# The Microbial Activity of Glutaraldehyde in Chain Conveyor Lubricant Formulations

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*ABSTRACT*

*Water-based chain conveyor lubricants are biosusceptible to microbial contamination, including environmental pathogens. Glutaraldehyde was evaluated as a control agent in use-diluted lubricant formulations against dairy and beverage field isolates, including Gram-positive and Gram-negative bacteria and fungi. Further efficacy was established against* Listeria monocytogenes, *a ubiquitous environmental pathogen in dairy, meat and related processed food operations. Field tests were carried out in dairy applications comparing untreated and treated conveyor line contamination levels. The results of laboratory and field studies indicate that glutaraldehyde can be considered an effective biocide in this area of application.*

## INTRODUCTION

The function of chain conveyor lubricants in the food, dairy and beverage industry is to lubricate chain surfaces, reduce friction, and ensure product and container transfer throughout the operation. Environmental contamination from air, water and product spillage can result in slime buildup, offensive odour, unpleasant appearance and corrosion (Rossmoore & Lichorat, 1986). In addition conveyor lubricants may harbour human pathogenic organisms. *Listeria monocytogenes* has been isolated from dairy chain conveyor lubricant. This organism is a recently recognized food-borne pathogen, the presence of which cannot

be tolerated in the dairy and food industry environment.

The purpose of this study was to find an effective biocide in order to (1) increase bioresistance of the diluted conveyor lubricant and (2) control environmental contamination *in situ* along the conveyor line, thus minimizing the potential for the distribution of contaminants. The list of candidate biocides is very limited. In addition, to satisfy compatibility and EPA approval, the candidate should be effective within the 'resident' time (i.e. between 30 min and 1 h).

The chemical that seemed to fit these requirements was a commercial preparation of glutaraldehyde which, at alkaline pH, is a potent microbicidal agent (Russell & Hopwood, 1976). It has good resistance to neutralization by organic matter when compared to other biocides (Gelinas & Goulet, 1983).

Glutaraldehyde, besides having a broad spectrum, has a reasonably fast rate of kill and is classified as a chemosterilant. Factors influencing activity have been reviewed (Gorman & Scott, 1980). For the present application, the level is at least 10 times less than the requirement for medical purposes (Rubbo *et al.*, 1967).

## METHODS

### Microbiological media

Tryptic soy agar (TSA), sabouraud agar (SAB), brain heart infusion agar (BHI) and the neutralizing buffer were obtained from DIFCO Laboratories, Detroit, Michigan. Neutralizing buffer with 1% glycine was used for swab sample treatment.

### Test organisms

All of the biodeteriogens were isolated from dairy conveyor lubricants, and identification was carried out by pure culture isolation technique on TSA, SAB and BHI prepared with 1% conveyor lubricant. Identification of pure cultures of bacteria and fungi was based on microscopic examination, Gram staining, and commercial biochemical test systems (API 20E, API 20C, Analytab Products; and Rapid NFT, DMS Laboratories, Inc.). All incubation was at 28°C for a minimum of 48 h and up to 4 days for fungi. Representative members of field isolates were selected for future efficacy studies; in addition, *L. monocytogenes* (ATCC 8766), *Zygosaccharomyces bailii* (ATCC 8766) (a resistant 'wild' yeast in the beverage industry), and *Staphylococcus aureus* (ATCC 6538) were

added to the biodeteriogen list. The list of field isolates includes *Pseudomonas fluorescens, Klebsiella oxytoca, Serratia marcescens, Micrococcus* sp., *Geotrichum candidum*, and *Candida lambica. L. monocytogenes* was kept and plated on BHI agar.

## Inoculum preparation

Bacterial and fungal inocula were prepared from TSA and SAB slants, respectively. After two transfers, the growth was washed off with 2 ml of phosphate buffer and diluted to 50 ml, 2 ml of which were inoculated into 250 ml French square bottles containing TSA or SAB with 3% agar. The cultures were incubated at 32°C for 24 h (bacteria) and at 25°C for 48 h (fungi). The surface growth was washed off with 3 ml of phosphate buffer, pH 7·2, and diluted so as to yield $10^7$ cfu/ml (bacteria) and $10^5$–$10^6$ cfu/ml (fungi). The bacterial inoculum was adjusted using a Spectronic 20 Photometer (Bausch & Lomb) at 500 nm.

## Compatibility of lubricant formulations with glutaraldehyde

Eight lubricant formulations were tested at their use dilution levels, and 250 ppm of glutaraldehyde was added to 500 ml of each. At 0, 10, 30 min and at 1, 3, 5, 24 h 50-ml aliquots were taken and inoculated with $5 \times 10^7$ *Klebsiella oxytoca*. After 10 min contact time, the activity loss was tested by plating on TSA; inactive systems did not give microbial control. A good compatibility lubricant formula (L-4) was selected for laboratory efficacy studies (Table 1).

**TABLE 1**
Antibacterial Efficacy of Glutaraldehyde[a] in a Compatible Lubrication Formulation

| *Test organisms* | *Inoculum (cfu/ml)* | *Log reduction in 30 min*[b] |
|---|---|---|
| *Pseudomonas fluorescens* | $2 \times 10^7$ | >5 |
| *Klebsiella oxytoca* | $8 \times 10^7$ | >5 |
| *Serratia marcescens* | $5 \times 10^8$ | >5 |
| *Staphylococcus aureus* | $4 \times 10^7$ | >5 |
| *Listeria monocytogenes* | $4 \times 10^7$ | >5 |

[a]Glutaraldehyde minimum effective concentration: 25 ppm.
[b]Based on $\log_{10}$ reduction.
Efficacy was tested in lubricant alone and in the presence of 1% beer and 1% milk, respectively. All permutations were equally effective.

**Efficacy of glutaraldehyde**

Diluted lubricant (100 ml) was inoculated and glutaraldehyde was added. At the appropriate intervals, 1 ml was taken into 9 ml of neutralizer solution and plated on TSA for bacterial and SAB for fungal counts. In addition, the lubricants were further stressed with 1% milk or 1% beer to simulate the dairy or brewery environment.

**Field efficacy studies**

Two dairy field studies were conducted with compatible lube systems, and one of these studies is presented here. Surfaces were sampled by swab using two cotton applicator sticks pre-wet with neutralizer prior to use. Five links (60 $cm^2$) were swabbed at each of the 21 sampling sites along the floor conveyor line. After swabbing, the applicator was placed into 10 ml neutralizer for transporting to the laboratory in ice and plated in 24 h to assess microbial population levels. The line was sampled before biocidal treatment ('base line' contamination) and repeated after 2 weeks of continual biocidal treatment ('treated' line contamination). The glutaraldehyde concentration was 85 ppm at the dosing pump at the time of the field test.

## RESULTS AND DISCUSSION

The microflora of the dairy conveyor lubricant was very similar to the microflora of the brewery conveyor lubricant (Rossmoore & Lichorat, 1986). The results of fluid compatibility tests indicated good to poor stability of glutaraldehyde in different formulations.

Considering the relatively short time needed for this special application, the systems that showed longer than 24 h of biological activity were considered good compatibility systems; between 5 and 24 h activity was considered fair; and less than 30 min activity was classified as poor. The results of biological compatibility of fluids were in good correlation with the results on chemical compatibility of glutaraldehyde. Monoethanolamine is a major factor of chemical incompatibility in lube systems. Cross-link formation with glutaraldehyde results in subsequent activity loss.

The results of laboratory efficacy studies indicate that glutaraldehyde is effective at a dose level of 25 ppm against deteriogens and pathogenic bacteria in compatible fluid (Table 1). A higher level and longer contact time are needed for the control of fungi (Table 2).

**TABLE 2**
Antifungal Efficacy of Glutaraldehyde[a] in a Compatible Lubricant Formulation

| *Test organisms* | *Inoculum (cfu/ml)* | *Log reduction in 30 min*[b] |
|---|---|---|
| *Geotrichum candidum* | $1 \times 10^5$ | >3 |
| *Zygosaccharomyces bailii* | $9 \times 10^6$ | >3 |
| *Candida lambica* | $2 \times 10^6$ | >3 |

[a]Glutaraldehyde minimum effective concentration: 250 ppm.
[b]Based on $\log_{10}$ reduction.
Efficacy was tested in lubricant alone and in the presence of 1% milk and 1% beer, respectively. All permutations were equally effective.

The results of field studies show that 85 ppm of glutaraldehyde reduced surface contamination along the dairy floor conveyor an average 4·4 $\log_{10}$ for bacteria and 3·4 $\log_{10}$ for fungi per 60 $cm^2$ sampled. Greater effectiveness under field conditions against fungi could be attributed to a much longer contact time.

Visual evidence of successful biocide treatment is shown in Fig. 1. Lubricant spray heads have a different appearance after glutaraldehyde treatment and is indicative of limitation of biofilm formation (Eagar *et al.*, 1986).

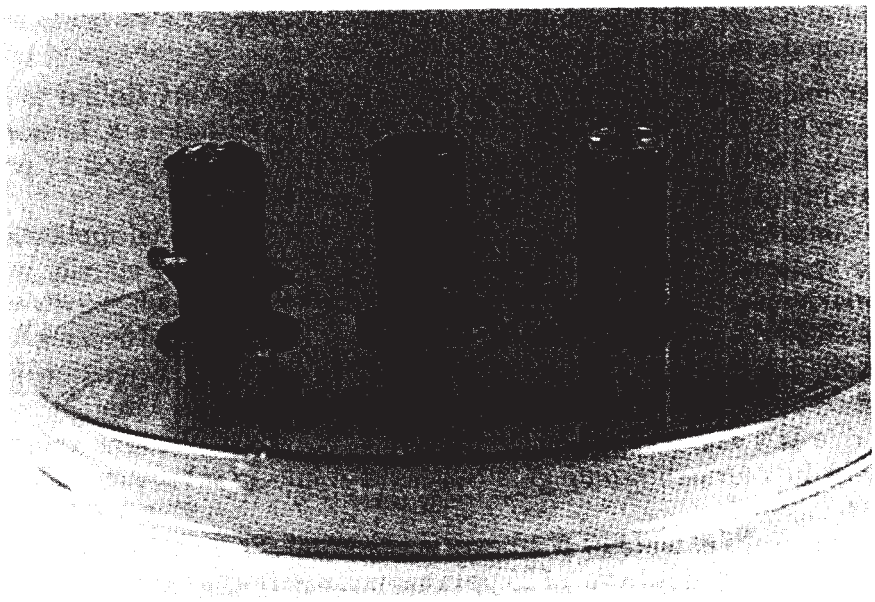

**Fig. 1.** Lubricant spray nozzles from treated (right) and untreated (left and center, respectively) lubricant.

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