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STLE PRESENTATION

Reassessing The Health Risks Associated With Employee Exposure To Metalworking Fluid Microbes©

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Metalworking fluids provide an excellent environment for the growth and proliferation of a variety of bacteria and fungi. Historically, the incidence of infectious disease outbreaks at metalworking facilities has been rare. Consequently the primary focus of microbial contamination control efforts has been to prevent fluid biodeterioration. Research conducted over the past decade increasingly supports an argument for reevaluating microbial contamination control strategies. Although communicable disease risk remains low, there is likely to be an increased risk of toxin and allergen exposure for metalworking facility personnel routinely exposed to metalworking fluid aerosols.

This paper summarizes current knowledge and suggests direction for further research on health risks associated with exposure to the biological constituents of metalworking fluid aerosols.

KEY WORDS

Antimicrobial Additives; Biocides; Fungicides; Degradation; Hygiene; Safety; Toxicology; Microbial; Coolants

INTRODUCTION

All recirculating metalworking fluid (MWF) systems provide aeration and surface area, both of which are conducive to microbial colonization and proliferation (Passman, (1988)).

As discussed by Rossmoore (Rossmoore, (1979)), some of the earliest reports on metalworking fluid microbiology (Bennett, et al., (1954), Tant, et al., (1956), Samuel-Maharajah, et al., (1956)) list potentially pathogenic bacteria that have been recovered from used emulsifiable oils. In 1956, Tant and Bennett (Bennett, et al., (1954)) reported their survey of metalworking fluids for potential pathogens. They defined potential pathogens as isolates representative of microbial genera that included bacteria known to cause disease, even if the isolated species were not known pathogens. Table 1, taken from the Tant and Bennett (Bennett, et al., (1954)) report illustrates the taxonomic diversity of MWF isolates. Many of the microbes listed in Table 1 are recovered rarely. Significantly, MWF taxonomic diversity is similar to that of public water supplies.

Tant and Bennett made no attempt to link MWF isolates with any disease reports, nor did they attempt to determine which isolates were able to proliferate and which were more likely to be incidental contaminants unlikely to persist. However, Wheeler (Bennett, et al., (1954)) did contract typhoid fever while working with the *Salmonella typhosa* (typhi) culture he isolated from a MWF sample.

Table 2 lists fungi that have been recovered from MWF. *Acremonium, Aspergillus, Candida, Fusarium, Penicillium and Saccharomyces* species are relatively common fungal isolates. The other fungi listed in Table 2 have been recovered infrequently.

There's no debate regarding the potential pathogenicity of some of microbes recovered from MWF. Despite considerable speculative and circumstantial evidence (Holden, (1977), Hill, et al., (1979), Cox-Ganser, et al., (1998)) that has been presented since 1956, there is little evidence for any direct cause and effect relationship between MWF exposure and infectious disease. However, there **is** a growing body of literature supporting hypotheses of toxic and allergic effects from MWF microbe exposure (Bernstein, et al., (1998), Robbins, et al., (1996)).

This paper summarizes the potential health risks associated with employee exposure to MWF microbes, and then describes the challenges confounding attempts to assess the actual risks.

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TABLE 1—POTENTIALLY PATHOGENIC BACTERIA ISOLATED FROM USED METALWORKING FLUID EMULSIONS (TANT, et al., (1956)

MICROORGANISM	FREQUENCY OF OCCURANCE, %
Pseudomonas oleovorans	64
Pseudomonas aeruginosa	57
Paracolobacterium spp.	47
Proteus vulgaris	46
Escherichia coli	41
Klebsiella pneumoniae	32
Pseudomonas spp. (not listed elsewhere)	32
Staphylococcus aureus	17
Achromobacter spp.	12
Streptococcus pyogenes	6
Bacillus cereus	7
Escherichia freundii (now *Citrobacter freundii*)	7
Proteus morganii (now *Morganella morganii*)	6
Proteus spp.	4
Staphylococcus albus	4
Bacillus subtilis	3
Escherichia intermedium (now *Enterobacter intermedius*)	2
Aerobacter cloacae (now *Enterobacter cloacae*)	1
Streptococcus pneumoniae	1
Micrococcus citreus	1
Sarcina spp.	1
Shigella dispar	1
Salmonella typhi	1

TABLE 2—FUNGI ISOLATED FROM USED METALWORKING FLUID EMULSIONS

Aspergillus spp.*
Aureobasidium spp.
Acremonium (formerly *Cephalosporium*) spp.
Candida albicans
Fusarium spp.*
Penicillium spp.*
Saccharomyces spp.
Trichoderma spp.
Trichosporon spp.

*These genera of fungi isolated from other sources, e.g. food, have demonstrated the ability for mycotoxin production (Fakih, et al., (1995).

MICROBIOLOGICAL HEALTH EFFECTS

With the exception of two conditions discussed below, a review of the various diseases caused by pathogens recovered from MWF is beyond the scope of this paper. For more information about diseases caused by specific microbes, the reader is referred to a manual of communicable disease (Chin, (2000)).

Microbes proliferating in MWF can cause three general types of health problems: disease, toxemia and allergy. Infectious disease occurs when a pathogenic microbe enters a susceptible host, proliferates and induces a body response. Examples of infectious disease include: salmonellosis, bacterial pneumonia and tuberculosis. Microbes that produce poisonous extracelluar substances cause toxemias. Microbe molecules and products can stimulate histamine responses in exposed individuals. This type of response is called an allergy.

Infectious Disease

Theoretically, a single pathogenic cell entering the body and settling at a suitable site can proliferate and lead to a disease state. In reality, a number of factors contribute to a microbe's ability to cause disease. These factors fall into two primary categories: *pathogenicity* and *host susceptibility*. A microbe's pathogenicity depends on its ability to cause damage to its host. *Virulence* is a semi-quantitative measure of a microbe's pathogenicity, and depends on the pathogen's biochemical, genetic and structural features. Host susceptibility reflects the host's inability to ward off infection. Generally speaking, healthy individuals are less susceptible to infection than are unhealthy individuals. The American Museum of Natural History has an excellent web site tutorial describing infectious disease (American Museum of Natural History (2001)). Kenneth Todar (Todar, (1998)) provides a detailed discussion of pathogenicity and susceptibility.

Pathogens that are particularly virulent are often called *frank pathogens*. Bacteria that are only pathogenic when the host is already compromised are called *opportunistic pathogens*. The distinction between frank and opportunistic pathogens is quite objective.

Legionella pneumophila, the etiologic agent for Legionnaire's Disease, is ubiquitous in the aquatic environment. Generally, *L. pneumophila* is considered to be an opportunistic pathogen. Most exposed individuals do not become ill. However, *L. pneumophila* untreated infections are fatal to approximately 5% to 30% of susceptible individuals who contract the disease. People who are diabetic or on immunosuppressive drugs are particularly susceptible to Legionellosis.

Pathogenicity is affected by route of entry also. For example, *Aspergillus* sp. are ubiquitous fungi that generally are not pathogenic. However, a few spores colonizing the exposed tissues of a surgery patient can be quite virulent, leading to death within days (Fakih, et al., (1995)).

Of the three potential health risks related to microbes in MWF, infectious disease is the most problematic, since pathogens can be communicable. A single infected employee can expose numerous co-workers to a virulent pathogen. People with sub-clinical (not accompanied by any obvious symptoms) or pre-clinical (symptoms have not yet appeared) infections may also infect colleagues.

Toxemias

Toxins and allergens, the products of some microorganisms, are not communicable. Toxins are poisons. For example *Clostridium botulinum*, produces seven different, potentially deadly neurotoxins. Poisoning occurs when someone eats food in which *C. botulinum* has grown and produced the toxin. There is no requirement for the victim to come into direct contact with the toxin-producing microbe. The effects of some toxins, such as the botulism group, are acute. Symptoms occur soon after exposure and, if they don't kill the poisoned individual, pass in a relatively short time (hours to days). In contrast, carcinogenic mycotoxins, produced by a variety of fungi, have a chronic effect on their victims. Chronic effects can linger for periods ranging from several months to the remainder of an individual's lifetime.

Although no studies have addressed MWF specifically, members of fungal genera routinely recovered from MWF are known to produce mycotoxins. Suttajit (Suttajit, (1998)) lists representative mycotoxins that are produced by fungi that are members of the same genera as those known to contaminate MWF. To date there is no evidence that mycotoxins accumulate in MWF. More research is needed to determine whether the absence of evidence reflects: a) an absence of mycotoxins; b) rapid denaturation of mycotoxins that are produced; or c) mycotoxins actually do accumulate in MWF but have been undetected due to experimental or analytical limitations.

Allergies

Allergens are substances that induce histamine production in an exposed individual. Histamine, a derivative of the amino acid histidine, is released as part of the body's response to insult from injury or exposure to certain antigens. Allergens may be natural or synthetic. They cause symptoms ranging from mild localized itching at the point of contact with an allergen, to anaphylaxis – a general, potentially lethal, whole body condition resulting from the body's rapid release of antibodies and histamine. Virtually any chemical substance can be allergenic to some members of the population. Plants, animals, foods, pollen, dander, bee stings and medications are common allergens. One characteristic that differentiates allergens from toxins is the susceptibility response range within an exposed population. Typically, only a small percentage of an exposed population responds to allergens. In contrast, dose response relationship variability for toxins typically is relatively narrow (e.g. a given dose will cause the same reaction in most exposed individuals). Although this paper focuses on microbial disease issues, it's important to note here that antimicrobial pesticides (MWF biocides) can also be allergenic (Rossmoore, (1995), Mathias, (1994)).

Although microbial health risks are classified into three categories, it's important to note that pathogenicity may be at least partially dependent on a microbe's toxin production within the host (for example anthrax and diphtheria toxins). Moreover, non-pathogenic microbes may induce allergic responses. Any chemical or substance that enters the body and causes an immune response (i.e. antibody production) is called an *antigen*. The antibodies produced in response to a particular antigen are very specific. By measuring specific antibody titers (concentrations in blood samples), immunologists can assess whether individuals have been exposed to particular antigens. The value of this immunological test will become apparent in the discussion of linking cause with effect, below.

In summary, pathogenic microbes may be frank or opportunistic pathogens. Microbes may cause infectious diseases, toxemias and allergies. Only microbes are infectious. Non-biological chemicals may cause toxemias and allergies.

LINKING CAUSE AND EFFECT

As recently as the mid-nineteenth century, the existence of a relationship between pathogenic microbes and the diseases they caused was debated hotly. During the period 1876 to 1882 a German physician, Robert Koch, proposed a set of four conditions that have become known as Koch's Postulates. To confirm that a particular microbe is responsible for a particular disease one must:

- Recover the suspect microbe from every instance of the disease;
- Grow the isolated, suspect microbe in pure culture, and maintain the culture over several microbial generations;
- Infect healthy, susceptible animals (hosts), with the microbe cultivated in the previous step, demonstrating that the intentionally infected hosts contract the disease; and
- Recover the suspect microbe from the intentionally infected hosts.

Although ethical and technical issues make it difficult to always fulfill all of Koch's Postulates when investigating the etiology of a suspected pathogen, clinical microbiology is still guided by these principles.

Koch focused his efforts on the frank pathogens responsible for anthrax, tuberculosis and cholera. His research demonstrated cause and effect relationships between the etiologic (causative) agent and their respective diseases unequivocally. More often, the relationship is more equivocal. In the case of opportunistic pathogens, only a small percentage of the exposed population becomes ill. During the 1970's and 1980's Professor Scott Clark, at the University of Cincinnati, investigated the relationship between pathogen exposure and disease incidence among municipal sewage and wastewater treatment plant operators. Clark (Clark, (1987)) found that despite exposure to elevated numbers of potentially pathogenic bacteria, experienced wastewater treatment plant operators did not become sick more often than did members of the control population (people who didn't work in high exposure environments). New employees did have an increased incidence of gastroenteritis during their first year of exposure to waste water treatment plant aerosols. Clark's work highlights the complexity of the pathogen-host relationship.

In industrial environments such as metalworking facilities, other aerosolized chemicals influence the possible effects of microbes and biomolecules. Multiple chemical sensitivity, or ecologic illness, is a chronic disorder characterized by a variety of symptoms that develop in response to direct, indirect (interaction) or both types of effects of exposure to environmental excitants (Sarnet, et al., (1992)). It's also well recognized that life-style choices (particularly smoking and alcohol consumption) can exacerbate individual worker responses to work environment sanitizers, allergens or immunotoxins (Robbins, et al., (1996)). This means that attempts to apply Koch's Postulates to link an etiologic agent to a particular disease in the metalworking shop environment are likely to be unsuccessful.

The recent outbreak of Legionnaires Disease at a Midwestern engine casting plant illustrates the challenge confronting industrial hygienists, clinical microbiologists, immunologists and epidemiologists. After four workers at the engine casting plant became ill, scientists from the Centers for Disease (CDC) investigated the origins of the outbreak (Allan, et al., (2001)). All four confirmed patients had pneumonia and laboratory evidence of *Legionella* spp. infection. Allan *et al.* collected 197 environmental samples and performed clinical tests (interviews, exams and serology) on 484 of the plant's 2,500 employees.

Legionella spp. isolates were recovered from 18 of the environmental samples (none from the building in which the four confirmed patients worked), but none of the isolates matched the clinical isolates by monoclonal antibody staining. Eleven of the survey participants had high anti-Legionella IgG antibody titers (levels) and at least two of the following clinical symptoms: cough, fatigue, fever, headache, myalgia (muscle pain) or shortness of breath. Another 105 participants qualified as controls. The remaining 368 were asymptomatic, but had detectable anti-Legionella IgG antibody titers. The spatially and temporally tight cluster of Legionnaire's Disease onset suggested that the workers were exposed at their job, but the follow-up investigation failed to substantiate this hypothesis. The authors speculated that the *Legionella* strain that made these workers ill was "short-lived and transient." Twenty years earlier, at an engine factory in Canada, the epidemiological data, based on serological evidence, were much less equivocal. During that outbreak, approximately 300 workers became ill with Pontiac fever, attributed to *Legionella feelii* (Herwaldt, et al., (1984)).

In summary, nearly 50 years of MWF microbiology literature suggest that infectious disease is a rare phenomenon in metalworking facilities. However, researchers are building a compelling case that machinists and other workers exposed to MWF aerosols are at increased risk to non-infectious microbial health hazards.

THE AUTHORS CURRENT UNDERSTANDING

Hypersensitivity Pneumonitis

Cause and effect dynamics become even more complicated in the case of chronic toxemias and allergies. Consider hypersensitivity pneumonitis (HP). This condition's etiology is not well understood. It's possible that HP may be induced by the irritant effect of respired particles (biological or non-biological) on alveoli (Merck, (2001)). Certain inhaled particles may be more toxic than others (Rose, (1996), Cormier, (1998)). Alternatively, HP may be an allergic response (Schuyler, (2001)).

Common HP symptoms include acute, flu-like illness; recurring pneumonia and chronic dyspnea (difficult respiration). Onset may occur within a few weeks or years after initial exposure to the causative agent(s). In its acute form, HP symptoms may resolve within a few days after exposure is terminated. The prognosis for patients with chronic HP is less favorable, with irreversible lung damage likely (Rose, (1996)). Depending on the industry affected, HP has a variety of alternative names including: farmer's lung (from exposure to fungus and actinomycete-colonized hay), bagassosis (from inhalation of moldy pressed sugarcane), malt worker's lung (from inhalation of moldy barley), humidifier lung, compost lung and others. Antigens linked to HP include *Alternaria* species, *Aspergillus* species, *Aureobasidium pullulans*, *Bacillus subtilis*, *Bacillus cereus*, *Acremonium* (formerly *Cephalosporium*) species, *Cryptostroma corticale*, *Enterobacter agglomerans*, *Mycobacterium* species, *Penicillium* species, *Saccharopolyspora* species, and *Thermoactinomyces* species (Cormier, (1998), Schuyler, (2001), Patterson, et al., (1981), Bernstein, et al., (1995), Dutkeiwicz, et al., (1985) and Muilenberg, et al., (1993)). Non-biological molecules, including trimellitic anhydride and a variety of isocyanates that are used in coatings, resins and foams, are also associated with HP (Cormier, (1998)). Since some of these microbes are recovered from MWF at least periodically, it's reasonable to hypothesize that workers exposed to MWF bioaerosols that contain individual or combinations of the aforementioned antigens are at increased risk for HP.

In 1993, Muilenberg *et al.* (Muilenberg, et al., (1993)) investigated an HP outbreak at an automotive parts plant. Ten workers had developed HP. Muilenberg *et al.* recovered 10^6 to 10^7 gram-positive, acid fast bacteria (both characteristics of *Mycobacteria* spp.) $\cdot$ ml^{-1} MWF from the sump near the affected workers. Another sump at the same plant but away from the area where workers became ill, yielded no acid-fast bacteria, but did contain 10^7 to 10^9 gram-negative bacteria $\cdot$ ml^{-1}, 10^5 yeasts $\cdot$ ml^{-1} and 10^4 *Fusarium* $\cdot$ ml^{-1} MWF. One of the 10 HP patients had an immunological response to MWF from the fluid contaminated with the gram-positive, acid-fast bacteria. This same worker was also diagnosed as having an "atypical *Mycobacterium* infection."

In 1995, Bernstein *et al.* (Bernstein, et al., (1995)) reported on six HP patients who worked at an automotive parts manufacturing facility. All six patients tested positive for antibodies to *Pseudomonas fluorescens* antigens and at least one of the following microbes: *Aspergillus niger*, *Bacillus pumilus*, an acid-fast *Rhodococcus* species, or *Staphylococcus capitas*. In contrast, eight of nine non-exposed control subjects tested negative for all of the aforementioned antigens. Although Bernstein and his colleagues hypothesized a causative role for *P. fluorescens*, they did not pursue the Koch process of trying to demonstrate a cause and effect relationship. Antibody concentrations are conserved long after antigen exposure. Bernstein's team examined blood samples from nine non-exposed individuals (one of whom tested positive for *P. fluorescens* antibodies) but didn't test other exposed machinists. Consequently there is no way to determine what percentage of *P. fluorescens* antibody-positive machinists suffered from HP. Moreover, Bernstein *et al.* reported recovery of an organism designated *Mycobacterium chelonae* (presumed now to be *Mycobacterium immunogenum*), but otherwise ignored it in their cause and effect analysis.

Subsequently, Kreiss and Cox-Ganser (Kreiss, et al., (1997)) have implicated *Mycobacterium* species and fungi as the most likely etiologic agents for HP in the MWF environment. Shelton *et al.* (Shelton, et al., (1999)) has reported that *M. immunogenum* (Wilson, et al., (2001)) is consistently recovered from MWF proximal to workers suffering from HP. Although *M. immunogenum* can also be recovered readily from coolant systems around which no HP has been reported, it appears to be a consistent component of MWF microbial communities associated with HP diagnosis (Sondossi, et al., (1999)). However, in reporting clinical findings for six machinists with HP, Zacharisen *et al.* (Zacharisen, et al., (1998)) did not find any serum precipitins to *Mycobacterium*. Instead, six of the seven affected workers exhibited serum precipitins to *Acinetobacter lwoffii*. This is the second report of serological (antibody) response to dominant gram-negative MWF bacteria. Lewis *et al.* (Lewis, et al., (2001)) reported that 35 % of an exposed population (177 individuals) and 25% of a non-exposed, control population (60 individuals), tested positive for *M. chelonae* antibodies. Based on this example of a serological response in the absence of clinical disease, Lewis and co-workers cautioned against interpreting antibody response data without adequate control and background data. In spite of the serological response reported for organisms associated with MWF, it is well known (Colston, (1996)) that mycobacterial immunity is associated with a cell-mediated phenomenon. This has not yet been demonstrated for HP/*M. chelonae* from MWF.

Although it's possible that inhalation exposure to *Mycobacterium* species contributes to HP in the metalworking environment, it is probable that *Mycobacterium* species exposure alone is not sufficient (Shelton, et al., (1999)). It's possible that, as Shelton *et al.* (Shelton, et al., (1999)) suggest, the risk of HP increases when *Mycobacterium* antigens are co-transported with other aerosolized MWF components, such as mineral oils, amines, or glycols. Sondossi *et al.* (Sondossi, et al., (1999)) have hypothesized that *Mycobacterium* species' hydrophobicity may contribute to their preferential aerosolization relative to other MWF contaminant microbes. More research is needed to determine the antigenic effect of non-biochemicals present in MWF aerosols as well as interactions among MWF aerosol antigens.

Increased awareness does not necessarily equate with increased disease incidence. Through 1997, there had been eight disease clusters affecting 98 metalworking industry workers (Reeve, et al., (1998)). Reeve *et al.* (Reeve, et al., (1998)) reviewed medical data for approximately 19,000 employees of an automotive manufacturer. Over a three-year period, from 1994 through 1996, there were 2.3 confirmed cases of HP annually per 10,000 workers (0.023% of the worker population). Reeve *et* al. did note that at one plant, not included in the general survey, 15 workers in a population of 3,000 contracted HP over a short period. At this plant the confirmed HP incidence rate was 0.5%. The two additional disease clusters that have been reported subsequently (Shelton, et al., (1999), Zacharisen, et al., (1998)) don't impact the industry-wide morbidity rate for HP. But it is noteworthy that HP occurs in sporadic, restricted clusters. Existing epidemiological data are insufficient to either support or refute a hypothesis that HP is on the rise among machine operators. However, as Hodgson (Hodgson, (1999)) has pointed out, multidisciplinary clinical, epidemiological and immunological strategies for better understanding the risk factors associated with HP have yet to be optimized.

The preceding discussion suggests two hypotheses that need further testing: a) HP is affecting an increasing number of workers who are exposed to MWF; or b) the relative number of MWF workers contracting HP has remained constant over the years while surveillance and reporting have improved. If the first hypothesis is valid, then metalworking industry personnel exposed to bioaerosols are at some increased HP risk. The dimensions of that risk and the factors contributing to that risk need to be better elucidated. Microbial taxa proliferating in current MWF formulations are qualitatively different than in the past (Rossmoore unpublished data). Coolant chemistry can affect both overall taxonomic diversity and the species composition. It's too early to determine whether increased recovery of gram positive, acid-fast bacteria reflects their increased abundance or the authors increased interest, although other gram-positive bacteria have been isolated from MWF in large numbers since the early 1980's (HWR unpublished data). It would be premature to conclude that strategies developed to reduce the incidence of biodeterioration inadvertently increased HP risk. However, if HP incidence is truly on the rise, current microbial contamination control strategies need to be reevaluated.

If the apparent increased HP incidence and *Mycobacterium* recovery rates are determined to be results of improved surveillance, the required research focus is likely to be different. Improved understanding of HP's etiology and patient susceptibility should illuminate the path towards improved practices for reducing HP risk. At this point it is not clear whether the historic incidence of chronic respiratory illness amongst MWF workers was documented adequately or if occurrence frequency has actually increased. Similarly, since focused testing for *Mycobacteria* followed Shelton *et al.* (Shelton, et al., (1999)) postulated link between HP and *Mycobacteria* recovery, the authors cannot say for certain whether *Mycobacteria* prevalence or their awareness has increased.

In summary, concern about HP has increased over the past decade. Circumstantial evidence suggests some role for *Mycobacterium* species. In at least one outbreak, *P. fluorescens*, and another, *A. lwoffii* were implicated, but the evidence for an HP role for either of these organisms is considerably weaker. However, there is as of yet insufficient information to make any unequivocal statements regarding the disease risk or the convergence of variables that result in an individual worker contracting HP.

Endotoxin Exposure

The term *endotoxin* refers to the lipopolysaccharide (LPS) component of gram-negative bacteria outer membranes. Todor (Todar, (1998)) provides an excellent discussion of LPS form and function. An LPS layer forms the outer surface of all gram-negative bacteria. Three moieties comprise the LPS molecule. An O-antigen outer region is linked to a core polysaccharide that is bound to the lipid component, called Lipid A. Lipid A appears to be LPS' toxigenic component. Purified Lipid A induces the body's immune system molecules that result in inflammation and fever (Raetz, (2000)).

Endotoxin inhalation induces systemic inflammatory response (Michel, et al., (1995)). Otherwise healthy individuals exposed to airborne LPS are likely to develop fever as well as bronchoconstriction (sick building syndrome) (Michel, (1999)). Asthma sufferers exposed to airborne LPS share these symptoms. Additionally their total lung capacity decreases (Michel, et al., (1989). Symptoms may occur upon inhalation or be delayed for several hours. Michel and coworkers investigated the dose response of nine healthy individuals to endotoxin inhalation (Michel, et al., (1997)). Their test subjects inhaled 0.5, 5 and 50 µg LPS. The lowest dose, 0.5 µg LPS induced changes in blood chemistry. Castellan, et al. (Castellan, et al., (1987)) computed the endotoxin no observable effect level (NOEL) to be 9 ng $\cdot$ m^{-3}, nearly three orders of magnitude below the lowest dose tested by Michel, *et al*.

Live gram-negative bacteria lose small amounts of LPS to their surroundings throughout their lives. However, major endotoxin release accompanies cell death (Laitinen, et al., (1999)).

Until recently (ASTM, (2001)) airborne endotoxin measurements could not be validated due to the absence of generally accepted sampling methodology with documented precision and accuracy terms. Having adopted a standard sampling practice, ASTM Subcommittee E34.50, Health and Safety of Metalworking Fluids, is now developing a guideline for endotoxin testing. A round-robin conducted in 1997 (Chun, et al., (2000)) demonstrated that for a given sample, test results varied significantly, depending on the test kit used. Variability due to analysis performed by different laboratories using the same type of test kit was also significant. Until test variability can be reduced, airborne endotoxin data should be treated as semi-quantitative. Chatigny, *et al.* (Chatigny, et al., (1989)) detail the factors that should be considered when developing a bioaerosol monitoring plan.

Lewis, *et al.* (Laitinen, et al., (1999)) reported concentrations of endotoxin ranging from non-detected (<0.005 EU $\cdot$ ml^{-1}) to > 1 x 10^6 EU $\cdot$ ml^{-1} in MWF samples and 0.5 EU $\cdot$ m^{-3} to 2.5 EU $\cdot$ m^{-3} in MWF system aerosols (one EU, or *endotoxin unit* equals 1.0 ng endotoxin). By comparison, Laitinen, *et al*. (Chatigny, et al., (1989)) surveyed 18 metalworking facilities and reported bulk fluid endotoxin concentrations ranging from 0.3 EU $\cdot$ ml^{-1} to 2.5 x 10^5 EU $\cdot$ ml^{-1} and airborne endotoxin concentrations ranging from <0.4 EU $\cdot$ m^{-3} to 1.4 x 10^3 EU $\cdot$ m^{-3}. Many of the air sample endotoxin concentrations were above the NOEL computed by Castellan, *et al*. (Castellan, et al., (1987). Laitinen, *et al.* computed the correlation coefficient for bulk MWF and aerosol endotoxin concentrations at one test site. The correlation coefficient, r, for 6 data pairs (n) was 0.60. Where n = 6, $r_{crit.,\ 0.05}$ = 0.811. This means that, perhaps counter-intuitively, at the 95% confidence level, the relationship between bulk fluid and aerosol endotoxin concentration was not statistically significant. Robbins, *et al.* (Robins, et al., (1997)) have reported similar results. This weak statistical relationship does not negate the prudent logic of minimizing bioburdens in the bulk fluid as part of a strategy to minimize airborne endotoxin exposure. Mattsby-Baltzer, *et al.* (Mattsby-Baltzer, et al., (1989)) report, the bioburden in MWF aerosols decreases logarithmically with distance from the source. This means that bioaerosol data must be interpreted in context with both source MWF bioburden data and the distance between the sampling point and mist source. More data are also needed to describe the relationship between qualitative and quantitative bioaerosol properties and mist generating activities. Investigating *A. fumigatus* aerospore generation associated with municipal sewage sludge composting, one of the authors (Passman, (1983)) found that increased concentrations of fungal spores were restricted both spatially and temporally around the most dust generating composting operations – pile turning and compost sifting (screening).

As noted earlier, major endotoxin release generally accompanies gram-negative bacteria lysis. Building on work reported by Brown and his colleagues (Browne, et al., (1970), (1976), (1978)) who had investigated the effect of the formaldehyde-releasing, chemotherapeutic agents, taurolin and noxythiolin, Douglas (Douglas, et al., (1990)) found that formaldehyde condensate microbicides and glutaraldehyde neutralized endotoxins. In contrast, non-formaldehyde condensate microbicides did not. In Douglas' research, at 1000 ppm (active ingredient – a.i.) each of the formaldehyde-condensate products neutralized up to 10 ng endotoxin $\cdot$ ml^{-1} of pH 7.0 buffered water. *Escherichia coli* contains approximately 32 fg endotoxin$\cdot$ $Cell^{-1}$ (1 fg = 10^{-15}g). *Pseudomonas* spp. and other Gram negative bacteria the commonly proliferate in MWF contain 300 to 1,000 fg endotoxin$\cdot$ $Cell^{-1}$ (Jay, (1989)). Consequently the endotoxin load associated with a 10^7 CFU $\cdot$ ml^{-1} bioburden can range from 0.3 to 10 x 10^3 ng endotoxin $\cdot$ ml^{-1}. This information has two important implications. First, the range of endotoxin content amongst MWF contaminating bacteria species explains the relatively weak correlation between total viable counts and endotoxin concentration. Second, it shows that although formaldehyde-condensate microbicides probably neutralize some endotoxin in MWF, the allowable microbicide dosages are insufficient to neutralize more than a fraction of the endotoxin present.

RESEARCH NEEDS

Research conducted over the past decade suggests that the chronic toxic and allergenic effects of MWF microbes constitute a significant risk to workers routinely exposed to MWF aerosols. There is still a need for more comprehensive data collection from which to build accurate risk models.

Traditionally, the principal means of quantifying airborne microbes were viable count methods. As with any test method, viable count procedures have both advantages and limitations relative to alternatives. On one hand, cells that proliferate on growth media are unequivocally viable. Moreover, colonies provide cells that can be isolated and investigated further. Within the context of currently available alternative technologies, viable count detection limits are typically lower (more sensitive) than those of either microscope direct-count observations, or methods that quantify either a cell constituent or byproduct. However, some cells that are viable within the system that is being sampled, may not elaborate into colonies on the enumeration media. For a variety of reasons, beyond the scope of this article to discuss, viable count data typically underestimate the number of viable cells in the system (Roszak, et al., (1987)). To describe an MWF's system adequately, it may be advantageous to augment viable count data with additional data. There are a number of methods that are used routinely in medical and public health microbiology applications (for example see White, et al., (1997), Stahl, (1997), Burlage, (1997)). Additionally, a variety of new methods show promise (van der Gast, et al., (2001)). Chemical methods that test for specific cell constituents or other biomarkers may need to be refined or otherwise adapted for MWF applications. For any method, including those currently used routinely, standardization and variability control are essential prerequisites for any method intended for general surveillance and monitoring purposes. To be of value, a test method must meet a number of criteria (ASTM, (2001)). First, it must detect and measure the target parameter with acceptable accuracy (e.g., reliably detecting the microbe or biomarker it's intended to measure) and precision (e.g. providing acceptable variation among replicate analyses of a single sample). To find broad industrial application, a non-conventional test method should be easy to perform and should provide data nearly instantaneously (e.g., comparable to pH, coolant concentration or other chemical parameter data).

It's a given that the qualitative and quantitative composition of bioaerosols will reflect the microbial community composition in the bulk MWF from which the aerosols are generated. Sterilization remains impractical, but a better understanding of MWF microbial ecology might enable fluid management stakeholders to develop strategies that best balance the sometimes conflicting objectives of maximum fluid life, minimum health risk and maximum production rates.

Though essential, detecting microbes in bulk fluids and their associated aerosols is insufficient to better understand the factors contributing to individual worker's disease susceptibility. There are many studies documenting the presence of gram-negative bacteria in MWF. There is a growing literature on *Mycobacterium* and endotoxin distribution in the metalworking environment. Studies performed within the metalworking industry as well as other industrial settings consistently demonstrate that only a small fraction of exposed individuals become ill. The industry needs more multidisciplinary survey work in which microbiological, immunological and epidemiological data are collected and analyzed holistically. Such surveys will enable the authors industry to chart the path towards improved industrial hygiene practice.

CONCLUSIONS

Despite rare clusters of infectious disease, there's little evidence that MWF microbes represent an incremental risk factor for the transmission of communicable diseases. However, research conducted over the past twenty years strongly supports the hypothesis that airborne biomolecules do represent an increased risk for chronic diseases such as HP, acute respiratory distress and general malaise. Although the detailed relationships between biomolecule exposure, non-biological mist exposure and other variables (lifestyles, genetics, etc.) remain to be elucidated, current knowledge is sufficient to warrant increased attention to bioaerosol exposure. The NIOSH recommendation to reduce exposure limits to MWF aerosols to 0.5 $mg \cdot M^{-3}$ (total particulate mass) (Patterson, et al., (1981)) should also reduce bioaerosol exposure risk. However, unlike chemical constituents, the dose-response relationship between exposure and disease for microbes and cell constituents is not well understood. Threshold concentrations for allergens to cause allergies aren't defined, nor are minimum infectious doses for Legionellosis. Improved control of diseases due to bioaerosol exposure may require qualitative changes in mist control in addition to the volume reduction initiatives currently in progress.

Over the past half-century, the metalworking industry has made considerable progress towards MWF biodeterioration control. The path ahead calls for better bioaerosol control and a better understanding between MWF chemistry and microbiology.

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