

Evaluation of an Unpleasant Odor at an Aircraft Ejection Seat Manufacturer

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The employer is required to post a copy of this report for 30 days at or near the workplace(s) of affected employees. The employer must take steps to ensure that the posted report is not altered, defaced, or covered by other material.

The cover photo is a close-up image of sorbent tubes, which are used by the HHE Program to measure airborne exposures. This photo is an artistic representation that may not be related to this Health Hazard Evaluation. Photo by NIOSH.

Highlights of this Evaluation

The Health Hazard Evaluation Program received a request from the employer at a facility where aircraft ejection seats are made. Employees were concerned about a lingering odor that was first noticed in April 2013 and continued to be a problem. The odor remained in materials and goods after they left the facility.

What We Did

- We visited the facility in May 2014.
- We interviewed employees about their work and health.
- We examined the ventilation systems and measured ventilation airflow and comfort parameters in some areas.
- We took air samples to analyze for metalworking fluid, volatile organic compounds, bacteria, and endotoxin.
- We took bulk samples of metalworking fluid from machining reservoirs to analyze for microbial diversity and endotoxin.
- We looked at work practices and conditions.

What We Found

- It is likely that 2-methoxy-3,5-dimethylpyrazine (3,5-MDMP, CAS 92508-08-2) is causing the odor in the facility. This chemical may be produced by some of the bacteria found in the metalworking fluid.
- The most common work-related symptoms reported by employees were fatigue, headache, eye irritation, and runny nose or congestion, and cough.
- Ventilation systems and water diversion systems were not well-maintained. Condensation pans in air handling units had standing water and debris. Gutters were rusted through, causing them to be ineffective.
- Air recirculation rates were high in some departments indicating poor general ventilation.
- Levels of metalworking fluid exposures in air reached about half of the NIOSH recommended limit.
- Genetic material from unexpected types of bacteria was found in the metalworking fluid and in the air. Endotoxin concentrations were high in the metalworking fluid.

We investigated an odor at an aircraft ejection seat manufacturer. We observed work processes and collected air samples for contaminants. Poor ventilation and poor bacterial control in metalworking fluid reservoirs could be contributing to the odor. We found 2-methoxy-3,5-dimethylpyrazine (3,5-MDMP) in the air and in the metalworking fluid. It is likely that this chemical, which can be produced by bacteria, is causing the odor. We recommend decontaminating the metalworking machines, improving metalworking fluid maintenance, and improving ventilation.

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- Employees who wore gloves used latex gloves when working with metalworking fluid.
 - Metalworking fluid was not well contained around the stock metal cutting machine.

What the Employer Can Do

- Repair and maintain building ventilation and water diversion systems. Modify ventilation systems that recirculate indoor air to introduce outdoor air.
- Isolate the computer numerical control department from the rest of the facility with barrier walls or relocate it to a separate room.
- Install a dedicated ventilation system in the computer numerical control department.
- Implement maintenance programs for managing metalworking fluid in the machines and recycling system.
- Develop and carry out a plan for metalworking machine decontamination.
- Improve metalworking fluid containment at the stock metal cutting machine.
- Provide non-latex gloves to employees to prevent the development of latex allergy.

What Employees Can Do

- Wear non-latex gloves when handling goods or doing tasks that require contact with metalworking fluids.
- Wash your skin with soap and water if you get metalworking fluid on your skin and after removing gloves.
- Wear heavy non-latex gloves to protect your hands from cuts when handling metal pieces.

Abbreviations

µg/mL	microgram per milliliter
µL	microliter
µm	micrometer
BLAST	Basic Local Alignment Search Tool
C	Celsius
CFR	Code of Federal Regulations
CFU/mL	Colony forming units per milliliter
CLIA	Clinical Laboratory Improvement Amendments
CNC	Computer numerical control
CO ₂	Carbon dioxide
DNA	Deoxyribonucleic acid
EU	Endotoxin units
EU/mL	Endotoxin units per milliliter
EU/m ³	Endotoxin units per cubic meter
F	Fahrenheit
FDA	Food and Drug Administration
ITS	Internal Transcribed Spacer
3,5-MDMP	2-methoxy-3,5-dimethylpyrazine (CAS 92508-08-2)
3,6-MDMP	2-methoxy-3,6-dimethylpyrazine (CAS 19846-22-1)
mg/m ³	Milligrams per cubic meter
mL	Milliliter
NCBI	National Center for Biotechnology Information
NIOSH	National Institute for Occupational Safety and Health
OEL	Occupational exposure limit
OSHA	Occupational Safety and Health Administration
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
PEL	Permissible exposure limit
rDNA	Ribosomal deoxyribonucleic acid
REL	Recommended exposure limit
spp.	Species
TWA	Time-weighted average
VOC	Volatile organic compound

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Introduction

The Health Hazard Evaluation Program received a request from the employer at a facility where aircraft ejection seats are made. Employees were concerned about a lingering odor in the facility that they first noticed in April 2013 and health symptoms thought to be related to it. The odor reportedly permeated and remained in materials and goods of employees after they left the facility. We evaluated indoor environmental quality; sampled air for volatile organic compounds (VOCs), metalworking fluid, endotoxin, and microbial contaminants; analyzed bulk metalworking fluid for microbial diversity and endotoxin; and interviewed employees about their work and health during our site visit in May 2014.

The single-story, 35,000-square-foot facility was built in the 1970s for use as a roller skating rink. It housed an electronics manufacturing company during the 1990s. The ejection seat manufacturing company has operated in the building since 2001. The company employed 15–20 people from 2001 to 2005. The company increased production and the number of workers in 2005. It employed 134 workers at the time of our evaluation and operated 24 hours per day, 5 days per week; employees worked 8-hour shifts. Most employees worked between the hours of 7:30 a.m. and 4:00 p.m. The company added 17,000 square feet of production space onto the original structure during the summer of 2013. This additional space was unoccupied at the time of our site visit. Dropped tile ceilings throughout the building varied in height from about 8 feet to 20 feet. Exterior walls consisted of two layers of steel with insulating foam between them. In office areas, drywall was attached to the steel walls by wooden studs. Most administrative offices were carpeted until early 2014. All carpet in the facility had been removed by the time of our site visit.

The production process began with cutting and machining stock aluminum into smaller parts in enclosed metalworking machines. The facility had four fully enclosed computer numerical control (CNC) metalworking machines (three vertical machines and one horizontal machine) and one stock metal cutting machine. One machine, DHP4000, was equipped with a mist collector. The company used semi-synthetic water-miscible cutting oils to lubricate, cool, and remove chips during machining and cutting. The aluminum parts were heat treated after machining. Rough edges were smoothed in a tumbler with abrasive powders. Fabric treated with flame retardant was cut with a laser cutting machine equipped with local exhaust ventilation. Sewers used machines to sew fabric for the seats. Aluminum seat parts were painted in a down-draft paint booth. Ejection seats and component parts were inspected at multiple points during the production process. Seats were hand-assembled in the crashworthy department. Employees repaired flawed parts or seats in component repair. Parachutes for the ejection seats were packed in the paraloft department.

Odors

Odors are organic or inorganic compounds that trigger the sense of smell and can be perceived as pleasant or unpleasant. Some, but not all, compounds that cause odors can be health hazards. Some odorless substances are very hazardous to health, for example, carbon monoxide. The presence of odors can cause some people to suspect harmful exposures. Odors in a building are not always a sign that occupants are overexposed to chemicals,

however. Some chemicals or compounds have a very low odor threshold, which means people can smell them even at very low levels.

The odor in this building was initially described as a musty smell by some and a chemical smell by others. The odor reportedly absorbed into organic material, paper, cardboard, leather, clothing, and hair. It also reportedly lingered for weeks on objects after they left the facility.

Metalworking Fluids

Metalworking fluids are complex mixtures used to cool, lubricate, and remove metal chips from tools and parts during machining of metal stock. Metalworking fluids often contain other substances, including biocides, corrosion inhibitors, metal fines, tramp oils, and biological contaminants [NIOSH 1998; Burton et al. 2012]. Inhalation of metalworking fluid aerosols may irritate the throat, nose, and lungs and has been associated with chronic bronchitis, asthma, hypersensitivity pneumonitis, and worsening of pre-existing respiratory problems [Burton et al. 2012]. The National Institute for Occupational Safety and Health (NIOSH) recommends limiting exposures to metalworking fluid aerosols to 0.4 milligrams per cubic meter (mg/m^3) for the thoracic particulate mass, as a time-weighted average (TWA) concentration, for up to 10 hours per day during a 40-hour workweek [NIOSH 1998]. Detailed information on occupational exposure limits (OELs) is presented in Appendix B.

Skin contact with metalworking fluids may cause allergic contact dermatitis or irritant contact dermatitis, depending on the chemical composition, additives and contaminants, type of metal being machined, and the exposed individual's tendency for developing allergies [WISHA 2001]. Synthetic, semisynthetic, and soluble oil metalworking fluids are diluted with water, so excess bacteria may grow if metalworking fluid is not properly monitored and maintained. The Health and Safety Executive in the United Kingdom states that well-maintained metalworking fluids should have bacterial concentrations below 10^3 colony forming units per milliliter (CFU/mL) of fluid [HSE 2006]. Concentrations between 10^3 and 10^6 CFU/mL indicate reasonable control, and concentrations greater than 10^6 CFU/mL indicate poor control [HSE 2006]. The outer cell walls of Gram-negative bacteria may release lipopolysaccharide compounds called endotoxins when the bacteria die or multiply. Endotoxin is believed to cause adverse respiratory effects such as chronic bronchitis and asthma. Endotoxin concentrations are reported in endotoxin units (EU) per cubic meter (EU/m^3). In 2010, the Dutch Expert Committee on Occupational Safety recommended a health-based OEL for airborne endotoxin of $90 \text{ EU}/\text{m}^3$ [DECOS 2010]. No OELs for endotoxin have been established in the United States.

Methods

Our primary objectives were to evaluate the indoor environmental quality in the facility and identify potential sources of the offending odor. Our work included: (1) evaluation of the physical building and ventilation systems, (2) document review, (3) air sampling for metalworking fluid, endotoxin, microbial diversity, and VOCs, (4) microbial analysis of the bulk metalworking fluid, (5) observations of work practices, and (6) confidential medical interviews with employees.

Building and Ventilation Evaluation

We inspected the air-handling units, exhaust fans, gutters, downspouts on the roof, and the plenum above the dropped ceiling tiles. We looked for evidence of past or current water damage, water incursion, or fungi inside and outside of the building. We measured supply and exhaust airflow rates in the painting preparation and paint booth area with a TSI Model 8371 AccuBalance™ Air Capture Hood. We measured carbon dioxide with a TSI Model 8554 Q-TRAK Plus™ Indoor Air Quality Monitor.

Document Review

We reviewed metalworking fluid maintenance records, industrial hygiene reports, and metalworking fluid safety data sheets. We reviewed environmental sampling results and consultants' reports regarding the odor and mold sampling. The consultants had identified mold growth in the conference room and heat treat area, which the company remediated before our site visit. The company hired a laboratory to analyze air filters for mycotoxins. They also hired a laboratory to analyze biological samples from employees and their family members for mycotoxins. We did not evaluate these mycotoxin analytical reports for reasons described in Appendix C. We reviewed maintenance and recycling best-practice protocols provided by the metalworking fluid manufacturer. We also reviewed company policies regarding metalworking fluid use and maintenance.

Air Sampling for Metalworking Fluid, Endotoxin, and Volatile Organic Compounds

We collected four full-shift, personal air samples to analyze for thoracic particle mass and extracted metalworking fluid mist in the CNC department. Thoracic sized particles have an aerodynamic diameter of 10 micrometers or less. These particles reach deeper into the lungs than particles larger than 10 micrometers. Thoracic particles sampled in this manner include all metal dusts, other particulates, and aerosols in addition to the aerosolized metalworking fluid itself. We noted the type of engineering controls and the employees' locations and shift tasks. We collected eight area air samples for endotoxin with use of endotoxin-free sampling media. We collected eight area air samples with thermal desorption tubes, a qualitative sampling method to detect and identify VOCs. Details of the sampling and analysis methods are in Appendix A.

Bulk Metalworking Fluid Sampling for Viable Bacteria and Endotoxin

We collected three bulk samples of metalworking fluid, one each from the supply reservoir of three CNC machines. A commercial laboratory analyzed the bulk metalworking samples for bacteria, mycobacteria, and fungi using viable culture methods. When organisms grew in the cultures, the laboratory tested further to determine the species. Details on the culture media, incubation temperature, and incubation times used for the cultures are in Appendix A. The bulk samples from the machine reservoirs were also analyzed for endotoxin, which

is a component of the cell wall of Gram-negative bacteria. Additionally, we analyzed the air space in the jars containing bulk metalworking fluid for VOCs.

Microbiological Diversity

We sent the three bulk metalworking fluid samples, each from a different CNC machine, and the 12 air samples for microbial diversity analysis. First, we amplified microbiological deoxyribonucleic acid (DNA) present in the air samples and in bulk metalworking fluids. Then we sequenced the DNA to identify which varieties of bacteria were present in these samples. We clustered the sequence data into operational taxonomic units (OTUs), a flexible term that refers to taxonomic placement (e.g., order, genus, or species) when analyzing DNA sequence data. Using sequences representative of each OTU, we searched for them within a database operated by the National Center for Biotechnology Information (NCBI) [Schloss et al. 2009]. This process resulted in a list of types of bacteria found in the air and bulk metalworking fluid. Details of the sampling and microbiological diversity analysis are in Appendix A.

Observations

We observed work practices and procedures in the facility, particularly in the CNC department. We noted handling practices and use of personal protective equipment.

Confidential Medical Interviews

We randomly selected 60/134 (45%) employees to participate in individual, semi-structured, confidential medical interviews. Employees who were not randomly selected were informed by the company via email that they could also be interviewed if they wished. During the interviews, we discussed their work, pertinent medical history, symptoms, and health concerns they related to the building.

Results and Discussion

Building and Ventilation Evaluation

Ducted supply airflow was provided to the building through 10 rooftop-mounted air-handling units. Four units were located on the west half of the roof, and six units were on the east half of the roof. We inspected three of the air-handling units. We observed standing water under the air intake of one of the units (Figure 1). Although it had rained in the previous 24 hours, debris in the water, biological growth, and discoloration of the roof under the intake suggested that water had been pooled at this location for an extended period of time. We also observed standing water and an extensive amount of rust in the condensate drain pan of another air-handling unit. The condensate drain pan for a different air-handling unit was damp and contained rotted plant debris. The drain line for both of these drain pans appeared to be partially or completely plugged. Pooling of water under the air-handling unit intakes or in condensate drain pans for prolonged periods may increase the likelihood of fungal or bacterial growth in the water. A filter for the air-handling unit serving the main offices on

the west side of the building had evidence of water damage, most likely due to condensation from the air-conditioning coils that did not drain properly into the drain pan.

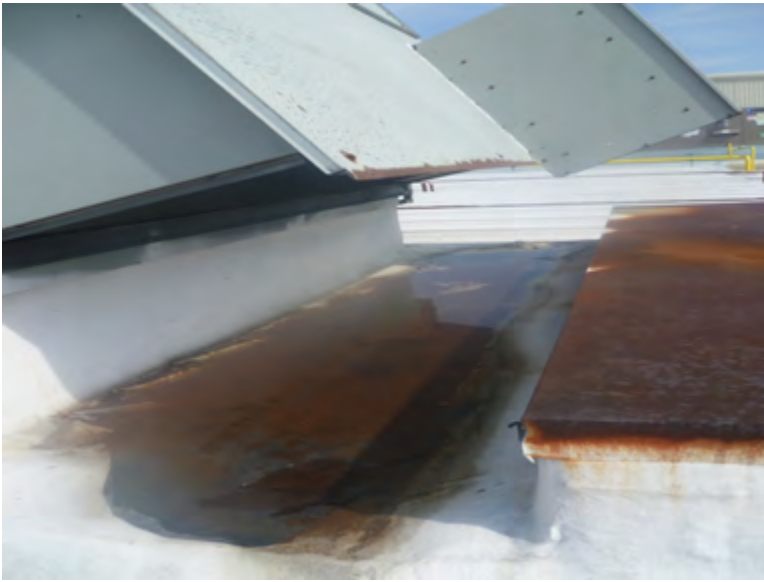


Figure 1. Standing water under intake of an air-handling unit on the roof. Photo by NIOSH.

In October 2013, the company had two large constant-volume exhaust fan units installed across the center of the roof. Exhaust air was not ducted to these units but was exhausted from the plenum space above the ceiling tiles. Some ceiling tiles had been replaced with plastic honeycomb tiles to provide a pathway for entry of air into the plenum space. However, ceiling tiles were missing at several locations, thus allowing unplanned pathways for air into the plenum space. The exhaust air from the fans was released through the top of the units, about 3 feet above the roof surface. Because of the low release point and proximity of the air-handling units on the roof, exhaust air could be recaptured by the outdoor air intakes, depending on prevailing wind speed and direction. The company did not have information on the supply rate of the air-handling units or exhaust rate of the fans.

We observed some sections of roof gutters and downspouts that had completely rusted through, making them ineffective for collecting and properly diverting water away from the building (Figure 2). The gutters and downspouts on the west side of the building drained directly to the ground near the foundation. Because grading of the ground on the west side of the building sloped toward the building, water draining from the roof or down the slope did not effectively drain away from the building and sometimes pooled near the foundation. Employees reported at least one incident of substantial water intrusion into the building through the lower part of the west-side walls during the previous winter. Employees also reported regular water incursion into the building at other locations. We saw evidence of water damage to walls and ceiling tiles at several locations, indicating leaks through the roof. We also saw several locations where the roof had been patched to repair leaks.



Figure 2. Gutter with a large hole rusted through. Photo by NIOSH.

Company personnel had found fungal contamination on some walls of the building. In June 2013, the company hired a contractor to have fungal contamination remediated in the conference room and in the heat-treat department. In September 2013, the same contractor remediated fungal contamination in the building entry area and removed the vestibule from the front of the building. According to the employer, the contractor's remediation involved (1) removing visible fungus from the walls and removing drywall when fungi was behind it, (2) spraying affected areas with a disinfectant to remove fungal colonies and dispersal structures such as spores, and (3) applying a fungal-resistant clear coat after treated areas were dry.

The company started using carbon-impregnated air filters in the air-handling units in April 2013 to help reduce the odor. Maintenance staff reportedly replaced the filters every 3 months. The company also installed hydrogen peroxide ionizers in the ventilation ducts and throughout the facility. Employees reported no reduction in odors after installation of the air filters and ionizers.

Outdoor temperatures were 75.2–80.5 degrees Fahrenheit (F), and the average relative humidity outdoors was 40% on the days of our evaluation. Indoor temperatures were 69.3°F–77.5°F, and the indoor relative humidity levels were 36%–56%. Carbon dioxide (CO₂) concentrations ranged from 1,107–1,820 ppm in the paraloft, component repair, document control, and inspection departments. We found that each of these departments was ventilated with an independent air-handling unit, which drew make-up air from inside the building rather than incorporating outdoor air. Recirculating only indoor air can cause an increase in the relative concentration of indoor air contaminants, such as the VOCs used or created during the manufacturing process and CO₂, which is sometimes used as a marker of ventilation effectiveness because it is a natural product of human respiration.

Measurements of supply and exhaust airflow in the paint-preparation room indicated that the air supply was substantially greater than its air exhaust. Because of this supply-to-exhaust

imbalance, the paint-preparation room was under positive pressure relative to surrounding areas, increasing the likelihood that solvent and paint vapors would move to the surrounding areas.

Air Sampling for VOCs

Qualitative sampling revealed the presence of styrene and methyl ethyl ketone in all sampling locations. Although the sampling methods were not quantitative, we found that indoor concentrations were relatively higher than outdoor concentrations. These findings were not surprising. Methyl ethyl ketone was used to clean and remove excess oil from machined parts in multiple departments. Styrene was an ingredient of the abrasive product used in the tumbler in the heat-treat area. Other VOCs found on the sampling media include benzaldehyde, methyl vinyl ketone, dimethylpyrazine, phenol, decamethylcyclopentasiloxane, phenoxyacetone, ethyl hexanol, acetone, and isopropanol.

A consultant, who reported the findings of air sample testing in April 2014, identified the odor as 2-methoxy-3,6-dimethylpyrazine (3,6-MDMP, CAS 19846-22-1). Methoxypyrazines are known to have low odor thresholds and can have a variety of odors, including musty, earthy, moldy, acrid, and “chemical” [Contis et al. 1998]. In our area air samples and headspace of bulk samples of metalworking fluid, we identified 2-methoxy-3,5-dimethylpyrazine (3,5-MDMP, CAS 92508-08-2) as the primary isomer. We also identified 3,6-MDMP, but at much lower levels. No research studies have reported on the toxicity of 3,5-MDMP. However, like other methoxypyrazines, this isomer has a very low odor threshold and its odor has been characterized as disagreeable and musty. The isomer has also been associated with odiferous metalworking fluids [Mottram et al. 1984; Muller and Rappert 2010].

On the basis of these findings and odor characteristics, it is likely that 3,5-MDMP is causing the unpleasant, persistent odor in the facility. Additionally, NIOSH investigators who visited the worksite noted that the odor of the 3,5-MDMP standard used in the NIOSH laboratory smelled remarkably similar to the facility odor. A variety of bacteria produce dialkyl methoxypyrazines, including 3,5-MDMP. Some bacteria have been demonstrated to produce 3,5-MDMP specifically, including *Rhizobium excellensis*, *Serratia odorifera*, and *Chondromyces crocatus* [Chatonnet et al. 2010]. These organisms were first identified as the source of 3,5-MDMP in tainted wine corks, with *Rhizobium excellensis* believed to be the main source [Chatonnet et al. 2010]. *Rhizobium* bacterial species generally are found in soil. Several bacteria within the phyla Proteobacteria, Bacteroides, Firmicutes, and Actinobacteria are known to produce an array of pyrazines, including 3,5-MDMP. For additional information, see Appendix D for various bacterial species that produce 3,5-MDMP and related methoxypyrazines.

Air Sampling for Metalworking Fluid

Personal thoracic metalworking fluid exposures ranged from 0.08 to 0.20 mg/m³ and did not exceed the NIOSH recommended exposure limit (REL) for thoracic metalworking fluid mist of 0.4 mg/m³. These concentrations equate to 20%–50% of the NIOSH REL. All of the sample concentrations were above the minimum detectable concentration. However, three of the four sample concentrations were between the minimum detectable concentration and

minimum quantifiable concentration, which means there is more uncertainty associated with these values.

Our full-shift, area air samples for metalworking fluid mist showed concentrations ranging from 0.27–0.74 mg/m³ in the CNC area and 0.22 mg/m³ in a room adjacent to the CNC area, where the CNC programmer worked. These samples demonstrated that metalworking fluid migration is not well-controlled and mist moves from production areas into non-production areas.

Microbials in Bulk Metalworking Fluid

The total bacterial count was 1.7×10^7 CFU/mL to 4.1×10^7 CFU/mL in the three bulk samples of metalworking fluid. Several bacteria were isolated in the metalworking fluid samples. The microbiology laboratory only reported the three bacteria with the highest counts for each sample. Across the three samples, the primary bacteria isolated were *Cupriavidus metallidurans*, *Corynebacterium* species (spp.), *Brevundimonas diminuta*, and *Alcaligenes faecalis*.

Bacteria can flourish within metalworking fluid systems, especially within water-miscible metalworking fluids. The sample concentrations we measured were in the Health and Safety Executive category of poor control of bacterial contamination [HSE 2006]. According to the metalworking fluid manufacturer, this system of metalworking fluid was expected to develop a culture of one specific bacterial species, *Pseudomonas oleovorans* [Kuenzi et al. 2014]. However, this bacterium was not one of the top three species found in our evaluation. This indicates poor control of the microbiota of the metalworking fluid system.

Mycobacterium species organisms were not detected in any of the samples. *Fusarium* species of fungi, common in soil, were detected in two samples at low concentrations (5 CFU/mL). The laboratory found no fungus in the third metalworking fluid sample.

Microbial Diversity in Air and Bulk Metalworking Fluid

We found relatively low levels of fungal DNA in the air and bulk metalworking fluid samples. We selected bacteria for further sequencing analysis on the basis of the preliminary results and low yield of fungal DNA from these samples.

We clustered the DNA sequences into 152 individual OTUs and identified 148 unique bacterial OTUs. Within the bacterial OTU dataset, 76% were $\geq 97\%$ identical to reference 16S bacterial sequences in the NCBI database. Additionally, we placed OTUs in Plantae ($n = 4$). We identified a large number of OTUs in metalworking fluid control samples, field blanks, and reagent controls because of bacterial contaminants from the environment, supplies, or reagents used throughout the sample collection and extraction processes. This is a common limitation associated with these genomic approaches to assessing bacterial diversity and indicates why it is important to utilize appropriate controls during extraction. All OTUs identified from the controls were removed and not included in the analysis of air and metalworking fluid samples.

Overall, sequencing analysis identified 23 clones in the bulk used metalworking fluid.

Samples from DHP4000 (n = 8) and DMV5025 (n = 10) CNC machines had the greatest number of clones; in contrast, samples from HP9 (n = 2) and HP11 (n = 3) CNC machines had the fewest. The clones were derived from the bacterial phyla Proteobacteria (39%), Firmicutes (39%), and Actinobacteria (22%). In the air sample sequence analysis, we identified a total of 48 clones, and they were derived from the bacterial phyla Proteobacteria (33%), Firmicutes (29%), Actinobacteria (25%), and Bacteroidetes (13%). Samples from the outdoors (n = 17), the crashworthy area (n = 5), and the front office (n = 8) had the greatest number of clones, compared to those identified in the 9 other air samples (range: 0–4 clones).

The preliminary Sanger sequencing analysis demonstrated that bacteria were present in both air and metalworking fluid samples. The diversity of bacteria appeared to be broader in air samples, in which greater numbers of clones and bacterial phyla were identified than in metalworking fluid samples. Compared with the culturable bacteria datasets reported above, Sanger sequencing data identified *Brevundimonas diminuta* in metalworking fluid samples but not *Cupriavidus metallidurans*; however, in the metalworking fluid sequencing analysis, we identified a variety of other betaproteobacteria in the same family (*Burkholderiaceae*). The Sanger sequencing analysis identified other culturable bacteria, including *Corynebacterium* spp. and *Alcaligenes faecalis*, but these were also present in several internal controls included in the analysis so they may not have originated from the workplace samples. We did not identify sequences from the primary bacteria species expected in the metalworking fluid, *Pseudomonas oleovorans*. This, along with the results from the cultured samples, suggests that the metalworking fluid was not populated with *Pseudomonas oleovorans*, the bacterium expected by the manufacturer.

Review of the scientific literature suggests that many bacterial orders produce odor-causing pyrazines (Appendix D). Pyrazine production by these organisms can vary, depending on the culture conditions. The Proteobacteria species known to produce 3,5-MDMP, including *Rhizobium excellensis* (Rhizobiales order), *Serratia odorifera* (Enterobacteriales order), and *Chondromyces crocatus* (Myxococcales order), were all identified in tainted wine corks [Chatonnet et al. 2010]. We did not identify these specific organisms in the Sanger sequencing analysis. However, we found sequences derived from the *Proteobacteria* order, *Rhizobiales*, as well as orders thought to produce other types of pyrazines (*Bacteriales*, *Actiniomycetales*, and *Sphingobacteriales*), in both air and metalworking fluid samples.

Biofilms are aggregates of microorganisms, including bacteria. Frequently, the bacteria produce a protective extracellular polymeric substance and adhere to each other or surfaces. In this case, biofilms can adhere to components of the metalworking fluid systems including the CNC interior, metalworking fluid reservoirs, recycling system containers, and even the surfaces of the machinery. Biofilms are very complex and can be difficult to remove once they are established [Lucchesi et al. 2012; Trafny 2013]. Bacteria within the biofilm may or may not be represented in bulk fluid samples. Biofilms can provide inoculating bacteria, which repopulate fresh metalworking fluid after cleaning or maintenance [Trafny 2013].

Endotoxin in Air and Bulk Metalworking Fluid

We took eight area air samples for endotoxin throughout the facility and outdoors.

Endotoxin is an indicator of bacterial contamination of the metalworking fluid. Endotoxin concentrations, shown in Table 1, ranged from below the limit of detection to 21 EU/m³. Airborne endotoxin was highest in the main office area and in the metal machining (CNC) department, at 18 EU/m³ and 21 EU/m³, respectively. No samples exceeded the Dutch Expert Committee on Occupational Safety–recommended limit of 90 EU/m³ [DECOS 2010].

Table 1. Area air sample results for endotoxin on May 8, 2014

Location	Time (min)	Volume (m ³)	Endotoxin concentration (EU/m ³)
Paraloft	411	0.82	1.3
Outdoors	490	0.96	ND
Crashworthy assembly	501	1.00	ND
Heat treat	495	0.98	ND
Office, main, desk	524	1.05	18
CNC department desk	503	1.00	21
Stockroom	500	1.00	ND
Conference room	544	1.09	1.7

ND = not detected; the minimum detectable concentrations ranged from 0.50 to 0.52 EU/m³.

We analyzed the bulk metalworking fluid samples from four machine reservoirs for endotoxin. Endotoxin concentrations in the bulk metalworking fluid samples were 77,300–527,000 endotoxin units per milliliter (EU/mL). The concentrations of endotoxin were high compared to those reported in the scientific literature. Simpson and associates found endotoxin ranging from the limit of detection to 1,870,000 EU/mL in 154 water-based metalworking fluid samples [Simpson et al. 2003]. The median endotoxin concentration for these samples was 8,039 EU/mL [Simpson et al. 2003]. In a study of three facilities, Cyprowski and associates [2007] found that the average concentration of bacterial endotoxins in the used metalworking fluids was 773 EU/mL. Endotoxin concentrations ranged from 220 to 1,700 EU/mL in a manufacturing facility for steel roller bearings [NIOSH 2006].

Document Review

At the time of the evaluation, the facility used a water-miscible, mineral oil-based metalworking fluid that contained 45%–65% mineral oil, 30%–50% emulsifiers, 1%–5% polar additives, 5%–15% chlorinated paraffin, and 1%–5% stabilizer and inhibitors, per the product safety data sheet [Blaser Swissslube Inc. 2010].

According to maintenance documentation, at the time of our evaluation the metalworking fluid in each of the four CNC machines had been replaced one time since February 2012. The metalworking fluid in two machines (HP9 and HP11) had been replaced in February 2012; the other two machines (DHP4000 and DMV5025) underwent metalworking fluid changes in April 2013 and August 2013. At the time of our evaluation, the metalworking machines had reservoirs that utilized skimmers to remove tramp oils. Metalworking fluid was recycled by a gravitational recycling system in the CNC department.

Consultants who evaluated metalworking fluid exposures at the facility in July 2012 noted

that airborne metalworking fluid was poorly controlled, especially when the mist collector was not used.

Metalworking fluid was added regularly to the CNC machines, but the additions were not documented. The pH of the metalworking fluid was reportedly measured and tracked every day. In May 2014, the fluid manufacturer's laboratory tested the metalworking fluid. The results showed that the odor of the metalworking fluid was unpleasant and that magnesium levels were higher than company-established tolerance limits. All other measures, including pH, sulfide, conductivity, calcium, calcium carbonate, chloride, sulfate, nitrate, nitrite, and aerobic bacteria, were within tolerance limits. The test showed aerobic bacteria concentrations less than 10^7 CFU/mL (the manufacturer's recommended range is to maintain concentrations greater than 10^5 CFU/mL). In contrast, the United Kingdom Health and Safety Executive recommends a limit of 10^3 CFU/mL for metalworking fluids [HSE 2006].

Observations

We observed that most employees used powder-free latex gloves when handling metal and metalworking fluid. Employees noted that the gloves protected them against cuts from sharp edges and prevented dermal exposure to metalworking fluid. We observed one employee with bare hands, handling metal coated in metalworking fluid, while using the metal-cutting machine. Although it is prudent to use gloves when working with metalworking fluid, latex can cause sensitization and result in local and systemic allergic reaction [OSHA 2008], so other materials should be selected.

Oil from the stock metal cutting machine splashed onto the ground, creating a slip hazard (Figure 3). Employees put sorbent pads on the ground to absorb excess metalworking fluid and prevent slips. Used sorbent pads were put into a trash can to drain off metalworking fluid. Once the metalworking fluid was removed, sorbent pads were reused. The trash can was full during our evaluation. According to the company, this practice was discontinued after our site visit in May 2014.



Figure 3. Stock metal cutting machine. Photo by NIOSH.

We observed one employee wearing a Moldex® (model 2740R95) filtering facepiece respirator. The employee wore the respirator voluntarily because of concern about exposure to an unknown agent causing the odor. The employee did not recall being provided with Appendix D from the Occupational Safety and Health Administration (OSHA) respiratory protection standard 29 CFR 1910.134 (Information for Employees Using Respirators When Not Required Under Standard). However, managers reported that all employees had a signed Appendix D form in their personnel files.

Confidential Medical Interviews and Reported Health Symptoms

We interviewed 81 (60%) of 134 employees during the visit. This included 53 (88%) of the 60 randomly selected employees and an additional 28 employees who asked to be interviewed. The remaining seven randomly selected employees were not working or were not available on the interview days.

The median age of interviewed employees was 50 years (range: 21–75 years). Forty-seven (58%) were male; 34 (42%) were female. The median amount of time worked at the facility was 6.75 years (range: 6 months to 14 years). The median number of hours worked per week at the facility was 40 hours (range: 36–60 hours). Interviewed employees included production, administrative, and managerial workers.

During the interviews, we asked employees if they were aware of any current water leaks, moisture problems, or mold problems in the facility. Seventy-three (90%) of employees reported they were aware of such problems. Reported problems included roof leaks throughout the building and mold problems in the cafeteria, conference room, and heat-treat area.

We also asked employees if they had noticed unusual odors in the facility in the 4 weeks prior to the interview. All but three employees (96%) reported noticing unusual odors during this period. Most employees reported they first noticed the odor in April 2013. Reported odor locations included the parking lot and throughout the building. Stronger odors were noted in the front vestibule, conference room, accounting office, CNC area, and maintenance area. Thirty-eight interviewed employees (49%) reported not knowing what the source of the odor was. Hypothesized sources by the other 40 employees included mold, stagnant water, soil/groundwater, and metalworking fluid from the CNC machines.

Of the 81 interviewed employees, 7 (9%) reported no symptoms in the previous 4 weeks that they thought were related to working in the facility. Of the 74 (91%) who did report symptoms, the most commonly reported were fatigue (80%), headache (64%), eye irritation (64%), and runny nose/congestion (63%). Employees who were randomly selected for interviews and employees who asked to be interviewed had similar reported prevalence of any symptom and also the most commonly reported symptoms above.

Twenty-three interviewed employees (28%) reported skin rash; they represented production, administrative, and managerial staff and worked in various primary locations including the sewing department, crashworthy department, accounting office, main office, and CNC department. Seventeen interviewed employees (21%) reported having experienced

psychological and/or emotional distress related to the odor experienced in the building in the past 4 weeks. Table 2 summarizes the work-related symptoms reported during medical interviews.

Table 2. Work-related symptoms in the previous 4 weeks, reported by interviewed employees

Work-related symptom	No. (%) of employees (N = 81)
Fatigue	65 (80)
Headache	52 (64)
Eye irritation	52 (64)
Runny nose or congestion	51 (63)
Cough	44 (54)
Sinus problems	43 (53)
Sore throat	40 (49)
Dizziness/lightheadedness	35 (43)
Shortness of breath	35 (43)
Muscle aches	30 (37)
Skin rash	23 (28)
Wheezing	18 (22)
Nosebleed	17 (21)
Chest pain	13 (16)
Loss of appetite	10 (12)
Other*	32 (40)

*The most common other symptoms included a metallic taste and dry or itchy skin.

We asked employees about underlying medical conditions. Thirty-two interviewed employees (40%) reported having one or more conditions consistent with atopy, a predisposition to allergic disease (defined as history of asthma, allergic rhinitis/hay fever, or eczema). Specifically, 28 (35%) reported a history of hay fever/allergic rhinitis, 7 (9%) reported a history of asthma, and 2 (2%) reported a history of eczema. Seventeen employees (21%) reported being a current smoker.

Many of the symptoms reported by these employees, such as fatigue, headache, eye irritation, and runny nose/congestion, are common in the general population [Lipscomb et al. 1992; Barsky and Borus 1995; Heyworth and McCaul 2001]. Nevertheless, over 90% of interviewed employees reported symptoms they experienced in the previous 4 weeks that they believed to be work-related. It is likely these symptoms are multifactorial in origin. First, the symptoms in some employees may be attributed to allergic rhinitis or hay fever from mold, dust, pollen, or other allergens; 35% of the interviewed employees reported a history of allergic rhinitis/hay fever. Second, it is also possible that insufficient outdoor air introduced through the ventilation system into the building could be contributing to or exacerbating symptoms. Too little outdoor air mixing in with indoor air can further concentrate existing contaminants, including chemicals used in and created during the

manufacturing process. For example, styrene and methyl ethyl ketone are known skin and mucous membrane irritants. Third, it is also possible that symptoms in some employees could be associated with the 3,5-MDMP odor.

Odors may produce health symptoms by three mechanisms. First, symptoms can be induced by exposure to odorants at levels that also cause irritation. Therefore, irritation, rather than the odorant, is the cause of the symptoms. Second, health symptoms from odorants at nonirritant concentrations, such as hydrogen sulfide, can be due to innate or learned aversions. Third, symptoms may be due to a co-pollutant, such as endotoxin, that is part of an odorant mixture [Schiffman and Williams 2005]. It is possible that symptoms reported by facility employees could be associated with all three mechanisms but also could be associated with non-occupational factors. In persons with existing health problems, such as asthma or chronic respiratory problems, odors can also worsen pre-existing symptoms. Odors have been found to affect the physiological and psychological responses of individuals with asthma [Beach et al. 1997; Jaén and Dalton 2014].

We found that 28% of interviewed employees reported a skin rash in the previous 4 weeks they believed was work-related. Skin rash was reported by production, administrative, and managerial employees. However, since we did not ask about specific diagnoses or specific locations of the rashes on the body in our interviews, it is difficult to hypothesize the cause.

Employees were concerned that the presence of mycotoxins could be causing their symptoms. During our visit, we discussed the limitations and risks of biological testing for mycotoxins. Despite our recommendation against proceeding with this type of testing, prior to our site visit, the company had arranged mycotoxin testing of employee urine specimens through a contract laboratory. Because of the problems with this type of testing, we do not believe that the results can be used to say anything about employee symptoms and exposures at the facility (Appendix C).

Conclusions

2-Methoxy-3,5-dimethylpyrazine (3,5-MDMP) was the primary isomer found in area air and bulk samples of metalworking fluid and is the likely cause of the persistent, unpleasant odor. 3,5-MDMP and other methoxypyrazines have been found to cause bad odors in metalworking fluid in the past, and a few species of bacteria are known to produce 3,5-MDMP. Bacteria belonging to the same order that produce 3,5-MDMP were identified in our analysis of air and bulk metalworking fluid samples, and may be contributing to the high concentrations of endotoxin found in the metalworking fluid. We found metalworking fluid mist in the air including in areas outside of where the fluids are used. Throughout the facility we found methyl ethyl ketone, styrene and other VOCs. These chemical and biological contaminants and odors may be contributing to health symptoms reported by employees although it is likely that the reported symptoms are multifactorial in origin.

Recommendations

On the basis of our findings, we recommend the actions listed below. We encourage the company to use a labor-management health and safety committee or working group to discuss our recommendations and develop an action plan. Those involved in the work can best set priorities and assess the feasibility of our recommendations for the specific situation at the facility.

Our recommendations are based on an approach known as the hierarchy of controls (Appendix B). This approach groups actions by their likely effectiveness in reducing or removing hazards. In most cases, the preferred approach is to eliminate hazardous materials or processes and install engineering controls to reduce exposure or shield employees. Until such controls are in place, or if they are not effective or feasible, administrative measures and personal protective equipment may be needed.

Engineering Controls

Engineering controls reduce employees' exposures by removing the hazard from the process or by placing a barrier between the hazard and the employee. Engineering controls protect employees effectively without placing primary responsibility of implementation on the employee.

1. Isolate the CNC department to prevent metalworking mist from migrating to adjacent areas. Possible methods may include (1) spatial isolation, such as constructing walls or moving equipment to a less central location, and (2) providing additional ventilation. Do not recirculate air from the CNC department into other areas of the facility. Maintain negative pressure relative to adjacent areas once the CNC department is isolated.
2. Modify the facility ventilation systems to incorporate outdoor air supply to the paraloft, component repair, document control, and inspection departments.
3. Repair the roof, gutters, and downspouts to ensure water is diverted away from the building. Excavate and change the grade on the west side of the building to move rainwater away from the building foundation and prevent flooding.
4. Increase the height of the exhaust fan units above the roofline to decrease the likelihood of re-entrainment of building exhaust air.
5. Evaluate the ventilation in the paint room and paint booth. Bring the paint room under negative pressure relative to adjacent areas. Do not recirculate air from this department to other areas of the facility.
6. Install containment around the stock metal cutting machine to prevent metalworking fluid from dripping onto the ground, creating a slip hazard.

Administrative Controls

Administrative controls are employer-dictated work practices and policies to reduce or prevent hazardous exposures. Their effectiveness depends on employer commitment and employee acceptance. Regular monitoring and reinforcement are necessary to ensure that policies and procedures are followed consistently.

1. Develop a decontamination plan for the CNC machines (including tramp oil removal equipment), metalworking recycling system containers, and all instrumentation that contacts metalworking fluid during normal operations. Consult with the metalworking fluid manufacturer for guidance. When developing a decontamination plan, consider the persistence potential of biofilms that may re-inoculate fresh metalworking fluid. Once all components of the metalworking fluid system have been decontaminated, all used metalworking fluid should be disposed of and replaced with new metalworking fluid.
2. Institute the metalworking fluid management plan that was distributed in July 2014. Consult with the metalworking fluid manufacturer to identify best practices for metalworking fluid maintenance and recycling, including change-out and cleaning schedules as well as troubleshooting odors. A good metalworking fluid management plan includes procedures for maintaining metalworking fluid, guidelines for metalworking fluid testing and analysis, procedures for mist collector maintenance, and development and maintenance of employee training plans and records. The management plan should include the maintenance and monitoring of metalworking fluids undergoing the recycling process. If not properly maintained, the recycling reservoirs can harbor undesirable and unexpected bacteria. The metalworking fluid manufacturer recommends cleaning coolant systems and replacing used metalworking fluid annually [Blaser Swissslube Inc. 2013].
3. Develop and adhere to an air handler and ventilation system maintenance schedule. This should include replacing water-damaged and rusted parts and inspecting air filters, belts, and drip pans.
4. Do not store used, saturated sorbent pads indefinitely. Employ a procedure for removing metalworking fluids and returning the pads to use quickly or disposing of them. Extended storage of saturated sorbent pads can contribute to uncontrolled microbial growth and odors. Consider using a spinner rather than gravitational methods to reduce metalworking fluid removal time. Discard metalworking fluid removed from sorbent pads.
5. Implement a medical monitoring program for employees who are exposed to metalworking fluid mist at half of the REL. Consider including all employees who are exposed to metalworking fluid in the air or on their skin in the medical monitoring program. A medical monitoring program helps prevent, identify, and manage skin and respiratory disease among included employees. It includes preplacement or initial examination, periodic examination, detailed examination for a subset of employees, physician's reports and follow-up evaluations. More information about medical monitoring can be found in the NIOSH document "What You Need to Know About Occupational Exposure to Metalworking Fluids" at <http://www.cdc.gov/niosh/pdfs/98-116.pdf>.

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6. Conduct periodic air sampling for metalworking fluids on CNC employees and others who work in adjacent areas. Additionally, repeat air sampling when process or equipment changes occur. Personal monitoring, rather than area air monitoring, should be performed to obtain exposure data that can be compared to OELs.
 7. Evaluate the use of methyl ethyl ketone and other solvents in the manufacturing process. Develop protocols for using methyl ethyl ketone and keep use records in every department where it is being used. Use these records to identify where solvent use can be reduced or eliminated or ventilation improvements can be made to reduce the ambient concentration of VOCs in the facility.
 8. Encourage employees with work-related health concerns to seek medical care from qualified medical professionals. Certification by medical specialty boards can be found by checking the American Board of Medical Specialties website at <http://www.abms.org>.
 9. Inform employees about the limitations and potential risks of nonstandard medical tests and treatments. Refrain from participating in nonstandard medical testing and treatments without full knowledge and informed consent of risks and benefits.
 10. Encourage employees to evaluate the quality of the health information that they find. It is important to ensure that health information is reliable, up-to-date, and unbiased. The National Library of Medicine and the National Institutes of Health offer guidelines for evaluating the quality of health information on the Internet (<http://www.nlm.nih.gov/medlineplus/evaluatinghealthinformation.html>).

Personal Protective Equipment

Personal protective equipment is the least effective means for controlling hazardous exposures. Proper use of personal protective equipment requires a comprehensive program and a high level of employee involvement and commitment. The right personal protective equipment must be chosen for each hazard. Supporting programs such as training, change-out schedules, and medical assessment may be needed. Personal protective equipment should not be the sole method for controlling hazardous exposures. Rather, personal protective equipment should be used until effective engineering and administrative controls are in place.

1. Provide non-latex gloves and require all employees to use them when handling materials that are covered with metalworking fluid and when using methyl ethyl ketone to clean parts.
2. Offer heavy-duty non-latex protective gloves to CNC operators for hand protection from cuts and metalworking fluid. Thick nitrile gloves are a suitable alternative.
3. Ensure employees who voluntarily wear respirators understand the requirements of voluntary respirator use per Appendix D (Information for Employees Using Respirators When Not Required Under Standard) from the OSHA respiratory protection standard [29 CFR 1910.134].

Appendix A: Methods

Metalworking Fluid Sampling and Analysis

Air samples for metalworking fluid analyses were collected by using 37-mm closed-faced three-piece cassettes containing a pre-weighed 2-micrometer-pore-size polytetrafluoroethylene filter and support pad. The sampling train consisted of a BGI thoracic cyclone, 37-millimeter cassette, and Tygon® tubing connecting the sampling assembly to SKC Air Check® 2000 air-sampling pumps. A sampling rate of 1.6 liters per minute was used to collect the thoracic fraction of the aerosol. Each pump was calibrated before and after use. The sampling medium was attached to the employee's lapel within the breathing zone (defined as an area in front of the shoulders with a radius of 6 to 9 inches). The samples were analyzed by gravimetric analysis for the thoracic fraction of metalworking fluid particulates per NIOSH Method 5524 [NIOSH 2015]. After the filter was gravimetrically weighed, a ternary solvent blend was used to extract the metalworking fluid fraction from each sample.

Endotoxin Sampling and Analysis

Air samples were collected by using an endotoxin-free three-piece 37-millimeter closed-face cassette, preloaded with 0.45-micrometer-pore-size polycarbonate filters. Samples were collected with AirCheck2000® personal air-sampling pumps calibrated at 2 liters per minute. Each pump was calibrated before and after use. Samples were analyzed for endotoxin content with the kinetic-chromogenic procedure using the limulus amoebocyte lysate assay [Cambrex 2005]. For these analyses, one EU was equivalent to 0.053 nanograms of endotoxin. The limit of detection was 0.50 EU per sample.

Viable Microbe Sampling and Analysis

We collected bulk metalworking fluid samples using a sterile pipette to fill 4-ounce sterile bottles, leaving at least 1 inch of headspace. These samples were kept on ice and shipped within 1 day to the laboratory for analysis. Each sample was concentrated by a 30-minute centrifuge, and excess fluid was poured off. The concentrate was vortexed for 1 minute and then plated to the appropriate media.

For aerobic bacteria, the media consisted of tryptic soy agar with polysorbate 80 and lecithin and buffered charcoal yeast extract. Plates were incubated at 23 ± 2 degrees Celsius (C) for 5 to 7 days and read daily. The media for fungi were yeast malt extract, inhibitory mold agar with gentamicin and chloramphenicol, and buffered charcoal yeast extract. Plates were incubated at $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 10 days as needed. Each plate was read on day 3 to see if it was overgrown and then read again on day 5 or 7 and day 10. The media for mycobacteria consisted of buffered charcoal yeast extract, Middlebrook 7H10, and Mitchison 7H11S. Plates and broth were incubated at $32^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in 7%–10% CO_2 for 4 weeks. Cultures were read at 3–5 days and 7 days. If specimens were overgrown, additional dilutions were made. Broths were Ziehl-Neelsen-stained at 2–3 weeks and 4 weeks [MSI 2011].

Volatile Organic Compound Sampling and Analysis

Area air was sampled for VOCs with thermal desorption tubes attached to SKC Inc. Pocket Pumps® calibrated at 100 cubic centimeters per minute. The thermal desorption tubes contained three beds of sorbent material: (1) 90 milligrams of Carbopack™ Y, (2) 115 milligrams of Carbopack B, and (3) 150 milligrams Carboxen™. After sampling, the thermal desorption tubes were stored in a cooler and then qualitatively analyzed for VOCs according to NIOSH Method 2549 [NIOSH 2015].

Microbial Diversity in Air and Metalworking Fluids Sampling and Analysis

Media Sampling for Microbial Diversity Analysis

We collected aerosols using a two-stage sampler with two cyclones depositing into microcentrifuge tubes and onto a mixed cellulose ester filter. We used AirCheck2000® personal air sampling pumps calibrated at 2 liters per minute. Each pump was calibrated before and after use. The bioaerosol samplers allowed for the collection of particles across three size fractions: ≥ 4.1 micrometers, 1.0–4.1 micrometers, and < 1.0 micrometer aerodynamic diameter. The three size cut samples taken with each bioaerosol sampler were aggregated for genomic DNA analysis.

We collected bulk metalworking fluid samples using a sterile pipette to fill a 4-ounce sterile bottle. These samples were kept on ice and shipped within 1 day to the laboratory for analysis.

Genomic DNA Extraction from Sample Media for Analysis

Air and metalworking fluid samples were processed separately for fungal and bacterial DNA extraction with use of the Roche High Pure polymerase chain reaction (PCR) template kit, as previously described [Rittenour et al. 2012; Rittenour et al. 2014]. Metalworking fluid samples, including internal controls, were centrifuged at 5,000 revolutions per minute for 10 minutes at 4°C. The supernatant fluid was then decanted, and the pellet was resuspended in 1 milliliter (mL) filter-sterilized (0.45 micrometer [μm]) phosphate buffered saline (pH 7.4), transferred to a 1.5-mL polypropylene microcentrifuge tube, and centrifuged at 14,000 revolutions per minute for 5 minutes. The supernatant fluid was then decanted and the pellet was resuspended in 1 mL phosphate buffered saline and centrifuged for a further 5 minutes, at 14,000 revolutions per minute. The supernatant fluid was decanted and the pellet resuspended in 500 microliter (μL) phosphate buffered saline; 20 μL of each metalworking fluid sample was added to a 2-mL bead-beater tube containing 300 mg glass beads (212–300 μm). The High Pure PCR kit lysis buffer was then added to each tube (330 μL) and placed in a BioSpec Products bead beater for 30 seconds at high speed. The tubes were centrifuged for 1 minute at 20,000 x g, and the supernatant was transferred to a 1.5-mL microcentrifuge tube and incubated with 25 μL Cell Lytic B lysis reagent for 15 minutes at 37°C. The kit's binding buffer (200 μL) and proteinase K (40 μL) were then added, and the solution was incubated at 70°C for 10 minutes. The sample was washed and eluted in 100 μL according to the manufacturer's instructions.

For air samples, including field blank and internal controls, each stage from the NIOSH BC251 air sampler was combined prior to DNA extraction. The after filter was sectioned into six pieces with a scalpel using aseptic methods. These pieces were placed into a 2 mL bead-beater tube containing 300 mg glass beads as described above. The tubes were placed in liquid nitrogen for 30 seconds and processed in a bead beater for 30 seconds. This process was repeated three times. The High Pure PCR Template kit lysis buffer (650 μ L) was then sequentially added to the first and second stage tubes and vortexed in order to collect the fungal and bacterial particles from the samples. The lysis buffer was added to the 2 mL bead-beater tube containing the macerated filter material. These tubes were processed with a bead beater for 30 seconds and then centrifuged for 1 minute at 20,000 x g. The supernatant was collected and incubated with 40 μ L Sigma Aldrich Cell Lytic B lysis reagent for 15 minutes at 37°C. The sample was mixed with the kit's binding buffer (400 μ L) and proteinase K (40 μ L) and incubated at 70°C for 10 minutes. The sample was then washed and eluted in 100 μ L as recommended by the manufacturer.

Fungal ITS and Bacterial 16S rDNA Amplification, Cloning, and Sanger Sequencing

Fungal ribosomal deoxyribonucleic acid (rDNA) was targeted for PCR amplification as previously described [Rittenour et al. 2012; Rittenour et al. 2014]. Briefly, fungal rDNA sequences were amplified with the primer pair Fun18Sf (TTGCTCTTCAACGAGGAAT) and ITS4 (TCCTCCGCTTATTGATATGC). Fungal internal transcribed spacer-1 (ITS1) and ITS2 regions were then amplified with Platinum Taq DNA polymerase (Invitrogen) according to the methods previously described [Rittenour et al. 2012; Rittenour et al. 2014]. For fungal amplification, 5 replicate PCR reactions (50 μ L) were run for each sample by using 5 μ L of DNA template. These replicates were then combined, and the rDNA amplicons were purified with a Qiagen PCR purification kit, according to the manufacturer's instructions. Purified product (8 μ L) was then run on a 1% agarose gel containing 1 microgram per milliliter (μ g/mL) ethidium bromide and examined for amplicons with ultraviolet light.

Bacterial 16S rDNA sequences were amplified with use of the highly conserved primer pair p8FPL (AGTTTGATCCTGGCTCAG) and p806R (GGACTACCAGGGTATCTAAT) [McCabe et al. 1999]. Bacterial 16S rRNA genes were amplified with Invitrogen Platinum Taq DNA polymerase by a modified method of McCabe et al. [1999]. The PCR conditions included initial denaturation at 95°C for 4 minutes, followed by 33 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 2 minutes, and completion with a final extension at 72°C for 10 minutes. Three 50- μ L replicate PCR reactions were run for each sample, with use of 5 μ L of DNA template. These replicates were then combined and the rDNA amplicons were purified with a Qiagen PCR purification kit according to the manufacturer's instructions. Purified product (8 μ L) was then run on a 1% agarose gel containing 1 μ g/mL ethidium bromide and examined for amplicons with ultraviolet light.

On the basis of the low yield of fungal DNA captured during extraction, bacteria were selected for further cloning and sequencing. Bacterial amplicons were separately cloned into the pDRIVE vector using a Qiagen PCR cloning kit. Clone libraries were generated by

transforming cloned plasmids into chemically competent *Escherichia coli* cells as previously described [Rittenour et al. 2012; Rittenour et al. 2014]. Positive colonies (as determined colorimetrically by the inactivation of the lacZ gene) were selected and cultured for 16 hours at 37°C in liquid Luria-Bertani medium containing 100 µg/mL ampicillin. Resultant cells were centrifuged at 1,800 x g and the pellet was resuspended in 200 µL of 15% glycerol and sent to Genewiz Inc. for Sanger sequencing of the bacterial 16S insert. Inserts were sequenced in both directions, allowing for sequence analysis of the 16S region.

Sequencing results were downloaded as “.ab1” chromatogram files from Genewiz Inc. Vector sequence data were trimmed and forward and reverse sequences were assembled using Biomatters Geneious R7 Software. Then we sequenced the DNA to identify which varieties of bacteria were present in the air. Sequence data were then clustered into OTUs with MOTHUR software version 1.32.1 using a 97% similarity cutoff as described in previous publications [Rittenour et al. 2012; Rittenour et al. 2014]. Sequences representative of each OTU were then used in a Basic Local Alignment Search Tool (BLAST) search against the NCBI database [Schloss et al. 2009].

Appendix B: Occupational Exposure Limits and Health Effects

NIOSH investigators refer to mandatory (legally enforceable) and recommended OELs for chemical, physical, and biological agents when evaluating workplace hazards. OELs have been developed by federal agencies and safety and health organizations to prevent adverse health effects from workplace exposures. Generally, OELs suggest levels of exposure that most employees may be exposed to for up to 10 hours per day, 40 hours per week, for a working lifetime, without experiencing adverse health effects. However, not all employees will be protected if their exposures are maintained below these levels. Some may have adverse health effects because of individual susceptibility, a pre-existing medical condition, or a hypersensitivity (allergy). In addition, some hazardous substances act in combination with other exposures, with the general environment, or with medications or personal habits of the employee to produce adverse health effects. Most OELs address airborne exposures, but some substances can be absorbed directly through the skin and mucous membranes.

Most OELs are expressed as a TWA exposure. A TWA refers to the average exposure during a normal 8- to 10-hour workday. Some chemical substances and physical agents have recommended short-term exposure limit or ceiling values. Unless otherwise noted, the short-term exposure limit is a 15-minute TWA exposure. It should not be exceeded at any time during a workday. A ceiling limit should not be exceeded at any time.

In the United States, OELs have been established by federal agencies, professional organizations, state and local governments, and other entities. Some OELs are legally enforceable limits; others are recommendations.

- The U.S. Department of Labor OSHA permissible exposure limits (29 CFR 1910 [general industry]; 29 CFR 1926 [construction industry]; and 29 CFR 1917 [maritime industry]) are legal limits. These limits are enforceable in workplaces covered under the Occupational Safety and Health Act of 1970.
- NIOSH RELs are recommendations based on a critical review of the scientific and technical information and the adequacy of methods to identify and control the hazard. NIOSH RELs are published in the *NIOSH Pocket Guide to Chemical Hazards* [NIOSH 2010]. NIOSH also recommends risk management practices (e.g., engineering controls, safe work practices, employee education/training, personal protective equipment, and exposure and medical monitoring) to minimize the risk of exposure and adverse health effects.
- Other OELs commonly used and cited in the United States include the threshold limit values, which are recommended by the American Conference of Governmental Industrial Hygienists, a professional organization, and the workplace environmental exposure levels, which are recommended by the American Industrial Hygiene Association, another professional organization. The threshold limit values and workplace environmental exposure levels are developed by committee members of these associations from a review of the published, peer-reviewed literature. These OELs are not consensus standards. Threshold limit values are considered voluntary exposure

guidelines for use by industrial hygienists and others trained in this discipline “to assist in the control of health hazards” [ACGIH 2015]. Workplace environmental exposure levels have been established for some chemicals “when no other legal or authoritative limits exist” [AIHA 2014].

Outside the United States, OELs have been established by various agencies and organizations and include legal and recommended limits. The Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (Institute for Occupational Safety and Health of the German Social Accident Insurance) maintains a database of international OELs from European Union member states, Canada (Québec), Japan, Switzerland, and the United States. The database, available at <http://www.dguv.de/ifa/Gefahrstoffdatenbanken/GESTIS-Internationale-Grenzwerte-für-chemische-Substanzen-limit-values-for-chemical-agents/index-2.jsp>, contains international limits for more than 1,500 hazardous substances and is updated periodically.

OSHA requires an employer to furnish employees a place of employment free from recognized hazards that cause or are likely to cause death or serious physical harm [Occupational Safety and Health Act of 1970 (Public Law 91–596, sec. 5(a)(1))]. This is true in the absence of a specific OEL. It also is important to keep in mind that OELs may not reflect current health-based information.

When multiple OELs exist for a substance or agent, NIOSH investigators generally encourage employers to use the lowest OEL when making risk assessment and risk management decisions. NIOSH investigators also encourage use of the hierarchy of controls approach to eliminate or minimize workplace hazards. This includes, in order of preference, the use of (1) substitution or elimination of the hazardous agent, (2) engineering controls (e.g., local exhaust ventilation, process enclosure, dilution ventilation), (3) administrative controls (e.g., limiting time of exposure, employee training, work practice changes, medical surveillance), and (4) personal protective equipment (e.g., respiratory protection, gloves, eye protection, hearing protection). Control banding, a qualitative risk assessment and risk management tool, is a complementary approach to protecting employee health. Control banding focuses on how broad categories of risk should be managed. Information on control banding is available at <http://www.cdc.gov/niosh/topics/ctrlbanding/>. This approach can be applied in situations where OELs have not been established or can be used to supplement existing OELs.

Below we provide the OELs and surface contamination limits for the compounds we measured, as well as a discussion of the potential health effects from exposure to these compounds.

Metalworking Fluids

NIOSH recommends that exposures to metalworking fluid aerosols be limited to 0.4 mg/m³ for the thoracic particulate mass, as a TWA concentration for up to 10 hours per day during a 40-hour workweek [NIOSH 1998]. The NIOSH REL is intended to prevent or greatly reduce respiratory disorders associated with metalworking fluid exposure. In addition, limiting dermal (skin) exposure is critical to preventing allergic and irritant disorders related to metalworking fluid exposure. NIOSH recommends that all employees exposed

to metalworking fluids at over half the REL receive medical monitoring, and all employees with exposure to metalworking fluid may benefit from medical monitoring [NIOSH 1998]. Supervision of the medical monitoring program should be done by a physician or other health professional with expertise in the identification and management of metalworking fluid-related respiratory conditions and skin diseases. Contaminated water in metalworking fluids may also contain fungi. Some fungi may infect susceptible hosts, such as immune compromised persons, and exposure to some fungi may result in adverse health effects such as hypersensitivity pneumonitis, allergic sensitization, and asthma. At this time, health data are insufficient to recommend a specific limit for fungal contamination in metalworking fluids.

Appendix C: Mycotoxins

Fungi can be found both indoors and outdoors. Fungi can cause adverse health effects in humans through three processes: (1) allergy, (2) infection, and (3) toxicity. About 40% of the population are atopic and express high levels of allergic antibodies to inhaled allergens. Of these, 25%, or 10% of the population, have allergic antibodies to common inhaled fungi [Horner et al. 1995]. While indoor fungi are well-recognized allergens, outdoor molds are generally more important [ACOEM 2011].

Most fungi are generally not pathogenic to healthy humans. Exposure to fungi indoors is generally not a specific risk factor for fungal infections. Exceptions include fungi that can cause superficial infections on the skin or mucosal surfaces of healthy people. These fungi include *Trichophyton*, *Epidermophyton*, and *Microsporum* species, which can cause infections of the epidermis and dermis. Other exceptions include dimorphic fungal pathogens such as *Blastomyces*, *Coccidioides*, and *Histoplasma*, which have areas of endemicity and are not normally found growing in residential or work environments such as this facility [ACOEM 2011].

Some species of fungi are capable of producing toxins, known as mycotoxins. Some mycotoxins such as penicillin and cyclosporine have a valuable clinical use. Mycotoxins are produced by a number of fungal genera, including *Alternaria*, *Aspergillus*, *Claviceps*, *Fusarium*, *Stachybotrys*, and *Penicillium* [Marin et al. 2013]. Most described human poisonings by mycotoxins have involved the eating of moldy foods and inhalation exposures of agricultural workers to spoiled grain products containing high concentrations of fungi and bacteria [Ciegler and Bennett 1980; Pohland 1993; Wu et al. 2014].

Critical reviews of the scientific literature have determined that there is currently no conclusive evidence of an association between mycotoxin exposure in the indoor environment and human illness [Menzies et al. 1997; Fung et al. 1998; Robbins et al. 2000; Sudakin 2000; Page and Trout 2001; Terr 2001]. No causal relationship has been established between health complaints and indoor exposures to specific molds such as *Stachybotrys chartarum* [ACOEM 2011].

Although commercially available and offered at many different sites, mycotoxin tests are considered to be “nonstandard tests.” Uncertainty exists as to how to interpret the results of these biologic tests for mycotoxins. These toxins can be found at different levels in the blood and urine of healthy people who do not report adverse health symptoms. The meaning of a “high level” versus a “low level” is unknown. These tests, when repeated, cannot be depended upon to give consistent results. Therefore, these tests are not considered to be valid or reliable.

Biologic tests for mycotoxins are not approved by the Food and Drug Administration (FDA). Although testing may be done at a laboratory certified under the Clinical Laboratory Improvement Amendments (CLIA), the tests themselves are not CLIA certified. Therefore, testing in a CLIA-certified laboratory is not necessarily approved by the FDA, and clinicians do not necessarily use the test results appropriately for diagnosis. CLIA certification of a laboratory indicates that the laboratory meets a set of basic quality standards. It is important

to note, however, that the CLIA program does not address the clinical validity of a specific test (i.e., the accuracy with which the test identifies, measures, or predicts the presence or absence of a clinical condition in a patient). FDA clearance/approval of a test, on the other hand, provides assurance that the test itself has adequate analytical and clinical validation and is safe and effective [Nelson et al. 2014].

Appendix D. Bacterial Species that Produce Pyrazines

Table D1. Bacterial species that produce pyrazines

Organism	Family	Order	Class	Phylum	Pyrazine(s)
<i>Chondromyces crocatus</i>	Polyangiaceae	Myxococcales	Deltaproteobacteria	Proteobacteria	Hydroxyalkylmethoxy-pyrazines, 3,5-MDMP, 3,6-MDMP [Chatonnet et al. 2010]
<i>Serratia odorifera</i>	Enterobacteriaceae	Enterobacteriales	Gammaproteobacteria	Proteobacteria	3,5-MDMP [Chatonnet et al. 2010]
<i>Rhizobium excellens</i>	Rhizobiaceae	Rhizobiales	Alphaproteobacteria	Proteobacteria	3,5-MDMP [Chatonnet et al. 2010]
<i>Pseudomonas perolens</i>	Pseudomonadaceae	Pseudomonadales	Gammaproteobacteria	Proteobacteria	2-methoxy-3-isopropylpyrazine, 2-sec-butyl-3-methoxy-pyrazine [Chatonnet et al. 2010]
<i>Serratia</i> spp.	Enterobacteriaceae	Enterobacteriales	Gammaproteobacteria	Proteobacteria	2-methoxy-3-isopropylpyrazine, 2-sec-butyl-3-methoxy-pyrazine [Chatonnet et al. 2010]
<i>Cedecea</i> spp.	Enterobacteriaceae	Enterobacteriales	Gammaproteobacteria	Proteobacteria	2-methoxy-3-isopropylpyrazine, 2-methoxy-3-(1-methylpropyl)-pyrazine [Chatonnet et al. 2010]
<i>Novosphingobium subarcticum</i>	Sphingomonadaceae	Sphingomonadales	Alphaproteobacteria	Proteobacteria	Detected in 3,5-MDMP tainted corks [Prat et al. 2009]
<i>Sphingobacterium</i> spp.	Sphingobacteriaceae	Sphingobacteriales	Sphingobacteria	Bacteroidetes	
<i>Acinetobacter Iwoffi</i>	Moraxellaceae	Pseudomonadales	Gammaproteobacteria	Proteobacteria	
<i>Pseudomonas</i> spp.	Pseudomonadaceae	Pseudomonadales	Gammaproteobacteria	Proteobacteria	Several methylpyrazines [Baferas et al. 2013]
<i>Pseudomonas taetrolens</i>	Pseudomonadaceae	Pseudomonadales	Gammaproteobacteria	Proteobacteria	2-methoxy-3-isopropylpyrazine [Chatonnet et al. 2010]
<i>Bacillus cereus</i>	Bacillaceae	Bacillales	Bacilli	Firmicutes	Tetramethylpyrazine [Seitz 1994]
<i>Bacillus subtilis</i>	Bacillaceae	Bacillales	Bacilli	Firmicutes	2,5-Dimethylpyrazine, Trimethylpyrazine, Tetramethylpyrazine, 2-ethyl-5-methylpyrazine, 2-methylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine [Larroche et al. 1999]
<i>Corynebacterium glutamicum</i>	Corynebacteriaceae	Actinomycetales	Actinobacteria	Actinobacteria	Tetramethylpyrazine [Seitz 1994]
<i>Halomonas venusta</i>	Halomonadaceae	Oceanospirillales	Gammaproteobacteria	Proteobacteria	2-methoxy-3-(1-methylpropyl)-pyrazine [Bungert et al. 2001]
<i>Paenibacillus polymyxa</i>	Paenibacillaceae	Bacillales	Bacilli	Firmicutes	Several isopropylpyrazines [Beck et al. 2003]
<i>Serratia ficaria</i>	Enterobacteriaceae	Enterobacteriales	Gammaproteobacteria	Proteobacteria	Several methylpyrazines
<i>Serratia rubidaea</i>	Enterobacteriaceae	Enterobacteriales	Gammaproteobacteria	Proteobacteria	[Gallois and Grimont 1985]
<i>Cedecea daviseae</i>	Enterobacteriaceae	Enterobacteriales	Gammaproteobacteria	Proteobacteria	

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