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Investigating a persistent odor at an aircraft seat manufacturer

Kendra Broadwater[a], **Marie A. de Perio**[a], **Jennifer Roberts**[b], **Nancy C. Burton**[a], **Angela R. Lemons**[c], **Brett J. Green**[c], and **Scott E. Brueck**[a]

[a]Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Surveillance, Hazard Evaluations and Field Studies, Hazard Evaluations and Technical Assistance Branch, Cincinnati, Ohio

[b]Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Division of Applied Research and Technology, Chemical Exposure and Monitoring Branch, Cincinnati, Ohio

[c]Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Health Effects Laboratory Division, Allergy and Clinical Immunology Branch, Morgantown, West Virginia

Abstract

An aircraft seat manufacturing company requested a NIOSH health hazard evaluation to help identify a strong odor that had persisted throughout the facility for over a year. Employees reported experiencing health effects thought to be related to the odor.

We collected and analyzed area air samples for volatile organic compounds, endotoxin, bacterial and fungal metagenome, and metalworking fluid aerosol. Bulk metalworking fluid samples were analyzed for endotoxin, bacterial and fungal metagenome, and viable bacteria and fungus. We also evaluated the building ventilation systems and water diversion systems. Employees underwent confidential medical interviews about work practices, medical history, and health concerns.

Based on our analyses, the odor was likely 2-methoxy-3,5-dimethylpyrazine. This pyrazine was found in air samples across the facility and originated from bacteria in the metalworking fluid. We did not identify bacteria known to produce the compound but bacteria from the same *Proteobacteria* order were found as well as bacteria from orders known to produce other pyrazines. Chemical and biological contaminants and odors could have contributed to health symptoms reported by employees, but it is likely that the symptoms were caused by several factors.

We provided several recommendations to eliminate the odor including washing and disinfecting the metalworking machines and metalworking fluid recycling equipment, discarding all used metal-working fluid, instituting a metalworking fluid maintenance program at the site, and physically isolating the metalworking department from other departments.

Contact Kendra Broadwater kbroadwater@cdc.gov National Institute for Occupational Safety and Health, Health Hazard Evaluation Program, 1090 Tusculum Ave, MS: R-11, Cincinnati, OH 45226.

Keywords

2-methoxy-3,5-dimethylpyrazine; aircraft manufacturing; metalworking fluids; odor; pyrazines

Introduction

In 2013, employees working at an aircraft ejection seat manufacturing facility began to experience an odor described as musty, moldy, and/or chemical-like. Employees were concerned about eye and throat symptoms thought to be related to the odor. The odor had an unknown origin and reportedly permeated manufacturing and packing materials as well as employees' personal effects. Because the company had been unsuccessful in identifying and resolving the odor, in 2014 management requested that the National Institute for Occupational Health and Safety (NIOSH) conduct a health hazard evaluation.

The manufacturing facility housed aircraft ejection seat production that consisted of simultaneous processes in seven distinct production departments or areas. Raw metal was cut and machined in the (1) Computer Numerical Control (CNC) department, heated and tumbled with abrasive powers in the (2) heat treat department, and (3) painted in a separate area. The company used semi-synthetic water-miscible cutting oils to lubricate, cool, and remove metal chips during machining and cutting. Fabric was cut with a laser cutting machine and sewn into seats in the (4) sewing department. Seats were hand-assembled in the (5) crashworthy department. Ejection seats and component parts were inspected at multiple points during the production process. Flawed parts or seats were repaired in (6) component repair. Parachutes for the ejection seats were packed in the (7) paraloft department. In addition to these manufacturing operations, the building contained offices for accounting, engineering, and project management.

The primary objectives of the evaluation were to identify potential sources of the offending odor and assess the indoor environmental quality in the facility.

Methods

Five components were included in this evaluation: (1) evaluation of the building ventilation systems; (2) analysis of air samples for volatile organic compounds (VOCs), metalworking fluids (MWFs), microbiological metagenome, and endotoxin; (3) analysis of bulk MWF samples for microbial burden and endotoxin; (4) review of MWF maintenance records; and (5) confidential medical interviews with employees. The full description of the methods and results can be found in the NIOSH health hazard evaluation report.[1]

Building and ventilation evaluation

The rooftop air-handling units and air filters rooftop exhaust fans, and plenum above the dropped ceiling tiles were inspected. The company provided change out schedules for carbon-impregnated filters used in the air handling units. Carbon dioxide was measured using a TSI Model 8554 Q-TRAK Plus™ Indoor Air Quality Monitor both indoors and outdoors.

Air sampling

Eight area air samples, including one outdoors, were collected for VOC analysis using thermal desorption tubes attached to SKC Inc. Pocket Pumps® calibrated at 100 cm^3 per minute. The thermal desorption tubes contained 90 milligrams (mg) of Carbopack Y, 115 mg of CarbopackB, and 150mg Carboxen 1003. After sampling, the thermal desorption tubes were stored in a cooler with ice packs and then qualitatively analyzed for VOCs according to NIOSH Method 2549.[2]

Four full-shift, personal air samples collected in the CNC department were analyzed for thoracic particle mass and extracted MWF mist per NIOSH Method 5524.[2] Thoracic particles sampled in this manner may include metal dusts, other particulates, and biological and non-biological aerosols in addition to the aerosolized MWF itself. The NIOSH recommended exposure limit (REL) is based on thoracic-sized metalworking fluid mist. Thoracic sized particles have an aerodynamic diameter of 10 μm or less.

Eight area air samples were collected across the facility and analyzed for endotoxin, an indicator of Gram negative bacteria. Samples included one outdoor air sample for comparison. Air samples were collected using an endotoxin-free, three-piece 37-mm closed-face cassette with a 0.45-μm-pore-size polycarbonate filter. Samples were collected with AirCheck2000 personal air sampling pumps calibrated at 2 L/min and analyzed for endotoxin content with the kinetic-chromogenic procedure using the limulus amebocyte lysate assay.[3] For these analyses, one endotoxin unit (EU) was equivalent to 0.053 ng of endotoxin. The limit of detection (LOD) was 0.50 EU per sample.

Twelve area air samples for biological aerosols were collected throughout the facility using a two-stage cyclonic aerosol sampler that was fabricated at NIOSH.[4] This cyclone aerosol sampler deposits bioaerosols into two cyclones followed by a filter; the first cyclone collects the non-respirable fraction, the second cyclone collects the respirable fraction, and the filter collects particles <1 μm.[4] AirCheck2000 personal air sampling pumps were used and calibrated at 2 L/min. Samples were collected over 75–115 min. The samples taken with each two-stage cyclonic aerosol sampler were aggregated for genomic deoxyribonucleic acid (DNA) analysis.

Bulk MWF sampling

Bulk samples of MWF were collected from the supply reservoir of the three CNC machines using a sterile pipette to fill a 4-oz sterile plastic container. These samples were kept cold with ice packs and shipped within 1 day of collection to the laboratory for analysis. Bulk MWF samples were analyzed for bacteria, mycobacteria, and fungi, which were identified using viable culture and culture-independent methods.[1,5] The bulk samples from the machine reservoirs were also analyzed for endotoxin. Additionally, the air space in the bulk MWF sample bottles was analyzed for VOCs using NIOSH method 2549.

Sanger sequencing analysis

Three bulk MWF samples, each from a different CNC machine, and eight air samples were evaluated for bacterial sources using Sanger sequencing. First, microbiological DNA present in the air samples and in bulk MWFs was extracted and amplified using universal fungal and bacterial primers previously described.[6–8] Due to high yields of bacterial DNA and relatively low yields of fungal DNA, the bacterial DNA was selected for sequence analysis. Amplified polymerase chain reaction products were cloned into *Escherichia coli*; clones containing bacterial DNA were selected and submitted for Sanger sequencing. The resulting sequences were clustered into operational taxonomic units (OTUs) with MOTHUR software version 1.32.1 using a 97% similarity cutoff as previously described.[6,7] Sequences representative of each OTU were then used in a Basic Local Alignment Search Tool (BLAST) search operated by the National Center for Biotechnology Information (NCBI) to identify the varieties of bacteria present in air and bulk MWF samples.[9]

Confidential medical interviews

A random sample of sixty (45%) of 134 employees participated in individual, semi-structured, and 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 the employees' work tasks, pertinent medical history, symptoms, and health concerns they related to the building.

Results

Building and ventilation evaluation

No outdoor air intakes were closer than the minimum recommended separation distance between air intake and building exhaust set forth by ASHRAE (15 ft for significantly contaminated exhaust and 30 ft for noxious or dangerous exhaust).[10] The two closest outdoor air intakes were 37 ft and 44 ft from building exhaust locations. We observed that the low height of the rooftop exhaust fans, 3 ft above the roof surface, could lead to recapture of building exhaust air, depending on outdoor weather conditions. During our inspection of three rooftop air handling units, we found standing water under an air intake and in a condensate drain pan, rotted plant debris in a condensate drain pan, plugged drain lines, and a water damaged air filter. Rooftop-mounted air handling units supplied air to most of the building through ventilation ducts. Maintenance staff reportedly replaced filters in the air handling units every 3 months. Employees reported no reduction in odors after the company switched to carbon-impregnated filters in an attempt to address the odor. The paraloft, component repair, document control, and inspection rooms were ventilated with independent air handling units that recirculated air from inside the building rather than incorporating outdoor air. Carbon dioxide concentrations in these areas were elevated, ranging from 1107–1820 parts per million, compared to those in areas that received outdoor air where levels were 450–950 parts per million. Outdoor carbon dioxide was about 400 parts per million.

Air sampling for VOCs and MWF

Semi-quantitative air sampling revealed the presence of styrene and methyl ethyl ketone in all sampling locations. The indoor concentrations of styrene and methyl ethyl ketone were higher than outdoor concentrations. 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. Since these chemicals were used in production, it is unlikely they were related to the relatively sudden development of the odor.

While the health hazard evaluation was underway, the company sent items from the facility to a consultant. The consultant performed off gassing sampling to identify the odor. The consultant subsequently identified the odor as 2-methoxy-3,6-dimethylpyrazine (3,6-MDMP, CAS 19846-22-1), two weeks prior to the NIOSH site visit. We identified 2-methoxy-3,5-dimethylpyrazine (3,5-MDMP, CAS 92508-08-2) as the primary isomer in all indoor area and bulk headspace samples, a compound that is structurally related but distinct from that proposed by the consultant. We also identified 3,6-MDMP in the area air samples but at much lower levels. Methoxypyrazines are known to have low odor thresholds and have odors described as musty, earthy, moldy, acrid, and "chemical."[11] Additionally, NIOSH investigators noted that the odor of the 3,5-MDMP standard used in the laboratory for analytical comparison was remarkably similar to the odor in the facility.

Full-shift personal thoracic MWF exposures ranged 0.08–0.20 mg/m^3 and did not exceed the NIOSH REL for thoracic MWF mist of 0.4 mg/m^3. These concentrations equate to 20–50% of the NIOSH REL.

Area air samples for MWF mist showed concentrations ranging from 0.27–0.74 mg/m^3 in the CNC area and 0.22 mg/m^3 in a room adjacent to the CNC area, where the CNC programmer worked.

Viable cultures bulk MWF

The total bacterial count ranged 1.7×10^7 – 4.1×10^7 colony forming units per milliliter (CFU/mL) in the three bulk samples of MWF. Across the three samples, the primary bacteria that were isolated included *Cupriavidus metallidurans, Corynebacterium* species, *Brevundimonas diminuta*, and *Alcaligenes faecalis*. Colony forming units derived from the fungal genus *Fusarium* were detected in two samples at low concentrations (5 CFU/mL). The laboratory found no fungal CFUs in the third MWF sample.

Concentrations of bacteria measured in MWF samples were in the United Kingdom Health and Safety Executive category for poor control of bacterial contamination in MWF.[12] According to the MWF manufacturer, this system of MWF was expected to develop a culture of one specific bacterial species, *Pseudomonas oleovorans*.[13] However, this bacterium was not one of the most populous three species cultured from the bulk MWF in this evaluation.

Sanger sequencing of fungal and bacterial DNA in air and bulk MWF

A relatively low yield of fungal DNA compared to bacterial DNA was found in the air and bulk MWF samples. Based on these preliminary results, bacterial DNA underwent further Sanger sequencing analysis. DNA sequences were clustered into 152 individual OTUs and identified 148 unique bacterial OTUs. Within the bacterial OTU dataset, 76% were ≥ 97% identical to reference 16S bacterial sequences in the NCBI database. A large number of bacterial OTUs were identified in MWF 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. All OTUs identified from the media controls, reagent controls, and field blanks were removed from the analysis of air and MWF samples. *Corynebacterium* spp. and *Alcaligenes faecalis,* which were among the most populous culturable bacteria, were among the excluded bacteria.

Overall, sequencing analysis identified 23 clones in the bulk used MWF. 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. These were derived from the bacterial phyla Proteobacteria (33%), Firmicutes (29%), Actinobacteria (25%), and Bacteroidetes (13%). Samples from the outdoors, had the highest number of clones (n = 17) relative to those identified in the other air samples (range: 0–8 clones).

The preliminary Sanger sequencing analysis demonstrated that bacteria were present in both air and MWF samples. Compared with the culturable bacteria datasets reported above, Sanger sequencing identified *Brevundimonas diminuta* but not *Cupriavidus metallidurans* in MWF samples. However, in the MWF sequencing analysis, we identified a variety of other betaproteobacteria in the same family *(Burkholderiaceae)*.

Sequences derived from the primary bacteria species expected by the manufacturer in the MWF, *Pseudomonas oleovorans*, were not identified in the Sanger sequencing analysis. Interestingly, sequences derived from the Proteobacteria order, Rhizobiales, as well as orders thought to produce other types of pyrazines (Bacteriales, Actin-iomycetales, and Sphingobacteriales) were identified in both air and MWF samples.

Endotoxin in air and bulk MWF

Endotoxin concentrations ranged from below the LOD to 21 endotoxin units per cubic meter (EU/m^3). Airborne endotoxin was highest in the main office area and in the CNC department, at 18 EU/m^3 and 21 EU/m^3, respectively. No samples exceeded the Dutch Expert Committee on Occupational Safety–recommended limit of 90 EU/m^3.[13] No occupational exposure limits for endotoxin have been established in the United States. Endotoxin concentrations in the bulk MWF samples were 77,300–527,000 EU per milliliter.

Document review

The company did not follow a MWF maintenance schedule. According to maintenance documentation, at the time of our evaluation, the MWF in each of the four CNC machines had been replaced one time during the two years prior to the NIOSH evaluation. The MWF in two machines had been replaced within the two years before the NIOSH evaluation; the other two machines underwent MWF changes the year before the NIOSH evaluation. The metalworking machines had reservoirs that utilized skimmers to remove tramp oils. MWF was recycled using a gravitational recycling system in the CNC department. MWF was added to the CNC machines when needed, but the dates and amounts of the MWF additions were not documented. The pH of the MWF was reportedly measured every day, but written records could not be provided by the company.

Medical interviews

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. Interviewed employees included production, administrative, and managerial workers. All but three employees (96%) reported noticing unusual odors in the 4 weeks prior to the interview. Hypothesized sources by employees included mold, stagnant water, soil/groundwater, and MWF from the CNC machines.

Of the 81 interviewed employees, 28 (35%) reported a history of hay fever/allergic rhinitis, and 7 (9%) reported a history of asthma. In total, 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%). Skin rash (28%) and respiratory symptoms such as cough (54%), shortness of breath (43%), and wheezing (22%) were also commonly reported. Symptom prevalence was similar among employees who were randomly selected for interviews and those who asked to be interviewed.

Discussion

MWFs are complex mixtures used to cool, lubricate, and remove metal chips from tools and parts during machining of metal stock. MWFs often contain other substances, including biocides, corrosion inhibitors, metal fines, tramp oils, and biological contaminants.[15,16] On the basis of the analytical findings and odor characteristics, it is likely that 3,5-MDMP being produced in MWFs was causing the unpleasant, persistent odor in the facility. No research studies have reported on the toxicity of 3,5-MDMP. However, like other methoxypyrazines, this isomer has a very low odor threshold (at part per billion concentrations) and its odor has been characterized as disagreeable and musty. The isomer has also been associated with malodorous MWFs.[17,18]

Bacteria belonging to Proteobacteria, the same Gram negative phylum that produces 3,5-MDMP, were identified in our analysis of air and bulk MWF samples, and may be contributing to the high concentrations of endotoxin found in the MWF. We also found MWF mist in the air including in areas outside of where the fluids are used, demonstrating that MWF migration is not well controlled and mist moves from production areas into non-production areas. 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*.[18] These organisms were identified as the source of 3,5-MDMP in tainted wine corks, with *Rhizobium excellensis* believed to be the main source.[19] *Rhizobium* bacterial species are generally 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. The compound found by the consultant, 3,6-MDMP, does not have any known microbial source.

Over 90% of interviewed employees reported symptoms they experienced in the previous 4 weeks that they believed to be work-related. Many of the symptoms reported by these employees, such fatigue, headache, eye irritation, and runny nose/congestion, are common in the general population.[20–22] 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.[23] 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.[24,25]

This evaluation had three primary limitations. First, biofilms were not sampled. Biofilms are very complex and can be difficult to remove once they are established.[26,27] Bacteria within the biofilm may or may not be represented in bulk fluid samples. Biofilms can provide inoculating bacteria, which repopulate fresh MWF after cleaning or maintenance.[27] Second, the environmental sampling took place over 2 days and may not be representative of daily conditions in the facility. Third, only a subset of employees at the facility were interviewed. Thus, the self-reported health symptoms may not be generalizable to the entire employee population.

Conclusion and recommendations

2-Methoxy-3,5-dimethylpyrazine (3,5-MDMP) was found in area air and bulk headspace samples of MWF and was the likely cause of the persistent, unpleasant odor. 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. As part of the evaluation, several recommendations were provided to the employer. Following are the subset of recommendations that are related to metalworking and MWF maintenance and that may address odor prevention and remediation.

We recommended that the company implement several engineering controls. First, they needed to isolate the CNC department from other areas in the facility to prevent fugitive metalworking mist from migrating to adjacent areas. Possible isolation methods may be spatial isolation, such as constructing walls or moving equipment to a less central location in the plant and providing additional dedicated ventilation. Avoid recirculation of air from the CNC department into other areas of the facility. Maintain negative pressure relative to adjacent areas once the CNC department is isolated. The company should modify the facility ventilation systems to incorporate outdoor air supply to paraloft, component repair, document control, and the inspection departments. Last, increasing the height of the exhaust air release point could be raised above the roofline to decrease the likelihood of reentrainment of building exhaust air.

Beyond engineering controls, the company should institute administrative controls to improve the quality of the metalworking fluid and prevent future contamination. We recommended they develop a decontamination plan for the CNC machines, recycling system containers, and all instrumentation that contacts MWF during normal operations. When developing a decontamination plan, the company needed to consider the persistence potential of biofilms that may re-inoculate fresh MWF. Once all components of the MWF system have been decontaminated, all used MWF should be disposed of and replaced with new MWF. To prevent future contamination, the company should roll out a MWF management plan. They can consult with the MWF manufacturer to identify best practices for MWF maintenance and recycling, including change out and cleaning schedules as well as procedures for troubleshooting odors. A good MWF management plan includes procedures for maintaining MWF, guidelines for MWF testing and analysis, and employee training plans and records. The management plan should include maintaining and monitoring MWFs undergoing the recycling process. If not properly maintained, the recycling reservoirs can harbor undesirable and unexpected bacteria.

In the interest of reducing odors and maintaining quality of the ventilation system, the company should 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 in the air handlers.

Finally, we recommended that the company encourage employees with work-related health concerns to seek medical care from qualified medical professionals.

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