

BY MICHAEL D. GERNON AND
BRUCE C. HEMMING



MYSTERIES OF MYCOBACTERIA

F

or a number of years, the metalworking fluid industry has suspected that an occupational lung disease known as Hypersensitivity Pneumonitis may be caused by exposure to metalworking fluid mists. In the past decade, a number of clusters of illnesses at metal removal plants have caught the attention of

researchers, union representatives and plant operators.

As a case in point, in November 2001 the Centers for Disease Control reviewed a situation that involved the hospitalization for respiratory illness of three machinists from an automobile brake manufacturing facility in Ohio. A review of the facility person-



Research Delves into Metalworking Fluid Risks



part-time work duties in the machining portion of the plant; the median length of time working at the plant was 18 years (range: 3 to 32 years).

Initial symptom onset for this cluster occurred during October 2000 with continued cases being reported through April 2001. The peak reporting frequency occurred in December 2000. From February through July 2001, multiple samples of bulk metalworking fluid from all central plant systems were analyzed. This testing revealed the predominant growth of a newly proposed species of the *Mycobacterium abscessus* / *Mycobacterium chelonae* group, with the name of *Mycobacterium immunogenum*. This bacterium was found at population levels of up to 10^6 (1 million) bacteria per milliliter.

Based on this and related incidents, a limited number of scientists and industrial health-care professionals have proposed a link between *M. immunogenum* and metalworking fluid related Hypersensitivity Pneumonitis (HP). However, the link between *Mycobacterium* and HP remains a hypothesis at this point with a number of research groups around the

world starting to work on definitively proving or disproving the connection.

What are Mycobacteria?

Arguably, the most notorious *Mycobacterium* known to man is the tuberculosis-causing organism *Mycobacterium tuberculosis*. Tuberculosis is a major global health problem plaguing hundreds of millions of people worldwide. The equally sinister but somewhat better controlled pathogenic member of the genus *Mycobacterium* is the leprosy-causing organism *M. leprae*. Almost everyone has a visceral reaction to any mention of leprosy despite the fact that the disease has been virtually eradicated except for a few isolated areas, such as certain parts of Brazil and India.

Species of the genus *Mycobacterium* are broadly grouped into two major categories on the basis of pathogenicity for animals and humans. Since the isolation and characterization of the causal agent of tuberculosis by Koch in 1882, many species were rapidly identified. *M. avium* was associated with a tuberculosis-like disease in fowl (1891); *M. paratuberculosis* was

nel records found that 107 (27 percent) of 400 workers had been placed on work restriction by their physicians during the preceding 11 months because of respiratory conditions; 37 (35 percent) of these 107 workers remained on medical leave and 70 (65 percent) had returned to work. Thirty-two workers had either full- or

isolated from cattle and sheep with chronic enteritis (1895); and *M. lepraemurium*, the rat leprosy bacillus, was described in 1903. During this same period, a number of saprophytic species, such as *M. phlei* and *M. smegmatis*, were also identified. (“Saprophytic” denotes an organism that feeds on dead organic matter.)

Beyond this, the genus *Mycobacterium* is fairly innocuous. The organism *M. marinum* causes a form of tuberculosis in fish that plagues aquarium keepers, and a variety of environmental mycobacteria have from time to time been implicated in minor disease outbreaks.

The International Journal of Systematic Microbiology now recognizes more than 30 species of mycobacteria, more than half of which were not identified until after 1950. Not all of these produce disease, so a distinction must be made between potential pathogens and commonly saprophytic species. The extremely pathogenic organisms *M. tuberculosis* and *M. leprae* are obligate parasites (they must transfer directly from host-to-host without residence in the environment), but the majority of species — such as *M. smegmatis* and *M. terrae* — are ubiquitous in the environment.

The advent of HIV infections in the early 1980s made it necessary to understand and mitigate the pathogenic potential of environmental mycobacteria in immuno-compromised individuals, but outside the realm of the immuno-suppressed population (such as recent organ transfer recipients, AIDS patients, sepsis patients, etc.) environmental mycobacteria are fairly innocuous. However, as mentioned above, the species *M. immunogenum* has been implicated as a cause of the infrequent but serious lung condition Hypersensitivity Pneumonitis.

The occurrence of HP has been strongly correlated with chronic exposure to metalworking fluid mists, and the bulk of the metalworking industry

has started to examine methods for the control of mycobacteria with an aim toward elimination of HP.

Seeking Answers Given the ubiquitous nature of the genus *Mycobacterium*, one might ask: Just how dangerous are environmental mycobacteria? How does one detect and quantify mycobacteria? Why would mycobacteria proliferate in preference to other environmental bacteria? In order to feel more comfortable in a world inundated with environmental mycobacteria, it would help to have a basic understanding of these ubiquitous organisms.

The mycobacteria are unicellular, aerobic, weakly Gram-positive but still acid-fast bacteria (i.e., once stained with one of the basic dyes such as fuchsin, they resist decolorizing with mineral acids or with acidified organic solvents).

The cell walls of mycobacteria are rich in mycolic acids (long-chain fatty acids about 40-97 carbons in length). This lipid-rich cell wall limits movement of dye either into or out of the cell. Stain may be taken up uniformly, but cells usually appear beaded or granular, with heavily stained areas separated by non-stained spaces.

The early subdivision of the “atypical” mycobacteria into “groups”, on the basis of pigment production and speed of growth, was proposed to provide a more systematic basis for study and discussion of these organisms. Even today, many clinical laboratories and global reference laboratories find it convenient to subdivide the mycobacteria on the basis of pigment production and growth rate, thus enabling a more rational selection of the key tests needed to precisely identify an unknown *Mycobacterium* species.

Although most mycobacteria are not very fastidious once they have adapted to in vitro growth, many investigators still feel that a “richer”

medium is necessary for primary isolation. In terms of cellular morphology, *Mycobacterium* colonies on egg-potato or serum-agar base formulations often appear granular.

How to rapidly quantify the microbial population is a fundamental problem in the investigation of the occurrence of *Mycobacterium* in complex environments like metalworking fluids. *Mycobacterium* are slow-growing organisms that are difficult to quantify with traditional culture techniques. The problem of accurate measurement is so fundamental to this field that an entire symposium has been scheduled for Dec. 5, 2004, in Tampa, Fla. The symposium, “Recovery and Enumeration of Mycobacteria from the Metalworking Fluid Environment,” is jointly sponsored by ASTM Committee E34.50 on Health and Safety of Metalworking Fluids and Committee D02.L.1 on Metalworking Fluids. (See www.astm.org.)

Tools and Tests Recently, the analysis of *Mycobacterium* species has been fostered by HPLC (High Pressure Liquid Chromatography), via a procedure that isolates, separates and compares each sample’s mycolic acid profile with a library of known profiles obtained from a range of mycobacteria. However, mycolic acid-HPLC separations must be performed at elevated temperatures, and it is essential that separation and detection temperatures be precisely and reproducibly controlled. So, while HPLC offers an improvement over traditional culture techniques, it is more likely that investigations using modern methods based on immunological techniques or DNA will ultimately prove or disprove the link between *Mycobacterium sp.* and HP.

One well-known immunological technique is called ELISA (Enzyme-Linked ImmunoSorbent Assay). In this multi-step assay, high-affinity “capture” antibodies specific to a particular strain of mycobacteria are

used to indicate the concentration of those mycobacteria in a given sample. ELISA is relatively fast and easy to perform in most medical laboratories, but its accuracy can be affected by how selective the antibody is to the mycobacteria in question.

More recently, DNA technology has initiated a new era in environmental microbiology. Nucleic acid based

response to a change in the environment. The authors of this article have been applying these methods to *Mycobacterium* detection, identification and population size determinations in metalworking fluids.

The undisputed success of detection assays based on the polymerase chain reaction has been largely due to PCR's speed in comparison with

rate assessment of the connection between *Mycobacterium* and HP will ultimately be made.

Arkema (formerly Atofina Chemicals) is currently conducting several statistical studies of the influence of our high-performing Synergex™ amine additives as inhibitors of mycobacteria. The next generation of Synergex™ additives will be optimized for mycobacterial control. New additives will be available by June 2004.

Larger and more thorough studies aimed at a comprehensive understanding of the real danger of *Mycobacterium* contamination in metalworking fluids are being carried out by a number of other groups, too, such as NIOSH. With a combination of commercial and public efforts, the role of *Mycobacterium* species in metalworking fluid related respiratory health issues will ultimately be understood and controlled. ■

Dr. Michael D. Gernon, a senior research scientist with Taminco

in King of Prussia, Pa., has worked on metal finishing and metal forming problems for over 20 years. He has over 50 patents and publications in diverse areas related to the chemistry and composition of formulated products, and can be reached at Michael.Gernon@Taminco.com.

Dr. Bruce C. Hemming is president, CEO and founder of St. Louis, Mo., based Microbe Inotech Laboratories, a specialized microbial analysis laboratory serving the environmental, food, pharmaceutical and industrial markets. A well-known speaker, author and editor, he has conducted R&D in the microbiological arena for over 30 years. E-mail him at BHEemming@MicrobeInotech.com, or phone (800) 688-9144.

For information about this article and the influence of fluid components on microbiological growth, call Conor Dowling of Taminco at (610) 366-6730.

Comparing Mycobacteria Assay Methods

Typical Sensitivities and Time

Method	Detection level	Time to complete assay
Culture	10 ⁷ -10 ⁸	4-10 days
ELISA	10 ⁵ -10 ⁷	48 hr
DNA Probes	10 ⁶	48-72 hr.
Polymerase Chain Reaction (PCR)	10 ² -10 ³	24 hr

Source: Atofina Chemicals

methods provide specific, sensitive detection of microorganisms from a variety of environments. Information can be obtained about the kinds of organisms present (phylogenetic assessment) and/or about the specific capabilities of the organisms present (functional assessment). This technology has become an invaluable tool for detecting specific organisms and/or their functional genes.

One widely used nucleic acid technology is polymerase chain reaction, or PCR. The only traditional limitation of PCR was that it was not quantitative, but as technology has advanced, this limitation has been overcome, and quantitative (real-time) PCR is now possible.

Quantitative PCR (qPCR) is possible through the combined use of specialized PCR reagents (e.g., TaqMan) and refined instrumentation. This advance is particularly useful in environmental microbiology because the population size — the amount of a particular organism — can be determined, and thus population changes can be tracked over time or in

many conventional diagnostic methods, such as cultures (see table).

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 with respect to specificity and sensitivity demands 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. The PCR technique can be used to detect, identify and differentiate microbial agents present in either clinical or environmental samples.

With PCR based techniques, a thorough and complete analysis of suspect metalworking fluids has become possible. Through statistical analysis of a large number of metalworking fluids, along with clinical analysis of any associated lung disease, an accu-