Report of results of the study
„Existence of mycobacteria in water-mixed metalworking fluids“

(Translation of the german study:
Ergebnisbericht zur Studie “Vorkommen von Mycobakterien in wassergemischten Kühlschmierstoffen“)

Client:
Süddeutsche Metall-Berufsgenossenschaft
(Institution for statutory accident insurance and prevention in the metalworking industry in southern Germany)
Section: Occupational Safety and Health
Subject area: Biological noxious substances
Responsibility: Dr. Isabel Warfolomeow

Research execution:

National Reference Centre for mycobacteria (NRZ)
Research Centre Borstel
Direction: Dr. S. Rüscher-Gerdes

Companies involved in the project:

Blaser Swisslube AG
Castrol Industrie GmbH
Fuchs Schmierstoffe GmbH
Oemeta Chemische Werke GmbH
DaimlerChrysler AG

Issue: April 2004
Definition of question and introduction

For several years, the USA have repeatedly been recording cases of Hypersensitivity Pneumonitis (HP) by mycobacteria, primarily Mycobacterium chelonae and Mycobacterium immunogenum (both risk group 2 according to TRBA 466) in connection with the application of water-mixed metalworking fluids. This was, particularly in the motorcar and component supplying industry. The different health organizations in the USA pay great attention to this subject and in the mean time, different bodies in this country as well turned to the Berufsgenossenschaften with regard to possibly existing risks. Since mycobacteria are not examined routinely in metalworking fluid samples due to the difficult detection procedure, no statements could have been made to this subject up to now.

The genus mycobacterium belongs to the order of actinomycetales and to the family of mycobacteriaceae. According to infectiological und epidemiological criteria, the genus mycobacterium can be classified into three groups:

- Mycobacterium tuberculosis complex
- Mycobacterium leprae
- Non-tuberculous mycobacteria (former term: „atypical mycobacteria“)

Mycobacteria are aerobic Gram-positive unregularly formed, slightly branched cells. A principle characteristic is their „acid resistance“ which is based on the high amount of mycol acids at the cell membrane which makes the cells wax-like and consequently highly hydrophobic. Whereas pathogenic kinds have to rely on complex nutrient substrates and grow very slowly, many ubiquitous existing mycobacteria don’t require additional substances and grow relatively fast.

Non-tuberculous mycobacteria may be found all over the world in the environment (water, ground) and they represent a component of the mucous membrane flora of human beings and animals. Many of them live saprophytically on organic material such as metabolic products of other microorganisms or dead biomass. Therefore, it seems a likely supposition that a high number of colonies, e. g. in water-mixed metalworking fluids represent favorable growth preconditions.

Infections by non-tuberculous mycobacteria are rare and are favoured by a reduced cellular immunity. They are found in large numbers with particular malignoms, patients with immune suppressive therapy and with AIDS-patients, as well as with existing bronchopulmonal diseases such as bronchiectases, chronic obstructive bronchitis, silicosis or the state after pulmonary tuberculosis.

The most frequently described symptoms by non-tuberculous mycobacteria are pulmonary infections (M. kansasii, M. marinum, M. avium-intracellulare, M. xenopi, M. fortuitum), abscesses at skin and soft parts (M. marinum, M. chelonae, M. fortuitum) and lymphatic diseases (M. scrofulaceum, M. szulgai). The disease is spread through inhalation or direct contact. Man to man infection is not known.

The interpretation of a positive result of non-tuberculous mycobacteria with regard to the clinical significance is difficult, since the pathogens are ubiquitous and occur with healthy persons as well. The unique proof taken from sputum, urine or bronchial secretion is not sufficient to make a diagnosis; only 10% of all persons having positive results actually have a mycobacteriosis.

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1 Technical rules for biological working material (TRBA) 466: „Classification of bacterium in risk groups“
Mycobacteriosis caused by non-tuberculous mycobacteria are not notifiable; recognitions as occupational disease have not occurred so far.

*Mycobacterium immunogenenum* was described for the first time in 1998 and is classed as M. chelonae-complex. It concerns non-tuberculous fast growing mycobacteria which were primarily described in connection with subcutaneous abscesses. Diseases of the respiratory tract by species of the mycobacterium chelonae-group were not known so far.

As cause for an exogenous allergic alveolitis, i.e. an inflammation of the alveols by immune complexes with subsequent formation of a scar and resulting reduction of the pulmonary functions is possible. Microbial allergenes are also considered to be the cause besides a large number of animal, plant, chemical and medicine allergenes. Primarily the thermophilic actinomycetes are counted among the reliably proven allergenes (e.g. symptoms of the farmer’s lung or humidifier lung), different mold fungus, such as aspergilli-, penicillium-species, mushroom spores or yeast, such as trichosporon, rhodotorula. Mycobacteria were neither mentioned as probable nor as suspicious allergenes in this connection.

With machine operators in the USA, interstitial changes of the lung and reductions of the pulmonary functions were detected in the sense of an EAA at simulatneous existance of specific IgG-antibodies and a correspondig positive result of mycobacteria (mainly *M. chelonae* and *M. immunogenenum*) in water-mixed metalworking fluids. A definite connection of the course of the disease in the sense of an occupation related cause has not been proven up to now.

Since mainly Hexahydrotriazine (HHT) were applied as biocide substances in the indicated metalworking fluid products, an insufficient strength towards mycobacteria was presumed. Even a specific promoting growth at an overdose of HHT was discussed as a possibility. This assumption finally led to a decree by the U.S. car manufacturers, to apply no longer all biocides on formaldehyde depot base for the pre-conservation of metalworking fluids, which had not inconsiderable consequences for the German market as well.

In the scope of this project it should have been examined at the first step, whether mycobacteria can be detected in different samples of water-mixed metalworking fluids, which species they belong to and if their occurrence is significant.

Moreover, different manufacturers of preservation-additives presently carry out studies concerning the effectiveness of different biocide-formulations about mycobacteria.
Material and methods

Pretests

Due to the long generation time of mycobacteria, the following must be taken into account: For the suppression respectively for the destruction of the accompanying flora - which would inevitably lead to an overgrowth of the cultures - a pretreatment of the material to be examined is required. According to the DIN-regulation 58 943 part 3 \(^2\) the pretreatment is carried out with N-Acetyl-L-Cystein-NaOH. However, it can be assumed with this, that approx. 10% of the mycobacteria can die by the pretreatment as well. In this respect, a more precise quantifying is not possible.

- Due to the long generation time of mycobacteria (16-20 hours) very long culture times are required on solid media. (3-4 weeks). By applying a liquid medium and indicators for the growth of the microorganisms, the sensitivity and the detection time is considerably reduced.

- The decontamination of the examined material with N-Acetyl-L-Cystein-NaOH and a combination of liquid and solid media has proven to be the optimum way for delivering the cultural proof of mycobacteria and is regarded today as standard method.

<table>
<thead>
<tr>
<th>Method of pretreatment:</th>
<th>N-Acetyl-L-Cystein-NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied nutrient media</td>
<td></td>
</tr>
<tr>
<td>Solid nutrient media:</td>
<td>Löwenstein-Jensen; Stonebrink; Middlebrook 7H10</td>
</tr>
<tr>
<td>Liquid nutrient media:</td>
<td>MGIT 960</td>
</tr>
<tr>
<td>Incubation temperature:</td>
<td>31° and 37° C</td>
</tr>
</tbody>
</table>

50 ml of a freshly prepared metalworking fluid sample had been each mixed with approx. \(10^4 \) – \(10^5\) CFU\(^3\)/ml *Mycobacterium chelonae* resp. *Mycobacterium avium* and were detected after successful cultivation.

Results of the pretests:

| Metalworking fluid sample without bacteria addition (blank test) | negative |
| Metalworking fluid sample mixed with *M. chelonae*                    | growth detectable |
| Metalworking fluid sample added with *M. avium*                      | growth detectable |

The pretests showed that there is no inhibition by the pretreatment with N-Acetyl-L-Cystein-NaOH due to possible interactions with the metalworking fluid and that the procedure chosen for the detection of mycobacteria from metalworking fluid samples can be applied.

\(^2\) DIN 58 943 part 3: Medical microbiology; tuberculosis diagnostics, cultural methods for isolation

\(^3\) CFU = Colony-forming-unit
Main test series

A total of 40 metalworking fluid samples (factory samples) of four metalworking fluid manufacturers were tested (BLASER SWISSLUBE AG, CASTROL INDUSTRIE GmbH, FUCHS SCHMIERSTOFFE GmbH, OEMETA CHEMISCHE WERKE GmbH) from different metalworking companies. Particular emphasis was laid on having a wide range of different samples, both with regard to the applied metalworking fluids as well as to the machining process and internal factory conditions. More detailed information concerning the tested metalworking fluid samples are listed in the annex.

The factory samples (10 samples per manufacturer) were made anonymous and sent to the NRZ for mycobacteria in Borstel, Germany.

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<td>Solid nutrient media:</td>
<td>Löwenstein-Jensen; Stonebrink; Middlebrook 7H10</td>
</tr>
<tr>
<td>Liquid nutrient media:</td>
<td>MGIT 960 (for incubation at 37° C) BACTEC 460 TB (for incubation at 31° C)</td>
</tr>
<tr>
<td>Incubation temperature:</td>
<td>31° and 37° C</td>
</tr>
<tr>
<td>Type:</td>
<td>Sequence analysis of 16S rRNA</td>
</tr>
</tbody>
</table>

The media had been incubated 6-8 weeks and once per week a control of the growth of mycobacteria was made. The evaluation and identification was made by microscopic analysis as well as by conventional cultivating procedures and molecular-diagnostic methods via Realtime-CR (Light Cycler). The cultural proof was carried out for both tuberculosis and non-tuberculous mycobacteria (NTM).

Results

39 of the 40 tested metalworking fluid samples were negative, i.e. no mycobacteria could have been detected. Mycobacterium immunogenenum was detected in one sample only (no. 25).

Assessment and discussion

As to the specifications of the individual metalworking fluid samples it has to be mentioned that these are only taken from the information given by the individual metalworking fluid manufacturer. They are not standardized with regard to the applied detection method. Therefore, a direct comparison of the data is only possible to a limited extent. The information should rather serve as a general review on the tested factory samples. A detailed list can be found in the annex to this report.
The majority of the tested metalworking fluid samples were mineral oil based products coming in about equal shares from the operational working stations (n = 22) and central plants (n = 17). More than half of the samples were free of bactericides; with the remaining samples, mainly formaldehyde depot substances (n = 18) were applied for the preconservation. Nearly all samples contained fungicides; Na-Pyrrithione (n = 16) and Iodine-Carbamate (n = 19) were applied with approximately equal frequency. The average sump-life when taking a sample was at 18.5 months; shortest sump-life one month, longest “sump-life” 150 months (12.5 years !?!). Grinding, milling and drilling represented the most frequent working procedures, steel and cast iron were the most frequently used materials.

The average concentration of the metalworking fluid samples at the determination via refractometer was at 7.4 % (n = 34) (lowest concentration 2.6%, highest concentration 14.0 %). Determination of concentration by emulsion splitting flask (DIN 51 368) and acid titration partly showed considerably deviating values, probably due to a high share of foreign oil. The pH-value was between 7.8 and 9.4 at an average pH of 8.8 (n = 39).

The nitrite concentration with an average of 3 ppm (n = 32) for nearly all of the samples was very low, resp. below the detection limit. Only with two samples, a nitrite concentration of 20 ppm was detected. A correlation of nitrite content to the TCFU was not ascertained.

The information as to the total-colony-forming-units of the samples was lying between 0 and 152 million bacteria (n = 40). More than half of the samples (n = 24) had more than 10⁶ CFU/ml; fungus was detected in 11 samples.

Central systems were charged on average with 3.0 x 10⁶ CFU/ml (Median 1.0 x 10⁴ CFU/ml), operational working stations with 1.6 x 10⁷ CFU/ml (Median 1.5 x 10⁶ CFU/ml).

If the average values are calculated separately according to manufacturers, the following values are achieved:

<table>
<thead>
<tr>
<th></th>
<th>All samples</th>
<th>Central systems</th>
<th>Operational working stations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average values</td>
<td>Median CFU/ml</td>
<td>Average values</td>
<td>Median CFU/ml</td>
</tr>
<tr>
<td>CFU/ml</td>
<td></td>
<td>CFU/ml</td>
<td></td>
</tr>
<tr>
<td>Manufacturer 1</td>
<td>3,1 x 10⁷</td>
<td>3,6 x 10⁶</td>
<td>-</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manufacturer 2</td>
<td>1,1 x 10⁸</td>
<td>5,0 x 10⁷</td>
<td>1,4 x 10⁸</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Manufacturer 3</td>
<td>6,1 x 10⁶</td>
<td>1,0 x 10⁷</td>
<td>4,0 x 10⁸</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Manufacturer 4</td>
<td>8,0 x 10⁵</td>
<td>1,0 x 10⁶</td>
<td>3,4 x 10⁸</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
<td></td>
<td>(n = 3)</td>
</tr>
</tbody>
</table>

Test samples nos. 24 and 25 (annex) are metalworking fluid samples taken from two different plants from a company in the USA; all other samples came from Germany.

In one single sample only, number 25, mycobacteria, namely mycobacterium immunogenum was detected as only species.

As already indicated in the introduction, M. immunogenum belongs to the non-tuberculous mycobacteria (NTM), which are very common in the environment. Due to the classification in risk group 2 according to TRBA 466, a potential infection risk must be assumed, although, M. immunogenum is often estimated to be non-pathogene.
Metalworking fluid sample number 25 was a preconserved metalworking fluid on an ester oil base (synthetic fatty acid ester) from a central system with a formaldehyde depot (O-Formal) and Na-Pyrithione as biocides. The pH-value was 8.7 and therefore in the normal range, as well as the total-colony-forming-units with $10^4$ CFU/ml; mould fungus were detectable. More conclusions with regard to an infection with mycobacteria cannot be drawn from comparing the sample data.

Metalworking fluid samples of the same product-- in this case the samples 24 (USA), 28 and 29 – however, were negative with regard to mycobacteria. This at least implies that there are no products which are somehow „predestined“ as far as a contamination with mycobacteria is concerned.

This applied as well for the assumption that a high number of colonies and with that the provision of organic material and metabolic products are likely to be growth promoting for mycobacteria. This could not be confirmed by the tests. In spite of a mainly high CFU – with the exception of sample no. 25 – neither pathogene nor apathogene mycobacteria were detected in the factory samples.

Comparable tests of users and manufacturers confirm these results: In eight samples taken from a factory of a large-size motorcar manufacturer – six different water-mixed metalworking fluids from central plants, a watermiscible quenching fluid and a recooling plant – no mycobacteria could have been detected.

In addition to that, another test which was also carried out at the NRZ for mycobacteria in Borstel by order of a metalworking fluid manufacturer in a so called „inoculation-test“ of a fresh metalworking fluid emulsion spiked with *Mycobacterium chelonae* (initial concentration $10^4$ CFU/ml). That within a period of 14 days, the number of detectable mycobacteria in the inoculated emulsion considerably decreases, the water-mixed metalworking fluid offers obviously no adequate growth conditions.

The question remains what might have caused the described contamination of water-mixed metalworking fluids with mycobacteria is to be traced back in the USA. Non-tuberculous mycobacteria are widespread in the environment (water, ground) and they represent a component of the mucous membrane flora of human beings and animals. Even in tap water systems there might be occasional contaminations with mycobacteria.

According to the guidelines concerning the drinking water regulation the tap-water in Germany is mainly disinfected by UV rays or ozone treatment; finally an additional chlorination might be carried out, if necessary. Although, no mycobacteria test is specified in the drinking water regulation, it can be assumed that due to strict regulations and intensive water treatment, the growth of other microorganisms is impeded.

The application of service-water - which is subjected to a higher microbial contamination - as mixing water for metalworking fluids is very rare in Germany.

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4 ($\leq 100$ CFU/ml, no detection of *E. coli*, total coliforms and fecal streptococci in 100 ml)
Whereas in the USA, the disinfection of drinking water is mainly performed by chlorination. Due to their particular cell membrane structure, mycobacteria have proven to be very resistant against environmental and chemical influences. May be the cause for a contamination of water-mixed metalworking fluids can be found at that stage, i.e. due to a microbial infection of the tap-/mixing water?! A possible increased application of contaminated service water for mixing the metalworking fluid must be taken into account as possible cause for an increased infection with mycobacteria.

Summary

Even if the 40 tested metalworking fluid samples do not represent a statistically validated result, the results show nevertheless that no significant occurrence of mycobacteria in water-mixed metalworking fluids has to be expected in this country. Whether this is due to a missing import of mycobacteria, for instance through the mixing water or missing growth preconditions in the metalworking fluid samples cannot be answered on the basis of the testing results. The present test results provide no reasons for a ban of formaldehyde-depots for the conservation of water-mixed metalworking fluids.

Therefore, it can be assumed that a risk for the employees due to mycobacteria when dealing with water-mixed metalworking fluids is not given in this country.

Data-table of the metalworking fluid samples (company samples): Click here!
Bibliographical reference

- „Mikrobiologische Diagnostik“, Burkhardt, Thieme-Verlag
- „Exogen Allergische Alveolitis“, Sennekamp, Dustri-Verlag Dr. Karl Feistle

Subject literature

- „White Paper – Hypersensitivity Pneumonitis: Is There an Association with Triazine Biocides and Mycobacteria in Metalworking Fluids?“, Independent Lubricant Manufacturers Association (ILMA), USA
- „Hypersensitivity Pneumonitis“, UAW; Occupational Health & Safety, Number 3, 2002
- „Respiratory Illness in Workers Exposed to Metalworking Fluid Contaminated with Nontuberculous Mycobacteria “, CDC; MMWR, April 26, 2002/ 51(16); 349-352
- „Mycobacterium sp. as a Possible Cause of Hypersensitivity Pneumonitis in Machine Workers“, Shelton et. al., Emerging Infectious Diseases Vol 5 Number 2, 1999
- „Mycobacterium immunogenum sp. nov., a novel species related to Mycobacterium abscessus and associated with clinical disease, pseudoutbreaks and contaminated metalworking fluids: an international cooperative study on mycobacteria taxonomy“, Wilson et. al., International Journal of Systematic and Evolutionary Microbiology (2001), 51, 1751-1764