Field Evaluation of Ochthinolone as a Fungicide For Metalworking Fluids®

A Case History

ANDREW B. RYAN (MEMBER, STLE)
Roth and Haas Co.
Research Division
Spring House, PA 19477

SUMMARY

Ochthinolone (2-acetyl-4-isothiocyanato-3-one) was evaluated for its ability to control microbial contamination in the fluid of two automotive metalworking fluid systems. Prior to the addition of ochthinolone, both systems displayed heavy slime accumulations primarily in and around the fluid fluxes. An initial dose of 50 ppm ochthinolone, followed by maintenance doses of 20 ppm every 2 to 14 days was found to provide excellent control of the fungal contamination. Dramatic improvement was noted in the appearance of the fluxes, wells and the surfaces bordering the systems as the fungal fouling was brought under control. An important additional benefit resulting from control of the fungal contamination in one of the systems was a significant reduction in the amount of fluid make up required for that system.

INTRODUCTION

Ochthinolone (2-acetyl-4-isothiocyanato-3-one) has been employed for several years as a mildewicide for paint films, laundry, plasticized vinyl and leather. Recently, it has been shown to be effective for controlling fungi in metalworking fluids. Results of laboratory studies on ochthinolone in metalworking fluids were presented at the 42nd Annual Meeting of the STLE in May 1987 (1). Subsequent to these studies, field trials were carried out on ochthinolone in two metalworking fluid systems in which fungi had become a problem. Metaphenylphenyl isothiocyanate (MCI) was being employed as the preservative agent in both of these systems. MCI, which usually provides good control as well as bacterial control was found to be somewhat incompatible with the fluid in one of the systems allowing fungi to proliferate. In one of the other systems, ochthinolone was effective in maintaining low bacterial and fungal populations in the fluid itself, but heavy biofilm accumulations had developed on the machines being supplied by the system in the splash areas bordering the system. This article presents the results of these trials carried out to observe the fungicidal performance of ochthinolone under conditions where MCI was ineffective.

Trial #1

Field Trial #1 was carried out in a 20,000 gallon metalworking fluid system of a large automobile manufacturer. The synthetic fluid used in the system contained a biocide in the concentrate, but in 1983 the plant began adding metaphenylenedinitrophenyl isothiocyanate (MCI) directly to the system because of an increase in the fungal population of the fluid. Apparently these organisms were not being controlled by the biocide present in the concentrate. Sometime later the plant reported that heavy fungal mats had formed on the fluxes and filter wets of the system. They observed that even though MCI was added to the fluid every week or every two weeks, the chlorinated component of the MCI could not be detected by HPLC beyond three days after biocide addition. This suggested incompatibility of the MCI with the metalworking fluid employed in the system. Incompatibility between the MCI and the metalworking fluid was subsequently confirmed in the laboratory (Table 1). Earlier laboratory studies reported by Lashen and Laut (2) had shown ochthinolone to have good chemical stability and to provide good fungicidal activity in several other metalworking fluids in which MCI was known to be incompatible. In view of this earlier work, the chemical stability of ochthinolone was evaluated in the laboratory in both used fluid taken from the problem system and in the same fresh fluid diluted from the concentrate. The results of these evaluations (Table II) showed ochthinolone to be very stable in both the freshly diluted and used fluid. Therefore, since ochthinolone was stable in the fluid, and since the problem in the system was claimed to be due to fungi rather than bacteria, a field trial on ochthinolone was initiated at this site. The purpose of the trial was to determine if ochthinolone would eradicate or establish a baseline problem under actual field conditions.

1. MATERIAL AND METHODS

A. System Characteristics

1. Size - 20,000 gallon
2. Type of Fluid - Synthetic (containing a biocide)
3. pH of Fluid - 9.0-9.2
4. Temperature of Fluid - Approximately 29°C
5. System Turn-Over - Approximately 4 times per year

B. Water and Fluid Make Up

Make-up water was added as required to maintain the volume of the system. Fluid concentrate was added when necessary as indicated by daily analysis of the condition of the fluid.
### TABLE I

**CHEMICAL STABILITY OF MCI IN METALWORKING FLUID IN SYSTEM USED FOR FIELD TRIAL #1**

<table>
<thead>
<tr>
<th>State of Fluid</th>
<th>0.1 ppm</th>
<th>1 Day</th>
<th>2 Days</th>
<th>7 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>From Tank System</td>
<td>7 ppm</td>
<td>5.3</td>
<td>2.2</td>
<td>7.5</td>
</tr>
<tr>
<td>Freshly Diluted</td>
<td>7 ppm</td>
<td>4.4</td>
<td>2.3</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Chlor. Comp. = Chlorinated and Principal Active Component of MCI
Unchlorinated = Unclorinated and Significantly Less Active Component of MCI
Total A.I. = Total Active MCI

### TABLE II

**CHEMICAL STABILITY OF OXETHELONE IN METALWORKING FLUID IN SYSTEM USED FOR FIELD TRIAL #1**

<table>
<thead>
<tr>
<th>State of Fluid</th>
<th>23 ppm</th>
<th>Zero Time</th>
<th>1 Day</th>
<th>3 Days</th>
<th>7 Days</th>
<th>14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>From Tank System</td>
<td>25 ppm</td>
<td>22.0 ppm</td>
<td>21.7 ppm</td>
<td>21.4 ppm</td>
<td>20.5 ppm</td>
<td>13.4 ppm</td>
</tr>
<tr>
<td>Freshly Diluted</td>
<td>23 ppm</td>
<td>22.9 ppm</td>
<td>22.4 ppm</td>
<td>22.5 ppm</td>
<td>21.9 ppm</td>
<td>21.7 ppm</td>
</tr>
</tbody>
</table>

### TABLE III

**RATE OF LOSS OF OXETHELONE FOLLOWING DOSING (TRIAL #1)**

<table>
<thead>
<tr>
<th>Dose No.</th>
<th>Ochthelone Concentration Immediately After Dose</th>
<th>Number of Days Required for 50% Loss of Ochthelone to 5 ppm</th>
<th>Number of Days Required for 67.5% Loss of Ochthelone to 10 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 ppm</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>31 ppm</td>
<td>10.5</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>31 ppm</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>30 ppm</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>31 ppm</td>
<td>15.5</td>
<td>13</td>
</tr>
</tbody>
</table>

Dose of ochthelone given following decision to allow levels to drop from 30 ppm to 5 ppm.
** = Plant shut down for two weeks vacation immediately after this dose (fluid still circulated).

C. Sampling Schedule

Fluid samples were taken daily except on weekends and during shutdowns. In addition to routine analyses such as pH, corrosion inhibitor concentration, etc., samples were examined for ochthelone and MCI concentration and microbial contamination levels.

D. Biocide Analyses of the Fluid Samples

Analyses for ochthelone and MCI content were performed by HPLC using reverse phase gradient separation on an octadecylsilane column with a UV detector (2).

E. Determination of Microbial Populations in the Fluid and Biocide Samples

Dilutions of both fluid and of 'biocide' scrappings were plated on Trypticase Soy Broth Agar (TSBA) to determine the number of bacterial colony forming units present, and on Sabouraud Dextrose Agar (SDA) or Rose Bengal Agar (RBA) containing chloramphenical, to determine the number of fungal colony forming units present. Plates were incubated at 25°C. The TSBA plates were observed for bacterial colonies after 3 days incubation, and the SDA or RBA plates were observed for the number of fungal colonies present after 10 days incubation.

F. Dosing

1. Ochthelone

   After an initial dose of 50 ppm, the ochthelone concentration was maintained at 25 ppm throughout the first one and one half months of the five month trial. During the last three and one half months the ochthelone level was allowed to drop to approximately 10 ppm before it was restored to 30 ppm by the addition of 20 ppm ochthelone.

2. MCI

   Prior to the trial, a dose of 7 ppm MCI had been added to the test system every week or every two weeks. During the five months of this trial MCI was added to the system only four times, twice when the bacterial population appeared to increase slightly, and twice when ammonia odors were detected which were suspected to be of biological origin.

II. RESULTS AND DISCUSSION

A. Ochthelone Concentration

   During the last three and one half months of the trial it was observed that it required approximately twelve to sixteen days for the ochthelone concentration to drop from 30 ppm to 5 ppm. This suggested that a dosing regimen of 20 ppm ochthelone every two weeks was appropriate for this system (Table III).

B. Effect on Bacterial and Fungal Populations in the Fluid

   At the onset of the trial the bacterial population of the fluid was between $10^5$ and $10^6$ colony forming units (C.F.U.) per ml. and the fungal population was between 4000 and 8000 colony forming units per ml., as indicated by microbial plate counts. It should be pointed out, however, that microbial plate counts of fluid in a system do not always reflect the actual microbial population of that system especially if there are biocide accumulations on the walls of the flumes and associated apparatuses of the system.

   During the five months of this trial, the bacterial population of the fluid fluctuated between $10^5$ and $10^6$ C.F.U./ml. Following the initial dose of ochthelone, added at the onset of the trial, the fungal population of the fluid dropped from approximately $5x10^5$ C.F.U./ml. to $<10^5$ C.F.U./ml. and remained there through the rest of the trial.

   The failure of MCI to prevent the buildup of fungi on the walls of the flumes and on the filter weirs appeared to be due to its incompatibility with the fluid (Table II). The growth of bacteria in the fluid was apparently inhibited by either the innate resistance of the fluid to bacterial proliferation, or the formation of the biocide contained in the concentrate, MCI, and ochthelone, or a combination of all of these factors. The laboratory studies run prior to the trial had shown the fluid which was employed in this system to be relatively resistant to bacterial growth but very susceptible to fungal growth.

C. Effect on the Existing Fungal Biocide Accumulations

   Five days prior to initiation of the trial some of the accumulated biocide was removed from several areas of the system by brushing. However, by the time the trial was initiated (five days later) there was already noticeable fungal regrowth in the scrubbed areas. Large slime accumulations (pods), which had broken off from the filters, were observed floating on the surface of the fluid (Figure 1).

---

Fig. 1 - Biocide Accumulations On The Weirs Of The Heavy Filtration System Prior To Initiation Of Trial #1 On Ochthelone

Journal of the Society of Tribologists and Lubrication Engineers 413
prior to the first addition of ochtillium, a sample of the slime mat covering the weirs through which the fluid flowed after it left the Henry filtration system was examined. This sample showed a bacterial population of 4.0 x 10^8 CFU/g (gram) and a fungal population of 1.6 x 10^7 CFU/g (gram). Scarcities taken from the same general location part way through the trial, showed a bacterial population of only 1.6 x 10^7 CFU/g (gram), and no visible fungi. Micromscopical examination of this latter sample showed it to be composed primarily of nonviable encrusted fungal mycelia.

Throughout the trial, plant personnel observed a gradual loss of the integrity of the fungal mats which covered the walls of the flumes and weirs of the system accompanied by a gradual sloughing off of this material. It appeared that the ochtillium had disrupted the integrity of these mats by killing their fungal constituents thereby exposing the previously protected bacterial inhabitants to attack by the biocide present in the concentrate and MCI. By the time the trial was completed, these mats had essentially disappeared from the walls of the flumes and weirs and only a small moldy mat remained flowing over the surface of the Fluid (see Fig. 2). There was also no evidence of any further regrowth of fungi in the areas on the flumes and weirs cleared prior to the trial, or of slime on or around the machines.

D. Effect on the Amount of Make Up Fluid Required by the System

According to plant personnel, during the first five months of this field trial approximately 30 percent less make up fluid was required to maintain the desired characteristics of the fluid than was normally required by this system. It is theorized that the heavy biofouling accumulations covering the flumes and weirs of the system, prior to initiation of the field trial, created a condition similar to that found in "trickle filters" used to degrade industrial waste. In such filters the industrial waste solution is allowed to trickle repeatedly over the large rocks completing the filter. These rocks are covered with microscopic slime which very efficiently degrade the organic material in the waste solution passing over them. We suggest that the heavy biofilm mats which existed in the test system prior to this trial were responsible for the need for frequent additions of make up fluid to replenish the fluid degraded by the microorganisms present in these mats. Destruction of the organisms and removal of the mats through the use of ochtillium eliminated this significant cause of fluid degradation.

Examining the volumes of make up fluid additions before and during the trial, and including the cost of the ochtillium employed, it has been estimated that the potential savings from reduced fluid use for this particular system amount to over $500,000 per year.

TRIAL #2

Trial #2 was carried out in a 28,000 gallon metalworking fluid system of another large automobile manufacturer. Prior to this trial MCI biocide was being used. Both the bacterial and fungal populations of the fluid were very low. It was observed, however, that heavy biofouling accumulations were present in the splash areas along the walkways bordering the flumes and once around the machines supplied by the system. According to plant personnel these accumulations always reformed within a very short time after being removed by washing and scraping.

Laboratory analyses showed ochtillium to have excellent stability in the fluid employed in this system. The purpose of this trial was to determine if ochtillium, as recommended use concentrations could prevent microbial slime build up in the splash areas surrounding a large industrial metalworking fluid system.

I. MATERIAL AND METHODS

A. System Characteristics
1. Size: 28,000 gallons
2. Type of Fluid - Synthetic
3. pH of Fluid: 8.0 - 8.2
4. Temperature of Fluid: Approximately 80°C

B. Sample Schedule

A sample of fluid from the system was taken prior to the first additions of ochtillium. Samples of the slime accumulations present on the floor of the plant were also taken prior to the cleaning of those areas in preparation for the trial.

During the trial, fluid samples were taken daily (except on weekends) for the first three weeks. Following this and up until the last month of the trial, samples of the fluid were taken on average of twice a week. During the last month fluid samples were taken weekly.

C. Biocide Analysis of the Fluid Samples

The HPLC analyses for ochtillium and MCI were performed in the same manner as in Trial #1 (2).

D. Determination of Microbial Populations in the Fluid and Biofouling Samples

Dilutions of the fluid samples and of the biofouling samples were plated as described in Section 1.8 of Trial #1 to determine the number of bacterial and fungal colony forming units present.

II. RESULTS AND DISCUSSION

A. Ochtillium

1. System

The system was dosed with approximately 50 ppm ochtillium at zero time and again one week later. It will be noted in Table III that there was only a small rapid loss of biocide from the system immediately following the first and second doses of the biocide than there was during the rest of the trial. Greater than 90 percent of the ochtillium added in the first dose was lost within the first three days. The loss of ochtillium within the three days after the second dose was about 50 percent.

In all subsequent doses the loss of ochtillium was at a much slower rate. It is thought that the loss of ochtillium from the initial dose was due to the demand of the system.

Following the initial doses of 50 ppm active biocide, weekly doses of approximately 35 ppm ochtillium were given for the next month. Subsequent doses of 35 ppm active ingredient were given every ten to twelve days until the end of the three month trial.

2. MCI

Prior to the trial, a dose of 20 ppm MCI had been added to the system every two weeks. The system had also been dosed with MCI the preceding trial which lasted and the additional dose of 20 ppm was added two weeks into the trial. At our request no more MCI was added for the duration of the trial.

HPLC analyses for MCI concentrations in the fluid for the first four weeks of the trial demonstrated its presence for approximately one week following each of the two additions.

B. Effect on Bacterial and Fungal Populations in the Fluid

At the outset of the trial, the bacterial population of the fluid in the system was 15 CFU/ml and no fungi could be detected.

No fungi were detected in any of the fluid samples submitted during the trial and the bacterial populations of those samples ranged from 15 to 100 CFU/ml, with an average of only 15 CFU/ml.

C. Effect on Biofouling Accumulations

Just prior to initiation of this field trial all of the biofouling accumulations along the edges of the flumes and around the machines were removed by scrubbing. A sample of fluid was taken at that time from the plant floor alongside one of the housing machines was found to contain a fungal population of 2.5 x 10^7 CFU/g (gram) and a bacterial population of 63 x 10^7 CFU/g (gram). Microscopic examination of this biofouling indicated that it consisted primarily of fungal mycelia, microbial slime and bacteria.

During the three months of this trial there was no evidence of any reformation of biofouling accumulations in any of the areas where it had previously flourished. There were no other areas of the system. Plant personnel commented that prior to this trial, heavy biofouling accumulations had always reappeared in the splash areas of the system within a week or two following their removal by scrubbing. Ochtillium was found to keep the splash areas free from fungal growth and biofouling for the duration of the three month trial.

CONCLUSIONS

From the results of these two field trials we conclude that ochtillium is an effective fungicide for tankside addition to metalworking fluid systems at concentrations ranging from 10-50 ppm. The results also suggest that ochtillium at these levels can maintain low fungal populations in metalworking fluid use dilutions, eradicate existing fungal based biofouling accumulations, and prevent buildup of fungal slime in splash areas outside sumps and flumes. It is also suggested that the use of ochtillium to eradicate fungal organism growing in parts or in heavily fouled metalworking fluid systems may result in a significant savings in fluid make up costs due to the elimination of this source of fluid degradation.

REFERENCES