

Occupational asthma and rhinitis due to detergent enzymes in healthcare

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Background	The use of proteolytic enzymes to improve the cleaning efficacy of washing powders was introduced in the mid 1960s. Many microbial enzymes are known to be potent respiratory sensitizers but previously there has been only one case of occupational asthma associated with workplace exposure in a health-care worker.
Aims	To report two cases of occupational asthma associated with exposure to biological enzymes in health-care workers and related occupational cases.
Methods	Reporting of clinical case reports from three different work places.
Results	One case of occupational asthma and three other cases with work-related asthma or rhinitis occurred in one workplace. A single case of probable occupational asthma presented at a second workplace with another case of work-related asthma at a third workplace. Exposures occurred in areas used for cleaning medical instruments and endoscopy suites. Hygiene measurements confirmed the potential for exposure. Control measures were not in place and recognition of the hazard was missing in these workplaces.
Conclusions	Detergent enzymes when used in healthcare settings should be recognized as potential respiratory sensitizers. Healthcare institutions and professional bodies that recommend the use of detergent enzymes should review their risk assessments to ensure that the most appropriate methods for preventing or reducing exposure are in place.
Key words	Asthma; enzymes; healthcare workers; respiratory; rhinitis; sensitizers.

Introduction

The use of proteolytic enzymes to improve the cleaning efficacy of washing powders was introduced in the mid 1960s [1]. The enzymes used were alkaline serine proteases produced from the growth of the *Bacillus* species *subtilis*, *lentus*, *amyloliquefasciens* and *licheniformis* being generically termed subtilisins. By 1969, there were reports of occupational asthma and sensitization due to subtilisin exposure [2,3]. Area levels of exposure in the workplace before intervention were likely to have been in the range hundreds of nanograms to low micrograms per cubic metre [4]. Subtilisins were initially used in a powdered form, however, their use in this form was discontinued because of the problems with allergy. With encapsulation technology in the 1970s, these products were reintroduced [1] and in the UK, there is now a workplace exposure limit (WEL) of 40 ng/m³ for airborne subtilisins [5]. Recently, Delclos *et al.* [6] constructed a job-exposure matrix for occupational risk factors and asthma in healthcare workers and noted the use of subtilisins as a component of some products used

in medical instrument cleaning. Although unaware of any recorded cases of occupational asthma linked to these compounds, the authors, recognizing that tasks associated with cleaning and/or disinfection of medical instruments were associated with an increased prevalence of reported asthma, called for more research. Subsequently, we noticed that a single case report of occupational asthma associated with medical equipment cleaning had been reported in a 41-year-old male subject who had both a positive skin prick test to the diluted enzyme solution (Klenzyme™) and a positive inhalation specific challenge [7].

We report two cases of occupational asthma and four other cases of possible occupational asthma/rhinitis associated with the use of detergent enzymes for cleaning medical instruments (Table 1) from three different health care organizations.

Workplace 1

A 56-year-old sterile services department (SSD) technician (Case A) was referred to occupational health by management because of absence recorded as asthma

Table 1. Case characteristics of detergent enzyme symptoms in healthcare workers

Case	Asthma	Rhinitis	Age	Gender	Duration of exposure	Atopy	Cigarette smoking
A	History, PEFs	History	56	Female	6 years	No	Ex-smoker
B	History	No	27	Female	4 years	Yes	Never
C	History	History	46	Female	6 years	Yes	Never
D	No	History	55	Female	3 years	No	Never
E	History, PEFs	No	32	Female	2 years	Yes	Never
F	History, PEFs	No	28	Female	15 months	Yes	Never
Lemière <i>et al.</i> [7]	Specific bronchial challenge	No	41	Male	1 year	Yes	Ex-smoker

and sinus problems. She had worked in SSD for 16 years and had developed asthma symptoms 4 years previously with a history of rhinitis for 2 years preceding this. Her nasal symptoms were worse at work and she reported using her reliever salbutamol inhaler only when at work. She had stopped smoking 14 years earlier.

The workplace was divided into three areas: the 'dirty area' which received used (i.e. 'dirty') surgical instruments, the 'clean room' for packing washed instruments and the 'autoclave area'. A workplace visit investigated whether any potential astmagens were present in SSD. Three different enzyme detergent products were in use. One product 3-Ezyme™ was added to an ultrasonic bath, another Enzycare 2™ was dosed volumetrically into the surgical instrument washer driers as was 3-Ezyme™. A third Neozyme™ was used as a foam spray to aid the manual cleaning of instruments. Staff said Neozyme™ had also been used on hard surfaces in SSD such as workbenches. The manufacturer's data sheets held in SSD did not identify the substances as respiratory sensitizers. Spillage of chemicals with visible crust formation was noted (Figure 1a–c). No other allergens were identified and aldehyde disinfectants were not used.

Her symptoms were reported as worse when in the dirty area in which enzyme detergents as a pre-cleaning treatment had been introduced 6 years previously. Occupational hygiene measurements for surface and airborne subtilisin were performed. The floor around the enzyme dosing containers was found to be heavily contaminated with subtilisin-like enzymes, 7475 ng/100 cm². The evidence of enzyme contamination was based on the use of a substrate assay (fluorescently quenched) to quantify protease activity with reference to a subtilisin standard (from *Bacillus licheniformis*) ranging from 0 to 25 ng/ml. Enzyme activity was measured as increases to fluorescence intensity (excitation 485 nm, emission 530 nm) using a Tecan Evolution 384 multifunctional plate reader. Personal samplers (IOM) were used to collect air samples (sampling rate 2 l/min) on Glass Fibre A filters. Wipe sampling of surfaces was carried out using a standard procedure with a 100 cm² mask and absorbent wipes containing a buffer and mild extraction detergent. Levels of enzyme in the sample were expressed as equivalent to units of subtilisin activity; for air samples, these were expressed as nanograms per cubic metre and

for wipe samples as ng/100 cm². Airborne levels of subtilisin were low at <10 ng/m³ although some changes to the processes and substances used had occurred by the time samples were taken. During a visit to another department at this workplace, cystoscopes were found being washed in detergent enzymes (Prozyme™) before transport to the dirty area of SSD where they were unpacked and checked before processing.

Specific IgE to Maxatase, alpha Amylase and Alkalase (Pharmacia Unicap™) were negative. Serial peak flow records (PEFs) included two work periods during which she had a marked decrease in PEF, increased diurnal variation and a positive OASYS work effect index of 4.0 (range is 1–4, a value >2.5 suggests occupational asthma) [8,9] (Figure 2). A diagnosis of probable occupational asthma was made and reported under the UK Reporting of Diseases and Dangerous Occurrences Regulations 1995 (RIDDOR).

She was relocated to a stores area and her symptoms improved. The use of enzyme detergents in the SSD was discontinued and replaced by alkaline cleaning chemicals. She returned to work in SSD starting in the autoclave area: serial PEFs showed no work effect (OASYS work effect index 1.18) and remained the same when she included the clean room in her work. However, on returning to the dirty area, a work-related reduction in PEFs with an OASYS work effect index of 3.75 was seen (Figure 2). She then had a 3-week absence from work due to an asthma exacerbation which was treated with oral steroids. She was advised not to return to work until a cause for the deterioration was established. It was later recognized that she had potentially been exposed to enzymes on the prewashed cystoscopes. Recommendations for re-measurement of airborne and surface subtilisin were made. These measurements confirmed the presence of subtilisin on the cystoscopes (18 ng/100 cm² on a wipe sample), on the cystoscope transport box (71 ng/100 cm²), on the bare hands of SSD workers handling the prewashed cystoscopes (2.98 ng/100 cm²) and on a number of surfaces (4.75 ng/100 cm²). No airborne levels were detected. She was permanently relocated with improvement of her symptoms.

Two other members of the SSD were seen in the occupational health department. A 27-year-old never-smoker (Case B) with pre-existing asthma who had



Figure 1. (a) An ultrasonic bath for cleaning surgical instruments. (b) The area for volumetric dosing of enzymes (note: absorbent material had been used to catch spilt chemicals). (c) Enzyme containers inside a surgical instrument washer drier machine.

work-related worsening of her asthma. Due to the clinical scenario, it was not possible to obtain objective confirmation of occupational asthma although reported as such for RIDDOR on the basis of the history and workplace findings. The other worker (Case C) was a 46-year-old SSD technician, never-smoker with a complaint of rhinitis and frontal headaches, which were worse at work. She reported chest tightness and some wheeze with her nasal symptoms and had previously had treatment for atopic asthma from her mid-teens to early twenties with a salbutamol inhaler. Her symptoms resolved on moving from this work area.

A fourth case (Case D) occurred in the same workplace in a 55-year-old female support worker who had worked in urology theatres. Detergent enzymes were used for washing cystoscopes. She had bloodstained nasal mucus on working days associated with nasal sinus pain and a reoccurrence after temporary removal from the workplace. Airborne levels of subtilisin were measured at 10.22 ng/m^3 with a static sampler placed near the cleaning sink. Extensive surface contamination was present with a wipe sample from a shelf ($199\,403 \text{ ng/100 cm}^2$), the sink taps (2083 ng/100 cm^2), the sink edge (1581 ng/100 cm^2) and the floor (131 ng/100 cm^2). Specific IgE to Savinase, Maxatase and alpha Amylase were negative in all these cases.

Workplace 2

A 32-year-old female healthcare worker (Case E) in a gastro-enterology unit presented to occupational health with a history of a raised urticarial rash on the left side of her neck and around the left eye. These symptoms disappeared when she was away from the ward for any significant amount of time but wearing a stethoscope around her neck appeared to aggravate the rash. Her general practitioner had prescribed topical and oral steroids with limited benefit. The rash resolved completely when she was away from work but seemed to recur when assisting with endoscopy. At times, the rash was severe with marked excoriation. She had eczema as a child and there were no hobbies likely to be contributing to her dermal symptoms.

She had a past history of asthma, which had been well controlled; medication was inhaled budesonide $200 \mu\text{g}$ twice daily and salbutamol MDI $200 \mu\text{g}$ as needed. She had never smoked. Some weeks after the skin symptoms began, she had noticed increased asthmatic symptoms requiring doubling of her inhaled steroid dose and regular use of salbutamol. After a 2-week period off-work, her skin symptoms resolved and her asthma stabilized. Within 2 days of returning to work, her skin had flared and she was sent home because of asthma. On three further occasions when she returned to work following days off to recover, both her dermal and respiratory symptoms recurred.

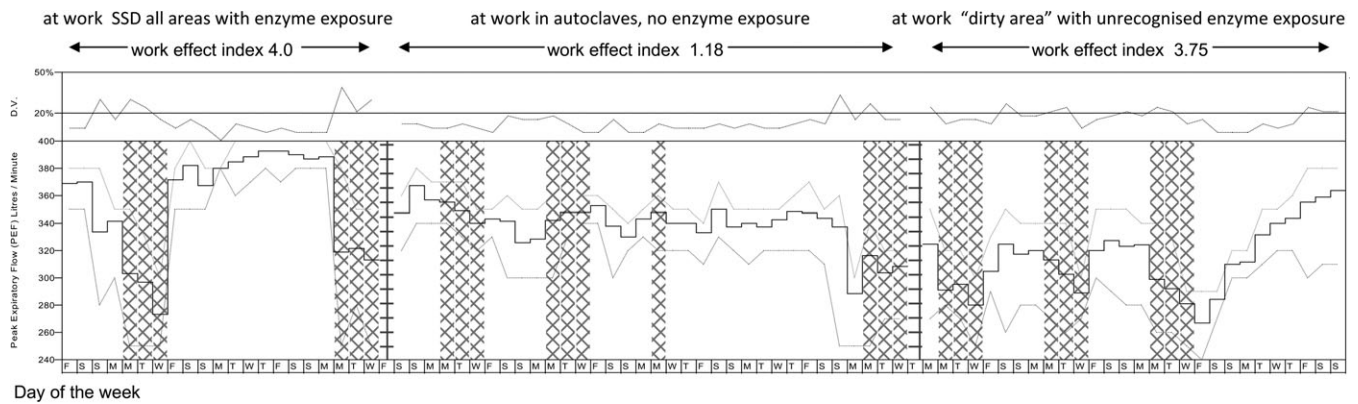


Figure 2. Case B OASYS plot of serial peak expiratory flow rate for three work periods. Hashed areas indicate work periods, in this case night work. The daily mean (solid black line), minimum and maximum PEF is shown in the lower plot with the diurnal variation in the upper plot.

A workplace visit revealed that the enzyme-based detergent Klerzyme™ was used to clean endoscopes. Diluted enzyme was kept in open-top bowls; after use, endoscopes were drawn through the bowls and then wiped with cloths in the endoscopy suite before being taken for disinfection. Hygiene monitoring as part of work to be reported elsewhere showed airborne subtilisin-like enzyme activity levels above the UK WEL and showed a similar pattern of surface contamination as in Case D.

Skin patch tests were positive to nickel, cobalt chloride, potassium dichromate and methyldibromoglutaronitrile but were negative to glutaraldehyde. Total IgE was within the normal range at 99 kU/l. Specific IgE to house dust mite was high (20.8 kU/l) with elevated levels against horse, grasses and dog. Specific IgE antibodies to amylase, Savinase and detergent termamyl were negative.

When seen in clinic during a period away from work her forced expiratory volume in 1 s (FEV₁) was 4.0 l and forced vital capacity 4.2 l, both 115% predicted. Serial two hourly peak expiratory flow rates ranged between 370 and 460 l/min with a pattern suggestive of work-related asthma (OASYS work effect index of 1.43, Figure 3).

A presumptive diagnosis of work-related sensitization to Klerzyme™ causing occupational asthma was made. Adaptations were put in place in the workplace to allow the individual to return to work but not in any areas associated with endoscopy. She was successfully redeployed to alternative duties where no further exposure to Klerzyme™ occurred with resolution of her work-related dermal and respiratory symptoms.

In a third workplace, a 28-year-old endoscopy nurse (Case F) with pre-existing asthma developed a rash on her hands and legs 3 months after starting work. She then developed significant worsening of her asthma with reported drops in PEF from around 450 to <200 l/min which occurred only when working in the endoscopy suite. She worked with an enzyme detergent Klerzyme™ in the same way as Case D. Her work-related symptoms resolved on removal from exposure.

Discussion

We have identified six healthcare workers with work-related symptoms to detergent enzymes. The diagnosis of occupational asthma in two cases was made on the basis of symptom patterns, improvement in symptoms on removal from exposure and PEF showing a work-related effect. Two cases with work-related asthma symptoms on the basis of history alone and two with possible occupational rhinitis, one of whom also reported work-related asthma symptoms that could not be confirmed.

There are no simple objective measures to diagnose occupational rhinitis. Challenge tests that can provide absolute evidence of an adverse reaction were not undertaken in any of these subjects as per usual UK practice [8]. Immunological confirmation of sensitization was sought but not found in these subjects, but there are several difficulties associated with such testing. The commercially available specific IgE assays for these enzymes were originally developed to detect antibodies to an earlier generation of enzymes, whereas those now in use are produced through protein modification to enhance activity and use over a range of pH and temperature conditions. In addition, the suppliers of formulated enzyme products have in our experience not been able to readily inform us of the type of bacterial or fungal enzyme(s) used. The sensitivity of serum-specific IgE tests may also be low, ranging from 57 to 86% [4] but is also dependent on the allergen being correctly characterized. Another consideration is that the detergent enzymes in particular proteases may be causing these symptoms by direct irritation [4]. Nevertheless, the diagnosis of six individuals in a short period of time, in three workplaces, with good histories and symptom improvement when removed from exposure suggest that occupational asthma/rhinitis to detergent enzymes in the UK health service may be common.

Detergent enzymes have been used in endoscope cleaning for around 20 years [7]. This raises not only the possibility of misattribution of the cause of occupational

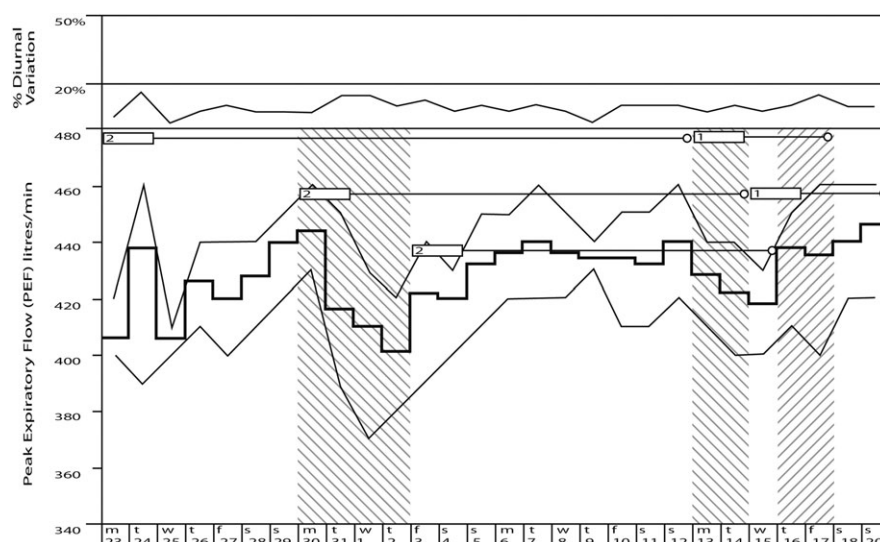


Figure 3. Case E OASYS plot of serial peak expiratory flow rate. Hashed areas indicate work periods. The daily mean (solid black line), minimum and maximum PEF is shown in the lower plot with the diurnal variation in the upper plot.

asthma in healthcare workers for some cases of supposed glutaraldehyde and other chemical disinfectant allergy but also the possibility that detergent enzyme exposure may have been a confounder in epidemiological studies in this area.

Concurrent exposure to a mixture of enzymes is likely to have a synergistic effect in enhancing respiratory allergy [1] and this has been demonstrated in animal studies. The enzyme detergent manufacturing industry work to best practice guidelines lower than the mandatory levels, achieving exposures $<15 \text{ ng/m}^3$ [4]. This was confirmed by Cullinan *et al.* [10] who found geometric mean levels of enzyme of 4.25 ng/m^3 in such industries with just 5% of results exceeding 15 ng/m^3 . The reason for adopting these lower levels is given as the recognition that enzyme allergy and asthma has been reported to be worse at detergent manufacturing sites than at enzyme manufacturing sites [4]. Surfactant has been reported to act as an adjuvant for the immune response to enzymes in animal studies [4] with bacillary amylase being a more potent allergen than proteases either innately or through potentiation. This mechanism would fit with the findings of epidemiological studies [10]. Enzyme detergents of the type used in healthcare settings are therefore likely to represent a hazard greater than that of subtilisin alone because of the multi-enzyme formulation and surfactant properties.

Jia *et al.* [11] investigated the effect of a non-ionic surfactant on the crystallization of the enzyme lysozyme and found that promotion of crystallization can occur and suggested a general mechanism. It is therefore plausible that particles of a size likely to become airborne and inhaled are more likely to arise on drying of detergent enzyme liquids because of surfactant action affecting particle nucleation and dispersion.

The case report by Lemièrre *et al.* [7] is particularly interesting because the authors supported the diagnosis by specific bronchial challenge using the Pepys method of inhaling from the air above a bowl of liquid while in an enclosed cabinet [12]. The use of this method and the positive results would imply that enzyme is able to become airborne with minimal or no agitation of the detergent liquid. The interface between air and surfactant monolayers is, however, a complex area with evaporation and aerosolization being affected by the nature of the surfactant, solvent, other additives and physical factors. This may be an aspect of potential occupational exposure deserving further study.

The current methods of handling detergent enzymes in healthcare settings could certainly be improved from our observations. We have witnessed staff decanting enzyme solutions, wiping down surfaces with enzyme detergents, spillages on floors during the transfer of enzyme pre-cleaned endoscopes to the automated washing equipment, drips of enzyme from tubes inside washing equipment dried to a chemical crust, spillages left uncleaned, enzyme decanted into pump bottles and atomisers to spray on the tips of endoscopes. The use of enzyme formulations in ultrasonic baths is common and promoted by manufacturers leading to conditions likely to cause aerosol formation. In addition, material safety data sheets for these products often do not identify the agents as posing any hazard.

We have reported two cases of occupational asthma and four others with respiratory symptoms related to detergent enzyme exposure. These cases add to the single report by Lemièrre *et al.* [7] and support the prescient risk assessment of Delclos *et al.* [6] that these substances could cause problems in the healthcare sector. Detergent

enzymes when used in healthcare settings should be recognized as potential respiratory sensitizers. In use, the levels of airborne subtilisins must be maintained below statutory levels, in the UK 40 ng/m³, and we believe should be kept at or below the guidance levels achieved in the detergent manufacturing industry. The manufacturers of these formulations should provide adequate safety information to allow the recognition of the potential respiratory hazards. Healthcare institutions and professional bodies that recommend the use of detergent enzymes should review their risk assessments to ensure that the most appropriate methods for preventing or reducing exposure are in place.

Key points

- Work-related respiratory symptoms and allergy to detergent enzymes in the healthcare sector may be unrecognized.
- Health surveillance for work-related allergy should be considered for exposed healthcare workers.
- Workplace exposure limits for subtilisins must not be exceeded and operating to lower levels may be appropriate.

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Conflicts of interest

None declared.

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