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**Powertrain Occupational Respiratory Disease
Outbreak: Report of Immunological Investigation**

MU/06/01

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PRIVACY MARKING:

Available to the public.

HSL report approval:	Andrew Curran
Date of issue:	March 2007
Job number:	JS2100876
Registry file:	MU RE 2004 22886
Electronic file name:	Medical Unit_SOELICE/Work/HSE (projects)/support/active projects/powertrain/report/Powertrain Final Report.doc

ACKNOWLEDGEMENTS

We would like to thank the members of the Health and Safety Executive/Laboratory, including Marcia Davies, Nick Ratty, Georgina Speake, Dr Ian Gardner, David Price, Kath Heywood, Dr Alan Scott, Dr Roger Rawbone, Donald Adey, Barbara Riley, James Barrett, Andy Fisher, Steve Cottrell and Mike Burd; the Birmingham Heartlands Hospital, including Prof. Sherwood Burge, Dr Alastair Robertson, Wendy Robertson, Dr Maaritta Jakkola, Cedd Burge and Vicky Moore; the Health Protection Agency, including Dr Sue Ibbotson, Dr David Hunt, Andrew Kibble, John Dyer and Dr Roger Gajraj; the Department of Health including Dr Rashmi Shukla, Rowena Clayton, Chris Spencer-Jones (Director of Public Health - South Birmingham PCT) and Dr Mary Kinoulty for all their help with this investigation. We would also like to thank Powertrain and Houghtons Plc staff for their cooperation with this work.

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EXECUTIVE SUMMARY

Background

Powertrain Limited, (a Phoenix Venture Holdings Limited company, of Longbridge, Birmingham, UK) produced engine components in aluminium and cast iron. However, the company went into administration in April 2005, and ceased operations later that year. The work involved machining metal using a wide variety of machine tools and cleaning worked components in washing machines. Water-mix metalworking fluids (MWF) were used in operations at the plant, which were either supplied to the machines from large central sumps or from individual sumps attached to individual machines. The amount of mist in the factory from machining was variable, and depended on whether the roof vents were closed, levels of tramp oil (machine, lubricating or gearbox oil leaking into MWF), changes to operations and changes to fluids, additives and oils. There were 20 large washing machines at Powertrain, using water-mix wash fluids for removing swarf and cleaning components, which contributed to overall levels of mist in the factory.

In March 2004, HSE was informed by Birmingham Heartlands Chest Clinic of a number of cases of extrinsic allergic alveolitis in Powertrain employees (referred in this report as the 'Index cases'), prompting further investigation. By March 2006 the cases of probable and definite work-related respiratory disease had reached 102¹ mainly of occupational asthma (OA) and extrinsic allergic alveolitis (EAA).

Objectives

The objective for this study was to investigate the degree to which exposure to microbial contaminants of metalworking and wash fluids may have contributed to the outbreak of occupational respiratory disease at the Powertrain worksite.

Main Findings

- The worker cohort under investigation consisted of groups of workers with either a clinical diagnosis of EAA or OA, a group of workers with self reported symptoms which did not fit the EAA/OA case definition criteria, a group of asymptomatic but potentially exposed workers and a group of non-exposed controls from another worksite.
- Two species of bacterium, *Ochrobactrum* and *Acinetobacter* were identified by DNA analysis in the samples of sump oil taken. A variety of other bacterial species (including *Pseudomonas*, *Citrobacter*, *Bacillus*, *Arthrobacter* and *Stenotrophomonas spp.*) were also cultured from samples of MWF and washfluids taken from the workplace after the initial clinical investigation.
- An initial scoping investigation of blood precipitins was undertaken on 11 index EAA cases (diagnosed at the Birmingham Heartlands Hospital), 11 asymptomatic but potentially exposed workers and a sample of non-exposed controls from another worksite. This enabled the project team to identify which microbiological species to use to screen the main study population. Those bacterium species that showed precipitating antibodies in the serum of EAA cases, but not controls, were selected for inclusion in

¹ The number of defined and probable cases is derived from the most inclusive definitions (Robertson, submitted).

the main study. This narrowed down the species selection to the two originally identified bacterial species, *Ochrobactrum* and *Acinetobacter*.

- Species of *Mycobacterium* and common environmental fungi, were included in the test panel to enable comparison with the results of other published studies.
- Evidence of altered immune responses were observed in a subsample of workers in the factory cohort, particularly in cases with a physician diagnosis of work related EAA. Over half of EAA cases (10/17 or 59%) had precipitating antibodies to at least one of the microbial species investigated or to used sump oil, the majority (7) testing positive to *Acinetobacter*.
- Clearly it is not possible to comment whether EAA cases may have had precipitating antibodies to other bacteria species not included in the screening panel, although it is likely that immune responses to other bacteria would have been documented had more bacteria species tested in the scoping study been included in the main investigation. Consistent with this, all 11 EAA cases investigated in the scoping study had at least one positive IgG precipitin to at least one bacteria species detected in the workplace and included in the test panel. It is possible that immune responses to these bacteria were relevant in the aetiology of EAA observed in the study population.

Conclusion

Although the aetiology of EAA and occupational asthma in this occupational setting remains to be fully elucidated, the findings of this investigation are in keeping with previous studies which have implicated bacterial contamination of MWF as the most likely relevant factor. In addition, the study findings relating to precipitating antibodies for the EAA cases may lend further weight to any arguments advanced in Robertson *et al.* 2006 regarding the working practices and worksite exposures that caused or contributed to the Powertrain disease outbreak.

Recommendations

- COSHH and other risk assessments must recognise the increased risk of serious respiratory disease, such as Extrinsic Allergic Alveolitis (EAA) and Occupational Asthma (OA) arising from contaminated metalworking and wash fluid;
- Awareness of the risk of serious respiratory disease from metalworking and wash fluids has to be raised among all involved - employees, employers, as well as occupational health and health and safety practitioners;
- The application of COSHH criteria for respiratory health surveillance to those exposed to metalworking and wash fluid mist should not be dependent upon guidance values, as there is no agreed health-based limit for exposure to mist;
- There are links between bacterial contamination in metalworking and wash fluids and serious respiratory disease, such as OA and EAA, and this risk needs to be assessed and controlled
- Further research is required to investigate the exact etiological mechanism of respiratory disease relating to this exposure.

1 INTRODUCTION

1.1 BACKGROUND

Metal working fluid, also referred to as suds, coolant, slurry or soap, is a generic term covering a wide variety of fluids, which are used as lubricants or coolants for metal machining processes such as drilling, milling and turning. They tend to be relatively complex mixtures, which may change in use and which also contain a number of additives to ensure performance and stability. Some constituents in some fluids may have the potential to cause adverse health effects. There are two broad types of MWF, neat oils and water-mix fluids. Neat oils are highly refined mineral oils. In recent times, due to economic, technical and health reasons, there has been a steady trend towards the use of water-mix fluids. The UK Lubricants Association estimates that current UK annual usage is approximately 20,000 tonnes of neat oil and 12,000 tonnes of water-mix fluid concentrate (which on dilution equates to something like 240,000 tonnes in the workplace) (BLF 2000, BLF 2002).

MWF, particularly water-mix fluids, owing to the ready supply of nutrients and water they provide, are particularly conducive for the growth of bacteria and fungi. Biocides may be added to combat microbial growth. Previous studies by HSL for HSE have shown that typical levels of bacteria in MWF range from <10 to 195 million colony forming units (CFU) per ml (Simpson *et al.*, 2003). Endotoxin, a cell wall component of bacteria that can elicit immunotoxicological response such as inhalation fever, typically may be present in MWF in concentrations ranging from <0.05 to 1.8 million endotoxin units (EU) per ml (Simpson *et al.*, 2003). MWF are often colonised by *Pseudomonas* species of bacteria, which are a common Gram-negative bacteria that have been shown in previous studies at HSL and elsewhere to elicit immune response in exposed workers (Fishwick *et al.* 2005). Other related species, such as *Acinetobacter*, have also given rise to similar responses in exposed workers (Zacharisen *et al.*, 1998). *Pseudomonas* and related genera are common environmental bacteria in soil, water and dust with a non-fastidious growth requirement and as such grow readily in MWF. In more recent years, investigations of respiratory ill health in MWF exposed workers in USA have implied that non-tuberculous *Mycobacterium* species are the cause of respiratory sensitisation leading to hypersensitivity pneumonitis (termed extrinsic allergic alveolitis in the UK) (CDC 1998, 2002, Kreiss and Cox-Ganser 1997). These species include the *Mycobacterium fortuitum/chelonae* complex and the recently characterised *Mycobacterium immunogenum*, which was specifically isolated from US MWF in an outbreak investigation.

1.2 FACTORS AFFECTING EXPOSURE

Exposure to MWF can occur by direct contact with the skin or by inhalation of MWF mist generated during machining. As supported by several published studies, the greatest risk of respiratory symptoms is in workers working with water-mix MWF, as opposed to neat oil (Greaves *et al.* 1997, Kennedy *et al.* 1999), despite those working with neat oils having been found to have higher exposures than those working with water based fluids in a study dealing with small machine shops [Piacitelli *et al.* (2001)]. With regards the kind of work being done, the highest exposures were found for metal machining activities involving grinding² and hobbing³. In addition, mists originating from straight oils were found to consist of larger aerosol particles than those from water-mix fluids. These results suggest that it may be certain

² A machining process to perform either of two effects: (1) to shape components that are too hard to be machined by conventional methods such as hardened tool steels and case hardened components, or (2) to obtain a high degree of dimensional accuracy and surface finish.

³ A special gear-cutting process in which the gear blank and hob rotate together as in mesh during the cutting operation.

components or characteristics of water-mix fluids that principally underlie respiratory symptoms, for example their greater ability to support bacterial contamination. Mist from washing machines may also pose risks of respiratory disease, particularly if the wash fluids are contaminated with bacteria.

1.3 EFFECTS OF RESPIRATORY EXPOSURE

Various respiratory symptoms have been attributed to exposure to MWF mist, notably cough, phlegm, wheeze, chest tightness, breathlessness and nasal irritation (Oudyk *et al.* 2003; Greaves *et al.* 1997). Further adding to the evidence base, similar studies that have investigated levels of exposure to MWF have documented exposure-response relationships between respiratory symptoms and total aerosol (Sprince *et al.* 1997). Other studies have found MWF exposure to be associated with across shift decrements in forced expiratory volume in one second (FEV₁) (Kriebel *et al.*, 1997), increased airway responsiveness (Kennedy *et al.*, 1999) and toxicological responses to endotoxin (Thorne and DeKosker, 1996). Kriebel's study reported dose-response relationships between MWF exposure and reduced lung function, which was confined to workers exposed to water-mix MWF rather than neat oil. Consistent with the findings of Kriebel, Kennedy *et al.* (1999) found increased bronchial hyperresponsiveness (BHR) to be associated with duration of exposure to both synthetic and soluble MWF, but a positive BHR status was associated with duration of exposure to synthetic MWF only. In contrast to both Kriebel *et al.* and Kennedy *et al.*, Eisen *et al.* (2003) reported an effect of MWF exposure in workers exposed to neat oils, but not water-mix fluids.

1.3.1 Immunological effects

Numerous studies have documented evidence of specific antibody responses to MWF, principally to specific bacterial species colonising the MWF, such as *Pseudomonas*, *Ochrobactrum*, *Acinetobacter* and *Mycobacterium* species (Fishwick *et al.*, 2005, Laitinen *et al.*, 1999, Fox *et al.* 1999, Mattsby-Baltzer *et al.*, 1989, Travers-Glass and Crook, 1994). Fox *et al.* investigated, by way of a case-control study, the factors contributing to an outbreak of hypersensitivity pneumonitis (HP) at a US engine manufacturing plant. Serum precipitin reactions to both used and unused MWF's, 3 culturable bacteria spp., as well as a range of biocides and detergents used at the factory, were investigated in 18 HP cases and 51 asymptomatic controls, also working at the factory. The study documented an almost complete absence of positive precipitin reactions to the unused MWF, biocides, detergents and hydraulic oils, but in contrast observed strong associations between HP and positive precipitin to used MWF. However, the study was unable to determine which contaminant in the MWF was the most likely cause. Fishwick *et al.* (2005) in a study of respiratory symptoms and serology in workers performing machining activities at a small metal working factory, investigated the prevalence of precipitating IgG to neat MWF extract used at the factory and micro-organisms isolated from the MWF. 11 of 21 exposed workers at the plant were included in the study. Bacterial and fungal species identified from various locations around the site included *Pseudomonas* and *Fusarium* spp. Precipitating IgG to the MWF used at the plant was found in 3/11 individuals and of these, 2 reported work related respiratory symptoms. In addition, precipitating IgG to the bacteria *Pseudomonas* spp. was found in 4/11 individuals, with 2 reporting work related respiratory symptoms. Laitinen *et al.* (1999) investigated exposure to microbes in MWF in workers who used synthetic fluid, mineral oil or rapeseed oil in grinding, turning or drilling work. The most common microbial species detected in the MWF were *Comamonas testosteroni* and *C. acidovorans*. Colonies of *Ochrobactrum anthropi*, *Pantoea agglomerans* and *Stenotrophomonas maltophilia* were also detected. In addition, fungi such as *Aspergillus*, *Cladosporium* and *Penicillium* species were identified in air, but not in the MWF.

Positive IgG antibodies were found in the sera of 22/25 of the workers examined. Antibodies against *S. maltophilia*, *P. agglomerans* and *C. acidovorans* were most commonly found, occurring in 72%, 64% and 64%, respectively, of workers. The results of these studies indicate a clear association between exposure to contaminated MWF and precipitating IgG. In addition, the results of Fox *et al.* and Fishwick *et al.* in particular suggest that contaminated MWF may have had at the very least a contributory role in the cases of occupational respiratory disease observed in the workforces investigated.

1.3.2 Specific disease effects

The main respiratory diseases associated with exposure to metalworking fluid mist are occupational asthma (Rosenman *et al.*, 1997), extrinsic allergic alveolitis (Freeman *et al.*, 1998), and work-related chronic bronchitis (Rosenman *et al.*, 1997). Studies have also found mineral oil mists, especially low viscosity oils, in the presence of high concentrations of mineral oil or hydrocarbon vapour, to be associated with a range of potential respiratory effects, including lipoid pneumonia and fibrosis (Skyberg *et al.*, 1986 and 1992), although these effects appear to require prolonged exposure. In a study from the USA, Rosenman *et al.* reported MWF to be the second most common cause of work related asthma reported to Michigan Department of Public Health (as part of mandatory surveillance for occupational disease), with most of the cases of disease reported over the period 1988-1994 coming from the automobile industry. In recent years (1995-2003) SWORD (Survey of Work-related and Occupational Respiratory Disease) have reported an estimated U.K. annual average of 14 cases of respiratory disease related to MWFs, 13 of which occupational asthma and <1 extrinsic allergic alveolitis (see Table 1, data taken from THOR⁴). Possible causes of occupational asthma include pine oil based re-odorants (Hendy *et al.*, 1985, Robertson and Weir 1988), ethanolamine (Vallieres *et al.*, 1977) and methyl esters of fatty acids (Spallek, 1989).

Table 1: Number of estimated and actual cases of respiratory disease attributed to MWFs reported to SWORD during the period 1995 to 2003

Year	Cases of respiratory disease reported to SWORD					
	All respiratory disease		Asthma		Extrinsic allergic alveolitis	
	Estimated	Actual	Estimated	Actual	Estimated	Actual
1995	32	10	28	6	-	-
1996	8	8	8	8	-	-
1997	35	13	34	12	-	-
1998	3	3	1	1	1	1
1999	14	3	14	3	-	-
2000	4	4	4	4	-	-
2001	5	5	5	5	-	-
2002	16	5	16	5	-	-
2003	6	6	5	5	1	1

⁴ <http://www.medicine.manchester.ac.uk/coeh/thor>

1.4 PRELIMINARY INVESTIGATIONS AND MICROBIOLOGICAL SURVEILLANCE

The factory floor at the Powertrain Plant was about 600 metres long and 200 metres wide. Transfer machines performing a number of sequential machining operations dominated the northern half. Metalworking fluids from large sumps of 210,000, 55,000 and 19,000 litres capacity lubricated these machining operations. Individual metalworking and transfer machines with their own sumps predominated in the southern half of the factory. Components were washed after machining in about 20 dedicated washing machines spread around the factory. 'Mist' from the sumps and machining operations was reportedly more often noticeable in the northern area of the factory, particularly near the largest common sump, known as the Mayfram, and associated machining, centring on what was known as "Prismatic" (non-moving engine parts) operations. This had long been acknowledged to be a nuisance within the factory with possible, but unspecified, health implications. 'Mist' concentrations varied from day to day, depending mainly on whether the automatic roof ventilation louvres were open, the amount of tramp oil in the metalworking fluid (oil leaking into the metalworking fluid from machine lubricating and hydraulic oil) and the machining operations being carried out.

At the start of the outbreak investigation in early 2004, when it became apparent that several workers were suffering respiratory ill health as a result of their work at Powertrain, the potential involvement of microbiological contamination was immediately suspected. Microbiological investigations at Powertrain included the collection (by HSL Field Scientists and HSE OH inspectors or by HSL Microbiology staff) of samples from MWF systems sumps, component wash fluid sumps and other environmental samples, including air samples in the factory. Initial results from samples taken from the main MWF sumps (large central sump systems feeding several machines) revealed little bacterial or endotoxin contamination, which was consistent with highly controlled systems with rigorous biocide usage at the time of sampling. Samples of workers' sera were also sent to HSL for immunoassay at this time. Also supplied was a sample of MWF from the large common sump (the Mayfram). It was recognised that the fluid management controlled to very low levels the live bacteria in the MWF, but attempts were made to determine whether non-culturable bacteria were present by extracting bacterial DNA which was subjected to DNA sequencing. This yielded DNA from two identified bacteria; an *Ochrobactrum* sp. (a species related to *Pseudomonas*) and an *Acinetobacter* sp. This suggested either that the systems had previously been contaminated with these bacteria but that contamination was now controlled, however with some residues remaining, or that bacterial contamination by these bacteria was present elsewhere in the factory and the DNA present was from cross contamination. Pure cultures of both species were obtained from a culture collection and extracts prepared for immunoassay.

It was recognised that although investigations had focused on the main MWF systems, other systems within the factory were possibly less stringently managed and could be a source of contamination. A further sampling exercise was initiated, followed soon after by a factory visit involving HSE and HPA. The results of the sampling exercise identified several machines where heavy bacterial and endotoxin contamination was present, up to 10 million bacteria per ml in certain samples and up to 55,000 Endotoxin Units per ml. Further follow up samples confirmed this. Prevalent bacterial isolates were identified by gene sequence analysis, which confirmed that among the range of bacteria, both *Ochrobactrum* and *Acinetobacter* were present, as well as *Pseudomonas* spp. DNA based tests revealed no evidence of the presence of non tuberculous *Mycobacterium* spp. in any of the 125 samples. Fungal contaminants were present infrequently and in small numbers.

Interventions initiated by HSE following microbiological surveillance included clean up of the contaminated MWF and wash systems. Samples taken after this intervention from sites previously showing high bacterial levels subsequently showed a progressive decline in bacteria and endotoxin. Most recent samples, taken in September 2005, have shown few bacteria and moderate levels of endotoxin. The results of microbiological investigations were reported previously in a series of laboratory reports. The reader is referred to these reports for more information.

2 OBJECTIVE AND METHODOLOGY

The objective for this study was to investigate the degree to which exposure to microbial contaminants of metalworking or wash fluids may have contributed to the outbreak of occupational respiratory disease at the Powertrain worksite. The report brings together the results of a number of microbiological and hygiene surveys and clinical investigations carried out by the Health and Safety Executive, the Health and Safety Laboratory and the Birmingham Heartlands Hospital, following the reporting of cases of EAA to HSE in March 2004. The study was initiated through the formation of an incident investigation team consisting of representatives from the Health and Safety Executive⁵, the Health and Safety Laboratory⁶, the Birmingham Chest Clinic⁷ and Warwick University Medical School⁸. This facilitated discussion and agreement on the study design, data analysis and case definitions.

2.1 CLINICAL INVESTIGATION

Clinical investigation was carried out in four main phases:

Phase 1 – A respiratory screening questionnaire was self-completed by 808/836 workers (96.7%) in May 2004.

Phase 2 – 481 Powertrain employees with at least one respiratory symptom identified on the screening questionnaire were invited for clinical assessment at the factory in June 2004. The assessment included a detailed questionnaire, spirometry, blood and clinical opinion. Fifty asymptomatic Powertrain employees, randomly selected using payroll number, were also invited for clinical assessment. 454/481 (94.4%) symptomatic employees⁹ and 48/50 (96%) asymptomatic employees agreed to participate.

Phase 3 – Based on the results of the initial clinical assessment, employees who required further investigation (including appointment at the Birmingham Chest Clinic and/or peak flow recording) were identified. 158 workers were seen for more detailed investigation at the Birmingham Chest Clinic and 198 workers returned peak flow records for analysis.

Phase 4 - Case definitions for extrinsic allergic alveolitis (EAA) and occupational asthma (OA) were applied in order to strictly define a set of firm cases who met predefined objective criteria with onset of disease after January 2003. The case definition groups for EAA and OA were non-mutually exclusive which resulted in workers potentially being assigned to more than one group.

The reader is referred to Robertson *et al.* (2007) for further information on the clinical assessments carried out. Case definitions are also summarised below.

⁵ Field Operations Directorate

⁶ Health and Safety Laboratory, an agency of the Health and Safety Executive, Harpur Hill, Buxton, Derbyshire, SK17 8JN

⁷ Professor Sherwood Burge OBE, and Dr Alastair Robertson, of Birmingham Heartlands & Solihull NHS Trust (Teaching) Department of Respiratory Medicine, Birmingham Chest Clinic, 151 Great Charles Street, Queensway, Birmingham B3 3HX

⁸ Wendy Robertson, Warwick Medical School, Division of Health in the Community, Warwick Medical School, Coventry CV4 7AL

⁹ these included the original 11 index cases that presented to Birmingham Chest Clinic reporting symptoms of work related respiratory disease and who were subsequently diagnosed with disease.

2.2 CASE DEFINITION CRITERIA

To ensure uniformity of diagnoses for EAA and OA and the identification of case series' whose disease post dated January 2003, strict case criteria were predefined and applied to all symptomatic workers who had undergone clinical assessment.

Extrinsic Allergic Alveolitis

The case definition for EAA was based on that reported by Fox *et al.* (1999). Workers with onset of disease since 2003 and meeting at least four out of seven criteria for EAA, were eligible as cases. The seven case criteria were as follows:

Onset of disease after December 2003 and

- 1) Physician diagnosis of EAA (probable or definite).
- 2) Onset of at least two pulmonary symptoms (cough, wheeze, chest tightness, shortness of breath) and one systemic symptom (fever, weight loss)
- 3) A history of symptoms improving regularly on days away from work and deteriorating on return to work.
- 4) Restrictive pattern on spirometry – FVC <80% predicted, and FEV₁/FVC >70%
- 5) Pulmonary diffusing capacity (TLCO) less than 80% predicted
- 6) Chest X ray or CT showing interstitial, reticulonodular or mosaic pattern
- 7) Biopsy evidence of non-caseating granulomas and/or lymphocytosis on bronchoalveolar lavage

Occupational Asthma

Diagnostic PEF record in 2003-5, i.e. peak flow record with an OASYS¹⁰ score ≥ 2.67 and/or a mean day interpreted difference between work and rest days ≥ 16 l/min

2.2.1 Definition of study case and control groups

Case definitions were applied to all workers with a possible, probable and definite clinical diagnosis of EAA and those who had completed a peak flow record. Of these, 19 met the case definition criteria for EAA and 74 for OA (comprising 85 workers in total, i.e. 8 workers were classed both as an EAA and OA case). The remaining 369 workers, while symptomatic, did not meet either case definition. A sample of 72 who had attended the Birmingham Chest Clinic and had provided a blood sample for assessment were selected from this group to act as a further case group (termed the exposed, symptomatic, non-diagnosed group). Two comparison groups were also established, a group of 48 asymptomatic but potentially exposed workers from the plant (randomly selected from the 327 employees not reporting respiratory symptoms on screening), and an external control group, consisting of 65 volunteer office workers from a different worksite. The derivation of the study case and control groups is summarised in Table 3.

¹⁰ *Oasys-2 is a computer program that plots and interprets serial peak expiratory flow (PEF) readings of patients suspected as having occupational asthma or work-related asthma. Oasys-2 uses discriminant analysis to score a peak flow record between 1 and 4. An Oasys score above 2.5 has been shown to have 94% specificity for diagnosis of occupational asthma and 75% sensitivity when evaluated against gold standard diagnostic methods independent of PEF (Gannon et al. 1996, Anees et al. 2004).*

Table 3: Summary of study case and control groups

Phase 1 Screening		Phase 2 Clinical Assessment	Phase 3 Further Assessment	Phase 4 Application of case definitions	Precipitin and Specific IgE Testing¹¹
808/836 workers screened	481 with lower respiratory symptoms	481 invited for clinical assessment (454 accepted)	198 returned PEF records, 158 further seen for more detailed clinical assessment	Case definitions applied to all workers with a clinical diagnosis of EAA and who had a completed peak flow record, 19 classed as EAA cases, 74 as OA cases, remaining 369, although symptomatic, met either case definition criteria	Carried out on 17/19 EAA cases, 70/74 OA cases and 72/368 symptomatic workers who met neither the EAA or OA case definition
	327 with no lower respiratory symptoms	50 invited for clinical assessment (48 accepted)	Not undertaken		Carried out on 42/48 asymptomatic workers
Not undertaken					Carried out on 65/65 external controls

¹¹ Precipitin and specific IgE tests were carried out just for those workers providing blood; Of the 85 EAA and OA cases, 11 were index cases; 10 index cases provided blood; 17 of the 19 EAA cases provided blood; 62 of the 74 OA cases provided blood

2.3 IMMUNOLOGICAL INVESTIGATION

Blood samples were requested from all workers invited for clinical assessment. These were used to determine the presence of specific IgE antibodies to commercially prepared common environmental allergens and common fungi associated with a contaminated environment. In addition, extracts of material collected from the worksite and bacteria identified within workplace metalworking and wash fluids subsequently cultured, were prepared for use in determining precipitating antibodies in workers' serum. 258 workers in total provided a blood sample, 17/19 EAA cases, 70/74 OA cases, all 72 symptomatic workers who met neither the EAA or OA case definition, 42/48 asymptomatic workers and all 65 external controls. More information on the methods used to determine the presence of precipitating and IgE antibodies can be found in Appendix B.

2.3.1 Choice of immunological tests

The decision regarding which microbiological species to use for screening the main study population was informed based on the results of an initial scoping investigation of blood precipitins undertaken on 11 index EAA cases diagnosed at the Birmingham Heartlands Hospital, 11 workers without symptoms, (but for whom exposure was possible) seen by the Birmingham Heartlands, and a sample of non-exposed office worker controls recruited from another worksite. Immunoassays were performed with extracts of two species of bacterium, *Ochrobactrum* and *Acinetobacter*, which were both identified by DNA analysis in the sample of metalworking fluid taken from the largest sump, the Mayfram, along with a variety of other bacterial species (including *Pseudomonas*, *Citrobacter*, *Bacillus*, *Arthrobacter* and *Stenotrophomonas spp.*) cultured from samples of metalworking and wash fluids taken from the workplace after the initial clinical investigation. The EAA cases and comparison groups were investigated for precipitating antibodies to these species (see Tables 9 and 10 in Appendix B). Due to resource constraints, only those bacterium species that showed precipitating antibodies in the serum of EAA cases, but not controls, were selected for inclusion in the main study. This narrowed down the species selection to the two bacterial species, *Ochrobactrum* and *Acinetobacter*, which had been identified in the original metalworking fluid sample and had also been isolated from other MWF and wash fluid samples subsequently taken from the factory. In addition, species of *Mycobacterium* (specifically, *Mycobacterium immunogenum*, *Mycobacterium chelonae* and *Mycobacterium fortuitum*) were also included in the test panel to enable comparison with the results of USA studies, even though there was no evidence of their presence in workplace samples¹². While no detectable fungal contamination of the metalworking and wash fluid samples tested was measured, the study team decided to test workers' sera for specific IgE responses to a range of common fungal allergens using a commercially prepared mixed disc of allergens. The rationale for this was that previous studies on workplace outbreaks of allergic alveolitis (hypersensitivity pneumonitis), e.g., humidifier fever in printing work have implicated fungi as likely aetiological agents (Pal *et al.* 1997).

2.3.2 Statistical analysis

Data were analysed to determine the association of immunological responses to the sample of metalworking fluid taken from the large Mayfram sump with either a clinical diagnosis of OA, EAA or reported respiratory symptoms in the absence of a clinical diagnosis. The study dataset consisted of information on disease status/respiratory symptoms, precipitin and specific IgE results for 258 workers who consented to provide a blood sample. Seven workers in total sent more than one blood sample for analysis, and for these workers the results from the earliest blood sample were used in statistical analyses. Four of the blood samples analysed had one or

¹² Two index (EAA) cases tested positive to a mixture of two *Mycobacteria* species (specifically *M. chelonae* and *M. fortuitum*) in the initial scoping study

more missing results for the precipitating antibodies or specific IgE responses due to insufficient blood sample. In order to test for statistically significant differences in precipitin and specific IgE results between disease groups and control groups, the data was formulated into 2 x 2 contingency tables and Fisher's exact tests were carried out. Fisher's exact test is suited to the analysis of categorical data where sample sizes are small. The test reports a P-value where P is the probability of observing the results by chance. A cut-off probability of 5% was applied to determine statistical significance.

3 RESULTS OF IMMUNOLOGICAL INVESTIGATION

3.1 DEMOGRAPHIC CHARACTERISTICS OF WORKFORCE

Demographic characteristics of the factory cohort as a whole (i.e. excluding the non-exposed office worker controls from the other worksite) are summarised in Table 11, Appendix C. Data is presented just for those workers who provided a blood sample (n=193). The mean age of factory workers (n=193) was 45 years (range=19-63 years). 92% of workers were male and 8% female. 23% were current smokers, 32% ex smokers and 45% had never smoked. The mean duration of employment at the worksite was 14 years (range=1-45 years), and the mean no. of hours worked per week 37 hours (range=32-50 hours). 87% of workers in the study cohort reported working in manufacturing areas. The demographic characteristics of the individual case (symptomatic, OA, EAA) and control groups are also detailed in Table 9. The age and gender characteristics and smoking habits of the groups making up the factory cohort were similar. In addition, the mean duration of employment and hours worked varied little across the groups. However, more of the OA and EAA cases worked in manufacturing areas than both the asymptomatic and symptomatic workers.

3.2 COMPARISON OF PRECIPITINS AND SPECIFIC IGE TEST RESULTS

Results of serological testing for precipitating antibodies and specific IgE are summarised for the worker groups in Tables 4 to 8. Precipitin results are also summarised via Venn Diagram in Figures 1 and 2.

3.2.1 Tests for Precipitating Antibodies

No workers tested positive for precipitating antibodies to *Mycobacteria* species. The greatest number of positive tests were observed for *Ochrobactrum* (3/17 EAA cases, 2/70 OA cases, 2/42 asymptomatic workers and 3/72 symptomatic workers – not EAA/OA), followed by *Acinetobacter* (7/17 EAA cases, 2/70 OA cases). There were 7 positive precipitins tests to used metalworking fluid (3/17 EAA cases and 4/70 OA cases). All of the 7 positive tests for *Acinetobacter*, 3 of the 7 for the used metalworking fluid, and 3 of the 10 for *Ochrobactrum*, met the case definition criteria for EAA, while the remaining 4 positive results for used metalworking fluid were categorised as having OA. There were 5 workers in total not meeting the case definition criteria for either EAA or OA, but who tested positive for precipitating antibodies to *Ochrobactrum*, 3 of which reporting respiratory symptoms (but not formally diagnosed with any disease). There were no positive precipitin results for the non-exposed control group. Of the case groups investigated, evidence for an association between work related respiratory disease and contaminated MWF exposure in the study cohort was strongest for EAA. Taking the EAA case group in total, over half (10/17) showed positive precipitins to at least one of the microbial species or used metalworking fluid. In contrast to the EAA cases, few of the OA cases showed a positive precipitin test to the microbial species or used metalworking fluid (7/70 only). Results just for the index cases are shown in Appendix C, Tables 12 and 13.

Table 4: Results of precipitin tests, grouped by case definition

	Used sump oil		Ochrobactrum	
	Negative	Positive	Negative	Positive
Asymptomatic ¹	41	0 (0%)	39	2 (5%)
EAA	14	3 (18%)	14	3 (18%)
Symptomatic (not EAA/OA)	72	0 (0%)	69	3 (4%)
OA	66	4 (6%)	68	2 (3%)
Control	65	0 (0%)	65	0 (0%)

Missing data (due to absence of/insufficient blood):

¹ Used metalworking fluid (1), Ochrobactrum (1)

Table 5: Results of precipitin tests, grouped by case definition

	Acinetobacter		Mycobacteria*	
	Negative	Positive	Negative	Positive
Asymptomatic ¹	40	0 (0%)	40	0 (0%)
EAA ²	10	7 (41%)	15	0 (0%)
Symptomatic (not EAA/OA)	72	0 (0%)	72	0 (0%)
OA	68	2 (3%)	69	0 (0%)
Control	65	0 (0%)	65	0 (0%)

* mixture of three species (specifically Mycobacterium immunogenum M. chelonae and M. fortuitum)

Missing data (due to absence of/insufficient blood):

¹ Acinetobacter (2), Mycobacteria (2)

² Mycobacteria (2)

Table 6: Results of significance tests across disease and comparison groups

		EAA		OA	
	n/N	n/N	P Value	n/N	P Value
USED SUMP OIL		3/17		4/70	
Asymptomatic	0/41		0.02		0.15
Control	0/65		<0.01		0.06
OCHROBACTRUM		3/17		2/70	
Asymptomatic	2/41		0.14		0.47
Control	0/65		<0.01		0.26
ACINETOBACTER		7/17		2/70	
Asymptomatic	0/40		<0.01		0.40
Control	0/65		<0.01		0.26
MYCOBACTERIA		0/15		0/69	
Asymptomatic	0/40		1.00		1.00
Control	0/65		1.00		1.00

3.2.2 Tests for Specific IgE

There was little difference in the prevalence of positive specific IgE responses to common environmental allergens across the worker groups (OA – 26/70, Symptomatic not EAA/OA – 34/72 or 47%, Asymptomatic – 20/42, Control – 34/65), with the exception of the EAA group (2/15), which had significantly lower rates ($P < 0.05$) relative to all other groups. The prevalence of specific IgE responses to mixed fungi, in contrast, differed little ($P > 0.05$) across all groups (EAA – 1/17, OA – 4/70, Symptomatic not EAA/OA – 5/72, Asymptomatic – 1/42, Control – 3/65).

Table 7: Results of Specific IgE tests, grouped by case definition

	Atopy		Mixed Fungi	
	Negative	Positive	Negative	Positive
Asymptomatic ¹	21	20 (49%)	40	1 (2%)
EAA ²	13	2 (13%)	14	1 (7%)
Symptomatic (not EAA/OA)	38	34 (47%)	67	5 (7%)
OA	43	26 (38%)	65	4 (6%)
Control	31	34 (52%)	62	3 (5%)

Missing data:

¹ Atopy (1), Mixed fungi (1)

² Atopy (2), Mixed fungi (2)

Table 8: Results of significance tests across disease and comparison groups

		EAA		P Value	OA	
		n/N	n/N		n/N	P Value
ATOPY		n/N	n/N		n/N	P Value
	Asymptomatic	20/41	2/15	0.01	26/69	0.17
	Control	34/65		<0.01		0.06
MIXED FUNGI		n/N	n/N		n/N	P Value
	Asymptomatic	1/41	1/15	0.46	4/69	0.38
	Control	3/65		0.57		0.53

Figure 1: Positive precipitins tests to Acinetobacter, Ochrobactrum and used sump oil in EAA cases

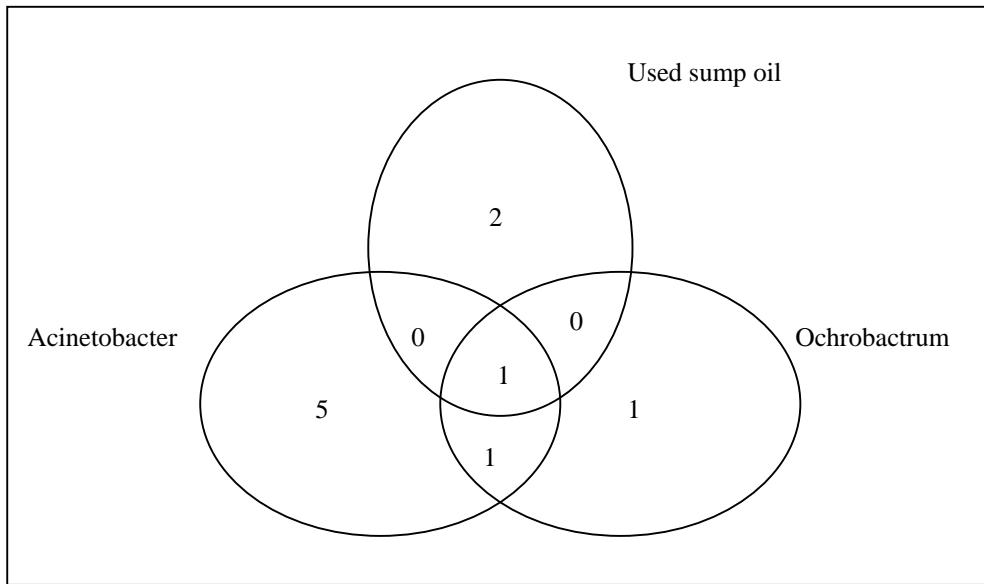
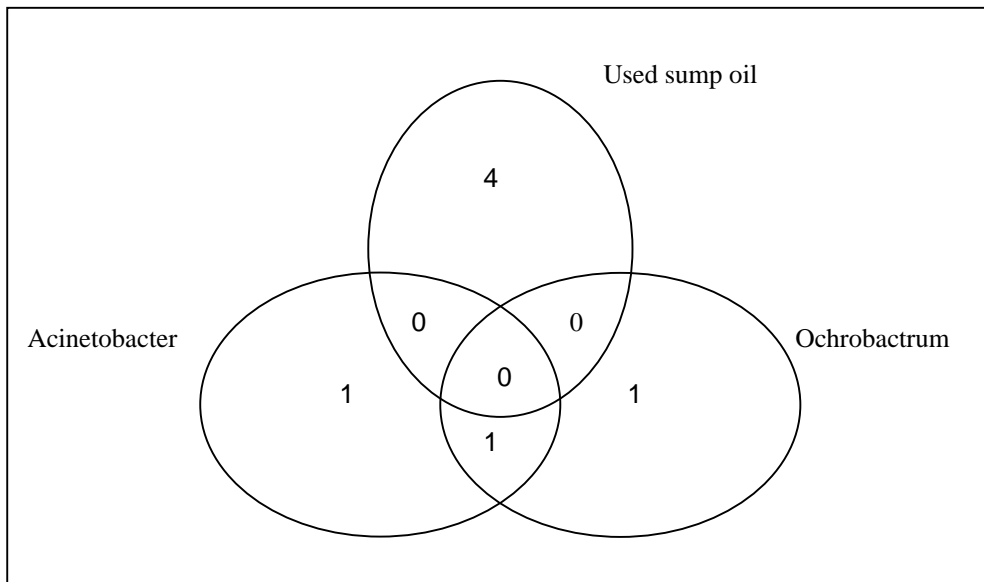


Figure 2: Positive precipitins tests to Acinetobacter, Ochrobactrum and used sump oil in OA cases



4 DISCUSSION

There is compelling evidence in the scientific literature suggesting that workers exposed to metal working fluid mist experience more respiratory symptoms than comparable non-exposed controls (Jarvholm *et al.* 1982; Robertson *et al.* 1988; Kennedy *et al.* 1989; Sprince *et al.* 1997). The evidence also suggests that MWF mist can cause asthma and alveolitis (Friend *et al.* 1977; Hendy *et al.* 1985). Studies have postulated a number of possible aetiological mechanisms. Historic indications from the scientific literature have pointed to certain species of bacteria containing endotoxins in their cell walls as one of the prime candidates. These have been shown to cause acute and chronic respiratory symptoms in addition to causing or exacerbating specific occupational respiratory diseases, such as occupational asthma (OA) in some susceptible individuals (Laitinen *et al.* 1999). It has also been suggested that exposure to toxic components in MWF such as pine oil based re-odorants (Robertson and Weir 1988), ethanalamine (Vallieres *et al.* 1977) and methyl esters of fatty acids may be linked with occupational asthma (Robertson *et al.* 1988, Vallieres *et al.* 1977). More recent studies have suggested a link between contaminant bacteria in MWF and extrinsic allergic alveolitis (hypersensitivity pneumonitis) (Freeman *et al.*, 1998). For example non-tuberculous *Mycobacterium* species have been cited as aetiological agents in various cases of extrinsic allergic alveolitis (EAA) in the United States of America (CDC 1998, 2002, Kreiss and Cox-Ganser 1997).

Several studies that have investigated the respiratory health effects of exposure to microbial contaminants of MWFs have utilised the presence of precipitating antibodies in blood sera as a proxy measure of microbial exposure (Laitinen *et al.* 1999). The presence of precipitating antibodies to a biological agent is indicative of prior exposure and subsequent immunological response. Immunological responses, when localised in the lungs, may manifest (in certain susceptible individuals) as the physiological changes and clinical symptoms that characterise respiratory diseases such as asthma and alveolitis. However, precipitating antibodies to a biological agent can be present in the sera of individuals with no reported symptoms simply due to exposure. This was clearly identified in the scoping study where asymptomatic HSL workers demonstrated specific IgG to common environmental bacteria (see Appendix A). Clearly, the association between respiratory symptoms and the presence of precipitating antibodies is complex.

The report of Robertson *et al.* (2007) describes the clinical investigation of the occupational respiratory disease outbreak at the Powertrain plant. With the gradual recognition that an abnormal burden of respiratory disease was occurring at the plant, a reactive investigation was instigated by HSE. This investigation involved a retrospective assessment of the management of metal working and wash fluids at the plant, an investigation of other aspects of work hygiene, and a more detailed clinical and immunological investigation of workers with respiratory symptoms as well as those with no symptoms but who were likely to have been exposed. It needs to be recognised that this retrospective assessment has placed constraint on identifying causative agents and circumstances. Consequently, the blood precipitin test results in this report need to be considered together with the clinical and epidemiological data, which were also collected as part of this investigation.

The objective for this study was to investigate the degree to which exposure to microbial contaminants of MWFs may have contributed to the outbreak of occupational respiratory disease at the Powertrain worksite. The cohort under investigation consisted of groups of workers with either a clinical diagnosis of EAA, or OA, a group of workers with self reported symptoms that did not fit the EAA/OA case definition criteria, a group of workers without symptoms but for whom exposure was possible, and a group of non exposed office workers acting as controls.

If the presence of precipitating antibodies in patient sera merely reflected exposure to contaminated metalworking or wash fluid mist then one may expect there to be an approximately equal proportion of exposed workers showing positive test results in the case groups and workers without symptoms. Alternatively, any evidence for higher rates of exposure and positive precipitin tests in cases relative to workers without symptoms is supportive of the view that exposure to contaminated metalworking or wash fluid mist is somehow associated, (perhaps causally or otherwise), with the disease observed in the cases.

Before attempting an interpretation of study results, it is useful to highlight a number of key methodological issues relating to the organisation of this investigation that may impact on the interpretation of the precipitin test results for the cases versus asymptomatic group.

There were changes of wash fluid for aluminium washing in 2003, along with a change of hydraulic oil for all metal working machines (which leaks as tramp oil into metalworking fluid, and thence to washing machines). However, there is no clear evidence to suggest these changes were significant.

The decision regarding which microbiological species to use for screening the main study population was informed based on the results of an initial scoping investigation of blood precipitins undertaken on 11 index EAA cases diagnosed at the Birmingham Heartlands Hospital, 11 workers without symptoms, (but for whom exposure was possible) selected by the Birmingham Heartlands, and a sample of non exposed controls recruited from another worksite. Immunoassays were performed with extracts of two species of bacterium, *Ochrobactrum* and *Acinetobacter*, which were both identified by DNA analysis in the sample of used metalworking fluid taken from the common Mayfram sump, along with a variety of other bacterial species (including *Pseudomonas*, *Citrobacter*, *Bacillus*, *Arthrobacter* and *Stenotrophomonas spp.*) cultured from samples of MWF and washfluids taken from the workplace after the initial clinical investigation. The EAA cases and comparison groups were investigated for precipitating antibodies to these species. Due to resource constraints, only those bacterium species that showed precipitating antibodies in the serum of EAA cases, but not comparison workers, were selected for inclusion in the main study. This narrowed down the species selection to the two bacterial species, *Ochrobactrum* and *Acinetobacter*, which had been identified in the original sump oil sample and had also been isolated from other MWF and wash fluid samples subsequently taken from the factory. In addition, species of *Mycobacterium* (specifically, *Myco. immunogenum*, *Myco. chelonae* and *Myco. fortuitum*) were also included in the test panel to enable comparison with the results of USA studies, even though there was no evidence of their presence in workplace samples.

A greater proportion of workers in the EAA and OA groups reported working in a manufacturing area compared to workers without symptoms (OA – 99%, EAA – 100%, asymptomatic – 62%). Other demographic factors such as the duration of employment at the worksite, the number of hours worked per week, age and gender mix, were equally distributed across the groups. More comparative information on the worker groups can be found in the accompanying report by Robertson *et al.* 2007, which communicates the results of the clinical and epidemiological investigations carried out as part of this study. The greater proportion of EAA and OA cases exposed to MWF will obviously contribute to any differences in precipitating antibodies observed across the case and comparison groups.

The investigation, perhaps unsurprisingly, observed significantly higher rates of atopy in OA than EAA cases. Atopy describes the tendency to exhibit IgE-mediated allergic reactions to common environmental allergens. The immune profile underlying cases of extrinsic allergic alveolitis in contrast is driven by IgG rather than IgE and a different subset of T-cells and cytokines (Shuyler 1993). However, perhaps rather spuriously, the rate of atopy in the non-

exposed controls was higher than in the OA case group (52% versus 38%). This is higher than the rate of around 30% typically observed in general populations (Wardlaw 1993). Lower rates of atopy found in cases than control groups, is often attributed to the former reflecting a survivor group. However, the underlying high rate of atopy in the control group in this study remains unexplained.

Evidence of altered immune responses were observed in a subsample of workers in the factory cohort, particularly in cases with a physician diagnosis of work related EAA. Over half of EAA cases (10/17 or 59%) had precipitating antibodies to at least one of the microbial species investigated or to used sump oil, the majority (7) testing positive to *Acinetobacter*. In contrast to the EAA cases, few of the OA cases (7/70) showed precipitating antibodies to one or more of the antigens tested for, most (4) of the positive cases testing positive to used sump oil. The finding of higher rates of precipitins in EAA than OA cases is in keeping with the aetiology of the two diseases. Clearly it is not possible to comment whether EAA cases may have had precipitating antibodies to other bacteria species not included in the screening panel, although it is likely that immune responses to other bacteria would have been documented had more bacteria species tested in the scoping study been included in the main investigation. Consistent with this, all 11 EAA cases investigated in the scoping study had at least one positive IgG precipitin to at least one bacteria species detected in the workplace and included in the test panel. It is possible that immune responses to these bacteria were relevant in the aetiology of EAA observed in the study population.

In summary, although the aetiology of EAA and occupational asthma in this occupational setting remains to be fully elucidated, the findings of this investigation are in keeping with previous studies which have implicated bacterial contamination of MWF as the most likely relevant factor. In addition, the study findings relating to precipitating antibodies for the EAA cases may lend further weight to any arguments advanced in Robertson *et al.* 2007 regarding the working practices and worksite exposures that caused or contributed to the Powertrain disease outbreak.

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6 APPENDIX A - RESULTS OF SCOPING STUDY

Table 9: Precipitin Scoping Study – No. of positive results/No. of valid cases

Test extract	Powertrain coolant oil	Powertrain Acinetobacter	Powertrain Ochrobactrum	PT20-61	PT22-62	PT33-63	PT36-64	PT17-65	PT27-66
Worker group									
Index cases n=11	4/11	7/11	2/11	2/10	8/10	5/10	1/10	1/10	3/10
Asymptomatic workers n=11	0/11	4/11	3/11	0/11	9/11	2/11	0/11	2/11	1/11
HSL controls N=6	0/5	0/5	0/5	0/4	2/4	2/4	0/4	1/4	2/4

Notes: PT20-61 – *Ochrobactrum anthropi*, PT22-62 – *Citrobacter freundii*, PT33-63 – *Ochrobactrum*, PT36-64 – *Bacillus*, PT17-65 – *Athrobacter*, PT27-66 – *Stenotrophomonas*

Table 10: Precipitin Scoping Study – No. of positive results/No. of valid cases

Test extract	PT31-67	Oil No26 op60	Oil No28 op100	New wash culture PT24	Mycobacteria species*	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas pseudoalcaligenes</i> 000/106	<i>Pseudomonas alcaligenes</i> 000/107
Worker group								
Index cases n=11	0/10	0/10	1/10	0/10	2/10	0/10	1/10	0/5
Asymptomatic workers n=11	0/11	0/11	0/11	0/11	0/11	-	-	-
HSL controls n=6	0/4	0/4	0/4	0/4	0/4	0/6	1/6	0/6

Notes: PT31-67 – *Citrobacter*, *mixture of two species (specifically *Mycobacterium chelonae* and *M. fortuitum*)

7 APPENDIX B - METHODOLOGIES FOR IMMUNOLOGICAL INVESTIGATION

Extracts were prepared using an adapted method described by Lewis *et al.* (2001). Briefly, extracts from fluids collected from site visits were chilled for several hours at 4 degrees. The lower fraction was removed (avoiding contamination from the oily surface emulsions) and centrifuged for 1 hour at 22,000g and 4 degrees. The lower fraction from this spun sample was again removed and stored at -20°C until required.

Acinetobacter and *Ochrobactrum* were grown on Nutrient Agar plates (Oxoid). *Mycobacterium* spp. were grown on Stonebrink egg pyruvate agar (Oxoid). Bacteria were harvested by scraping from the surface of the agar and re-suspending in PBS. Samples were then frozen and thawed before sonication. The extracts were rotated overnight and then centrifuged for 30 minutes (11,500g). The supernatant was removed and stored at -20°C until used.

Total protein estimation on all the allergen extracts was performed using a bicinchoninic acid protein assay; (BCA) (Smith *et al* 1985) which was automated using the Cobas Fara (Roche, Welwyn Garden City, UK). Protein determination reagent consisted of 5ml bicinchoninic acid with 100 μl of copper (II) sulphate. The principle of the assay is that the protein in the sample will reduce alkaline Cu (II) to Cu (I). The bicinchoninic acid is a chromogenic reagent for Cu (I), and produces a purple complex with a maximum absorbance at 562nm. The protein standard used was bovine serum albumin (1mg/ml) and quality control samples were prepared from human serum albumin dissolved in distilled H_2O . The assay had a between assay coefficient of variation of 3.0 % at 528.9mg/ml, and of 2.0 % at 267.5mg/ml.

Agar double diffusion plates (Microgen Bioproducts Ltd., Surrey, UK) were prepared to determine the presence of precipitating antibodies to the prepared extracts of metalworking and wash fluids and bacteria, using the Ouchterlony double diffusion technique as described by Hudson and Hay (1976). Approximately 100ul of each test or control serum was placed in the outer wells of specialised agar plates and a 30ul sample of extract (working concentrations for all extracts of approximately 0.5mg/ml were used) was placed in the surrounding wells. Plates were incubated in an enclosed humid container for 36 hours. Following incubation, the plates were washed with phosphate buffered saline (PBS) (3 changes over 8 hours) and then with distilled water. Plates were removed from their containers and placed onto Whatman filter paper (Whatman, London, UK). Several sheets of this paper were then pierced with a needle and placed on top of the gel followed by additional absorbent paper. Heavy weight was placed on top and the gels left to compress and dry overnight. The compressed gels were returned to containers and washed with GelCode Blue stain (Pierce, UK) for 15 minutes and rinsed with distilled water. In cases of a positive result, bands of precipitating antibodies were visible between wells containing serum and antigen extract. All gels were scored, countersigned and photographed as a record.

Specific IgE levels to common environmental allergens (phadiatop – atopy) and to mixed fungi (mix 1 – *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus* and *Alternaria alternata*) were determined for individuals participating in the study using the automated UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden). Allergens were pre-covalently coupled to ImmunoCaps and incubated with patient serum samples according to the manufacturers protocol. Following a wash step, enzyme labelled anti-human IgE was added to the Immunocap which complexes to the already bound IgE from the patients sera. Following an incubation step, the unbound anti-human IgE was removed by washing and a developing agent was incubated with the bound complex. The fluorescence of the eluate was measured and

compared directly against a reference dilution curve. The test sample was assigned a specific IgE class from 0-6 representing a range from undetectable to very high levels of specific IgE. Class scores greater than two were assumed to be positive. The assay had a maximum within assay CV of 6%, a between assay CV of 11% and was reported to exhibit no cross reactivity to other common allergens.

8 APPENDIX C - RESULTS OF IMMUNOLOGICAL INVESTIGATION

Worker Demographics

Table 11: Worker Demographics and Employment History

Total no. in group	All ¹ n=193	External controls n=65	Asymptomatic n=42	Symptomatic n=72	OA cases n=70	EAA cases n=17
Characteristics						
Age (years)						
Mean (SD)	44.52 (8.01)	37.71 (9.96)	43.60 (9.79)	45.63 (7.12)	44.04 (7.75)	46.36 (7.67)
Range	19-63	21-62	19-63	32-59	29-61	29-61
n	193	65	42	72	70	17
Gender No. (%)						
Male	177/193 (92%)	26/65 (40%)	38/42 (90%)	68/72 (94%)	64/70 (91%)	15/17 (88%)
Female	16/193 (8%)	39/65 (60%)	4/42 (10%)	4/72 (6%)	6/70 (9%)	2/17 (12%)
Smoking No. (%)						
Current	44/188 (23%)	-	6/38 (16%)	21/71 (30%)	15/70 (21%)	3/17 (18%)
Ex	60/188 (32%)	-	12/38 (32%)	24/71 (34%)	21/70 (30%)	6/17 (35%)
Never	84/188 (45%)	-	20/38 (53%)	26/71 (36%)	34/70 (49%)	8/17 (47%)
Duration of employment at East Works (years)						
Mean (SD)	12 (8)	-	11 (8)	13 (9)	13.41 (8.65)	11 (11)
Range	1-45		1-35	2-41	1.27-45.15	2-45
n	190		42	70	69	17
Hours worked						
Mean (SD)	37 (1.7)	-	38 (2.7)	37 (1.3)	37.28 (1.39)	37 (0.7)
Range	32-50		36-50	32-45	34-47	37-39
n	190		42	70	69	17
Work in manufacturing Areas No. (%)	168/193 (87%)	-	26/42 (62%)	64/72 (89%)	69/70 (99%)	17/17 (100%)

Percentages expressed relative to no. in group (excluding missing data)

¹Excluding external controls

Precipitin and specific IgE results for index cases

Table 12: Precipitin results of the 10 index cases

Patient	Sump Oil	Ochrobactrum	Acinetobacter	Mycobacteria
1	1	1	1	0
2	0	0	1	0
3	0	0	1	0
4	0	0	1	0
5	0	0	1	Insufficient blood
6	0	0	1	0
7	1	0	0	0
8	0	0	0	0
9	0	1	1	0
10	1	0	0	Insufficient blood

Table 13: Specific IgE results of the 10 index cases

Patient	Atopy	Mixed fungi
1	1	0
2	0	0
3	1	0
4	0	1
5	Insufficient blood	Insufficient blood
6	0	0
7	0	0
8	0	0
9	0	0
10	Insufficient blood	Insufficient blood

9 APPENDIX D – INVESTIGATION OF THE POTENTIAL FOR DROPLET DISPERSION AROUND THE FACTORY FOR ENGINE PARTS WASHING MACHINES

Towards the end of the investigation (July 2005), Mike Burd (the HSE Project Officer) contacted HSL's Exposure Control Section about investigating the potential for droplets released from the exhaust ducts of the engine parts washing machines' to reach areas of the factory where the reported cases worked.

Two main options were discussed during an initial site visit:

- 1) The use tracer gas and/or fluorescent tracer particles emitted from the washing machines, and measurements of gas and aerosols in the areas of the factory where the reported cases worked.
- 2) Measurement of the size and velocity of droplets emitted from the washing machine exhaust ducts, prediction of the final particle size, how long they will remain airborne and modelling of dispersion to areas of the factory where the reported cases worked.

During the discussions, option 1 was discounted because of the likely day-to-day variability in the ventilation in the factory. With roof vents, ventilation ducts, opening doors and complicated structure associated with the various machining lines, the airflows in the factory are likely to be very complicated. Whilst a positive tracer reading would indicate transport from the washers, a negative reading would not be sufficient to be able to state that there would not be transport on different days when the ventilation conditions may have changed. To be sure that particles from the washing machines could not be transported to the relevant areas of the factory would require a considerable monitoring effort over different seasons when the air velocity distributions in the factory is likely to be different. This was not possible because of the impending closure of the factory

Option 2 was therefore explored further and arrangements made to measure the velocity and size of droplets emitted from the engine block washer and the camshaft washer. This involved constructing scaffolding around the exhaust ducts of the two washers and the rental for one day of a Malvern Spraytech laser diffractometer to measure the droplet size and the use of a TSI Velocicalc hot wire anemometer to measure the emission velocity. Unfortunately, on the day of the tests, one washer was not in operation and there were no parts being washed by the other washer and so no droplets were emitted. Mean velocities of 2.2 and 4.8 m s⁻¹ were measured from the two ducts of the washer tested.

No further tests were thought worthwhile as the plant involved and linked processes had not been in use for several months, pending its removal to China. It was thought impossible therefore to recreate the actual production conditions, which had existed during the outbreak, without the unreasonable expenditure of time, money and effort. However, the exercise demonstrated that HSL has the capability to carry out tests and modelling to investigate the possible transport of aerosols released from a source in a factory to possible sensitive locations in other parts of the factory. This could be used in studies in other machine workshops and other industrial sectors where there is the potential for droplet release.