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VIA HAND DELIVERY

Dockets Management Branch (HFA-305)
Food and Drug Administration
12420 Parklawn Drive, Room 123
Rockville, MD 20857

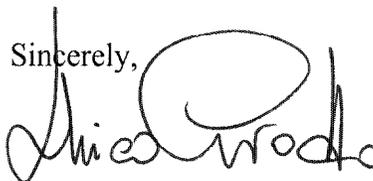
RE: Amuchina International Inc.'s Citizen's Petition to Amend the Tentative Final Monograph for OTC Antimicrobial Drug Products (Docket #75N)

Dear Sir or Madam:

Enclosed please find an original and three copies of Amuchina International Inc.'s Citizens Petition to amend the Tentative Final Monograph for OTC Health-Care Antiseptic Products with supporting documents. This submission is made pursuant to the Federal Food, Drug and Cosmetic Act and 21 C.F.R. §§ 10.30, 10.31, 330 and 333 to request that the Commissioner include the active ingredient, sodium hypochlorite 0.05 to 0.5 percent, in the monograph for OTC.

Note that Amuchina International, Inc. has changed the corporate name to Alcavis International, Inc., but we have chosen to leave the Amuchina name in the petition because many tests have been done with this name.

Any correspondence regarding this petition should be directed to Ludovico Giavotto.

Sincerely,


Ludovico Giavotto
President
Amuchina International Inc.

Enclosure

75N-183H

CP13



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**CITIZEN'S PETITION TO AMEND THE TENTATIVE FINAL MONOGRAPH FOR
OTC HEALTH-CARE ANTISEPTIC PRODUCTS**

Amuchina International Inc. respectfully submits this petition, pursuant to the Federal Food Drug and Cosmetic Act ("FFDCA"), and 21 C.F.R. §§ 10.30, 10.31, 330 and 333 to request the Commissioner to amend the Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule (1) to include the active ingredient sodium hypochlorite 0.05 to 0.5 percent. To that end, Amuchina requests that the Commissioner reopen the administrative record for this TFM, and consider the marketing, safety and effectiveness information for sodium hypochlorite enclosed in this petition. Amuchina has also submitted on January 27, 1999, a petition to reopen the administrative record of the OTC First Aid Antiseptic Drug Product, (Docket #75N 183F). Reference will be made to this document in this petition.

I. ACTION REQUESTED

Amuchina requests that the administrative record for the TFM for Health-Care Antiseptic Drug Products be reopened for receipt of information on an antiseptic ingredient not previously reviewed, that is, sodium hypochlorite. Based upon this information, Amuchina has requested that sodium hypochlorite be added to the First Aid Antiseptic TFM as an active ingredient and is now requesting that the information in that petition, as well as additional material, added here be considered for the TFM for Health-Care Antiseptic Drug Products. Amuchina requests that, based upon the information and data in this petition, the proposed 21 C.F.R § 333 and 369 be amended as follows:

- A. 21 C.F.R. § 333.412 be amended to add a new clause “(s)”:

“Sodium hypochlorite 0.10 to 0.5 percent.”

- B. 21 C.F.R. § 333.460 be changed to include labeling of products containing sodium hypochlorite, 0.10 to 0.50 percent as a preparation for access site preparation for procedures that will conform to labeling requirements under § 333.460 (1) (a), (b), (c), (e), and (i) and (ii)

II. STATEMENT OF GROUNDS

Sodium hypochlorite, a topical antimicrobial with a long history of safety and effectiveness, has not been considered under rulemaking for the TFM for Health-Care Antiseptic Drug Products. Inclusion of this ingredient would be in the public interest, since sodium hypochlorite offers a unique combination of a non-sensitizing topical antimicrobial with significant antimicrobial activity against a broad spectrum of microorganisms and also with low toxicity. Further, many of the active ingredients listed under this TFM are not used to a

significant degree for skin and access site preparation, and the most widely used antimicrobial, povidone-iodine, is a known skin sensitizer. Sodium hypochlorite offers an effective alternative that is needed in the marketplace. Effective non-sensitizing alternatives to povidone iodine are few. Further, the topical effectiveness of sodium hypochlorite has been substantiated in the submission of a Petition to the OTC First Aid Antiseptic Drug Products TFM submitted by Amuchina. Therefore, it would be in public interest to reopen the administrative record, and review the information submitted for this safe and effective ingredient. In this petition, we provide information on sodium hypochlorite's chemical activity, history of use, and demonstrated safety and effectiveness concerning its use as a topical skin and access site preparation product..

A. Background

Sodium hypochlorite, NaOCl, has long been recognized for its effectiveness as an antiseptic and a disinfectant. In his historic pioneering work, in which he settled in a convincing clinical trial, the importance of hand disinfection, Semmelweis used sodium hypochlorite as a hand-wash and disinfectant, to reduce mortality from childbed fever (2). In another historic discovery, Carrel and Dakin introduced 0.45 to 0.5 percent buffered sodium hypochlorite solution for the treatment of traumatic wounds during the First World War (3,4,5). This solution, known as "Dakin's solution," revolutionized the treatment of traumatic wounds, and was used extensively during the War and after, and until the widespread introduction of antibiotics (6). Despite the advent of antibiotics, Dakin's and other hypochlorite solutions have continued to be used for wound treatment (7,8). Sodium hypochlorite is also widely used for other antiseptic and

disinfectant purposes, which include uses as an antiseptic for burn treatment (9); as an antiseptic for pressure sores and deep ulcers (10); as an endodontic rinse (11); and as a disinfectant for dialysis equipment (12). Numerous non-medical uses of sodium hypochlorite include almost universal use as a water treatment chemical (13,14,15), a bleaching agent, a sanitizer for food processing equipment (16), and as a produce wash for fresh fruits and vegetables (17).

The antimicrobial activity of sodium hypochlorite is attributed to the presence of the chlorine. Chlorine in its native form as a gas is not usable as an antiseptic therefore a chlorine-releasing compound such as sodium hypochlorite is used instead. In solution, sodium hypochlorite dissociates to sodium hydroxide (NaOH) and hypochlorous acid (HOCl).



Hypochlorous acid further dissociates to hydrogen ions (H^+) and hypochlorite ions (OCl^-).



It is common industry practice to use the word “chlorine” or “available chlorine” to signify active chlorine compounds. The actual accepted meaning of the term is as, “aqueous solution of active chlorine compounds consisting of a mixture of OCl^- , Cl_2 , and HOCl .” The activity of hypochlorite-containing compounds is also often expressed as “available chlorine,” which is often defined in terms of the equivalent amount of elemental chlorine in the compound. However, the effectiveness of sodium hypochlorite solutions depends on more than the total amount of “available chlorine.” Effectiveness varies among solutions that contain the same percentage of sodium hypochlorite depending on the equilibrium between hypochlorous acid and

hypochlorite ions. Solutions that have an increased concentration of undissociated hypochlorous acid are more effective. The most effective method of increasing the relative concentration of undissociated hypochlorous acid is to decrease the pH.

While the mechanism for the increased antimicrobial activity of sodium hypochlorite has not been determined, Piacenza hypothesized that undissociated hypochlorous acid is more effective as an antimicrobial agent because it lacks an ionic charge, and is similar in size and structure to water molecules (18). Because of this similarity, it is able to penetrate into bacterial cells with comparative ease. In contrast, hypochlorite ions are negatively charged, and do not penetrate bacterial cell walls as easily. Hypochlorous acid can penetrate the cell wall and deliver chlorine to the interior of the bacterial cell, and this is thought to help explain the antimicrobial activity of sodium hypochlorite.

Sodium hypochlorite has antimicrobial activity against a broad spectrum of microorganisms, including gram-positive and gram-negative bacteria, fungi, and viruses. A common rationale for its antimicrobial activity is through the inhibition of essential enzymatic systems as a consequence of oxidation of the $-SH$ groups (18).

Common commercial sodium hypochlorite solutions used as disinfectants and bleaches are manufactured and marketed at a pH between 11.5 and 12 to ensure stability. Higher pH values can increase irritation to the skin, and these solutions are not appropriate for topical application. Sodium hypochlorite solutions for antiseptic use are usually formulated at a lower pH. Amuchina has determined that the optimal balance between stability and antiseptic effectiveness is achieved at a pH between 10.0 and 10.7 for its undiluted 1.1 percent sodium hypochlorite solution (the pH is slightly lower after dilution to 0.11 and .055 percent).

B. Sodium Hypochlorite is not a New Drug

Section 201(p) of the FDCA defines a “new drug” as one that is either not generally recognized as safe and effective for its labeled uses, or is generally recognized as safe and effective but has not been used for a material time or to a material extent. The courts have therefore interpreted section 201(p) to mean that to be an “old drug,” and to avoid the preapproval requirements of a “new drug,” the proponent of the drug must establish; that the drug product is generally recognized as safe and effective by qualified experts under the conditions of its use; and that the drug has “been used to a material extent “ and “for a material time” under such conditions (19,20). This section will address the second part of the “old drug” test, that sodium hypochlorite has been used for a material time and to a material extent. General recognition of safety and effectiveness will be discussed later in this petition.

C. Sodium Hypochlorite has Been Used for a Material Time and to a Material Extent

The criteria that a drug be used for a material time and material extent was likely added to the statute to ensure that drugs not subject to preapproval had a history of safe use. If a drug had been used for a material time and extent, there would be ample opportunity for potential safety issues to surface. The substantial lack of adverse effects for a drug product with sodium hypochlorite’s extensive history of use demonstrates the safety of this substance. In order to qualify as an “old drug,” an ingredient’s history of use should be for conditions similar to the product’s current intended use. Sodium hypochlorite would be used as an antiseptic for minor cuts, scrapes, and burns, and the most relevant historical use of the substance has been for treatment of wounds and burns. While there is considerable information regarding the use of

sodium hypochlorite for these purposes, Amuchina believes that the history of use for other purposes is relevant to the demonstration of the safety of sodium hypochlorite.

The history of the use of hypochlorite as a disinfectant and antiseptic goes back hundreds of years. It was used for the treatment of wounds and burns even before the revolutionary work of Lister and Koch. Among early uses, the Marquis de la Motte used a hypochlorite solution for the treatment of gangrene in 1732 (2); and Paris surgeons used it for the treatment of burns, operative wounds, and ulcers (2). As noted in the background section, Semmelweis used hypochlorite as an antiseptic hand wash to reduce the very high incidence of puerperal fever (childbed fever) during childbirth in a Vienna hospital. He ensured that his hands, and the hands of his assistants, were washed in a hypochlorite solution. He also insisted that a hypochlorite solution be used on any instruments likely to come in contact with the vaginal canal. While Semmelweis' technique resulted in a drastic decrease in the death rate from puerperal fever, his contemporaries largely ignored his work (2,5). Koch reported the antiseptic properties of hypochlorites in 1880 (7); however, the widespread acceptance of hypochlorite, and recognition of its activity in wounds, would await the work of Carrel and Dakin. The conditions of trench warfare during the First World War resulted in large numbers of casualties with wounds contaminated by soil and human and animal excrement. These conditions led to a high incidence of wound infection and gangrene (3). Existing antimicrobial compounds such as phenol, mercuric chloride and tincture of iodine proved to be unsuitable for antiseptic treatment of large traumatic wounds. These compounds could not be used in the volume necessary to debride and disinfect the wounds without producing toxic or highly irritating effects (4). To combat the high mortality that resulted from the wound infections of war, Nobel Laureate, Dr. Alexis Carrel enlisted the aid of a noted chemist, Henry Dakin, to formulate a nonirritating solution that had

significant antiseptic effect (5). Dakin examined over 200 substances in his search for a solution that met Carrel's requirements (21). Among the substances examined were ingredients that FDA now considers to be category I under the First Aid Antiseptics TFM: phenol, hydrogen peroxide and tincture of iodine. Dakin rejected these substances as either too toxic or irritating (phenol and iodine) or because of insufficient antimicrobial activity (hydrogen peroxide) (4). Dakin determined that sodium hypochlorite at concentrations of 0.45 to 0.5 percent, in a buffered solution, had the best combination of nonirritating properties and antimicrobial effectiveness.

Carrel used Dakin's solution in a specific treatment regimen that involved, among other things, irrigating debridement and use of large volumes of their hypochlorite solution on the wounds (22). As a result of this "Carrel/Dakin" method, Carrel's hospital in France had the lowest infection and amputation rate of any hospital in France during World War I; however, politics and personal rivalries prevented the method from being widely adopted by French surgeons during the war. Advocates of the Carrel/Dakin method claimed that it would have saved at least 150,000 allied soldiers had it been made the standard treatment for traumatic wounds (3). The method was demonstrated in the United States in 1917 at the New Rockefeller Demonstration Hospital for War Training located on the grounds of Rockefeller Institute in New York (5,22). The method was well received in the United States, and was used in civilian as well as military surgical practice (3,5).

Dakin's solution was used again during the Second World War, when the Dakin/Carrel treatment method was employed for the treatment of both wounds and burns (6,22). With the advent of antibiotics, and the belief that wound infection would soon be a thing of the past, the use of Dakin's solution declined; however, the medical community later realized that antibiotics

had limitations (23). Antibiotics often did not reach bacteria in deep wounds or necrotic tissue, and often had activity against only a limited spectrum of organisms. Additionally, with widespread use of antibiotics, many resistant strains of bacteria began to appear. Because of the limitations of antibiotics, today, topical antiseptics have again increased in use (24). Recently, McDonnell noted that “[t]here now appears to be yet another resurgence in the clinical use of Dakin’s solution” (23). Recent antiseptic uses of sodium hypochlorite cited in published literature include use for burns, wounds, pressure sores and deep ulcers.

In an article that discusses the safety of 0.1 to 0.5 percent sodium hypochlorite for the treatment of burns, Cotter notes the use of 0.05 to 0.2 percent sodium hypochlorite during the Second World War, and the recent usage of 0.08 percent buffered sodium hypochlorite (25). An article by Wright, and a letter by Thomas both note recent use of sodium hypochlorite as a burn antiseptic (9,26). Bloomfield discussed the use of 0.125 and 0.25 percent sodium hypochlorite by hospitals for wounds, pressure sores and ulcers (27). Articles by Lineaweaver and Kozol, and letters by Raffensperger and Barese also note recent use of sodium hypochlorite for wound treatment (8,28,29,30). An article by Slahetka describes the use of 0.45 to 0.5 percent sodium hypochlorite solution for the treatment of deep ulcers in geriatric patients (10).

Evidence of sodium hypochlorite’s long use is also recorded in several standard pharmaceutical references. The following sample of these references is not exhaustive. The Merck Index, 7th Edition (1960) and 11th Edition (1989) contain entries for “Sodium Hypochlorite Solution, Diluted and Modified Dakin’s Solution” (31,32). These entries describe a 0.45 to 0.5 percent solution of sodium hypochlorite used as an antiseptic for wound irrigation. The American Pharmaceutical Association’s (“APhA”) Handbook of Nonprescription Drugs, 5th

Edition (1977), 6th Edition (1979) and 7th Edition (1982) all list 0.5 percent sodium hypochlorite solution as a topical antiseptic ingredient (33,34,35). Remington's Practice of Pharmacy, 8th Edition (1936) and 9th Edition (1961) both describe the method of preparation for 0.45 to 0.5 percent buffered sodium hypochlorite solution (36,37). Remington's refers to this solution as "Modified Dakin's Solution," and describes it as "an inexpensive but efficient germicide for the treatment of wounds." The United States Pharmacopoeia ("USP") 10th Decennial Revision (1926) includes a monograph for "Surgical Solution of Chlorinated Soda;" and the USP 11th Decennial Revision (1936) includes a monograph for "Diluted Solution of Sodium Hypochlorite" (38,39). Both of these monographs give the alternate name "Modified Dakin's Solution," and describe a solution of 0.45 to 0.5 percent sodium hypochlorite.

Because of the relative ease of formulation, Dakin's and other medical hypochlorite solutions are often formulated by hospital pharmacies rather than purchased in finished form; however, OTC marketing of these solutions has existed since at least 1934. The 1934 Modern Drug Encyclopedia contains a listing for "Hyclorite," a 4 percent solution of sodium hypochlorite sold by Bethlehem Laboratories (40). This solution was sold as an antiseptic, deodorant, and disinfectant, for use in a variety of situations including chronic ulcers, empyema, abdominal infections, and burns. Hyclorite was a concentrate that was to be diluted prior to use, with final sodium hypochlorite concentrations from 0.00025 to 0.5 percent. The recommended topical concentration was 0.225 to 0.5 percent sodium hypochlorite. Listings for Hyclorite are also found in the 1941 Modern Drug Encyclopedia, the 1946 Blue Book, the 1945-1946 Red Book, and the 1947 Physicians Desk Reference (41,42,43,44).

Sodium hypochlorite solutions remain on the OTC market. Century Pharmaceutical's one half-strength and full-strength Dakin's solutions are listed in the Red Book as far back as 1992 (45). Century's Dakin's solutions are also listed in APhA's Handbook of Nonprescription Drugs, 10th Edition (1993) and Nonprescription Products: Formulations & Features 97-98, as first aid antiseptics (46,47).

Amuchina currently markets sodium hypochlorite solutions in a number of concentrations, for a variety of antiseptic and disinfectant purposes. These solutions have been sold in a number of countries, including the United States, where introduction took place in 1986.

As noted previously, sodium hypochlorite has been used for a variety of medical uses. It is one of the most widely used of all endodontic irrigating solutions. Concentrations of 2 to 5.25 percent have been recommended for this purpose (11). In his study of 5 solutions, Berutti noted that "[a]lthough numerous endodontic irrigant solutions have been proposed, sodium hypochlorite has been shown to be the most effective" (48).

Sodium hypochlorite solutions in general, and those produced by Amuchina in particular, have been used effectively as patient preoperative disinfectants. Rovenda noted this use in an article that examined the effectiveness of Amuchina's 0.11 percent sodium hypochlorite solution (49).

Amuchina's sodium hypochlorite solution is commonly used as a disinfectant for dialysis equipment in the United States and other countries. In the United States, it is regulated for this purpose through medical device pre-market notifications, or 510(k)s (50,51)

In addition to many medical uses, sodium hypochlorite has also been widely used for a number of non-medical uses, notably for water purification. Since its introduction, chlorination has become one of the most widely used, and effective methods for providing safe drinking water to the world's population. (13,14)

The long historical use of sodium hypochlorite for wounds, burns and other medical indications, as well as the recent and current use of the ingredient as a topical antiseptic, demonstrate that sodium hypochlorite has been used for a material time and a material extent. Further assurance of the ingredient's safety is provided by its long history of use for a variety of other medical and non-medical purposes. Therefore, sodium hypochlorite should be considered to have been used for a material time, and to a material extent, and included in the review of ingredients for use as a first aid antiseptic and as a health-care antiseptic.

D. Safety

1. Toxicology

The toxicity and carcinogenicity of sodium hypochlorite has been examined extensively. Both published and unpublished studies have repeatedly demonstrated the ingredient's safety, and failed to raise any significant questions regarding acute toxicity or carcinogenicity. In this section Amuchina presents a number of studies that examine sodium hypochlorite's carcinogenic and mutagenic potential by both in vitro and in vivo methods; its toxicity after several methods of exposure; and the development of blood levels of sodium hypochlorite after topical application.

a. Carcinogenicity/Mutagenicity Studies

One of the most common worldwide uses of chlorine and chlorine containing compounds (including sodium hypochlorite) is as a disinfectant for drinking water. This use has led a number of investigators to examine the carcinogenicity of chlorinated water.

Hasegawa examined the effects on rats of a 104-week administration of sodium hypochlorite in drinking water at levels of 0.05 to 0.2 percent (52). No significant increase in incidence of any tumors was observed, leading the investigators to conclude that sodium hypochlorite showed no carcinogenic potential.

Kurokawa examined the carcinogenicity of long term exposure to sodium hypochlorite in both rats and mice (53). The rats were given a 104-week administration of sodium hypochlorite in drinking water at levels of 0.05 to 0.2 percent, whereas the mice underwent a 103-week administration of sodium hypochlorite in drinking water at levels of 0.05 to 0.1 percent. The authors also concluded that sodium hypochlorite was not carcinogenic in rats or mice.

Robinson examined hyperplasia in mouse skin after dermal exposure to sodium hypochlorite, hypochlorous acid, and to the hypochlorite ion (54). The stated goal of this study was to examine the potential for these compounds to promote skin cancer. The study consisted of exposing the skin of SENCAR mice to concentrations of 0.001 to 0.1 percent of various chlorine compounds. All of these compounds resulted in some degree of hyperplasia. The author's decision to use the ability of sodium hypochlorite, and its derivatives, to induce hyperplasia (thickening of the skin) as an indicator of the substance's tumor promoting capacity was based upon the "excellent correlation" between the hyperplasiogenic activity and tumor

promotion among phorbol esters. However, the author admits that this correlation does not hold true for compounds of chemical classes other than phorbol esters. Given that the correlation between skin hyperplasia and tumor promotion appears to be limited to phorbol esters, and that sodium hypochlorite is unrelated to this compound, one questions the selection of this assay as a valid indicator of sodium hypochlorite's possible carcinogenicity. One cannot conclude any positive information concerning carcinogenicity with an assay of this dubious nature.

In another study using an assay of dubious relevance, Meier examined the effect of interperitoneally administered sodium hypochlorite and its derivatives on the production of sperm-head abnormalities in mice (15). In this study, three different *in vivo* tests designed to look for chromosomal damage were used to examine various disinfectant chemicals. In one of these tests, the sperm-head abnormality assay, hypochlorite ions showed a weak positive response. According to the author, this indicated that the substance could be mutagenic. It is worth noting that this weak positive response for hypochlorite ions was detected in only one of three sample groups, and was not found for other sodium hypochlorite derivatives. Further, three different assays for mutagenic substances were used in the study, and only the sperm-head abnormality assay showed any indication of mutagenic activity. Additionally, the author admits that the meaning of a positive result in this assay was the subject of scientific debate. Therefore, a weak positive result to interperitoneally administered hypochlorite ions, in an unproven assay for mutagenic activity, has little bearing on the safety of sodium hypochlorite for topical use.

Amuchina commissioned two *in vitro* studies to examine the possible mutagenic potential of its "Amuchina - Electrolytic Chloroxidizer," a sodium hypochlorite disinfectant/antiseptic.

One study by Pirvano was conducted using one of the most widely used of all tests for mutagenesis, the Ames assay (55). This assay measures the influence of a chemical compound on the spontaneous mutation rate of Salmonella typhimurium. In this test, mutagenic compounds showed an increase in the rate of mutations over the background or control rate. Amuchina's sodium hypochlorite solution did not increase the mutation rate, and therefore, it can be concluded that it does not have mutagenic potential in tests that are often difficult when the chemical is an antimicrobial.

Pirvano also conducted a second study (56). This study was similar to the first, but used the yeast, Saccharomyces cerevisiae, in place of S. typhimurium as an indicator organism. Again, Amuchina's solution did not show an increase in the mutation rate, and did not show mutagenic potential.

In sum, many studies with sodium hypochlorite have not found any demonstrated mutagenic or carcinogenic potential. Two long-term feeding studies in rats and one in mice showed no increase in the rate of tumor formation. In two standard in vitro assays, sodium hypochlorite did not show any mutagenic potential.

b. Acute Toxicity Studies

Amuchina has commissioned a number of studies to examine the acute toxicity of sodium hypochlorite solutions by various routes of administration, including oral, intravenous, intraperitoneal, and topical dermal application. These studies performed by Gnemi at the Istituto di Ricerche Biomediche, in Italy were conducted on Amuchina's 1.1 percent sodium hypochlorite solution.

A single dose oral toxicity study in rats was conducted by administering various amounts of 1.1 percent sodium hypochlorite solution resulting in dosages of 256 to 400 mg/Kg (57). The LD₅₀ was calculated to be 290 mg/Kg, with 95 percent confidence limits of 267 and 315 mg/Kg.

A single dose intravenous toxicity study in rats was conducted by injecting various amounts of 1.1 percent sodium hypochlorite solution resulting in dosages of 26.2 to 124.8 mg/Kg (58). The LD₅₀ for intravenous administration was calculated to be 33.3 mg/Kg, with 95 percent confidence limits of 28.2 and 39.3 mg/Kg.

A single dose intraperitoneal toxicity study in rats was conducted by intraperitoneal administration of various amounts of 1.1 percent sodium hypochlorite solution resulting in dosages of 26.2 to 150 mg/Kg (59). The LD₅₀ for intraperitoneal administration was calculated to be 87.7 mg/Kg, with 95 percent confidence limits of 79 and 97.5 mg/Kg.

A single dose acute dermal toxicity study in rats was conducted by exposing shaved skin to 1.1 percent sodium hypochlorite solution in a dose of 50 mg/Kg (60). The test was performed by taping gauze patches to the test site, applying the solution to the patches, and leaving the patches in place for 24 hours. After 14 days of observation, none of the animals showed any signs of systemic toxic effect or local irritation. The author concluded that the LD₅₀ for dermal application is higher than 50 mg/Kg.

Clementi examined acute dermal toxicity using rabbits instead of rats (61). This four-week study examined the effects of 5 and 10 percent concentrations of Amuchina's Electrolytic Chloroxidizer (0.055 and 0.11 percent sodium hypochlorite) on intact and abraded skin. After

daily administration of the test compounds for the four-week study period, no systemic toxic effect or local irritation were observed.

These various studies indicate that sodium hypochlorite is essentially non-toxic, especially at the proposed use concentration, and for the proposed indications. For example, the oral LD₅₀ for sodium hypochlorite is 290 mg/Kg. If this amount were to be extrapolated to a human dose, it would require that a 100 Kg man consume approximately six liters of a 0.5 percent solution, or approximately 50 liters of Amuchina's 0.057 percent solution. Also note that the acute dermal studies demonstrated no toxic effects after the topical application of sodium hypochlorite.

c. Blood Levels

The 1978 Tentative Final Monograph for OTC Topical Antimicrobial Products stated that where appropriate, safety information should include studies to show expected blood levels after the use of an OTC product (62). Sodium hypochlorite breaks down almost instantaneously on contact with blood and blood components, and therefore would not be expected to be found in the blood stream. Therefore, information on blood levels of sodium hypochlorite is not technically feasible. Carrel and Keen both recognized early that sodium hypochlorite solution lost its antimicrobial activity soon after contact with the open wound (21,22). More recently, Cotter noted that "because NaOCl reacts with protein and other cellular debris, its antimicrobial activity is depleted during treatment" (25).

Zanolo examined this disappearance of activity by measuring the levels of the available chlorine in sodium hypochlorite solutions exposed to high concentrations of human and dog

blood (63). To measure this disappearance, 8 ml of either human or dog blood was added to 2 ml of 1.1 percent sodium hypochlorite, and the combination was mixed for five seconds. After five seconds, the reaction was stopped with a neutralizer, and the remaining chlorine assayed. The decomposition of the sodium hypochlorite after exposure to the blood was so rapid, that no available chlorine could be measured after the five seconds of mixing.

General knowledge of sodium hypochlorite's rapid decomposition after it contacts wounds, as well as the results of the Zanolo's study, indicates that no sodium hypochlorite would be found in the blood stream as a result of topical application of sodium hypochlorite solution. Therefore, measuring blood levels would not be appropriate for this compound.

d. Toxicology Summary

In all these studies, no adverse toxicological effects were found with sodium hypochlorite. Therefore, based on these studies and on a lack of negative toxicological information during the long history of use of sodium hypochlorite, it should be considered non-carcinogenic, non-mutagenic, and essentially non-toxic for the proposed use, at the proposed concentrations.

2. Topical Safety

a. Wound Healing and Irritation

Long history of use, and published and unpublished studies, have established that buffered sodium hypochlorite solutions do not inhibit wound healing, and are essentially non-irritating. Studies have also shown that any irritating properties that sodium hypochlorite

solutions may have are less pronounced than those exhibited by other ingredients, such as phenol and povidone-iodine, classified as category I under the First Aid Antiseptic TFM and in the Health-Care Antiseptic Products TFM and in the 1978 TFM for OTC Topical Antimicrobial Products.

As noted previously, while examining the acute dermal toxicity of sodium hypochlorite solution, both Gnemi and Clementi also looked for signs of skin irritation (60,61). Gnemi found no evidence of dermal reaction on intact rat skin after a 24-hour exposure to 1.1 percent sodium hypochlorite. Clementi noted that four weeks of exposure to 0.11 percent sodium hypochlorite solution did not produce significant irritant effect on either intact or abraded rabbit skin.

Gnemi also examined the acute eye irritation effects of sodium hypochlorite (64). The study was conducted by administering 0.1 ml of 0.11 percent sodium hypochlorite solution to the eyes of rabbits. No reactions were noted during the 72-hour observation period. The author concluded that 0.11 percent sodium hypochlorite was not irritating to the eye.

Thé examined inflammatory responses from subcutaneous exposure to sodium hypochlorite in guinea pigs (11). In these studies, open-ended tubes containing 0.9 to 8.4 percent sodium hypochlorite solution were placed under the skin of guinea pigs. After 7 and 14 days, there was no significant difference in inflammatory response between any of the sites treated with sodium hypochlorite, and the negative control sites treated with saline solution.

Cotter studied the effect of 0.1 and 0.5 percent buffered sodium hypochlorite solutions on the viability of basal cells of guinea pig skin (25). The basal cells of the skin were exposed to the 0.5 percent solution and showed no reduction in viability after one week, but showed a 15

percent decrease in viability after two weeks. The cells exposed to the 0.1 percent solution showed no loss in viability after two weeks. Cotter concluded that both 0.1 and 0.5 percent solutions would be well tolerated by patients.

Billhimer conducted a human primary irritation patch test in a clinical examination of the irritation potential of sodium hypochlorite (65). In this study, each test subject was exposed to three consecutive 24-hour applications of 0.11 percent sodium hypochlorite solution with observations taken after each application. Only transient, slight to moderate irritation was observed during the study.

Mian conducted a comparative clinical study of topical antiseptics for treatment of burn patients (66). Burn patients were treated with either a 1 percent silver sulfadiazine cream (a standard treatment) or a 0.05 to 0.11 percent sodium hypochlorite solution. The patients treated with the sodium hypochlorite solution tolerated their treatment with less pain, had a lower incidence of dermatitis, and showed faster wound healing than those treated with silver sulfadiazine cream.

Heggers conducted both *in vivo* and *in vitro* examinations of the effects of sodium hypochlorite on wound healing (67). The *in vivo* portion of this investigation consisted of making full-thickness incisions in rats, and then examining the effect of 0.25 and 0.025 percent sodium hypochlorite solutions on these wounds. The rats were sacrificed after 3, 7 and 14 days of treatment and examined. The wounds treated with sodium hypochlorite showed little or no difference from those treated with the saline controls. In the *in vitro* testing, mouse fibroblasts were exposed to 0.25, 0.025, and 0.0125 percent sodium hypochlorite solutions. The cells exposed to 0.25 percent solution demonstrated cell death and disruption; the cells exposed to

0.025 percent solution remained viable, but exhibited some cellular damage; and the cells exposed to 0.0125 showed no adverse effects. This study clearly illustrates that in vitro results do not always correlate well with in vivo results. This theoretical extension of the direct in vitro effect of sodium hypochlorite on fibroblasts cells an effect on wounds healing, clearly is not borne out in this study.

Cooper examined the in vitro effects of three topical antiseptics on fibroblasts and keratinocytes (68). The test solutions were 0.125 sodium hypochlorite, 0.5 percent povidone-iodine, and 0.25 percent acetic acid. The cells were exposed to various dilutions of the antiseptic solutions. Toxicity to the fibroblasts was measured by a test system that recorded the rate of thymidine incorporation and the rate of neutral red uptake. Toxicity to the keratinocytes was measured by the rate of neutral red uptake. With this test system, sodium hypochlorite was toxic only at the highest concentration. Sodium hypochlorite was shown, in this test, to be the least toxic to fibroblasts and keratinocytes of the three tested antiseptic solutions.

Spangberg examined the in vitro toxicity and antimicrobial effectiveness of seven commonly used dental endodontic antiseptic solutions (69). The antiseptics tested were sodium hypochlorite, chlorhexidine, three different iodine compounds, parachlorophenol, and formocresol. The toxicity was measured by exposing tissue cultures of HeLa cells to various concentrations of the solutions. The antimicrobial effectiveness was determined by mixing the antiseptic solutions with various microorganisms in the presence of calf serum. All of the compounds tested proved to be toxic to the tissue culture cells at concentrations below the effective antimicrobial concentration. Sodium hypochlorite was toxic to cell culture cells at 0.05 percent, while it was not effective against all the test microorganisms used at less than 0.24

percent. It should be noted that the antimicrobial testing was done in the presence of 25 percent calf serum, yet the toxicity assay did not contain it. This addition maximizes potential toxicity and minimizes effectiveness because of the presence of the protein. The antimicrobial testing in this study will be more thoroughly discussed in the efficacy section of this petition.

Lineaweaver examined the effects of four topical antiseptics on wound healing by both in vivo and in vitro methods (8). The antiseptics were 0.5 percent sodium hypochlorite, 1.0 percent povidone-iodine, 0.25 percent acetic acid, and 3.0 percent hydrogen peroxide. In the in vivo portion of this study, 4 cm incisions were made on the backs of rats, and these wounds were irrigated with each one of the antiseptic solutions three times a day, for four days. After a given treatment, its effect on wound healing was measured by two testing methods; the tensile strength of the wound and determination of the rate of wound epithelialization. All of the solutions, except hydrogen peroxide, showed some inhibition of the wound healing process. The in vitro portion of the study consisted of treating cultured human fibroblasts with various dilutions of the four antimicrobial solutions. After 24 hours of exposure, all of the solutions were toxic to the fibroblasts at full strength. Sodium hypochlorite was toxic as defined in this study at 0.025 percent, but not at 0.005 percent. Non-toxic levels of other solutions tested were at 0.001 percent for povidone-iodine, 0.0025 percent for acetic acid, and 0.003 percent for hydrogen peroxide. Lineaweaver also examined the bactericidal effectiveness of the four test solutions. While all four were found to be effective as antimicrobials, only sodium hypochlorite and povidone-iodine were antimicrobial at concentrations that were not toxic to the fibroblasts after direct application to the cells.

In addition, in an in vitro study by Kozol, the effect of sodium hypochlorite solution on neutrophil migration was used as an assay (28). Sodium hypochlorite solutions down to a concentration of 0.00025 percent inhibited greater than 90 percent of neutrophil function.

The studies by Lineaweaver and Kozol provided the suggestion that sodium hypochlorite, along with other topical antimicrobials (some of which are category I under the First Aid Antiseptic TFM), showed in vitro effects and may cause some adverse effects on wounds and wound healing. However, this evidence must be weighed together with some other relevant information and conclusions cannot be drawn from differing assay techniques extended to rationalize effects on wound healing. From the clinical information accumulated over the years, it may be reasonably assumed that these studies place too much emphasis on in vitro methods. Carrel's early clinical work with sodium hypochlorite solutions was criticized by a Dr. Almoroth Wright, who based his criticisms on results from vitro experiments. Carrel replied that "[e]xperiments must be made under the real clinical conditions of the treatment, if sound conclusions are to be reached" (5). History tells us that Carrel was right. As it turns out, the treatment developed by Wright, highly regarded on the basis of his in vitro observations, was unsuccessful in practice.

Further, Heggars recognized that the in vitro model was insufficient to provide a complete picture of the wound healing process. His study showed a drastic difference in toxicity between in vitro and in vivo models. Therefore, "individual tissue culture toxicity assays may be misleading in that the wound milieu consists of a polycellular environment that consists of a mixture of cell types ... which all contribute to the wound healing process" (67). Heggars also recognized that "the cellular constituents provide a protective substance or mechanism that

neutralizes the toxicity of NaOCl.” Barese and Cuono also recognized the shortcomings of extrapolating results from in vitro experiments to effects in an actual wound (30). In a letter critical of Kozol’s conclusions described above, the authors note the contradiction between sodium hypochlorite’s successful clinical use and its reported adverse effects on tissue cell cultures. Like Heggars, they believed that this apparent contradiction could be explained by the difference between real wounds, and Kozol’s model, and in their words, “which bears little similarity to a real wound milieu.” As noted previously, sodium hypochlorite breaks down rapidly once exposed to blood serum, or other components of the wound environment.

Further, Barese and Cuono also questioned the composition of the “Dakin’s solution” tested by Kozol. They noted that the study did not state whether the solution was buffered, or if buffered, what buffer was used. They further noted that unbuffered sodium hypochlorite is less effective, and far more irritating (due to high alkalinity) than the buffered solution. Interestingly, Kozol does not address this issue in his reply, but rather attacks the relevance of sodium hypochlorite’s historical use (70). The Lineaweaver study also fails to detail the composition of the tested sodium hypochlorite solution (8).

It is quite likely that Kozol and Lineaweaver did, in fact, test unbuffered solutions. Many of the references that discuss the formulation of “Dakin’s solution” and “Modified Dakin’s solution,” including the original works by Dakin (4) and Carrel (21), Remington’s practice of Pharmacy (36,37), the Modern Drug Encyclopedia (41), as well others, describe a buffered sodium hypochlorite solution. However, the USP from at least 1937 on describes “Modified Dakin’s solution” as a 0.45 to 0.5 percent sodium hypochlorite solution, with no reference to any buffering. Therefore it is possible that Kozol and Lineaweaver actually examined the effects of

an unbuffered sodium hypochlorite solution instead of properly formulated Dakin's solution. Dakin himself recognized the irritating potential of unbuffered sodium hypochlorite, and developed his formulation accordingly (4). Further, in a clinical study by Bloomfield (recognized expert in chlorine compounds), unbuffered sodium hypochlorite (also known as Milton solution) demonstrated a higher score for skin irritancy than buffered sodium hypochlorite solutions (27).

Farrow also criticized the use of sodium hypochlorite solution in the report "The Place of Eusol in Wound Management" (7). This report discussed the controversy around the use of an antiseptic, "Eusol" or "Edinburgh University Solution of Lime," and concluded that it should no longer be used for wound treatment. The report contains a number of flaws, not the least of which is that it misidentifies the antimicrobial component of Eusol. The report states that "Eusol consists of a weak solution of sodium hypochlorite, buffered by boric acid." In fact, Eusol is a solution of chlorinated lime, and not sodium hypochlorite. The British Pharmacopoeia contains a monograph for "Chlorinated Lime and Boric Acid Solution," and lists "Eusol" as a synonym (71). This monograph lists the ingredients for Eusol as 12.5 g of chlorinated lime and 12.5 g of boric acid in 1000 ml of purified water. Chlorinated lime is not sodium hypochlorite, but rather an indefinite, undefined mixture of chlorine-containing calcium compounds such as $\text{Ca}(\text{OCl})_2$, CaCl_2 , and $\text{Ca}(\text{OH})_2$ (32). When Farrow's report discusses the wrong active ingredient, it becomes of questionable value in evaluating the safety of sodium hypochlorite.

The studies discussed above indicate clearly that sodium hypochlorite is not a skin irritant and does not inhibit wound healing. All but one of the *in vivo* studies of sodium hypochlorite support this conclusion. The one study to the contrary, the Lineaweaver study, does not specify

whether the protocol used a buffered sodium hypochlorite solution, or a more irritating unbuffered solution. A few in vitro studies indicate that sodium hypochlorite may have adverse effects on some cell types; however, these studies fail to account for the significant differences between in vitro conditions, and the environment of an actual wound. Furthermore, even these in vitro studies demonstrate that sodium hypochlorite is no more irritating, and often less irritating than other category I first aid antiseptics or category I or III antimicrobials. The original Tentative Final Monograph for OTC Topical Antimicrobial Products recognized that a product that “causes slight irritation or delays wound healing for a relatively short period can be generally recognized as safe and effective if those side effects are offset by a compensating benefit” (62).

b. Sensitizing Potential

The fact that sodium hypochlorite is not a sensitizer is a significant advantage that sodium hypochlorite solutions have over some other topical antiseptics. To clarify sensitization potential, Amuchina commissioned Hazelton Laboratories to examine 1.1 percent sodium hypochlorite solution using a standard test for predicting sensitization or allergic reactions, the Guinea Pig Maximization Test (72). This test consisted of dermal and intradermal application of various concentrations of test solutions of sodium hypochlorite as an induction dose; followed two weeks later by a challenge dose of sodium hypochlorite solution. No reaction to the challenge dose was observed with sodium hypochlorite. The authors concluded that sodium hypochlorite solution was not a skin sensitizer in guinea pigs. This test has often been used as a screening test for human use and has been predictive of potential sensitization in humans.

c. Topical Safety Summary

Sodium hypochlorite has been shown to be essentially non-irritating, and not an inhibitor of the wound healing process. Further, it has not shown any potential as a skin sensitizer. Therefore, sodium hypochlorite should be found safe for the proposed indications at the proposed concentrations.

E. Efficacy

Sodium hypochlorite has a long history of demonstrated efficacy as an antiseptic and disinfectant. Its effectiveness has been demonstrated by in vitro studies, controlled clinical studies, and clinical experience.

1. In Vitro Effectiveness

The First Aid Antiseptic TFM provides a proposed in vitro test procedure for demonstration of an ingredient's effectiveness (73). This proposed procedure requires that the antiseptic ingredient show a 3-log reduction in cultures of the test organisms in 10 minutes. The organisms to be tested are Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The antimicrobial action of sodium hypochlorite has been examined extensively in many published and unpublished studies that demonstrate effectiveness; however, many of these studies were conducted prior to the publication of FDA's proposed in vitro method. Nevertheless, the methods used in the studies reported demonstrate effectiveness with protocols and methods that are equal to or exceed that required by the FDA proposed method. Sodium hypochlorite's effectiveness has been demonstrated against a wide variety of microorganisms, including bacteria, fungi, and viruses. Sodium hypochlorite has also been found effective under the stringent antimicrobial standards used by the French and Swiss governments for the

evaluation of health care disinfectants and antiseptics, and has been found effective under the tests required by the Environmental Protection Agency for General Purpose Disinfectants (Hospital Disinfectants).

a. Published Studies

Perhaps the earliest example of in vitro examination of sodium hypochlorite is found in Dakin's original work on the properties of various antiseptics (4). Various concentrations of a number of antiseptics, including sodium hypochlorite solution, were tested for antimicrobial activity, both with and without 50 percent added blood serum. Sodium hypochlorite killed Staphylococcus aureus at a concentration of 1:500,000 (or 0.0002 percent) in the absence of blood serum, and at a concentration of 1:1500 (or 0.067 percent) in the presence of blood serum. Bacillus pyocyaneus (P. aureginosa) was killed at a concentration of 1:100,000 (or 0.001 percent) in the absence of blood serum and at a concentration of 1:2500 (or 0.04 percent) in the presence of blood serum. It is worth noting that sodium hypochlorite demonstrated antimicrobial effectiveness in the presence of 50 percent blood serum. This represents a much greater organic load than the 10 percent serum level proposed in the First Aid Antiseptic TFM.

More recent published in vitro studies also establish the bactericidal effectiveness of sodium hypochlorite. Heggors demonstrated that a 0.025 percent solution of sodium hypochlorite killed a 1×10^7 inoculum of ten different organisms isolated from burn patients, including the three species listed in the First Aid Antiseptic TFM proposed testing procedures (67).

Cotter examined the antimicrobial activity of 0.5 and 0.1 percent sodium hypochlorite solutions (25). In an attempt to replicate the conditions of topical application, Cotter used cadaveric skin as a substrate for the test microorganisms, S. aureus, P. aeruginosa, and Candida albicans. After inoculation with the test organisms, the skin samples were treated with 0.1 and 0.5 percent sodium hypochlorite, and then sampled at various time points. Application of the 0.5 percent solution resulted in kills with an average 6-log reduction of all three test organisms after 10 minutes. A 10 minute exposure to the 0.1 percent solution resulted in a complete kill (7-log reduction) of S. aureus, a 4-log reduction of P. aeruginosa, and a 3-log reduction of C. albicans. This study is significant as it demonstrates that sodium hypochlorite solution exhibits significant antimicrobial activity against microorganisms on a protective substrate such as the skin.

Lineaweaver examined the antimicrobial properties of several disinfectants against S. aureus (8). A 1×10^6 inoculum of the organism was used to inoculate various concentrations of four different antiseptics, and bacterial survival was examined after a 24-hour exposure. Sodium hypochlorite in a 0.005 percent solution killed the test organism (6-log reduction). This experiment illustrates the effectiveness of sodium hypochlorite as compared to other antiseptic ingredients considered to be Category I by the First Aid Antiseptic TFM. Sodium hypochlorite killed the test organism at 0.005 percent, or one-tenth of the lowest recommended use concentration, while the Category I ingredient, hydrogen peroxide, had no antiseptic activity at one-tenth of its recommended use level (0.3 percent).

Spangberg examined the in vitro toxicity and antimicrobial effectiveness of seven commonly used endodontic antiseptics in order to determine the optimal concentration for use as an endodontic rinse (69). This effectiveness testing was conducted using S. aureus, P.

aeruginosa, Streptococcus faecalis, and C. albicans as test organisms. The method used in this study consisted of adding cultures of the test organisms to various concentrations of each tested antiseptic in the presence of 25 percent calf serum. After either five or ten minutes, a portion of the test solution was placed in a neutralizing medium and incubated for four days. Any growth in the neutralizing medium during the four-day incubation meant that the antiseptic did not eliminate the test organism. With this method, the number of cells in the test cultures was not determined, the exposure time was not clearly stated, and the number of cells remaining after exposure was not measured. Therefore, the results of this study cannot be directly compared to the results of more quantitative methods that give results in terms of log reduction over a certain time period. Nevertheless, the study found the effective concentration for sodium hypochlorite in the presence of 25 percent serum to be either 0.24 or 0.12 percent, depending upon test organism.

Three studies have evaluated the effectiveness of Amuchina's sodium hypochlorite solution as a disinfectant for dialysis equipment. In the study by Baldini, the disinfection of both naturally and artificially contaminated dialysis units was examined (12). The bacterial levels in the naturally contaminated units were not high enough to provide useful information. The artificially contaminated units were inoculated with 1×10^6 levels of P. aeruginosa, E. coli, and Proteus vulgaris, incubated for 24 to 48 hours, and then washed, leaving bacterial levels of between 1×10^6 and 1×10^5 . The units were then disinfected with various concentrations of sodium hypochlorite solution. A 15-minute treatment with a 0.0057 percent killed all P. aeruginosa. A 15-minute treatment with 0.033 percent solution eliminated E. coli and P. vulgaris.

Buoncristiani examined the effectiveness of sodium hypochlorite solution as a peritoneal dialysis disinfectant (74). Different concentrations were examined for both total antimicrobial effect, as well as activity in the presence of dialysis solution. A preliminary screen demonstrated that a 0.057 percent solution killed 1×10^8 concentrations of a wide variety of microorganisms. Further studies found that 0.008 percent solution killed a 1×10^9 inoculum of P. aeruginosa in 10 minutes, and a 0.05 percent solution did the same in 30 seconds.

In another study of peritoneal dialysis disinfectants, Bianchi examined the effects of three disinfectants against P. aeruginosa, Proteus mirabilis, and S. aureus in saline, broth, and broth with blood (75). Five-minute treatments with sodium hypochlorite solutions at concentrations of 0.017 percent or less killed 1×10^4 cultures of the test organisms in the presence of broth. Concentrations of 0.275 percent killed a 1×10^4 inoculum of the test organisms in the presence of broth with 25 percent blood added.

The proposed in vitro test procedure outlined in the First Aid Antiseptics TFM only evaluates effectiveness against bacteria. Sodium hypochlorite solutions have also been tested for fungicidal and virucidal effectiveness.

Narang examined the virucidal properties of a number of common hospital disinfectants, including "Chloros," an 11 percent sodium hypochlorite solution (76). Several common viruses were exposed to various dilutions of the disinfectants. The lowest tested concentration of the sodium hypochlorite solution, 0.11 percent, was completely effective against all tested viruses in the shortest contact time tested, 2 hours.

A 1:10 to 1:00 dilution of 5 percent sodium hypochlorite (commercial bleach) has been recommended by Centers for Disease Control for use on blood spills, and in clean-up where HIV virus is suspected (77).

Lee examined the effects of various concentrations of sodium hypochlorite on Mycoplasma mycoides (14). The study used both a suspension test and a carrier test. In the suspension test, inoculum was added directly to the test solution. In the carrier test, the test organisms were inoculated onto porcelain carriers to simulate hard surface disinfection. Both tests were conducted with and without an organic load. In the suspension test, a 15-second exposure to a 0.0025 percent solution killed a 1×10^6 inoculum of the microorganism in the absence of an organic load. When 1 percent BSA (bovine serum albumin) was added, a 15-second exposure to a 0.04 percent solution, or a 5-minute exposure to a 0.005 percent solution resulted in no survivors. The porcelain carriers provided considerable protective effect to the test organisms. A total kill on the carrier test required a 30-minute exposure to a 0.0125 percent solution in the absence of an organic load, and a 90-minute exposure to a 0.02 percent solution in the presence of 1 percent BSA.

Sodium hypochlorite has also demonstrated some capacity to detoxify bacterial endotoxins. Butler used both in vitro and in vivo methods to determine the effects of sodium hypochlorite on endotoxins from E. coli 0127:B8 and Salmonella typhosa 0901 (78). In the in vitro part of the study, a 0.58 percent solution detoxified 100ng/ml of the endotoxins. Sodium hypochlorite was not as effective in the in vivo portion of the study, where only the highest tested concentration, 5.20 percent, exhibited any effect on the endotoxins.

b. Unpublished Studies

Sodium hypochlorite solution was tested for effectiveness using the official methods required by the French government for health care disinfectants and antiseptics. Unlike the United States, in France there is no distinction in approval between disinfectants used on hard surfaces and topical antiseptics; the same standards are used for both. These methods, the AFNOR Standard Test Methods (Association Francaise de Normalisation) are also used by the Swiss authorities as one criteria for disinfectant effectiveness. The AFNOR standards are discussed in detail in Cremieux's chapter in Disinfection, Sterilization, and Preservation (87). For disinfectants, the essential antimicrobial tests are suspension tests that place the test solution in contact with the test organism for a specified time and assess viable recovery much like the First Aid Antiseptic TFM proposed test method.

French law requires that studies conducted for AFNOR approval be conducted in France by a "French Expert." Amuchina commissioned J. C. Darbord to conduct testing of Amuchina's sodium hypochlorite solutions under the AFNOR requirements. To meet these requirements, Darbord tested 0.055 percent sodium hypochlorite according to the test methods for effectiveness in the absence of and in the presence of interfering substances.

The test for effectiveness in the absence of interfering substances is under AFNOR norm NF T 72150 (24). This standard requires that the test solution produce a 5-log reduction in the specified bacterial population in five minutes at 20°C. Amuchina's sodium hypochlorite solution was tested against P. aeruginosa CIP A22, E. coli CIP 54127, S. aureus CIP 53154, Enterococcus hirae CIP 5855, and Mycobacterium smegmatis CIP 7326. The test solution showed the required 5-log reduction for all test organisms (79,80,81,82).

The test for effectiveness in the presence of interfering substances is under AFNOR norm NF T 72170 (24). This standard requires that the test solution cause a 5-log reduction in the specified bacterial population in five minutes at 20°C in the presence of serum and hard water. The same test organisms as used for AFNOR norm NF T 72150 were used. Again Amuchina's 0.055 percent sodium hypochlorite solution caused the required 5-log reduction for all test organisms (83).

Darbord also examined Amuchina's sodium hypochlorite solution for effectiveness against a variety of clinical isolates under AFNOR norm NF T 72170. The test organisms included isolates of E. coli, Proteus vulgaris, S. aureus, Staphylococcus captitis, Streptococcus pyogenes, Streptococcus agalactiae, Haemophilus influenza, Acinetobacter calcoaceticus, and Mycobacterium chelonae. The test solution caused the necessary 5-log reduction within the required 5-minute time period for all organisms tested (84).

Fungicidal activity of 0.055 sodium hypochlorite was examined in accordance with AFNOR norm NF T 72200, which requires a 4-log reduction of the fungal spores or yeast cells in 15 minutes (24). The organisms tested were Penicillium verrucosum CIP 1231-80, Cladosporium cladosporioides CIP 1232-80, Aspergillus niger CIP 1431-83, and Candida albicans ATCC 2091. The test solution produced the required 4-log reduction for all test organisms (85).

Darbord also examined the effectiveness of Amuchina's 0.055 percent sodium hypochlorite solution against poliovirus under AFNOR norm NF T 72180 (24). The test solution was found to be virucidal against this test organism (86).

Darbord prepared a summary of his findings for submission to the French authorities, which is included (87).

In a study to meet Swiss standards, Pappalardo used the AFNOR membrane filtration suspension test (AFNOR NF T 72-151) (24) to evaluate the effectiveness of sodium hypochlorite against ten different microorganisms (88). A one-minute exposure with a 0.057 percent solution killed 1×10^8 cultures of all test organisms except Bacillus subtilis (spore former). A five-minute exposure produced a 3-log reduction of this organism.

Sodium hypochlorite's effectiveness as an antiseptic and disinfectant is clearly demonstrated in the results of the tests conducted to meet AFNOR standards.

Amuchina also commissioned a series of studies to comply with the EPA registration requirements for a Hospital Disinfectant (also now designated a General Purpose Disinfectant) (89). These studies used the AOAC Use Dilution Method (90) to evaluate the effectiveness of various concentrations of Amuchina's sodium hypochlorite solution against a variety of microorganisms. The Use Dilution Method was created in an attempt to simulate conditions of hard surface disinfection. The method is performed by inoculating stainless steel "carriers" with broth cultures of test organisms, transferring the carriers to the test solution, and then transferring the carriers to neutralizing broth tubes. If the test solution is effective, no growth is observed in the neutralizing broth. Demonstration of effectiveness with the Use Dilution Method requires that a large number of carriers (720) show no growth after exposure to the test disinfectant. In evaluating these studies, one must consider that the organisms in the Use Dilution Method are inoculated onto the protective substrate of the carriers, and they are significantly harder to kill

than organisms added directly to an antiseptic solution in the typical "suspension" type test such as the one proposed in the First Aid Antiseptic TFM.

In one study, the Use Dilution Method was employed to evaluate the effectiveness of 3 percent Amuchina (0.033 percent sodium hypochlorite) against S. aureus, Salmonella choleraesuis and P. aeruginosa (91). Each test organism was used to inoculate 60 carriers to a level of between 1×10^4 and 1×10^5 organisms per carrier. Each carrier was subjected to a 15-minute exposure to the 0.033 percent sodium hypochlorite test solution, and then transferred to neutralizing broth. No growth was observed in any of the neutralizing broth tubes. The author concluded that the test solution was germicidal against the test organisms.

Additional Use Dilution Method studies evaluated the effectiveness of 5 and 10 percent Amuchina (0.055 and 0.11 percent sodium hypochlorite) against P. aeruginosa, in the presence of a 5 percent soil load (92,93). In each study, 10 carriers were inoculated with 1×10^5 cells. No growth was observed in the neutralizing broth tubes after the carriers were exposed to the test solutions for 15 minutes. The author concluded that both concentrations of the test solution were germicidal against P. aeruginosa in the presence of a soil load.

Two studies evaluated 5 percent Amuchina (0.055 percent sodium hypochlorite) for effectiveness against S. aureus and S. choleraesuis in the presence of a 5-percent soil load (94,95). No growth was observed in the neutralizing broth tubes after the carriers were exposed to the test solutions for 15 minutes. The author concluded that the test solutions were germicidal against S. aureus and S. choleraesuis in the presence of a soil load.

The EPA registration also involved an examination of 5 percent Amuchina solution (0.055 percent sodium hypochlorite) for fungicidal properties (96). Carriers inoculated with Trichophyton mentagrophytes were exposed to 0.055 percent sodium hypochlorite solution for 10 minutes. No growth was observed in any of the neutralizing tubes after the carriers were transferred from the test solution. The author concluded that the test solution was fungicidal against T. mentagrophytes.

Bechara conducted a study on Amuchina's virucidal properties as a part of the EPA registration process (97). The virucidal effectiveness test was performed using the accepted ASTM protocol (98). A 0.033 percent sodium hypochlorite solution was found to be effective against the Herpes simplex type 1 virus.

Bechara also examined Amuchina's effectiveness against Mycobacterium bovis, which is used as a surrogate for Mycobacterium tuberculosis (human tuberculosis) in laboratory testing (99). The study was conducted using the Confirmatory AOAC Tuberculocidal Test method (24). A 25-minute exposure to 0.11 percent sodium hypochlorite solution was effective against M. bovis.

The Pasteur Institute conducted a study for EPA registration that examined three concentrations of Amuchina's sodium hypochlorite solution for inactivation of HIV-1 (100). The dilutions tested were 0.11 percent, 0.055 percent and 0.0165 percent sodium hypochlorite. All dilutions demonstrated the complete inactivation of HIV-1

The studies presented in this section demonstrate the effectiveness of sodium hypochlorite against a wide variety of microorganisms under worst case test conditions. The

results of these studies go well beyond the requirements of First Aid Antiseptic TFM for demonstration of effectiveness. They are included here as a basis for effectiveness as an Access Site Preparation in the Health-Care Antiseptic Drug Products TFM. Additional clinical evidence of the effectiveness of sodium hypochlorite is also presented and several studies are added for this specific petition to demonstrate the effectiveness of sodium hypochlorite for this indication.

2. Clinical Effectiveness

Clinical information provides excellent evidence of the antimicrobial activity of sodium hypochlorite in situations relevant to the product's actual intended use. This section discusses three studies that examine sodium hypochlorite use as a preoperative antiseptic, one that examines use as a gynecological wash, and one that examines use as a burn antiseptic.

In a published study conducted by Jones, 0.11 percent sodium hypochlorite solution was compared to 10 percent povidone-iodine solution for use as a preoperative antiseptic (101). Each solution was used to treat sites on both the axilla and the abdomen. Effectiveness was determined by measuring the reduction in total bacterial count. On the abdominal site, both test solutions reduced the baseline bacterial count by approximately 2 logs. On the axillary sites, sodium hypochlorite was more effective, reducing the baseline count by 2 logs, compared to a 1-log reduction with povidone-iodine.

Rovenda conducted a similar study, with similar results. This study also compared 0.11 percent sodium hypochlorite solution with 10 percent povidone-iodine solution (49). Again, both solutions were tested on axillary and the abdominal sites. In this case, the sodium hypochlorite solution showed a 1.5-log reduction from baseline on the abdominal sites, and a 2-

log reduction in the axillary sites. In comparison, povidone-iodine reduced baseline counts by 1.5-logs on the abdominal sites, and by 1-log in the axillary sites.

There has been some discussion about the use of axillary sites for testing pre-operative preparations. The axillae have been used in a number of studies where two sites have been requested in the Health-Care Antiseptic Drug Products monograph publications. The selection of sites has developed from the desire to test sites that have a normal flora with both Gram positive and Gram negative bacteria. These are usually moist and occluded, such as, the axilla, groin and sub- mammary spaces. The abdominal area has been used because many surgeries are performed on this body area; however, the normal flora of the abdomen is often sparse, making accurate reduction estimation difficult. Further, the axillae are more easily sampled than the groin area. The groin area often has very high counts, but these can be variable because of unavoidable contamination with urine and feces complicated with the presence of hair. Doubts about the use of the axillae as a test area have been raised because of the use of widespread antiperspirants which are antimicrobial. However, a washout period is included in the protocol, which is effective in re-establishing a complex normal flora. The flora is not as variable as in the groin, and the counts are as high.

Cruz also examined the use of 0.11 percent sodium hypochlorite solution and 10 percent povidone-iodine solution as preoperative antiseptics (49b). Both solutions exhibited similar effectiveness, with a 1-log reduction from baseline levels on the abdominal sites, and a 2-log reduction on the axillary sites.

Carabelli compared the effectiveness of Amuchina's 0.055 percent sodium hypochlorite solution to an unidentified mercurial antiseptic for the treatment of vaginal itching (102). Over

the course of a three-day treatment schedule, both solutions were effective in reducing the severity of the initial symptoms, but the sodium hypochlorite solution proved to be significantly more effective than the mercurial antiseptic in decreasing microbial counts.

As previously discussed, Mian conducted a comparative clinical study on topical antiseptics for treatment of burn patients (66). Patients were treated with either a 1 percent silver sulfadiazine cream or 0.11 to 0.055 percent sodium hypochlorite solution. Patients treated with sodium hypochlorite exhibited a significantly lower incidence of sepsis.

3. Clinical Case Studies

Case studies also provide valuable information about the effectiveness of sodium hypochlorite (21). Carrel's description of the use of Dakin's solution in traumatic wounds contains a number of detailed case studies. These case studies describe the course of bacterial infections in both serious and superficial wounds treated with sodium hypochlorite and other solutions. Invariably, these cases demonstrated the effectiveness of sodium hypochlorite in preventing and treating wound infection.

Slahetka describes the use of sodium hypochlorite for the treatment of deep ulcers in three geriatric patients (10). In all three cases, the patients had badly infected chronic ulcers. A 0.5 percent sodium hypochlorite solution effectively treated the infection and permitted wound healing. The author observed that the solution not only successfully destroyed bacteria, but it also dissolved necrotic tissue and removed detritus. She hypothesized that sodium hypochlorite promoted wound healing by stimulating circulation.

4. Effectiveness and Additional Dermal Toxicity

The earlier petition for topical use of sodium hypochlorite in concentrations ranging from 0.05 to 0.5 percent establishes the historical use of this product topically. The dramatic history of use by Carrel in caring for traumatic wounds, often contaminated with dirt and animal feces from the trenches, has been recorded in several references (3,5,6). These historical uses are directly related to the current changes with the increased use of medical devices and the increasing need for use of catheters; from preparation for catheter access, central venous access, peripheral catheters, and injection to repeated access for dialysis.

In 1916, Carrel commissioned Dakin to find an effective antimicrobial that he could use in wound care in his French Hospital in World War I. Dakin settled on sodium hypochlorite and Carrel developed a care method using wound debridement immediately on admission of the casualties from the fields and trenches. Wounds were left open and a series of small glass tubes were rigged in a wound so that the wound perfusing treatment could be repeated. Carrel treated the skin and wound margin with hypochlorite prior to insertion of his tube apparatus. Wounds were not closed until a sample was bacteriologically negative. This was a luxury we cannot repeat today and his clinical results reflected this care. These procedures were adapted and used after the war and published in detail (6, 21, 22). This is clearly an early use of Dakin's or sodium hypochlorite solution as a skin preparation for subsequent treatment. Other applications in clinical situations that apply to topical situations include the prior references to ulcer and wound debridement, endodontic use in dentistry and the treatment of burns. (7,9). All of these topical applications are meant to reduce skin or mucous tissue flora prior to a treatment or manipulation.

As suggested, the treatment developed by Carrel used repeated application of a volume of hypochlorite. To increase acceptance, a colleague of Dakin changed (reduced) the alkalinity of Dakin's solution. After glass tubes were inserted, the wound was saturated every two hours. Many lives and limbs were saved using these procedures. Eventually clean wounds, which were not necessarily bacteria free when closed became the practice, differing from Carrel's procedure. With the use of antibiotics, after World War II, there was decreasing interest in this treatment. With today's increasing incidence and range of antibiotic resistance, especially with topical treatment, there is a resurgence of use of chemical antimicrobials such as sodium hypochlorite, which have shown little or no development of resistance.

Dakin recommended the solution ultimately named after him because of its superior antiseptic qualities when tested in blood serum. Dakin as well as many others recognized its debriding activity and assistance in the rapid dissolution of necrosed tissue. In further development, Dakin produced his solution by electrolysis on the battleship, Aquitania. Dakin's solution was also used during and after World War II. When the resistance development to antibiotics became severe in the late 1960s and 1970s, many revived the use of Dakin's solution for debridement and wound treatment.

In the 1980s there were controversial publications concerning deleterious effects on wound healing. These effects have been disputed and when Dakin's solution or sodium hypochlorite is used in appropriate concentrations in a clinical and "real life" setting, it is both effective and non-toxic. As mentioned above, there has been a resurgence in use in this current time frame because of its antiseptic and debridement effects and low resistance development.

Specific concentrations of sodium hypochlorite solution have been used in burn treatment, often in reduced concentrations so as not to impede in any way the burn wound healing (67).

Over the years since the end of World War II, iodophor solutions and sodium hypochlorite have been used in many clinical applications, often not reported, literally for irrigation in every imaginable application (in World Congress on Antisepsis, Weisenhofer et al. for example, 105). As a result of the OTC review of antimicrobials (begun in 1972), a specific protocol was developed for the testing of pre-operative preparations, which has had many iterations, and just recently, the ASTM E 35.15 Subcommittee has passed another revision to include access site testing. Some aspects of this method will be critiqued later. Speed and convenience were often prime considerations in testing prior to specific tests developed during the OTC Review. A very generalized examination of the activity of potential prep products was often used. This OTC methodology seemed appropriate at the time, but was not necessarily the best one especially for the clinical setting. Many investigators of surgical procedures have chosen to use contact plates (such as Rodac) or other "in situ" sampling procedures (Geelhoed, 106, 107 and others, 108) in the "live" surgical setting. These studies have been designed for sampling surgical sites before and after prepping and after the surgical procedure at closure.

The selection of a test site for pre-operative, and currently, access sites is deceptively complicated. In development of a protocol by OTC, surgical sites commonly found in surgery were selected as test sites. Prior to this, the forearm had often been used in skin testing, but currently, the abdomen, the groin (high count area), the axillae and often, the back have been used as sites. There are arguments for all of these. We have come to realize that the microflora of the various areas of the body is highly variable in numbers and types of organisms. Not only

do sites on the body vary; individuals vary greatly in the numbers and types of organisms they maintain as normal flora. It has proven very difficult to find test volunteers who fit within the count range for different sites that has been suggested in the OTC protocol. A test site must have a consistent, reliable count containing a large enough population that a specified reduction can reasonably be tested. Drier areas of the skin can have low counts depending on the temperature and relative humidity of the environment. The back, the forearm and the abdomen have historically been recognized as usually having low counts. (109, 110)

In the design and analysis of these studies, there in fact, must be a significantly greater reduction than, say the two-log or three-log reduction, specified in various protocols in order to statistically conclude that this specified reduction has occurred. When a two-log reduction is required and the baseline or pre-treatment count is only skirting three-logs (say on the abdomen), it may become difficult to show that the reduction actually occurred, especially when other sources of variation in the study are considered. Further, when low counts must be recovered, accuracy declines.

Toxicity and Effectiveness Data with Amuchina's ExSept Plus

The discussion presented above describes the in vitro data collected to show the effectiveness of sodium hypochlorite for antiseptic/disinfectant use as formulated in ExSept (0.055 percent sodium hypochlorite). Toxicity data were presented showing the safety of use of this product.

The ExSept Plus product by Amuchina contains 0.10 percent sodium hypochlorite. This petition is for the use of this product with an increased concentration of sodium hypochlorite (over

ExSept, but within the original range of the petition) and is indicated for preparation/treatment of access sites for injection, catheterization and dialysis.

5. Additional Toxicity Data

Dermal Toxicity

Additional skin toxicity testing was performed with this formulation.

1. A Cumulative Irritation Patch Study was conducted on the Amuchina 0.11 percent sodium hypochlorite, then called Amu-Skin (103). The formulation and the vehicle were both tested for its potential to cause irritation and/or sensitization to the skin of normal volunteer subjects using a blinded, randomized, semi-occlusive 21-day cumulative irritation patch study with challenge. There was no significant irritation in the irritation phase of the study. There was no evidence of sensitization to any of the products evaluated in the challenge phase of the study.
2. A Repeated Insult Patch Study was also performed by Amuchina (TKL Laboratories performed the test, 104). In this test, 0.1% sodium hypochlorite (Amu-Skin) was evaluated neat to determine its ability to sensitize the skin of normal volunteer subjects using a blinded, randomized, occlusive, repeated insult patch study. Under the conditions employed in this study, using one hundred ninety-four subjects, there was no evidence of sensitization to Amu-Skin or to the vehicle.

Background effectiveness as a pre-operative, pre-catheterization, and pre-infection preparation was discussed in the prior discussion of significant in vitro data in the petition dealing with ExSept or 0.05% sodium hypochlorite.

6. Skin Flora and Effectiveness Criteria

As far as the microbial flora of the skin is concerned, we have had a history of early years of harsh scrubbing with pig bristle brushes to the present day, when brushing the hands during scrubbing has evolved from plastic brushes to the elimination of the brush entirely. This is based on the realization that this scrubbing brings up the deeper flora and can be irritating. When prepping is done, even today, there is an emotional and psychological mind-set that washing and rubbing make the treated area cleaner for surgery. Comparative results of tests show that the elimination of the brush in hand washing does not affect the bacterial reduction.

Other areas of the body such as the sites used to gain access to the body for catheters, tubes and connectors have highly variable skin microflora. In the development of a suggested protocol for examining pre-operative preparations, the overwhelming drive was to include testing areas of the body with a Gram-negative population along with other areas of the body with predominately Gram-positive normal flora.

The axillae, the groin area and sub-mammary folds are usually recognized as having a variable flora that usually includes Gram negatives. When the axillae is used, any residual antiperspirant can be easily eliminated by wash-out before testing. This site can be fairly uniform with respect to distribution of the flora. When the groin is used as a site, the degree of extraneous contamination cannot be determined because of unpredictable contamination from urine, feces or blood further complicated with the presence of hair.

In a suggested protocol recently approved as a Standard Test Method by the ASTM E-35.15 (112) Subcommittee on Antimicrobial and Antiviral Agents, the pre-test control for testing in the groin is selected in the area of the Abdominal Crevice closest to genitals, but this

establishes a questionable base to use as a standard for reduction since this site often has the highest count but also, may be the most variable. Testing in the groin can be physically difficult, and in fact, the area chosen for testing is the Abdominal Crevice area rather than actually in the groin.

When this protocol is executed, significant effort in design and data analysis is based on a highly variable site and population. Sampling is executed with considerable agitation and a “stripping” solution. This area is rich in hair follicles (as is the axillae), and this methodology (cup-scrub) samples flora not only at the skin surface but deep in the skin and hair follicles, which may have no part in wound contamination or migration of bacteria into the catheter or connector site or to the wound margin. When studying pre-operative preps for effectiveness, many investigators in a clinical setting have chosen to use contact or in situ sampling (Geelhoed, 106, 107, 108), because these methods sample surface flora and can be used in a clinical setting. These investigators have shown effectiveness of pre-op products and have compared products using this methodology. Bruch (113) has discussed the questions of in situ sampling and the difficulties occurring when the deeper flora is moved to the surface. When the cup-scrub is used as recommended in the OTC and ASTM protocols for investigation of pre-op and site access preparations, large numbers of bacteria are moved up from the deeper layers. This chapter entitled, “Preparation of the Skin for Surgery and Site Access...Testing’s Stepchild” is in Press.

There is variability when scrubbing/stripping solution is used to work up deeper flora in sampling. Comparatively, in situ sampling samples the superficial flora of the surgical wound. Prep solution including the antimicrobial usually remains on the skin after application as contrasted with a surgical scrub which is washed off. The microflora of the skin is not uniformly

distributed over the skin surface, but occurs as microcolonies, irregularly spaced. Some contact or in situ samplers, such as contact plates or velvet blocks will reflect this pattern. Scrubbing and standard swabbing will break up microcolonies resulting in exaggerated counts. Two types of prep products are available: one usually with soap or detergent is used with an antimicrobial to physically remove bacteria from the skin and then, the initial application is often repeated. It may be rinsed or not. Rinsing occurs sometimes, but not uniformly, so the detergent may remain. Sampling for recovery then further agitates the skin during sampling. Another type of prep product is applied to the skin in situ with some agitation (usually mild agitation and no rinsing). Sampling for detergent based products is now usually done with washing techniques. An in situ sampling method, for instance, contact sampling, does not disturb the deeper flora. It is the scrubbing and agitation with a surfactant that agitates the deeper flora.

Hands are really different from other parts of the body (see experts on microbial flora of the skin, Mary Marples and William Noble, 109, 110). The applied clinical studies described here used the currently described pre-operative prep test in the OTC Antiseptic Health Care Products Tentative Final Monograph or an adaptation of an earlier protocol and have used iodophor as a comparative control. As described in the attached Chapter by Bruch (113), the various skin sampling methods and the location of the flora, are described. She notes that the most universally used prep in hospitals, an iodophor solution is considered effective if it is perceived by the hospital staff as effective. Alcohol is also often used in combination with an antimicrobial solution or an alcoholic paint is used, especially by plastic surgeons. Iodophors and alcohol are regulated under the OTC Monograph system. The criteria for effectiveness of a pre-op prep suggested in the Tentative Final Monograph are a three-log reduction at the groin site and two-log reduction at an abdominal site. Data in the literature Ayliffe and Rotter (114,

repeatedly showed a two-log or slightly higher reduction for these antimicrobials (iodophors or ethanol). The test methodology (112) requires a post-exposure sample at ten minutes, thirty minutes and six hours. In actual use, the six-hour sample has meaning for surgical preparation when surgery could last for six hours. The relevance for site preparation for access relies on immediate and possibly, effectiveness for the ten or thirty minutes following application. The techniques used are essentially the same protocol as for a pre-op prep and access site that have been adopted in a recent procedures accepted in the ASTM Standard Method (112).

It is important to ask what criteria should be met for a product when used as indicated.

1. For a pre-operative prep, the bacterial contaminations on the skin (transients and some residents) which could migrate or be transferred into the surgical wound by manipulation are considered the most important (Rhodeheaver, 116). An antimicrobial that acts rapidly and is broad spectrum is also important. When the prepping is done, there is agitation to the skin and some of the deep flora is forced to the surface of the skin. Most antimicrobials used delay the repopulation of the skin. The criteria for a preop prep indicated in the TFM for Antiseptic Health-Care Products is a three-log reduction at the six-hour sample. There are only two sites (the groin and the axillae) that can provide a site contaminated to a 10^5 - 10^6 level required for finding the target reduction to satisfy the statistical requirement because dealing with human subjects and their skin flora is fraught with variation. Ultimately, each of the chemicals is used as preparation for access and/or surgical procedures. Most products will produce a level of reduction as measured in a currently accepted study protocol of two-plus logs. Chlorhexidine will in many testing situations achieve a three-log reduction at the six-hour period, but does not do so consistently. The reference product used in many tests is a detergent preparation of

chlorhexidine, which is almost never used as a prep because of the presence of a concentrated detergent which may be irritating. The data to be presented shows great comparability between the products tested and permits a conclusion that these products are effective as preparation for access and surgical procedures.

2. Currently there are no criteria specified for effectiveness of prep products for access sites. Some have made assumptions that it should be the same as for preoperative preps. However, there is a difference in usage. The access procedures last a relatively short time compared to surgical ones. The groin and the axillae are not common insertion sites, and certainly not for dialysis equipment so the innate microbial level of usual insertion sites is not high. The real meaning of log reduction of bacteria on the skin should be clarified. If a groin or axillary site is populated with $1-5 \times 10^5/\text{cm}^2$ for example, there are 100,000 bacteria per square centimeter; when reduced three-logs, this becomes 100 bacteria. Similarly if we are dealing with other body sites, there may be a maximum (for instance the abdomen, inner arm or back) of $10^3/\text{cm}^2$. A two-log reduction leaves 10 cells. Since the reduction in reality has to be greater than the target level to be achieved, only a very tenuous conclusion from these studies can be made reliably. This is further complicated by trying to recover low bacterial counts consistently.
3. The current TFM for Health-Care Antiseptics does address the subject of skin preparation for injection (1). In this reference, a one-log reduction is considered the criteria for effectiveness. No test method for this determination is mentioned in the TFM.

7. Clinical Testing

Three studies using Pre-operative testing techniques have been performed with ExSept Plus (0.11 percent sodium hypochlorite).

1. Study performed by Besselaar Labs (now part of Hill Top Biolabs). June 1994.

Evaluation of the Quantitative Skin Degerming Activity of 10% Amuchina Used as a Pre-Operative Skin Preparation. The procedure used in this study (117) does not conform to the TFM protocol but produced some interesting results since this study compared a saline control to the two test products. The effect of physical removal is shown using this procedure. Normally the effectiveness of the two-test products is compared directly and the saline control not included. Nonetheless, it was shown in this study that the 10 percent povidone iodine product is comparable to Amuchina 0.1% (ExSept Plus). Effectiveness as a pre-op is usually shown by the reduction of the count on the test site compared to the control. This design showed the effect of the use of saline alone used in a prepping manner. The microbial flora of the test sites was enhanced by covering with Saran Wrap occlusion for 48-hours. This is an adaptation of a procedure described by Leyden et al. (118). Many areas of the body especially exposed areas, have low and variable counts. Wrapping or covering test skin areas with occlusive plastic expands the flora present on the skin. Thus the level for the initial pre-treatment count is higher than usual.

The conclusion important to this petition is that ExSept and the iodophor gave equivalent results and today, iodophors are the most widely used prep product by far.

This was a parallel, randomized, placebo controlled study in which the evaluator was blinded with respect to treatment assignment. Thirty subjects (3 groups of 10) participated in the study

for 17 days which included a 14 day conditioning period followed by a 3 day test period. The test material resident time was different in each group (i.e., 10 minutes, 30 minutes, and 4 hours). Each subject served as his own control. To enhance the normal bacterial flora, an occlusive patch was applied to the test area 48 hours prior to treatment. Each subject had three test areas on each forearm and three test areas on each side of the abdomen for a total of 12 test sites. Subjects were randomly assigned Amuchina and Povidone Iodine to contralateral sides of the body. Each side (L forearm, R forearm, L abdomen, R abdomen) also included an untreated control site and a treated control site.

Treatment:

Each patient received Amuchina and Povidone Iodine treatment on each forearm and abdominal areas according to the randomization code. Within each treatment area, the active test material was randomly assigned to 1 to 3 sites. The remaining two sites on each test area served as the baseline untreated control and the treated control (saline) sites. Each site (left or right) was randomly assigned an active treatment.

Each Treatment site was scrubbed for one minute using a cotton swab (2 scrub periods of 30 seconds each) saturated with the appropriate product. After scrubbing, each site was covered for four minutes with a sterile gauze pad (2"x2") saturated with 5 ml of the assigned product.

The data was analyzed as the reduction in bacterial counts following treatment as compared to the untreated and saline control sites. Statistical analysis was performed on log transformed log₁₀ data using an analysis of variance (ANOVA) with a 95% level of confidence.

Thirty subjects completed sampling, which included treatment of both the abdomen and the forearm with both Amuchina and Povidone Iodine. The summary results are presented in

Appendix F1 for the forearm and Appendix F2 for the abdomen. Results for individual subjects are attached. It should be noted that the patching procedure prior to treatment allowed levels of bacteria at all sites on the abdomen and forearm to permit analysis of treatment differences. The CFU/ml for control sites for both the abdomen and the forearm are similar and average at least 1×10^7 CFU/ml of sample for the untreated control and at least 4×10^5 CFU/ml for the saline treated control site. Amuchina and Providone Iodine treated sites after 10, 30 and 240 minutes gave average counts from 10^3 to 10^4 CFU/ml. This resulted in a reduction of at least 99% (2 logs) when compared to the control and untreated sites.

Both Amuchina and Providone Iodine were equally effective in reducing bacterial counts when compared to the saline control. This effect was noted in both the forearm and abdomen regions at all time points evaluated.

Similarly, both of these treatments were significantly better than the untreated control sites. However, on the forearm region, Providone Iodine was more effective than Amuchina at the 30 minutes sampling interval, but less effective at the 240 minute sampling interval. In addition, on the abdomen sites, both treatments exhibited a significantly greater efficacy at the 10 minute sampling interval when compared to efficacy at 30 minutes, but this difference was not evident at the 240 minute interval. It is unlikely that these differences have any clinical value.

Therefore it is concluded there is no clinically significant difference between Amuchina and Povidone treatments after 10, 30 and 240 minutes.

Amuchina was shown to be effective in reducing the bacterial flora on the skin and this effectiveness was comparable to the standard Povidone Iodine 10% Solution in this clinical trial. Amuchina was also shown to be safe with no adverse experiences resulting from treatment.

References

Williamson, P. and Kligman, A.M. A new method for the Quantitative Investigation of Cutaneous Bacteria, J. of Invest. Derm. 45: 498-502 (1965).

Leyden, J.J. Stewart, R. and Kligman, A. updated in vivo Methods for Evaluating Topical Antimicrobial Agents on Human Skin. J. of invest. Derm. 72: 165-170 (1979).

2. Quantitative Skin Degerming Evaluation of Pre-Operative Skin Preparations Study performed by Hill Top Biolabs Inc., Miami, OH.

Three test products were included, in this test (119),

The objective of this study was to determine the skin degerming activity of pre-operative skin preparation for its ability to reduce the resident aerobic flora on the abdomen and in the axilla.

This report presents results of an investigation of three treatments, a test drug, placebo drug and a reference drug. The test and placebo drugs were received from Amuchina. The investigator provided the reference drug. The materials are identified below:

<u>HTB Code</u>	<u>Use</u>	<u>Sponsor's Identification</u>
A	Test Drug	Amu-skin (Amuchina 10%) 250 ml Lot 2257- 01-10-93
B	Placebo	Amu-skin Placebo Lot TF 05-04-94
C	Reference Drug	Betadine Solution Lot 5DF Exp. 10/96

The study was an independent design with three treatments assigned to randomly selected subjects. Treatments were applied to contralateral skin areas on the abdomen 1-2 inches above the navel and in the axilla area. Abdominal treatments were applied to a 5 cm x 5 cm area on the

left and right side in the vicinity above the navel. Axillary treatments were applied to a 5 cm x 5 cm area in the left and right axilla. There was an untreated control site (baseline site) as well as a treatment site of the same area adjacent to each other.

Treatments were applied to one or two different anatomical locations, sites located on the abdomen and sites in the axilla.

Abdominal treatment sites, 5 cm x 5 cm, were defined on a subject's left and right side in the vicinity below the umbilicus. Axillary treatment sites also measured 5 cm x 5 cm, and one was located in each axilla.

Treatments were applied over each 5 cm x 5 cm area of skin as defined below after collection of the second baseline sample.

1. Amu-Skin (Amuchina 10% Solution)-A sterile proctoscopic swab was saturated with Amu-Skin and the treatment site scrubbed in a circular fashion for one minute. After scrubbing, the area was covered for four minutes with a sterile gauze pad which has been saturated with Amu-Skin. This was considered the end of the treatment. Timing was begun for post-treatment collection at this time.
2. Placebo was applied same as Amu-Skin (Amuchina 10%)
3. Betadine solution was applied according to these same directions.

Ten (10) minutes after treatment, one site in the axilla and or one abdominal site on subjects in each treatment group was randomly chosen and sampled using the cup scrub technique described under MICROBIOLOGICAL METHODS.

Thirty (30) minutes post-treatment, the remaining treated contralateral sites on approximately half the subjects in each treatment group were sampled. The sites on the remaining subjects were sampled 4 hours post-prep.

The following table summarizes the results of the study. This table shows the mean log₁₀ reduction achieved by each treatment at each sampling interval. Also shown is the number of subjects (n) from which the data was derived.

MEAN LOG₁₀ REDUCTION FROM BASELINE

Test Article	Axillary Sites Time Post-Treatment			Abdominal Sites Time Post-Treatment		
	10 min. (n)	30 min. (n)	4 hrs. (n)	10 min. (n)	30 min. (n)	4 hrs. (n)
A	1.2147 (13)	1.7273 (6)	1.0601 (7)	1.1090 (3)	1.0110 (1)	2.1650 (2)
B	0.7639 (11)	0.9136 (5)	0.8721 (6)	0.6467 (4)	-1.5172 (1)	0.7607 (3)
C	1.4950 (13)	1.4345 (6)	1.5935 (7)	3.0653 (4)	2.9027 (1)	1.5141 (3)

The statistical analysis of the axillary data indicates that the post-treatment data for Test Materials "A"(Amu-Skin) and "C"(Betadine Solution) were significantly different from baseline after each sampling (10 minutes, 30 minutes and 4 hours). The analysis indicated that the post-treatment data for Test Material "B"(Saline) was significantly different from baseline after the 10 minute and 30 minute samplings.

When the Test Materials were compared at the individual treatment intervals, it was determined the Test Materials "A" had significantly better reduction from baseline than Test Materials "B" following the 30 minute sampling.

No statistical analysis was made on the abdominal data because of the limited number of sites that were tested.

The log reductions determined, again show comparability with the Betadine solution (iodophor). The data show the variability of the baseline and recovery counts. The results from abdominal sites, were not extensively analyzed because a full complement of subjects could not be found with the target population (the required levels and reductions have since changed).

3. Report for Comparison of Two Procedures for Pre-operative prepping with Amu-Skin (ExSept Plus, .10% sodium hypochlorite, 10% Amuchina).

Study by: Hill Top Research Inc., Miamiville, OH.

Many operative prep procedures involve cleaning and/or treatment of the skin with alcohol prior to surgery or as part of the formulation and use of the prep. This study (120) used a cleanser or alcohol prior to prepping with soaked gauze pads followed by covering with a saturated pad for 5 minutes.

The objective of this study was to compare the antimicrobial effectiveness of two pre-operative skin prepping procedures using Amu-Skin (Amuchina electrolytic chloroxidizer, 10% solution). The study was a within-subjects, paired comparison of two methods of pre-operative skin prepping. Bacterial reduction at 10 minutes and 30 minutes was determined on abdominal and groin sites. The treatment and sampling configuration resulted in 12 samplings of each treatment at two time intervals after application.

The test articles used in this study were: AMU-SKIN, Lot: 4396 (HTR Code A), 70% ethanol (HTR Code B), and AMU-SOF, Lot: 07 (HTR Code C, cleansing agent).

There was no statistically significant difference in the effectiveness of the two pre-operative skin prepping procedures used to apply Amu-Skin Lot 4396. When evaluated for use on abdominal sites, prepping procedure Test Method A provided a 2.2977 \log_{10} reduction. The proposed FDA Criteria for a patient pre-operative skin prep on an abdominal site requires greater than a two \log_{10} reduction.

When Amu-Skin Lot 4396 was evaluated for efficacy as a pre-operative skin prep for the groin area, neither Test Method A or Test Method B provided the minimum 3 \log_{10} reduction that has been proposed by the FDA. Test Method A provided a 2.658 and Method B, a 2.5272 \log_{10} reduction.

The raw data showing actual bacterial counts for each subject selected for the study are shown in the attached study.

Appendix VIII-A Baseline Bacterial Counts and Post-Treatment Bacterial Counts

Appendix VIII-B Baseline Bacterial Counts for the Excluded Subjects

When comparing prep Test Method A for applying Amu-Skin Lot 4369 to prep Test Method B as pre-operative skin prep, procedures Test Method A provided slightly greater log reductions than Test Method B though no statistically significant differences were found between \log_{10} reductions achieved by the two Test Methods on either abdominal site or groin sites.

Clearly, the two prepping methods increased the log reduction when compared to reductions achieved with other prepping techniques.

These clinical studies show comparability with the most commonly used prep in hospitals; iodophor solution. ExSept Plus provides rapid, broad spectrum reduction of the bacterial flora on areas of the skin for site access.

The accumulation of data from in vitro studies, historical clinical use and clinical studies using protocols delineated by FDA in their Tentative Final Monograph show that ExSept Plus is safe and effective for use as an access site preparation product.

The Commissioner should reopen the record for the Antiseptic Health-Care Products TFM to include sodium hypochlorite 0.11 percent as an access site preparation.

Prevention of Infection Clinical Testing

Many experts, infectious disease personnel and other physicians (often without microbiological experience) have often suggested that prospective prevention of infection studies be performed to assure the effectiveness of topically applied presurgical antimicrobial products. This has included surgical scrubs, pre-op preps, hand washing products and other antiseptics products for many uses. The idea is good, but appropriate execution is difficult.

Semmelweis really answered the question of the effect of hand washing and topical application with his groundbreaking studies of childbed fever in 1889 (2). This work was done with an extremely high infection rate with death as an outcome and when antibiotics weren't used. In the U.S., we have been successful in reducing hospital infection rates over the last few decades. It is often hard to know the exact infection rate for reasons of public health and legal implications. From many sources, it may be assumed that it ranges from 2 to 4 percent. In order

to show the effect of any single element in preventing infection, test groups would have to be in the thousands (with hexachlorophene use and prevention of neonatal infection, the estimate by CDC for a proposed study was 10,000 subjects per group). Of course, if the infection rate is often increased, say doubled, the task is easier. The studies by Taplin (121) and Allen (122) and Taplin (123) showed a significant reduction of superficial topical infection rate in a closed population when compared to a placebo. It would be virtually impossible to determine differences if two active products were compared.

The development of infection (superficial, topical or surgical site) is most often in patients who are already ill and many of the patients themselves contribute several pre-disposing factors to infection development. We no longer have a simple one-factor cause and effect world. The cause of these infections is multi-factorial.

In the CDC Guideline for Prevention of Surgical Site Infection, 1999 (124), the very extensive set of factors to be considered in surgical site infection are elaborated. The microbiological effects of surgical preparation includes hand and arm washing as well as skin preparation. These are only two elements considered. Risk factors for infection include diabetes, nicotine use, steroid use, malnutrition, length of hospital stay, pre-operative transfusion, and colonization of the anterior nares with Staphylococcus aureus.

Some operative characteristics that affect surgical site infection include antiseptic showering, hair removal, and antimicrobial prophylaxis. The operating room environment, surgical attire and drapes, surgical technique and incision care can all affect infection.

These huge number of variables that defy constancy for subjects who might be selected for a clinical trial. These factors combined with the number of subjects required to determine a significant difference make the study of a single factor highly unreliable. In clinical trials of this type, it would be unethical to eliminate pre-operative preparation in an untreated control group.

CDC has promulgated a revision of their 1995 "Guideline for Prevention of Intravascular Device Related Infections" (125). This guideline and the Surgical Site Guideline (124) contain extensive references concerning prevention of infection studies (many inconclusive and flawed) and the many extraneous factors involved in either prospective or case control studies. This new guideline may be changed since the results of comments will be incorporated into the final.

There have been many control factors initiated to prevent these site infections and clearly, the antiseptic preparation of the skin and hands is merely one factor among many.

Conclusion

The data presented in this petition have shown the effectiveness and safety of sodium hypochlorite, 0.11 percent for inclusion in the TFM for Health-Care Antiseptic Products. Comparative effectiveness to ingredients already included in the monograph such as iodophors and chlorxylenol (PCMX) has been shown.

As set forth in this petition, sodium hypochlorite has been used for a material time and to a material extent as a wound antiseptic and has been demonstrated to be safe and effective. Therefore, sodium hypochlorite meets the requirements for marketing without approval of a New Drug Application. Amuchina respectfully requests the inclusion of 0.05 to 0.5 percent sodium

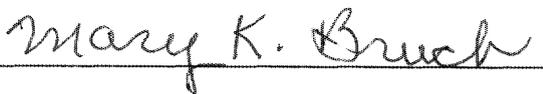
hypochlorite as a generally recognized safe and effective ingredient in the Health-Care Antiseptic Products TFM.

III. ENVIRONMENTAL IMPACT

In accordance with 21 C.F.R. § 25.31(b), an environmental impact analysis is not required.

IV. CERTIFICATION

The undersigned certifies that, to the best of the undersigned's knowledge and belief, this petition includes all information and views on which the petition relies. Further, this petition includes representative data, favorable and unfavorable, that are presently available to the petitioner.



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Note: Please note that Amuchina has changed the name of the company to Alcavis International, Inc.

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