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미생물 성장을 억제하기 위하여 수용성 절삭유에 과다하게 첨가한 붕소와 아민 사례 연구

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Ethanolamine and boron abuse to limit microbial growth in water-synthetic metalworking fluids

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This study was conducted to examine whether a specific synthetic metalworking fluid (MWF), "A", in use for 10 months without replacement, displayed microbial resistance and to identify the additives associated with the control of microbial growth. Three synthetic MWF products ("A", "B", and "C") were studied every week for two months. Microbial deterioration of the fluids was assessed through evaluation by endotoxin, bacteria and fungi levels in the MWFs. In addition, formaldehyde, boron, ethanolamine, and copper levels were also studied to determine whether they influence microbial growth in water-based MWFs. Throughout the entire study in the sump where MWF "A" was used, bacteria counts were lower than 103 CFU/mL, and endotoxins never exceeded 103 EU/mL. These levels were significantly lower than levels observed in sumps badly deteriorated with microbes. Boron levels in MWF "A" ranged from 91.7 to 129.6 ppm, which was significantly higher than boron levels found in other MWF products. The total level of ethanolamine (EA) in MWF "A" ranged from 35,595 to 57,857 ppm (average 40,903 ppm), which was over ten times higher than that found in other MWFs. Monoethanolamine (MEA), diethanolamine (DEA) and triethanolamine (TEA) concentrations in MWF "A" were also significantly higher than seen in other MWFs. However, although EA and boron might improve anti-microbial performance, their abuse can pose a serious risk to workers who handle MWFs. From an industrial hygiene perspective, our study results stress that the positive synergistic effect of boron and EA in reducing microbial activity in MWF must be balanced with the potentially negative health effects of such additives. Our study also addresses the disadvantage of failing to comprehensively report MWF additives on Material Safety Data Sheets (MSDS). Future research in MWF formulation is needed to find the best level of EA and boron for achieving optimal synergistic anti-microbial effects while minimizing employee health hazards.

Key Words :synthetic metalworking fluids, monoethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA), microbial resistance, boron, and endotoxin.

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Ⅰ. Introduction

Water-miscible metalworking fluids (MWFs) provide an excellent environment for the growth of a large variety of microorganisms (Bennett, 1972; Rossmoore et al., 1980), causing deterioration of the fluids, equipment corrosion, and physical blockage of flow lines. In addition, the appearance of slime and the generation of foul odors can cause serious concomitant problems affecting the worker' s physical condition (Rossmoore, 1981).

Exposure to microbes is associated with several respiratory health ailments, such as allergic reactions, extrinsic allergic alveolitis, and various kinds of infections (NIOSH, 1998).

The spoilage of MWF continues to be a major industrial problem, costing the metalworking industry several million dollars each year in the U.S. (Rossmoore, 1981) Many strategies have been offered to prevent microbial deterioration in water-based MWF. One of the chief concerns that confront the MWF formulator is to achieve a formulation that maximizes microbial inhibition while minimizing health risks.

A specific synthetic MWF (designated as MWF "A"), imported from Japan, was promoted by the fluid supplier as having excellent anti−microbial properties. One machine shop, which manufactures automotive parts, used this fluid for ten straight months, prior to this study, without changing the fluid. A dip slide test in the workplace showed no microbial contamination. However, the owner of the machine shop wanted the health risk of MWF "A" to be assessed before it was introduced to other sumps.

In general, it has been reported that additives inhibiting microbial growth in water- based MWF are: formaldehyde-releasing agents, (Rossmoore, 1981) copper preservatives, (Law et al., 1991) boron-amine interaction, (Rossmoore, 1981; Hernandez et al., 1984) and alkanolamines. (Sandin et al., 1990; Steiner et al., 1993; Bennett, 1979; Beyer et al., 1983) The Material Safety Data Sheets (MSDS) on MWF "A" indicated only the presence of carboxylic salts, synthetic oil, and alkanolamines. The specific amount and type of alkanolamines were not reported due to confidential trade secrets. Therefore, the MSDS was not a useful reference for establishing the identification, or relative concentration of anti−microbial components in MWF "A".

In this study, factors known to promote anti-microbial performance were investigated and compared among various sumps and fluids. In particular, the study was initiated to examine whether MWF "A" was associated with anti-microbial performance, and, if so, to identify the components responsible for its microbial resistance. Factors indicating significant differences among the fluids were extensively studied.

Ⅱ. Methods and materials

MWF Types and Sumps Studied

Three synthetic MWFs (MWF "A", "B", and "C") were studied every week for two months (from June 1 to July 30) in a single machine shop. MWF "A" was first charged to one sump, called MT 01, which has a 20 L volume capacity, on June 1.

This MWF was used, unchanged, until the end of this study. Two other MWFs ("B" and "C") were used in six other sumps the same periods as MWF "A", again without changing the fluid. About 2−3 L of a 20:1 dilution of bulk MWF with water was added to the sumps each day to compensate for loss due to evaporation. Management of MWF use in the test workplace was typically the responsibility of the machine operators.

MWF Sump Bulk Sample Collection and Measurement

One fluid sample from each sump was taken once every week from a flowing stream, at the cutting points of each machine, while the circulation system was in operation and placed into a 50−mL sterile, tissue−culture grade, centrifuge tube (cat. no. 05 - 538 - 55, Fisher Scientific, Pittsburgh, PA). The temperature and pH level of the MWFs were measured in the field using a thermometer (Model 230, Portable Meter, Orion, Beverly, Mass.). In addition, the MWF concentration was measured in the field using a refractometer (Model N20E, Atago, Tokyo, Japan). Information on the replacement of old MWF with fresh, the addition of fluid to compensate for MWF losses, and biocide use was sought from interviews with shop personnel. The MWF samples were then brought to the laboratory in a refrigerated container, where total alkalinity was measured according to standard methods (APHA et al., 1995).

Formaldehyde Analysis

Formaldehyde, released from the formaldehyde−liberating biocide in the bulk sample, was quantified by reaction with 2,4 - DNPH, which resulted in the formation of formaldehyde - 2,4 - DNPH derivatives. After desorption in 1 ml of HPLC grade acetonitrile (Merck, Darmstadt, Germany), the derivative was analyzed with an HPLC UV−VIS absorbance detector (Waters 2489, Waters Corporation, Milford, Mass). To quantify

Table 1. Summary of Levels in Bulk Sump Samples of Water-Based Metalworking Fluids

	MWF "A"		MWF "B"		MWF "C"		ANOVA	
	Sample No.	Average (SD)	Sample No.	Average (SD)	Sample No.	Average (SD)	F-ratio	p-value
MWF pH	8	9.14(0.12)	16	8.64(0.50)	32	8.92(0.42)	4.25	0.019
MWF Temperature (℃)	8	22.6(3.6)	16	24.0(3.18)	32	23.4(3.09)	0.261	0.773
MWF Concentration (%) Alkalinity, mg/L	8	6.2(0.65)	16	5.4(1.49)	32	7.0(2.56)	3.003	0.055
Alkalinity, mg/L	8	13,838(941)	16	2,869(695)	32	3,497(964)	480.9	0.000
Boron, mg/L	8	105.1(13.9)	13(3) [A]	12.7(8.9)	28(4) [A]	14.4(13.9)	171.3	0.000
Copper, mg/L	1 (7) [A]	0.2	16	12.4(6.9)	30(2) [A]	25.9(17.6)	5.265	0.009
Formaldehyde, mg/L	8	285(58)	32	311(150)	32	95(112)	0.451	0.991
Monoethanolamine (MEA), ppm	8	4,036(687)	5 (11) [A]	680(437)	20(12) [A]	1,296(614)	73.86	0.000
Diethanolamine (DEA), ppm	8	2,386(384)	0 (16) [A]	Not detected	2(30) [A]	971(583)	7.053	0.017
Triethanolamine (TEA), ppm	8	34,480(6,537)	15 (1) [A]	520(377)	32	1,229(651)	680.2	0.000
Total ethanolamine, ppm	8	40,903(6,888)	15 (1) [A]	622(714)	32	2,003(1,378)	742.8	0.000

MWF "A" used in sump MT 01, MWF "B" in two sumps (NC 01 & NC 02), and MWF "C" in four sumps (MT02, ML04, ML06 & DM10)
Sample No: Number of sumps where each type of MWF was used x 8 measurements (1 per week per sump)
A : Sample number in parentheses = number of samples not detected; average and standard deviation were based only on results of samples detected.

the formaldehyde in the MWF, a standard curve was constructed using serial dilution of formaldehyde - DNPH stock solution (100 ㎍/mL in acetonitrile, cat no. 4 - 7177, Supelco, Bellefonte, PA, USA).

Boron and Copper Analysis

The MWF bulk sample was pretreated by microwave digestion (MDS - 2000, CEM Corp., Matthews, NC). Copper and boron compounds in the MWFs were quantified as elements using Inductively Coupled Plasma analysis (Optima 3000 DV, PerkinElmer, Wellesley, Mass.).

Ethanolamine Analysis

A portion of the MWF bulk sample was filtered using a micro-syringe, with a 0.4 ㎛ pore size filter. Mono-(MEA), di-(DEA) and triethanolamine (TEA) in fluid filtered were quantified by ion chromatography (Water 717 plus auto sampler, Waters Corporation) using an IC PAK ™ C M/D guard column (Alltech, Lexington, KY), an IC PAK ™ Cation M/D column (3.9 x 150 mm WAT 036570, Alltech) and a 432 conductivity detector (Waters Corporation). The eluent (0.1 mM EDTA and 2 mM nitric acid) was filtered through a 47 mm diameter, 0.45 um pore size, Super 450 membrane filter (Waters Corporation), and sonicated (Model 3210, Branson, Danbury, Conn.) for 40 minutes to remove dissolved air. Samples for quantification were diluted to achieve optimum levels for analysis.

Microbial Analysis

A MWF bulk sample was mixed thoroughly by gentle inversion. For viable

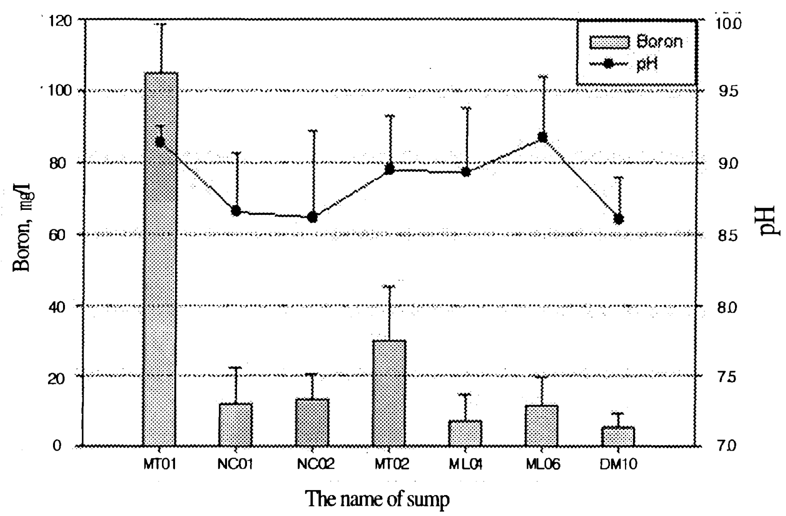

Fig. 1. Comparison of pH and boron concentration by sump
(MT01 ; MWF "A", NC01 & NC02 ; MWF " B", MT02, ML04, ML06 & DM10; MWF "C").

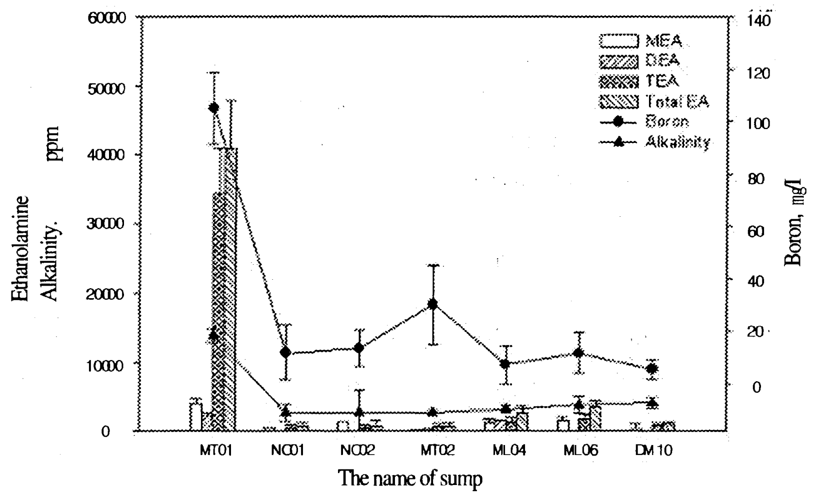

Fig. 2. Comparison of the ethanolamine, boron and alkalinity concentration by sump
(MT01 ; MWF "A", NC01 & NC02 ; MWF " B", MT02, ML04, ML06 & DM10; MWF "C").

counts, 10-fold serial dilutions of the suspension fluid in phosphate-buffered saline were prepared, and 0.1 mL of the diluted samples was plated on Trypticase Soy Agar for aerobic bacteria growth and Malt Extract Agar for fungi growth. The plates were incubated for 48 hours at 35 ℃ (for bacteria) and 4 days at 35 ℃ (for fungi), and then counted.

Ⅲ. Results

The temperature, pH, and concentration of the MWFs in all sumps were found to be within appropriate ranges for microbial growth, as shown Table 1. The pH level of MWF "A" ranged from 9.01 to 9.38 throughout the entire study, and showed the smallest variation when compared to other sumps. Total alkalinity ranged from 14,700 ppm to 15,200 ppm, which was far higher than the range seen in other sumps, 1,300 ppm to 5,200 ppm. The high alkalinity in MWF "A" was likely deliberate to keep its pH high, as well as stable. Elemental concentrations of boron were also substantially higher in the MWF "A" when compared to other MWFs (Fig. 1). Differences of formaldehyde and copper concentrations were not detected among the three MWFs studied.

The average of total EA in MWF "A" was 40,903 ppm (range: 35,595 ppm to 57,857 ppm), which was over ten times higher than the average concentration in MWF "B" or "C". Furthermore, individual concentrations of MEA, DEA and TEA were also significantly higher in MWF "A" than in the other fluids evaluated. In particular, TEA accounted for 84% of the total EA (Table 1 and Fig. 2). ANOVA analysis found that pH, alkalinity, boron, MEA, DEA and TEA in MWF "A"were significantly higher than found in the other MWF types studied (Table 1).

In sump MT 01 where MWF "A" was used, bacteria and fungi counts were lower than 103 CFU/mL throughout the entire study, which was greatly lower than the levels observed in the other sumps. In addition, the concentrations of fungi in MWF "A" were below the limit of detection until five weeks into the study. However, the other six sumps, containing MWF "B" or MWF "C", were badly contaminated with bacteria and fungi. MWF "A' was found to be contaminated with microbes after 6 weeks. However, the level of contamination was not severe until 8 weeks (Table 2).

Ⅳ. Discussion

Although the synthetic MWF "A" had been used, unchanged, for 12 months, including the period of this study, the fluid was not seriously contaminated with microbes. The obvious inhibition of

Table 2. Bacteria and Fungi Levels in Bulk Sump Samples by Week

Week	MWF "A" CFU/mL		MWF "B" CFU/mL		MWF "C" CFU/mL	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
1	<10	<10	4.80×10^5 ~ 1.64×10^7	<10	1.00×10^4 ~ 2.90×10^7	<10 ~ 80
2	<10	<10	4.40×10^7~ 5.10×10^7	<10	1.00×10^6 ~ 4.20×10^8	<10 3
3	<10	<10	1.00×10^5 ~ 1.02×10^7	<10 ~ 69	1.08×10^7 ~ 8.04×10^7	<10 ~ 2.40×10^2
4	<10	<10	4.80×10^6	39 ~ 1.05×10^3	1.00×10^3 ~ 4.60×10^8	26 ~ 3.08×10^2
5	<10	<10	1.60×10^7 ~ 2.14×10^7	60 ~ 363	100 ~ 2.13×10^8	<10 ~ 20
6	<10	<10	3.10×10^5 ~ 1.16×10^7	1.02×10^3~ 1.69×10^3	1.00×10^4 ~ 9.16×10^6	20 ~ 50
7	<10	<10	1.00×10^4 ~	60 ~ 80	2.00×10^5 ~ 2.62×10^8	30 ~ 4.50×10^2
8	56	<10	2.52×10^7 ~ 3.26×10^7	5.00×10^5	2.40×10^5 ~ 7.90×10^7	70 ~ 1.38×10^3
Range	<10 ~ 56	1.00×10^4 ~ 5.10×10^7	1.00×10^4 ~ 5.10×10^7	<10 ~ 5.00×10^5	1.00×10^3 ~ 4.60×10^8	<10 ~ 1.38×10^3

MWF "A" used in sump MT 01, MWF "B" in two sumps (NC 01 & NC 02), and MWF "C" in four sumps (MT02, ML04, ML06 & DM10)

microbial growth in the synthetic MWF "A" is presumably attributable to particular fluid additives with strong anti microbial performance. This study was focused on identifying the components that contribute to anti microbial activity in this fluid, and on examining whether there are perceived risks from exposure to these components.

As noted in the study results, there were three components or factors that were significantly increased in MWF "A" compared to the other two MWFs evaluated: alkalinity, and EA and boron concentrations. The addition of much EA dosage to the MWF "A" formulation might have resulted in the increased level of alkalinity.

Based on these elevated levels, a possible assumption is that a high total EA level in combination with elevated borate levels at pH range of 9.01-9.38 is responsible for the anti microbial performance seen in MWF "A". This assumption is further supported by the results from studies conducted in the laboratory (Hernandez et al., 1984; Sandin et al., 1990; Steiner et al., 1993; Bennet, 1979; Beyer et al., 1983; Rossmoore, 1993; Oppong et al., 1989).

Sandin et al. (1990) found that the anti-microbial effects of alkanolamines, such as DEA, dimethylamino-methyl-propanol, and butylethanolamine, are greatly enhanced at a high pH. Compared with uninhibited growth, a reduction of more than 10^5 CFU/mL was found at pH 9.1 at a concentration of 76 mM (7900 ppm) DEA(Sandin et al., 1990).

Hernandez et al., (1984) reported the anti-microbial performance of boron-amine interaction with three types of synthetic formulations (Hernandez et al., 1984). A 1:30 dilution of synthetic MWF containing 14% boric acid and 16% EA, showed a marked synergism in the ability to control microbial growth, limiting the bacterial and mold count to 10^4 CFU/mL throughout the entire eight-week test. However, neither boron nor EA alone produced the desired anti-microbial results. Rossmoore (1993) found that two fluids, both amine/borate-based, one with a pH of 9.8 and the other with a pH of 9.2, showed complete control of bacterial growth for eight weeks. (Rossmoore, 1993) These findings further support the author's assumption that EA and boron work in a synergistic manner to inhibit microbial growth and that this effect was observed at high than 9 of pH.

However, from an industrial hygiene perspective, the advantage of reduced microbial activity must be weighed against the potentially adverse effects of the microbial inhibitors. The TEA average level (34,480 ppm) in synthetic MWF "A" greatly exceeded the Independent Lubricating Manufacturing Association (ILMA) recommendation of 2.5 %. The MEA and DEA levels also exceeded this threshold. The range of total EA in MWF "A" in the study was 35,595 ppm to 57,857 ppm, which often exceeded the recommended level set by Beyer et al., who reported that in products like MWFs, intended for prolonged contact with skin, the concentration of EA should not exceed 5% (Beyer et al., 1983). Also, although high levels of EA might improve anti microbial performance, excessive amounts of EA can cause adverse health effects. Regardless of the controversies concerning the carcinogenicity of DEA (NTP, 1999) or TEA (NTP, 1999; Savonius et al., 1994) , EA has the potential to act as a respiratory irritant and a sensitizing agent(Rossmoore, 1981). Furthermore, the nitrosation of EA may result in the formation of nitrosamines, such as N-nitrosodiethanolamine (NDELA), which has been shown to be carcinogenic in laboratory animals(Beyer et al., 1983). Therefore, from an industrial hygiene perspective, a balance must be achieved between the level of EA and boron required to reduce microbiological growth and the level that is safe in the workplace.

Unfortunately, we could not conduct exposure assessment with airborne concentrations that regarding upper respiratory irritation or dermal irritation that would lead to validate the concern of this study. Thus, this study couldn't find an adequate discussion of the significant health risks or effects caused by the use of MWF "A".

To date, the optimal concentration of TEA and borate, that can provide both microbiological resistance in use and yet is nontoxic and easily disposed, has not been studied.

Therefore when developing MWFs, formulators have to design MWFs that can be both microbiologically resistant, as well as nontoxic. Consequently, microbial contamination control in MWFs must be considered from both an operational and an industrial hygiene perspective. Formulating MWFs which satisfy these dual goals will pose a considerable challenge to developers.

There are countries, such as Korea, with no regulations or guidelines for the safe use of MWF, additives, or microbiocides. In addition, MWF fluid suppliers provide only limited information on their fluid additives.

Many products have been introduced into MWFs as biocide substitutes, generally with euphemistic designations, such as reodorants, rejuvenators, or stabilizers. Those who are responsible for the selection of a MWF should be thoroughly aware of the claims made by fluid manufacturers, that their fluids are resistant and therefore, do not require biocide additions. However unfortunately, with the limited information provided on the MSDS concerning additives, it is technically impossible for fluid users to evaluate a manufacturer's claims. Since there are no regulatory requirements for the use of microbiocides in MWF, the excessive addition of potentially hazardous chemicals may be a common practice. Considering the potential health risk posed by MWF additives, MSDS providers should be mandated to furnish sufficient additive information and, regulations or guidelines for their use should be established, to allow for the proper selection and safe use of MWFs.

V. Conclusions

Our case study found that excessive amount of boron and EA were added by the fluid supplier with the intention of arresting microbial growth in MWF. Our conclusion that boron and EA caused anti microbial activity in MWF is in agreement with the results reported by several previous studies conducted in the laboratory. Excessive addition of specific chemicals could be common in a country without a regulatory requirement for the use of microbiocides. In addition, considering the lack of additive information provided on the MSDS, it is technically impossible for fluid users to recognize, not only a general consideration for the safe use of microbiocides, but also a risk due to abuse of specific chemicals.

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