An investigation of the Biokker Photocatalytic Oxidation system used to reduce airborne allergens and microorganisms from an animal unit cage wash

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Summary

As more and more resources are invested in both workplace wellbeing and health and safety, it is still difficult to put viable solutions in some working environments. This is especially true with laboratory animals where the highest risk is from airborne Laboratory Animal Allergens (LAAs). LAA exposure, particularly to Mouse and Rat urinary proteins (Mus m1 & Rat n1) can lead to sensitisation, rendering those exposed unable to work with the animal species that they have become sensitized to. Animal technicians and staff often have to wear full Personal Protection Equipment (PPE) when working with the animals, particularly during procedures, changing cages or for staff in the cage wash areas. The PPE worn during activities with high-risk of exposure to LAA or for those with allergies include scrubs/ disposable coveralls, disposable gloves and a facemask or filtered helmet.

Wearing full PPE often restricts free movement when carrying out certain precision procedures and may impact on the practicality of conducting some intricate experiments and procedures. To reduce LAAs in high risk areas the Biokker PCO (Photocatalytic Oxidation) system has been utilised to oxidise airborne pathogens such as LAAs, airborne bacteria and fungi. The aim of this short report is to determine the ability of the system to reduce or remove airborne contaminants in a high throughput area. The location chosen was the dirty side of the cage wash facility at The University of Manchester's main animal unit.

Air quality is becoming increasingly important, it is common knowledge that the European Union are looking at new air quality levels for many Volatile Organic Compounds (VOCs) such as formaldehyde and glutaraldehyde, to bring the UK in line with many other countries. Short Term Exposure Limits (STELs) are significantly lower in European Union Guidelines than those of the Health and Safety Executive (HSE) in the UK. With all this activity in many areas of air quality and staff welfare, it is therefore unusual that within the Laboratory Animal (LA) sector we do not have firm levels set by the government.

In April 2007 Gary Childs BSc (Hons) FIAT, RAnTech, Mandy Thorpe, MIAT, RAnTech and Seth Jethwa produced a paper to highlight the issues of high exposure levels of LAAs and a potential solution to the problem at that time. This paper was until recently the point of reference used to determine “acceptable working levels” of allergens. Sensitization often results from an accumulated exposure to urinary proteins in the air, so in the latter part of the exposure process, it may only take a fraction of what is considered a “safe level”, to trigger the sensitisation. A safe exposure level is considered within the UK LA sector to be under 2.5ng/m^3 of air sampled, but as already stated no level is 100% safe. A recent paper published, August 2017 “Airborne exposure to laboratory animal allergens”, Howard J Mason * and Laura Willerton (of the HSE) cites a level of under 3 ng/m^3 to be safe.

Due to the constant inflow of solid cages into the room levels of LAA were never totally removed. As cages are continually brought in, stacked and held in the room, allergens can and will be released into the air. Other dynamics also come into play such as; air inlet / extract along with the movement of people within the room may create eddies of air releasing any allergens within the area.

It was determined by the data that even with the Biokker unit switched on there were still some allergens and pathogens in the air; however, LAA levels were reduced as a whole to under 3 ng/m^3 when the Biokker PCO system was on.

Introduction

Laboratory Animal Allergen (LAA) sensitisation is the most common cause of ill health within the research sector. It is estimated that 20% of those exposed will continue to develop symptoms and about 10% of all those who work with laboratory animals will develop the serious symptoms of asthma. The main, but not the only, sources of animal allergens are urine, fur, hair, dander, saliva, droppings and serum. The majority of cases of allergic disease among laboratory animal workers are caused by rats and mice, probably because these are the animals most commonly used in experimental work. Other species such as guinea pigs, cats, insects and shellfish can also cause respiratory and skin allergies in some individuals. The HSE document EH76 refers to this. http://www.hse.gov.uk/pubns/eh76.pdf

EH76 also offers advice on exposure reduction and advice to employers on adequate controls to reduce exposure to LAAs as required by COSHH regulations (Control of Substances Hazardous to Health 2002 amended). This document was produced after consultation with the Home Office and took into account the health and welfare requirements of animals used under the Animal (Scientific Procedures) Act 1986. COSHH sets out a hierarchical system to:

- Eliminate the hazard.
- Substitute the substance for a safer alternative.
- Introduce physical or engineering controls to reduce the substance at source to provide general overall protection in preference to that of any given individual.
- Alter the job design.
- Personal Protective Equipment (PPE) is seen as the last resort.

This study took place in the cage scraping (dirty side) of the cage washing area at The University of Manchester animal unit. Many local engineering and management controls have been introduced to moderate the levels of LAA being emitted into the atmosphere. These include automated robotic cage dumping, the use of individually ventilated cages (IVCs), ventilated rooms and a cage storage area with approximately 20 air changes per hour. Dirty cages are stored for a minimal amount of time prior to loading onto the conveyor into the robotic scraper. Developed and improved by Bioseguridad Integral S.L.U.Poligono Industrial Casarrubios – C/Argentina 2, Na 28806 Alcala de Henares, Madrid, Spain. Biokker has further improved on AiroCide technology developed by National Aeronautics and Space Administration (NASA) for use on their space stations and approved by the U.S. Food and Drug Administration. The Biokker is specifically designed to remove or substantially reduce airborne micro-organisms, allergens, odours and volatile organic compounds (VOCs) from the immediate environment (Fig. 1).

Figure 1. The Biokker is mounted on the opposite wall to the cage scraping robot. It is designed to significantly reduce the airborne allergens and pathogens in the working area 24/7.

The Biokker 40S (standard) system has an air flow of 15cfm / .425 m^3 / minute, therefore, it will treat up to 612 cubic metres of air per 24 hours. The system destroys the organic material within the cells of particles passing through a matrix of titanium dioxide (TiO_2) and zirconium dioxide (ZrO_2) that is irradiated by Ultra Violet C (254nm bandwidth) light. The photocatalytic reaction between the UV light and titanium dioxide produces a 'mist' of hydroxyl and super-oxide radicals that oxidise the organic material within the cell of airborne organisms, allergens and organic contaminants, producing traces of carbon dioxide and water vapour (Fig. 2).

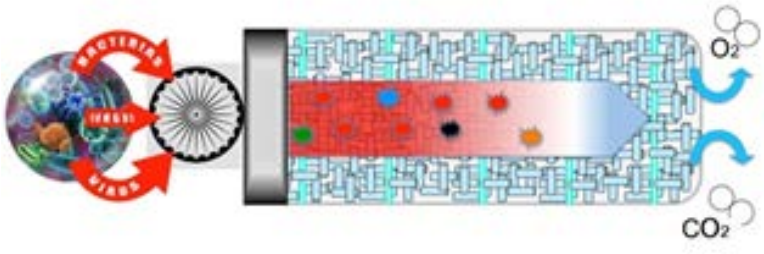

Figure 2**.** Contaminated air is drawn into the Biokker, this air then passes through a matrix of TiO_2 (Titanium Dioxide) and ZrO_2 (Zirconium dioxide) both naturally occurring materials that make up the catalyst in the system which is irradiated by Ultra Violet C lamps. The bound organic material is oxidised resulting in CO_2 / H_2O as the only by-product.

This study was designed to test the efficiency of the Biokker system in the reduction and or removal of LAA's and pathogens from the working environment.

Methodology

The sampling area was the dirty cage wash side of the large animal unit at the University of Manchester. This room is served by one door leading directly to the central corridor, so all dirty cages from the facility come in through this here. In this area, there are approximately 12-15 air changes per hour. It should also be noted that the building is an older type of building, built between 1969 and 1972. Temperatures during the investigation period were in the range of 16 – 24ºC with a relative humidity of 42-60%.

The facility uses Tecniplast IVC racks and the associated cages, both green and red types. These come into the room individually or pre-stacked by animal technicians, the lids are removed and placed on a cage rack for auto washing in the large rack washer. The dirty cage bases are stacked into one another to a height of 14 cages and stored on a dolly in the middle of the room ready for loading onto the automatic robotic cage scraping system. When the dolly is loaded onto the system, photo sensors recognise the dolly and cage type. The robot lifts the top cage, travels 45 degrees anticlockwise,

rotates the cage over a macerator which dumps the majority of dirty bedding in the water vacuum system. The robot taps and scrapes the dirty bases before placing them onto the conveyor belt of the automatic cage washing system. Dirty bedding travels through the vacuum system into waste bins held in an external bin shed on a lower level. The robot is housed behind a safety interlocked perspex screen which has a 12 inch (300mm) gap to the floor and is open topped.

In the room is also a yellow bulk waste bin on wheels with a hinged top into which large waste products in the form of transit cages etc. are disposed of. This is located on the right-hand wall as you look at the robot. Hydro pack water bags are also disposed of in the cage wash area, with empty pouches being stored just to the left of the door.

One Biokker 40 is mounted on the opposing wall to the Robot and next to the entry doors to the room. It is hung 1.65m from the floor and 3.65m from the robot enclosure and has a protective steel crash guard around it to protect it from impact damage from cage wash racks.

Dirty cages from both rat and mouse rooms contain soiled bedding (aspen chips supplied by Datesand), excess food (the main diet used is BK001 provided by SDS (Special Diet Services), several other diets are used on a smaller scale, these include RM1, RM3 and several experimental diets), sizzle nest and cardboard play tunnels.

The locations of all samples taken within the room were constant and this was decided to be the pillar in the middle of the room and at a height of 200cm from the floor. This was the only practical location the samples could be taken as the constant movement of racking and cages meant anywhere else would be susceptible to moving the sampling equipment or compromising the sample by dirty racks being parked right next to the sampling equipment.

LAA monitoring

We carried out static LAA sampling using: The SKC , (SKC Ltd, Blandford Forum, Dorset, UK) “Sidekick” sampling pump which was calibrated to a constant flow rate of 2 litres per minute. This calibration was carried out on an SKC Rotameter through an SKC IOM sampling head and tygon flexible tubing. Inserted into the IOM sampling head carrier was a 0.2µm Flouropore™ PTFE filter (Merck Millipore, Tullagreen, Co cork Ireland.) (Fig. 3).

Figure 3. Sampling equipment used to test airborne LAA levels.

These pumps were manually switched on at 09:00hrs each weekday and switched off at 15:00hrs allowing a runtime of 6 hours covering any activity in the room. No samples were taken at weekends as there was no activity in the cage wash room over the weekends.
The flow rate and time of each sample were noted so that a sample volume could be calculated. Each filter was eluted in 2ml of buffer (PBS with 1% v/v Tween 20) for a minimum of 24 hours. The buffer was then split into 2 x 1ml aliquots. One was stored and the other used for the analysis. The samples were analysed using quantitative Enzyme Linked Immuno-Sorbant Assay (ELISA) tests, (INBIO Inc, Virginia, USA), to determine the levels of MUP and RUP that had been captured. The mass of urinary proteins was then divided by the sample volume so that an airborne concentration could be calculated.

Active Air Microbiology

Active air microbiology tests were carried out using a Parratt Microbio MB2 (Cantium Scientific, Dartford, Kent.) scientific sampler placed at a height of 200cm avoiding moving equipment (Fig. 4). The sampler was set to intake 515 litres at a sampling speed is 1.6 L/second. TSA and SAB agar plates were used as growth media. Plates were then incubated and colony numbers counted and where possible, identification carried out. Microbiological sampling was conducted between 12:00 – 13:00hrs each working day of the three week investigation period.

Multiple-jet impactors, typically with 220 or 400 holes, are used widely for collecting aerosols of living bacteria and fungi. In this type of impactor, the air jets impinge directly onto nutrient agar in a petri dish which is incubated after sampling until collected cells multiply into colonies. Colony counts were adjusted to account for the probability that more than one viable particle was collected through a sampling hole and merged with other microorganisms at an impaction site to produce a single colony.

Figure 4. Parratt Microbio MB2 sampler used to measure air microbiology.

Results

LAA

Results show higher levels of LAA in weeks 1 and 3 for MUP (Fig. 5 & 6). Levels of MUP were at an average level of 10.96 ng/m^3 during the first week of testing when the Biokker system was off. Levels quickly lowered to an average of 1.2ng/m^3 when the system was turned on during the second week of testing. Levels in the third week rose as expected to an average of 4.54ng/m^3 but did not reach the same high levels that were observed in the first week. Levels of RUP (Fig 5 & 6) are lower than MUP due to the small comparable number of rats in the facility, however having made that observation it can clearly be noticed that the levels during week one were quickly reduced from an average of 1.88ng/m^3 in week one to 0.24ng/m^3 in week two when the Biokker was switched on. The actual average of RUP in week two may be lower than the 0.24ng/m^3 stated here as tests used to analyse the amount of urinary protein captured by each filter can only be detected down to 0.2ng/m^3. Therefore levels of RUP for days 6, 7, 9 and 10 may be lower than the 0.2ng/m^3 that was used to calculate the average for week 2. Again the levels of RUP creep back up to an average of 1.36ng/m^3 in week 3 when the system was switched off again.

System on/ off	Date	ng MUP/m^3	ng RUP/m^3
	06/08/2018	9.3	2.8
	07/08/2018	11.4	2.1
Off	08/08/2018	12.6	2.4
	09/08/2018	11.8	1
	10/08/2018	9.7	1.1
	13/08/2018	1.3	<0.2
	14/08/2018	1.5	<0.2
On	15/08/2018	1.2	0.4
	16/08/2018	1.5	<0.2
	17/08/2018	0.5	<0.2
	20/08/2018	3.8	1.2
	21/08/2018	4.1	2.1
Off	22/08/2018	4.8	0.8
	23/08/2018	5.1	1.9
	24/08/2018	4.9	0.8

Figure 5. Results from LAA screening with corresponding dates.

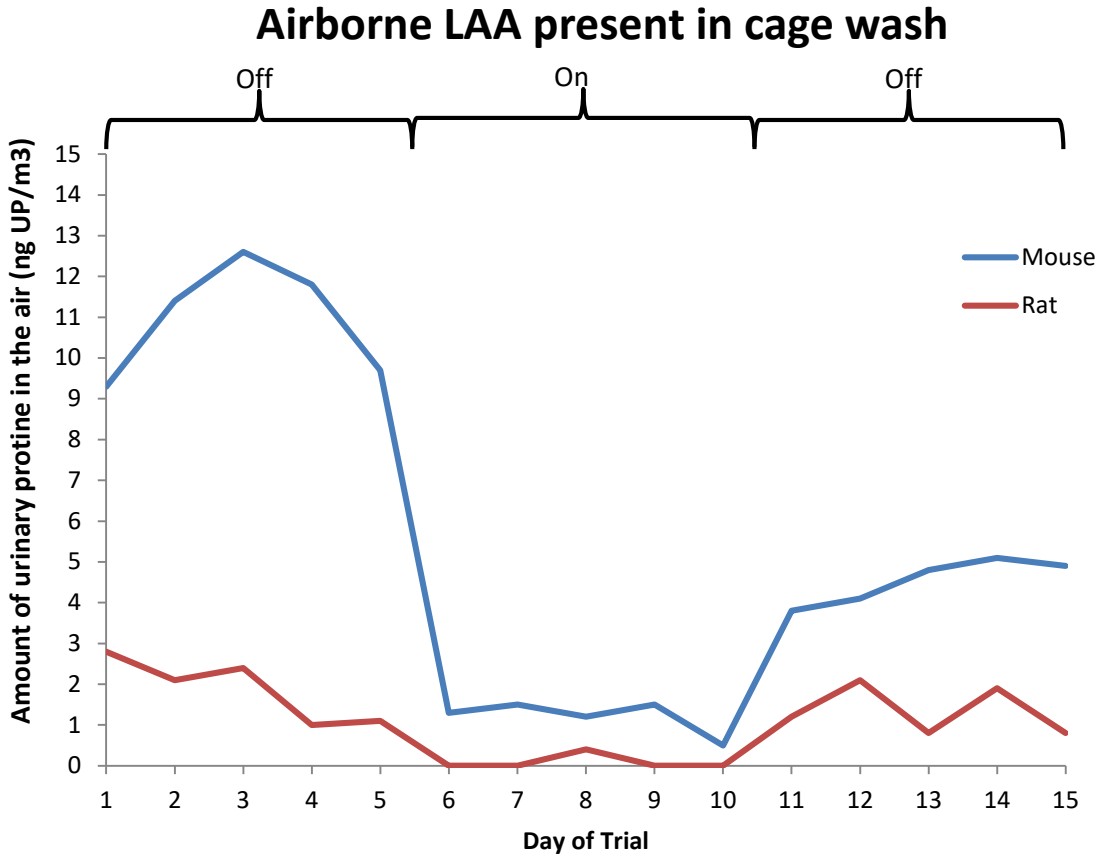

Figure 6. The amount of mouse and rat urinary proteins present in the air during trial days.

The amount of mouse and rat urinary proteins present in the air during trial days. The Biokker machine was off during days 1-5 when mouse and rat urinary proteins were at their highest with a daily average of 10.96ng/m^3 for mice and 1.88ng/m^3 for rats. There was a drop in levels on days 6-10 when the machine was on with a daily average of 1.2ng/m^3 for mice and 0.24ng/m^3 for rats. Urinary proteins began to rise again after day 11 when the machine was turned off with a daily average levels rising to 4.54ng/m^3 for mice and 1.36ng/m^3 for rats.

Microbiology

As can be seen (Fig. 7 & 8) in week one when the Biokker was switched off, the levels of environmental material are high with average daily CFUs of 220.4. During week 2 when the Biokker was switched on, the levels were much lower with a daily CFU average of only 43. CFU were not totally eliminated as environmental bacteria, yeasts and moulds can be carried into a room in the bedding of animals, on the clothing or feet of personnel entering the room and even on everyday items such as brushes and vacuum cleaning equipment etc. So, levels shown during week 2 may be peaks due to the throughput of people coming and going into the room with the associated contamination. In week 3 when the Biokker was switched off again, we can clearly see a slight increase in environmental growth. The types of bacteria and fungi identified represent no health risk to animals or personnel, they are commonly found in animal facilities across the UK.

Date	TSA Adjusted bacteria	SAB Adjusted yeast	SAB Adjusted mould	Total weekly CFU	Average daily CFU
6.8.18	216	11	2		
7.8.18	20	16	0		
8.8.18	324	149	0		
9.8.18	313	16	0		
10.8.18	26	9	0	1102	220.4
13:08:18	29	16	0		
14.08.18	32	21	0		
15.8.18	29	3	0		
16.8.18	14	5	0		
17.8.18	58	8	0	215	43
20.8.18	16	12	0		
21.8.18	32	10	1		
22.8.18	89	16	1		
23.8.18	150	16	1		
24.8.18	27	13	0	384	76.8

Figure 7. Data collected to determine the Biokkers effectiveness at fighting microbiological agents.

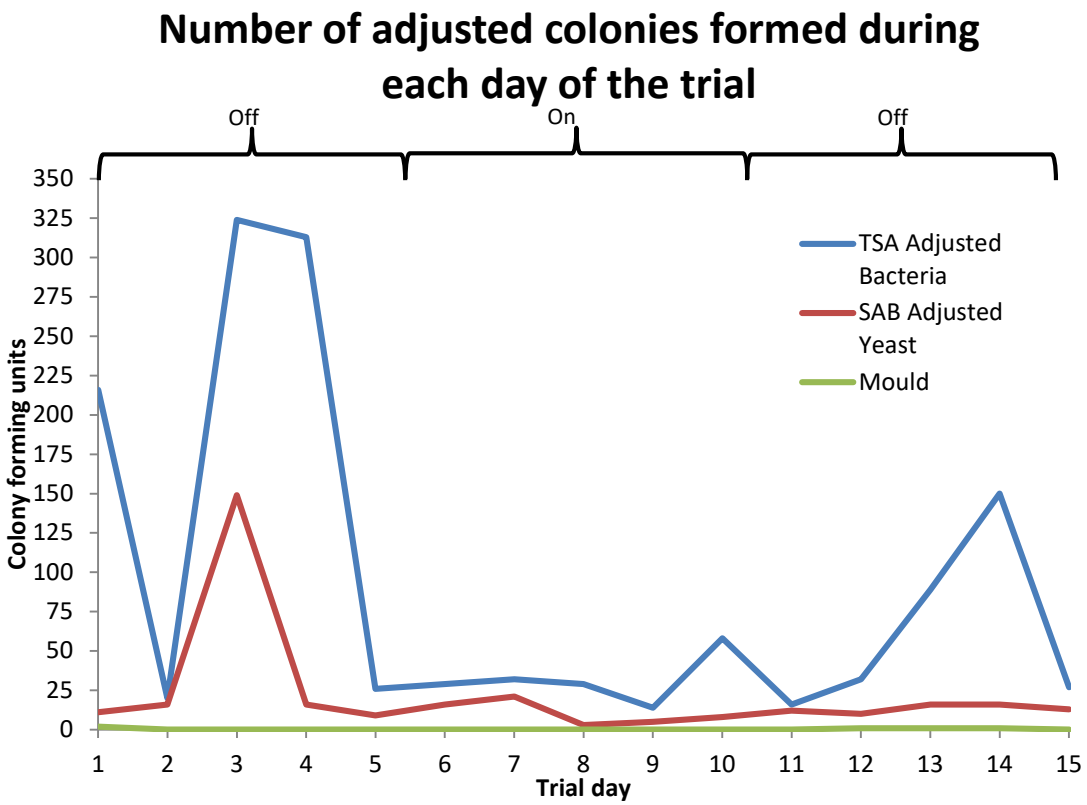

Figure 8. Number of adjusted CFU produced on each day of the trial.

Discussion

Wherever there are animals there are airborne allergens with all the associated risks that involves. Many areas of the animal unit can reduce LAA exposure risk though the use of specialist equipment such as laminar flow hood, IVCs and air handling units, such equipment is not useful in the cage wash environment where soiled cages have to be exposed in order to be cleaned. Whilst health and safety measures such as the installation of the automated cage was robot have been taken, LAA levels may regularly be above the 2.5ng/m^3 industry recognised safety level. This was clearly shown in the data from week one with the highest reading being 12.6ng/m^3 of mouse urinary protein, 5 times the recommended safe limit.

Regular exposure to high levels such as those found are not good as exposures leading to sensitivity are cumulative and even small repeated doses may result in an individual becoming sensitised. When staffs jobs rest on their ability to interact and work with animals daily becoming sensitised to LAAs would be disastrous. What we have demonstrated in this report is that the Biokker Photocatalytic Oxidation system can reduce airborne allergen levels in a high risk area to an industry recognised safe level. This ensures the health and wellbeing of the workforce minimising the risk of staff sensitisation.

The Biokker PCO system was also shown to reduce the number of microorganisms in the air with a decrease in CFU during week two of the trial. The reduction of bacteria and fungus in the air not only upholds the health and safety of the workforce but also may aid in the protection of spread of disease through the animals in the unit.

The LAA and microbiological burden of the air was never brought down to zero completely. This is unlikely to ever occur due to the size of the Manchester University's animal unit and the resultant constant though put of cages, equipment and staff. Whilst zero airborne LAA may never be achievable the lowest possible burden should be aimed for to ensure staff health and prolonged employment.

It may also be mentioned that whilst the machine was switched on, the cage wash staff did notice an odour reduction. This is a common factor when speaking with other facilities using this technology. This also shows that the Biokker PCO system not only helps safeguard health but also helps to create a more pleasant working environment.

With the installation of the Biokker Photocatalytic Oxidation system into an area where there are high burdens of airborne Mouse or Rat urinary proteins or pathogens, it is evident that the system is effective at reducing these materials to acceptable levels. In this investigation, we have demonstrated the system's ability to reduce the allergen levels significantly and the microbiological levels similarly.

As such the Biokker Photocatalytic Oxidation system can be considered one of the few if not the only, cost-effective method to reduce LAA's through an engineered solution. The Biokker Photocatalytic Oxidation systems small installation area and effectiveness make it an attractive solution to enhance the occupational health of all staff exposed to allergens.

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Health and safety executive, Control of laboratory animal allergy, guidance note EH76 **http://www.hse.gov.uk/pubns/eh76.pdf.**