Effectiveness of Cleaners and Sanitizers in Killing Spore-forming Bacteria

A Senior Project

presented to

the Faculty of the Dairy Science Department

California Polytechnic State University, San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Bachelor of Science

by

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March, 2013

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Abstract

The objective of this study was to determine the effectiveness of the selected cleaners and sanitizers in killing spore-formers by preventing, treating or reducing the effects of problem spores in the dairy export industry. The chemicals used in this experiment to test against the spore-forming bacteria were Chlorine (Kem-Tek chlorinating liquid), Mandate Plus (Ecolab Clean-in-Place acid sanitizer), HD Acid PL-10 (Ecolab acid detergent for pipeline and bulk tank equipment), and Principal (Ecolab chlorinated mechanical and CIP cleaner for protein soils). The \textit{Bacillus licheniformis} concentrated solution was provided by the Dairy Products Technology Center at California Polytechnic State University. Data on the effectiveness of selected cleaners and sanitizers were derived from 1:1 concentrations and 10^{-1} solutions that were isolated and put onto the 3M Petrifilm for observation and records. A series of dilutions were made of the sanitizers and cleaners from 1:1 to 1:10,000, but the 1:1 and 10^{-1} were selected to test the optimum effectiveness of each chemical. A One-way ANOVA (Analysis of Variance) was used to analyze the results, and test for differences in the 3M Petrifilm counts of 1:1 Chlorine, 10^{-1} Chlorine, 1:1 Mandate Plus, 10^{-1} Mandate Plus, 1:1 HD Acid PL-10, 10^{-1} HD Acid PL-10, 1:1 Principal and 10^{-1} Principal; statistically significance difference was set at P< .05. There was no statically significant difference between all samples. The effectiveness of Chlorine, Mandate Plus, HD Acid PL-10 and Principal at 1:1 10^{-1} and 10^{-2} was, although significant, inconclusive due to there being to many microorganisms to count. The effectiveness of the four selected chemicals at 10^{-1}, with further dilutions of 10^{-1} and 10^{-2}, contained positive results with the \textit{Bacillus licheniformis}, indicating which of the four chemicals was the most effective against a spore-forming bacteria. After an incubation period, the 3M Petrifilms showed results of chlorine 10^{-1} having no visible growth on either dilutions of 10^{-1} or 10^{-2}, denoting that Chlorine
(sodium hypochlorite 10.0% and inert ingredients 90.0%) was the most effective of the four chemicals and sanitizers selected. There was only one chemical, which made had no significant difference in either the 1:1, $10^{-1}$ and $10^{-2}$, or $10^{-1}$, with further dilutions of $10^{-1}$ and $10^{-2}$. In conclusion, the addition of chlorine (sodium hypochlorite) reduces or eliminates the growth of psychrotrophic bacteria (spore-formers).

**Key words:** cleaners, sanitizers, spore-forming bacteria, sodium hypochlorite
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Introduction

A primary goal for any food-processing corporation is to provide their customers with a product that not only has the highest quality but also exceptional food safety. In order to have outstanding food quality and safety, it is vital to control the onset of bacterial growth in the food product and the processing equipment. In the food industry, usually an effective cleaning and sanitation program is part of the process to inactivate the microorganism and to prevent the accumulation of microbial cells and biofilms as well as particulates on the surface of equipment (Dunsmore et al., 1981). Generally the sanitation agents and disinfectants are developed on the basis of studies utilizing the so-called naked bacteria, which are quite different from the biofilm microbes due to their altered physiological status (Wirtanen et al., 1992). Inactivation and removal of microbial cells, especially those capable of attaching to the surface of process equipment and forming biofilm, deserves much more attention (Chou et al., 2002). Biofilms are of concern for the food industry because of their high resistance to cleaning procedures, which allows bacteria within the biofilm to detach and cross-contaminate the food product (Wirtanen et al., 1992).

*Bacillus cereus* and *Bacillus licheniformis* are the most common *Bacillus spp.* are found in milk at all stages of processing (Anderton et al., 2008). *Bacillus cereus* is a well-known food poisoning organism that can produce enterotoxin (Granum et al., 1997). Spore-forming bacteria, such as *Bacillus cereus*, are considered to be one of the most important organisms impairing the preservation of pasteurized milk and milk products (Crielly et al., 1994). The occurrence of widespread spore-forming bacteria belonging to the genus *Bacillus spp.* has often been reported in raw milk (Faille et al., 2001). Furthermore, spores of *Bacillus cereus* are reported to possess a pronounced ability to adhere to the surface of stainless steel, a common food processing plant
material (Chou et al., 2002). Once adhesion of the *Bacillus* microorganism to the surface has occurred, colonization of the bacteria can transpire with favored conditions. The attached *Bacillus cereus* cells may subsequently form a biofilm on the surface of stainless steel and present a major problem for the food industry (Chou et al., 2002).

*Bacillus* species (*B. subtilis*, *B. licheniformis* and *B. pumilis*) are frequently isolated from various heat-treated milk products such as pasteurized milk, UHT-processed milk (135°C for 10 seconds), milk powder, cheese and yoghurt (Johnson et al., 1984). Unfortunately, the presence of *Bacillus* spores in food may be deleterious; indeed, *B. cereus* is often implicated in food-borne gastroenteritis but may also give rise to common milk spoilage such as bitty cream, sweet curdling and various off-flavors (Faille et al., 2001). Regrettably, both spores and bacteria embedded in biofilms are of concern to the food industry because of their strong adherence and high resistance to cleaning procedures, and also because of their increased resistance to cleaning and disinfection procedures (Wirtenan et al., 1992). Moreover, adhering bacteria may detach and a further cross-contamination of products during processing may occur (Faille et al., 2001).

Due to these facts, it is apparent that with the presence of *Bacillus spp.*, spore-forming bacteria, on the food processing equipment and the food product it amplifies the prominence of having the proper sanitizers and cleaners. The objective of this study was to determine the effectiveness of the selected cleaners and sanitizers in killing spore-formers by preventing, treating or reducing the effects of problem spores in the dairy export industry.
Literature Review

Spore-formers and Their Effect in the Dairy Industry

This study is particularly focusing on *Bacillus* spore-formers; *Bacillus* refers to a specific genus of bacteria. The family *Bacillaceae* are all gram-positive, rod-shaped bacteria which forms endospores, the focus being on the aerobic or facultatively anaerobic spore-forming bacteria (Andersson et al., 1999). *Bacillus* endospores are resistant to hostile physical and chemical conditions, but in addition various *Bacillus* species have a wide range of physiologic adaptations, which enable them to survive or thrive in harsh environments (Andersson et al., 1999). Spores have a tough outer covering made of keratin, which makes them highly resistant to heat and chemicals (Andersson et al., 1999). *Bacillus* food poisoning usually occurs because heat-resistant endospores survive cooking or pasteurization and then germinate and multiply when the food is inadequately refrigerated (Andersson et al., 1999). Raw milk is the usual source of spore-forming bacteria in finished dairy products (Lendenbach et al., 2008).

As quality of pasteurized milk improves because of reduction in levels of post-pasteurization contamination, the presence of heat-resistant psychrotrophic bacteria in the milk supply will assume a greater importance (Champagne et al., 1994). Spore-forming microorganisms in the modern milk supply are significant because of their prevalence, their relatively high heat resistance, and because of current trends in the dairy industry towards higher pasteurization temperatures and extended storage times for both raw and pasteurized dairy products (Harper et al., 1963). However, an increase in pasteurization temperature does not necessarily correlate into an increase in shelf life again because of activation of spores at higher temperatures (Champagne et al., 1994). Gram-positive spore-formers are the leading contributors to the spoilage of milk and milk products manufactured under good hygiene conditions and in
which a long storage period is expected (Champagne et al., 1994). Among the spore-forming microorganisms, *Bacillus licheniformis* and *Bacillus cereus* are the most commonly isolated species of *Bacillus* found in milk at all stages of processing (Anderton et al., 2008). *Bacillus licheniformis* was ubiquitous in the farm environment, and counts in raw milks heat-treated in the laboratory were higher during the winter months, while *B. cereus* is associated with cattle feed throughout the year, and tended to be more common in raw milks during the summer months (Anderton et al., 2008). *Bacillus cereus* has been identified as being associated with causing human illness since 1969, but in recent years attention has shifted to other species that have been identified as having an association with human illness: *B. licheniformis, B. subtilis, B. pumilis,* and *B. brevis* (Champagne et al., 1994).

Defects in milk and milk products can occur in four distinct sources: growth of psychrotrophic bacteria prior to pasteurization, activity of thermo-resistant enzymes, growth of thermo-resistant psychrotrophs, and post-pasteurization contamination (Champagne et al., 1994). But, due to varying changes in “on the farm” milk compilation and storage time the chance of contamination increases (Honer et al., 1981). In situations such as this, psychrotrophic bacteria will develop and generate a variety of defects in dairy products (Champagne et al., 1994). Normally, most psychrotrophic bacteria would be eliminated during proper pasteurization, but their extracellular enzymes such as lipases and proteases are heat resistant and are not deactivated by pasteurization (Champagne et al., 1994). In order for a protease to begin its activation, it requires an organic nitrogen source, and amino acids such as glutamine, asparagine, and aspartic acid (Fairbairn et al., 1986). Upon the activation of the proteases, the enzymes degrade the protein integrity of milk and milk products (Champagne et al., 1994). Protease synthesis by psychrotrophic bacteria in milk is proceeded by a dramatic drop in oxygen tension.
(Gilmour et al. 1982). When oxygen tension of the medium is maximized by aeration, protease production decreases (Champagne et al., 1994). The effect that psychrotrophs contribute to in dairy products is indicated in Table 1.

Table 1. Effects on the quality of dairy products due to the growth of psychrotrophs in raw milk before heat treatment (Sorhaug et al. 1997).

<table>
<thead>
<tr>
<th>Product type</th>
<th>Log CFU/mL psychrotrophs in raw milk</th>
<th>Effects of quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHT Milk</td>
<td>6.9-7.2</td>
<td>Gelation after 2-10 weeks; gradual development of lack of freshness; slightly stale, unclean, bitter flavor</td>
</tr>
<tr>
<td>Milk Powder; freeze-dried milk</td>
<td>6.3-7</td>
<td>Reduced heat stability; increased foaming capacity of reconstituted milk</td>
</tr>
<tr>
<td>Pasteurized Milk</td>
<td>7-8</td>
<td>Shorter shelf life; increased fouling in heat exchanger</td>
</tr>
<tr>
<td>Hard cheeses</td>
<td>7.5-8.3</td>
<td>Different flavor defects, predominantly rancidity and soapy taste; reduced cheese yield</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>5-7.8</td>
<td>Significant correlation between psychrotrophic counts in raw milk and bitter taste</td>
</tr>
<tr>
<td>Butter</td>
<td>ND</td>
<td>Faster development of rancidity in butter made from cold-stored milk than in that made from fresh milk; lipase from Pseudomonas was active in frozen butter</td>
</tr>
<tr>
<td>Yogurt</td>
<td>7.6-7.8</td>
<td>Bitter, unclean or fruity flavors, depending on the specific microflora</td>
</tr>
</tbody>
</table>

ND, not determined

Except for *Bacillus cereus*, pathogenic psychrotrophs are rarely implicated in technological or flavor defects of dairy products (Champagne et al., 1994). However, these pathogenic psychrotrophs contribute to a critical element in the quality of dairy products
(Champagne et al., 1994). *Bacillus cereus* is a common contaminant of raw milk. In a survey conducted by Griffiths and Phillips for raw milk, they isolated spores of psychrotrophic *Bacillus* spp. from 58% of their samples; of those obtained isolates, 39% were identified as *Bacillus cereus* (Griffiths et al., 1990). The highest levels of *Bacillus* spp. occur within the late summer and early fall (Champagne et al. 1994). The organism can be present in milk deriving from a variety of sources, but psychrotrophic strains of *Bacillus* spp. are introduced into milk as spores from pasture or as a result of improper cleaning of bulk tanks (Phillips et al., 1990).

**Chemicals**

Chemicals can be used in two ways in order to prevent psychrotrophic bacteria contamination problems in dairy foods (Champagne et al. 1994). First, they can act as a sanitizing agent on dairy surfaces, thus killing cells on equipment and preventing the contamination of milk (Cousins et al. 1977). Secondly, they can be added into raw milk in order to prevent the development psychrotrophic microflora (Champagne et al. 1994). The chemicals used in this experiment are used as a sanitizing agent on dairy surfaces before and after product is used. Mandate Plus is an EPA registered product that was designed for automated clean-in-place (CIP) systems, containing acetic acid 15%, nitric acid 14%, phosphoric acid 8%, 1-octanesulfonic acid 5-20%, pelargonic acid 6.30 and capric acid 1.09; a full product description of Mandate Plus can be found in Appendix A (Ecolab). HD PL-10 Plus is a concentrated, non-foaming, tri-blend detergent, which helps remove alkaline films from milking equipment surfaces; containing 32.8% nitric acid and phosphoric acid 27%. A full product description of HD PL-10 Plus can be found in Appendix B (Ecolab). Principal is a liquid, chlorinated detergent formulated for circulation, spray and soak cleaning of dairy, food and beverage processing equipment;
containing 15% sodium hydroxide and 3% sodium hypochlorite. A full product description of Principal can be found in Appendix C (Ecolab). Kem-Tek chlorine is a chlorinating liquid contains 10.0% sodium hypochlorite; a full product description of Chlorine can be found in Appendix D (Kem-Tek).
Materials and Methods

The spore-former that was used for this experiment was a concentrated solution of *Bacillus licheniformis*. The chemicals used to test against the growth of *B. licheniformis* were chlorine (Kem-Tek chlorinating liquid), mandate plus (Ecolab CIP acid sanitizer), HD Acid PL-10 (Ecolab acid detergent for pipeline and bulk tank equipment), and Principal (Ecolab chlorinated mechanical and CIP cleaner for protein soils). Dilutions of the sanitizers and cleaners were made from 1:1 to 1:10,000 in glass, capped test tubes; five dilutions of each chemical were made using Gilson pipetman calibration PM. Before dilutions were added each test tube contained 9 milliliters of deionized water. Then 1 milliliter of chemical solution was added to each test tube. Then 20 microliters of dense spore solution were added to each test tube. Next, 9 milliliters of media were added to each test tube containing the 1-milliliter of chemical solution and 20 microliters of dense spore solution. All the test tubes were then heat shocked for 30 minutes at 80°C (176 °F) on a Corning PC-620D hot plate. After the heat shock all of the test tubes were then incubated for 12 hours at 55°C (131 °F) in a Stabil-Therm Dry Type Bacteriological Incubator with gravity convection. Once the 12-hour incubation was done, 1 milliliter from each test tube was plated onto a 3M Petrifilm, and put back into the 55°C (131 °F) incubator overnight. Only 1:1 concentrations and 10⁻¹ solutions were isolated and put onto the 3M Petrifilm for observation. The next day the plates were hand-counted and recorded.

The procedure for the stock spore solution contained *Bacillus licheniformis*. Aseptically, transfer single loop of each spore stock solution to sterile TSB broth tubes, then vortex or agitate them gently to mix. Then activate the spores with heat shock of mesophilic or psychrotrophic cultures at 80°C ± 1°C for 12 minutes (Wehr et al., 2004) and thermophilic cultures at 100°C ± 1°C for 10 minutes (Jimenez et al., 2012) in a water bath. Then agitate once during heat shock to
mix the broth. Immediately cool the samples in an ice bath for about 10 minutes (Jimenez et al., 2012). Next, streak for isolation on a TSA plate with 0.2% soluble starch, and incubate at the appropriate temperature for optimum growth (Jimenez et al., 2012).

Table 2. Spore Solution with their optimum growth temperature (Jimenez et al., 2012).

<table>
<thead>
<tr>
<th>Culture</th>
<th>Growth Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 23857 B. subtilis</td>
<td>26</td>
</tr>
<tr>
<td>ATCC 12759 B. licheniformis</td>
<td>30</td>
</tr>
<tr>
<td>ATCC 8186 B. brevis (Brevibacillus parabrevis)</td>
<td>30</td>
</tr>
<tr>
<td>ATCC 14579 B. cereus</td>
<td>30</td>
</tr>
<tr>
<td>ATCC 4513 B. circulans</td>
<td>30</td>
</tr>
<tr>
<td>ATCC 14581 B. megaterium</td>
<td>30</td>
</tr>
<tr>
<td>ATCC 301 B. polymyxa (Paenibacillus polymyxa)</td>
<td>30</td>
</tr>
<tr>
<td>ATCC 72 B. pumilus</td>
<td>30</td>
</tr>
<tr>
<td>ATCC 23842 B. amyloliqufaciens</td>
<td>37</td>
</tr>
<tr>
<td>ATCC 7050 B. coagulans</td>
<td>37</td>
</tr>
<tr>
<td>ATCC 14580 B. licheniformis</td>
<td>37</td>
</tr>
<tr>
<td>ATCC 23059 B. subtilis</td>
<td>37</td>
</tr>
<tr>
<td>ATCC 1298 B. stearothermophilus (Geobacillus..)</td>
<td>55</td>
</tr>
<tr>
<td>Difco B. stearothermophilus</td>
<td>55</td>
</tr>
</tbody>
</table>

Plates were checked after 24 hours. Once adequate growth was observed a representative isolated colony was carefully selected to inoculate sterile TSB broth (Jimenez et al., 2012). All tubes were incubated until turbid (24-48 hours) at appropriate incubation temperatures (Jimenez et al., 2012). Cultures in the broth were streaked for heavy, diffuse growth across AK agar #2 plates (4 plates each) using a sterile swab (Jimenez et al., 2012). Plates were placed in bags to prevent dehydration of media before incubation at appropriate temperatures (Jimenez et al., 2012). Then the plates were checked after 7 days of incubation to evaluate the extent of sporulation using phase contrast microscopy (Jimenez et al., 2012). Once sporulation was observed, plates were
placed at 4°C overnight to induce further sporulation with the objective to achieve at least 90% sporulation (Jimenez et al., 2012). If minimal sporulation was observed at the 7-day check then plates were put back in the incubator for up to 7 days continued incubation (Jimenez et al., 2012). Spores were scraped from the surface of the agar plates using sterile hockey sticks into sterile 50 milliter falcon tubes. Sterile, deionized water was used to wash the agar surface (Jimenez et al., 2012). Cells were pelleted at 4000 rpm for 30 minutes (Jimenez et al., 2012). The supernatant was discarded. Cell pellets were re-suspended in approximately 10 milliters of sterile, deionized water and heat-shocked at 70°C for 30 minutes (Richardson et al., 1985) to kill vegetative cells. Then 9 milliters of filter sterilized lysozyme solution (0.9 mg/ml lysozyme, 15 mM MgCl₂, 0.1 M K-phosphate buffer; pH 7.0) was added to each tube to digest remaining vegetative cells (Jimenez et al., 2012). Samples were vortexed and incubated for 1 hour at 37°C (Jimenez et al., 2012). After incubation, spores were pelleted at 4000 rpm for 30 minutes and the supernatant was decanted (Jimenez et al., 2012). Sterile, deionized water (10 ml) was added to each pellet and the four samples were combined. To wash the spore pellets, the combined samples were centrifuged at 4000 rpm for 30 minutes and the supernatant was discarded (Jimenez et al., 2012). This procedure was repeated three times (Jimenez et al., 2012). Sterile deionized water (30 ml) was added to the final spore pellet (Jimenez et al., 2012). The concentration of the spore stock solution was determined using direct microscopic count (hemocytometer) and recorded on each tube; spore stock solution was maintained at 4°C (Jimenez et al., 2012).
**Statistical Analysis**

All samples were plated in duplicate using 3M Petrifilm. A One-way ANOVA (Analysis of Variance) was used to analyze the results, and test for significant differences. Statistically significant difference was set at P< .05, and Minitab 16 software (version 16.0, Minitab Inc., State College, PA) was used for all analyses. The initial hypothesis ($H_0$: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = \mu_8 = \mu_9 = \mu_{10} = \mu_{11} = \mu_{12} = \mu_{13} = \mu_{14} = \mu_{15} = \mu_{16}$) was set for all means were equal. The alternative hypothesis ($H_1$: Not all 16 population means are equal) was set for the opposing argument that not all samples are equal. The overall mean ($\mu_i$) was to test the mean effectiveness for all samples that received *B. Licheniformis*. The Levene’s Test could not be used because that it relies on large sample sizes (>30); this test only contained 16 samples. Upon testing for equal variance, we can assume that equal variances appears feasible, because p-value= 0.979>.05. We have failed to reject the hypothesis ($H_0$). When checking for normality, the hypothesis ($H_0$) is assumed to be from a normal population. The alternative hypothesis ($H_1$) is assumed to be not from a normal population. When checking for normality we always assume that the level of significance is $\alpha= .01$. The p-value from the normality test indicated that these samples were from a normal population; because the p-value 0.357 > .01, we failed to reject $H_0$. We can assume that normality is feasible.
Results and Discussion

After a 24-hour incubation period in 55°C (131 °F), in a Stabil-Therm Dry Type Bacteriological Incubator with gravity convection, all four chemicals used (chlorine, Mandate Plus, Principal and HD Acid PL-10) with a concentration of 1:1 $10^{-1}$ and $10^{-2}$ had a solid pink shade over the 3M Petrifilm; providing it with too many to count (TMTC). But, fortunately when the four chemicals used had a concentration of $10^{-1}$ with further dilutions of $10^{-1}$ and $10^{-2}$, there were measureable results. After the incubation period, the 3M Petrifilms showed results of chlorine $10^{-1}$ having no visible growth on either dilutions of $10^{-1}$ or $10^{-2}$. Mandate Plus $10^{-1}$ exhibited growth at $10^{-1}$ with 538 counts/mL and at $10^{-2}$ 168 counts/mL, Principal $10^{-1}$ revealed complete saturation growth giving an entire pink shade for both dilutions of $10^{-1}$ and $10^{-2}$, and HD Acid PL-10 $10^{-1}$ presented some growth with 457 counts/mL and 427 counts/mL, respectively.

These results suggest that chlorine (sodium hypochlorite 10.0% and inert ingredients 90.0%) is the most effective in reducing or killing spore-forming bacteria such as *Bacillus cereus* and *Bacillus licheniformis*. The second most effective chemical was Mandate Plus (6.30% nonanoic acid, 1.09% decanoic acid and 92.6% inert ingredients), a non-iodine acid sanitizer, which demonstrated that it had a significant reduction along with its dilutions. The third most effective chemical was HD Acid PL-10 (Phosphoric acid detergent), which produced a slight reduction with its dilutions. Finally, the least effective chemical on the concentrated solution of *Bacillus licheniformis* was Principal (sodium hydroxide and sodium hypochlorite), which is a chlorinated sanitizer, which had too many colonies to count (TMTC). The results of this experiment suggest that for the most effective treatment in reducing or killing spore-forming bacteria (*Bacillus cereus* and *Bacillus licheniformis*) the use of chlorine (sodium hypochlorite...
10.0% and inert ingredients 90.0%) or Mandate Plus (6.30% nonanoic acid, 1.09% decanoic acid and 92.6% inert ingredients), a non-iodine acid sanitizer, would yield the best results out of the industry chemicals tested.

Sodium hypochlorite and Quaternary ammonia compound (QAC) are some of the most common and widely used sanitizers in the food industry (Petrocii et al., 1983). As a strong oxidizing agent, hypochlorite is known to be very active in killing most bacteria, fungi and viruses (Chou et al. 2002). QAC are hydrophilic, cationic molecules. It was reported that QAC readily adsorb to bacterial surface, which is hydrophilic and negatively charged, penetrate the cell wall, and disrupt the cytoplasmic membrane (Petrocii et al., 1983). Chou et al. 2002, an approximately 5.5 log CFU/ml decrease in number was noted with these planktonic cells (Bacillus cereus) after only a 15 second exposure to hypochlorite as shown in table 2 (Chou et al. 2002).

Table 3. Inactivation of B. cereus cell by sodium hypochlorite (Chou et al. 2002)

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Cell type</th>
<th>Population reduction (log CFU/ml or chip) after time of exposure (s)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>25</td>
<td>planktonic</td>
<td>5.52</td>
</tr>
<tr>
<td></td>
<td>attached single cell</td>
<td>4.04B</td>
</tr>
<tr>
<td></td>
<td>biofilmd</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>milk-biofilmc</td>
<td>–</td>
</tr>
<tr>
<td>50</td>
<td>planktonic</td>
<td>5.54</td>
</tr>
<tr>
<td></td>
<td>attached single cell</td>
<td>4.30B</td>
</tr>
<tr>
<td></td>
<td>biofilm</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>milk-biofilm</td>
<td>–</td>
</tr>
</tbody>
</table>

A. Obtained by subtracting final population (log cfu/ml or chip) after treatment from original population (log CFU/ml or chip) B. Common letters within the same column for the same treatment denote results that are not significantly different (P>0.05; N=5) C. Not determined D. B. cereus biofilm developed on clean stainless steel chip after 8 days' incubation in SLB. E.B. cereus biofilm developed on milk pre-soiled stainless steel chip after 8 days' incubation in SLB.
Chambers et al. 1956 and William et al. 1984 indicate that sanitizers are considered to be effective if they inactivate bacteria by at least at 5.0 log cycles in 30 seconds. With this information it is presumed that chlorine (sodium hypochlorite 10.0% and inert ingredients 90.0%) is the best chemical sanitizer to use when reducing or killing a spore-forming bacteria is needed. Mosteller and Bishop et al. 1993 found that the efficacy of sanitizers including iodophor, hyphohlorite, acid anionic, peroxyacetic acid, fatty acid and quaternary ammonium compound versus the suspensions of Pseudomonas fluorescens, Yersinia enterocolitica and Listeria monocytogenes resulted in a >5.0 log-cycle reduction while the same concentrations were relatively ineffective against the attached cells (Bishop et al. 1993). On the other hand, Mustapha and Liewen et al. 1989 have indicated that L. monocytogenes is more resistant to the lethal action of sodium hypochlorite on a stainless steel surface than in vitro. Although sodium hypochlorite is successful in reducing/ killing spore-forming bacteria, such as Bacillus spp., it is not as effective with other spoilage organisms (Chou et al. 2002). Indicating that other cleaners and sanitizer must be used to properly disinfect the processing equipment.
Fig. 1 Counts of (a) *Bacillus cereus* DLS vegetative cells and spores and *Pseudomonas fluorescens* M2 cells in (b) single and (c) binary species biofilms before and after daily treatment with a chlorine dioxide-containing sanitizer over 6 d. □, Vegetative count of *B. cereus* DLS before sanitizer treatment; □, vegetative count of *B. cereus* DLS after sanitizer treatment; D, spore count before sanitizer treatment; •, spore count after sanitizer treatment; □, count of *P. fluorescens* M2 before sanitizer treatment; □, count of *P. fluorescens* M2 after sanitizer treatment (Lindsay et al. 2002)

Based on the successfulness of chlorine (sodium hypochlorite), why was Principal, which contains sodium hypochlorite, not as effective? This is likely caused by the sodium hydroxide, which is also present in Principal, which alters the effectiveness of the hypochlorite. Another theory as to why Principal was less effective than Chlorine would be because it was not as concentrated with sodium hypochlorite as Chlorine was. This study is aimed to determine the most effective food industry chemical against spore-forming bacteria; further testing is needed to
examine the potential potency of the Chlorine and Principal solutions to determine the cause of one having the lesser effectiveness.
Conclusion

This study provides a detailed description of the effectiveness of selected cleaners and sanitizers in killing or reducing spore-forming bacteria. Chlorine (sodium hypochlorite 10.0% and inert ingredients 90.0%) is the most effective in reducing or killing spore-forming bacteria such as *Bacillus cereus* and *Bacillus licheniformis*. The addition of chlorine (sodium hypochlorite) after proper clean-in-place (CIP) procedures, cleaning with a chlorinated sanitizer and a phosphoric acid detergent, can reduce or kill the occurrence of spore-forming bacteria in the food processing facility by a 5.5 log CFU/mL reduction (Chou et al. 2002). The results obtained would be of significant value to the food processing industry, as it is directly applicable in determining the proper cleaners and sanitizers to be used.
Acknowledgements

The author would like to thank Dr. Rafael Jimenez-Flores and staff members, of the Dairy Products Technology Center at California Polytechnic State University, for allowing the use of their facilities and equipment, as well as their assistance and guidance throughout the project. Moreover, thank you for providing the materials and methods needed in order for this research to be completed.
Appendix A

Material Safety Data Sheet

1. Product and company identification
   Trade name of product: MANDATE PLUS
   Product use: Sanitizer
   Supplier's information: Ecolab Inc. Food & Beverage Division 370 N. Wabasha Street St. Paul, MN 55102 1-800-392-3392
   Code: 902730-03:
   Date of issue: 29-November-2012
   EPA Registration No: 1677-194
   EMERGENCY HEALTH INFORMATION: 1-800-328-0026
   Outside United States and Canada CALL 1-651-222-5352 (in USA)
   Product dilution information: Up to 1 oz/3 gal in water

2. Hazards identification
   Product AT USE DILUTION
   Liquid. CAUTION!
   MAY CAUSE EYE IRRITATION.
   Avoid contact with eyes. Wash thoroughly after handling.
   Physical state: Liquid.
   Emergency overview: Danger!

   CAUSES RESPIRATORY TRACT, DIGESTIVE TRACT, EYE AND SKIN BURNS. MAY BE FATAL IF ABSORBED THROUGH SKIN. HARMFUL IF INHALED OR SWALLOWED.

   Do not ingest. Do not get in eyes, on skin or on clothing. Do not breathe vapor or spray. Keep container closed. Use only with adequate ventilation. Wash thoroughly after handling.

   Potential acute health effects
   Product AS SOLD

   Product AT USE DILUTION
   Moderately irritating to eyes.
   No known significant effects or critical hazards. No known significant effects or critical hazards.
   No known significant effects or critical hazards.

   Eyes: Corrosive to eyes.
   Skin: Corrosive to the skin. MAY BE FATAL IF ABSORBED THROUGH SKIN.
   Inhalation: HARMFUL IF INHALED. Corrosive to the respiratory system.
   Ingestion: Harmful if swallowed. Causes burns to mouth, throat and stomach.

   Product AS SOLD
   See toxicological information (Section 11)
3. Composition/information on ingredients

Product AS SOLD

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS number</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACETIC ACID</td>
<td>64-19-7</td>
<td>15</td>
</tr>
<tr>
<td>nitric acid</td>
<td>7697-37-2</td>
<td>14</td>
</tr>
<tr>
<td>PHOSPHORIC ACID</td>
<td>7664-38-2</td>
<td>8</td>
</tr>
<tr>
<td>1-octanesulfonic acid, sodium salt</td>
<td>5324-84-5</td>
<td>5 - 20</td>
</tr>
<tr>
<td>PELARGONIC ACID</td>
<td>112-05-0</td>
<td>6.30</td>
</tr>
<tr>
<td>CAPRIC ACID</td>
<td>334-48-5</td>
<td>1.09</td>
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</tbody>
</table>

16MANDATE PLUS 29 November 2012

3. Composition/information on ingredients

Product AT USE DILUTION

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS number</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitric acid</td>
<td>7697-37-2</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

4. First aid measures

Product AT USE DILUTION

In case of contact, immediately flush eyes with plenty of water. Remove contact lenses and flush again. Get medical attention if irritation persists.

No known effect after skin contact. Rinse with water for a few minutes.

No special measures required. Treat symptomatically.

Get medical attention if symptoms occur.

5. Fire-fighting measures

Eye contact: In case of contact, immediately flush eyes with cool running water. Remove contact lenses and continue flushing with plenty of water for at least 15 minutes. Get medical attention immediately.

Skin contact: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention immediately. Wash clothing before reuse. Clean shoes thoroughly before reuse.

Inhalation: If inhaled, remove to fresh air. If exposed person is not breathing, give artificial respiration or oxygen applied by trained personnel. Get medical attention immediately.

Ingestion: If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Do not induce vomiting. Never give anything by mouth to an unconscious person. Get medical attention immediately.

Product AS SOLD

Hazardous thermal decomposition products: sulfur oxides phosphorus oxides metal oxide/oxides

Fire-fighting media and instructions: Use an extinguishing agent suitable for the surrounding fire.
Dike area of fire to prevent runoff. In a fire or if heated, a pressure increase will occur and the container may burst.

**Special protective equipment for fire-fighters:** Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

6. **Accidental release measures**

**Product AT USE DILUTION**
Use personal protective equipment as required.

Avoid contact of large amounts of spilled material and runoff with soil and surface waterways.

**Personal precautions:** Initiate company's spill response procedures immediately. Keep people out of area. Put on appropriate personal protective equipment (see section 8). Do not touch or walk through spilled material.

**Environmental precautions:** Avoid contact of spilled material and runoff with soil and surface waterways.

6. **Accidental release measures**

Use a water rinse for final clean-up.

7. **Handling and storage**

**Product AT USE DILUTION**
Avoid contact with eyes. Wash thoroughly after handling.

Keep out of reach of children. Store in a closed container.

**Methods for cleaning up:** Follow company's spill procedures. Keep people away from spill. Put on appropriate personal protective equipment (see section 8). Absorb/neutralize liquid material. Use a tool to scoop up solid or absorbed material and put into appropriate labeled container. Use a water rinse for final clean-up.

**Handling:** Do not ingest. Do not get in eyes, on skin, or on clothing. Do not breathe vapor or spray. Keep container closed. Use only with adequate ventilation. Wash thoroughly after handling. Do not mix with bleach or other chlorinated products - will cause chlorine gas.

**Storage:** Keep out of reach of children. Keep container tightly closed.

Do not store below the following temperature: -10°C

**Engineering measures:** Use only with adequate ventilation. If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. Provide suitable facilities for quick drenching or flushing of the eyes and body in case of contact or splash hazard.

8. **Exposure controls/personal protection**

**Product AT USE DILUTION**
Good general ventilation should be sufficient to control worker exposure to airborne contaminants.

No protective equipment is needed under normal use conditions.

No protective equipment is needed under normal use conditions.

No special protection is required.

**Personal protection:** Wear suitable protective clothing.

**Eyes:** Use chemical splash goggles. For continued or severe exposure wear a face shield over the
goggles. **Hands:** Use chemical-resistant, impervious gloves. **Skin Respiratory:** Wear appropriate respirator when ventilation is inadequate and occupational exposure limits are exceeded.

### Ingredient Exposure limits

**ACETIC ACID**  
*ACGIH TLV (United States, 2/2010).*  
STEL: 37 mg/m³ 15 minute(s). STEL: 15 ppm 15 minute(s). TWA: 25 mg/m³ 8 hour(s). TWA: 10 ppm 8 hour(s).  
*OSHA PEL (United States, 6/2010).*  
TWA: 25 mg/m³ 8 hour(s). TWA: 10 ppm 8 hour(s).

**nitric acid**  
*ACGIH TLV (United States, 2/2010).*  
STEL: 10 mg/m³ 15 minute(s). STEL: 4 ppm 15 minute(s). TWA: 5.2 mg/m³ 8 hour(s). TWA: 2 ppm 8 hour(s).  
*OSHA PEL (United States, 6/2010).*  
TWA: 5 mg/m³ 8 hour(s). TWA: 2 ppm 8 hour(s).  
*NIOSH REL (United States, 6/2009).*  
TWA: 2 ppm 10 hour(s). TWA: 5 mg/m³ 10 hour(s). STEL: 4 ppm 15 minute(s). STEL: 10 mg/m³ 15 minute(s).

8. **Exposure controls/personal protection**

**PHOSPHORIC ACID**  
*ACGIH TLV (United States, 2/2010).*  
STEL: 3 mg/m³ 15 minute(s).  
TWA: 1 mg/m³ 8 hour(s).  
*OSHA PEL (United States, 6/2010).*  
TWA: 1 mg/m³ 8 hour(s).  
*NIOSH REL (United States, 6/2009).*  
TWA: 1 mg/m³ 10 hour(s). STEL: 3 mg/m³ 15 minute(s).

9. **Physical and chemical properties**

**Product AT USE DILUTION**  
**Physical state:** Liquid  
**Flash point:** > 100°C  
**Color:** Colorless [Light]  
**Odor:** Faint odor  
**pH:** 1 to 3  
**Relative density:** 1.17

10. **Stability and reactivity**

**Stability:** The product is stable. Under normal conditions of storage and use, hazardous polymerization will not occur.  
**Reactivity:** Highly reactive or incompatible with the following materials: alkalis. Reactive or incompatible with the following materials: metals. Slightly reactive or incompatible with the
following materials: moisture. Do not mix with bleach or other chlorinated products - will cause chlorine gas.

**Hazardous decomposition:** Under normal conditions of storage and use, hazardous decomposition products should not be produced.

**Hazardous polymerization:** Under normal conditions of storage and use, hazardous polymerization will not occur.

11. **Toxicological information**

**Product AT USE DILUTION**

**Potential acute health effect:**

**Eyes:** Corrosive to eyes.

**Skin:** Corrosive to the skin. MAY BE FATAL IF ABSORBED THROUGH SKIN.

**Inhalation:** HARMFUL IF INHALED. Corrosive to the respiratory system

**Ingestion:** Harmful if swallowed. Causes burns to mouth, throat and stomach.

**Other adverse effects**

**Target organs:** Contains material which may cause damage to the following organs: blood, lungs, upper respiratory tract, teeth, testes.

12. **Ecological information**

**Ecotoxicity:** Not reported

13. **Disposal considerations**

**Product AT USE DILUTION**

Diluted product can be flushed to sanitary sewer. Discard empty container in trash.

**Waste disposal:** Avoid disposal. Attempt to use product completely in accordance with intended use. Disposal should be in accordance with applicable regional, national and local laws and regulations.

**RCRA classification:** Unused product is D002 (Corrosive)

14. **Transport information**

Certain shipping modes or package sizes may have exceptions from the transport regulations. The classification provided may not reflect those exceptions and may not apply to all shipping modes or package sizes.

**Product AS SOLD**

**DOT**

**DOT Classification:** UN1760

**DOT Proper shipping name:** Corrosive liquids, n.o.s. (acetic acid, nitric acid)

Class: 8

Packing group: 11

**IMO/IMDG**

**IMO/IMDG Classification:** UN1760

**IMO/IMDG Proper shipping name:** CORROSIVE LIQUID, N.O.S. (nitric acid, acetic acid)

Class: 8

Packing group: 11

For transport in bulk, see shipping documents for specific transportation information.

**Product AT USE DILUTION**

23
Not intended for transport.

15. Regulatory information

Product AT USE DILUTION
Irritating material

Product AS SOLD
HCS: Corrosive material; Target organ effects

Classification
Product AS SOLD
U.S. Federal regulations
TSCA 8(b) inventory: All components are listed or exempted.
EPA Registration No.: 1677-194
SARA 302/304/311/312 extremely hazardous substances: nitric acid
SARA 302/304 emergency planning and notification: nitric acid
SARA 313
Form R - Reporting requirements
California Prop. 65
Product name
: nitric acid : No listed substance
CAS number
7697-37-2
Concentration
14.07
5/6

MANDATE PLUS

16. Other information

Product AS SOLD
Hazardous Material: Information System (U.S.A.)
* 3

29 November 2012
Product AT USE DILUTION
Data not available - Refer to Product AS

0

National Fire Protection Association (U.S.A.)
Health 3
Date of issue Responsible name
0

: Flammability Instability/Reactivity
Special
: 29-November-2012 : Regulatory Affairs 1-800-352-5326
0

SOLD
Flammability 0 Instability/Reactivity
Special
Health

Indicates information that has changed from previously issued version.

Notice to reader

The above information is believed to be correct with respect to the formula used to manufacture the product in the country of origin. As data, standards, and regulations change, and conditions of use and handling are beyond our control, NO WARRANTY, EXPRESS OR IMPLIED, IS MADE AS TO THE COMPLETENESS OR CONTINUING ACCURACY OF THIS INFORMATION.

6/6
Material Safety Data Sheet

1. Product and company identification

Trade name of product: HD ACID PL-10
Product use: Cleaning product
Supplier's information: Ecolab Inc. Food & Beverage Division
370 N. Wabasha Street
St. Paul, MN 55102 1-800-392-3392

HD ACID PL-10
Code: 969345-02
Date of issue: 13-April-2011 EMERGENCY HEALTH INFORMATION: 1-800-328-0026
Outside United States and Canada CALL 1-651-222-5352 (in USA)

Product dilution information: Up to 0.1 oz/gal or 0.8 mL/L in water

2. Hazards identification
Product AT USE DILUTION

Physical state: Liquid.
Emergency overview: DANGER!
CAUSES DIGESTIVE TRACT, EYE AND SKIN BURNS. CAUSES RESPIRATORY TRACT IRRITATION.
Do not ingest. Do not get in eyes, on skin or on clothing. Avoid breathing vapors, spray or mists. Keep container closed. Use only with adequate ventilation. Wash thoroughly after handling. CAUTION!
No specific hazard. No specific hazard.

Potential acute health effects
Eyes: Corrosive to eyes.
Skin: Corrosive to the skin.
Inhalation: Irritating to respiratory system.
Ingestion: Causes burns to mouth, throat and stomach.

Product AS SOLD:
See toxicological information (section 11)

3. Composition/information on ingredients

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS number</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHOSPHORIC ACID</td>
<td>7664-38-2</td>
<td>27</td>
</tr>
</tbody>
</table>

Product AT USE DILUTION
Within the present knowledge of the supplier, this product does not contain any hazardous ingredients in quantities requiring reporting, in accordance with local regulations.
1/6HD ACID PL-10 13 April 2011

4. First aid measures
Product AT USE DILUTION
No known effect after eye contact. Rinse with water for a few minutes.
No known effect after skin contact. Rinse with water for a few minutes.
No special measures required. Treat symptomatically.
Get medical attention if symptoms occur.

5. Fire-fighting measures

**Eye contact:** In case of contact, immediately flush eyes with cool running water. Remove contact lenses and continue flushing with plenty of water for at least 15 minutes. Get medical attention immediately.

**Skin contact:** In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention immediately. Wash clothing before reuse. Clean shoes thoroughly before reuse.

**Inhalation:** If inhaled, remove to fresh air. Get medical attention if irritation persists.

**Ingestion:** If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Do not induce vomiting. Never give anything by mouth to an unconscious person. Get medical attention immediately.

**Hazardous thermal decomposition products:** Decomposition products may include the following materials: phosphorus oxides

**Fire-fighting media and instructions:** Use an extinguishing agent suitable for the surrounding fire.

**Special protective equipment for fire-fighters:** Dike area of fire to prevent runoff. In a fire or if heated, a pressure increase will occur and the container may burst. Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

6. Accidental release measures

**Product AT USE DILUTION**
Use personal protective equipment as required.
Avoid contact of large amounts of spilled material and runoff with soil and surface waterways. Use a water rinse for final clean-up.

**Personal precautions:** Initiate company's spill response procedures immediately. Keep people out of area. Put on appropriate personal protective equipment (see section 8). Do not touch or walk through spilled material.

**Environmental precautions:** Avoid contact of spilled material and runoff with soil and surface waterways.

**Methods for cleaning up:** Follow company's spill procedures. Keep people away from spill. Put on appropriate personal protective equipment (see section 8). Absorb/neutralize liquid material. Use a tool to scoop up solid or absorbed material and put into appropriate labeled container. Use a water rinse for final clean-up.

**Product AS SOLD**

2/6

*HD ACID PL-10*  13 April 2011

7. Handling and storage

**Handling:** Do not ingest. Do not get in eyes, on skin, or on clothing. Avoid breathing vapors, spray or mists. Keep container closed. Use only with adequate ventilation. Wash thoroughly after handling. Do not mix with bleach or other chlorinated products - will cause chlorine gas.

Do not store above the following temperature: 40°C
Storage: Wash thoroughly after handling. Keep out of reach of children.

8. Exposure controls/personal protection

Engineering measures: Use only with adequate ventilation. If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. Provide suitable facilities for quick drenching or flushing of the eyes and body in case of contact or splash hazard

Personal protection

Eyes: Use chemical splash goggles. For continued or severe exposure wear a face shield over the goggles.

Hands: Use chemical-resistant, impervious gloves.

Skin: Use synthetic apron, other protective equipment as necessary to prevent skin contact.

Respiratory: Avoid breathing vapors, spray or mists.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Exposure limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHOSPHORIC ACID</td>
<td>ACGIH TLV (United States, 2/2010).</td>
</tr>
<tr>
<td></td>
<td>STEL: 3 mg/m3 15 minute(s).</td>
</tr>
<tr>
<td></td>
<td>TWA: 1 mg/m3 8 hour(s).</td>
</tr>
<tr>
<td></td>
<td>OSHA PEL (United States, 6/2010).</td>
</tr>
<tr>
<td></td>
<td>TWA: 1 mg/m3 8 hour(s).</td>
</tr>
</tbody>
</table>

9. Physical and chemical properties

Product AT USE DILUTION

Physical state: Liquid.
Flash point: > 100°C
Color: pink
Odor: Faint odor
pH: 0.5 to 1 [Conc. (% w/w): 100%]
Relative density: 1.148 to 1.178
Solubility: Easily soluble in the following materials: cold water and hot water.

Liquid. > 100°C Pale color Faint odor 1 to 2

3/6

HD ACID PL-10 13 April 2011

10. Stability and reactivity

Product AS SOLD

Stability: The product is stable. Under normal conditions of storage and use, hazardous polymerization will not occur.

Reactivity: Highly reactive or incompatible with the following materials: alkalis. Reactive or incompatible with the following materials: metals. Slightly reactive or incompatible with the following materials: organic materials and moisture. Do not mix with bleach or other chlorinated products - will cause chlorine gas.

Hazardous decomposition products: Under normal conditions of storage and use, hazardous decomposition products should not be produced.
Hazardous polymerization: Under normal conditions of storage and use, hazardous polymerization will not occur.

11. Toxicological information
Potential acute health effects
Eyes: Corrosive to eyes.
Skin: Corrosive to the skin.
Inhalation: Irritating to respiratory system.
Ingestion: Causes burns to mouth, throat and stomach.

Product AS SOLD

Other adverse effects
Target organs: Contains material which may cause damage to the following organs: lungs, upper respiratory tract.

12. Ecological information
Ecotoxicity: Not reported

13. Disposal considerations
Product AT USE DILUTION
Diluted product can be flushed to sanitary sewer. Discard empty container in trash.

Waste disposal: Avoid disposal. Attempt to use product completely in accordance with intended use. Disposal should be in accordance with applicable regional, national and local laws and regulations.

RCRA classification: Unused product is D002 (Corrosive)

Product AS SOLD

14. Transport information
Certain shipping modes or package sizes may have exceptions from the transport regulations. The classification provided may not reflect those exceptions and may not apply to all shipping modes or package sizes.

Product AS SOLD
DOT: UN1805
Classification

DOT Proper shipping name: Phosphoric acid solution
Class: 8
Packing group: III
For transport in bulk or using IMDG regulations, see shipping documents for specific transportation information.

HD ACID PL-10
14. Transport information
Product AT USE DILUTION
Not intended for transport.

15. Regulatory information

Product AS SOLD
HCS Classification: Corrosive material; Target organ effects

Product AS SOLD

U.S. Federal regulations
Product AT USE DILUTION
Not regulated.

TSCA 8(b) inventory: All components are listed or exempted. SARA 302/304/311/312 extremely hazardous substances: No listed substance SARA 302/304 emergency planning and notification: No listed substance SARA 313 Form R - Reporting requirements: No listed substance

California Prop. 65: No listed substance

Product name
CAS number Concentration

16. Other information

Product AS SOLD
Hazardous Material: Information System (U.S.A.)

*3 0

Product AT USE DILUTION

National Fire Protection Association (U.S.A.)
Health 3

Date of issue Responsible name
0

: Flammability Instability/Reactivity
Special
: 13-April-2011 : Regulatory Affairs
0

Flammability 0 Instability/Reactivity
Special
0

Health 0

1-800-352-5326 Indicates information that has changed from previously issued version.

Notice to reader
5/6

HD ACID PL-10 13 April 2011

16. Other information
The above information is believed to be correct with respect to the formula used to manufacture the product in the country of origin. As data, standards, and regulations change, and conditions of use and handling are beyond our control, NO WARRANTY, EXPRESS OR IMPLIED, IS MADE AS TO THE COMPLETENESS OR CONTINUING ACCURACY OF THIS INFORMATION.
Appendix C

Material Safety Data Sheet

1. Product and company identification
Trade name of product: PRINCIPAL
Product use: Cleaning product
Supplier's information: Ecolab Inc. Food & Beverage Division
370 N. Wabasha Street
St. Paul, MN 55102 1-800-392-3392

Code: : 948463
Date of issue: 12-December-2011 EMERGENCY HEALTH INFORMATION: 1-800-328-0026
Outside United States and Canada CALL 1-651-222-5352 (in USA)
Product dilution information: Up to 0.75 oz/gal or 5.8 mL/L in water

2. Hazards identification
Physical state: Liquid.
Emergency overview: DANGER!
CAUSES RESPIRATORY TRACT, DIGESTIVE TRACT, EYE AND SKIN BURNS.
Do not ingest. Do not get in eyes, on skin or on clothing. Do not breathe vapor or spray. Keep container closed. Use only with adequate ventilation. Wash thoroughly after handling.

Potential acute health effects
Product AT USE DILUTION
Eyes: Corrosive to eyes.
Skin: Corrosive to the skin.
Inhalation: Corrosive to the respiratory system.
Ingestion: Causes burns to mouth, throat and stomach.

Product AS SOLD
See toxicological information (Section 11)

3. Composition/information on ingredients
Product AS SOLD

<table>
<thead>
<tr>
<th>Name</th>
<th>CAS number</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODIUM HYDROXIDE</td>
<td>1310-73-2</td>
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</tr>
<tr>
<td>sodium hypochlorite</td>
<td>7681-52-9</td>
<td>3</td>
</tr>
</tbody>
</table>

Product AT USE DILUTION
Within the present knowledge of the supplier, this product does not contain any hazardous ingredients in quantities requiring reporting, in accordance with local regulations.
1/6PRINCIPAL 12 December 2011

4. First aid measures
Eye contact: In case of contact, immediately flush eyes with cool running water. Remove contact lenses and continue flushing with plenty of water for at least 15 minutes. Get medical
attention immediately.

**Skin contact:** In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention immediately. Wash clothing before reuse. Clean shoes thoroughly before reuse.

**Inhalation:** If inhaled, remove to fresh air. If exposed person is not breathing, give artificial respiration or oxygen applied by trained personnel. Get medical attention immediately.

**Ingestion:** If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Do not induce vomiting. Never give anything by mouth to an unconscious person. Get medical attention immediately.

---

**Product AS SOLD**

5. Fire-fighting measures

**Product AS SOLD**

**Hazardous thermal decomposition products:** Decomposition products may include the following materials: carbon dioxide carbon monoxide halogenated compounds metal oxide/oxides

**Fire-fighting media and instructions:** Use an extinguishing agent suitable for the surrounding fire.

Dike area of fire to prevent runoff.

In a fire or if heated, a pressure increase will occur and the container may burst.

**Special protective equipment for fire-fighters:** Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

---

6. Accidental release measures

**Product AT USE DILUTION**

Use personal protective equipment as required.

Avoid contact of large amounts of spilled material and runoff with soil and surface waterways.

Use a water rinse for final clean-up.

**Personal precautions:** Initiate company's spill response procedures immediately. Keep people out of area. Put on appropriate personal protective equipment (see section 8). Do not touch or walk through spilled material.

**Environmental precautions:** Avoid contact of spilled material and runoff with soil and surface waterways.

**Methods for cleaning up:** Follow company's spill procedures. Keep people away from spill. Put on appropriate personal protective equipment (see section 8). Absorb/neutralize liquid material. Use a tool to scoop up solid or absorbed material and put into appropriate labeled container. Use a water rinse for final clean-up.

---

**Product AS SOLD**

2/6

*PRINCIPAL* 12 December 2011

7. Handling and storage

**Product AT USE DILUTION**
Wash thoroughly after handling.
Keep out of reach of children.

**Handling:** Do not ingest. Do not get in eyes, on skin, or on clothing. Do not breathe vapor or spray. Keep container closed. Use only with adequate ventilation. Wash thoroughly after handling.

**Storage:** Keep out of reach of children. Keep container tightly closed. Do not store above the following temperature: 40°C

### 8. Exposure controls/personal protection

**Product AT USE DILUTION**

Good general ventilation should be sufficient to control worker exposure to airborne contaminants.

No protective equipment is needed under normal use conditions.

No special protection is required. **Engineering measures:** Use only with adequate ventilation. If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. Provide suitable facilities for quick drenching or flushing of the eyes and body in case of contact or splash hazard.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Exposure limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>SODIUM HYDROXIDE</td>
<td>ACGIH TLV (United States, 1/2008).</td>
</tr>
<tr>
<td></td>
<td>CEIL: 2 mg/m3</td>
</tr>
<tr>
<td></td>
<td>OSHA PEL (United States, 11/2006).</td>
</tr>
<tr>
<td></td>
<td>TWA: 2 mg/m3 8 hour(s).</td>
</tr>
<tr>
<td>sodium hypochlorite</td>
<td>AIHA WEEL (United States, 1/2008).</td>
</tr>
<tr>
<td></td>
<td>STEL: 2 mg/m3 15 minute(s).</td>
</tr>
<tr>
<td>Chlorine</td>
<td>ACGIH TLV (United States, 1/2008).</td>
</tr>
<tr>
<td></td>
<td>STEL: 2.9 mg/m3 15 minute(s). STEL: 1 ppm 15 minute(s). TWA: 1.5 mg/m3 8 hour(s).</td>
</tr>
<tr>
<td></td>
<td>OSHA PEL (United States, 11/2006).</td>
</tr>
<tr>
<td></td>
<td>CEIL: 3 mg/m3 CEIL: 1 ppm</td>
</tr>
</tbody>
</table>

### 9. Physical and chemical properties

**Personal protection**

**Eyes:** Use chemical splash goggles. For continued or severe exposure wear a face shield over the goggles.

**Hands:** Use chemical-resistant, impervious gloves.

**Skin:** Use synthetic apron, other protective equipment as necessary to prevent skin contact.
**Respiratory:** Wear appropriate respirator when ventilation is inadequate and occupational exposure limits are exceeded.

**Physical state:** Liquid.

**Flash point:** > 100°C

**Color:** Yellow [Light]

**Odor:** chlorine

**pH:** 12.5 to 13.5 [Conc. (% w/w): 100%]

**Relative density:** 1.237 to 1.25

**Product AS SOLD**

**Product AT USE DILUTION**

Liquid. > 100°C Pale color [Light] Faint odor 11.7 to 12.7

---

**9. Physical and chemical properties**

**10. Stability and reactivity**

**Product AS SOLD**

**Stability:** The product is stable. Under normal conditions of storage and use, hazardous polymerization will not occur.

**Reactivity:** Extremely reactive or incompatible with the following materials: acids. Reactive or incompatible with the following materials: moisture. Slightly reactive or incompatible with the following materials: metals. Mixing this product with acid or ammonia releases chlorine gas.

**Hazardous decomposition products:** Under normal conditions of storage and use, hazardous decomposition products should not be produced.

**Hazardous polymerization:** Under normal conditions of storage and use, hazardous polymerization will not occur.

**11. Toxicological information**

**Product AT USE DILUTION**

No known significant effects or critical hazards.

**Product AS SOLD**

No known significant effects or critical hazards.

**Product AS**

No known significant effects or critical hazards.

**Potential acute health effects:**

**Eyes:** corrosive to the eyes.

**Skin:** corrosive to the skin.

**Inhalation:** corrosive to the respiratory system.

**Ingestion:** Causes burns to mouth, throat and stomach.

**Product AS SOLD**

**Other adverse effects**

**Target organs:** Contains material which may cause damage to the following organs: upper respiratory tract.

---

**12. Ecological information**
Ecotoxicity: Not reported

13. Disposal considerations
Product AT USE DILUTION
Diluted product can be flushed to sanitary sewer. Discard empty container in trash.

Waste disposal: Avoid disposal. Attempt to use product completely in accordance with intended use. Disposal should be in accordance with applicable regional, national and local laws and regulations.

RCRA classification: Unused product is D002 (Corrosive)

Product AS SOLD

14. Transport information
Certain shipping modes or package sizes may have exceptions from the transport regulations. The classification provided may not reflect those exceptions and may not apply to all shipping modes or package sizes.

Product AS SOLD
DOT Classification: UN1824
DOT Proper shipping name: Sodium hydroxide solution
Class: 8
Packing group: II
For transport in bulk or using IMDG regulations, see shipping documents for specific transportation information.

4/6

PRINCIPAL

14. Transport information
Product AT USE DILUTION
Not intended for transport.

15. Regulatory information
Product AS SOLD
HCS: Corrosive material
Classification Target organ effects

Product AS SOLD
U.S. Federal regulations
12 December 2011
Product AT USE DILUTION
Not regulated.
TSCA 8(b) inventory: All components are listed or exempted.
SARA 302/304/311/312 extremely hazardous substances: No listed substance
SARA 302/304 emergency planning and notification: No listed substance
SARA 313
Form R - Reporting requirements
California Prop. 65
Product name
No listed substance

CAS number    Concentration

16. Other information

Product AS SOLD

Hazardous Material   : Information System (U.S.A.)

*3  0

Product AT USE DILUTION

0

0 0

0

National Fire Protection Association (U.S.A.)

Health    3

Date of issue Responsible name

0

Flammability Instability/Reactivity

Special

:    12-December-2011:    Regulatory Affairs 1-800-352-5326

0

Flammability 0 Instability/Reactivity

Special

0

Health  0

Indicates information that has changed from previously issued version.

Notice to reader

5/6

PRINCIPAL    12 December 2011

16. Other information

The above information is believed to be correct with respect to the formula used to manufacture the product in the country of origin. As data, standards, and regulations change, and conditions of use and handling are beyond our control, NO WARRANTY, EXPRESS OR IMPLIED, IS MADE AS TO THE COMPLETENESS OR CONTINUING ACCURACY OF THIS INFORMATION.
Appendix D  
Material Safety Data Sheet Chlorine

SECTION I - COMPANY AND PRODUCT INFORMATION

Item No.: .................. 331
EPA Reg. No.: ............... 7616-45
Manufacturer's Name: .......... Chem Lab Products, Inc.
Emergency Telephone No.: .... (909) 390-9912 ......................... (800)4249300(CHEMTREC)
Street Address: ............. 5160 E. Airport Drive
City/State/Zip code: ........... Ontario, CA 91761
Chemical Name and Synonyms: Sodium Hypochlorite, Bleach
Trade Name and Synonyms: KemKlor II Chlorinating Liquid
Chemical Family: ............. Alkali Hypochlorite
Formula: .................. NaOCl

NFPA Rating: Health: 2 Flammability: 0 Reactivity: 1 Special:
DOT Proper Shipping Name:..... Consumer commodity
DOT ID Number: ................ N/A
DOT Hazard Class: ............. ORM-D
Package Group: ............... III

SECTION II - HAZARDOUS INGREDIENTS

SODIUM HYPOCHLORITE (CAS No. 7681-52-9;UN1791) ........ 10%
SODIUM HYDROXIDE (CAS No. 1310-73-2;UN1823) ............ 0.8%

SECTION III - PHYSICAL DATA

FREEZING POINT ............. 0°F
BOILING POINT .............. 224°F
SPECIFIC GRAVITY ......... 1.160-1.180
pH ................................ 12
SOLUBILITY IN WATER........ N/A
VAPOR PRESSURE (cm Hg.).. Variable
% VOLATILE BY VOLUME .... Variable
MOLECULAR WEIGHT ......... 74.45

ANALYSIS

EVAPORATION RATE (____=1)...... N/A
VAPOR DENSITY (Air = 1)...... N/A
ODOR.................. Chlorine odor

SECTION IV - FIRE AND EXPLOSION HAZARD DATA

FLASH POINT .................. Non-flammable
FLAMMABLE LIMITS .......... N/A
EXTINGUISHING MEDIA ....... Flood with water or Carbon Dioxide (CO2).
SPECIAL FIRE FIGHTING PROCEDURES: Must wear NIOSH certified gas mask with
analyzer for chlorine. UNUSUAL FIRE AND EXPLOSION HAZARDS: Material is strong
oxidizer; contact with combustibles may initiate or promote combustion. Acid and heat
accelerate decomposition. Decomposition Products may include chlorine.

SECTION V - HEALTH HAZARD DATA

THRESHOLD LIMIT VALUE: ......... N/A
EFFECTS OF OVEREXPOSURE:
INGESTION - Causes irritation of the mouth, throat, and stomach.
SKIN - severe irritation.
INHALATION - severe irritation. EYE - severe irritation.
EMERGENCY AND FIRST AID PROCEDURES:
IF SWALLOWED: Drink large quantities of milk or gelatin solution, or if these are not available, drink large quantities of water. Do not give vinegar or other acids. Do not induce vomiting. Get prompt medical attention. IF IN EYES: Flush with a steady, gentle stream of water for 15 minutes. Get medical attention. IF ON SKIN: Wash with plenty of soap and water. IF INHALED: Remove victim to fresh air. Call physician if exposure is severe.

SECTION VI - REACTIVITY DATA
STABILITY: This Product is stable
CONDITIONS TO AVOID:
Light, heat, fire, low pH, metallic impurities, naturally decomposes with age.
INCOMPATIBILITY: (Materials to avoid)
Acid, ammonia, amines, cyanides, chlorinated isocyanurates, combustibles, detergents, reducing agents, oxidizable materials.
HAZARDOUS DECOMPOSITION PRODUCTS: Contact with acid will releases chlorine gas; natural decomposition Products is oxygen. Thermal decomposition or burning may produce HCl.
HAZARDOUS POLYMERIZATION: ............. WILL NOT OCCUR
CONDITIONS TO AVOID ................. None Known

SECTION VII - SPILL OR LEAK PROCEDURES
STEPS TO BE TAKEN IN CASE OF MATERIAL RELEASED OR SPILLED:
Material is toxic to fish. Contain in diked area. Neutralize with sodium sulfite, sodium thiosulfate, or ferrous salt solutions. Dilute copiously with water.
WASTE DISPOSAL METHOD: This material is toxic to fish. Do not contaminate water by cleaning equipment or disposal of wastes. Untreated effluent should not be discharged where it will drain into lakes, streams, or ponds. Dispose of waste in facility permitted for non-hazardous waste.

SECTION VIII - SPECIAL PROTECTION INFORMATION
RESPIRATORY PROTECTION: (Specify type)
Use MSHA-NIOSH approved respirator for dusts, mists, and fumes with TLV not less than .05 mg/m³
VENTILATION:
LOCAL EXHAUST: Desirable.
PROTECTIVE GLOVES: Rubber Gloves
EYE PROTECTION: Use chemical goggles
OTHER PROTECTIVE EQUIPMENT: Rubber splash apron. Safety shower and eyewash should be located nearby.

SECTION IX - SPECIAL PRECAUTIONS
PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING:
Store upright in cool, day area away from direct sunlight and excessive heat. Do not store near other incompatible household Products. Do not store next to Acid. OTHER PRECAUTIONS:
Rinse empty container thoroughly with plenty of water. Do not contaminate food, feed, or water by store, disposal, or cleaning of equipment. Wash contaminated clothing before reuse. Discard contaminated shoes.
This data is offered in good faith as typical values and not as a product specification. No warranty, either expressed or implied, is hereby made. The recommended industrial hygiene and safe handling procedures are believed to be generally applicable. However, each user should review these recommendations in the specific context of the intended use and determine whether they are appropriate.

Revised: January 5, 2006
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