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# Report on Respiratory and Dermal Conditions among Machine Shop Workers

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DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Centers for Disease Control and Prevention



**The employer shall post a copy of this report for a period of 30 calendar days at or near the workplace(s) of affected employees. The employer shall take steps to insure that the posted determinations are not altered, defaced, or covered by other material during such period. [37 FR 23640, November 7, 1972, as amended at 45 FR 2653, January 14, 1980].**

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## ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
CNC	Computer numerical control
DNA	Deoxyribonucleic acid
HHE	Health Hazard Evaluation
L	liters
mg/m <sup>3</sup>	milligrams per cubic meter of air
MWF	metalworking fluid
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
REL	recommended exposure limit
TWA	time-weighted average
PCR	polymerase chain reaction
LAL	Limulus amoebocyte lysate

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# HIGHLIGHTS OF THE NIOSH HEALTH HAZARD EVALUATION

**NIOSH received a confidential request to conduct a health hazard evaluation at Superior Industries International, Inc. in Pittsburg, Kansas. Workers reported respiratory and skin problems that they related to the metalworking fluid (MWF), or coolant, used in the machine shop.**

## ***What NIOSH Did:***

- Conducted telephone interviews with workers, company management and safety officials, treating physicians, and the director of the company's referral occupational health clinic
- Reviewed medical records
- Reviewed records of MWF and air monitoring conducted by the company
- Tested samples of MWF collected in the machine shop

## ***What NIOSH Found:***

- Some workers in the machine shop have had work-related respiratory and skin problems that have been shown in the scientific literature to be associated with exposure to MWF
- Workers are hesitant to share health and safety concerns with management
- Workers reported not receiving training on the health risks associated with exposure to MWF
- Culture tests showed MWF had no or low growth of bacteria, fungi, and mycobacteria
- Additional non-culture tests showed MWF contained products of fungi and mycobacteria, specifically *Mycobacterium immunogenum*
- Ventilation in the machine shop is limited to general exhaust
- Workers in the machine shop are not in the facility's respiratory protection program
- Workers in the machine shop can be seen at the referral occupational health clinic, but are not in a medical surveillance program

## ***What Superior Industries International Managers Can Do:***

- Foster open communication with workers about health and safety issues
- Provide workers with training on MWF, including information on symptoms associated with exposure to MWF
- Continue air monitoring and include personal sampling

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## HIGHLIGHTS OF THE NIOSH HEALTH HAZARD EVALUATION (CONTINUED)

**NIOSH found that workers in the machine shop have respiratory and skin exposure to MWF. Exposure to MWF has been shown in the scientific literature to be associated with respiratory and skin conditions. NIOSH recommends that management reduce workers' exposure to MWF by installing local ventilation and providing workers with personal protective equipment, including respirators and gloves.**

- Continue monitoring the in-use MWF
- Add local exhaust ventilation to machines using MWF
- Include machine shop workers in the company's respiratory protection program
- Provide protective clothing including gloves to all workers who have skin contact with MWF
- Establish a medical surveillance program for workers exposed to MWF

### ***What Superior Industries International Workers Can Do:***

- Use personal protective equipment such as respirators and gloves
- Participate in a medical surveillance program
- Report respiratory and skin problems to safety officials and the referral occupational health clinic

**On May 25, 2007, NIOSH received a confidential HHE request from workers at Superior Industries International, Inc. in Pittsburg, Kansas. Workers reported respiratory and skin problems that they related to the metalworking fluid (MWF), or coolant, used in the machine shop. The NIOSH investigation found that workers in the machine shop have respiratory and dermal exposure to MWF and symptoms consistent with that exposure. NIOSH recommends that management provide training on MWF to exposed workers, conduct environmental monitoring that includes personal sampling, implement local ventilation, provide personal protective equipment including respirators and gloves, and establish a medical surveillance program.**

On May 25, 2007, the National Institute for Occupational Safety and Health (NIOSH) received a confidential Health Hazard Evaluation (HHE) request from workers at the Superior Industries International, Inc. facility in Pittsburg, Kansas. Workers reported recurrent pneumonias, asthma, and other respiratory symptoms as well as rashes and skin irritation that they related to the metalworking fluid (MWF), or coolant, used in the facility's machine shop. Exposure to MWF is associated with respiratory conditions, including asthma, bronchitis, and hypersensitivity pneumonitis, as well as with dermatitis [NIOSH 1998]. NIOSH has established a Recommended Exposure Limit (REL) for MWF in the air of 0.4 mg/m<sup>3</sup> (thoracic particulate mass), as a time-weighted average [TWA] for up to 10 hours. This level corresponds to 0.5 mg/m<sup>3</sup> for total particulate mass.

NIOSH investigators conducted telephone interviews with workers, treating physicians, company management and safety officials, and the director of the company's referral occupational health clinic. They reviewed medical records and environmental monitoring conducted by the company. They also conducted microbiological tests on samples of MWF collected from the machine shop.

The investigators found that workers' symptoms and diagnoses were consistent with those associated with exposure to MWF. Workers in the machine shop reported not receiving training on the health hazards of MWF and not being provided respiratory protection; furthermore, they are not in a medical surveillance program. Operations are enclosed, but ventilation is limited to general exhaust and workers handling the automobile wheels have skin contact with MWF. Environmental monitoring conducted by the company showed MWF air levels above the NIOSH REL, but no or low growth of bacteria and fungi in the MWF. Analyses of MWF by NIOSH confirmed the minimal microbial growth, but did demonstrate the presence of mycobacterial DNA and fungal products.

NIOSH recommends that management provide training on MWF to exposed workers, conduct environmental monitoring that includes personal sampling, implement local ventilation, provide personal protective equipment including respirators and gloves, and establish a medical surveillance program aimed at early identification of MWF-related respiratory and dermal conditions.

**Keywords:** metalworking fluid, occupational asthma, hypersensitivity pneumonitis, dermatitis, mycobacteria

On May 25, 2007, the National Institute for Occupational Safety and Health (NIOSH) received a confidential Health Hazard Evaluation (HHE) request from workers at Superior Industries International, Inc. in Pittsburg, Kansas. The requesters described recurrent pneumonias, asthma, and other respiratory symptoms as well as rashes and skin irritation among workers in the facility's machine shop. They expressed concern about respiratory and skin exposures to the metalworking fluid (MWF), or coolant, used in the machine shop.

Occupational exposure to MWF is associated with respiratory illnesses including lipid pneumonia, legionellosis, hypersensitivity pneumonitis, asthma, and chronic bronchitis [NIOSH, 1998]. Lipid pneumonia and legionellosis have been reported rarely in recent decades. However, hypersensitivity pneumonitis, an allergic pneumonia, has been the subject of more recent reports of workers exposed to MWF [CDC 1996; Kreiss, Cox-Ganser 1997; Freeman et al. 1998; Zacharisen et al. 1998; Fox et al. 1999; Shelton et al. 1999; Hodgson et al. 2001; CDC 2002; Bracker et al. 2003; Trout et al. 2003; Beckett et al. 2005; Dawkins et al. 2006; Gupta, Rosenman 2006; Robertson et al. 2007]. In some recent investigations of outbreaks of MWF-associated hypersensitivity pneumonitis, other respiratory illnesses, including asthma and chronic bronchitis, have been found in co-workers [Kreiss, Cox-Ganser 1997; Zacharisen et al. 1998; Hodgson et al. 2001; Robertson et al. 2007].

It is not certain which components or contaminants of MWF are responsible for the development of respiratory illness in exposed workers. For hypersensitivity pneumonitis, evidence points to organisms that grow in MWF, such as bacteria, mycobacteria, and fungi [Kreiss, Cox-Ganser 1997; NIOSH 1998; Fox et al. 1999; Shelton et al. 1999; CDC 2002; Beckett et al. 2005; Robertson et al. 2007]. When inhaled, these organisms or their products may cause an allergic response in the lungs of some workers. Organisms have typically been identified through their growth in culture, although allergic sensitization to bacteria is independent of their culturability [Veillette et al. 2004]. In a recent investigation of 19 cases of hypersensitivity pneumonitis at a manufacturing facility, bacteria did not grow in the facility's MWF, but an association was found between illness and bacterial genetic material (deoxyribonucleic acid, or DNA) detected in the facility's MWF [Robertson et al. 2007]. For asthma, which may be irritant or allergic, evidence points to organisms, as well as to

MWF components and additives or by-products, including amines, chlorine, and formaldehyde [NIOSH 1998].

NIOSH recommends keeping the concentration of MWF in the air to 0.4 mg/m<sup>3</sup> (thoracic particulate mass), as time-weighted average (TWA) for up to 10 hours, corresponding to 0.5 mg/m<sup>3</sup> for total particulate mass [NIOSH 1998]. This recommended exposure limit (REL) reduces, but does not eliminate, respiratory illnesses associated with MWF, as some workers have developed hypersensitivity pneumonitis and asthma when exposed to MWF at lower concentrations [NIOSH 1998]. Because respiratory illness can develop even at levels below the REL, medical monitoring of workers exposed to MWF is recommended [NIOSH 1998; OSHA 1999; Cohen, White 2006].

Workers with exposure to MWF are also at risk for skin conditions [NIOSH 1998]. Components and contaminants of MWF may cause irritant contact dermatitis or allergic contact dermatitis. Reducing skin exposure to MWF is essential to preventing MWF-associated dermatitis [NIOSH 1998; OSHA 1999].

### Process Description

Superior Industries International, Inc. supplies cast and forged aluminum road wheels for the original equipment automotive industry. The facility in Pittsburg, Kansas is one of nine manufacturing facilities operated by Superior Industries International, Inc. in the United States, Mexico, and Europe. In addition to the machine shop, the facility includes a foundry, where an aluminum alloy is melted and cast, and a coating shop, where wheels are painted and clear coated. The company is a participant in the OSHA Voluntary Protection Program.

The machine shop consists of 29 lines where computer numerical control (CNC) lathes and drilling machines are used to shape the wheels. Wheels are loaded and unloaded by robots and the automated machining operations are enclosed. After automated machining, workers deburr the wheels by hand. Ventilation in the machine shop consists of a general exhaust system with 17 exhaust fans. Individual machines do not have local exhaust systems. At the time of the investigation, the machine shop operated 24 hours per day, with two 12-hour shifts. There were approximately 100 workers in the machine shop, divided between the two shifts. Workers in the machine shop are not in a respiratory protection program.

Each machine has an individual MWF reservoir that is connected to a common system, with the exception of one machine (29), which has its own sump not connected to the common system. MWF is used as a lubricant and coolant during the automated machining and as a means of collecting pieces of aluminum cut from the wheels. Such pieces are carried via canals (“sharks”) under the machine shop to a filtration area, the chip recovery system (labeled “pre-melt” in Appendices B, C, and D), where the aluminum is recovered. The canals are visible through overlying grating in the machine shop floor. The capacity of the system is approximately 70,000 gallons. Since 2004, the facility has been using a soluble mineral oil MWF that is promoted as minimizing microbial growth and the need for the addition of biocides.

The facility monitors the in-use MWF to evaluate the performance characteristics of the MWF. The facility provides a sample to the manufacturer weekly and receives a report that includes MWF concentration, pH, chloride concentration, and bacterial and fungal counts as well as recommendations for MWF management. The amount of bacteria and fungi in the sample is determined by using dip slides with bacterial growth agar on one side and fungal growth agar on the other. The dip slide is coated with the MWF sample and incubated for several days, after which counts are determined from a colorimetric assay. The dip slide tests are not designed to identify mycobacteria or other bacteria and fungi that require unusual growth factors or prolonged incubation. Since the introduction of this MWF at the facility in 2004, the manufacturer has made recommendations to add MWF but has not recommended addition of biocides or change-out of the MWF. The facility most recently monitored the air in the machine shop in 2004 and 2006. This monitoring was conducted by the corporate industrial hygienist. Analysis was conducted for oil mist, metals including aluminum, and several organic compounds.

A physician, industrial hygienist, and epidemiologist from the NIOSH Division of Respiratory Disease Studies conducted the investigation. The investigators interviewed workers, company management and safety officials, and treating physicians by telephone to assess the machine shop layout, work processes, potential exposures, and health problems encountered by workers. They reviewed results from MWF and air monitoring conducted by the company. They also communicated with investigators conducting a concurrent Occupational Safety and Health Administration (OSHA) inspection of the facility; identifying worker information was not shared with OSHA. The investigators discussed medical surveillance of workers with the director of the occupational health clinic at Mt. Carmel Regional Medical Center, which was identified by company safety officials as the facility's referral occupational health clinic.

The NIOSH investigation included tests on MWF collected at the facility to assess for the presence of microbes, such as bacteria and fungi. Two types of tests were conducted: those that detect organisms by seeing if they grow under laboratory conditions (culture tests) and those that detect organisms by seeing if some unique material made by the organism is present (non-culture tests). On June 28, 2007, samples were collected for NIOSH by a resident physician rotating at NIOSH, with the assistance of company representatives. On November 28, 2007, additional samples were collected for NIOSH by an OSHA industrial hygienist conducting a concurrent inspection of the facility. On both occasions, samples were collected from the following machine shop locations: the reservoirs of machines (lathes) 1, 8, 13, 20, 25, 26, 27, 28, and 29; the east and west sharks; and the chip recovery system (labeled "premelt" in Appendices B, C, and D). The samples were shipped overnight directly to the laboratories and chain of custody was followed.

The samples collected on June 28, 2007, were analyzed by culture for bacteria, mycobacteria (a special type of bacteria), and fungi by EMLab P&K, a commercial laboratory. If organisms grew in culture, speciation techniques were used to determine which species were present. Details on the culture media, incubation temperature, and incubation times used for the cultures are contained in Appendix B. EMLab P&K also conducted a non-culture test to determine the level of endotoxin, a compound produced by gram negative bacteria, in each sample. For this test, the lab used the *Limulus amoebocyte lysate* (LAL) chromogenic

kinetic assay. Information about the endotoxin test can be found in Appendix B. In addition to these tests, NIOSH laboratory specialists conducted a non-culture test to determine the level of (1→3)-β-D-glucan (“glucan”), a cell wall component of fungi, using a glucan-specific LAL assay. The glucan-specific LAL assay allows measurement of glucan without interference from endotoxin. Endotoxin indicates the presence of gram negative bacteria and glucan indicates the presence of fungi, even if the organisms themselves do not grow in culture.

The samples collected on November 28, 2007, were analyzed for mycobacterial DNA using polymerase chain reaction (PCR) amplification by Microbe Inotech Laboratories (MiL), Inc., a commercial laboratory. DNA is the genetic material of mycobacteria. If mycobacterial DNA was detected, further tests (melt curves) were used to determine which species were present. Details on the PCR techniques and melt curves are contained in Appendix D. PCR can detect the presence of mycobacterial DNA, even if the mycobacteria themselves cannot be cultured.

NIOSH investigators interviewed six machine shop workers by telephone. Workers described a “haze” or “fog” of MWF that is continuously visible in the machine shop, but worse in winter months when less outdoor air is introduced. They indicated that skin and clothing contact with MWF occurs during deburring and other machine shop tasks.

Five of the workers described one or more respiratory symptoms, including cough, wheeze, chest tightness, chest discomfort, and shortness of breath, that get better when away from the machine shop. Two workers have been diagnosed by a pulmonologist with occupational asthma.

One worker experienced recurrent pneumonias. In one episode, after time away from the machine shop, this worker experienced chest discomfort, cough, and shortness of breath that began 4-5 hours into a shift. These symptoms were followed by fever, chills, and sweats. This constellation of symptoms was concerning to the worker’s pulmonologist for hypersensitivity pneumonitis.

Four of the workers described skin irritation and rash that get better when away from the machine shop or when they use personal protective equipment that reduces skin contact with MWF, such as gloves and aprons. Several workers also described work-related eye irritation and nasal symptoms.

Workers described a lack of trust in the management and safety officials at the facility with regard to health and safety issues. They reported receiving no training on the symptoms and illnesses associated with occupational exposure to MWF. Workers who did ask questions of safety officials about health risks described being provided with misinformation.

The company’s lack of open communication about possible health effects of MWF exposure has contributed to a climate of suspicion among machine shop workers. In the absence of accurate information, workers have turned to speculation about changes to the concentration of the fluid, addition of biocides or other chemicals, overgrowth of bacteria, and activities that occur during the annual facility shut-down.

Records of MWF monitoring conducted by the facility and MWF manufacturer on a weekly basis from May 2004 through June 2007 demonstrate that the MWF concentration varied from 3.7%

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## RESULTS (CONTINUED)

(on January 18, 2006) to 11.3% (on January 23, 2007) (Appendix A). Most readings fell in the range of 7-9%, close to the 8% recommended by the MWF manufacturer. The pH during this period varied little, staying close to 9.0. These records also show no or low growth of bacteria and no growth of fungi.

Records of air monitoring show that sampling was conducted on January 29, 2004 and October 31, 2006 (Appendix A). The records do not indicate where in the facility the samples were taken. The corporate industrial hygienist reported that samples were collected using NIOSH sampling protocols over 8 hours. The 2004 samples had oil mist concentrations of 1.133 mg/m<sup>3</sup> and <0.11 mg/m<sup>3</sup>. The 2006 samples had oil mist concentrations of 0.802 mg/m<sup>3</sup> and 0.653 mg/m<sup>3</sup>. While all samples were below the OSHA permissible exposure limit (PEL) of 5 mg/m<sup>3</sup>, all samples but one were in excess of the NIOSH REL.

None of the 12 samples of MWF collected for the NIOSH HHE on June 28, 2007, grew mycobacteria or fungi (Appendix B). Each sample grew bacteria, ranging from 200 colony forming units per milliliter (CFU/ml) (2 colonies) from the machine 29 sample to 2700 CFU/ml (27 colonies) from the east shark sample. These concentrations represent very low concentrations of bacteria. Bacteria included *Bacillus* species (generally gram-positive organisms), other gram-positive bacilli, and gram-positive cocci. Most of the bacteria that were cultured are commonly found in soil and/or on human skin. None of the bacteria that were cultured typically causes infection in humans under normal conditions, although some may occasionally cause infection in persons with weakened immune systems or under unusual circumstances, such as in the setting of traumatic injury. *Bacillus cereus* can cause toxin-mediated food poisoning if food on which it is growing is ingested.

Endotoxin levels ranged from 52 endotoxin units per milliliter (EU/ml) in machine 29 sample to 150 EU/ml in the samples taken from machines 8 and 26, the east and west sharks, and the premelt (Appendix B). These endotoxin levels are very low, consistent with the lack of growth of gram-negative bacteria in the cultures. Eleven of the samples were positive for the presence of glucan; the machine 29 sample was negative for glucan (Appendix C). The average glucan level in the 11 positive samples was 185.0 ng/ml, with a range of 133.8 to 266.1 ng/ml. These glucan levels

indicate the presence of fungi in the MWF.

Of the 12 samples collected on November 28, 2007, 11 were positive for the presence of mycobacterial DNA, specifically *Mycobacterium immunogenum* (Appendix D). The machine 29 sample was negative for mycobacterial DNA. *Mycobacterium immunogenum*, a rare species of mycobacteria related to *Mycobacterium chelonae*, has been found in MWF in some investigations of work-related hypersensitivity pneumonitis [Kreiss, Cox-Ganser 1997; Fox et al 1999; Shelton et al. 1999; Wilson et al 2001; Trout et al 2003; Beckett 2005; Gupta, Rosenman 2006].

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## CONCLUSIONS

Occupational exposure to MWF is known to be associated with respiratory illnesses and skin problems. It is not certain which compounds in MWF are responsible. Possible causes include exposure to MWF components, additives, by-products, and microbes, such as bacteria, mycobacteria, and fungi. In the absence of certainty about the cause of symptoms and the nature of the dose-response relationship, reducing workers' exposure to MWF through engineering controls and personal protective equipment is necessary. Both NIOSH and OSHA provide guidelines for reducing workers' exposure to MWF [NIOSH 1998; OSHA 1999]. Furthermore, because respiratory disease has occurred with exposures below the NIOSH REL, ongoing monitoring of workers for symptoms is prudent [NIOSH 1998; OSHA 1999].

Workers in the machine shop at the Superior Industries International, Inc. facility in Pittsburg, Kansas have had work-related respiratory and skin problems consistent with those associated with exposure to MWF. Management has been proactive about conducting routine monitoring of in-use MWF to detect, among other things, gross overgrowth of bacteria and fungi. The lack of growth on these tests is an encouraging outcome that reflects good MWF maintenance practices. However, these results do not represent an exhaustive microbial characterization of the in-use MWF in the machine shop and should not be interpreted to mean that no MWF-related health risk exists. A study comparing culture to the direct count method showed that the culture method identified less than 1% of the microbial mass present in MWF [Veillett et al. 2004].

The detectable glucan and mycobacterial DNA indicate that both fungal and mycobacterial products were present in the in-use MWF. This finding suggests that viable fungi and mycobacteria may have been present in the past and may be currently present in the in-use MWF, despite the lack of growth of these organisms by culture tests. Regardless of the original source of organisms, once they have become established in a MWF system, it is difficult to eliminate such organisms by changing the MWF [Veillette et al. 2004]. Adding biocides in attempt to eliminate such organisms may expose workers to additional health risks related to the biocides themselves [NIOSH 1998]. Thus the reduction of exposures through engineering controls and personal protective equipment should be emphasized. Currently in the machine shop, ventilation is limited to general exhaust and workers are not provided with respiratory protection.

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## CONCLUSIONS (CONTINUED)

Open communication with workers about the health risks associated with exposure to MWF and training on the ways to reduce risk is recommended by both NIOSH and OSHA [NIOSH 1998; OSHA 1999]. Workers in the machine shop reported that they do not receive training on the health risks associated with exposure to MWF and that they are hesitant to share health and safety concerns with management. Workers who have shared concerns described receiving inaccurate information from management and safety officials.

The best evidence that MWF exposures are being controlled may be that workers do not experience MWF-related symptoms. However, even if most workers experience improvement in their symptoms after controls are instituted, and new workers remain free of symptoms, some workers with allergic conditions may not show improvement. Because their immune systems may continue to react to very small amounts of substances to which they are allergic, such individuals may have to avoid exposure to MWF even after otherwise successful controls are introduced. An individualized management plan (such as assigning an affected worker to a different work location) is sometimes required, depending upon medical findings and recommendations of the individual's physician.

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## RECOMMENDATIONS

### 1) Communication and Training:

Foster open communication among management, safety officials, and workers about the health risks of MWF. Training about MWF should include accurate information about the adverse health effects associated with exposure to MWF and how exposure can be reduced. Training about MWF should be provided at the time of initial job assignment, to current workers who have not been previously trained, whenever a new and significantly different MWF is introduced, and whenever a new way of protecting workers is introduced [OSHA 1999]. Details on designing a MWF training program can be found in the OSHA MWF Best Practices manual ([http://www.osha.gov/SLTC/metalworkingfluids/metalworkingfluids\\_manual.html#f](http://www.osha.gov/SLTC/metalworkingfluids/metalworkingfluids_manual.html#f)).

### 2) Environmental Monitoring:

Continue to conduct monitoring of air and in-use MWF. The goal of air monitoring is to ensure a more healthful work environment where worker exposures do not exceed the NIOSH REL. However, because adverse health effects can occur below the REL, lower exposures are desirable whenever feasible. The initial air sampling survey should collect representative personal samples for the entire work shift. All routine personal samples should be collected in the breathing zones of the workers.

Surveys should be repeated annually and whenever any major process changes take place. More frequent monitoring should be undertaken in workers with higher exposure. Airborne exposure measurements should be taken at least every six months for workers whose exposures are one-half or more of the REL. For workers exposed to MWF at concentrations above the REL, more frequent monitoring should be maintained until at least two samples indicate that the workers' exposure no longer exceeds the REL. All workers should be notified of monitoring results and of any control actions being undertaken to reduce their exposures. Further details on environmental monitoring can be found in the NIOSH Criteria Document (<http://www.cdc.gov/niosh/98-102.html>).

### 3) Engineering controls:

Implement engineering controls to reduce workers' exposure to MWF. The current machine enclosures serve to reduce the

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## RECOMMENDATIONS (CONTINUED)

amount of MWF in the air in the machine shop. However, air levels remain above the NIOSH REL, demonstrating that local exhaust ventilation for each machine is needed. Automated or power-assisted handling equipment to reduce workers' skin contact with MWF while handling wet wheels also should be considered.

#### 4) Personal protective equipment:

Engineering controls should be the primary means of reducing workers' exposure to MWF. However, in the event of airborne exposures that exceed the NIOSH REL or of skin contact with MWF, personal protective equipment should be provided to machine shop workers.

While engineering controls are being instituted and for intermittent tasks that expose workers to concentrations above the NIOSH REL, respiratory protection should be provided. A formal respiratory protection program that adheres to the requirements of the OSHA Respiratory Protection Standard (29 CFR 1910.134) is required. The program administrator for the program must have adequate training and experience to run it and regularly evaluate its effectiveness. Details on the Respiratory Protection Standard and on how a company can set up a respiratory protection program are available on the OSHA website (<http://www.osha.gov/SLTC/respiratoryprotection/index.html>).

For tasks that result in skin contact with MWF, protective clothing should be provided. Workers should wear face shields or goggles, protective sleeves, aprons, trousers, caps, and gloves as needed to protect skin. For gloves, data indicate that nitrile affords the most chemical resistance of chemical protective materials and provides flexibility and resistance to abrasion, tears, and punctures [NIOSH 1998].

#### 5) Medical Surveillance:

Establish a medical surveillance program for machine shop workers and any other workers exposed to MWF, for the early identification of workers who develop symptoms of MWF-related conditions such as asthma, hypersensitivity pneumonitis, and dermatitis. Medical surveillance should be directed and supervised by a qualified and licensed physician who periodically reviews a worker's health status. This review should include a worker-completed questionnaire addressing respiratory and dermal symptoms and their work-

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## RECOMMENDATIONS (CONTINUED)

relatedness, as well as a physical examination directed at the lungs and skin. Pulmonary function testing also can be included. Workers identified by medical surveillance as having respiratory or skin problems potentially related to MWF should undergo further medical evaluation. Medical surveillance and follow-up medical evaluations should be provided at no cost to workers.

Newly hired or transferred workers should undergo a pre-placement evaluation to determine a baseline status. All workers in the medical surveillance program should undergo periodic evaluations. Annual evaluation is reasonable in the absence of new MWF-related symptoms. However, if medical surveillance reveals that one or more workers has developed lung or skin problems related to MWF, evaluations should occur more frequently. Aggregate analyses of medical surveillance data can be useful to safety officials for identifying risks while maintaining the confidentiality of the results for individual workers.

Further information on establishing a medical surveillance program can be found in the NIOSH Criteria Document (<http://www.cdc.gov/niosh/98-102.html>) and in the OSHA MWF Best Practices manual ([http://www.osha.gov/SLTC/metalworkingfluids/metalworkingfluids\\_manual.html#f](http://www.osha.gov/SLTC/metalworkingfluids/metalworkingfluids_manual.html#f)).

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# APPENDIX A: ENVIRONMENTAL MONITORING DATA

Jun 21 2007 3:00PM

HP LASERJET FAX

5013622033

p. 1



*Air monitoring data*  
7 pgs.

**ANALYTICS CORPORATION**  
8040 VILLA PARK DRIVE, SUITE 250  
RICHMOND, VIRGINIA 23228  
804-264-7100 PHONE  
800-888-8061 PHONE  
804-264-8673 FAX  
WWW.ANALYTICSCORP.COM

Sup No. I034-042  
Account No. 03801005  
Report Date: 02/12/04

TIM BARRY  
SUPERIOR INDUSTRIES INT'L INC  
AUTOMOTIVE COMPONENTS DIVISION  
424 INDUSTRIAL PARK RD  
HEBER SPRINGS, AR 72543

*Pittsburg  
Coating Machine  
Shotblast, casting*

\*\*\*\* FINAL REPORT \*\*\*\*

Date Received: 02/03/04  
Sample Type: 9 - Air Sample(s)  
Project: SUP. PITTSBURG

PO Number: 957434

*1-04*

## Analytical Results

Lab	Parameter	Volume	Amount	LOQ	Concentration	Analysis
001	1-1-29-04 Oil Mist	Samp Date: 01/29/04 600 L	679.8 ug	0.8 micron MCE filter 50 ug	1.133 mg/M3	02/06/04
002	2-1-29-04 Aluminum Chromium Copper Iron Magnesium Manganese Nickel	Samp Date: 01/29/04 455 L 455 L 455 L 455 L 455 L 455 L 455 L	20.9 ug 2.58 ug 1.45 ug 773 ug 3.35 ug 6.64 ug < 2.00 ug	0.8 micron MCE filter 2 ug 2 ug 1 ug 2 ug 2 ug 2 ug 2 ug	0.046 mg/M3 0.006 mg/M3 0.003 mg/M3 1.70 mg/M3 0.007 mg/M3 0.015 mg/M3 < 0.004 mg/M3	02/05/04 02/05/04 02/05/04 02/05/04 02/05/04 02/05/04 02/05/04
003	3-1-29-04 Total Dust Oil Mist	Samp Date: 01/29/04 455 455 L	0.235 mg < 50.0 ug	5um Preweighed PVC Filter .05 mg 50 ug	0.516 mg/M3 < 0.11 mg/M3	02/04/04 02/06/04
004	4-1-29-04 HCHO-Net Total	Samp Date: 01/29/04 15.90	4.84 ug	UMEX Formaldehyde Badge .2 ug	0.25 ppm	02/05/04
005	5-1-29-04 HCHO-Net Total	Samp Date: 01/29/04 17.16	8.20 ug	UMEX Formaldehyde Badge .2 ug	0.39 ppm	02/05/04
006	6-1-29-04 VMP Naph-Total	Samp Date: 01/29/04 18.46	< 75.0 ug	3M 3500 ORGANIC POVM 75 ug	< 4.10 mg/M3	02/05/04
007	7-1-29-04 THCH-Total	Samp Date: 01/29/04 14.56	< 30.0 ug	3M 3500 ORGANIC POVM 30 ug	< 2.06 mg/M3	02/06/04
008	8-1-29-04	Samp Date: 01/29/04		0.8 micron MCE filter		

Page 1

# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

Jun 21 2007 3:00PM

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**ANALYTICS CORPORATION**  
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Group No. I034-042  
Account No. 03801005  
Report Date: 02/12/04

IM BARRY  
SUPERIOR INDUSTRIES INT'L INC  
AUTOMOTIVE COMPONENTS DIVISION  
24 INDUSTRIAL PARK RD  
WEBER SPRINGS, AR 72543

Final Report

Date Received: 02/03/04

Sample Type: 9 - Air Sample(s)

Project: SUP.PITTSBURG

PO Number: 957434

## Analytical Results

Lab	Parameter	Volume	Amount	LOQ	Concentration	Analysis
-	Aluminum	543 L	12.8 ug	2 ug	0.024 mg/M3	02/05/04
009	9-1-29-04 Samp Date: 01/29/04			3M 3500 ORGANIC	POVM	
-	DPGME-Total	14.07	< 30.0 ug	30 ug	< 0.35 ppm	02/06/04

Mineral oil used as standard reference.

Abbreviations: ug = micrograms, mg = milligrams, mg/M3 = milligrams per cubic meter of air, g = grams, ug/M3 = micrograms per cubic meter of air, L = liters, w/w = percent weight basis, all Volumes given in liters, ppm = parts per million, ppb = parts per billion, Areas given in square feet; ND = Not Detected; ug/wp = ug/wipe; NVG = No Volume Given.

Page 2

# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

Jun 21 2007 3:00PM

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p.3



**ANALYTICS CORPORATION**  
 8040 VILLA PARK DRIVE, SUITE 280  
 RICHMOND, VIRGINIA 23228  
 804-264-7100 PHONE  
 800-888-8061 PHONE  
 804-264-8873 FAX  
 WWW.ANALYTICSCORP.COM

Group No. I034-042  
 Account No. 03801005  
 Report Date: 02/12/04

TIM BARRY  
 SUPERIOR INDUSTRIES INT'L INC  
 AUTOMOTIVE COMPONENTS DIVISION  
 424 INDUSTRIAL PARK RD  
 HEBER SPRINGS, AR 72543

Final Report

## Summary of Analytical Methods

Compound Name	Analytical Method	Abbreviation
Aluminum	NIOSH 7300	----
Chromium	NIOSH 7300	----
Copper	NIOSH 7300	----
Dipropylene glycol methyl ether	NIOSH Method 1403M	DPGME
Iron	NIOSH 7300	----
Formaldehyde-Net Total	NIOSH Method 2016M	HCHO-Net Total
Magnesium	NIOSH 7300	----
Manganese	NIOSH 7300	----
Nickel	NIOSH 7300	----
Oil Mist	NIOSH 5026	----
Total Hydrocarbons as Hexane	NIOSH Method 1500	THCH
Total Dust	NIOSH 0500	----
M&P Naphtha	NIOSH Method 1550	VMP Naph

Notes

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# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

Jun 21 2007 3:00PM HP LASERJET FAX

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p. 4



**ANALYTICS CORPORATION**  
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Group No. I034-042  
Account No. 03801005  
Report Date: 02/12/04

TIM BARRY  
SUPERIOR INDUSTRIES INT'L INC  
AUTOMOTIVE COMPONENTS DIVISION  
24 INDUSTRIAL PARK RD  
WEBER SPRINGS, AR 72543

Final Report

Date Received: 02/03/04

Sample Type: 9 - Air Sample(s)

Project: SUP.PITTSBURG

PO Number: 957434

Attached are the results we obtained on the analysis of your samples. Any Chains-of-Custody associated with this sample group are also enclosed. Air concentrations are calculated as a convenience to the client and the overall accuracy of this result depends on both the accuracy of the air volume and the amount found by analysis. Theoretical Air Volumes for passive monitors are calculated using the sampling time submitted and the manufacturer's listed sampling rate for each compound.

For blanks and non-detects the results indicated with a '<' value represents the reporting limit for that analysis. Unless otherwise noted results are not corrected for blank values.

Unless the signature of the appropriate manager(s) appears on the final page of this report, this report should be considered PRELIMINARY and is subject to change.

We appreciate your confidence in allowing Analytics to be your testing laboratory. Any questions regarding this report can be addressed by calling our client services department (800-888-8061).

  
James A. Calpin, CIH  
Laboratory Director

End of Report  
Page 4

# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

Jun 21 2007 3:00PM

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p. 5



**ANALYTICS CORPORATION**  
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Group No. I034-042  
Account No. 03801005  
Report Date: 02/12/04

TIM BARRY  
SUPERIOR INDUSTRIES INT'L INC  
AUTOMOTIVE COMPONENTS DIVISION  
424 INDUSTRIAL PARK RD  
HEBER SPRINGS, AR 72543

Final Report

---

## 8-Hour Time-weighted Average Summary

---

Parameter	8-Hr TWA
-----------	----------

---

TWA includes sample(s) 3-1-29-04

Total Dust	.489	mg/M3
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\* Note: All unsampled time is assumed at zero (0) exposure.

# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

Jun 21 2007 2:57PM

HP LASERJET FAX

5013622033

p. 1



**ANALYTICS CORPORATION**  
 10929 Stony Run Lane  
 Ashland, Virginia 23005  
 804-365-3000 Phone  
 800-888-8081 Phone  
 804-365-3002 Fax  
 www.analyticscorp.com

Op No. K310-070  
 Count No. 03801005  
 Report Date: 11/08/06

TIM BARRY  
 SUPERIOR INDUSTRIES INT'L INC

74 WALKER DRIVE  
 HEBER SPRINGS, AR 72543

\*\*\*\* FINAL REPORT \*\*\*\*

Date Received: 11/06/06  
 Sample Type: 4 - Air Sample(s)  
 Project: PITT 10\_06 PO Number: 976588

**Analytical Results**

Lab	Parameter	Volume	Amount	LOQ	Concentration	Analysis
-001	2-10-31-6 Oil Mist	Samp Date: 10/31/06 950 L	5um PVC Filter 762.0 ug	50 ug	0.802 mg/M3	11/07/06
-002	3-10-31-6 Oil Mist	Samp Date: 10/31/06 970 L	5um PVC Filter < 50 ug	50 ug	< 0.052 mg/M3	11/07/06
-003	1-10-31-6 Oil Mist	Samp Date: 10/31/06 960 L	5um PVC Filter 626.9 ug	50 ug	0.653 mg/M3	11/07/06
-004	4-10-31-6 Oil Mist	Samp Date: 10/31/06 0 L	BLANK < 50 ug	5um PVC Filter 50 ug	--	11/07/06

Mineral oil used as standard reference.

Abbreviations: ug = micrograms, mg = milligrams, mg/M3 = milligrams per cubic meter of air, g = grams, ug/M3 = micrograms per cubic meter of air, L = liters, all Volumes given in liters, ppm = parts per million, ppb = parts per billion, Areas given in square feet; ND = Not Detected; ug/wp = ug/wipe; NVG = No Volume Given. NAG = No Area Given, LOQ = Limit of Quantitation.

*M. Mackin*  
*F. Kelly*

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# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

Jun 21 2007 2:57PM HP LASERJET FAX

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p. 2



## ANALYTICS CORPORATION

10329 Stony Run Lane  
Ashland, Virginia 23005

804-365-3000 Phone

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Group No. K310-070  
Account No. 03801005  
Report Date: 11/08/06

TIM BARRY  
SUPERIOR INDUSTRIES INT'L INC

74 WALKER DRIVE  
HEBER SPRINGS, AR 72543

Final Report

### Summary of Analytical Methods

Compound Name	Analytical Method	Abbreviation
Oil Mist	NIOSH 5026	----

### Notes

Results provided in this report relate only to the items tested.

Attached are the results we obtained on the analysis of your samples. Any Chains-of-Custody associated with this sample group are also enclosed. Air concentrations are calculated as a convenience to the client and the overall accuracy of this result depends on both the accuracy of the air volume and the amount found by analysis. Theoretical Air Volumes for passive monitors are calculated using the sampling time submitted and the manufacturer's listed sampling rate for each compound.

For blanks and non-detects the results indicated with a '<' value represents the reporting limit for that analysis. Unless otherwise noted results are not corrected for blank values.

Unless the signature of the appropriate manager(s) appears on the final page of this report, this report should be considered PRELIMINARY and is subject to change.

We appreciate your confidence in allowing Analytics to be your testing laboratory. Any questions regarding this report can be addressed by calling our client services department (800-888-8061).

  
James A. Galpin, CIH  
Laboratory Director

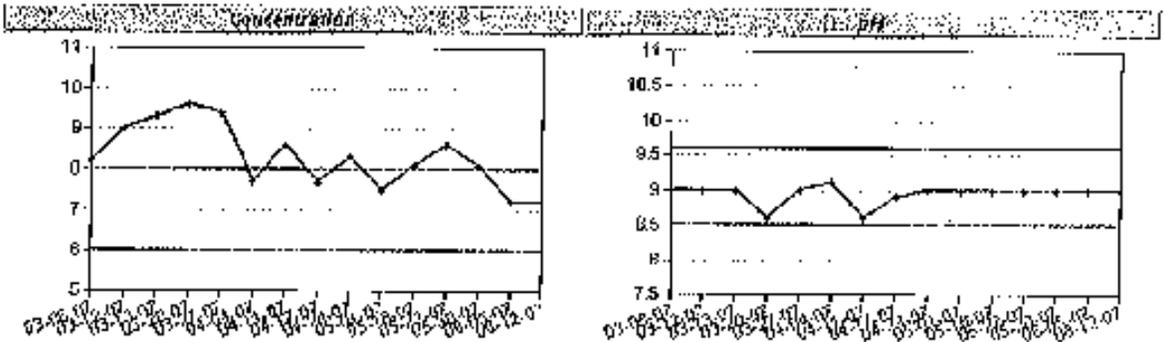
# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

Coolant monitoring data

## Coolant Management Report

<b>Customer Data:</b> SUPERIOR INDUSTRIES - PTTSRUNG 1530 EAST 27TH TERRACE PITTSBURGH, KS 66762-2757	<b>System Data:</b> Central  Capacity 70,000 gal/eng	<b>Report Number:</b> 407-2509 <b>Sample Description:</b> Central <b>Data Complete:</b> 08/18/2007
--	---	--

Sample ID	Date Taken	Date Received	Current Analysis	pH	Temp. Diff. Content in Solution (%)	Iron #105 (ppm)	Dil. 12 (ppm)	Chloride (ppm)	Water Concentration	Fluoride	Ammonia
407-2500	06/13/07	06/19/07	12	8.0	0.5	270	250	575	15.1	120	0
407-2532	06/20/07	07/02/07	22	9.0	0.0	280	190	620	19.7	101	0
407-2534	05/29/07	05/31/07	11	8.0	0.0	280	190	560	14.7	0	0
407-2127	05/22/07	05/23/07	18	9.0	0.1	260	350	450	1.01	100	0
407-2140	05/16/07	05/16/07	11	9.0	0.2	280	320	445	16.8	0	0
407-1856	05/01/07	04/23/07	05	9.0	0.0	255	180	490	10.5	0	0
407-1896	02/27/07	04/23/07	28	9.0	0.0	270	14	460	15.1	0	0
407-1481	04/10/07	04/16/07	11	8.0	0.0	290	70	490	21.5	0	0
407-1082	04/10/07	04/12/07	16	8.5	0.0	280	280	510	12.1	100	0
407-1226	04/04/07	04/05/07	17	8.1	0.7	265	160	560	13.8	0	0
407-1187	03/29/07	03/29/07	14	9.0	0.1	255	110	490	16.3	0	0
407-1215	03/06/07	03/23/07	28	9.0	0.7	280	180	445	10.7	0	0
407-1190	03/12/07	03/14/07	09	9.0	0.5	280	280	445	14.7	100	0
407-0030	02/12/07	03/29/07	30	9.0	0.0	260	270	390	15.1	100	0
407-0835	02/05/07	03/06/07	17	9.0	0.0	290	200	460	17.5	100	0

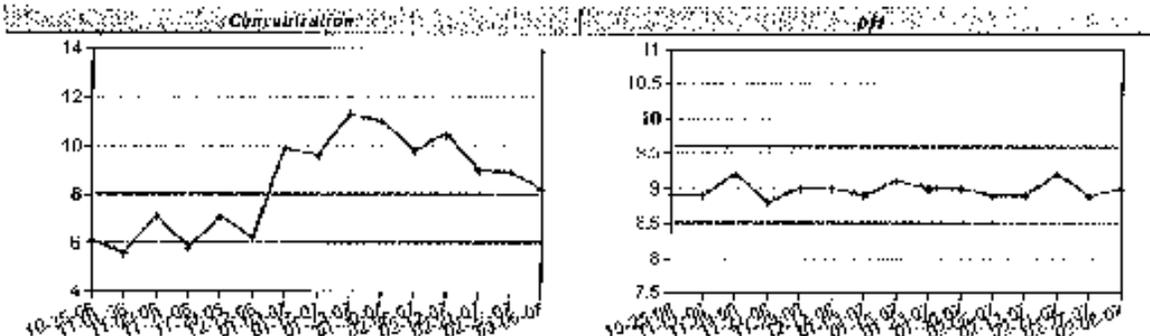


Comments & Recommendations

# Coolant Management Report

<b>Customer Data :</b> SULPHUR INDUSTRIES - PITTSBURG 1500 EAST 27TH TERRACE PITTSBURG, KS 66782-2757	<b>System Data :</b> Central Capacity 70,000 gallons	<b>Report Number :</b> 497-0935 <b>Sample Description :</b> Central <b>Date Complete :</b> 03/13/2007
--	--	---

Sample ID	Date Taken	Date Received	Concentration J-S	PH 3.0-9.0	Trans Oil Content n. Solids G	Hardness PPM	Oil-1.1 micron PPM	Chloride ppm	Fiber Concentration	Residue 0-1000000	Dispers 0-100
497-0460	08/08/07	09/09/07	3.2	3.0	0.6	170	357	415	116	100	0
497-0461	09/26/07	09/24/07	3.8	3.8	0.4	210	300	450	127	0	0
497-0462	09/27/07	09/26/07	4.0	4.7	0.0	210	420	490	123	0	0
497-0463	09/28/07	09/28/07	10.0	9.0	0.4	330	121	490	10.1	100	0
497-0441	02/16/07	02/07/07	8.8	8.0	1.0	320	670	410	110	150	0
497-0442	04/15/07	02/03/07	11.0	9.0	0.1	330	390	420	0	0	0
497-0419	01/22/07	01/26/07	11.5	9.0	0.0	360	170	320	0	0	0
497-0423	01/15/07	01/17/07	9.8	9.1	0.1	290	80	380	0	1000	0
497-0424	02/07/07	01/04/07	5.0	8.0	0.1	280	312	330	0	100	0
497-0437	01/20/07	12/19/06	8.2	6.0	0.0	...	460	...	...	100	0
497-0439	1/10/07	1/10/07	7.7	6.0	0.0	260	460	430	...	100	0
497-0438	1/20/07	1/12/07	8.8	8.4	0.0	290	380	440	...	10000	0
497-0437	1/10/07	1/10/07	7.1	7.2	0.0	380	90	400	...	0	0
497-0438	1/10/07	1/10/07	8.8	8.7	0.0	250	390	410	...	100	0
497-0432	08/08/07	08/21/07	6.1	8.9	0.2	250	870	450	...	100	0

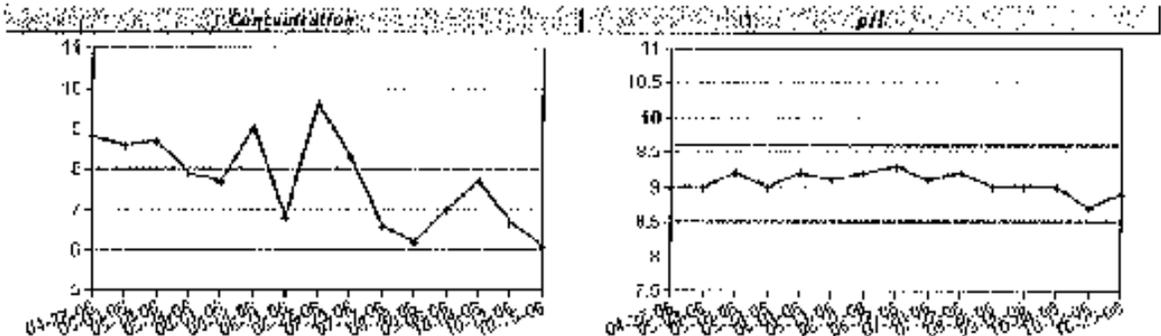


Comments & Recommendations :  
Adjust to recommended concentration.

# Coolant Management Report

<b>Customer Data :</b> SUPERIOR INDUSTRIES - PITTSBURG 1650 EAST 27TH TERRACE PITTSBURG, KS 66762-2757	<b>System Data :</b> Central Capacity 70000 gallons	<b>Report Number :</b> 008-4852 <b>Sample Description :</b> Central <b>Date Complete :</b> 10/01/2006
---	---	---

Sample Id	Date Taken	Date Received	Concentration g - g	pH 8.5 - 9.5	Tramp Oil Content in Soluble Oils max 2.5	Hardness ppm	Chloride ppm	Oil 1.2 micron ppm	Bacteria 0 1000000	Fungus 0 100
406-4062	10/10/06	10/26/06	8.1	8.8	0.2	250	450	570	100	0
406-4206	10/12/06	10/12/06	8.7	8.7	0.1	210	515	290	0	0
406-4093	10/04/06	10/06/06	7.7	8.0	0.0	210	485	450	0	0
406-4204	09/06/06	09/08/06	7.0	8.0	0.0	270	470	100	0	0
406-4044	06/28/06	09/29/06	6.2	8.0	0.1	230	430	200	120	0
406-3601	07/27/06	07/28/06	6.6	8.2	0.0	270	500	310	0	0
406-3605	07/20/06	07/21/06	8.2	8.1	0.0	315	540	130	0	0
406-3262	06/27/06	06/28/06	8.6	8.3	0.0	260	525	780	0	0
406-3167	06/14/06	06/22/06	6.0	8.2	0.0			670	0	0
406-3074	06/14/06	06/15/06	9.0	8.1	0.0	190	640	1320	100	0
406-3386	06/01/06	06/02/06	7.7	9.2	0.5	290	905	280	0	0
406-3317	05/24/06	06/26/06	7.9	9.0	0.0	280	905	380	0	0
406-3096	05/18/06	06/19/06	8.7	9.2	0.0	220	870	10	0	0
406-2406	06/03/06	06/04/06	8.6	9.0	0.0	410	575	410	0	0
406-2406	04/27/06	04/28/06	8.8	9.0	0.0	170	575	460	100	0

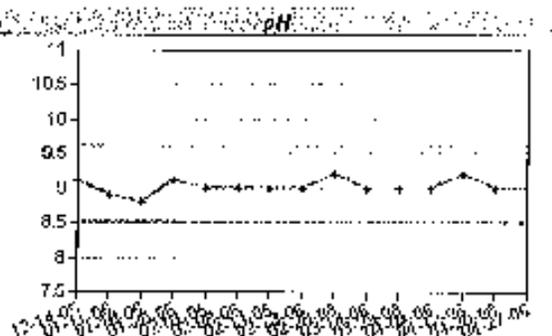


Comments & Recommendations :

# Coolant Management Report

<b>Customer Data :</b> SUPERIOR INDUSTRIES - PITTSBURG 1500 EAST 27TH TERRACE PITTSBURG, KS 65762-2737	<b>System Data :</b> Central Capacity 70,000 gallons	<b>Report Number :</b> 406-2400 <b>Sample Description :</b> Central <b>Data Complete :</b> 05/02/2006
---	--	---

Sample ID	Date Taken	Date Received	Concentration	pH	Tramp Oil Content in Soluble-Oils	Hardness ppm	Chloride	Dist 1.2 micron ppm	Bacteria	Fungus
			0 - 1	8.5 - 9.0	max 2.0				0 - 1000cfu	0 - 100
406-2400	04/27/06	04/28/06	8.8	8.0	0.0	170	575	440	100	0
406-2397	04/20/06	04/24/06	8.0	8.0	0.5	320	540	450	1000	0
406-2202	04/12/06	04/12/06	9.1	8.2	0.3	300	515	370	1000	0
406-2088	04/04/06	04/06/06	8.0	8.0	0.0	320	535	370	0	0
406-1979	03/28/06	03/30/06	8.6	8.0	0.0	335	510	350	0	0
406-1752	03/14/06	03/15/06	8.1	8.0	0.0	260	470	280	1,000	0
406-1644	02/28/06	03/01/06	8.8	8.2	0.0	280	540	400	0	0
406-1401	02/23/06	02/24/06	8.4	9.0	0.0	300	505	300	0	0
406-1361	02/14/06	02/16/06	8.8	9.0	0.0	270	515	340	100	0
406-1270	02/08/06	02/09/06	9.2	9.0	0.0	320	500	300	100	0
406-1175	02/01/06	02/02/06	8.4	9.0	0.5	140	465	300	100	0
406-1050	01/26/06	01/27/06	3.8	9.1	0.0	220	660	500	0	0
406-0356	01/18/06	01/20/06	3.7	8.8	0.0	110	450	1250	0	0
406-0183	01/11/06	01/12/06	7.1	8.8	0.1	140	350	150	100	0
405-0183	12/14/05	12/15/05	6.3	8.1	0.0	120	295	240	1000	0



Comments & Recommendations :  
Adjust to recommended concentration.

# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

## COOLANT MANAGEMENT REPORT

CUSTOMER: SUPERIOR INDUSTRIES  
 LOCATION: PITTSBURG, KS

E-MAIL: SUPERIOR PITTSBURG

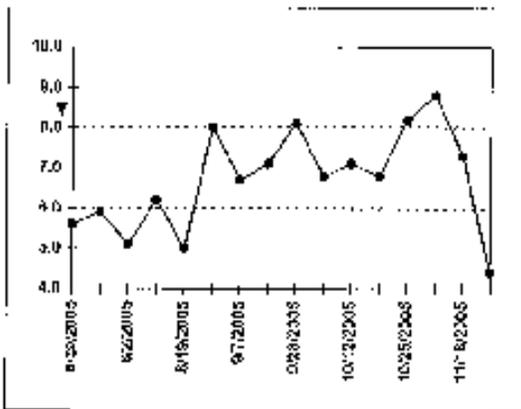
SYSTEM: CENTRAL  
 CAPACITY: 70,000 GALLONS

PRODUCT:  
 REC. CONC: 8%

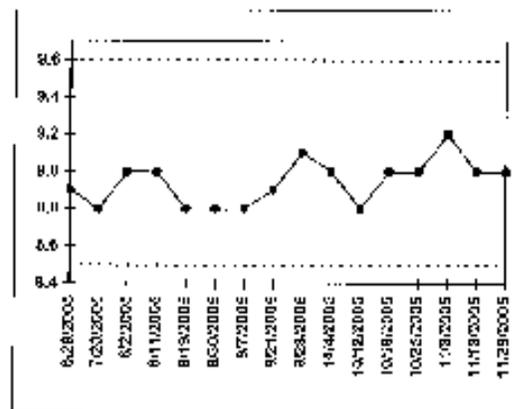
DATE CHARGED:

TAKEN	RECEIVED	CONC.	PH	TRAMP OIL	HARDNESS PPM	BACTERIA	FUNGI
6/29/2005	6/30/2005	5.6	8.9	1.6	100	0	0
7/20/2005	7/21/2005	5.9	8.8	1.0	210	0	0
8/2/2005	8/3/2005	5.1	9.0	0.3	70	1,000	0
8/11/2005	8/12/2005	6.2	9.0	0.0	250	0	0
8/19/2005	8/22/2005	5.0	8.8	1.6	100	0	0
8/30/2005	8/31/2005	8.0	8.8	0.2	150	1,000	0
9/7/2005	8/8/2005	6.7	8.8	0.8	140	0	0
9/21/2005	9/22/2005	7.1	8.9	0.9	160	1,000	0
9/28/2005	9/29/2005	8.1	8.1	0.2	180	0	0
10/4/2005	10/5/2005	6.8	9.0	1.3	170	1,000	0
10/12/2005	10/13/2005	7.1	8.8	0.7	90	0	0
10/16/2005	10/19/2005	6.8	9.0	1.9	110	0	0
10/25/2005	10/28/2005	8.2	9.0	0.5	130	1,000	0
11/8/2005	11/9/2005	8.8	9.2	0.0	250	1,000	0
11/18/2005	11/21/2005	7.3	9.0	0.0	110	1,000	0
11/29/2005	11/30/2005	4.4	8.0	0.0	170	1,000	0

CONCENTRATION



PH



### RECOMMENDATIONS / COMMENTS

11/29/05 INCREASE TO RECOMMENDED CONCENTRATION  
 11/29/05 PPM CHLORIDE - 240  
 11/28/05 PPM DIRT (1.2 MICRON FILTER) - 70

877080

# APPENDIX A: ENVIRONMENTAL MONITORING DATA (CONTINUED)

## COOLANT MANAGEMENT REPORT

CUSTOMER: SUPERIOR INDUSTRIES  
 LOCATION: PITTSBURG, KS

E-MAIL: SUPERIOR PITTSBURG

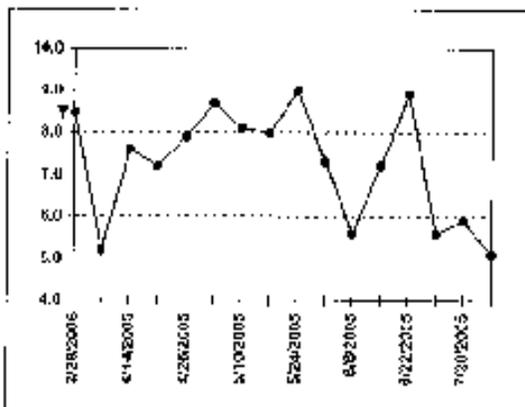
SYSTEM: CENTRAL  
 CAPACITY: 70,000 GALLONS

PRODUCT:  
 REC. CONC: 8%

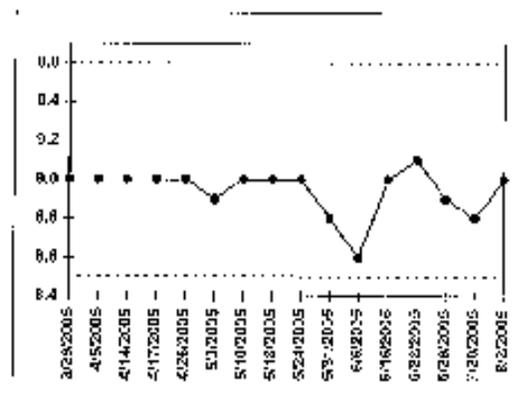
DATE CHARGED:

TAKEN	RECEIVED	COND.	PH	TRAMP OIL	HARDNESS PPM	BACTERIA	FUNGI
3/28/2005	3/30/2006	8.5	9.0	1.1	290	0	0
4/5/2005	4/6/2005	8.2	9.0	0.3	210	1,000	0
4/14/2005	4/15/2005	7.6	9.0	0.0	250	0	0
4/17/2005	4/20/2005	7.2	9.0	0.1	200	1,000	0
4/28/2005	4/27/2005	7.9	9.0	0.7	260	0	0
5/3/2005	5/4/2005	8.7	8.9	0.5	230	1,000	0
5/10/2005	5/11/2005	8.1	9.0	1.0	220	0	0
5/18/2005	5/19/2005	8.0	9.0	0.0	420	0	0
5/24/2005	5/25/2005	9.0	9.0	0.0	230	1,000	0
5/31/2005	6/1/2005	7.3	8.8	0.0	230	1,000	0
6/6/2005	6/7/2005	8.6	8.6	1.8	190	0	0
6/16/2005	6/17/2005	7.2	9.0	1.2	240	0	0
6/22/2005	6/23/2005	8.9	9.1	0.0	160	1,000	0
6/29/2005	6/30/2005	8.6	8.9	1.6	100	0	0
7/20/2005	7/21/2005	8.9	8.8	1.0	210	0	0
8/2/2005	8/3/2005	8.1	9.0	0.3	70	1,000	0

CONCENTRATION



PH



### RECOMMENDATIONS / COMMENTS

8/2/05 INCREASE TO RECOMMENDED CONCENTRATION  
 8/2/05 PPM CHLORIDE - 610  
 01/2/05 PPM DIRT (1.2 MICRON FILTER) - 340

677500

**COOLANT MANAGEMENT REPORT**

TAKEN	RECEIVED	CONC.	PH	TRAMP OIL	HARDNESS	BACTERIA	FUNGI
5/13/2004	5/14/2004	9.4	9.3	0.4	120	1,000	0
5/19/2004	5/20/2004	8.8	9.2	0.0	140	0	0
5/24/2004	5/25/2004	7.7	9.2	0.0	120	0	0
6/2/2004	6/3/2004	8.3	9.1	0.0	130	0	0
6/9/2004	6/10/2004	8.5	9.2	0.0	150	1,000	0
6/14/2004	6/15/2004	7.9	9.2	0.3	120	0	0
6/21/2004	6/22/2004	8.8	9.2	0.1	110	0	0
6/28/2004	6/29/2004	7.9	9.1	0.3	110	0	0
7/8/2004	7/9/2004	8.5	9.2	0.3	110	0	0
7/19/2004	7/20/2004	7.1	9.1	0.9	120	0	0
7/26/2004	7/27/2004	8.0	9.0	0.6	140	0	0
8/12/2004	8/13/2004	10.0	9.1	0.0	130	0	0
8/20/2004	8/23/2004	9.0	9.1	1.9	120	0	0
8/25/2004	8/27/2004	7.4	9.2	1.9	110	1,000	0
8/31/2004	9/1/2004	7.6	9.0	0.5	110	1,000	0
9/9/2004	9/10/2004	7.4	9.0	0.3	110	0	0
9/16/2004	9/17/2004	7.2	9.0	0.0	110	0	0
9/23/2004	9/24/2004	7.9	9.0	0.3	100	0	0
9/30/2004	10/1/2004	8.2	9.0	0.9	130	0	0
10/8/2004	10/11/2004	7.8	9.2	0.4	120	0	0
10/14/2004	10/15/2004	8.1	9.0	0.6	160	1,000	0
10/21/2004	10/25/2004	8.0	9.1	1.2	130	0	0
10/28/2004	10/29/2004	7.9	9.2	1.1	150	0	0
11/4/2004	11/5/2004	8.2	9.0	0.0	140	0	0
11/11/2004	11/12/2004	8.1	9.0	0.6	150	1,000	0
11/18/2004	11/19/2004	8.3	9.0	0.0	160	0	0
12/2/2004	12/3/2004	8.3	9.0	0.4	170	1,000	0
12/8/2004	12/9/2004	8.5	9.0	0.3	170	1,000	0
12/15/2004	12/16/2004	8.4	8.9	0.2	140	0	0
12/21/2004	12/22/2004	8.3	9.0	0.8	170	1,000	0
1/10/2005	1/11/2005	6.6	8.9	0.4	160	1,000	0
1/24/2005	1/25/2005	8.1	9.0	0.0	180	1,000	0
1/31/2005	2/1/2005	9.3	9.0	0.0	200	0	0
2/7/2005	2/8/2005	9.0	9.0	0.0	220	1,000	0
2/15/2005	2/16/2005	7.9	9.0	0.0	210	1,000	0
2/22/2005	2/23/2005	9.0	9.0	0.7	240	1,000	0
3/1/2005	3/2/2005	8.7	9.0	0.5	230	1,000	0
3/8/2005	3/9/2005	8.2	9.2	0.0	220	0	0
3/15/2005	3/16/2005	7.8	9.0	0.0	220	0	0
3/22/2005	3/23/2005	9.1	9.1	0.0	230	0	0

## APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES



Wednesday, July 11, 2007

Randy Boylstein  
NIOSH  
1095 Willowdale Road, Room H2517  
Morgantown, WV 26505



Re: Project Number: 750-706-0867 - HETA 2007-0263

Dear Randy Boylstein:

P&K Microbiology is pleased to provide the enclosed report of analyses for samples received 06/29/2007. This cover letter and accompanying pages are an integral part of this report. All analyses are performed in our AIHA EMLAP accredited laboratory using the Best Laboratory Practices. The data generated in this report are based on the samples and accompanying information provided and represent concentrations at a point in time under the conditions sampled. Results can vary with site conditions. P&K Microbiology employees did not collect samples for this project, and may provide limited interpretation of this data as it relates to the overall investigation. For ecological information on fungi and bacteria identified in this report, please consult our publication, "The Ecology and Classification of Common Fungi and Bacteria Found in Indoor Environments". The latest edition of this publication can be ordered by calling a P&K Microbiology Project Manager toll free at 1 (866) 871-1984.

### Quality Assurance

P&K Microbiology is staffed with over 35 professionals, including PhD's, microbiologists, and mycologists with over 20 years of experience. The reliability of test results depends on many factors such as the personnel performing the tests, environmental conditions, selection and validation of test methods, equipment functioning, measurement traceability, as well as the sampling, storage and handling of test items, all of which are a reflection of the laboratory's overall quality system.

P&K Microbiology has modeled its quality system after ISO 17025 guidelines, one of the most stringent sets of standards in the industry, to ensure that its customers receive the high standard of accuracy, reliability, and impartiality that they have come to expect from a leader in the environmental industry. P&K Microbiology's adherence to the standards set forth in the ISO 17025 guidelines has been validated and formally recognized through accreditations granted by the American Industrial Hygiene Association (AIHA). As an additional measure to demonstrate its competency to perform the analyses it offers to its clients, P&K Microbiology also participates in a variety of different proficiency testing programs, including the Environmental Microbiology Proficiency Analytical Testing Program (EMPAT) sponsored by the American Industrial Hygiene Association.

As part of its continuous commitment to excellence, P&K Microbiology is inspected by governmental agencies, independent commercial groups, and internal oversight personnel; these audits are in addition to those already mentioned above. Below you will find additional information regarding the specific analyses requested for this project.

# APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)



P&K 100, 102, 105, 105A, 103, 103A, 104, 106, 106A

### Culture Analyses for Fungi and Bacteria

Culturable microorganisms are those that are viable when media are inoculated, and will grow on the selected media and at the selected temperature.

The type of media and incubation temperature can vary depending on the scope of the survey. Isolates are identified to the service level requested. Typical analysis includes identification of most fungi to the species level, except for species of *Cladosporium* and *Penicillium*. Identification to the species level can be performed if requested in advance. General incubation parameters are summarized below. Incubation times can vary depending on specific growth characteristics. Samples submitted for culture analysis using Malt Extract Agar (MEA), DG-18, Commeal Agar (CMA) or Cellulose Agar are cultured for 7-10 days.

P&K Microbiology has published several excellent resources on culture analysis of fungi. Please refer to the following technical fact sheets: "Fungi in the Air: What do results of fungal air samples mean?"

Test	Incubation Temperature (° C)	Minimum Incubation Time
Environmental Bacteria	25	7-10 days
Total Fung	25	7-10 days
Thermophilic fungi	35	7-10 days
Thermophilic Actinomycetes	55	5-7 days

### Common Culture Media

Acronym	Name
BAP	Tryptic Soy Agar with 5% Sheep Blood
PCA	Plate Count Agar
BCYE	Buffered Charcoal Yeast Extract Agar
PDA	Potato Dextrose Agar
MEA	Malt Extract Agar
DG-18	Dichloran Glycerol Agar
SAB	Sabouraud's Dextrose Agar
RRA	Rose Bengal Agar
CMA	Commeal Agar

P&K 120

### Endotoxins

This analysis utilizes the response of *Limulus Amebocyte Lysates* or LAL to endotoxin. The most sensitive of these techniques is a chromogenic kinetic assay that compares samples to standard endotoxin concentrations. The recent advent of adding zwitterions eliminates  $\beta$ -glucan interference. For more information, please refer to [www.aerotechpk.com](http://www.aerotechpk.com) and the technical fact sheet entitled "Endotoxins".



## Data Qualifiers

The *Data Qualifiers* identify issues or events that are relevant to your analytical results. A data qualifier includes information about the validity, the source of the data whether calculated, entered or estimated, and the value of an observation. In each case the data qualifiers provide significant information vital to the interpretation of the laboratory data.

This communication is intended only for the individual or entity to which it is directed. It may contain information that is privileged, confidential, or otherwise exempt from disclosure under applicable law. Dissemination, distribution, or copying of this communication by anyone other than the intended recipient, or a duly designated employee or agent of such recipient, is prohibited. If you have received this communication in error, please notify us immediately by telephone at 1 (866) 871-1984, and delete this message and all attachments thereto.

For additional information, or if you have any questions regarding this report, please do not hesitate to call.

Sincerely,

A handwritten signature in cursive script that reads "Nan-Sea Rovegno".

Nan-Sea Rovegno  
Project Manager  
P & K Microbiology  
856-489-4455

## Analytical References

1. Medically Important Fungi: A Guide to Identification, 3rd ed., ASM, 1995.
2. Standard Methods for the Examination of Water and Wastewater, 19th ed., APHA, 1995.
3. Sampling and Identifying Allergic Pollens and Molds, Blewstone, 1990.
4. Identifying Filamentous Fungi: A Clinical Laboratory Handbook, Star, 1996.
5. Manual of Clinical Microbiology, 7th ed., ASM, 1999.
6. A Laboratory Guide to Common *Aspergillus* Species and their Teleomorphs, CSIRO, 1994.
7. Bioaerosols: Assessment and Control, ACGIH, 1999.

# APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

Client: KDCOR, Morgantown, WV 26609

Project ID: HETA 2007-0263

Date Sampled: 06/26/2007

Contact: Randy Boychuk

PKC Report Number: 758-706-0867

Date of Evaluation: 07/03/2007

Date Analysis Completed: 08/13/2007

## Bacterial Analysis (Culture Method)

### Swab Liquid Samples

PKC Sample ID Client Sample ID Location	Volume (ml)	Medium Used	Dilution Factor	Bacterial ID	Colony Counts	Conc. ** (CFU/ml)	Percentage*
1 L1 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Bacillus species Strom Positive Cocci	3 12	300 1,200 Total: 1,500	20 80 100
2 L4 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Bacillus species Strom Positive Cocci	1 18	100 1,800 Total: 1,900	5 95 100
3 L13 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Bacillus species Strom Positive Cocci	2 18	200 1,800 Total: 2,000	10 90 100

The sample(s) in this report was/were received in acceptable condition.

\* Percentage of each group of fungi/bacteria in total population.

\*\* Concentration is rounded to two significant digits. Concentration is in CFU/sample if sample amount/area is NA.

Media types: Actinomycete Isolation Agar (AIA), cornmeal agar (CMA), 2% yeast extract agar (YEA), inhibitory mold agar (IMA), pseudomonas isolation agar (PIA), rose bengal agar (RBA), enhanced dechloro agar (EDA), tryptic soy agar (TSA), nutrient agar (NTA), blood agar (BA), staphylococcus medium 110 (Staphy), phenylethyl alcohol agar w/ 5% sheep blood (PEA), plate count agar (PCA). The detection limit of fungal and bacteria analysis using culture methods is one colony. The quantitative limits vary from analysis to analysis and from processing procedure to processing procedure. Contact us to determine your quantitation limits.

*Wych...*

Lab Review

*Manzba Koregno*

Final Review

# APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

Client: KDCOR, Morgantown, WV 26505

Project ID: HETA 2007-0263

Date Sampled: 06/26/2007

Contact: Randy Boychuk

PKC Report Number: 758-706-0867

Date of Evaluation: 07/03/2007

Date Analysis Completed: 08/13/2007

## Bacterial Analysis (Culture Method)

### Swab Liquid Samples

PKC Sample ID Client Sample ID Location	Volume (ml)	Medium Used	Dilution Factor	Bacterial ID	Colony Counts	Conc. ** (CFU/ml)	Percentage*
4 L20 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Basilus species Strom Positive Cocci	4 18	400 1,800 Total: 2,200	18 82 100
5 L25 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Basilus species Strom Positive Cocci	2 11	200 1,100 Total: 1,300	15 85 100
6 L26 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Basilus species Strom Positive Cocci	2 13	200 1,300 Total: 1,500	13 87 100

The sample(s) in this report was/were received in acceptable condition.

\* Percentage of each group of fungi/bacteria in total population.

\*\* Concentration is rounded to two significant digits. Concentration is in CFU/sample if sample amount/area is NA.

Media types: Actinomycete Isolation Agar (AIA), cornmeal agar (CMA), 2% yeast extract agar (YEA), inhibitory mold agar (IMA), pseudomonas isolation agar (PIA), rose bengal agar (RBA), enhanced dechloro agar (EDA), tryptic soy agar (TSA), nutrient agar (NTA), blood agar (BA), staphylococcus medium 110 (Staphy), phenylethyl alcohol agar w/ 5% sheep blood (PEA), plate count agar (PCA). The detection limit of fungal and bacteria analysis using culture methods is one colony. The quantitative limits vary from analyte to analyte and from processing procedure to processing procedure. Contact us to determine your quantitation limits.

*Wych...*

Lab Review

*Manzba Koregno*

Final Review

# APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

Client: KDCOR, Westborough, NY 26849

Project ID: HETA 2007-0263

Date Sampled: 05/26/2007

Contact: Randy Boychuk

PKC Report Number: 758-706-0867

Date of Evaluation: 07/03/2007

Date Analysis Completed: 08/13/2007

## Bacterial Analysis (Culture Method)

### Walk Liquid Samples

PKC Sample ID Client Sample ID Location	Volume (ml)	Medium Used	Dilution Factor	Bacterial ID	Colony Counts	Conc. ** (CFU/ml)	Percentage*
7 L27 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Bacillus species Strom Positive Cocci	3 17	300 1,700 Total: 2,000	15 85 100
8 L28 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Bacillus species Strom Positive Cocci	3 12	300 1,200 Total: 1,500	20 80 100
9 L29 REB	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Bacillus species	2	200 Total: 200	100 100

The sample(s) in this report was/were received in acceptable condition.

\* Percentage of each group of fungi/bacteria in total population.

\*\* Concentration is rounded to two significant digits. Concentration is in CFU/sample if sample amount/area is NA.

Media types: Actinomycetes Isolation Agar (AIA), enriched agar (CPM), 2% yeast extract agar (PEA), inhibitory mold agar (IMA), pseudomonas isolation agar (PIA), rose bengal agar (RBA), enhanced clostridia agar (EDA), tryptic soy agar (TSA), nutrient agar (NTA), blood agar (BA), staphylococcus medium 110 (Staphy), phenylethyl alcohol agar w/ 5% sheep blood (PEA), plate count agar (PCA). The detection limit of fungal and bacteria analysis using culture methods is one colony. The quantitative limits vary from analyte to analyte and from processing procedure to processing procedure. Contact us to determine your quantitation limits.

*Wycha--*

Lab Review

*Manzba Koregno*

Final Review

# APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

Client: KDCOR, Westport, NY 20049

Project ID: HETA 2007-0263

Date Sampled: 05/26/2007

Contact: Randy Boychuk

PK Report Number: 750-706-0067

Date of Evaluation: 07/03/2007

Date Analysis Completed: 08/13/2007

## Bacterial Analysis (Culture Method)

### Walk Liquid Samples

PK Sample ID Client Sample ID Location	Volume (ml)	Medium Used	Dilution Factor	Bacterial ID	Colony Counts	Conc. ** (CFU/ml)	Percentage*
10 BRANK EAST	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Gram Positive Cocci	27	2,700 Total: 2,700	100 100
11 BRANK WEST	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Gram Positive Cocci Gram Positive Bacilli Gram Positive Cocci	1 2 28	100 200 2,800 Total: 3,100	4 8 87 100
12 PREPILT	1.00	PEA	100	Fungi No Growth	n/a	Total: <100	n/a
	1.00	TBA	100	Bacteria Gram Positive Cocci Gram Positive Cocci	2 13	200 1,300 Total: 1,500	10 90 100

The sample(s) in this report was/were received in acceptable condition.

\* Percentage of each group of fungi/bacteria in total population.

\*\* Concentration is rounded to two significant digits. Concentration is in CFU/sample if sample amount/area is NA.

Media types: Actinomycete Isolation Agar (AIA), cornmeal agar (CMA), 2% yeast extract agar (YEA), inhibitory mold agar (IMA), pseudomonas isolation agar (PIA), rose bengal agar (RBA), enhanced dechlorase agar (EDA), tryptic soy agar (TSA), nutrient agar (NA), blood agar (BA), staphylococcus medium 110 (Staphy), phenylethyl alcohol agar w/ 5% sheep blood (PEA), plate count agar (PCA). The detection limit of fungal and bacteria analysis using culture methods is one colony. The quantitative limits vary from analyte to analyte and from processing procedure to processing procedure. Contact us to determine your quantitation limits.

*Wycha--*

Lab Review

*Manzba Koregno*

Final Review

# APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

**EMLab P&K**  
 1936 Olney Avenue, Cherry Hill, NJ 08003  
 (866) 871-1984 Fax (856) 489-4085 www.emlab.com

**Client:** NIOSH, Morgantown, WV  
**Project ID:** HETA 2007-0263  
**Date Sampled:** June 28, 2007  
**Date of Inoculation:** July 3, 2007  
**Samples Submitted By:** Randy Hoylestein  
**Date Analysis Completed:** August 14, 2007  
**EMLab ID Number:** 750-706-0867

## Bacteria Analysis (Culture Method)

### Milk Liquid Samples

P&K Sample ID Client Sample ID Location	Vol. used (ml)	Medium used	Dilution Factor	Bacterial ID	Colony counts	Conc. ** (CFU/ml)	Percentage*
750-706-0867-01 L1 MBB	1.0	TBA	100	Bacteria			
				<i>Bacillus</i> OC group 2E	1	100	7%
				<i>Bacillus</i> spizizenii	2	200	13%
				<i>Staphylococcus</i> xylosum	12	1,200	80%
						Total: 1500	100%
750-706-0867-02 L4 MBB	1.0	TBA	100	Bacteria			
				<i>Bacillus</i> megaterium	1	100	5%
				<i>Lactococcus</i> lactis	1	100	5%
				<i>Staphylococcus</i> xylosum	9	900	60%
						Total: 1800	100%
750-706-0867-03 L15 MBB	1.0	TBA	100	Bacteria			
				<i>Bacillus</i> strophium	1	100	5%
				<i>Bacillus</i> megaterium	1	100	5%
				<i>Staphylococcus</i> xylosum	16	1,600	80%
						Total: 2000	100%
750-706-0867-04 L20 MBB	1.0	TBA	100	Bacteria			
				<i>Bacillus</i> flavus	3	300	14%
				<i>Bacillus</i> thuringiensis	1	100	5%
				<i>Staphylococcus</i> xylosum	16	1,600	82%
						Total: 2200	100%
750-706-0867-05 L25 MBB	1.0	TBA	100	Bacteria			
				<i>Bacillus</i> cereus	1	100	8%
				<i>Erwinia</i> <i>Bacillus</i> pasteuris	1	100	8%
				<i>Staphylococcus</i> xylosum	11	1,100	85%
						Total: 1300	100%

## APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

P&K Sample ID (Client Sample ID Location)	Vol. used (µl)	Medium used	Dilution Factor	Bacterial ID	Colony counts	Conc. ** (CFU/ml)	Percentage*
750-706-0897-06 L25 REB	1.0	TBA	100	Bacteria Bacillus cereus Bacillus sphaericus Staphylococcus xylosum	1 1 19	100 100 1,900 Total: 2100	5% 5% 90% 100%
750-706-0897-07 L27 REB	1.0	TBA	100	Bacteria Bacillus cereus Bacillus sphaericus Staphylococcus xylosum	2 1 17	200 100 1,700 Total: 2000	10% 5% 85% 100%
750-706-0897-08 L28 REB	1.0	TBA	100	Bacteria Bacillus cereus Bacillus sphaericus Staphylococcus xylosum	1 2 12	100 200 1,200 Total: 1500	7% 13% 80% 100%
750-706-0897-09 L29 REB	1.0	TBA	100	Bacteria Bacillus sphaericus	2	200 Total: 200	100% 100%
750-706-0897-10 Black East	1.0	TBA	100	Bacteria Staphylococcus xylosum	27	2,700 Total: 2700	100% 100%
750-706-0897-11 Black West	1.0	TBA	100	Bacteria Bacillus cereus Klebsiella sibirica Staphylococcus xylosum	1 2 20	100 200 2,000 Total: 2300	4% 9% 87% 100%

## APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

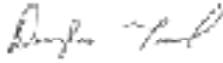
P&K Sample ID Client Sample ID Location	Vol. used (ml)	Medium used	Dilution Factor	Bacterial ID	Colony count	Conc. ** (CFU/ml)	Percentage*
758-706-0867-12 Fremont	1.0	TBA	100	Bacteria			
				Bacillus cereus	1	100	5%
				Parabacillus polygonum	1	100	5%
				Staphylococcus xylosum	19	1,900	90%
						Total: 2100	100%

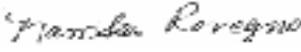
The sample(s) in this report was/were received in acceptable condition.

\* Percentage of each group of microorganisms in total population.

\*\* Concentration is recorded to two significant digits. Concentration is in CFU/sample if sample concentration is NA.

Media types: Actinomycete Isolation Agar (AIA), casein agar (CMA), 2% yeast extract agar (MEA), inhibitory mold agar (IMA), penicillium isolation agar (PIA), casein hydrolysis agar (CHA), sulfonamide antibiotic agar (SIA), tryptic soy agar (TSA), nutrient agar (NTA), blood agar (BA), staphylococcus aureus 110 (Staphy), phenylethyl alcohol agar w/ 2% sheep blood (PEA). The detection limit of fungal and bacteria analysis using culture methods is one colony. The quantitative limits vary from analysis to analysis and from processing procedure to processing procedure. Contact us to determine your quantitative limits.

Approved by:   
 Douglas Tool, Ph.D. Laboratory Director

Quality control checked by: 

# APPENDIX B: P&K CULTURE AND ENDOTOXIN ANALYSES (CONTINUED)

## Test Report - Endotoxin Analysis (Kinetic Chromogenic Method)

Client: NIOSH / DRDS / PSE, Morgantown, WV  
Project ID: HETA 2007 - 0263  
Samples Submitted By: Randy Boylstein  
Date Sampled: June 14, 2007  
Date Samples Received: June 29, 2007  
Date Analysis Completed: July 11, 2007  
P&K Report No.: 750-706-0867

Sample Type: Metal Working Fluid

Sample ID	Sample Volume (ml)	Endotoxin Concentration*
L1 Res	1	130 EU/ml
L2 Res	1	150 EU/ml
L13 Res	1	140 EU/ml
L20 Res	1	140 EU/ml
L25 Res	1	140 EU/ml
L26 Res	1	150 EU/ml
L27 Res	1	140 EU/ml
L28 Res	1	140 EU/ml
L29 Res	1	53 EU/ml
Shank East	1	150 EU/ml
Shank West	1	150 EU/ml
EyeMelt	1	150 EU/ml

Instrument detection limit: 0.505 EU

\*Endotoxin concentration:  $\log = 6$  EU

The sample(s) in this report was/were received in acceptable conditions.

Reported results relate only to the portion of items tested.

Lab Review: \_\_\_\_\_

*Michael Berg*  
Michael Berg Ph.D., Manager Biochemistry

Final Review: \_\_\_\_\_

*Marcela Rovigno*

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## APPENDIX C: NIOSH GLUCAN ANALYSES

### Glucan Results

Samples collected June 28, 2007

Superior Industries International, Inc.

Pittsburg, Kansas

Sample Name	Glucan concentration (ng/ml)
Machine 1 Reservoir	135.0
Machine 13 Reservoir	133.8
Machine 25 Reservoir	156.3
East Shark	185.6
Machine 20 Reservoir	166.4
Machine 26 Reservoir	212.8
Premelt	159.8
West Shark	189.1
Machine 8 Reservoir	189.9
Machine 28 Reservoir	240.7
Machine 27 Reservoir	266.1
Machine 29 Reservoir	<i>(Assay not valid)</i>
Average (standard deviation)	185.0 (41.7)

Microbe Inotech Laboratories, Inc.  
Summary Report of Analysis  
[MILB – 5164A]

Randy Boylstein  
NIOSH  
1095 Willowdale Rd.  
Morgantown, WV 26505  
Telephone: 304-285-6062  
Fax: 304-285-5820  
E-mail: [zig1@cdc.gov](mailto:zig1@cdc.gov)

December 5, 2007

**Sample Description and Chain of Custody Record Information:**

Thursday, November 29, 2007 9:55am: Received twelve (12) samples of metal working fluid for Mycobacterium qPCR assays.

Sample #1 ID: L1 Res  
Sample #2 ID: L8 Res  
Sample #3 ID: L13 Res  
Sample #4 ID: L20 Res  
Sample #5 ID: L25 Res  
Sample #6 ID: L26 Res  
Sample #7 ID: L27 Res  
Sample #8 ID: L28 Res  
Sample #9 ID: L29 Res  
Sample #10 ID: Shark East  
Sample #11 ID: Shark West  
Sample #12 ID: Premelt

MIL, Inc. REPORT & Invoice Number: MILB-5164A  
Purchase Order #: Credit Card

**Overview of PCR Amplification Chemistry:**

For this sample, DNA was extracted from the samples using our Standard Operating Protocol (SOP): MIC-082-1 entitled "Microbial Genomic DNA Isolation Using Microbeads"

**Sample Preparation:**

40mL of the metal working fluid sample provided was centrifuged @ 5000xG for 60 minutes. The cells were re-suspended into a 5.0 mL aliquot by removal of the top layer of the sample by transfer pipet and the pellet re-suspended in 5.0 mL of remaining solution of which 2.0 mL was then transferred a 2.0 mL sterile Eppendorf tube and processed as indicated in the method of SOP MIC-082. These 2.0 mL were centrifuged @ 10,000xG and microbial genomic DNA extracted using microbeads SOP (MIC-082-2).

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The 50 $\mu$ L of each sample obtained from this method was then measured using the NanoDrop<sup>®</sup> ND-1000 as described below.

The sample retention system used by the NanoDrop<sup>®</sup> ND-1000 is probably its best feature. Not only does it enable the analysis of extremely small sample volumes (as small as one microliter), but it also eliminates the need for cuvettes and capillaries. This saves the cost of either disposable cuvettes or the time and effort spent in cleaning reusable ones.

### How It Works



With the sample apparatus open, a droplet of sample is pipetted onto the measurement pedestal.



When the sample apparatus is closed, the sample arm slightly compresses the droplet and a sample column is drawn. Surface tension alone holds the sample in place. The spectral measurement is then made and quantification is made based on the tightly controlled path length of 1 mm.

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When the measurement is complete, the sample apparatus is opened and the sample is simply wiped from both the sample arm and sample pedestal using an ordinary dry laboratory wipe. Since the sample is not contained in a secondary vessel, the sample directly wets the system optics, reducing the variations resulting from changing and/or repositioning cassettes. When the sample is removed, the optics can be easily cleaned making it possible to measure successive sample concentrations varying by more than 1000 fold in concentration with no carryover.

**General Description of the PCR Procedure:**

PCR represents a cyclic reaction where target DNA is amplified *in vitro* by a series of polymerization cycles. Each cycle includes three steps: a heating step at 91°–97°C, where the DNA template duplex is denatured (melted) to single strands, an annealing step usually at 40°–45°C where short oligonucleotide primers bind to the single-stranded DNA template, and an extension step at 68°–73°C where thermostable DNA polymerase catalyzes the synthesis of a new DNA strand by elongation of the primed strand. The reaction requires two short oligonucleotides (primers) flanking the target region to be amplified, which are present in large molar excess and hybridize to complementary segments of DNA. During the reaction, deoxynucleotide triphosphates (dNTP), i.e., dATP, dCTP, dGTP and dTTP, are bound to the free 3' hydroxyl end of the new strand. Only deoxynucleotide monophosphate is incorporated in the DNA chain, cleaving off a pyrophosphate group. Ideally, the number of DNA copies is doubled in each cycle. Therefore, a single copy of target DNA should theoretically be multiplied to 2<sup>30</sup>, i.e., 1.074 x 10<sup>9</sup>, copies after 30 cycles. In practice, however, the number of copies in the final reaction product is lower, mainly due to inhibitory effects, the influence of structural and methodological parameters as well as the exhaustion of reagents.

The undisputed success of detection assays based on the polymerase chain reaction (PCR) has been largely due to its speed in comparison to many conventional diagnostic methods. In addition, microbial agents that are difficult to propagate outside their natural host often remain undetected by techniques relying on cultural enrichment. The enormous potential of DNA amplification assays in respect to specificity and sensitivity would demand a continual eye on the current developments in this area. PCR has the ability to amplify specific DNA sequences in an exponential fashion by *in vitro* DNA synthesis. It is possible to produce millions of copies of a characteristic genomic segment starting from just a few molecules of template DNA. It is a technique, which is used to detect, identify and differentiate microbial agents present in either clinical or environmental samples. Target sequences used in PCR detection assays of microorganisms have included 16S rRNA, 18S rRNA, 16-23S intergenic spacer, 23S rRNA. The ribosomal (r) RNA gene region has emerged as the most prominent target in microbial detection because the region represents a versatile mix of highly conserved and moderately to highly variable segments and are now known for virtually all microorganisms of veterinary and human health interest. Many PCR assays targeting protein genes were developed in an effort to genetically replicate conventional typing methods based on phenotypic properties, such as serological reactivity, enzymatic or

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toxigenic activity. Methods have also been developed on largely universal housekeeping proteins, e.g., elongation factor EF-Tu, DNA repair enzymes, DNA-binding proteins, etc., that are present in all organisms and whose sequences are phylogenetically interrelated in a manner comparable to rRNA genes. Toxin genes naturally lend themselves as targets because, in many instances, they were among the first genes cloned from the respective microbes, thus they are usually well characterized. Other frequently used targets are the genes of surface antigens or outer membrane proteins. There are many reports of genes coding for cellular enzymes, essential transporters, DNA repair enzymes, heat shock proteins, invasion factors, and various virulence factors being used in PCR assays.

The sensitivity of the detection assay is connected with the nature of the target region via the efficiency of primer binding. The finding that different primer pairs for the same gene can exhibit up to 1000-fold differences in sensitivity illustrates the extent of this relationship. Some microorganisms possess repetitive sequences or insertion elements present in multiple copies, which can serve as targets. In combination with sequence-specific DNA capture prior to amplification, a detection limit of one mycobacterial genome was attained. The high sensitivity of PCR inevitably leads to a greater number of positive samples in comparison to conventional methods. As a rule, the agent will be detected over a longer period in the course of tracking. That PCR test may detect DNA from nonviable or dead microbial cells is occasionally interpreted as a weak point. The question of whether such a finding really represents a false positive result is difficult to answer unambiguously. On the other hand, microorganisms identified by PCR, even if nonviable or nonculturable, can provide important evidence on the presence of a species that would have remained undetected by other methods.

Since the invention of thermal cyclers in connection with thermostable DNA polymerases, real-time detection equipment has introduced the second major automation event into PCR technology. The real-time mode of amplification has basically abolished the need to open PCR tubes following amplification, which is the main source of what is known as "carryover contamination". It also facilitates the automation of the methods. In addition, application of solution hybridization probes in combination with fluorescence dyes can increase diagnostic specificity and sensitivity of PCR analysis. Automation has increased the throughput capacity of PCR laboratories substantially. Current instruments with which our laboratory is acquainted or has had direct experience include the Light Cycler (Roche), the iCycler (Bio-Rad), the Rotar-Gene, Centrifugal Real-Time DNA Amplification System (sold by Phasix Research Products, manufactured by Corbett Research) and the SmartCycler ([www.smart-cycler.com](http://www.smart-cycler.com), Cepheid, Sunnyvale, CA). The work of this project was completed on an iCycler.

**qPCR Results:**



**Microbe Inotech Laboratories, Inc.  
PCR Quantification Report**

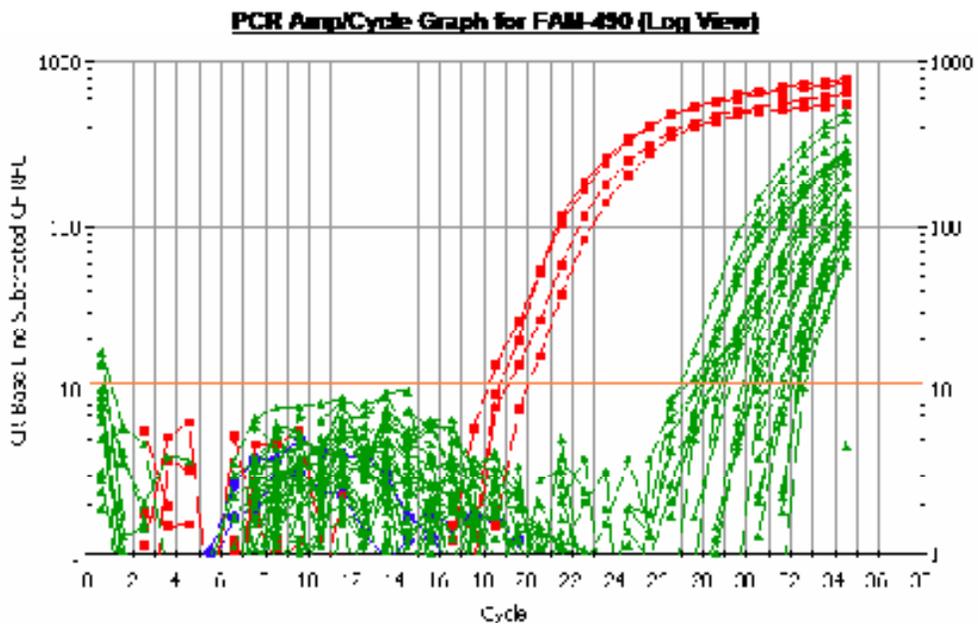
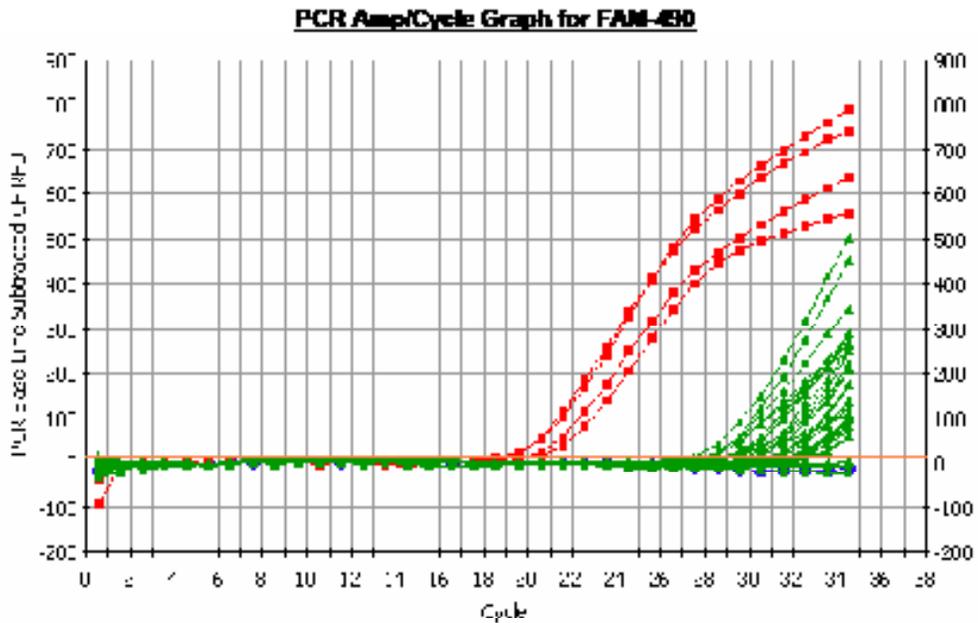
Current Date: 05-Dec-07 01:47 PM  
 Data generated on: 04-Dec-07 at 11:45 AM.  
 Optical data file name: Data 04-Dec-07 1145.opd  
 Plate Setup file used: 120407.pls  
 Protocol file used: Mycobacterium.2.tmo  
 Sample volume: 25.00 ul  
 Hot Start? No  
 Well factor collection: Experimental Plate

Comments

Protocol

Cycle 1: { 1X}		
Step 1:	95.0PC	for 03:00
Cycle 2: { 35X}		
Step 1:	95.0PC	for 00:30
Step 2:	52.0PC	for 00:30
Step 3:	72.0PC	for 01:00
Data collection enabled.		
Cycle 3: { 1X}		
Step 1:	72.0PC	for 05:00
Cycle 4: { 92X}		
Step 1:	54.0PC	for 00:20
Increase setpoint temperature after cycle 2 by 0.5PC		
Melt curve data collection and analysis enabled.		
Cycle 5: { 1X}		
Step 1:	4.0PC	HOLD

# APPENDIX D: MiL, Inc. PCR ANALYSES (CONTINUED)

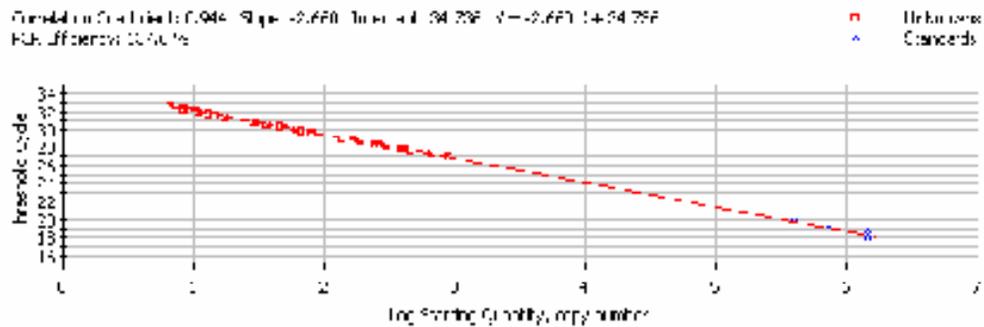


**Note:** Data for Mycobacterium DNA standards are shown in red squares. Data for background negative control are shown in blue circles. Unknown Sample data are shown in upward green triangles.

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## APPENDIX D: MiL, Inc. PCR ANALYSES (CONTINUED)

### Standard Curve Graph for FAM-450



### Data Analysis Parameters

Calculated threshold has been replaced by the user selected threshold 10.9.

Per-well baseline cycles have been determined automatically.

Data analysis window is set at 95.00% of a cycle, centered at end of the cycle.

Weighted Mean digital filtering has been applied. Global filtering is off.

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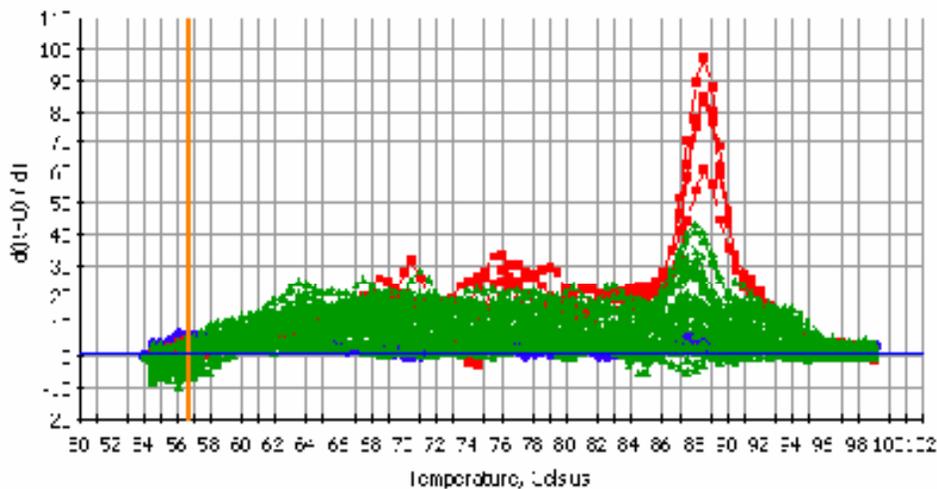
# APPENDIX D: MIL, INC. PCR ANALYSES (CONTINUED)

**Standard Curve Spreadsheet Data for FAM-490 Units: copy number**

Type	Identifier	Rep	Ct	Log SQ	SQ	SQ Mean	SQ SD	Ct Mean	Ct SD
A06 Standard	STD II	2	19.95	5.589	3.88E+05	3.88E+05	N/A	19.95	N/A
A08 Standard	STD III	3	19.03	5.843	6.97E+05	6.97E+05	N/A	19.03	N/A
A09 Standard	STD IV	4	18.16	6.143	1.38E+08	1.38E+08	0.00E+00	18.43	3.88E-01
A10 Standard	STD IV	4	18.70	6.143	1.38E+08	1.38E+08	0.00E+00	18.43	3.88E-01
C02 Unknown	51B4A-1	1	28.14	2.479	3.01E+02	2.18E+02	1.18E+02	28.61	6.58E-01
C03 Unknown	51B4A-1	1	29.07	2.129	1.35E+02	2.18E+02	1.18E+02	28.61	6.58E-01
C04 Unknown	51B4A-2	2	27.24	2.818	6.58E+02	3.37E+02	2.78E+02	28.25	8.85E-01
C05 Unknown	51B4A-2	2	28.85	2.211	1.63E+02	3.37E+02	2.78E+02	28.25	8.85E-01
C06 Unknown	51B4A-2	2	28.67	2.279	1.50E+02	3.37E+02	2.78E+02	28.25	8.85E-01
C08 Unknown	51B4A-3	3	30.22	1.698	4.56E+01	2.89E+01	2.93E+01	31.28	1.47E+00
C09 Unknown	51B4A-3	3	32.30	0.918	1.23E+00	2.89E+01	2.93E+01	31.28	1.47E+00
C10 Unknown	51B4A-4	4	29.88	1.788	6.13E+01	4.19E+01	1.73E+01	30.48	4.58E-01
C11 Unknown	51B4A-4	4	30.88	1.451	2.82E+01	4.19E+01	1.73E+01	30.48	4.58E-01
C12 Unknown	51B4A-4	4	30.59	1.558	3.61E+01	4.19E+01	1.73E+01	30.48	4.58E-01
E01 Unknown	51B4A-5	5	31.65	1.161	1.45E+01	1.19E+01	2.25E+00	31.89	2.10E-01
E02 Unknown	51B4A-5	5	31.97	1.038	1.09E+01	1.19E+01	2.25E+00	31.89	2.10E-01
E03 Unknown	51B4A-5	5	32.04	1.014	1.03E+01	1.19E+01	2.25E+00	31.89	2.10E-01
E04 Unknown	51B4A-8	6	29.68	1.901	7.97E+01	2.35E+02	1.53E+02	28.64	9.35E-01
E05 Unknown	51B4A-8	6	27.85	2.588	3.86E+02	2.35E+02	1.53E+02	28.64	9.35E-01
E06 Unknown	51B4A-8	6	28.40	2.380	2.40E+02	2.35E+02	1.53E+02	28.64	9.35E-01
E07 Unknown	51B4A-7	7	31.80	1.103	1.27E+01	1.50E+02	2.24E+02	30.13	2.08E+00
E08 Unknown	51B4A-7	7	27.79	2.811	4.89E+02	1.50E+02	2.24E+02	30.13	2.08E+00
E09 Unknown	51B4A-7	7	30.81	1.478	2.99E+01	1.50E+02	2.24E+02	30.13	2.08E+00
E10 Unknown	51B4A-8	8	31.45	1.238	1.72E+01	1.06E+01	5.77E+00	32.11	5.88E-01
E11 Unknown	51B4A-8	8	32.32	0.908	1.11E+00	1.06E+01	5.77E+00	32.11	5.88E-01
E12 Unknown	51B4A-8	8	32.57	0.814	6.51E+00	1.06E+01	5.77E+00	32.11	5.88E-01
G05 Unknown	51B4A-10	10	28.30	2.418	2.62E+02	1.38E+02	1.75E+02	29.97	2.38E+00
G06 Unknown	51B4A-10	10	31.63	1.168	1.47E+01	1.38E+02	1.75E+02	29.97	2.38E+00
G07 Unknown	51B4A-11	11	26.88	2.952	3.55E+02	5.88E+02	2.68E+02	27.44	4.88E-01
G08 Unknown	51B4A-11	11	27.77	2.818	4.14E+02	5.88E+02	2.68E+02	27.44	4.88E-01
G09 Unknown	51B4A-11	11	27.66	2.858	4.56E+02	5.88E+02	2.68E+02	27.44	4.88E-01
G10 Unknown	51B4A-12	12	29.91	1.814	6.52E+01	3.99E+01	2.84E+01	30.81	1.20E+00
G11 Unknown	51B4A-12	12	32.17	0.983	1.18E+00	3.99E+01	2.84E+01	30.81	1.20E+00
G12 Unknown	51B4A-12	12	30.33	1.855	4.52E+01	3.99E+01	2.84E+01	30.81	1.20E+00
C01 Unknown	51B4A-1	1	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C07 Unknown	51B4A-3	3	N/A	N/A	N/A	N/A	N/A	N/A	N/A
G01 Unknown	51B4A-8	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A
G02 Unknown	51B4A-8	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A
G03 Unknown	51B4A-8	8	N/A	N/A	N/A	N/A	N/A	N/A	N/A
G04 Unknown	51B4A-10	10	N/A	N/A	N/A	N/A	N/A	N/A	N/A

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**Melt Curve Graph for FAM-490**



Note: Data for *Mycobacterium* DNA standards are shown in red squares. Data for background negative control are shown in blue circles. Unknown Sample data are shown in upward green triangles.

**Summary Comments and Conclusions:**

Sample #1 (ID: L1 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $2.18 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $1.74 \times 10^3$  cells/mL or  $6.98 \times 10^4$  cells/40mL.

Sample #2 (ID: L8 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $3.37 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $2.70 \times 10^3$  cells/mL or  $1.08 \times 10^5$  cells/40mL.

Sample #3 (ID: L13 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $2.89 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $2.31 \times 10^3$  cells/mL or  $9.25 \times 10^4$  cells/40mL.

Sample #4 (ID: L20 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $4.19 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $3.35 \times 10^3$  cells/mL or  $1.34 \times 10^5$  cells/40mL.

Sample #5 (ID: L25 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $1.19 \times 10^3$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $9.52 \times 10^3$  cells/mL or  $3.81 \times 10^5$  cells/40mL.

Sample #6 (ID: L26 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean

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## APPENDIX D: MiL, Inc. PCR ANALYSES (CONTINUED)

population level for duplicate readings of  $2.35 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $1.88 \times 10^2$  cells/mL or  $7.52 \times 10^4$  cells/40mL.

Sample #7 (ID: L27 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $1.50 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $1.20 \times 10^2$  cells/mL or  $4.80 \times 10^4$  cells/40mL.

Sample #8 (ID: L28 Res) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $1.06 \times 10^3$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $3.48 \times 10^3$  cells/mL or  $3.39 \times 10^5$  cells/40mL.

Sample #9 (ID: L29 Res) was negative for the presence of *Mycobacterium immunogenum*.

Sample #10 (ID: Shark East) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $1.38 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $1.10 \times 10^2$  cells/mL or  $4.42 \times 10^4$  cells/40mL.

Sample #11 (ID: Shark West) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $5.88 \times 10^2$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $4.70 \times 10^2$  cells/mL or  $1.88 \times 10^5$  cells/40mL.

Sample #12 (ID: Prenett) was positive for the presence of *Mycobacterium immunogenum* at a mean population level for duplicate readings of  $3.99 \times 10^3$  copy number/mL in the 5mL concentrated pellet which was derived from 40mL or an 8x concentration for a mean mycobacterium population of  $3.19 \times 10^3$  cells/mL or  $1.28 \times 10^5$  cells/40mL.

Respectfully submitted,  
[Signatures on hardcopy originals]

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## ACKNOWLEDGEMENTS AND AVAILABILITY OF REPORT

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This report was prepared by Kristin Cummings, Randy Boylstein, and Jean Cox-Ganser of RDHETAP, Division of Respiratory Disease Studies. Desktop publishing was performed by Nicole Edwards.

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