gathered from the study of populations working under 'traditional' conditions [3,4]. As these conditions have largely been replaced by alternatives, a process that is likely only to be consummated, we have to rethink almost everything we believed we knew about LAA.

## HOW LARGE IS THE EXPOSED POPULATION?

It is remarkably difficult, perhaps because it is a sensitive area, to obtain information on the number of people who work in laboratory animal research. Draper and colleagues [5] estimated that in the United Kingdom between 12 000 and 17 000 researchers and technicians worked with small laboratory animals, some 60% of them with mice, but in the intervening 15 years since their survey it is

likely that this proportion has increased significantly (see below). In China, it is claimed that over 100 000 staff work with laboratory animals, 84% of them in biomedical research; the source for this estimate is unclear [6].

Somewhat easier to find are estimates of the number of animals used in research and it is a reasonable assumption that these will correlate – in total and by species – with the exposed (human) population. National reporting systems vary significantly and comparisons between countries are fraught with difficulty; for example, two of the largest users, the USA [7] and Japan, provide no useful information on rodent experiments. A series of recent regional estimates are shown in Table 1 [2,8–11], ordered by year.

The data confirm the increasing primacy of the mouse; further demonstration is provided in Figure 1 [12] which displays the inexorable increase in experimental mouse (and matched decline in rat) procedures in Great Britain over a 17-year period.

## HOW HAVE EXPOSURES CHANGED?

The traditional animal facility housed mixed species of small mammals – rats, mice, hamsters, guinea pigs – in 'open-topped' cages with central systems of exhaust ventilation. The use of personal protective equipment by husbandry and scientific staff was haphazard and the arrangements for entry to and egress from the facility often were uncontrolled. Modern facilities have highly concentrated populations of carefully bred mice which are housed in sealed cages with local exhaust ventilation [individually ventilated cages (IVCs)]; an example is depicted in Figure 2. Protective equipment for staff – including the use of respiratory and hair protection – is more uniformly used and in some countries is mandated [13]; systems for staff movements in and out of the facility are tightly regulated and include changing of clothes and more or less elaborate methods of decontamination including the use of wet and dry-showers.

**Table 1.** Numbers of animals (procedures) used in research in five regions; percentages refer to the proportion of total animal procedures

|             | Year | Mice (%) | Rats (%) |
|-------------|------|----------|----------|
| UK [2]      | 2012 | 74       | 7        |
| Germany [8] | 2012 | 87       |          |
| EU [9]      | 2011 | 61       | 14       |
| Canada [10] | 2009 | 31       | 8        |
| EU [11]     | 2005 | 53       | 19       |

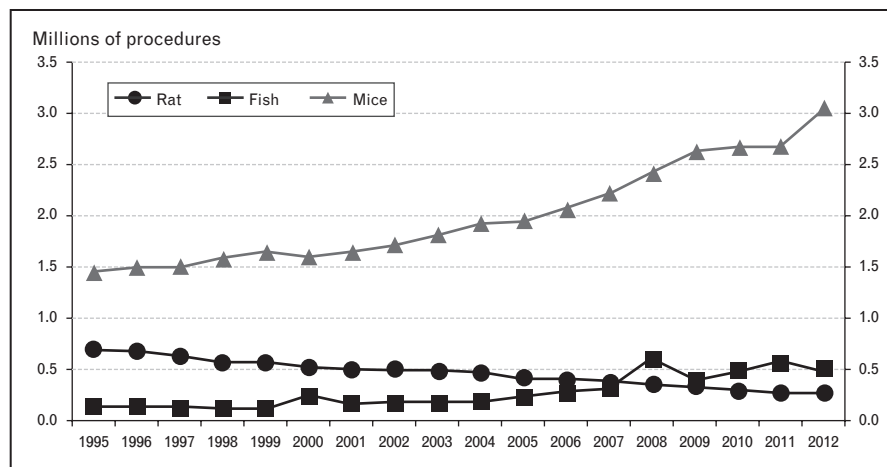


**FIGURE 1.** Research procedures using mice, rats or fish in Great Britain 1995–2012 [12].

To repeat, these arrangements were designed primarily to prevent the cross-infection of experimental animals. A small number of studies have examined the changes in airborne animal protein levels consequent on the early introduction of IVCs to animal houses. Gordon and colleagues, for example, reported substantial control of ambient levels of mouse urinary protein in the vicinity of high-efficacy IVCs operating under negative pressure [14]; similar findings have been reported in a Swedish animal facility [15,16], but the authors provided a warning against any complacency since exposures remain high during procedures such as cage changing which required direct animal handling. Surprisingly, no more current research in this area has been published.



**FIGURE 2.** Example of an individually ventilated cage and its airflow [courtesy of TECNIPLAST SPA, Buguggiate (VA), Italy].

## MOUSE VERSUS RAT ALLERGENS

An awareness of the characteristics of mice and rat allergens, and their similarities, is important in helping understand and interpret sensitization rates obtained from epidemiological surveys and when considering cross-reactivity in immune responses. A recent review has described in detail the allergens involved in laboratory animal allergy [17<sup>\*</sup>]. Rodent allergens are found in dander, hair, saliva, urine and serum; urine is traditionally considered to be the main source of allergenic proteins in both rats and mice [18]. As with Rat n 1, the primary mouse allergen Mus m 1 is produced in the liver under control of androgenic hormones explaining, in part, the observation that working with male rodents is an independent risk factor for development of LAA [19]; while adult male mice produce around 5–10 mg of mouse urinary protein per day, female adult mice produce four times less.

Both Mus m 1 and Rat n 1 are lipocalin proteins, sharing 64% of their amino acid structures. Mouse urinary protein has shown IgE cross-reactivity with rat urinary protein [20] and Equ c 1 (a major horse allergen) [21], probably because of the presence of several identical amino acids in the primary sequence located primarily at the terminal ends of the proteins which form a large epitope for potential IgE-binding. Rodent allergens are carried on a range of particle sizes, which can remain airborne (and respirable) for extended periods. Mouse allergen are most commonly found on particles measuring 3.3–10  $\mu\text{m}$  [22]; rat allergens can be found on larger particles but the majority are on similar sized particles ranging 1–7  $\mu\text{m}$  [23].

These similarities in physical properties of rat and mouse allergens are noted, but what is unclear is

whether their propensities to cause allergy are similar. By way of analogy, two major dog allergens, Can f 1 and Can f 4 have a similar capacity to sensitize, but 200 times more Can f 1 was found in dog dander than Can f 4, suggesting that the quantity in the animal source is not necessarily related to the capacity to sensitize [24].

Immunological sensitization to rodent allergens may be determined using skin prick tests or through immunoassays [ImmunoCAP or radioallergosorbent test (RAST) methods] to detect the presence of specific IgE antibodies in serum samples. Skin prick tests are generally believed to be more sensitive, particularly in individuals with low levels of circulating total IgE, but this will depend on which skin prick test allergen solution(s) are being used. There are, in some jurisdictions, severe restrictions on the ‘in-house’ preparation of skin test solutions, and the few commercial allergens that are available are unvalidated. We have reported discordance between the skin prick test and specific IgE testing suggesting that more than one immunological measurement should be used for determination of sensitization in cases where it is crucial to avoid false-negative results [25].

RATES AND DETERMINANTS OF SENSITIZATION

In the general population the prevalence of IgE sensitization to mouse appears to be very low (<1%) and is barely higher in those who report allergic symptoms – at least in the United States [26]; in some selected populations in the same country, the prevalence may be higher [27,28] but it is not clear how far this is generalizable. Thus, while it is probably safe to assume that any evidence of sensitization to rodents is because of exposure to domestic pets or occupational exposure knowledge of the background rates of sensitization in the local population would be helpful.

As mentioned above, there are very few published estimates of the prevalence of sensitization in laboratory workers exposed ‘only’ to mice; and none readily

precludes the likelihood that some workers will have had prior exposure to other species with the possibility of cross-reactivity between species. The published evidence is summarized in Table 2 and shows fairly consistent estimates of prevalence between 5 and 8% [29,30] with the egregious exception of one population in the United States where the incidence of sensitization over 2 years was 23% [31].

The reasons for this discrepancy are not immediately obvious but may reflect that the US study was carried out in a mouse breeding unit where stocking density is likely to be higher and practices may differ from those in a purely experimental facility. The largest study, which is also the most recent, has the advantage of being a multicentred study; however, overall the combined number of individuals in the studies is small (total *n* < 1000) and the results may not be entirely representative of the total exposed population. For example, surveys may preferentially be conducted (and their findings published) in facilities where management are advocates of protecting workers’ health and where exposure controls are consequently tighter. The evidence is derived wholly from studies of researchers in high-income countries and its generalizability to economically developing settings must be interpreted with caution. All epidemiological surveys are at risk of participator response bias, and, finally, there will be some healthy worker effect where those individuals who have become sensitized and symptomatic have left employment. Taken together these factors suggest the reported estimates are likely to be an underestimate of the true rates in the total exposed population.

Similar studies of those working with rats suggest a higher prevalence of sensitization [3], although it is difficult to draw exact comparisons. Any greater risk with rat exposure may reflect historical factors associated with higher aeroallergen exposure, such as different control measures, including the use of open cages, a lower frequency of cage cleaning, limited (if any) use of personal protective equipment, or may be because of factors directly related to the allergen and its potency to sensitize an exposed individual.

Table 2. Epidemiological studies of mouse sensitization in laboratory research settings

| Study                 | Country   | Year of publication | N surveyed | N (%) sensitized           | Definition of sensitization |
|-----------------------|-----------|---------------------|------------|----------------------------|-----------------------------|
| Schumacher [30]       | Australia | 1981                | 121        | 9 (7%)                     | SPT (≥4 mm)                 |
| Cullinan <sup>a</sup> | UK        | 1994                | 86         | 4 (5%)                     | SPT (≥3 mm)                 |
| Peng [31]             | USA       | 2011                | 179        | 23% incidence over 2 years | SPT (≥3 mm) and/or IgE      |
| Feary <sup>b</sup>    | UK        | 2015                | 512        | 41 (8%)                    | SPT (≥3 mm) and/or IgE      |

<sup>a</sup>Personal communication.  
<sup>b</sup>Unpublished data.  
SPT, skin prick test.

On the basis of studies of researchers working with rats, the established risk factors for developing LAA include the intensity of aeroallergen exposure [32,33], atopy [32,34–39], genetic susceptibility [34,40] and work with male rodents [41]. Exposure is believed to be the most important risk factor, although the dose-response relationship may be nonlinear [42,43] with attenuation of sensitization and symptoms to rats at high allergen exposure; and furthermore, variability of exposure pattern may play a key role [31]. The extent to which these factors operate in contemporary facilities remains largely unexplored.

## CONCLUSION

Driven by rapid changes in technology and scientific advances in genomic studies, the pace of change in laboratory animal research has been rapid and shows no signs of slowing. The occupational health research community has been slow to adapt and there are significant lacunae in our understanding of what LAA looks like in this new world. The extent to which we can apply the lessons learned in historical studies of researchers working in very different conditions is uncertain. As exposures to laboratory animal allergens fall, the importance of individual employee susceptibility will be magnified with significant implications for the protection of the health of a precious human resource. We suggest that the key, unanswered areas for research include the relationship between exposure(s) and outcome and the understanding of threshold exposures and of how these may be controlled; the most efficient methods for determining early sensitization and the prevention of clinical allergy; and the components of individual susceptibility that will allow individualized approaches to disease prevention.

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## Conflicts of interest

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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