To: USDA/AMS/NOP, Standards Division
Attention: National List Manager
1400 Independence Ave. SW
Room 2642-So., Ag Stop 0268
Washington, DC 20250-0268

Petitioner: Innovacyn, Inc.
3546 North Riverside Avenue
Rialto, CA 92377
Contact: Rebecca Lei / Scott van Winkle
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Petition to Include Hypochlorous Acid (generated by Electrolyzed Water) onto National List 7 CFR § 205.603 – Volume I

Background:

On September 11, 2015, the National Organic Program (NOP) issues a Policy Memorandum (PM 15-4), which updates the status of electrolyzed water under the U.S. Department of Agriculture (USDA) organic regulations at 7 CFR Part 205. The memorandum clarifies that electrolyzed water is a type of chlorine material that is allowed in organic production and handling. In particular, the NOP considers Hypochlorous Acid generated by electrolyzed water to be an allowable type of Chlorine material, which is currently allowed to be used in organic production and handling per the NOP 5026 guidance. However, the allowable uses of Chorine materials in organic production and handling does not include applications as topical treatment materials.

As Innovacyn, Inc. manufactures and uses Hypochlorous Acid solutions as a wound and pink eye treatment product for livestock animals, it has contacted Ms. Lisa Brines at the USDA NOP regarding this issue. Ms. Lisa Brines has reviewed the product labels of Innovacyn's Hypochlorous Acid-based wound and pink eye treatment products, and has given the following response on June 20th, 2016.

> *Hi Rebecca,*
>
> *Thank you for your call on Friday and for sending the product labels.*
>
> *As you are aware, the NOSB recently recommended hypochlorous acid as a chlorine material for 205.603(a). The current allowance for chlorine materials on section 205.603 of the National List states "Chlorine materials—disinfecting and sanitizing facilities and equipment" (i.e., no direct use on animals). In addition, treatment for pinkeye and livestock wounds is not addressed in the 2016 NOSB recommendation to add hypochlorous acid.*
>
> *Therefore, you would need to submit a petition for these additional labeled uses on livestock. Most medical treatments appear on section 205.603(a) of the National List, but the placement determination would need to be made by the NOSB. The petition guidelines only require that you indicate the section (i.e., 205.603), so you*

do not need to specify (a) or (b). In addition, I would suggest limiting the petition to hypochlorous acid, since that is the active ingredient indicated on both product labels.

Additional information on the petition process is available here: NOP 3011 - National List Petition Guidelines (PDF).

If you have any further questions, please let me know. Thanks!

Sincerely,

Lisa M. Brines, Ph.D.
National List Manager
National Organic Program
USDA Agricultural Marketing Service
Direct: 202-821-9683
lisa.brines@ams.usda.gov

Accordingly, Innovacyn hereby submits the enclosed petition. This petition is prepared by responding to the items under Section 4.2 of the National List Petition Guidelines, and does not contain any Confidential Business Information.

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Questions are in blue bold fonts and responses in black fonts.

Item A.1 — Indicate which section or sections the petitioned substance will be included on and/or removed from the National List. The current National List may be viewed at www.ams.usda.gov/NOPNationalList.

Please accept the enclosed petition to include ***Hypochlorous Acid (HOCl) generated by Electrolyzed Water*** into Section §205.603, Synthetic Substances Allowed for Use in Organic Livestock Production, of the National List. Specifically, the petition is made for the application of HOCl as a topical treatment substance for livestock animals.

Item A.2 — OFPA Category - Crop and Livestock Materials
For substances petitioned for use in crop or livestock production, eligible substances must contain an active synthetic ingredient in one of the following OFPA categories (7 U.S.C. § 6517(c)(1)(B)(i)):

- **Copper and sulfur compounds;**
- **Toxins derived from bacteria;**
- **Pheromones;**
- **Soaps;**
- **Horticultural oils;**
- **Fish emulsions;**
- **Treated seed;**
- **Vitamins and minerals;**
- **Livestock parasiticides and medicines; and**
- **Production aids.**

Petitioners should indicate which OFPA category applies to their petitioned material. The OFPA categories referenced above do not apply to materials petitioned for use in organic handling or processing.

The petitioned substance, Hypochlorous Acid, will be used as an active ingredient in the product, which will be for treatments of wound and eye irritation, and thus fit into the category of livestock parasiticides and medicines.

Item A.3 — Inert Ingredients
If the substance is a synthetic inert ingredient intended for use in a pesticide product, please see NOP Notice 11-6 for more information.

Not applicable, the substance, Hypochlorous Acid, will be used as an active ingredient.

Item B—Provide concise and comprehensive responses in providing all of the following information on the substance being petitioned.
For petitions to add or change an annotation for a substance that is already on the National List, items 5-11 are optional. Petitioners are encouraged to address these items if the information has changed since the NOSB's original review of the substance.

1. Substance Name

Hypochlorous Acid (HOCl); CAS # 7790-92-3

2. Petitioner and Manufacturer Information

Company Name:	Innovacyn, Inc.	
Corporate Address:	3546 North Riverside Avenue, Rialto, CA 92377	
Corporate Number:	(909)822-6000	
Contact Information:	Rebecca Lei	| Regulatory and Quality Manager
	Scott Van Winkle	| Vice President of Sales & Marketing
Phone Number:	Rebecca Lei:	(909)237-3716
	Scott Van Winkle:	(909)237-3428
Email:	Rebecca Lei:	rebeccal@aquaoxindustries.com
	Scott Van Winkle:	scottv@innovacyn.com
Mailing Address:	3546 North Riverside Avenue, Rialto, CA 92377	
Manufacturing Facility:	Innovacyn, Inc. 3546 North Riverside Avenue, Rialto, CA 92377	

3. Intended or Current Use

Describe the intended or current use of the substance, e.g., use as a pesticide, animal feed additive, processing aid, nonagricultural ingredient, sanitizer, or disinfectant. If the substance is an agricultural ingredient, the petition must provide a list of the types of product(s) (e.g., cereals, salad dressings) for which the substance will be used and a description of the substance's function in the product(s) (e.g., ingredient, flavoring agent, emulsifier, processing aid)

The intended use of the substance will be used as a topical treatment for wound management and for treatment of eye irritation for all animal and livestock species. Specific uses include the following:

3.1. For the management of skin abrasions, lacerations, minor irritations, cuts and intact skin;
3.2. For cleaning and debriding of wounds, cuts, abrasions, skin irritations, skin ulcers, post-surgical incision sites, burns, and rashes on external teat ends;
3.3. For treating irritated eyes to help relieve burning, stinging and itching by removing air pollutants, e.g. pollen, smog and other foreign materials, and for washing away mucus secretions and discharges.

4. Intended Activities and Application Rate

Provide a list of the crop, livestock, or handling activities for which the substance will be used. If used for crops or livestock, the substance's rate and method of application must be described.

4.1. The intended use of the substance will be for topical treatment for all livestock and animal species including:

- 4.1.1. Birds
- 4.1.2. Cats
- 4.1.3. Cows
- 4.1.4. Dogs
- 4.1.5. Goats
- 4.1.6. Horses
- 4.1.7. Pigs
- 4.1.8. Rabbits
- 4.1.9. Sheep

4.2. The concentration of the substance in the final product will range from 0.012 – 0.015%. The substance will be applied to the animals' infected areas for 3 – 4 times a day, or as necessary, through a dressing saturated with the substance onto the infected areas or directly spraying the substance to saturate the infected areas. No rinsing will be required after treatment.

5. Manufacturing Process

Provide the source of the substance and a detailed description of its manufacturing or processing procedures from the basic component(s) to the final product.

The Hypochlorous Acid solution is first produced by the following electrolysis process. The starting materials of this process are purified water and a brine solution containing 24 – 26% Sodium Chloride (NaCl). The electrolysis process is summarized below.

This page ends here.

Feed Solution

1) Purified water is produced from reverse osmosis (RO) system.

2) A saturated Sodium Chloride (NaCl) solution is generated and stored in a brine tank.

3) The diluted NaCl solution for electrolysis is produced by mixing the purified water with the brine.

Electrolysis

4) Electrolyte solution passes through electrolysis cells connected in series.

5) The feed solution enters the electrolytic cells with the intermediate Sodium Hydroxide (NaOH) being generated. Hydrogen gas (H_2) by-product is degassed from the system.

6) The NaOH solution undergoes treatment to form Hypochlorous Acid (HOCl) and Hypochlorite ions (OCl-). Oxygen gas (O_2) by-product is degassed from the system.

Collection

7) HOCl solution generated from electrolyzed water is produced and collected.

The electrolysis process entails the conversion of a Sodium Chloride solution to a Hypochlorous Acid solution. The feed solution for electrolysis is a dilute saline solution composed of less than 0.1% NaCl prepared by mixing a saturated brine solution containing 24 – 26% NaCl with reverse osmosis purified water. The feed solution then enters into an electrolysis process, details of which are proprietary information. The product solution is a mixture of both HOCl and Sodium Hypochlorite (NaOCl). The percentage of these two chlorine species in the product solution is driven by pH and temperature. The maximum percentage of HOCl is found when the solution is at pH 5 at 0°C. At such condition, the solution contains 99.85% HOCl and 0.15% NaOCl. Produced at a free available chlorine (FAC) ranging from 130 – 160 parts per million (ppm), pH 6.3 and 30°C, this product solution contains approximately 0.012 – 0.015 % HOCl and 0.001% NaOCl. The following equations illustrate the reactions that occur during the electrolysis process.

$$2\ Na^+ + 2H_2O \rightarrow 2\ NaOH + H_2\uparrow$$

$$2Cl^- \rightarrow Cl_2 + 2e^-$$

$$2\ H_2O \rightarrow O_2\uparrow + 4\ H^+ + 4e^-$$

$$Cl_2 + 2OH^- \rightarrow OCl^- + Cl^- + H_2O$$

The electrolyzed HOCl solution is then dosed with Monobasic Sodium Phosphate (NaH_2PO_4), Dibasic Sodium Phosphate (Na_2HPO_4), and a proprietary blend of Sodium Phosphates. The total concentration of these inert ingredients does not exceed 0.05% w/w of the final product

concentration. Due to the proprietary nature of the information, the exact concentration of these inert ingredients is not disclosed.

6. Ancillary Substances

For substances petitioned for use in organic handling or processing, provide information about the ancillary substances (including, but not limited to, carriers, emulsifiers, or stabilizers) that may be included with the petitioned substance, including function, type of substance, and source, if known.

The final product solution also contains less than 0.1% of Sodium Chloride and Blended Phosphates as inert ingredients. Sodium Chloride is residual salt from the electrolysis process and does not have any function in the formulation. The phosphate blend contains Monobasic Sodium Phosphate, Dibasic Sodium Phosphate, and a proprietary blend of Sodium Phosphates. The Monobasic and Dibasic Sodium Phosphates act as pH buffer in the formulation, while the proprietary Sodium Phosphate blend acts as a chelating agent which stabilizes the product. The product solution also contains an insignificant amount of Sodium Hypochlorite, i.e. < 0.001%, which is the counterpart of Hypochlorous Acid at that given pH and temperature.

Component	**Source**
Electrolyzed Water (H_2O)	Generated in-house
Hypochlorous Acid (HOCl)	Generated in-house
Sodium Hypochlorite (NaOCl)	Generated in-house
Sodium Chloride (NaCl)	Purchased from VWR
Blended Polyphosphate	Purchased from Aquaox Industries, Inc.
Sodium Phosphate, Monobasic (NaH_2PO_4)	Purchased from Spectrum Laboratory Products, Inc.
Sodium Phosphate, Dibasic (Na_2HPO_4)	Purchased from Spectrum Laboratory Products, Inc.

7. Previous Reviews

Provide a summary of any available previous reviews of the petitioned substance by State or private certification programs or other organizations. If this information is not available, this should be stated in the petition.
If the substance has been previously reviewed and rejected by the NOSB, the petition must provide new information that was not submitted in an earlier petition or provided for in the previous technical reports for the substance.

Per Policy Memo 14-3 issued by the NOP on June 9, 2014, Hypochlorous Acid is a synthetic substance that is not included on the National List and cannot be used on organic production or processing. On May 29th, 2015, Botanical Food Company Pty Ltd. has submitted a petition to include HOCl (as produced by the electrochemical activation of Sodium Chloride and water)

for the annotation of Chlorine Materials as per 7 CFR § 205.600 – 606. As a result, the NOSB has evaluated the case and has published formal recommendations for the addition of HOCl (generated from electrolyzed water) onto § 205.601(a), § 205.603(a)(7)(iv) and § 205.605(b) of the National List on April 26th, 2016. On September 11th, 2015, The NOP has also issued Policy Memo 15-4, which noted that the NOP considers HOCl generated by electrolyzed water as an allowable type of chlorine material. However, HOCl generated from electrolyzed water is still not allowed to be used for topical treatment applications under § 205.603(b).

8. Regulatory Authority

Provide information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers. The information provided must confirm that the intended use of the substance is permitted under EPA or FDA regulations, as applicable.

Per 40 CFR § 180.940, Hypochlorous Acid, when not exceeding 200 ppm determined as total available chlorine, is exempted from the requirement of a tolerance when used in accordance with good manufacturing practice as ingredients in an antimicrobial pesticide formulation on a semi-permanent or permanent food-contact surfaces, including those surfaces in public eating places, dairy-processing equipment, and food-processing equipment and utensils.

Hypochlorous Acid products have a long history as an antimicrobial wound care product. Innovacyn, Inc. has received a 510(k) clearance on the Puracyn Plus Skin and Wound (K133542), an HOCl-based wound management and cleansing solution intended for cleansing, irrigating, and debriding dermal wounds in addition to moistening and lubricating absorbent wound dressings. This Puracyn Plus product has exactly the same formulation as discussed in Question 6 above, except that it contains a higher HOCl concentration. In addition to the Puracyn Plus, the following lists some example products containing HOCl as active ingredient which have also been 510(k) cleared for similar wound care applications on human.

- K090206: Oculus Puracyn Antimicrobial Skin and Wound Cleanser, Oculus Innovative Sciences, Inc.
- K093697: Vashe® Wound Therapy Solution (OTC use), PuriCore, Inc.
- K123072: Vashe® Wound Therapy Solution (Professional use), PuriCore, Inc.
- K113693: Nixall™ Wound and Skin Care (OTC and Professional use), Seriously Clean Ltd.

Further, Hypochlorous Acid products are widely used as surface disinfectants and sanitizers. Aquaox, Inc., Innovacyn's sister company, has obtained an EPA registration on the same 510(k) cleared formulation (EPA # 85021-04). This product formulation is registered to be used as a one-step hard surface disinfectant. The following lists more examples of EPA registered disinfectants containing Hypochlorous Acid as active ingredients.

- EPA # 82341-4: Excelyte VET, IET, Inc.
- EPA # 89896-2: Clean Smart, Simple Science, Ltd.
- EPA # 91685-2: FloraFresh® Floral Quality Care Solution, PuriCore, Inc.
- EPA # 87518-3: HSP2O Pro Disinfectant, HSP USA, LLC.

For food ingredients and processing aids, the substance must be approved by FDA for the petitioned use. For pesticide active ingredients, the substance must have an EPA tolerance or tolerance exemption, as applicable. If this information does not exist or is not applicable, the petitioner should state this in the petition.

Not applicable, the substance being petitioned is not intended for use in food ingredients or processing aids.

9. Chemical Abstracts Service (CAS) Number and Product Labels

Provide the CAS number or other product numbers of the substance. If the substance does not have an assigned product number, the petitioner should state so in the petition. For food additives, the International Numbering System (INS) number should also be provided.

The CAS number of the petitioned substance, Hypochlorous Acid, is CAS # 7790-92-3. The part numbers of the product are 1000, 1002, 1004, 1007, 1008, 1100, 1101, and 1900, the different part numbers are due to the different container sizes and packaging configurations.

The CAS numbers of the other ingredients in the product solution are as following:

- Electrolyzed Water (H2O) 7732-18-5
- Sodium Chloride (NaCl) 7647-14-5
- Sodium Hypochlorite (NaOCl) 7681-52-9
- Sodium Chloride (NaCl) 7647-14-5
- Blended Polyphosphate (Proprietary Blend) 7758-29-4, 7320-34-5
- Sodium Phosphate, Monobasic (NaH2PO4) 7558-80-7
- Sodium Phosphate, Dibasic (Na2HPO4) 7558-79-4

This item should also include labels of products that contain the petitioned substance. If a product label does not apply to this substance, please provide a brief explanation. Product specification sheets, product data sheets, non-retail labels, or other product information may be substituted for the product label, if appropriate.

Product labels are attached in Appendix I below.

10. Physical and Chemical Properties

Provide the substance's physical properties and chemical mode of action including the following: (a) Chemical interactions with other substances, especially substances used in organic production; (b) Toxicity and environmental persistence; (c) Environmental impacts from its use and/or manufacture; (d) Effects on human health; and (e) Effects on soil organisms, crops, or livestock.

<u>Physical Properties:</u>

- Physical State: Liquid
- Color: Clear

- Odor: Slight Chlorine Odor
- pH: 6.2 – 7.2
- Relative Density (H_2O = 1 at 20°C): 1.00 – 1.06 at 20°C
- Viscosity: Comparable to Water
- Solubility: Complete in Water
- Boiling Point: Comparable to Water
- Evaporation Rate: ` Comparable to Water
- Melting Point: Not Applicable
- Flash Point: Not Applicable
- Flammability: Non-Flammable
- Upper Flammable Limit (UFL): Non-Flammable
- Lower Flammable Limit (LFL): Non-Flammable
- Upper Explosive Limit (UEL): Non-Explosive
- Lower Explosive Limit (LEL): Non-Explosive
- Partition Coefficient: Not Applicable
- Auto Ignition Temperature: Not Applicable
- Decomposition Temperature: Not Applicable
- Vapor Pressure: Not Applicable
- Vapor Density: Not Applicable

Mode of Action:

The petitioned substance acts as an active ingredient in the final product, in a concentration of 0.12 – 0.15 %. The final product is a clear hypotonic solution topically applied to skin and wound areas of animals to be treated. The product is a wound management and cleansing solution that is intended for cleansing, irrigating, and debriding dermal wounds in addition to moistening and lubricating absorbent wound dressings. The mechanical action of fluid moving across the wound provides for the mechanism of action and aids in the removal of foreign objects such as dirt and debris.

The active ingredient, Hypochlorous Acid, is a major inorganic bactericidal compound of innate immunity http://www.ncbi.nlm.nih.gov/pubmed/7487057, HOCl is produced by neutrophil granulocytes, the most abundant type of white blood cells in mammals. It is involved in the last step of the Oxidative Burst Pathway in fighting infection and foreign substance invasion. HOCl molecules are neutral and small in size, and is effective against a broad range of microorganisms. The antimicrobial activities of HOCl have been studied for long and its biocidal effects have been proposed to be contributed by the following actions, 1) by inhibiting glucose oxidation of the microbes, 2) through depletion of adenine nucleotides, 3) through inhibition of DNA replication, and 4) by causing protein unfolding and aggregation. All of the above four actions ultimately lead to the loss of viability of the microorganisms.
https://en.wikipedia.org/wiki/Hypochlorous_acid#Formation.2C_stability_and_reactions
The following journal further explains the mode of action and bactericidal action of HOCl, and its role in wound cleansing, irrigation and debridement. http://www.faim.org/the-use-of-hypochlorous-acid-solution-in-wound-management

According to a journal published by Wang and associates, a study has been done to demonstrate the potent antimicrobial activities using a stabilized form of HOCl against a wide

range of microorganisms. HOCl's *in vitro* cytotoxicity profile in L929 cells and the *in vivo* safety profile of HOCl in various animal models are characterized. This study concluded that on the basis of antimicrobial activity and the lack of animal toxicity, a stabilized form of HOCl has potential pharmaceutical applications in the control of soft tissue infection.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1853323/

(a) Chemical Interactions with other Substances:

Hypochlorous Acid does not react with any inorganic matters, but is instable in nature and breaks down readily upon exposure to any of the following conditions, 1) when exposed to strong acids, alkalis, oxidizing agents, organic and soil materials, and 2) when exposed to direct light exposure, freezing and high temperatures. In either case, the reactions involve the rapid utilization of HOCl, forming dilute saline solution as a harmless decomposition product. The degradation pathway of HOCl has been profiled Richard and associates, their study has concluded that HOCl decayed readily upon elevated temperatures and organic matters. http://www.antenna.ch/en/medias/Stability-of-sodium-hypochlorite-in-solution-after-adding-sodium-hydroxide_eng.pdf. The presense of light can also cause HOCl to decompose through a process known as photolysis. Another study by Feng et al. has shown the degradation profile of various chlorine species, including HOCl and OCl^- ions, when exposed to UV light. https://www.researchgate.net/publication/230745626_Photolysis_of_aqueous_free_chlorine_species_NOCl_and_OCl-_with_254_nm_ultraviolet_light. In fact, one of the reasons that cyanurates are used in swimming pools is to provide UV protection to the HOCl molecules. HOCl is also known to participate in numerous reactions with organic matter. Deborde et al. has done a study characterizing the reactions and reaction mechanisms of HOCl with various organic compounds and micropollutants.

Lastly, Innovacyn has done a spectrum of stability and material compatibility studies on the HOCl products, at the HOCl concentration range of our products, we have found no or insignificant material compatibility issues nor any chemical residues when using this product on various hardsurfaces.

(b) Toxicity and Environmental Persistence:

Due to the proprietary nature of the information, below is only an excerpt of the original report. Innovacyn, Inc. has performed a panel of ISO-10993 Biocompatibility Testing on the Puracyn Plus Skin and Wound Care product, which contains a higher HOCl concentration, i.e. 0.024%, than the product being petitioned. The testing program consisted of the following seven studies, an *in vitro* cytotoxicity study using L929 mouse fibroblast cells, a primary dermal irritation test in rabbits, an ocular irritation study in rabbits, a dermal sensitization study in guinea pigs, an acute oral toxicity study in rats, an acute inhalation toxicity test in rats, and a 28-day repeated dose dermal toxicity study in rats with intact and full thickness wounded skin. The study and summary results are given in the following table.

Test Type	Species	Route	Result	Testing Facility	Study Report Number
Cytotoxicity	L-929 Cells	*In vitro*	Not cytotoxic	NAMSA	13T-25324-03
Skin Irritation	Rabbit	Dermal	Not a skin irritant	NAMSA	13T-25324-05
Eye Irritation	Rabbit	Ocular	Not an eye irritatnt	NAMSA	13T-25324-04
Sensitization	Guinea Pigs	IC / Dermal	Not a sensitizer	NAMSA	13T-25324-07
Acute	Rat	Oral	Non-toxic	NAMSA	13T-25324-06
Acute	Rat	Inhalation	Non-toxic	IITRI	2420
28-Day Repeacted Dosing to Intact and Wounded Skin	Rat	Dermal	No local or systemic effects	NAMSA	13T-25462-02

As shown in the above table, he Puracyn Plus product is not cytotoxic, not a skin irritant, not an eye irritant, not a dermal sensitizer, not toxic, nor does it exhibit any local or systemic effects in the event of long term exposures. Therefore, the product being petitioned, which contains a less HOCl concentration, should be equally safe, if not more.

Furthermore, a toxicity waiver rationale has been performed on Aquaox's EPA registered surface disinfectant formulation, which contains 0.0275% Hypochlorous Acid as active ingredient. This rationale is performed following the EPA Health Effects Test Guidelines, OPPTS Series 870, and covers the following six areas, acute oral toxicity (870.1100), acute dermal toxicity (870.1200), acute inhalation toxicity (870.1300), primary eye irritation (870.2400), primary dermal irritation (870.2500), and dermal sensitization (870.2600). This studies presented in this assessment supports the 0.0275% HOCl disinfectant formulation to be of the EPA Toxicity Category IV, i.e. practically non-toxic and not an irritant, in all the six areas being assessed. The rationale report is attached in Appendix II.

The Final Report of the Sanizier Committee (2008 – 2010) regarding on-site generated Hypochlorous Acid and Sodium Hypochlorite also supports HOCl to be used for various disinfecting / sanitizing applications and treatment applications on humans including to treat acute and chronic wounds, as wound care irrigants and as endodontic cleansers. http://www.foodprotect.org/issues/packets/2010ScribePacket/attachments/III_005_a.pdf

Due to the instable nature of Hypochlorous Acid when exposed to veracious compounds, as discussed in response to Question 10(a) above, HOCl does not persist in the environment nor in the food chain because it decomposes readily upon exposure to light, freezing or heat, strong acids, alkalis, oxidizing agents, organic and soil materials, all of which are readily available in the environment. Its decomposition product, dilute saline solution, is harmless to practically all terrestrial and aquatic organisms. As discussed above, even if any residual HOCl were to persist for a short amount of time in the environment, it does not have any acute or chronic effects to the living macro-organisms at the concentration in this product.

(c) Environmental Impacts from its Use and/or Manufacture:

As aforementioned, Hypochlorous Acid is not an environmental persistent chemical due to its instable nature and thus does not last in the food chain nor in the ecosystem. Its decomposition product, dilute saline solution, is harmless to lifeforms. HOCl, at the

concentration in this product, does not pose any acute or chronic effects to lifeforms. Therefore, the use and manufacturing of HOCl poses negligible impact to the environment and lifeforms in general. The byproducts generated during from its manufacture include the Catholyte water which is of a pH of 11 – 12, Oxygen and Hydrogen gas. The Oxygen and Hydrogen gases readily diffuse in air and reaches equilibrium with other gas molecules, and thus do not pose any harm to the environment. The Catholyte water, which consists of approximately 0.02% Sodium Hydroxide (NaOH), is always ensured to be probably stored and neutralized before disposal per Innovacyn's manufacturing procedures. When neutralized, NaOH reacts readily with protic acids to produce water and the corresponding salts, both of which are harmless to the environment and living organisms.

(d) Effects on Human Health:

As discussed in response to Question 10(b), various *in vitro* and *in vivo* studies have shown that Hypochlorous Acid, at the concentration of this product, is practically not toxic, not an irritant, not a sensitizer, nor does it exhibit any local or systemic effects in the event of long term exposures. In addition, per 40 CFR § 180.940 as mentioned in response to Question 8 above, HOCl, when not exceeding 200 ppm determined as total available chlorine, are exempted from the requirement of a tolerance when used as ingredients in an antimicrobial pesticide formulation and may be applied to food-contact surfaces in public eating places, dairy-processing equipment, and food-processing equipment and utensils. Besides, as discussed in the same response, the FDA has given 510(k) clearances to various Hypochlorous Acid based products, at a concentration higher than the petitioned product, as wound care products. Hence, all of the above support that HOCl, at the concentration of the petitioned product, does not have any adverse effects against human and animal health, or against the whole ecosystem in general.

(e) Effects on Soil Organisms, Crops, or Livestock:

To further reiterate, Hypochlorous Acid does not have any negative impact on soil organisms, crops, or livestock due to its instable nature, safety profile, and harmless decomposition products. HOCl does not persist in the environment due its instable nature and due to the fact that the compounds causing HOCl to decay are readily available in the environment. Most importantly, HOCl does not have any acute or chronic toxicity effects to any macro-organisms as shown by the aforementioned study reports. All of these points have been discussed in previous sections.

Moreover, the NOSB has already recommended for Hypochlorous Acid (generated from Electrolyzed Water) to be listed in the following sections of the National List, § 205.601 synthetic substances allowed for use in organic crop production; § 205.603(a)(7)(iv) synthetic substances allowed for use in organic livestock production (particularly for disinfecting and sanitizing facilities and equipment); and § 205.605(b) nonagricultural (nonorganic) substances allowed as ingredients in or on processed products labeled as "organic" or "made with organic" of the National List.

As indicated above and in previous sections, Hypochlorous Acid (at the given concentration of this product) is not toxic, is not environmental persistant, does not pose

any harmful effects to the environment, human or animal health from its use or manufacture, and does not have any harmful effects on soil organisms, crops and livestock.

11. Safety Information

Provide safety information about the substance including a Material Safety Data Sheet (MSDS) and a substance report from the National Institute of Environmental Health Studies. If this information does not exist or is not applicable, the petitioner should state so in the petition.

Safety Data Sheet:

- Vetericyn Plus OTC Liquid Products Appendix III
- Vetericyn Plus VF Liquid Products Appendix IV

Substance report from the National Institute of Environmental Health Studies:

- Chloraminated water - M910056
  http://ntp.niehs.nih.gov/testing/status/agents/ts-m910056.html
- Chlorinated water - M910004
  http://ntp.niehs.nih.gov/testing/status/agents/ts-m910004.html

12. Research Information

This item should include research information about the substance. The research should include comprehensive substance research reviews and research bibliographies, including reviews and bibliographies that present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on or removal from the National List.

For petitions to include nonorganic agricultural substances on the National List for organic handling, this information should include research on why the substance should be permitted in the handling of an organic product, including the availability of organic alternatives.

If research information does not exist for the petitioned substance or for the contrasting position, the petitioner should state so in the petition.

Appendix V lists all literature research. Since the primary application of this product is for management of wound and eye irritation for animal and livestock species, the literature review has been focused on this area. Limited literature research can be found against the usage of Hypochlorous Acid on this application.

a) Environmental Assessment of Hypochlorous Acid Solution from HSP USA, LLC, for Food Contact Notification FCN No. 001176.
b) Environmental Decision Memo for Food Contact Notification No. 001176.
c) Environmental Assessment of Hypochlorous Acid Solution from Sterilox Food Safety/Div. of PuriCore for Food Contact Notification FCN No. 001470.
d) Environmental Decision Memo for Food Contact Notification No. 001470.
e) Hypochlorous Acid as a Potential Wound Care Agent. Part I. Stabilized Hypochlorous Acid: A Component of the Inorganic Armamentarium of Innate Immunity.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1853323/

f) Hypochlorous Acid as a Potential Wound Care Agent: Part II. Stabilized Hypochlorous Acid: Its Role in Decreasing Tissue Bacterial Bioburden and Overcoming the Inhibition of Infection on Wound Healing. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1853324/
g) Hypochlorous Acid: An Ideal Wound Care Agent with Powerful Microbicidal, Antibiofilm, and Wound Healing Potency. http://www.ncbi.nlm.nih.gov/pubmed/25785777
h) Hypochlorous acid: its multiple uses for wound care. http://www.o-wm.com/files/owm/pdfs/OWM_September2013_Liden.pdf
i) Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers. http://www.ncbi.nlm.nih.gov/pubmed/16669304
j) Conquering Chronic Non-Healing Wounds with Pure Hypochlorous Acid. http://novabay.com/wp-content/uploads/2013/05/Symposium-on-Advanced-Wound-Care-Conquering-the-Chronic-Non-Healing-Wounds-with-NeutroPhase%c2%ae.pdf
k) A Randomized Clinical Trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wound. http://www.iiste.org/Journals/index.php/JEP/article/viewFile/13072/13653
l) Use of Hypochlorous Acid Solution as a Disinfectant in Laboratory Animal Facilities. http://www.jscimedcentral.com/MedicalMicrobiology/medicalmicrobiology-1-1005.pdf
m) Living with a killer: the effects of hypochlorous acid on mammalian cells. http://www.ncbi.nlm.nih.gov/pubmed/11327319

Innovacyn has also collaborated with universities to perform the following studies showing the efficacy of the Hypochlorous Acid based products treating wounds, eye irritation, and hairy foot warts in animals. Particularly, the study performed by Auburn Univerisity explained the advantages of using HOCl over traditional antibiotics (oxytetracycline antibiotics) for treating pink eye in animals.

n) White Paper: Evaluation of Vetericyn Plus™ Pinkeye Spray as an aid in corneal healing and reduction of pain and infection of the cornea following experimentally induced Bovine Keratoconjunctivitis, Auburn University College of Veterinary Medicine, Auburn University.
o) Study Report: The Effect of Vetericyn® Technology (hypochlorous acid) on the Treatment of Hairy Foot Warts in Dairy Cows, California Polytechnic State University, San Luis Obispo.
p) Pilot Study Report: The Effect of Puracyn® Plus on Deep Dermal Infections, Miller School of Medicine, University of Miami.

On top of that, Innovacyn has performed the following antimicrobial effectiveness testing at NASMA following the USP<51> guidelines. The HOCl-based product being petitioned was able to obtain a >7 log of reduction on the following microbes in as short as 15 seconds of exposure time. Due to the proprietary nature of the information, only the result section of the study report is disclosed below.

Organisms	Theoretical Inoculum Concentration per mL of Test Article	CFU/mL Duplicate Average after Exposure	Log Reduction
Klebsiella pneumoniae	1.32×10^7	$< 1.00 \times 10^7$	> 7.21
Enterococcus faecalis	4.10×10^7	$< 1.00 \times 10^7$	> 7.61
Methicillin-resistant *Staphylococcus aureus* (MRSA)	6.55×10^7	$< 1.00 \times 10^7$	> 7.82
Staphylococcus epidermidis	5.80×10^7	$< 1.00 \times 10^7$	> 7.76
Acinetobacter baumannii	8.50×10^7	$< 1.00 \times 10^7$	> 7.93
Staphylococcus aureus	8.75×10^7	$< 1.00 \times 10^7$	> 7.94
Pseudomonas aeruginosa	5.20×10^7	$< 1.00 \times 10^7$	> 7.72
Escherichia coli	8.90×10^7	$< 1.00 \times 10^7$	> 7.95
Candida albicans	2.30×10^7	$< 1.00 \times 10^7$	> 7.36

13. Petition Justification Statement

This petition and the following petition justification statement is for A) the inclusion of a Synthetic on the National List, § 205.601, 205.603, 205.605(b). The following justification statement will:

- **Explain why the synthetic substance is necessary for the production or handling of an organic product.**
- **Describe any non-synthetic substances, synthetic substances on the National List or alternative cultural methods that could be used in place of the petitioned synthetic substance.**
- **Describe the beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support its use instead of the use of a non-synthetic substance or alternative cultural methods.**

Currently, the only synthetic materials that are listed under § 205.603, synthetic substances allowed for use in organic livestock production, and that are related to wound management are Copper Sulfate (§ 205.603(b)(1)) and Iodine (§ 205.603(b)(3)).

Copper Sulfate is notorious for its applications in treating hairy foot warts but is not generally used for wound treatment applications due to potential safety issues, i.e. Copper sulfate is moderately toxic upon oral exposure, is corrosive to the skin and eyes, and is a skin sensitizer. Copper sulfate is also known to be very toxic to fish, and to other aquatic invertebrate specifies such as crab, shrimp and oysters. Moreover, copper compounds can pose phytotoxicity or poisonous effects in plants by disrupting photosynthesis.
http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/copper-sulfate-ext.html#17

Iodine has been recently moved from List II to List I in the classification of illicit drugs regulated under the United States Drug Enforcement Administration (DEA). Iodine has been used for long as an antiseptic for both human and animal applications. Nonetheless, the following research, conducted by Mekkawy and associates, shows that Hypochlorous Acid has a superior biocidal efficacy when compared to Iodine.
http://www.iiste.org/Journals/index.php/JEP/article/viewFile/13072/13653 HOCl outweighs Iodine in their safety profile, the following research, performed by Paulíková et al., has also

shown the hidden risk of prolonged iodine consumption in ruminants. http://vri.cz/docs/vetmed/47-12-343.pdf

Tea tree oil is amongst the most widely used non-synthetic substance for wound healing, it has powerful antibacterial, antifungal and antiseptic properties. However, it is also known to be a skin sensitizer, and can cause skin irritation and swelling. Above all, its biocidal activity is also not as effective when compared to that of Hypochlorous Acid.

Given the above limited options, it is beneficial to include Hypochlorous Acid into § 205.603 of the National List, synthetic substances allowed for use in organic livestock production, in particular for the application as a topical treatment on livestock animals. HOCl is practically not toxic, not an irritant, not a sensitizer and does not pose any acute or chronic effects to human and animals. It is not environmentally persistent and does not last in the food chain due to its instable nature, and thus does not pose any harmful effects to human and animals, nor to the environment and ecosystem in general from the use or manufacture of it.

This page ends here.

Appendix I: Product Labels of Petitioned Substance

Stability: See below for date of expiration.

Disposal: No special disposal requirements. Vetericyn is environmentally friendly.

Ingredients: Active: Hypochlorous Acid (HOCl) (0.0120%) **Inactive:** Electrolyzed Water (H2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)

Storage: Store at room temperature away from direct sunlight and heat. **DO NOT ALLOW TO FREEZE** because product may lose its potency. After use, seal or close dispenser.

Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.

Made in the USA

Manufactured & Bottled by Innovacyn, Inc.
3546 N.Riverside Ave, Rialto CA 92377

vetericyn.com | 866.318.3116

Tell Us Your Story!

- *Non-irritating & non-sensitizing*
- *Safe if licked or ingested*
- *Works on all animal skin types*

Indications: Vetericyn Wound and Skin Care is intended for the OTC management of skin abrasions, lacerations, minor irritations, cuts and intact skin. Safe for use on all animal species.

Directions for use: Move excess hair from wound area. Clip if necessary. Adjust spray nozzle. Saturate the affected area. If dressing is required, saturate dressing with Vetericyn at each dressing change. Repeat 3-4 times per day until wound is healed. No rinsing necessary. Faster healing is achieved when wounds are cleaned and maintained moist. Safe for use around mouth, nose, ears and eyes.

Use Precautions: For external use only. Discontinue use if irritation develops and contact your veterinarian. **For animal use only. Not for human use.** Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.

Lot Number:

Expiration Date:

8 52009 00202 4

Part Number: 1000; 90003 v4.0

Vetericyn plus®

advanced cleanser *plus* therapy

Proven Safe & Effective

Wound & Skin Care

Veterinarian Recommended

Topical application for wounds, cuts, lacerations, abrasions, sores & skin irritations

Safe & non-toxic *No alcohol, steroids or antibiotics*

16 fl oz (473 ml)

Ingredients: Active: Hypochlorous Acid (HOCl) (0.0120%) **Inactive:** Electrolyzed Water (H_2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)

Storage: Store at room temperature away from direct sunlight and heat. **DO NOT ALLOW TO FREEZE** because product may lose its potency. After use, seal or close dispenser.

Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.

Made in the USA

Manufactured & Bottled by Innovacyn, Inc.
3546 N.Riverside Ave, Rialto CA 92377

vetericyn.com | 866.318.3116

Tell Us Your Story!

Lot Number:

Expiration Date:

8 52009 00201 7

- *Non-irritating & non-sensitizing*
- *Safe if licked or ingested*
- *Works on all animal skin types*

Indications: Vetericyn Wound and Skin Care is intended for the OTC management of skin abrasions, lacerations, minor irritations, cuts and intact skin. Safe for use on all animal species.

Directions for use: Move excess hair from wound area. Clip if necessary. Adjust spray nozzle. Saturate the affected area. If dressing is required, saturate dressing with Vetericyn at each dressing change. Repeat 3-4 times per day until wound is healed. No rinsing necessary. Faster healing is achieved when wounds are cleaned and maintained moist. Safe for use around mouth, nose, ears and eyes.

Use Precautions: For external use only. Discontinue use if irritation develops and contact your veterinarian. **For animal use only. Not for human use.** Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.

Stability: See left for date of expiration.

Disposal: No special disposal requirements. Vetericyn is environmentally friendly.

Part Number: 1002; 90006 v4.0

Vetericyn plus

advanced cleanser *plus* therapy

Proven Safe & Effective

Wound & Skin Care

Veterinarian Recommended

Topical application for wounds, cuts, lacerations, abrasions, sores & skin irritations

- *Safe & non-toxic*
- *No alcohol, steroids or antibiotics*

8 fl oz (237 ml)

Ingredients: Active: Hypochlorous Acid (HOCl) (0.01-0.20%) **Inactive:** Electrolyzed Water (H2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)

Storage: Store at room temperature away from direct sunlight and heat. **DO NOT ALLOW TO FREEZE** because product may lose its potency. After use, seal or close dispenser.

Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.

Made in the USA

Manufactured & Bottled by Innovacyn, Inc. 3546 N. Riverside Ave. Rialto CA 92377

vetericyn.com | 866.318.3116

Tell Us Your Story!

Lot Number:

Expiration Date:

8 52009 00230 7

- *Non-irritating & non-sensitizing*
- *Safe if licked or ingested*
- *Works on all animal skin types*

Indications: Vetericyn Wound and Skin Care is intended for the OTC management of skin abrasions, lacerations, minor irritations, cuts and intact skin. Safe for use on all animal species.

Directions for use: Move excess hair from wound area. Clip if necessary. Adjust spray nozzle. Saturate the affected area. If dressing is required, saturate dressing with Vetericyn at each dressing change. Repeat 3-4 times per day until wound is healed. No rinsing necessary. Faster healing is achieved when wounds are cleaned and maintained moist. Safe for use around mouth, nose, ears and eyes.

Use Precautions: For external use only. Discontinue use if irritation develops and contact your veterinarian. **For animal use only. Not for human use.** Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.

Stability: See left for date of expiration.

Disposal: No special disposal requirements. Vetericyn is environmentally friendly.

Part Number: 1004; 90026 v4.0

Vetericyn® plus

advanced cleanser *plus* therapy

Proven Safe & Effective

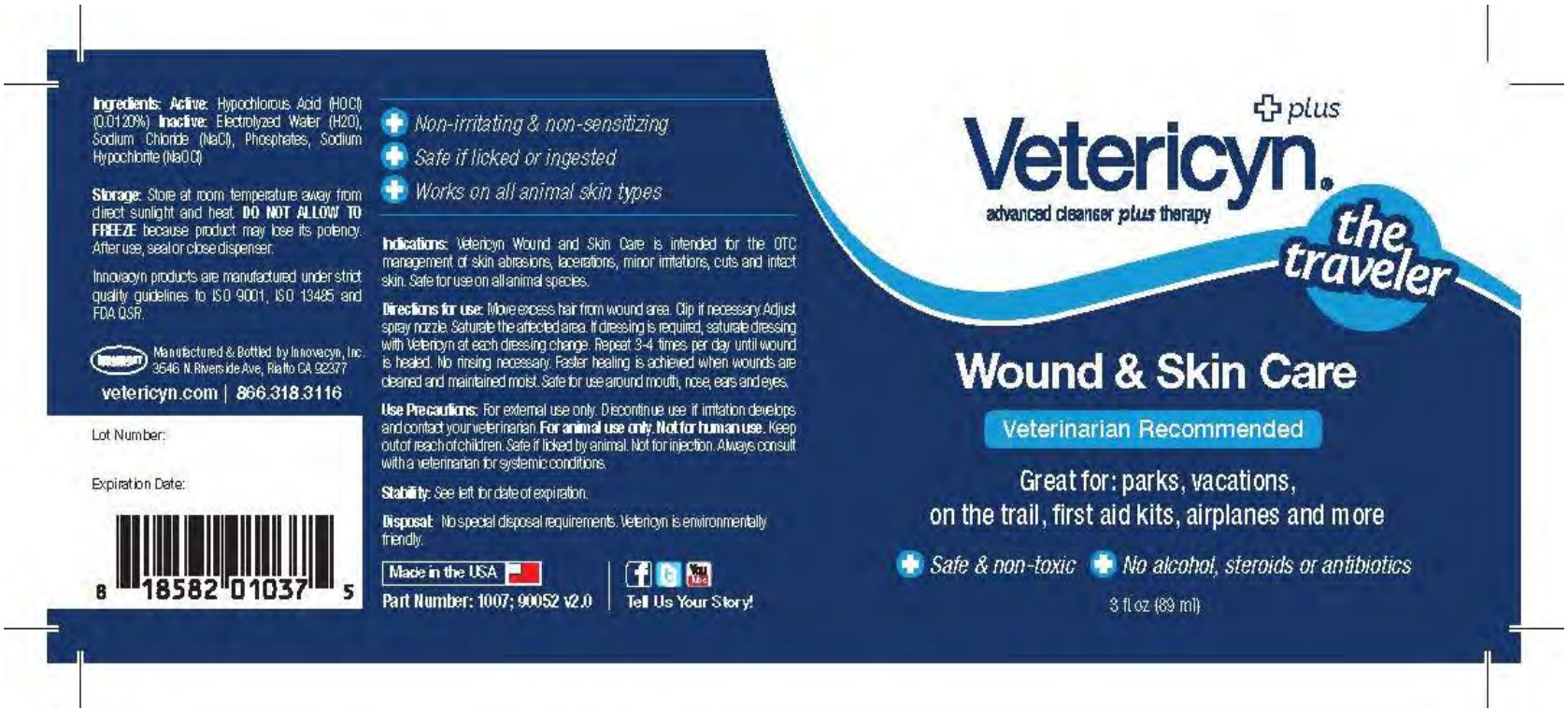
Wound & Skin Care

Veterinarian Recommended

Topical application for wounds, cuts, lacerations, abrasions, sores & skin irritations

Safe & non-toxic + *No alcohol, steroids or antibiotics*

4 fl oz (118 ml)


plus
Vetericyn.
advanced cleanser plus therapy
the traveler
Wound & Skin Care
Veterinarian Recommended
Great for: parks, vacations,
on the trail, first aid kits, airplanes and more
Safe & non-toxic
No alcohol, steroids or antibiotics
3 fl oz (89 ml)
Non-irritating & non-sensitizing
Safe if licked or ingested
Works on all animal skin types
Indications: Vetericyn Wound and Skin Care is intended for the OTC management of skin abrasions, lacerations, minor irritations, cuts and intact skin. Safe for use on all animal species.
Directions for use: Move excess hair from wound area. Clip if necessary. Adjust spray nozzle. Saturate the affected area. If dressing is required, saturate dressing with Vetericyn at each dressing change. Repeat 3-4 times per day until wound is healed. No rinsing necessary. Faster healing is achieved when wounds are cleaned and maintained moist. Safe for use around mouth, nose, ears and eyes.
Use Precautions: For external use only. Discontinue use if irritation develops and contact your veterinarian. For animal use only. Not for human use. Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.
Stability: See left for date of expiration.
Disposal: No special disposal requirements. Vetericyn is environmentally friendly.
Made in the USA
Part Number: 1007; 90052 v2.0
Tell Us Your Story!
Ingredients: Active: Hypochlorous Acid (HOCl) (0.0120%) Inactive: Electrolyzed Water (H2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)
Storage: Store at room temperature away from direct sunlight and heat. DO NOT ALLOW TO FREEZE because product may lose its potency. After use, seal or close dispenser.
Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.
Manufactured & Bottled by Innovacyn, Inc.
3546 N. Riverside Ave, Rialto CA 92377
vetericyn.com | 866.318.3116
Lot Number:
Expiration Date:
8 18582 01037 5

Stability: See below for date of expiration.

Disposal: No special disposal requirements. Vetericyn is environmentally friendly.

Ingredients: Active: Hypochlorous Acid (HOCl) (0.0120%) **Inactive:** Electrolyzed Water (H2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)

Storage: Store at room temperature away from direct sunlight and heat. **DO NOT ALLOW TO FREEZE** because product may lose its potency. After use, seal or close dispenser.

Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.

Made in the USA

Manufactured & Bottled by Innovacyn, Inc.
3546 N.Riverside Ave, Rialto CA 92377

vetericyn.com | 866.318.3116

Tell Us Your Story!

- *Non-irritating & non-sensitizing*
- *Safe if licked or ingested*
- *Works on all animal skin types*

Indications: Vetericyn Wound and Skin Care is intended for the OTC management of skin abrasions, lacerations, minor irritations, cuts and intact skin. Safe for use on all animal species.

Directions for use: Move excess hair from wound area. Clip if necessary. Adjust spray nozzle. Saturate the affected area. If dressing is required, saturate dressing with Vetericyn at each dressing change. Repeat 3-4 times per day until wound is healed. No rinsing necessary. Faster healing is achieved when wounds are cleaned and maintained moist. Safe for use around mouth, nose, ears and eyes.

Use Precautions: For external use only. Discontinue use if irritation develops and contact your veterinarian. **For animal use only. Not for human use.** Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.

Lot Number:

Expiration Date:

8 52009 00200 0

Part Number: 1008; 90041 v4.0

plus

Vetericyn®

advanced cleanser *plus* therapy

Proven Safe & Effective

Wound & Skin Care

Veterinarian Recommended

Topical application for wounds, cuts, lacerations, abrasions, sores & skin irritations

Safe & non-toxic *No alcohol, steroids or antibiotics*

16 fl oz (473 ml)

Indications: For the cleaning and debriding of wounds, cuts, abrasions, skin irritations, skin ulcers, post surgical incision sites, burns, and rashes on external teat ends. Safe for use on all animal species.

Directions for use: Move excess hair from wound area. Clip if necessary. Adjust spray nozzle. Saturate the affected area. If dressing is required, saturate dressing with Vetericyn at each dressing change. Repeat 3-4 times per day until wound is healed. Faster healing is achieved when wounds are cleaned and maintained moist. For teat application, use spray mode for better coverage. Saturate teat ends with Vetericyn and repeat at each milking or as needed. No rinsing necessary.

Use Precautions: For external use only. Discontinue use if irritation develops and contact your veterinarian. **For animal use only. Not for human use.** Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.

Storage: Store at room temperature away from direct sunlight and heat. **DO NOT ALLOW TO FREEZE** because product may lose its potency. After use, seal or close dispenser.

Stability: See below for date of expiration.

Disposal: No special disposal requirements. Vetericyn is environmentally friendly.

Ingredients: Active: Hypochlorous Acid (HOCl) (0.012%) **Inactive:** Electrolyzed Water (H2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)

Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.

Innovacyn

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866.318.3116 | www.innovacyn.com

Made in the USA

Tell Us Your Story!

Lot Number:

Expiration Date:

8 52009 00276 5

Part Number: 1101; 90048.v4.0

Vetericyn plus

advanced cleanser *plus* therapy

Proven Safe & Effective

Utility Spray

Veterinarian Recommended

Wound Care, Teat Spray, Debridement Solution

Topical application for wounds, cuts, abrasions, sores, minor burns & skin irritations

- Safe & non-toxic
- No alcohol, steroids or antibiotics
- Non-irritating & non-sensitizing
- Safe if licked or ingested
- Works on all animal skin types

Pint
16 fl oz (473 ml)


Use Precautions: For external use only. Discontinue use if irritation develops and contact your veterinarian. **For animal use only. Not for human use.** Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.
Ingredients: Active: Hypochlorous Acid (HOCl) (0.0120%) **Inactive:** Electrolyzed Water (H2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)
Storage: Store at room temperature away from direct sunlight and heat. **DO NOT ALLOW TO FREEZE** because product may lose its potency. After use, seal or close dispenser.
Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.
Made in the USA
Lot Number:
Expiration Date:
vetericyn.com | 866.318.3116
Non-irritating & non-sensitizing
Safe if licked or ingested
Works on all animal skin types
Indications: Vetericyn Wound and Skin Care is intended for the OTC management of skin abrasions, lacerations, minor irritations, cuts and intact skin. Safe for use on all animal species.
Directions for use: Move excess hair from wound area. Clip if necessary. Adjust spray nozzle. Saturate the affected area. If dressing is required, saturate dressing with Vetericyn at each dressing change. Repeat 3-4 times per day until wound is healed. No rinsing necessary. Faster healing is achieved when wounds are cleaned and maintained moist. Safe for use around mouth, nose, ears and eyes.
Manufactured & Bottled by Innovacyn, Inc.
3546 N. Riverside Ave, Rialto CA 92377
Tell Us Your Story!
Part Number: 1900; 90014 v4.0
plus
Vetericyn.
advanced cleanser plus therapy
Proven Safe & Effective
Wound & Skin Care
Veterinarian Recommended
Topical application for wounds, cuts, lacerations, abrasions, sores & skin irritations
Safe & non-toxic
No alcohol, steroids or antibiotics
SAMPLE NOT FOR RESALE 2 fl oz (59 ml)

Indications: For irritated eyes to help relieve burning, stinging and itching by removing air pollutants (pollen/smog) and other foreign materials. Use to wash away mucus secretions and discharges. Safe for use on all animal species.

Directions for use: Wipe away excess matter that may have collected in the corner of the eyes. Set nozzle to stream setting. Spray 3 times directly into each eye with Vetericyn. Apply 1-2 times per day until condition is no longer visible. No rinsing necessary. For superficial use with intact cornea.

Use Precautions: For external use only. Discontinue use if condition worsens and contact your veterinarian. **For animal use only.** Not for human use. Keep out of reach of children. Safe if licked by animal. Not for injection. Always consult with a veterinarian for systemic conditions.

Storage: Store at room temperature away from direct sunlight and heat. **DO NOT ALLOW TO FREEZE** because product may lose its potency. After use, seal or close dispenser.

Stability: See below for date of expiration.

Disposal: No special disposal requirements. Vetericyn is environmentally friendly.

Ingredients: Active: Hypochlorous Acid (HOCl) (0.0150%) **Inactive:** Electrolyzed Water (H2O), Sodium Chloride (NaCl), Phosphates, Sodium Hypochlorite (NaOCl)

Innovacyn products are manufactured under strict quality guidelines to ISO 9001, ISO 13485 and FDA QSR.

Innovacyn

Manufactured & Bottled by Innovacyn, Inc.
3546 N. Riverside Ave, Rialto, CA 92377
866.318.3116 | www.innovacyn.com

Made in the USA

Tell Us Your Story!

Lot Number:

Expiration Date:

8 52009 00251 2

Part Number: 1100; 90040 v4.0

Vetericyn plus

advanced cleanser *plus* therapy

Proven Safe & Effective

Pink Eye Spray

Veterinarian Recommended

Safe for use to clean and debride eyes when showing the following symptoms:

- ✔ Pink Eye
- ✔ Redness and Irritation
- ✔ Discharge and Drainage

Pint
16 fl oz (473 ml)

Appendix II: Waiver Rationales for Acute Toxicity Testing for HOCl Products

(Pages 2 – 3 have been removed due to the confidential nature of the information)

Title

Waiver Rationales for Acute Toxicity Testing of 275 TBD

Data Requirements

Health Effects Test Guidelines - OCSPP Series 870
Acute Oral Toxicity – OCSPP 870.1100
Acute Dermal Toxicity – OCSPP 870.1200
Acute Inhalation Toxicity – OCSPP 870.1300
Primary Eye Irritation – OCSPP 870.2400
Primary Dermal Irritation – OCSPP 870.2500
Dermal Sensitization – OCSPP 870.2600

Author

Gary Burin, PhD, MPH, DABT
Technology Sciences Group
1150 18th Street NW, Suite 1000
Washington, DC 20036

Date
April 29, 2016

Sponsored/Submitted by

SKV Scientific, Inc.
3546 North Riverside Avenue
Rialto, CA 92377

OCSPP Guideline: 870.1100
Data Requirement: Acute Oral Toxicity

The product is a solution of 0.0275% hypochlorous acid (HOCl) in water. No other ingredients are added to the formulation. The solution will be used for disinfectant purposes.

A chronic study of chlorinated drinking water was performed in rats by the National Toxicology Program (NTP, 1992). This study has been used to identify a Reference Dose for free chlorine that included a characterization of the hypochlorous acid content (EPA, IRIS). A No Observed Adverse Effect Level (NOAEL) was identified by the U.S. EPA for rats exposed to "free chlorine" in the NTP drinking water study (U.S. EPA, IRIS, NTP, 1992). This NOAEL was 14.4 mg/kg/day in female rats, the high dose tested was based on a concentration in the drinking water of 275 ppm (0.0275 %) of free chlorine. The NTP report noted: "The total concentrations of hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) are expressed as available atomic chlorine (free chlorine) and are used to express chlorine concentration in this report."

The amount of hypochlorous acid in a limit dose exposure for the acute oral toxicity study can be calculated based on the concentration of hypochlorous acid in the formulation. The limit dose exposure in the acute oral toxicity study is 5,000 mg /kg body weight. 5,000 mg/kg of the product results in a 1.375 mg/kg dose of hypochlorous acid. An acute oral toxicity study on chlorine that resulted in an LD_{50} greater than 1.375 mg/kg would equate to an LD_{50} greater than 5,000 mg/kg of the SKV product. The NOAEL in the NTP study was greater than the hypochlorous acid dose that would be administered in the acute oral limit dose study. The concentration of free chlorine was identical (275 ppm) to that found in the SKV product. A second study exposed rats to hypochlorous acid at concentrations of up to 14 mg/kg/day for 12 months in drinking water (Abdel-Rahman et al., 1984). No mortality and only minor systemic toxicity was observed.

A waiver is requested from the requirement of an Acute Oral Toxicity study for this product based on the oral NOAEL dose for hypochlorous acid being greater than that would be achieved in an acute oral toxicity limit dose study of this product. The NTP study supports an EPA Toxicity Category IV for acute oral toxicity.

OCSPP Guideline: 870.1200
Data Requirement: Acute Dermal Toxicity

The product is a solution of 0.0275% hypochlorous acid in water. A study in rats and mini-pigs to evaluate the toxicity of a solution of hypochlorous acid following dermal exposure to full-thickness wounds for 28 days (Wang et al., 2007) was performed. Rats and mini-pigs received daily applications of stabilized hypochlorous acid of 0.01%, 0.03 % or 0.1% w/v. The applications were followed by 24-hour covering with occlusive dressings after which time the dressing were removed and the skin washed. No evidence of systemic toxicity was reported at any dose. This study demonstrates that hypochlorous acid in water is not toxic by the dermal route at higher concentrations than would be present in the product.

A waiver from the requirement of an Acute Dermal Toxicity study is requested due to the fact that dermal application of hypochlorous acid at concentrations of 0.03% and 0.1% to rats and mini-pigs daily for 28 days did not cause any systemic effects. The data from the study by Wang et al. (2007) support an EPA Toxicity Category IV for dermal toxicity.

OCSPP Guideline: 870.1300
Data Requirement: Acute Inhalation Toxicity

The product is a solution of 0.0275% hypochlorous acid (HOCl) in water at a near neutral pH. The World Health Organization (WHO) confirmed that under physiological conditions (pH 7.4; temperature 37°C) chlorine reacts rapidly with water to produce hypochlorous acid (WHO, 1982). A number of inhalation studies have been conducted to evaluate the effects of chlorine in experimental animals (summarized by WHO, 1982). The results of these inhalation studies, conducted using chlorine as a test article, provide appropriate surrogate data for the product because the chlorine is rapidly converted to hypochlorous acid upon inhalation.

The amount of hypochlorous acid in a limit dose exposure for the acute inhalation toxicity study can be calculated based on the concentration of hypochlorous acid in the test article. The limit dose exposure in the acute inhalation toxicity study is 2 mg product/ L of air. 2 mg of product /L multiplied by the percentage of hypochlorous acid in the solution (0.0275%) is 0.00055 mg/L, or 0.5 µg/L hypochlorous acid. An inhalation toxicity study on chlorine that resulted in an LC_{50} greater than 0.5 µg/L would result in an LC_{50} greater than 2 mg/L of the product based on the amount of hypochlorous acid in the product.

An inhalation study in rats performed in 1977 reported an LC_{50} for chlorine of 850 µg/L (as summarized in WHO, 1982). The LC_{50} for inhalation exposure of rats to the product would be significantly greater than 2 mg/L. The data support an EPA Toxicity Category IV for inhalation toxicity for the product.

OCSPP Guideline: 870.2400
Data Requirement: Primary Eye Irritation

The product is a solution of 0.0275% hypochlorous acid in water. A study in rabbits is available that evaluated the potential of a solution of hypochlorous acid to cause eye irritation (Wang et al., 2007). New Zealand White rabbits received an instillation of hypochlorous acid in one eye at a concentration of 0.01%, 0.03 % or 0.1% w/v. No evidence of ocular irritation was reported at any concentration. A second study evaluated the effect of repeated instillation of a 0.013% hypochlorous acid solution in the eyes of Dutch pigmented rabbits every 8 hours for 72 hours. No evidence of eye irritation was observed after this repeated ocular exposure. The results of these studies demonstrate that an aqueous solution of hypochlorous acid at the concentration in the SKV product is not an eye irritant.

A waiver from the requirement of a Primary Eye Irritation study is requested based on the fact that ocular instillation of hypochlorous acid at concentrations of 0.03% and 0.1% in rabbits did not cause any evidence of irritation. Data cited above support an EPA Toxicity Category IV for eye irritation.

OCSPP Guideline: 870.2500
Data Requirement: Primary Skin Irritation

The product is a solution of 0.0275% hypochlorous acid in water. Dermal irritation from exposure to hypochlorous acid can be evaluated based on two studies by Wang et al. (2007). The first is a study in rats and mini-pigs that evaluated the effects of a solution of hypochlorous acid following dermal exposure to full-thickness skin wounds for 28 days. The rats and mini-pigs received daily dermal applications of hypochlorous acid at concentrations of 0.01%, 0.03 % or 0.1% w/v. The application sites were covered with occlusive dressings for 24-hours after each application. No evidence of irritation at any of the three concentrations, 0.01, 0.03 or 0.1 % hypochlorous acid. In a second study, no evidence of irritation was seen in Guinea pig sensitization assay conducted by the same authors with dermal doses of up to 0.1% hypochlorous acid.

A waiver is requested from the requirement of a Primary Skin Irritation study based on the fact that dermal application of hypochlorous acid at concentrations of 0.03% and 0.1% did not cause any evidence of skin irritation in laboratory animals. The data presented in above support an EPA Toxicity Category IV for potential skin irritation.

OCSPP Guideline: 870.2600
Data Requirement: Dermal Sensitization

The product is a solution of 0.0275% hypochlorous acid in water. The potential for dermal sensitization from exposure to hypochlorous acid was evaluated by Wang et al. (2007). A dermal sensitization study in guinea pigs was performed according to the standard Buehler design. Guinea pigs in the Buehler sensitization study showed no evidence of dermal reaction at any of the three concentrations of hypochlorous acid tested, 0.01, 0.03 or 0.1 %. The results of this study demonstrate that the solution of hypochlorous acid is not a sensitizing agent.

A waiver from the requirement of a dermal sensitization study is requested for the product based on the fact that a dermal sensitization assay is available for solutions of hypochlorous acid at concentrations of 0.03% and 0.1%. The study by Wang et al. (2007) supports the determination that the product is not a sensitizing agent.

References

Abdel-Rahman, MS, DH Suh and RJ Bull (1984). Abel-Rahman MS, Suh DH and Bull RJ. 1984. Pharmacodynamics and toxicity of chlorine in drinking water in the rat. J Appl Toxicol 4: 82-86.

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U.S. EPA . 1994. Drinking Water Criteria Document for Chlorine, Hypochlorous Acid and Hypochlorite Ion. Office of Research and Development. ECAO-CIN-D004. January 1994. PB94179884.

Wang, L. 2007. Hypochlorous acid as a potential wound care agent. Part I stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. Journal of Burns and Wounds. April 2007. 65-79.

Appendix III: Safety Data Sheets for Vetericyn Plus OTC Liquid Products

Innovacyn
Innovation Based on Science.
3546 N. Riverside Ave, Rialto, CA | 866.318.3116 | www.innovacyn.com

SAFETY DATA SHEET
Vetericyn® Plus AX130 OTC Liquid Products
Part# 60000

1. COMPANY IDENTIFICATION

Innovacyn Inc.
3546 N. Riverside Ave
Rialto, CA 92377
(866) 318-3116

Product Identification

Part Number	Size	Description
1000	16 fl. oz.	Vetericyn® Plus Wound & Skin Care
1002	8 fl. oz.	Vetericyn® Plus Wound & Skin Care
1003	8 fl. oz.	Vetericyn® Plus Wound & Skin Care
1004	4 fl. oz.	Vetericyn® Plus Wound & Skin Care
1005	4 fl. oz.	Vetericyn® Plus Wound & Skin Care
1006	4 fl. oz.	Vetericyn® Plus Reptile Wound & Skin Care
1007	3 fl. oz.	Vetericyn® Plus Wound & Skin Care – The Traveler
1008	16 fl. oz.	Vetericyn® Plus Wound & Skin Care
1010	8 fl. oz.	Vetericyn® Plus Hot Spot Spray
1011	4 fl. oz.	Vetericyn® Plus Hot Spot Spray
1012	8 fl. oz.	Vetericyn® Plus Poultry Care
1101	16 fl. oz.	Vetericyn® Plus Utility Spray
1900	2 fl. oz.	Vetericyn® Plus Wound & Skin Care-Sample
2075	2 fl. oz.	Vetericyn® Plus VF Ophthalmic Wash
0011002	237 ml	Vetericyn® Plus Skin Care (Soins de la peau)
0011004	118 ml	Vetericyn® Plus Skin Care (Soins de la peau)
0011007	90 ml	Vetericyn® Plus Skin Care (Soins de la peau)
0011008	473 ml	Vetericyn® Plus Skin Care (Soins de la peau)
0311002	250 ml	Vetericyn® Plus Huidverzoring Reinigt en Beschermt
0311004	120 ml	Vetericyn® Plus Huidverzoring Reinigt en Beschermt
0342075	60 ml	Vetericyn® Plus VF Lavado Oftálmico
0441000	500 ml	Vetericyn® Plus Wound & Skin Care
0441002	250 ml	Vetericyn® Plus Wound & Skin Care
0441004	120 ml	Vetericyn® Plus Wound & Skin Care
0611000	500 ml	Vetericyn® Plus Equine Wound Wash
0611002	250 ml	Vetericyn® Plus Equine Wound Wash

QA ORIGINAL

RECOMMENDED USE IDENTIFICATION

This products intended use is for the OTC management of minor skin wounds including minor lacerations, abrasions, irritations, cuts, burns and intact skin; in addition, to moistening and lubricating absorbent wound dressing.

Innovacyn
Innovation Based on Science.
3546 N. Riverside Ave, Rialto, CA | 866.318.3116 | www.innovacyn.com

RESTRICTIONS ON USE IDENTIFICATION
For external use only.

2. HAZARDS IDENTIFICATION

NOT HAZARDOUS to humans and animals.

Hazardous Material Identification System (HMIS) Rating:
Health: 0
Flammability: 0
Physical: 0; Reactivity: 0
Personal Protection Index: A

NFPA/HMIS Definitions:
0-Minimal Hazard
1-Slight Hazard
2-Moderate Hazard
3-Serious Hazard
4-Severe Hazard

The following values are obtained using the guidelines prepared by the National Fire Protection Association (NFPA) and American Coatings Association (HMIS)

HAZARDOUS AND/OR REGULATED COMPONENTS
None

HAZARDS DISCLOSURE
As defined by the OSHA Hazard Communication Standard 29 CFR 1910.1200 and defined under the Superfund Amendments & Reauthorization Act (SARA) 311 and 312, this product contains no known hazardous materials.

3. COMPOSITION / INFORMATION ON INGREDIENTS

Ingredients	Percentage	CAS #
Electrolyzed Water	99.916%	7732-18-5
Sodium Hypochlorite	0.001%	7681-52-9
Hypochlorous Acid	0.012%	7790-92-3
Sodium Chloride	0.031%	7647-14-5
Phosphates	0.040%	N/A

4 FIRST AID MEASURES

EYE CONTACT FIRST AID: Product is non-irritant, non-toxic. Flush with water, if wished.
SKIN CONTACT FIRST AID: Product is non-irritant, non-toxic. Flush with water, if wished.
INHALATION FIRST AID: Product is non-irritant, non-toxic. No known risks if inhaled.
INGESTION FIRST AID: Product is non-irritant, non-toxic.

5. FIRE-FIGHTING MEASURES

General Fire Hazards: None, Non-flammable
Hazardous Combustion Products: None, Non-flammable
Extinguishing Media: Product will not burn
Personal Protection: None required
Rate of Burning: Non-flammable

6. ACCIDENTAL RELEASE MEASURES

None required
Spill/Leak: Dike spill will inert absorbent materials to contain and soak up liquid. Place wastes into an appropriate waste disposal container. Product is non-hazardous, non-toxic.

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7. HANDLING AND STORAGE

RECOMMENDED STORAGE TEMPERATURE: Ideal storage is at room temperature.
HANDLING: No special handling requirements.
STORAGE: Store at room temperature away from direct sunlight or heat.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Permissible Exposure Limit (PEL): None
Threshold Limit Values (TLV): None
Engineering Controls: None
Personal Protective Equipment (PPE): None

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance: Liquid
Odor: Slightly chlorinated odor
Odor Threshold: Shelf life of product
PH: Balance between 6.2-7.2
Boiling Point: Same as water (212°F / 99.98°C)
Melting Point: Not applicable
Flash Point: Not applicable
Evaporation Rate: Not applicable
Flammability (solid, gas): Non-flammable
Upper Flammable Limit (UFL): Non-flammable
Lower Flammable Limit (LFL): Non-flammable

FLAMMABLE LIMITS IN AIR
Lower Explosive Limit (LEL): None
Upper Explosive Limit (UEL): None
Vapor Pressure: Not applicable
Vapor Density: Not applicable
Relative Density: 1.00-1.06
Solubility: Not applicable
Partition coefficient: Not applicable
Auto Ignition Temperature: Not applicable
Decomposition Temperature: Not applicable
Viscosity: Same as water

10. STABILITY AND REACTIVITY

Reactivity: Not applicable
Chemical Stability: Stable under recommended storage conditions
Possibility of hazardous reactions: Not hazardous
Conditions to avoid: Avoid freezing
Incompatible Materials: None
Hazardous decomposition products: None

11. TOXICOLOGICAL INFORMATION

EYE EFFECTS: No known hazards, non-toxic
SKIN EFFECTS: No known hazards, non-toxic
ORAL LD_{50}: >5000 mg/Kg. No known hazards, non-toxic
DERMAL LD_{50}: >5050 mg/Kg. No known hazards, non-toxic.
INHALATION LC_{50}: >2.16 mg/l. No known hazards, non-toxic

Potential Health Affects

EYE: No potential health affects; product is non-hazardous.
SKIN: No potential health affects; product is non-hazardous.
INHALATION: No potential health affects; product is non-hazardous.
INGESTION: No potential health affects; product is non-hazardous.
CARCINOGENICITY INFORMATION: No known cancer hazards.

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12. ECOLOGICAL INFORMATION

Presents no hazards to the environment
Ecotoxicity: non-hazardous
Persistence and degradability: Unknown
Bioaccumulative potential: Biodegradable, non-hazardous
Mobility in soil: Unknown
Other adverse effects (such as hazardous to the ozone layer): Unknown

13. DISPOSAL CONSIDERATIONS

No special disposal considerations are required. Follow local ordinance for waste or recycling.

14. TRANSPORTATION INFORMATION

UN number: None required
UN proper shipping name: Oxidized water
Transport (D.O.T.) hazard class(es): Not DOT regulated
Freight Packing group, if applicable: 70, PG III
Environmental hazards (e.g., Marine pollutant (Yes/No): NO
Transport in bulk (according to Annex II of MARPOL 73/78 and the IBC Code): Not applicable
Special precautions: None

15. Regulation

National Fire Protection Association (NFPA)
American Coatings Association (HMIS)
OSHA Hazard Communication Standard 29 CFR 1910.1200
Superfund Amendments & Reauthorization Act (SARA) 311 and 312
NMFC-National-Motor-Freight-Classification
The US Department of Transportation
Annex II of MARPOL 73/78 and the IBC Code
APPENDIX D TO §1910.1200 - SAFETY DATA SHEETS

16. Other Information

SDS: Vetericyn® Plus AX130 OTC Liquid Products Part# AX60000
Version: 3.0
Effective: April 22, 2015
DCO No. 254-15

QA ORIGINAL

Appendix IV: Safety Data Sheets for Vetericyn Plus VF Liquid Products

3546 N. Riverside Ave, Rialto, CA | 866.318.3116 | www.innovacyn.com

QA ORIGINAL

SAFETY DATA SHEET
Vetericyn® Plus AX160 VF Liquid Products
Part# AX62000

1. COMPANY IDENTIFICATION

Innovacyn Inc.
3546 N. Riverside Ave
Rialto, CA 92377
(866) 318-3116

Product Identification

Part Number	Size	Description
1100	16 fl. oz.	Vetericyn® Plus Pink Eye Spray
2000	16 fl. oz.	Vetericyn® Plus VF Wound & Skin Care
2001	8 fl. oz.	Vetericyn® Plus VF Wound & Skin Care
2002	2 fl. oz.	Vetericyn® Plus VF Wound & Skin Care
2003	4 fl. oz.	Vetericyn® Plus VF Wound & Skin Care
2023	4 fl. oz.	Vetericyn® Plus VF Otic Rinse
2900	2 fl. oz.	Vetericyn® Plus VF Wound & Skin Care-Sample
0012000	473 ml	Vetericyn® Plus VF Skin Care (Soins de la peau)
0012001	237 ml	Vetericyn® Plus VF Skin Care (Soins de la peau)
0112003	118 ml	Vetericyn® Plus VF Skin Care (Soins de la peau)
0112023	118 ml	Vetericyn® Plus VF Otic Rinse (Liquide de rinçage otique)
0312001	250 ml	Vetericyn® Plus VFHuidverzorging Reinigt en Beschermt
0312003	120 ml	Vetericyn® Plus VFHuidverzorging Reinigt en Beschermt
0342000	500 ml	Vetericyn® Plus VF Skin Care
0342002	60 ml	Vetericyn® Plus VF Skin Care
0342003	120 ml	Vetericyn® Plus VF Skin Care
0342023	120 ml	Vetericyn® Plus VF Lavado Ótico
0441100	500 ml	Vetericyn® Plus Livestock Eye Care
0442000	500 ml	Vetericyn® Plus VF Wound & Skin Care
0442001	250 ml	Vetericyn® Plus VF Wound & Skin Care
0442002	59 ml	Vetericyn® Plus VF Wound & Skin Care
0442003	120 ml	Vetericyn® Plus VF Wound & Skin Care
0612000	500 ml	Vetericyn® Plus VF Equine Wound Wash
0612001	250 ml	Vetericyn® Plus VF Equine Wound Wash
3532000	500 ml	Vetericyn® Plus VF Cut & Skin Care
3532003	120 ml	Vetericyn® Plus VF Cut & Skin Care

RECOMMENDED USE IDENTIFICATION

This products intended use is for the OTC management of minor skin wounds including minor lacerations, abrasions, irritations, cuts, burns and intact skin; in addition, to moistening and lubricating absorbent wound dressing.

RESTRICTIONS ON USE IDENTIFICATION

For external use only.

DCO No: 256-15

Innovacyn
Innovation Based on Science
3546 N. Riverside Ave, Rialto, CA | 866.318.3116 | www.innovacyn.com

2. HAZARDS IDENTIFICATION

NOT HAZARDOUS to humans and animals.

Hazardous Material Identification System (HMIS) Rating:
Health: 0
Flammability: 0
Physical: 0; Reactivity: 0
Personal Protection Index: A

NFPA/HMIS Definitions:
0-Minimal Hazard
1-Slight Hazard
2-Moderate Hazard
3-Serious Hazard
4-Severe Hazard

The following values are obtained using the guidelines prepared by the National Fire Protection Association (NFPA) and American Coatings Association (HMIS)

HAZARDOUS AND/OR REGULATED COMPONENTS
None

HAZARDS DISCLOSURE
As defined by the OSHA Hazard Communication Standard 29 CFR 1910.1200 and defined under the Superfund Amendments & Reauthorization Act (SARA) 311 and 312, this product contains no known hazardous materials.

3. COMPOSITION / INFORMATION ON INGREDIENTS

Ingredients	Percentage	CAS #
Electrolyzed Water	99.905%	7732-18-5
Sodium Hypochlorite	0.001%	7681-52-9
Hypochlorous Acid	0.015%	7790-92-3
Sodium Chloride	0.039%	7647-14-5
Phosphates	0.040%	N/A

4 FIRST AID MEASURES

EYE CONTACT FIRST AID: Product is non-irritant, non-toxic. Flush with water, if wished.
SKIN CONTACT FIRST AID: Product is non-irritant, non-toxic. Flush with water, if wished.
INHALATION FIRST AID: Product is non-irritant, non-toxic. No known risks if inhaled.
INGESTION FIRST AID: Product is non-irritant, non-toxic.

5. FIRE-FIGHTING MEASURES

General Fire Hazards: None, Non-flammable
Hazardous Combustion Products: None, Non-flammable
Extinguishing Media: Product will not burn
Personal Protection: None required
Rate of Burning: Non-flammable

6. ACCIDENTAL RELEASE MEASURES

None required
Spill/Leak: Dike spill will inert absorbent materials to contain and soak up liquid. Place wastes into an appropriate waste disposal container. Product is non-hazardous, non-toxic.

7. HANDLING AND STORAGE

RECOMMENDED STORAGE TEMPERATURE: Ideal storage is at room temperature.
HANDLING: No special handling requirements.
STORAGE: Store at room temperature away from direct sunlight or heat.

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8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Permissible Exposure Limit (PEL): None
Threshold Limit Values (TLV): None
Engineering Controls: None
Personal Protective Equipment (PPE): None

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance: Liquid
Odor: Slightly chlorinated odor
Odor Threshold: Shelf life of product
PH: Balance between 6.2-7.2
Boiling Point: Same as water (212°F / 99.98°C)
Melting Point: Not applicable
Flash Point: Not applicable
Evaporation Rate: Not applicable
Flammability (solid, gas): Non-flammable
Upper Flammable Limit (UFL): Non-flammable
Lower Flammable Limit (LFL): Non-flammable

FLAMMABLE LIMITS IN AIR
Lower Explosive Limit (LEL): None
Upper Explosive Limit (UEL): None
Vapor Pressure: Not applicable
Vapor Density: Not applicable
Relative Density: 1.00-1.06
Solubility: Not applicable
Partition coefficient: Not applicable
Auto Ignition Temperature: Not applicable
Decomposition Temperature: Not applicable
Viscosity: Same as water

10. STABILITY AND REACTIVITY

Reactivity: Not applicable
Chemical Stability: Stable under recommended storage conditions
Possibility of hazardous reactions: Not hazardous
Conditions to avoid: Avoid freezing
Incompatible Materials: None
Hazardous decomposition products: None

11. TOXICOLOGICAL INFORMATION

EYE EFFECTS: No known hazards, non-toxic
SKIN EFFECTS: No known hazards, non-toxic
ORAL LD_{50}: >5000 mg/Kg. No known hazards, non-toxic
DERMAL LD_{50}: >5050 mg/Kg. No known hazards, non-toxic.
INHALATION LC_{50}: >2.16 mg/l. No known hazards, non-toxic

Potential Health Affects

EYE: No potential health affects; product is non-hazardous.
SKIN: No potential health affects; product is non-hazardous.
INHALATION: No potential health affects; product is non-hazardous.
INGESTION: No potential health affects; product is non-hazardous.
CARCINOGENICITY INFORMATION: No known cancer hazards.

12. ECOLOGICAL INFORMATION

Presents no hazards to the environment
Ecotoxicity: non-hazardous
Persistence and degradability: Unknown
Bioaccumulative potential: Biodegradable, non-hazardous
Mobility in soil: Unknown
Other adverse effects (such as hazardous to the ozone layer): Unknown

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13. DISPOSAL CONSIDERATIONS

No special disposal considerations are required. Follow local ordinance for waste or recycling.

14. TRANSPORTATION INFORMATION

UN number: None required
UN proper shipping name: Oxidized water
Transport (D.O.T.) hazard class(es): Not DOT regulated
Freight Packing group, if applicable: 70, PG III
Environmental hazards (e.g., Marine pollutant (Yes/No): NO
Transport in bulk (according to Annex II of MARPOL 73/78 and the IBC Code): Not applicable
Special precautions: None

15. Regulation

National Fire Protection Association (NFPA)
American Coatings Association (HMIS)
OSHA Hazard Communication Standard 29 CFR 1910.1200
Superfund Amendments & Reauthorization Act (SARA) 311 and 312
NMFC-National-Motor-Freight-Classification
The US Department of Transportation
Annex II of MARPOL 73/78 and the IBC Code
APPENDIX D TO §1910.1200 - SAFETY DATA SHEETS

16. Other Information

SDS: Vetericyn® Plus AX160 VF Liquid Products Part# AX62000
Version: 1.0
Effective: April 24, 2015
DCO No. 256-15

From:	Rebecca Lei - SKV Scientific
To:	Walden, Jessica - AMS
Cc:	Brines, Lisa - AMS
Subject:	RE: Petition Status - hypochlorous acid
Date:	Tuesday, August 02, 2016 9:14:00 AM
Attachments:	#85021-4 EPA Stamped Label and Approval Letter AX275.pdf K133542_Puracyn Plus 510k Summary.pdf Puracyn Plus State Certificates.pdf
Importance:	High

Dear Ms. Jessica Walden,

Thank you for your email and thank you for your time on the phone yesterday. We acknowledge the receipt of your attached letter dated August 1, 2016. Below please find our response regarding information missing for Item B.8, Regulatory Authority.

We have not registered the Vetericyn Plus product with the EPA nor have we received a 510(k) clearance on this product because the use indications of this product are not regulated by either of the Agencies. However, as indicated in the petition document, this product formulation has received a 510(k) clearance under the brand name Puracyn Plus for the same use indications on human applications, and attached is the 510(k) summary for the Puracyn Plus product. In addition, this product formulation has also been registered with the EPA as a hard surface disinfectant, and attached is also the stamped label from the federal EPA. As explained in the petition document, both the 510(k) cleared formulation and the EPA registered formulation have the same active ingredient, Hypochlorous acid, as the formulation being petitioned in this document. Again, the reason why the petitioned formulation has not received any clearance or registration from either Agencies is because the use indications are not under these Agencies' jurisdiction.

Lastly, attached are the certificates granted by the Department of Agriculture/ Health of 7 out of the 48 contiguous states for the Vetericyn Plus products. The reason why we have only been granted certificates from 7 states is because some states do not require registration, e.g. Arizona and Delaware; while some other states have not responded to our inquires for the registration process, e.g. Alabama and Arkansas.

Thank you very much and please let me know should you need additional information.

Rebecca

From: Walden, Jessica - AMS [mailto:Jessica.Walden@ams.usda.gov]
Sent: Monday, August 01, 2016 8:24 AM
To: Rebecca Lei <rebeccal@aquaoxindustries.com>
Subject: Petition Status - hypochlorous acid

Dear Ms. Lei,

Please see the attached document that provides information on the status of the petition for hypochlorous acid. Please let me know if you have any questions.

Sincerely,

Jessica Walden
Materials Specialist, Standards Division
National Organic Program
Agricultural Marketing Services
United States Department of Agriculture
Tel: (202) 720-3252
Direct: 202-740-4923
jessica.walden@ams.usda.gov
www.ams.usda.gov/nop

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Antimicrobials Division (7510P)
1200 Pennsylvania Avenue NW
Washington, D.C. 20460

EPA Reg. Number:	Date of Issuance:
85021-4	SEP 10 2014

Term of Issuance: Unconditional

Name of Pesticide Product: Aquaox Disinfectant 275

NOTICE OF PESTICIDE:
x Registration
___ Reregistration

(under FIFRA, as amended)

Name and Address of Registrant (include ZIP Code):

Aquaox Inc
16155 Sierra Lakes Parkway-Suite 160-714
Fontana, CA 92336

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Antimicrobials Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act. Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This product (OPP Decision No. D-489002) is registered in accordance with FIFRA sec 3(c)(5) provided that you:

1. Submit and/or cite all data required for registration of your product under FIFRA sec. 3(c)(5) when the Agency requires all registrants of similar products to submit such data; and submit acceptable responses required for re-registration of your product under FIFRA section 4.

2. Submit one (1) copy of your final printed labeling before distributing or selling the product bearing the revised labeling.

Signature of Approving Official:

Demson Fuller
Product Manager 32
Regulatory Management Branch II
Antimicrobials Division (7510P)

Date: SEP 10 2014

If these conditions are not complied with, the registration will be subject to cancellation in accordance with FIFRA sec. 6(e). Your release for shipment of the product constitutes acceptance of these conditions.

A stamped copy of the label is enclosed for your records.

Sincerely,

Demson Fuller
Product Manager 32
Regulatory Management Branch II
Antimicrobials Division (7510P)

Enclosures: (Stamped Label)

ACCEPTED

09/10/2014

Under the Federal Insecticide, Fungicide and Rodenticide Act as amended, for the pesticide registered under EPA Reg. No. 85021-4

Aquaox Disinfectant 275

Hypochlorous Acid Solution Generated Electrochemically from Sodium Chloride

Aquaox Disinfectant 275 is:

- a cost-effective disinfecting solution;
- produced with low energy and low costs from water and salt;
- produced in a single-stage process by a simple electrolytic cell;
- produced for use in medical, institutional, industrial and commercial applications and
- produced with a controlled pH and controlled concentration of Free Available Chlorine (FAC).

ACTIVE INGREDIENT:	
Hypochlorous Acid	**0.0275%**
OTHER INGREDIENTS:	99.9725%
TOTAL:	100.0000%

Contains > 275ppm Free Available Chlorine (FAC)

KEEP OUT OF REACH OF CHILDREN

CAUTION

See Back Label for Precautionary Statements

Reg. No. 85021- Est. No. 85021-CA-001

Manufactured by:

AQUAOX INC.

16155 Sierra Lakes Pkwy Suite 160, Box 714
Fontana, CA 92336
Phone No.: +1(909)-829-1664
Email: info@aquaox.net

Aquaox Disinfectant must be used within 30 days after production OR Product must be tested with chlorine test kit provided by Aquaox. DO NOT USE PRODUCT when Chlorine concentration is below 248ppm.

DATE PRODUCED: _____

Container size: 1 gallon, 5 gallon, 30 gallon, 55 gallon, 275 gallon, 330 gallon, 660 gallon

Aquaox Disinfectant is a Hypochlorous Acid solution produced by passing an aqueous saline solution (brine) through 1 or more electrolytic cells. The current within the electrolytic cell(s) splits the sodium chloride compound into two separate fluids. One fluid is Hypochlorous Acid, a powerful oxidizing agent exhibiting antimicrobial properties.

Aquaox Disinfectant is produced at a near neutral pH, (approximately pH 6.5) where the predominant antimicrobial agent is Hypochlorous Acid, an efficient and efficacious species of chlorine. Hypochlorous Acid kills bacteria, fungi, molds, viruses and spores.

Aquaox Disinfectant properties are closely controlled by controlling the voltage and the current to the electrolytic cell(s), brine conductivity, temperature and flow rate through the cells as well as the pH of the Hypochlorous Acid generated in the cell(s).

Aquaox Disinfectant freezes at 32°F and boils at 212°F**.** It is a colorless and aqueous solution with a slight chlorine or ozone odor.

After production, **Aquaox Disinfectant** must be stored in a closed plastic container in a cool and dark area away from direct sunlight.

Aquaox Disinfectant is intended to be used soon after being produced.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

Hard, Non-Porous Surface Disinfection

To *[Clean and]* Disinfect *[and Deodorize]* Hard, Non-Porous Surfaces: For heavily soiled areas, a preliminary cleaning is required. Apply *[Wipe, Spray or Dip]* **Aquaox Disinfectant** to hard, non-porous surfaces with a cloth, wipe, mop, sprayer or sponge. Treated surfaces must remain wet for 10 minutes. Allow surfaces to air dry. Do not use on utensils, glasses or dishes.

This product is not to be used as a terminal sterilant / high level disinfectant on any surface or instrument that (1) is introduced directly into the human body, or (2) contacts intact mucous membranes but which do not ordinarily penetrate the blood barrier or otherwise enter normally sterile areas of the body. This product may be used to pre-clean or decontaminate critical or semi-critical devices prior to sterilization or high-level disinfection.

Pathogen	Strain	Contact Time
Pseudomonas aeruginosa	ATCC 15442	10 minutes
Staphylococcus aureus	ATCC 6538	10 minutes
Swine Influenza Virus (H1N1)	ATCC VR-333	10 minutes
Salmonella enterica	ATCC 10708	10 minutes

CLAIMS

+ Broad Spectrum Disinfectant
+ One-Step Cleaner / Disinfectant when Disinfection Directions are followed
+ Aids in the Reduction of Cross-Contamination between Treated Surfaces
+ This Disinfection Process assures Proper Strength, Product Effectiveness and Standardizes Technique
+ Formulated for Bacteria Fighting
+ Bactericide - or – Bactericidal
+ Bathroom Disinfectant
+ Nursery Disinfectant
+ Athletic Facility Disinfectant
+ Cleans and Disinfects Site(s) on Tables 1–4 below
+ Cleans and Disinfects Hard, Non-Porous Surfaces
+ Cleans, Deodorizes and Disinfects
+ Deodorizes by Killing Odor-Causing Bacteria
+ Disinfecting Formula
+ Disinfects and Deodorizes by Killing Bacteria and their Odors
+ Eliminates - or - Reduces Odors caused by Bacteria
+ Eliminates odors at their source; bacteria
+ Disinfects Hard, Non-Porous Surfaces on Site(s) on Tables 1–4 below
+ Easy and Convenient Disinfecting on Site(s) on Tables 1–4 below
+ Easy One-Step Cleaning and Disinfecting when Disinfection Directions are followed
+ Effective against - or - Kills Organism(s) mentioned in Table on Page 2 above
+ Effective against - or - Kills H1N1 Swine Influenza virus
+ Effectively Disinfects Hard, Non-Porous, Environmental Surfaces
+ Fight(s) - and/or - Kill(s) - and/or - Effective against *Salmonella enterica*
+ Fight(s) - and/or - Kill(s) - and/or - Effective against *Staphylococcus aureus*
+ Fight(s) - and/or - Kill(s) - and/or - Effective against *Pseudomonas aeruginosa*
+ Fight(s) - and/or - Stops - and/or - Prevent(s) Cross-Contamination on Hard, Non-Porous Surfaces on Tables 1–4 below
+ Kills Odor-Causing Bacteria mentioned in Table on Page 2 above
+ Kills - or - Effective against Bacteria mentioned in Table on Page 2 above
+ Multi-Purpose Disinfectant
+ One-Step Cleaner and Disinfectant when Disinfection Directions are followed
+ One-Step Cleaner and Disinfectant (when Disinfections Direction are followed) designed for General Cleaning and Disinfecting Hard, Non-Porous Environmental Surfaces in Health Care Facilities and on Sites listed on Tables 1–4 below
+ Pseudomonocidal
+ Staphylocidal
+ Ready-to-Use Hospital Disinfectant
+ The Answer to your Disinfecting Needs
+ The Easy - and/or - Convenient way to Disinfect
+ This Product controls Cross-Contamination on most Hard, Non-Porous Surfaces
+ This Product meets AOAC Efficacy Testing Requirements - or - Standards for Hospital Disinfection
+ Use in Public - or - Common Places where Bacteria may be of concern on Hard, Non-Porous Surfaces
+ Use where Control of the Hazards of Cross-Contamination between Treated Hard Non-Porous Surfaces is of Importance

GENERAL CLAIMS

+ Convenient
+ For General Use
+ For Use on Nursery Surfaces
+ Suitable for Hospital Use
+ Easy to Handle
+ For Use on Bathroom Surfaces
+ For Use in Athletic Facilities
+ For Use on Athletic Equipment
+ Will not Harm Surfaces listed on Tables
+ Will not Harm Hard, Non-Porous Inanimate Environmental Surfaces
+ Will not Harm Titanium-Coated, Medical Grade Stainless Steel

TABLE ONE: Medical Environments

USE SITES

+ Ambulances - or - Emergency Medical Transport Vehicles
+ Anesthesia Rooms - or - Areas
+ Assisted Living - or - Full Care Nursing Homes
+ CAT Laboratories
+ Central Service Areas
+ Central Supply Rooms - or - Areas Critical Care Units - or - CCUs
+ Dialysis Clinics
+ Emergency Rooms - or - RS (Registered Sanitarian) Health Care Settings - or Facilities
+ Home Health Care Settings
+ Hospitals
+ Intensive Care Units - or - ICU Laboratories
+ Medical - or - Physician's - or - Doctor's Offices Newborn - or - Neonatal Nurseries
+ Medical Clinics
+ Medical Facilities
+ Nursing - or - Nurses' Stations
+ Orthopedics
+ Outpatient Clinics
+ Patient Restrooms
+ Patient Rooms
+ Pediatric Examination Rooms - or - Areas
+ Pharmacies
+ Physical Therapy Rooms - or - Areas
+ Radiology - or - X-Ray Rooms - or - Areas
+ Surgery Rooms - or - Operating Rooms - or ORs

SURFACES (Applicable to Surface Materials listed on Page 9)

+ Bed pans
+ Exam - or - Examination Table:
+ External Surfaces of Medical Equipment - or - Medical Equipment Surfaces
+ External Surfaces of Ultrasound Transducers
+ Gurneys
+ Hard, Non-Porous Environmental Hospital - or - Medical Surfaces
+ Hospital - or - Patient Bed Railings - or - Linings - or - Frames
+ IV Poles
+ Patient Chairs
+ Plastic Mattress Covers
+ Reception Counters - or - Desks - or - Areas
+ Stretchers
+ Wash Basins
+ Wheelchairs

TABLE TWO: Dental Environment:

USE SITES

+ Dental - or - Dentist's Offices
+ Dental Operatory rooms

SURFACES (Applicable to Surface Materials listed on Page 9)

+ Dental Countertops
+ Dental Operatory Surfaces
+ Dentist - or - Dental Chairs
+ Hard, Non-Porous Environmental Dental Surfaces
+ Light Lens Covers
+ Reception Counters - or - Desks - or - Areas

TABLE THREE: Veterinary Environments:

Animal Premises: Remove all animals and feed from the premises, vehicles and enclosures. Remove all litter, droppings and manure from the floors, walls and surfaces of barns, pens, stalls, chutes and other facilities and fixtures occupied or traversed by animals. Empty all troughs, racks and other feeding and watering appliances. Thoroughly clean all surfaces with soap and/or detergent and rinse with water.

Apply **Aquaox Disinfectant** and saturate surfaces with solution for 10 minutes. Immerse all halters, ropes and other types of equipment used in handling and restraining animals as well as forks, shovels and scrapers used for removing litter and manure.
After application, ventilate buildings, coops and other closed spaces. Do not house animals or employ equipment until treatment has been absorbed, set or dried. Thoroughly scrub all treated feed racks, mangers, troughs, automatic feeders, fountains and waterers with soap or detergent and rinse with potable water before reuse.

USE SITES

+ Animal - or - Pet Grooming Facilities Kennels
+ Animal Housing Facilities
+ Animal Life Science Laboratories
+ Livestock - and/or - Swine - and/or - Poultry Facilities
+ Pet Areas
+ Pet Shops - or - Stores
+ Small Animal Facilities
+ Veterinary - or - Animal Hospitals
+ Veterinary Clinics - or - Facilities
+ Veterinary Offices

SURFACES (Applicable to Surface Materials listed on Page 9)

+ Animal Equipment Automatic Feeders
+ Cages
+ External Surfaces of Veterinary Equipment
+ Feed Racks
+ Fountains
+ Hard, Non-Porous Environmental Veterinary Surfaces
+ Pens
+ Reception Counters - or - Desks - or - Areas Stalls
+ Troughs
+ Veterinary Care Surfaces
+ Watering Appliances

TABLE FOUR: Miscellaneous / General Environments

USE SITES

+ Airplanes
+ Blood Banks
+ Boats
+ Bowling Alleys
+ Chillers
+ Churches
+ Colleges
+ Correctional Facilities
+ Cruise Lines
+ Day Care Centers
+ Dormitories
+ Factories
+ Funeral Homes
+ Gymnasiums - or - Gyms
+ Health Club Facilities
+ Hotels
+ Industrial Facilities
+ Laundromats
+ Laundry Rooms Locker Rooms
+ Manufacturing Facilities
+ Manufacturing Plants - or - Facilities
+ Military Installations
+ Motels
+ Preschool Facilities
+ Public Areas
+ Recreational Centers - or - Facilities
+ Restrooms - or - Restroom Areas
+ School Buses
+ Schools
+ Shelters
+ Shower Rooms
+ Storage Rooms - or - Areas
+ Trains
+ Universities
+ Wineries
+ Yachts

SURFACES (Applicable to Surface Materials listed on Page 9)

+ Bathroom Fixtures
+ Bath Tubs
+ Behind and under Counters
+ Behind and under Sinks
+ Booster Chairs
+ Cabinets Ceilings
+ Cellular - or - Wireless - or - Mobile - or - Digital Phones
+ Chairs
+ Computer Keyboards
+ Computer Monitors
+ Counters - or - Countertops
+ Cribs
+ Desks
+ Diaper - or - Infant Changing Tables
+ Diaper Pails
+ Dictating Equipment Surfaces
+ Doorknobs
+ Exterior - or - External Toilet Surfaces
+ Exterior - or - External Urinal Surfaces
+ Faucets
+ Floors
+ Garbage - or - Trash Cans
+ Hampers
+ Hand Railings
+ Headsets
+ Highchairs
+ Lamps
+ Linoleum
+ Playpens
+ Shelves
+ Showers - or - Shower Stalls
+ Sinks
+ Stall Doors
+ Tables
+ Telephones
+ Tiled Walls
+ Toilet Rims
+ Toilet Seats
+ Towel Dispensers
+ Toys
+ Vanity Tops - or - Vanities
+ Other Telecommunications Equipment Surfaces

SURFACE MATERIALS

+ Baked enamel
+ Chrome
+ Common Hard, Non-Porous Household - or - Environmental Surfaces
+ Formica
+ Glass
+ Glazed Ceramic Tile
+ Glazed Porcelain
+ Glazed Porcelain Enamel
+ Laminated Surfaces
+ Plastic Laminate
+ Stainless Steel
+ Synthetic Marble
+ Vinyl Tile
+ Similar Hard, Non-Porous Surfaces except those excluded by the label

Not Recommended For Use On - or - Avoid Contact With

+ Aluminum Brass
+ Chipped enamel
+ Clear plastic
+ Clothes
+ Copper
+ Fabrics
+ Gold
+ Natural marble
+ Natural rubber
+ Painted surfaces
+ Paper surfaces
+ Sealed granite
+ Silver
+ Unfinished wood
+ Wood

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

For Industrial and Commercial Use Packages:

Pesticide Storage: Store in a closed dark plastic container in a cool, dry area away from heat and sunlight. Do not store near easily oxidizable materials, acids and reducers. In case of spill, isolate container (if possible) and flood area with water to dissolve all material before discarding this container in trash.

Emergency Handling: In case of contamination or decomposition. Do not reseal container. Isolate in open, well-ventilated area. Flood with large amounts of water. Cool unopened containers in vicinity by water spray.

Pesticide Disposal: Pesticide wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility. Improper disposal of excess pesticide, spray mixture or rinsate is a violation of Federal Law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environment Control Agency, or the Hazardous Waste Representative at the EPA Regional Office for guidance.

Small packages (5 gallons or less):

Container Handling: Non-refillable rigid container. Do not reuse or refill this container. Triple-rinse container (or equivalent) promptly after emptying. Triple-rinse as follows: Empty the remaining contents into the application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Full the container ¼ with water and recap. Shake for 10 seconds. Pour rinsate contents into the application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure 2 more times. Then offer for recycling or reconditioning if available or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities, by burning. If burned, stay clear of smoke.

Container Handling: Refillable container. Refill this container with **Aquaox Disinfectant** only.
Do not reuse this container for any other purpose. Cleaning before refilling is the responsibility of the refiller. Cleaning the container before final disposal is the responsibility of the person disposing the container. To clean the container before final disposal, empty the remaining contents into the application equipment or a mix tank. Agitate vigorously or recirculate water with the pump for 2 minutes. Dispose of rinsate as pesticide waste. Repeat this rinsing procedure two more times. Then offer for recycling if available or puncture and dispose of in a sanitary landfill, or by incineration, or by procedures allowed by state and local authorities.

PRECAUTIONARY STATEMENTS

Physical or Chemical Hazards: **Aquaox Disinfectant 275** is not compatible with other chemicals such as acids and hydrogen peroxide**.**

Hazards to Humans and Domestic Animals

CAUTION

Causes moderate eye irritation. Avoid contact with eyes. When handling the product, wear safety glasses or goggles. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet.

FIRST AID **Call a poison control center or doctor for treatment advice. Have the product container or label with you when calling a poison control center, doctor or going for treatment. You may also contact the National Pesticide Information Center (NPIC) 1-800-858-7378 for emergency medical treatment information.**	
If in eyes	• Hold eye open and rinse slowly and gently with water for 15 – 20 minutes. • Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.

APR 2 8 2014

5.0 510(k) SUMMARY

5.1 Sponsor Information[1]

Company Information: Innovacyn, Inc.
3546 N. Riverside Ave.
Rialto, CA 92377

Contact Information: Dr. Fred Ma M.D., Ph.D.
Chief Medical Officer
909-349-3370, Ext. 375
fma@innovacyn.com

Date of Preparation: November 13, 2013

Date of Revision: April 3, 2014

5.2 Device Information

Common Name: Wound Cleanser

Trade Name: Puracyn Plus™ Skin and Wound Care

Classification Name: Dressing, wound, drug

Device Class: Unclassified

Device Code: FRO

Classification Panel: General and Plastic Surgery

5.3 Identification of Legally Marketed Device for Substantial Equivalence Comparison:

K093697: Vashe® Wound Therapy Solution (OTC use) manufactured by PuriCore, Inc.

K123072: Vashe® Wound Therapy Solution (Professional use) manufactured by PuriCore, Inc.

K113693: Nixall™ Wound and Skin Care (OTC and Professional use) manufactured by Seriously Clean Ltd.

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[1] Innovacyn has contracted with Aquaox Inc. and its subsidiary, Aquaox Industries, for the exclusive use of equipment and technology that has been developed by Aquaox Inc. for the manufacture of hypochlorous acid solutions such as those used in Puracyn Plus™ Skin and Wound Care (which is referred in the Aquaox documentation as AX250).

5.4 Device Description

Puracyn Plus™ Skin and Wound Care is a clear hypotonic solution topically applied to skin and wound areas. The subject device is a wound management and cleansing solution that is intended for cleansing, irrigating, and debriding dermal wounds in addition to moistening and lubricating absorbent wound dressings (e.g. gauze). The mechanical action of fluid moving across the wound provides for the mechanism of action and aids in the removal of foreign objects such as dirt and debris. Puracyn Plus™ Skin and Wound Care will be supplied in food grade 4 oz. plastic PET bottles with spray inserts and caps.

5.5 Intended Use

Puracyn Plus™ Skin and Wound Care is intended for over-the-counter use and professional use as follows:

OTC: Puracyn Plus™ Skin and Wound Care is intended for the OTC use of the management of minor skin wounds including minor lacerations, minor abrasions, minor irritations, minor cuts, minor burns and intact skin, in addition to moistening and lubricating absorbent wound dressings.

Professional Use: Puracyn Plus™ Skin and Wound Care is intended for use by healthcare professionals for cleansing, irrigating, moistening, and debriding to remove wound debris from acute and chronic dermal lesions that are partial or full thickness wounds such as 1st and 2nd degree burns, stage I – IV pressure ulcers, diabetic ulcers, stasis ulcers, abrasions and minor skin irritations, post-surgical wounds, grafted and donor sites, in addition to moistening and lubricating absorbent wound dressings.

These indications are similar to the predicate devices (Vashe® Wound Therapy Solution and Nixall™ Wound and Skin Care).

5.6 Device Technological Characteristics

Puracyn Plus™ Skin and Wound Care is a clear hypotonic solution to aid in the removal of debris and foreign material from the application site. This is accomplished through the flow of the solution moving across the application site with or without the assistance of a suitable wound dressing. Puracyn Plus™ Skin and Wound Care solution contains a preservative that may help inhibit the growth of microorganisms within the solution. Puracyn Plus™ Skin and Wound Care is manufactured under Good Manufacturing Practices (GMP) guidelines.

5.7 Performance Testing

ISO 10993 biocompatibility testing established the safety of Puracyn Plus™ Skin and Wound Care for its intended use. The overall biocompatibility testing results warrant Puracyn Plus ™ Skin and Wound Care product as a safe to use medical device, i.e. non-cytotoxic, non-

sensitizing, non-irritating, and non-toxic. The results of stability testing have demonstrated the product is stable for at least 11 months when stored at 25°C/60%RH±2% Stability Conditions.

5.8 Substantial Equivalence

Puracyn Plus™ Skin and Wound Care is substantially equivalent to the cited predicate devices based on similarity of use indications, functionality, chemical and physical characteristics, antimicrobial activity, and biocompatibility.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609

Silver Spring, MD 20993-0002

April 28, 2014

Innovacyn Incorporated
Fred Ma, M.D., Ph.D.
Chief Medical Officer
3546 North Riverside Avenue
Rialto, California 92377

Re: K133542
Trade/Device Name: Puracyn Plus™ Skin and Wound Care
Regulatory Class: Unclassified
Product Code: FRO
Dated: April 3, 2014
Received: April 4, 2014

Dear Dr. Ma:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

David Krause -S

for Binita S. Ashar, M.D., M.B.A., F.A.C.S.
Acting Director
Division of Surgical Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K133542

Device Name
Puracyn Plus™ Skin and Wound Care

Indications for Use *(Describe)*

OTC: Puracyn PlusTM Skin and Wound Care is intended for the OTC use of the management of minor skin wounds including minor lacerations, minor abrasions, minor irritations, minor cuts, minor burns and intact skin, in addition to moistening and lubricating absorbent wound dressings.

Professional Use: Puracyn PlusTM Skin and Wound Care is intended for use by healthcare professionals for cleansing, irrigating, moistening, and debriding to remove wound debris from acute and chronic dermal lesions that are partial or full thickness wounds such as 1st and 2nd degree burns, stage I – IV pressure ulcers, diabetic ulcers, stasis ulcers, abrasions and minor skin irritations, post-surgical wounds, grafted and donor sites, in addition to moistening and lubricating absorbent wound dressings.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☒ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

Jiyoung Dang -S

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

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STATE OF CALIFORNIA
DEPARTMENT OF FOOD AND AGRICULTURE
FEED, FERTILIZER, LIVESTOCK DRUGS, & EGG REGULATORY SERVICES
1220 N STREET
SACRAMENTO CA 95814

CERTIFICATE OF REGISTRATION FOR LIVESTOCK DRUGS

NON TRANSFERABLE

FIRM NO.

86911

Firm

INNOVACYN
3546 N RIVERSIDE AVE
RIALTO, CA 92377

is authorized to manufacture, deliver or sell in California the products listed below. Registration is not an endorsement or approval by the Department of Food and Agriculture of any product or any claim made for it. No reference may be made to the State of California, Department of Food and Agriculture in labeling or advertisements. Registration may be canceled after a hearing at any time for just cause. The composition of each product and the label used on it must be the same as those submitted by the registrant.

1. AQUACYN WOUND GEL, Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
2. AQUACYN WOUND WASH, Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
3. SUPER 7+ NAVEL DIP, Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017
4. VETERICYN FOAM CARE MEDICATED SHAMPOO, Status: Approved, Issued: Apr 6, 2016, Expires: Dec 31, 2017
5. VETERICYN HOT SPOT SPRAY-EQUINE &RABBIT, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
6. VETERICYN PLUS EAR RINSE, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
7. VETERICYN PLUS EYE WASH, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
8. VETERICYN PLUS EYE WASH (BOVINE), Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
9. VETERICYN PLUS HYDRO GEL WOUND &SKIN CARE (HORSE &LLAMA), Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
10. VETERICYN PLUS HYDRO GEL WOUND &SKIN CARE (HORSE), Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
11. VETERICYN PLUS HYDRO GEL WOUND &SKIN CARE (RABBIT), Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
12. VETERICYN PLUS OPHTHALMIC GEL, Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
13. VETERICYN PLUS PINK EYE SPRAY, Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
14. VETERICYN PLUS POULTRY CARE, Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
15. VETERICYN PLUS UTILITY GEL WOUND CARE UMBILICAL NAVEL &UDDER PROTECTION, Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
16. VETERICYN PLUS UTILITY SPRAY, Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
17. VETERICYN PLUS VF OTIC RINSE, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
18. VETERICYN PLUS WOUND &SKIN CARE, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
19. VETERICYN SELECT (HORSE &RABBIT), Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017
20. VETERICYN VF PLUS HYDRO GEL WOUND &SKIN CARE (HORSE #2040), Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
21. VETERICYN VF PLUS HYDRO GEL WOUND &SKIN CARE (HORSE #2041), Status: Approved, Issued: Aug 20, 2014, Expires: Dec 31, 2017
22. VETERICYN VF PLUS HYDRO GEL WOUND &SKIN CARE (HORSE #2043), Status: Approved, Issued: Apr 5, 2016, Expires: Dec 31, 2017
23. VETERICYN VF PLUS OPHTHALMIC WASH, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
24. VETERICYN VF PLUS WOUND &SKIN CARE, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
25. VETERICYN VF-OPTHALMIC WASH, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017

26. VETERICYN VF-OTIC RINSE, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
27. VETERICYN WOUND &SKIN CARE-EQUINE, Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017
28. VETERICYN WOUND &SKIN CARE HYDROGEL SPRAY- EQUINE &LLAMA, Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017
29. VETERICYN WOUND &SKIN CARE HYDROGEL SPRAY-RABBIT, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
30. VETERICYN WOUND &SKIN CARE- EQUINE, RABBIT, LLAMA, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
31. VETERICYN WOUND &SKIN CARE-RABBIT, Status: Approved, Issued: Apr 4, 2016, Expires: Dec 31, 2017
32. VETERICYN-EAR RINSE, Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017
33. VETERICYN-EYE WASH, Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017
34. VETERICYN-OPTHALMIC GEL, Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017
35. VETERICYN-PINK EYE SPRAY, Status: Approved, Issued: Apr 1, 2016, Expires: Dec 31, 2017

A CERTIFICATE OF REGISTRATION FOR LIVESTOCK DRUG IS NOT TRANSFERABLE. IF THERE IS A CHANGE IN BUSINESS OWNERSHIP, A NEW APPLICATION AND FEE(S) ARE NECESSARY.

NORTH DAKOTA
DEPARTMENT OF AGRICULTURE

COMMISSIONER
DOUG GOEHRING

STATE CAPITOL
600 E. BOULEVARD AVE. - DEPT. 602
BISMARCK, ND 58505-0020

www.nd.gov/ndda
Phone: (701) 328-2655
Toll Free: (800) 242-7535
FAX: (701) 328-4567
Email: doa-bah@nd.gov

INNOVACYN INC

3546 N RIVERSIDE AVENUE

RIALTO, CA 92377

Certificate Date:6/8/2016

NORTH DAKOTA CERTIFICATE OF LIVESTOCK MEDICATION REGISTRATION

Company Information

Company:	INNOVACYN INC	**Company ID:**	100230138
Contact:	KAREN ELDRIDGE	**Email:**	karene@burlingameindustries.com
Phone:	909-822-6000	**Fax:**	

Pursuant to N.D.C.C. 19-14-02, the following livestock medications(s) with a Status of "Active" are authorized for distribution in North Dakota.

Product Name	State ID	Status	Date Registered	Expire Date
EAR RINSE	600003300	Active	6/8/2016	6/30/2018
EAR RINSE (W/ALL ANIMALS)	600003304	Active	6/8/2016	6/30/2018
EYE WASH	600003306	Active	6/8/2016	6/30/2018
HOT SPOT SPRAY	600003298	Active	6/8/2016	6/30/2018
OPTHALMIC GEL	600003310	Active	6/8/2016	6/30/2018
OPTHALMIC WASH	600003322	Active	6/8/2016	6/30/2018
OTIC RINSE	600003320	Active	6/8/2016	6/30/2018
PINK EYE SPRAY	600003312	Active	6/8/2016	6/30/2018
TEAT SPRAY	600003314	Active	6/8/2016	6/30/2018
UMBILICAL, NAVAL & UDDER GEL	600003316	Active	6/8/2016	6/30/2018
VETERICYN SUPER 7+	600003538	Active	6/8/2016	6/30/2018
VETERICYN VF WOUND & INFECTION TREATMENT SPRAY GEL	4295	Active	6/8/2016	6/30/2018
VETERICYN WOUND & INFECTION TREATMENT	4293	Active	6/8/2016	6/30/2018
WOUND & INFECTION CARE	600003318	Active	6/8/2016	6/30/2018
WOUND & INFECTION CARE/HYDROGEL SPRAY	600003308	Active	6/8/2016	6/30/2018

Reviewed By:

Susan J Keller DVM

Susan J. Keller, DVM
State Veterinarian and Animal Health Division Director

Please keep the Department informed of any changes in address, contact information, ownership, or any other pertinent information. Any changes to product name and/or analysis must be submitted to the Department for review.

Product Renewal forms will be sent to your business, by the email address on file, prior to expiration.

All updated/revised labels must be submitted for review at: http://kellysolutions.com/erenewals/livestockmed/documentsubmit/

Form 1014 Rev 04/08
OREGON DEPARTMENT OF AGRICULTURE
635 CAPITOL ST NE
SALEM, OR 97301-2532
(503) 986-4550

POST IN A CONSPICUOUS PLACE

INNOVACYN INC
KAREN ELDRIDGE
3546 N RIVERSIDE AVE
RIALTO CA 92377

License No.: AG-R1010826VET
Date Issued: 05/27/2016
Date Expires: 06/30/2017
Printed: 05/31/2016

Veterinary Product Registration Certificate

Veterinary Products Registered in Oregon

	Product No.	UPC	Brand	Product Name
1	1034	8 52009 00217 8	Vetericyn	EYE Wash CAT
2	1030	52009 00208	Vetericyn Plus	Ear Rinse
3	1025	8 52009 00233 8	Vetericyn	Ear Rinse cat
4	90005	8 52009 00208 6	VETERICYN	Eye Wash
5	1030	52009 00208	vetericyn Plus	Eye Wash
6	1033	8 52009 00216 1	Vetericyn	Eye Wash dog
7	1036	8 52009 00256 7	VEtericyn	Eye Wash horse dog cat bunny
8	1010	8 52009 00206 2	VETERICYN	HOT SPOT SPRAY
9	90042	8 52009 00239 0	VETERICYN	Opthalmic Gel
10	2075	-	Vetericyn Plus VF	Opthalmic Wash
11	90046	8 52009 00275 8	VETERICYN VF	Otic Rinse
12	2023	52009 00275	Vetericyn Plus VF	Otic Rinse
13	90040	8 52009 00251 2	VETERICYN	Pink Eye Spray
14	7016	8 18582 01033 7	VETERICYN	SUPER 7+ UMBILICAL CORD DRY-OUT & PROTECTION
15	7001	8 18582 01033 7	VetERICYN	Super 7+ UMBILICAL CORD DRY-OUT & PROTECTION
16	90048	8 52009 00276 5	VETERICYN	Teat Spray
17	90047	8 52009 00277 2	VETERICYN	Umbilical, Navel & Udder Gel
18	1101	52009 00276	Vetericyn Plus	Utility Spray
19	1033	8 52009 00207 9	VETERICYN	WOUND & SKIN Care
20	90001	8 52009 00204 8	VETERICYN VF	WOUND & SKIN Care
21	90019	8 52009 00223 9	VETERICYN VF	WOUND & SKIN Care HydroSPRAY GEL
22	1002	8 52009 00201 7	VETERICYN	WOUND & SKIN Care cat dog horse bird

Form 1014 Rev 04/08
OREGON DEPARTMENT OF AGRICULTURE
635 CAPITOL ST NE
SALEM, OR 97301-2532
(503) 986-4550

POST IN A CONSPICUOUS PLACE

IN /ACYN INC — Veterinary Product Registration Certificate — License No.: AG-R1010826VET

Veterinary Products Registered in Oregon

	Product No.	UPC	Brand	Product Name
23	1042	8 52009 00210 9	Vetericyn	WOUND & skin Care, HydroGel Spray horse dog cat bird
24	1000	8 52009 00202 4	VETERICYN	Wound & Skin Care
25	2000	52009 00204	Vetericyn VF Plus	Wound & Skin Care
26	90003	8 52009 00202 4	VeTERICYN	Wound & Skin Care
27	2043	8 52009 00227 7	VETERICYN VF	Wound & Skin Care HydroGel Spray dog cat bunny bird
28	1005	8 52009 00229 1	VETERICYN	Wound & Skin Care cat
29	1008	8 52009 00200 0	Vetericyn	Wound & Skin Care horse dog cat bird

PRODUCTS REGISTERED: 29

Product Registration

South Dakota Department of Agriculture

Company Code: 2100

INNOVACYN INC
ATT: VICTOR TORCAT MALLEN
3546 N RIVERSIDE AVENUE
RIALTO CA 92377

Animal Remedy Products	Expiration Date	Status	Discontinued
Vetericyn Ear Rinse	12/31/2016	Active	No
Vetericyn Eye Wash	12/31/2016	Active	No
Vetericyn Hot Spot Spray	12/31/2016	Active	No
Vetericyn Opthalmic Gel	12/31/2016	Active	No
Vetericyn Pink Eye Spray	12/31/2016	Active	No
Vetericyn Plus Ear Rinse	12/31/2016	Active	No
Vetericyn Plus Eye Wash	12/31/2016	Active	No
Vetericyn Plus Hot Spot Spray	12/31/2016	Active	No
Vetericyn Plus Opthalmic Gel	12/31/2016	Active	No
Vetericyn Plus Pink Eye Spray	12/31/2016	Active	No
Vetericyn Plus Reptile Wound & Skin Care	12/31/2016	Active	No
Vetericyn Plus Utility Gel	12/31/2016	Active	No
Vetericyn Plus Utility Spray	12/31/2016	Active	No
Vetericyn Plus VF Opthalmic Wash	12/31/2016	Active	No
Vetericyn Plus VF Otic Rinse	12/31/2016	Active	No
Vetericyn Plus VF Wound & Skin Care	12/31/2016	Active	No
Vetericyn Plus VF Wound & Skin Care All Animals	12/31/2016	Active	No
Vetericyn Plus VF Wound & Skin Care HydroGel	12/31/2016	Active	No
Vetericyn Plus VF Wound & Skin Care Sample	12/31/2016	Active	No
Vetericyn Plus Wound & Skin Care	12/31/2016	Active	No
Vetericyn Plus Wound & Skin Care - The Traveler	12/31/2016	Active	No
Vetericyn Plus Wound & Skin Care Hydro Gel	12/31/2016	Active	No
Vetericyn Plus Wound & Skin Care Sample	12/31/2016	Active	No
Vetericyn Super 7+ Navel Dip	12/31/2016	Active	No
Vetericyn Super 7+ Ultra Green	12/31/2016	Active	No
Vetericyn Teat Spray	12/31/2016	Active	No
Vetericyn Utility Gel Umbilical Navel and Udder Gel	12/31/2016	Active	No
Vetericyn VF Opthalmic Wash	12/31/2016	Active	No
Vetericyn VF Otic Rinse	12/31/2016	Active	No
Vetericyn VF Wound and Skin Care	12/31/2016	Active	No
Vetericyn VF Wound and Skin Care HydroGel Spray	12/31/2016	Active	No

Vetericyn Wound and Skin Care	12/31/2016	Active	No
Vetericyn Wound and Skin Care HydroGel Spray	12/31/2016	Active	No

Company Product Counts

Not Discontinued:	**33**
Discontinued:	**0**
Total Products:	**33**

By Expiration Date

Expiration Date	Product Count
12/31/2016	33

VIRGINIA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES
OFFICE OF PLANT INDUSTRY SERVICES
102 GOVERNOR STREET, RICHMOND VA 23219

FEED PRODUCT REGISTRATION

CALENDAR YEAR 2016

REGISTRANT NO: 596723

INNOVACYN INC
VICTOR TORCAT MALLEN
3546 NORTH RIVERSIDE AVENUE
RIALTO, CA 92377

This certifies that the annual registration fee has been paid for the product(s) listed. The above registrant is hereby entitled to sell the registered product(s) in the Commonwealth of Virginia.

CATEGORY AR - ANIMAL REMEDIES

38 PRODUCTS REGISTERED

PROD NO	PRODUCT NAME
[illegible]12	VETERICYN EAR RINSE - CATS
630013	VETERICYN EAR RINSE - DOGS
630011	VETERICYN EAR RINSE - HORSES, DOGS, CATS, RABBITS
642456	VETERICYN EYE WASH - CATS
630014	VETERICYN EYE WASH - DOGS
627537	VETERICYN EYE WASH - HORSES, DOGS, CATS, RABBITS
627536	VETERICYN HOT SPOT SPRAY - DOGS, HORSES, CATS, RABBITS, HAMSTERS
627538	VETERICYN OPHTHALMIC GEL
648500	VETERICYN PINK EYE SPRAY
785640	VETERICYN PLUS EAR RINSE (W/DOG)
785642	VETERICYN PLUS EYE WASH (W/CAT)
785650	VETERICYN PLUS HOT SPOT SPRAY (W/ALL ANIMALS)
785644	VETERICYN PLUS OPTHALMIC GEL
785646	VETERICYN PLUS OTIC RINSE (W/ALL ANIMALS)
785654	VETERICYN PLUS PINK EYE SPRAY
785652	VETERICYN PLUS REPTILE WOUND & SKIN CARE
785656	VETERICYN PLUS UTILITY GEL
785658	VETERICYN PLUS UTILITY SPRAY
[illegible]026	VETERICYN PLUS VF OPTHALMIC WASH
785632	VETERICYN PLUS VF WOUND & SKIN CARE

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BY: .....

VIRGINIA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES
OFFICE OF PLANT INDUSTRY SERVICES
102 GOVERNOR STREET, RICHMOND VA 23219

FEED PRODUCT REGISTRATION

CALENDAR YEAR 2016

CONTINUATION OF REGISTRANT 596723 - INNOVACYN INC

CATEGORY AR - ANIMAL REMEDIES

38 PRODUCTS REGISTERED **** CATEGORY CONTINUED ****

PROD NO	PRODUCT NAME
785628	VETERICYN PLUS VF WOUND & SKIN CARE (W/ALL ANIMALS)
785630	VETERICYN PLUS VF WOUND & SKIN CARE HYDROGEL (W/CAT)
785624	VETERICYN PLUS VF WOUND & SKIN CARE SAMPLE
785638	VETERICYN PLUS WOUND & SKIN CARE (W/ALL ANIMALS)
785648	VETERICYN PLUS WOUND & SKIN CARE HYDROGEL (W/CAT)
785634	VETERICYN PLUS WOUND & SKIN CARE SAMPLE
785636	VETERICYN PLUS WOUND & SKIN CARE- THE TRAVELER
751438	VETERICYN SUPER 7+ NAVEL DIP
636424	VETERICYN TEAT SPRAY
636584	VETERICYN UMBILICAL, NAVEL & UDDER GEL
633152	VETERICYN VF OPHTHALMIC WASH
630015	VETERICYN VF OTIC RINSE - HORSES, DOGS, CATS, RABBITS
636583	VETERICYN VF WOUND & INFECTION CARE - HORSES, DOGS, CATS, BIRDS
633150	VETERICYN VF WOUND & INFECTION CARE HYDROGEL SPRAY - DOGS, CATS, RABBITS, BIRDS
627535	VETERICYN WOUND & INFECTION CARE - CATS
642193	VETERICYN WOUND & INFECTION CARE - CATS, DOGS, RABBITS, BIRDS
633151	VETERICYN WOUND & INFECTION CARE - HORSES, RABBITS, DOGS, CATS, LLAMAS
636423	VETERICYN WOUND & INFECTION CARE HYDROGEL SPRAY -DOGS, CATS, RABBITS, BIRDS

Washington State Department of Health

This organization

Vetericyn

is authorized by RCW 18.64 to have a

Pharmaceutical Wholesaler License

Operated by: **Vetericyn Inc**

Located at: **3546 N Riverside Ave**
Rialto, CA 92377-3802

Secretary

Status
ACTIVE

Effective Date
10/01/2016

Credential Number
PHWH.FX.60126366

Expiration Date
09/30/2017

DOH Contact Information
www.doh.wa.gov/hsqa/default.htm

Vetericyn
3546 N Riverside Ave
Rialto, CA 92377-3802

WYOMING DEPARTMENT OF AGRICULTURE
Cheyenne, WY 82002

Registering Company Number: 11901134

Amount
$20.00

OFFICIAL RECEIPT

INNOVACYN INC
3546 N RIVERSIDE AVE

RIALTO, CA 92377
Attn. KAREN ELDRIDGE

Phone 9098226000
Email KARENE@BURLINGAMEINDUSTRIES.C

Date Paid: 2/22/2016
BatchNo: 20

Certificate of Registration

Expiration Date
12/31/2016

is authorized to manufacture, deliver or sell in Wyoming the products listed below. Registration is not an endorsement or approval by the Department of Agriculture of any product or claim made for it. No reference may be made to registration in labeling or advertisements. Registration may be canceled after hearing at any time for just cause. The composition of each product and the label used on it must be the same as those submitted by the registrant. A certificate of registration may not be transferred if there is a change in business ownership but a new application and fee are necessary.

ANIMAL REMEDIES

056115 VETERICYN PLUS POULTRY CARE

Products Registered	1

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Licensing and Registration

To: USDA/AMS/NOP, Standards Division
Attention: National List Manager
1400 Independence Ave. SW
Room 2642-So., Ag Stop 0268
Washington, DC 20250-0268

Petitioner: Innovacyn, Inc.
3546 North Riverside Avenue
Rialto, CA 92377
Contact: Rebecca Lei / Scott van Winkle
Number: (909)237-3716 / (909)237-3428
Email: rebeccal@aquaoxindustres.com / scottv@innovacyn.com

Petition to Include Hypochlorous Acid (generated by Electrolyzed Water) onto National List 7 CFR § 205.603 – Volume II

This Page Ends Here.

Appendix V: Literature Research about Hypochlorous Acid

		Page
a)	Environmental Assessment of Hypochlorous Acid Solution from HSP USA, LLC, for Food Contact Notification FCN No. 001176.	**3**
b)	Environmental Decision Memo for Food Contact Notification No. 001176.	**11**
c)	Environmental Assessment of Hypochlorous Acid Solution from Sterilox Food Safety/Div. of PuriCore for Food Contact Notification FCN No. 001470.	**14**
d)	Environmental Decision Memo for Food Contact Notification No. 001470.	**29**
e)	Hypochlorous Acid as a Potential Wound Care Agent. Part I. Stabilized Hypochlorous Acid: A Component of the Inorganic Armamentarium of Innate Immunity. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1853323/	**35**
f)	Hypochlorous Acid as a Potential Wound Care Agent: Part II. Stabilized Hypochlorous Acid: Its Role in Decreasing Tissue Bacterial Bioburden and Overcoming the Inhibition of Infection on Wound Healing. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1853324/	**51**
g)	Hypochlorous Acid: An Ideal Wound Care Agent with Powerful Microbicidal, Antibiofilm, and Wound Healing Potency. http://www.ncbi.nlm.nih.gov/pubmed/25785777	**63**
h)	Hypochlorous acid: Its multiple uses for wound care. http://www.o-wm.com/files/owm/pdfs/OWM_September2013_Liden.pdf	**66**
i)	Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers. http://www.ncbi.nlm.nih.gov/pubmed/16669304	**70**
j)	Conquering Chronic Non-Healing Wounds with Pure Hypochlorous Acid. http://novabay.com/wp-content/uploads/2013/05/Symposium-on-Advanced-Wound-Care-Conquering-the-Chronic-Non-Healing-Wounds-with-NeutroPhase%c2%ae.pdf	**73**
k)	A Randomized Clinical Trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wound. http://www.iiste.org/Journals/index.php/JEP/article/viewFile/13072/13653	**75**
l)	Use of Hypochlorous Acid Solution as a Disinfectant in Laboratory Animal Facilities. http://www.jscimedcentral.com/MedicalMicrobiology/medicalmicrobiology-1-1005.pdf	**89**
m)	Living with a killer: the effects of hypochlorous acid on mammalian cells. http://www.ncbi.nlm.nih.gov/pubmed/11327319	**92**
n)	White Paper: Evaluation of Vetericyn Plus™ Pinkeye Spray as an aid in corneal healing and reduction of pain and infection of the cornea following experimentally induced Bovine Keratoconjunctivitis, Auburn University College of Veterinary Medicine, Auburn University.	**101**
o)	Study Report: The Effect of Vetericyn® Technology (hypochlorous acid) on the Treatment of Hairy Foot Warts in Dairy Cows, California Polytechnic State University, San Luis Obispo.	**105**
p)	Pilot Study Report: The Effect of Puracyn® Plus on Deep Dermal Infections, Miller School of Medicine, University of Miami.	**111**

Appendix V: Literature Research about Hypochlorous Acid

a) Environmental Assessment of Hypochlorous Acid Solution from HSP USA, LLC, for Food Contact Notification FCN No. 001176.

Environmental Assessment for (Hsp_2O)® Solution in Support of an FCN Regarding the Use of Hypochlorous Acid to Disinfect Water Used to Crisp Vegetables with Draining

1. Date: Prepared April 16, 2012

2. Name of Submitter: HSP USA, LLC

3. Address:

HSP USA, LLC
3111 Route 38, Suite 11, #310
Mount Laurel, NJ 08054

4. Description of Proposed Action:

a. Requested Action:

This FCN is seeking an approval for the use of a food-contact substance for re-hydrating fresh produce at retail and food service establishments:

- Introduction of 20 gals of a (Hsp_2O)® solution at up to 60 ppm available free chlorine (AFC) in a produce sink consistent with the preconditions outlined in the Food Code.
- Place 1 box of leafy greens or other whole uncut produce item in increments of five (5) pound loads into the sink to soak for a minimum of 5 minutes.
- Remove produce and set aside to drain
- Test (Hsp_2O)® solution for AFC and if above 25 ppm charge the sink with another five (5) pounds of produce. If AFC is below 25 ppm, drain the sink and re-fill with fresh (Hsp_2O)® solution.
- Continue until all the produce requiring crisping is complete.
- Produce may be used for display or prepared for consumption after 10 minutes of draining.

b. Need for action:

The intended technical effect of eliminating a potable water rinse is to avoid cross contamination of re-crisped product or products. Looking specifically at lettuce re-crisping as an example, if lettuce is re-crisped or rinsed by placing fresh-cut lettuce/leafy greens in containers with tap water or other water with a low level of chlorine, the chlorine present is quickly inactivated by the organic load presented by the lettuce/leafy greens. This will increase the potential for lettuce/leafy greens cross contamination particularly if additional lettuce/leafy greens are added to the container (Wachtel and Charkowski, 2002 Attachment A).

The ability of chlorine to prevent cross contamination in aqueous baths was illustrated with inoculated cantaloupes and Salmonella (Suslow 2004 Attachment B). This report clearly shows that Salmonella migrated when fruit was soaked in water. By analogy and experience, the treatment of lettuce will not fully disinfect the lettuce. All subsequent treatment of lettuce with water without benefit of chlorine will promote both cross contamination and spread of existing contamination within the head.

c. Locations of use/disposal:

$(Hsp_2O)^{®}$ solution for re-hydrating fresh produce at retail will generally be used in the backroom of the store where produce is prepared. Given that crisping is done in a sanitary sink, disposal will be through the sanitary sewer.

5. Identification of substances that are subject to the proposed action:

$(Hsp_2O)^{®}$ solution is a hypochlorous solution made from a tightly controlled chemical process (See Attachment K – Confidential Business Information. Hypochlorous acid (7790-92-3) is the active component and will generally be present at at or below 60 ppm for treatment.Residual sodium chloride (7647-14-5) is also present as a result of the reaction.

The formula for hypochlorous acid is HOCl. Its molecular weight is 52.46. Concentrated hypochlorous acid is greenish-yellow in solution. In its concentrated form hypochlorous acid is highly unstable and decomposes to hydrogen chloride and oxygen except in dilute solution. $(Hsp_2O)^{®}$ solution is generated on site as needed.

6. Introduction of substances into the environment:

a. Introduction of substances into the environment as a result of manufacture:

No extraordinary circumstances apply to the generation of $(Hsp_2O)^{®}$ solution. It is generated on site in accordance with demand. The generation process releases hypochlorous acid in a very weak brine solution

b. Introduction of substances into the environment as a result of

use/disposal:

The released materials from the procedure include sodium chloride brine, residual available chlorine, and low levels of by-products including chlorate, chlorite, and disinfection by-products that are associated with the use of the various forms of chlorine.

To estimate the potential discharge volumes associated with the proposed process, it is sufficient to have a projected number of units to be installed, a projected usage rate and the measured concentrations of the various components and by-products in the discharge. The calculations require simple multiplications of the concentrations, the number of 20 gallon cycles per day (See Confidential Business Information), the number of days per year (365) and the projected number of installations (See Confidential Business Information). The calculations and results are reported in Attachment K, Confidential Business Information.

The expected concentrations of the various constituents and by-products have been measured and reported in the various studies and other information associated with this FCN. The discharged weak brine will be the dominant component besides water at less than 0.005% sodium chloride.

The discharged brine solutions will also contain modest levels of free available chlorine depending on the amount of treated produce ranging from essentially zero to 60 ppm if no produce is treated. The average discharge is expected to be less than 25ppm based on the process guideline of reusing solutions greater than 25 ppm and discharges those solutions which are less than 25 ppm. This residual chlorine can be expected to rapidly react (less than 3 hours) with organic material in the waste stream producing mostly oxygen and chloride. For the minor components and by-products, the chlorite levels will be less than 0.3 ppm. This estimate is based on the detection limit as no chlorite was detected experimentally. The chlorate levels will be less than 2 ppm. And finally, the total trihalomethanes, representative of the disinfection by-products, will be between 20-50 ppb. These discharge concentrations are the same as those in FCN No. 692. These concentrations are used to estimate annual discharges which are reported in Attachment K, Confidential Business Information.

Virtually 100% of these materials will enter the waste stream via the sanitary sewer at the retail store.

7. Fate of substances released into the environment:

All of the components of $(Hsp_2O)^{®}$ solution and the degradation products are well known and with the exception of the sodium chloride are at levels approximating those found in drinking water. The proposed process does not increase or decrease the discharges from the allowed process.

This said, sodium chloride is exceedingly stable in the environment. It will become part of the total dissolved solids (TDS) in the effluent stream from the wastewater treatment facility. Furthermore, because oxychlorine species (hypochlorous acid, chlorite, chlorate and chlorine dioxide) readily react with the organic matter and microorganisms in water and soil (sediments) and will undergo ultimate degradation into chloride ion, we anticipate that the expected environmental concentrations for these oxychlorine species will be very small and thus will be of no environmental concern. **(**Attachment C**,** Supplement to the Environmental Information for Food Contact Notification No. 450, October 18, 2004, Tong Zhou, Ph.D., Environmental Toxicologist Environmental Review Group Division of Chemistry Research and Environmental Review)

Given that the $(Hsp_2O)^{®}$ solutions will be discharged to a sanitary sewer after use where the total stream is generally chlorinated, the discharges from this process will rapidly be lost in these larger pools. Furthermore, when a chlorinated effluent is released into receiving waters, free residual chlorine dissipates rapidly. It has a half-life of 1.3 to 5 hours (Attachment D, EPA RED for Chlorine Gas). The ultimate fate of chlorine-containing effluent is site specific, and depends on factors such as the chemical constituents of the receiving waters, their temperature, the dilution ratio and the intensity of sunlight (Attachment D, EPA RED for Chlorine Gas). The disinfection by-products such as the trihalomethanes are the same materials found in chlorinated drinking water, at levels well within the drinking water standard and will share the same fate as these larger pools of material in the wastewater effluent.

8. Environmental effect of released substances:

We reviewed information on www.pesticideinfo.org and have identified ecotoxicology studies on fish and zooplankton species for the expected effluent components. Among these are chlorite studies for opossom shrimp, sodium chloride studies on water flea (*Ceriodaphnia dubia*) as well as hypochlorous acid studies on rainbow trout. The findings are summarized in the table below:

No.	Compound	Organism	Effect	Measurement	Life Stage	Study Time	Endpoint	Toxic Dose (ug/L)
1	Hypochlorous Acid	Oncorhynchus mykiss	Mortality	Mortality	156-169 MM	2h	LC 50	200
2	Sodium Chloride	Ceriodaphnia dubia	Mortality	Mortality	<24h	7d	LC 50	330,000
3	Sodium Chlorite	Americamysis bahia	Mortality	Mortality	<24h	96h	LC 50	576 (mean)
4	Sodium Chlorate	Daphnia magna	Mortality	Mortality	NR	48h	LC 50	3,162,000

Uses of hypochlorous acid that are **not** regulated under the NPDES permit program, include swimming pool, aquaria and indoor use patterns (fruit and vegetable rinsing and food processing), should produce only intermittent discharges of minimal concentration into lakes or streams, resulting in minimal environmental exposure." (Attachment F EPA RED Facts Chlorine Gas)

The Estimated Environmental Concentrations (EECs) from the proposed use for all species will be many orders of magnitude less than the levels discharged to the sewer at the point of use. This estimate can be rationalized by assuming a retail store provides produce for up to 10,000 people (Estimate based on Milpitas, CA with about 70,000 people and 7 retail stores). The total water discharge in Milpitas is 18.5 million gallon per day (Attachment E, Clean Watershed Need Survey).

The Estimated Environmental Concentrations (EECs) are summarized as follows based on the assumptions above. See Attachment K, Confidential Business Information for detailed calculations.

No.	Compound	ECCs (µg/L)
1	Hypochlorous Acid	1.8
2	Sodium Chloride	3.8
3	Sodium Chlorite	0.023
4	Sodium Chlorate	0.15

With the 4 to 5 orders of magnitude of dilution, EECs are significantly below the endpoints for all four

concerned substances. In addition, the short half-life of hypochlorous acid will further reduce its EEC once it enters the sanitary sewer. The environmental effects of the released substances will be insignificant.

The data available strongly suggests that the amounts of the oxychlorine species which would be expected to be released into the environment as a bi-product of using $(Hsp_2O)^{®}$ solution to crisp leafy vegetables through the use and disposal would be so low as to pose no threat to either aquatic or terrestrial ecosystems.

9. Use of resources and energy:

The proposed change will have essentially no effect on resources or energy. The use of draining to remove residual chlorine will provide a very minor reduction in water use. All other factors would remain the same.

10. Mitigation measures:

No adverse situations requiring mitigation have been identified.

11. Alternatives to the proposed action:

No adverse environmental impacts remain to be addressed so alternatives are not required.

12. List of preparers:

Henry Dao
President and CEO
HSP USA, LLC
3111 Route 38, Suite 11, #310
Mount Laurel, NJ 08054
(856) 437-0688

Mr. Dao has over nineteen years of experience in the management consulting, process engineering and manufacturing. He has consulted with clients including healthcare, food and beverage, water treatment, industrial products and chemicals, and many other industrial or commercial fortune 500 companies. As the President and CEO, Mr. Dao has the overall responsibility in setting strategic direction and tactical management of the firm. In addition, Mr. Dao is passionate about and active in promoting clean technology and economic development focused initiatives.

13. Certification:

The undersigned official certifies that the information presented is true, accurate and complete to the best of the knowledge of HSP USA, LLC.

_4/17/12_____

(Date)

(Signature) Henry Dao, President / CEO

14. References:

A. Marian R. Wachtel and Amy O. Charkowski, Cross-Contamination of Lettuce with *Escherichia coli* O157:H7, Journal of Food Protection, Vol. 65, No. 3, 2002, Pages 465–470

B. Trevor V. Suslow, Minimizing the Risk of Food Borne Illness Associated with Cantaloupe Production and Handling in California, Regents of the University of California, 2004

C. Tong Zhou , Supplement to the Environmental Information for Food Contact Notification No. 450, October 18, 2004, Environmental Toxicologist Environmental Review Group Division of Chemistry Research and Environmental Review)

D. *Reregistration Eligibility Decision (RED): Chlorine Gas*; EPA738-R-99-001; Office of Pesticide Programs; US EPA: February 1999, http://www.epa.gov/oppsrrd1/REDs/4022red.pdf

E. Clean Watersheds Needs Survey – Discharge Database 2000 for Santa Clara County, Environmental Protection Agency, http://cfpub.epa.gov/cwns/rpt_discharge2_00.cfm

F. Registration Eligibility Decision (RED) Facts : Chlorine Gas, EPA-738-F-99-001, US EPA, February 1999 http://www.epa.gov/oppsrrd1/REDs/factsheets/4022fact.pdf

G. Chlorine Dioxide: Final Risk Assessment Case 4023; Docket ID No. EPA-HQ-OPP-2006-0328; U.S. Environmental Protection Agency, Antimicrobials Division: Washington, D.C., Aug 2, 2006.

H. James Ringo, Sodium chlorite environmental Assessment, Biocide International, August 27, 2004, http://www.fda.gov/downloads/Food/FoodIngredientsPackaging/FoodContactSubstancesFCS/UCM143218.pdf

I. Ambient Water Quality Criteria for Chloride-1988, EPA 440/5-88-001, US EPA , February 1988, http://www.epa.gov/waterscience/pc/ambientwqc/chloride1988.pdf

J. Ambient Water Quality Criteria for Chloroform EPA 440/5-80-033, US EPA, October 1980 http://www.epa.gov/waterscience/pc/ambientwqc/chloroform80.pdf

15. Attachments:

A. Wachtel and Charkowski, 2002, (The data was part of FCN 692 and is therefore not included)

B. Suslow, 2004 (The data was part of FCN 692 and is therefore not included)

C. Supplemental for FCN No. 450 (The data was part of FCN 692 and is readily available on the web and is therefore not included)

D. EPA RED for Chlorine Gas (The data was part of FCN 692 and is readily available on the web and is therefore not included)

E. Clean Watersheds Need - Santa Clara County (The data was part of FCN 692 and is readily available on the web and is therefore not included)

F. EPA RED Factsheet for Chlorine Gas (The data was part of FCN 692 and is readily available on the web and is therefore not included)

G. Chlorine Dioxide Final Risk Assessment (The data was part of FCN 692 and is readily available on the web and is therefore not included)

H. Sodium chlorite Environmental Assessment (The data was part of FCN 692 and is readily available on the web and is therefore not included)

I. Water Quality Criteria -Chloride (The data was part of FCN 692 and is readily available on the web and is therefore not included)

J. Water Quality Criteria -Chloroform (The data was part of FCN 692 and is readily available on the web and is therefore not included)

K. Confidential Business Information

Appendix V: Literature Research about Hypochlorous Acid

b) Environmental Decision Memo for Food Contact Notification No. 001176.

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

Environmental Decision Memo for Food Contact Notification No. 001176

Return to inventory listing: **Inventory of Environmental Impact Decisions for Food Contact Substance Notifications (http://www.accessdata.fda.gov/scripts/fdcc/?set=ENV-FCN)** or the **Inventory of Effective Food Contact Substance Notifications (http://www.accessdata.fda.gov/scripts/fdcc/?set=FCN)**.

See also **Environmental Decisions (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/default.htm)**.

April 30, 2012
From: Biologist, Regulatory Team 2, Division of Biotechnology and GRAS Notice Review (HFS-255)
Through: Annette M. McCarthy, Ph.D, Senior Science and Policy Staff____

To: Division of Food Contact Notifications (HFS-275)
Attention: Donna Robie, Ph.D.

Subject: FCN No. 1176 – Hypochlorous acid for use as an antimicrobial agent in a solution used for re-hydrating fresh fruits and vegetables including leafy green vegetables.

Notifier: HSP USA, LLC.

Attached is the Finding of No Significant Impact (FONSI) for FCN 1176. After this notification becomes effective, copies of this FONSI and the notifier's environmental assessment, dated April 16, 2012, may be made available to the public. We will post digital transcriptions of the FONSI and the environmental assessment on the agency's public website.

Please let us know if there is any change in the identity or use of the food-contact substance.

Leah D. Proffitt

Attachment: Finding of No Significant Impact

FINDING OF NO SIGNIFICANT IMPACT

A food-contact notification (FCN No. 1176), submitted by HSP USA, LLC., to provide for the safe use of hypochlorous acid for use as an antimicrobial agent in a solution used for re-hydrating fresh fruits and vegetables including leafy green vegetables.

The Office of Food Additive Safety has determined that allowing this notification to become effective will not significantly affect the quality of the human environment and, therefore, will not require the preparation of an environmental impact statement. This finding is based on information submitted by the notifier in an environmental assessment, dated April 16, 2012.

Prepared by _____Date: April 30, 2012
Leah D. Proffitt
Biologist
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

Approved by _____Date: April 30, 2012
Annette M. McCarthy, Ph.D.
Senior Science and Policy Staff
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration

More in Environmental Decisions (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/default.htm)

Decisions for Food-Contact Notifications (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/ucm105897.htm)

Decisions for Petitions (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/ucm105895.htm)

Definitions of Environmental Terms (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/ucm105934.htm)

Appendix V: Literature Research about Hypochlorous Acid

c) Environmental Assessment of Hypochlorous Acid Solution from Sterilox Food Safety/Div. of PuriCore for Food Contact Notification FCN No. 001470.

Environmental Assessment of Sterilox™ Hypochlorous Acid Solution

1. **Date:** October 2, 2014

2. **Name of Applicant:** PuriCore, Inc.

3. **Address:**

PuriCore, Inc.
508 Lapp Road
Malvern, PA 19355

All communication regarding this food contact notification (FCN) environmental assessment (EA) should be sent to the attention of the authorized representative:

Kim Carson
Exponent, Inc.
1150 Connecticut Ave, NW
Suite 1100
Telephone: 202 772 4952
Email: KCarson@Exponent.com

4. Description of Proposed Action

The action requested in this submission is the food contact notification of the use of the food contact substance (FCS) hypochlorous acid solution. Hypochlorous acid is intended to provide an antimicrobial component to water designated to crisp (*i.e.*, re-hydrate) whole and fresh cut fruits and vegetables.

Maximum Use Level:
Sterilox hypochlorous acid solution is formulated to deliver up to 60 ppm available free chlorine (AFC). Sterilox hypochlorous acid solution is generated through the electrochemical oxidation of chloride from salt brine by the Sterilox Food Safety System.

Food Types:
In addition to whole fruits and vegetables, as included in FCN 692, Sterilox hypochlorous solution is intended to be used on fresh cut fruits and vegetables. The fruit or vegetable may be cut prior to submerging in the Sterilox solution or cut after the fresh fruits and vegetables have soaked in the Sterilox hypochlorous acid solution.

Conditions of Use:

The process for the use of Sterilox hypochlorous acid solution is as follows:

- Fresh produce is placed into a sink containing Sterilox hypochlorous acid solution and soaked for a minimum of 90 seconds and maximum of 10 minutes. The produce is removed from the solution and set aside to drain.
- Alternatively, Sterilox hypochlorous acid solution is introduced by spraying the solution onto the fresh produce and allowing the solution to drain from the produce.
- The Sterilox hypochlorous acid solution application process continues until all the produce requiring hydrating or crisping is complete.
- Produce may be used for display in the store or prepared for consumption (*e.g.*, made-to-order salad) after 10 minutes of draining.

Controls:

- The hypochlorous acid solution must be between 25 and 60 ppm. If AFC is below 25 ppm, the sink is drained and re-filled with fresh Sterilox hypochlorous acid solution.

5. Identification of the Food Contact Substance

Typical Physical Properties of Sterilox Hypochlorous Acid Solution
Appearance: greenish-yellow in solution
pH: weak acid
Solubility: soluble in water
Stability: the hypochlorous chemical species is unstable and decomposes to halogenated chemical species and oxygen (*e.g.*, chlorine, chlorite, chlorate and trihalomethanes (THMs) including: bromodichloromethane, dibromochloromethane and bromoform).

Hypochlorous acid (HSDB)
CAS #: 7790-92-3
Formula: HOCl
Molecular weight: 52.46 g/mol
Water solubility: soluble
Dissociation constant (pKa): 7.53
Comment: The active oxychloric species in the solution; present at not more than 60 ppm in the end-use product solutions.

Sodium chloride (HSDB)
CAS#: 7647-14-5
Formula: NaCl
Molecular weight: 58.44 g/mol
Water Solubility: highly soluble
Dissociation constant (pKa): completely dissociated

Comment: Starting material. Food grade salt supplied by Morton Salt with a specification of 99.95-99.99%% sodium chloride. It contains no additives and less than 0.05% typical impurities including calcium sulfate, calcium carbonate and heavy metals (*e.g.*, copper).

Sodium hydroxide (HSDB)

CAS#: 1310-73-2
Formula: NaOH
Molecular weight: 40.00 g/mol
Water Solubility: highly soluble
Dissociation constant (pKa): completely dissociated
Comment: By-product from Sterilox hypochlorous acid solution

Chlorine (HSDB)

CAS#: 7782-50-5
Formula: Cl_2
Molecular weight: 70.91 g/mol
Water Solubility: soluble (aqueous form)
Vapor pressure: 5.83 E10 mm Hg @ 25^{o}C (gaseous form)
Comment: By-product from Sterilox hypochlorous acid solution, minimized under controlled pH environment; interchangeable chlorine species in final solution

Hydrogen chloride (HSDB)

CAS#:7647-01-0
Formula: HCl
Molecular weight: 36.46 g/mol
Water Solubility: soluble (aqueous form)
Vapor pressure: 3.54 E4 mm Hg @ 25^{o}C (gaseous form)
Comment: By-product from Sterilox hypochlorous acid solution, minimized under controlled pH environment; interchangeable chlorine species in final solution

Chlorite (HSDB)

CAS#: 7758-19-2 (sodium chlorite)
Formula: ClO_2- (ion form) ($NaClO_2$ salt)
Molecular weight: 90.44 g/mol ($NaClO_2$)
Water Solubility: very soluble
Oxidizer: Strong; readily reduced to chloride and chlorate
Solid partition coefficient (Kd): not measured or reported (EPA, 2006c)
Bioconcentration: not expected (USEPA 2006c)
Not readily biodegradable under aerobic conditions (EPA, 2006c)
Comment: By-product from Sterilox hypochlorous acid solution, minimized under controlled pH environment

Chlorate (HSDB)

CAS#: 7775-09-9 (sodium chlorate)

Formula: ClO_3- (ion form) ($NaClO_3$ salt)
Molecular weight: 106.44 g/mol ($NaClO_3$)
Water Solubility: very soluble
Vapor pressure: 'very low' (EPA, 2006b)
Bioaccumulation: 'low potential' (EPA, 2006b)
Oxidizer: Strong; readily reduced to chloride and chlorate Comment: By-product from Sterilox hypochlorous acid solution, minimized under controlled pH environment

Bromodichloromethane (HSDB)
CAS#: 75-27-4
Formula: $CHBrCl_2$
Molecular weight: 163.83 g/mol
Octanol Water Partition Coefficient (log Kow): 2
Water Solubility: soluble
Vapor pressure: 50 mm Hg @ 20^oC
Solid partition coefficient (Koc): 53 to 251
Henry's Law Constant: 2.12 E-3 atm-cu m/mole
Bioconcentration factor: 7 (estimated)
Comment: By-product formed in final solution

Chlorodibromomethane (HSDB)
CAS#: 124-48-1
Formula: $CHBr_2Cl$
Molecular weight: 162.08 g/mol
Octanol Water Partition Coefficient (log Kow): 2.16
Water Solubility: soluble
Vapor pressure: 5.54 mm Hg @ 20^oC
Solid partition coefficient (Koc): 84
Henry's Law Constant: 7.83 E-4 atm-cu m/mole
Bioconcentration factor: 9 (estimated)
Comment: By-product formed in final solution

Bromoform (HSDB)
CAS#: 75-25-2
Formula: $CHBr_3$
Molecular weight: 252.73 g/mol
Octanol Water Partition Coefficient (log Kow): 2.40
Water Solubility: soluble
Vapor pressure: 5.4 mm Hg @ 20^oC
Solid partition coefficient (Koc): 116, 126
Henry's Law Constant: 5.35 E-4 atm-cu m/mole
Bioconcentration factor: 14 (estimated)
Not readily biodegradable
Comment: By-product formed in final solution

AFC levels and other by-products in Sterilox™ hypochlorous solutions were measured in the study entitled, "Measurement of Disinfectant By-product Formation During Treatment of Cut Lettuce by Submersion in Hypochlorous Acid Solution," June 14, 2014 (PuriCore, 2014). These values are used for the environmental assessment as a worst-case estimate of environmental exposure for the residuals because cut lettuce increases cellular surface area, much of which was disrupted during cutting, thus potentially altering water chemistry, such as pH and solution activity. The hypochlorous acid solution used in the study dated June 14, 2014 was prepared from a hypochlorous acid concentration manufactured on May 27, 2014. The analysis for chlorine, chlorate, chlorite and THMs was conducted between May 28 through June 2, 2014. It should be noted that although a hypochlorous acid concentrate was used to produce the solution, the production of the concentrate was just days prior to the analysis and is representative of solution freshly generated electrochemically *in situ* as no differences in composition is expected, including residuals or by-products between a solution produced from a freshly made concentrate and a solution prepared electrochemically *in situ*. Table 1 lists the concentrations of residuals measured in the freshly prepared Sterilox solution following the treatment process described by the label and thus relevant to typical consumer use. The measured values of AFC in this study ranged from 41 to 56 ppm. To be conservative, the nominal concentration of AFC in Sterilox solution, 60 ppm, is used as a worst case estimate in the environmental exposure calculations.

Table 1. Measurements of the FCS and Residual Chemical Species in Hypochlorous Acid Solution

Chemical Species	Maximum Measured Hypochlorous Acid Solution [a]	
	ppm	%
Food Contact Substance		
Available free chlorine	60[b]	0.006
Residuals		
chlorite	<0.5[c]	0.00005
chlorate	<0.5[c]	0.00005
total trihalomethanes	0.0606	0.00000606

[a] Measurement of Disinfectant By-product Formation During Treatment of Cut Lettuce by Submersion in Hypochlorous Acid Solution, June 4, 2014. PuriCore 2014.
[b] Nominal concentration of hypochlorous acid in Sterilox solution.
[c] Less than detection limits of chlorite and chlorate.

6. **Introduction of Substances into the Environment**

6.a. As a result of manufacture/generation at site of production

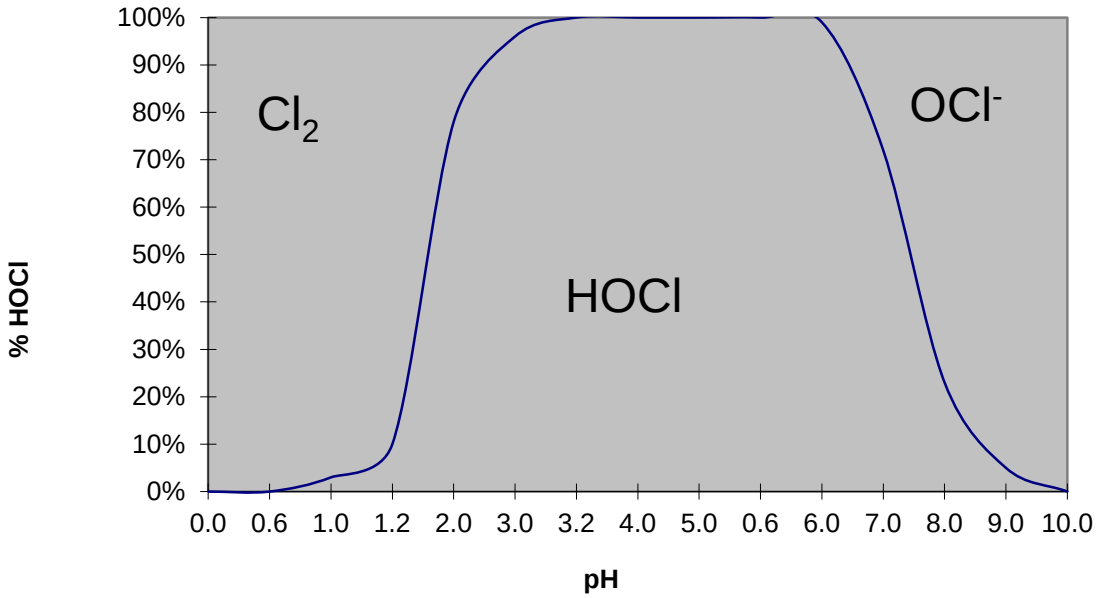
PuriCore offers Sterilox hypochlorous acid solution generated *in situ* by the customer at the point of application through an *in situ* device that utilizes an electrochemical chlor-alkali oxidation process from starting salt brine. The generation process requires sodium

chloride as a starting material and generates sodium hydroxide, hydrogen and oxygen gas due to the hydrolysis of water in the electrochemical cell. Due to the control of pH during production of the solution, trace amounts of hydrogen, oxygen and diatomic chloride gas are generated during the equilibrium reaction favoring hypochlorous acid solution and therefore release of these gases into the atmosphere at the site of production is negligible. Under 21 Code of Federal Regulations (CFR) § 25.40(a), an EA should focus on relevant environmental issues relating to the use and disposal from use, rather than the production, of FDA-regulated articles. Information available suggests no extraordinary circumstances suggesting an adverse environmental impact as a result of the manufacture of the antimicrobial agent. Consequently, information on the manufacturing site and compliance with relevant emissions requirements is not provided here.

6.b. As a result of use/disposal

Using Sterilox hypochlorous acid solution in the proposed crisping procedure introduces a salt starting material and various oxychloro, chloride and THM by-products into the environment. Relative proportions of each are determined by pH control of the Sterilox solution by the Sterilox Food Safety System. The effective oxychloro species, hypochlorous acid, exists interchangeably with other chlorine species, including chlorine, hydrogen chloride (aqueous and gaseous) and chlorite (1993 Letter to Dr. Michael Rose from Dr. Andrew Laumbach, FDA). This is supported by the equilibrium chemistry of active chlorine. In a controlled pH environment, hypochlorous acid will exist as the dominant chlorine species under pH conditions ranging from 5 to 8.4 (see diagram below).

Figure 1. Equilibrium Chemistry of Active Chlorine



The table below summarizes measured available free chlorine (includes diatomic chlorine,

hypochlorous acid and hypochlorite species) in Sterilox solution, which ranged from 93 to >98% depending on the pH.

Table 1. AFC in Sterilox Hypochlorous Acid Solution

Percent Concentration In Sterilox Hypochlorous Acid Solution Generated *in Situ*		
Chemical Species	At pH 5-6	At pH 8.4
Available free chlorine (includes diatomic chlorine, hypochlorous acid and hypochlorite)	93	>98

In addition to AFC (largely hypochlorous acid), residual chemicals in the final solution that may be released down the drain to publicly owned treatment works (POTWs) include the following: sodium chloride (starting material), sodium hydroxide (formed during the generation process), degradation oxycloro species (chlorate, chlorite) and THM formation by-products (bromodichloromethane, chlorodibromomethane and bromoform).

6.b.1. Maximum yearly market volume for proposed use

The maximum yearly market estimate per retail site for use of Sterilox hypochlorous acid solution is 146,657 kg. The per retail site usage is a projection based on 2012 and 2013 Sterilox sales data for all current and anticipated usage types (*i.e.*, soaking and misting of fresh whole and cut fruits and vegetables). This estimate is based on projections of large (*e.g.*, high volume) retail locations using 5 20-gallon cycles of Sterilox hypochlorous acid solution per day for 365 days per year. As the consumer consumption of fruits and vegetables is not expected to increase, the overall usage of Sterilox solution is not likely to increase but shift from usage on whole fruits and vegetables to usage on prepared or cut fruits and vegetables. The usage estimate is an overestimation as it assumes usage every day of the year and 100 gallons of solutions per day and is not adjusted for lower usage periods (*e.g.*, seasonality). Volume projections based on 2012-2013 sales data for smaller retails stores estimate less than 100 gallons per day. The anticipated use of Sterilox hypochlorous acid solution to mist fresh whole and cut fruits and vegetables is limited to traditional grocery stores and not expected to increase or expand to other grocery store formats (*e.g.*, warehouse) because of the cost associated with installing and maintaining misting systems. The use of misting is included in the projections. One gallon of Sterilox solution is equivalent to 4.018 kg.

$$\begin{aligned} &Maximum\ daily\ volume\ estimate\ of\ Sterilox\ solution\ at\ one\ site \\ &= 20\ gallons\ Sterilox\ soln\ x\ 5\ cycles\ per\ day\ x\ 4.018\ kg\ per\ gallon\ Sterilox\ soln \\ &= 401.80\ kg\ per\ day\ per\ retail\ site \end{aligned}$$

$$\begin{aligned} &Maximum\ yearly\ mass\ estimate\ of\ Sterilox\ solution\ at\ one\ site \\ &= 401.80\ kg\ Sterilox\ soln\ per\ day\ per\ site\ x\ 365\ days\ per\ year \\ &= 146{,}657\ kg\ Sterilox\ soln\ per\ year\ per\ retail\ site \end{aligned}$$

6.b.2. Percent of FCS and residual chemicals entering the down-the-drain waste stream at site of production/use

To estimate the release of the FCS and residual chemicals into the environment at each retail outlet site, the percentage of each chemical species (Table 2) is multiplied by the maximum daily volume estimate of hypochlorous acid solution released down the drain to POTWs daily and annually (i.e., 146,657 kg/year/retail site). Table 2 summarizes the release estimates.

Table 2. Daily and Annual Release Estimates of Free Chlorines and the Oxychloro and THM Residual Chemical Species from a Single Retail Outlet Site that Uses Hypochlorous Acid Solution to POTWs

Chemical Species			Estimated Release Down-The-Drain Daily (kg/day)[1]	Yearly (kg/yr)[2]
Chloro species	free chlorines (AFC)		2.411	879.9
	chlorite		0.02009	7.333
	chlorate		0.02009	7.333
		SUM	**2.451**	**894.6**
Trihalomethane species			**0.002435**	**0.8887**

[1] calculated by multiplying the percentage of the maximum measured chemical species in hypochlorous acid solution from Table 1 by the maximum daily volume estimate of Sterilox at one site
[2] calculated by multiplying the percentage of the maximum measured chemical species in hypochlorous acid solution from Table 1 by the maximum yearly volume estimate of Sterilox at one site

6.b.3. The mode of chemical introduction into the environment

The Sterilox hypochlorous acid solution is generated intermittently at the site of crisping in accordance with demand at each retail outlet. Solution is then disposed of down the drain to POTWs and ultimately into the environment.

6.b.4. Expected concentration of chemicals introduced into the environment

Due to the control of pH, only trace amounts of hydrogen, oxygen and hydrochloric acid gas are generated to make hypochlorous acid solution and therefore release of these substances into the atmosphere at the site of production is negligible. No solid waste is generated at the site of production.

The chemical species generated from the hypochlorous acid solution are aqueous and will be introduced into the aquatic environment via down-the-drain movement into POTWs for standard treatment processes before movement into aquatic environments. Chlorine and oxychloro species, such as hypochlorous acid, generated by the electrolysis of a brine solution are common sanitizers for potable water. Therefore, POTWs are designed to capture and minimize the impact of brines, sanitizers and their residual products on aquatic environments.

Because the pH is controlled in the Sterilox hypochlorous acid solution, the dominant oxychloro species is hypochlorous acid. Oxychloro species are strong oxidizers. Once the oxychloro species reach the POTWs, they readily react with oxidizable organic compounds (such as phenols, amino acid, proteins) and inorganic compounds (such as iron, manganese, sulfides), and progress to reduced chlorine species, *i.e.*, hypochlorite (ClO-, oxidation state I), chlorine dioxide (oxidation state IV), and the chloride anion (oxidation state -I); it is unlikely that a single reduced species exists (EPA, 2006). Therefore, we can assume 2.451 kg daily and 894.6 kg annually of the sum of the mass of the oxychloro species summarized in Table 2 are released from the POTW into the aquatic environment (Table 2). From there we may assume as a simplistic assumption that this mass is equally distributed between these reduced species. Note that this is considered a conservative approach since oxychlorine species are strong oxidizers and are expected to react readily with oxidizable compounds in the POTW before discharge to surface waters. Little is reported on the properties of the oxychloro species but it is assumed that they have low bioaccumulation potential, low volatility and do not readily biodegrade under aerobic conditions (HSDB, EPA, 2006a and 2006b). Therefore, the conservative assumption would be that the oxychloro species would not readily transition out of surface waters once introduced.

THMs are residuals formed in the final Sterilox hypochlorous acid solution as a result of halogen substitution and oxidation reactions of chlorine with naturally occurring organic matter in the presence of bromide. This same mechanism occurs in chlorinated drinking water. Upon reaching surface water, the THMs are expected to transition out of the aquatic environment within hours to days (HSDB). Based upon measured or estimated Koc values, bromodichloromethane and chlorodibromomethane are expected to have low to moderate mobility (Koc 53 and 84), while bromoform is expected to readily adsorb to suspended solids and sediment in aquatic environments (Koc 116) (HSDB). Volatilization from water surfaces is expected to be an important fate process for all three THM species based upon Henry's Law constants, which range from 5.35 E-4 to 2.12 E-3 (HSDB).

We anticipate that the expected environmental concentrations for the oxychloro and THM species will be very small and thus will be of no environmental concern. To confirm this, we elected to use the EPA screening-level exposure model, Exposure and Fate Assessment Screening Tool (E-FAST^{TM}) /Down-The-Drain to estimate aquatic ecosystem exposure release of the residual chemicals to the aquatic environment. The underlying principles, calculations and units incorporated in the E-FAST Down-the-Drain model are described in more detail in the E-FAST manual (EPA, 2007). E-FAST is a screening tool that requires minimal input data and generally provides highly conservative assessments.

Table 3. Estimated[1] Sum Concentration of Chloro Species and THM species in Aquatic Environments After Processing through POTWs

Chemical Species	E-FAST Data Input			Surface Water Concentration	
	BCF (L/kg)	WWT removal (%)	Production Volume (kg/yr)[2]	(µg/L)	(mg/L)
sum chloro species	--[3]	--[3]	894.6	2.17 E-2	2.17 E-5
sum trihalomethane species	--[3]	--[3]	0.8887	2.16 E-5	2.16 E-8

[1] E-FAST Down-The-Drain model, 10th percentile 7Q10 stream dilution descriptor
[2] Annual production volume listed in Table 2.
[3] '—' indicates a negligible contribution, thus '0' was included in the model.

7. **Fate of Substances Released into the Environment**

We have shown that negligible amounts of the oxychloro and trihalomethane chemical species will reach aquatic environments (<<1 ug/L) (Table 3). The majority of environmental depletion mechanisms, such as adsorption and oxidation-reduction reactions, will have occurred during processing through POTWs. These chemical species distribution assumptions are in agreement with EPA's re-registration eligibility document for chlorite and chlorate species (EPA, 2006a and 2006b, respectively).

8. **Environmental Effect of Released Substances**

Any number of reduced chlorine species, possibly including hypochlorite, chlorine dioxide, and the chloride anion, as well as THM species, including bromodichloromethane, chlorodibromomethane and bromoform, may be released down-the-drain through POTWs into aquatic environments during intermittent use of the Sterilox hypochlorous acid solution. The available toxicity endpoint ranges for chlorinated and THM species are summarized in Tables 4 and 5, respectively. Effects on terrestrial organisms are not expected from these residual chemicals produced by retail outlet use of hypochlorous acid as evaluated by comparing the toxicity endpoints to surface water exposure concentrations in Table 3. Additionally, negligible uptake by organisms is expected due to low estimated bioconcentration factors for all chemical species (HSDB). This demonstrates that the use of hypochlorous acid solution for crisping fruits and vegetables at retail outlets will have a negligible effect on the environment.

Table 4. Summary of Environmental Toxicity Endpoints for Available Chlorine Chemical Species.

Species	Chemical species	LC50 or EC50 (mg/L)	NOEC (mg/L)
Freshwater fish	chlorite[1]	50.6-420	32-216
	chlorate[2]	7.3-1100	600-1000
Freshwater invertebrates	chlorite	0.027-1.4	0.003-0.4
	chlorate	2100-4100	52-1000
Estuarine/marine fish	chlorite	75	13.9
Estuarine/marine invertebrates	chlorite	0.576-21.4	14.3
Aquatic plants	chlorite	1.32	<0.62
	chlorate	3.1-444	50-3137

[1]EPA, 2006a, [2]EPA, 2006c

Table 5. Summary of Environmental Toxicity Endpoints for Available THM Chemical Species.

Species	Chemical species	LC50 or EC50 (mg/L)	NOEC (mg/L)
Freshwater fish	chlorodibromomethane[1]	53-250	--[2]
	bromoform[1]	29-33	--
Freshwater invertebrates	bromoform	46-56	--
Estuarine/marine fish	bromodichloromethane[1]	67.4	--
	chlorodibromomethane	33.5	--
	bromoform	7.1-52.3	--
Estuarine/marine invertebrates	bromoform	1-24,400[2]	--

[1] data listed as summarized in HSDB, http://toxnet.nlm.nih.gov/cgi-bin/sis/search2, accessed 9/2/2014
[2] '—' indicates that no data was listed for this endpoint
[3] only approximately 30% of original concentration was still present

Additionally, discharges of chlorine to ambient waters are regulated by the National Pollutant Discharge Elimination System (NPDES) in which discharge permit limits are established to meet state water quality standards. These standards reflect federal ambient water quality criteria (WQC) established for the protection of aquatic life and human health (EPA, 2013). The WQC include the Criteria Maximum Concentration (CMC) which is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect and the Criterion Continuous Concentration (CCC) which is an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect. The CMC and CCC for chlorine in freshwater is 19 µg/L and 11 µg/L, respectively. The CMC and CCC for chlorine in saltwater is 13 µg/L and 7.5 µg/L, respectively. The surface water concentrations of the reduced sum chlorine species as estimated by the E-FAST™ Down-the-Drain screening model (Table 3) are below these concentrations and therefore meet water quality standards.

Given that the effluent of the proposed process will be discharged to POTWs where further treatment will occur, the environmental effects of the released substances will be insignificant. EPA has considered the environmental effects of using chlorine and has stated in their R.E.D FACTS, February 1999, Chlorine Gas:

> "In receiving waters from facilities using chlorine, if acute levels of concern are exceeded, a significant risk to aquatic organisms and endangered aquatic organisms can be expected. Levels of concern (equaling one-half of the EC_{50}) are 0.009 ppm for aquatic invertebrates, 0.023 ppm for freshwater fish, and 0.013 ppm for estuarine organisms. Levels of concern for endangered species (equaling one twentieth of the EC_{50}) are 0.85 ppb for aquatic invertebrates, 2.3 ppb for freshwater fish, and 1.3 ppb for estuarine invertebrates.
>
> Uses of chlorine that are **not** regulated under the NPDES permit program, including swimming pool, aquaria and indoor use patterns (fruit and vegetable rinsing and food processing), should produce only intermittent discharges of minimal concentration into lakes or streams, resulting in minimal environmental exposure."

Direct discharge of Sterilox hypochlorous acid solution to sensitive waterways needs to be avoided to avoid these potential environmental effects. Given the location and limited volumes involved in this application, such discharges are exceedingly unlikely.

9. **Use of Resources and Energy**

The proposed use will have essentially no negative effect on resources or energy associated with typical fresh fruit and vegetable hydration processes. The use of Sterilox hypochlorous acid solution will remove the need for a potable water rinse but this is expected to provide a minor reduction in overall water use. All other factors of a typical fresh fruit and vegetable hydration process would remain the same. Any energy savings associated with using industrially produced chlorine as opposed to generating Sterilox hypochlorous acid solution onsite will be lost in transportation and shipping costs given the requirements for shipping the hazardous concentrated materials.

10. **Mitigation Measures**

No adverse environmental impacts remain to be addressed so alternatives are not required.

11. **Alternatives to the Proposed Action**

No adverse environmental impacts remain to be addressed so alternatives are not required.

12. List of Preparers

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13. Certification

The undersigned official certifies that the information presented is true, accurate and complete to the best of her knowledge.

10-2-2014

Date

Wendy Hillwalker, Ph.D., Authorized Representative of PuriCore, Inc.

14. References

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Appendix V: Literature Research about Hypochlorous Acid

d) Environmental Decision Memo for Food Contact Notification No. 001470.

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

Environmental Decision Memo for Food Contact Notification No. 1470

Return to inventory listing: **Inventory of Environmental Impact Decisions for Food Contact Substance Notifications (http://www.accessdata.fda.gov/scripts/fdcc/?set=ENV-FCN)** or the **Inventory of Effective Food Contact Substance Notifications (http://www.accessdata.fda.gov/scripts/fdcc/?set=FCN)**. See also **Environmental Decisions (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/default.htm)**.

Date: November 4, 2014

From: Biologist, Regulatory Team 2, Division of Biotechnology and GRAS Notice Review (HFS-255)

Subject: FCN No. 1470 – Hypochlorous acid (CAS Reg. No. 7790-92-3), electrolytically generated in dilute solution, as an antimicrobial agent in water used to re-hydrate fresh and fresh-cut fruits and vegetables.

Notifier: Exponent Inc. on behalf of **PuriCore Inc.**

To: Elizabeth Petro, Ph.D., Division of Food Contact Notifications (HFS-275)
Through: Suzanne Hill, Environmental Team Lead, Office of Food Additive Safety, HFS-255

Attached is the Finding of No Significant Impact (FONSI) for FCN 1470. After this notification becomes effective, copies of this FONSI and the notifier's environmental assessment, dated October 2, 2014, may be made available to the public. We will post digital transcriptions of the FONSI and the environmental assessment on the agency's public website.

Please let us know if there is any change in the identity or use of the food-contact substance.

Mariellen Pfeil

Attachment:Finding of No Significant Impact

FINDING OF NO SIGNIFICANT IMPACT

A food-contact notification (FCN No. 1470), submitted by PuriCore Inc., to provide for the safe use of Hypochlorous acid (CAS Reg. No. 7790-92-3), electrolytically generated in dilute solution, as an antimicrobial agent in water used to re-hydrate fresh and fresh-cut fruits and vegetables.

The Office of Food Additive Safety has determined that allowing this notification to become effective will not significantly affect the quality of the human environment and, therefore, will not require the preparation of an environmental impact statement. This finding is based on information submitted by the notifier in an environmental assessment (EA), dated October 2, 2014, as summarized below.

This notification is intended to expand the currently approved use of on-site, electrochemically generated hypochlorous acid solution (the FCS) on whole fruits and vegetables[1] to include use on fresh-cut fruits and vegetables as well. Additionally, this notification removes the batch-size limitation, expands the contact time to a minimum of 90 seconds, and allows for a spray application. The intended maximum use level is 60 ppm, measured as available free chlorine.

In a study performed by the notifier, detailed in Attachment 7 to the notification, and discussed in the EA, the use of the FCS at retail food preparation facilities in the proposed crisping procedure introduces various available free chlorine (AFC; combined hypochlorous acid, hypochlorite, and chlorine residues), degradative oxychloro (e.g., chlorate and chlorite), and trihalomethane (e.g., bromodichloromethane, chlorodibromomethane and bromoform) by-products.[2] These residuals are summarized and quantified in Table 1 of the EA. The residuals are then discharged to publicly-owned treatment works (POTWs) and ultimately into the environment. Terrestrial application of FCS treated water is not anticipated due to the nature of the expected use (retail food preparation).

Based on 2012/2013 sales data and use projections the notifier provides estimates of the yearly release (in kilograms) of the environmentally pertinent total chloro (AFC plus degradative oxychloro) and trihalomethane FCS by-products (see Table 2 of the EA). The U.S. EPA Exposure and Fate Assessment Screening Tool (E-FAST™)[3] “Down-the-Drain” exposure model was used to estimate POTW aquatic ecosystem release of the total chloro and trihalomethane residues. According to the manual for the E-FAST exposure model the “Down-the-Drain” module was developed as a “*screening-level model for estimating concentrations of chemicals in surface waters that may result from the disposal of consumer products into household wastewater. The methodology assumes that household wastewater undergoes treatment at a local wastewater treatment facility and the treated effluent is subsequently discharged into surface waters.*”[4] The results of this analysis (10th percentile, 7Q10 stream dilution) are shown in EA Table 3 (reproduced below). The surface water concentration is equivalent to the estimated environmental concentration, EEC.

EA Table 3 (reproduced):
Estimated* Residues in Aquatic Environments after Processing through POTWs

Chemical Species	E-Fast Data Input			Surface Water Concentration (mg/L = ppm)
	BCF [a] (L/kg)	WWT removal [b] (%)	Production Volume [c] (kg/year)	

* E-Fast Down-the-Drain model, 10th percentile 7Q10 stream dilution descriptor.
[a] Bioconcentration factor; 0 = no bioconcentration
[b] Wastewater Treatment Removal percentage
[c] Annual production volume listed in Table 2 of the EA

Chemical Species	E-Fast Data Input			Surface Water Concentration (mg/L = ppm)
	BCF [a] (L/kg)	WWT removal [b] (%)	Production Volume [c] (kg/year)	
Total Chloro Species	0	0	894.6	2.17 x 10-5
Total Trihalomethanes	0	0	0.8887	2.17 x 10-8

* E-Fast Down-the-Drain model, 10th percentile 7Q10 stream dilution descriptor.
[a] Bioconcentration factor; 0 = no bioconcentration
[b] Wastewater Treatment Removal percentage
[c] Annual production volume listed in Table 2 of the EA

Evidence and discussion provided in the U.S. EPA re-registration eligibility documents for chlorite and chlorate species[5],[6] and described in the EA show that oxychloro species readily react with oxidizable organic compounds (i.e., phenols, amino acids, proteins) and inorganic compounds (i.e., iron, manganese, and sulfides) and progress to reduced chlorine species (i.e., hypochlorite, chlorine oxides and chloride anion) during POTW treatment. Discharges of chlorinated species into ambient waters are regulated by National Pollutant Discharge Elimination System (NPDES) permitting to meet established water quality standards which reflect U.S. EPA water quality criteria for chlorine including the Criteria Maximum Concentrations (CMCs) for acute effects and the Criterion Continuous Concentrations (CCCs) for chronic effects.[7] The estimated surface water chloro species concentration (or EEC) is several orders of magnitude lower than these CMC and CCC levels for both fresh and marine discharges.

Further, when compared to ecotoxicity endpoint data for fresh and marine fish and invertebrates and aquatic plants given in EA Table 4, the total chloro species surface water concentration (or EEC) presented in Table 3 (i.e., 2.17 x 10-5 ppm) is greater than three orders of magnitude lower than the most sensitive toxicity endpoint (0.027 ppm chlorite) LC50 for freshwater invertebrates (species not identified). With respect to the trihalomethane (THM) residuals, the trihalomethane surface water concentration (or EEC), 2.17 x 10-8 ppm, is greater than eight orders of magnitude lower than the most sensitive toxicity endpoint[8](1 ppm bromoform) LC50 for estuarine/marine invertebrates (species not identified). Toxnet HSDB[9] data described in the EA indicate there is rapid volatilization from water surfaces (all THMs) and adsorption (of primarily bromoform) to suspended solids and sediment in aquatic environments, further lowering the aquatic concentration. Additionally, THM residues are not expected to bioaccumulate.[10]

The proposed use is not anticipated to have significant impact on resources or energy associated with typical fresh fruit and vegetable hydration processes as all other factors associated with these processes will remain the same. The use of FCS solution will remove the need for a potable water rinse but this is expected to provide a minor reduction in overall water use.

Therefore, no significant environmental impacts are anticipated from the proposed use and disposal of the FCS.

Prepared by_____
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Approved by_____
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[1] Per FCN 692, Effective 5-24-2007, for re-hydrating fresh fruits and vegetables including leafy green vegetables (Sterilox Food Safety/Div. of PuriCore, Inc.)
[2] Hypochlorous acid (HOCl), Hypochlorite (OCl-), Chlorine (Cl2), Chlorate (ClO3) and Chlorite (ClO2)
[3] U.S. EPA, Office of Pollution Prevention and Toxics' (OPPT) Exposure and Fate Assessment Screening Tool (E-FAST) Version 2.0. United States Environmental Protection Agency, Washington, DC, USA. 2007. **http://www.epa.gov/opptintr/exposure/pubs/efast2man.pdf (http://www.epa.gov/opptintr/exposure/pubs/efast2man.pdf)**
[4] Ibid, footnote 3, page 72
[5] Reregistration Eligibility Decision (RED) for Chlorine Dioxide and Sodium Chlorite (Case 4023). Office of Prevent, Pesticides and Toxic Substances, EPA 738-R-06-007. August 2006.
[6] Reregistration Eligibility Decision (RED) for Inorganic Chlorates (Case 4023). Office of Prevent, Pesticides and Toxic Substances, EPA 738-R-06-014. July 2006.
[7] U.S. EPA. Office of Water. Current National Recommended Water Quality Criteria, Chlorine; **http://www.epa.gov/waterscience/criteria/wqcriteria.html (http://www.epa.gov/water-science/criteria/wqcriteria.html)** (accessed Nov. 8, 2013, verified 10-16-2014).
[8] As presented in EA Table 5.
[9] Toxnet. Hazardous Substances Database. Halomethanes; **http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB)** (accessed 9-2-2014; verified 10-14-2014)
[10] Ibid.

More in Environmental Decisions (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/default.htm)

Decisions for Food-Contact Notifications (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/ucm105897.htm)

Decisions for Petitions (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/ucm105895.htm)

Definitions of Environmental Terms (/Food/IngredientsPackagingLabeling/EnvironmentalDecisions/ucm105934.htm)

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Appendix V: Literature Research about Hypochlorous Acid

e) Hypochlorous Acid as a Potential Wound Care Agent. Part I. Stabilized Hypochlorous Acid: A Component of the Inorganic Armamentarium of Innate Immunity.

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Hypochlorous Acid as a Potential Wound Care Agent

Part I. Stabilized Hypochlorous Acid: A Component of the Inorganic Armamentarium of Innate Immunity

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Published April 11, 2007

Objective: Hypochlorous acid (HOCl), a major inorganic bactericidal compound of innate immunity, is effective against a broad range of microorganisms. Owing to its chemical nature, HOCl has never been used as a pharmaceutical drug for treating infection. In this article, we describe the chemical production, stabilization, and biological activity of a pharmaceutically useful formulation of HOCl. **Methods:** Stabilized HOCl is in the form of a physiologically balanced solution in 0.9% saline at a pH range of 3.5 to 4.0. Chlorine species distribution in solution is a function of pH. In aqueous solution, HOCl is the predominant species at the pH range of 3 to 6. At pH values less than 3.5, the solution exists as a mixture of chlorine in aqueous phase, chlorine gas, trichloride (Cl_3^-), and HOCl. At pH greater than 5.5, sodium hypochlorite (NaOCl) starts to form and becomes the predominant species in the alkaline pH. To maintain HOCl solution in a stable form, maximize its antimicrobial activities, and minimize undesirable side products, the pH must be maintained at 3.5 to 5. **Results:** Using this stabilized form of HOCl, the potent antimicrobial activities of HOCl are demonstrated against a wide range of microorganisms. The in vitro cytotoxicity profile in L929 cells and the in vivo safety profile of HOCl in various animal models are described. **Conclusion:** On the basis of the antimicrobial activity and the lack of animal toxicity, it is predicted that stabilized HOCl has potential pharmaceutical applications in the control of soft tissue infection.

A remarkable feature of the immune system is its ability to launch an effective response against invading pathogens by deploying a group of highly reactive chemicals, including oxidized halogens, oxidizing radicals, and singlet oxygen.[1,2]

As depicted in Figure 1, the precursor of these reactive oxygen species (ROS) is the oxygen radical (O_2), which is generated by specialized immune cells—neutrophils, eosinophils, mononuclear phagocytes, and B lymphocytes.[1–9] Production of ROS in these cells is

This work was fully supported by NovaBay Pharmaceuticals, Inc, Emeryville, CA.

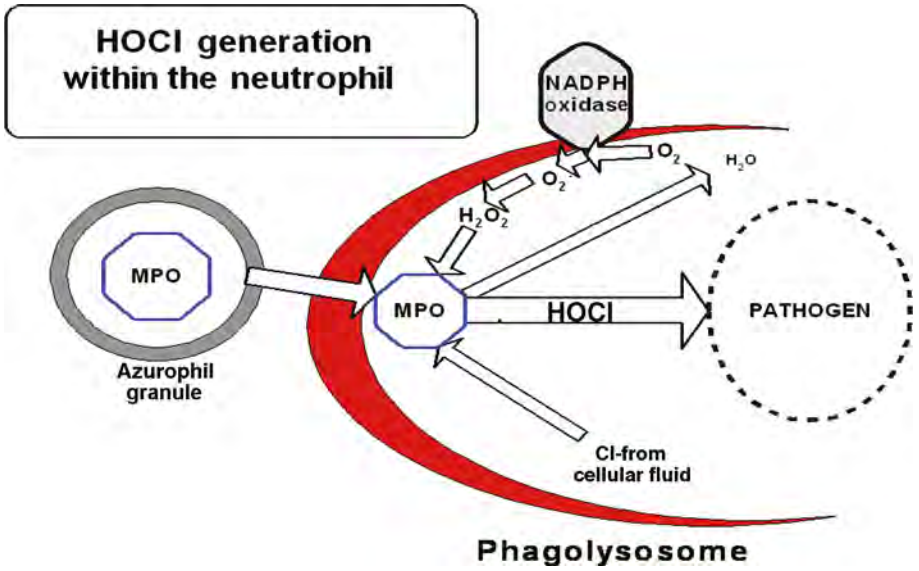

Figure 1. A schematic representation of hypochlorous acid (HOCl) production during the oxidative burst process. During this process, cells utilize O_2 and convert it to hydrogen peroxide (H_2O_2) using a mitochondrial-membrane–bound enzyme NADPHase. Then, myeloperoxidase catalyzes the reaction between H_2O_2 and Cl^- to generate HOCl. As deregulations take place, the lumen of the phagasome progressively becomes more acidic and leaves the bacterium within a vacuole (phagolysosome) containing MPOse and H_2O_2 in a medium containing 0.1 M Cl^- at estimated pH 4 to 6. During this process, conditions are optimal for MPOse-catalyzed generation of HOCl as depicted in this figure. On the basis of these principles, we set out to establish the conditions of generating the stable form of HOCl (NVC-101).

accompanied by a significant rise in oxygen consumption, a series of events collectively referred to as the oxidative burst. The primary enzyme responsible for ROS production is a mitochondrial-membrane–bound enzyme known as respiratory burst NADPH oxidase.[1] Patients with chronic granulomatous disease have oxidase defective genes, which makes them susceptible to repeated infection.[10,11] During a respiratory burst, neutrophils produce H_2O_2, which is converted to HOCl by the activity of the granule enzyme myeloperoxidase in the following reaction.[12]

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O$$

HOCl is known to be the major strong oxidant produced by neutrophils, and is a potent microbicidal agent within these cells.[2,10] Experimentally, it has been estimated that 10^6 neutrophils stimulated in vitro can produce 0.1 μM HOCl. This quantity of HOCl can kill 1.5×10^7 *Escherichia coli* in less than 5 minutes.[13] HOCl reacts readily with a range of biological molecules, particularly those with thiol, thiolether, heme proteins, and amino groups,[12] and may lead to tissue injury. Taurine, a nonessential amino acid naturally found at roughly 15 mM within neutrophils acts as a scavenger molecule for HOCl via the following mechanism, and effectively dampens the collateral damage to cellular macromolecules

caused by HOCl.[14]

$$HOCl + NH_2\text{-}\ CH_2\text{-}\ CH_2\text{-}\ SO_3H \rightarrow Cl_{(1or2)}\ N\text{-}CH_2\text{-}CH_2\text{-}SO_3H + H_2O$$

To date, pure HOCl has not been developed as a commercial pharmaceutical formulation presumably because of the challenge of maintaining storage stability. In this article, we describe a method for the preparation and stabilization of a pure form of HOCl (also referred to as NVC-101) for potential use as a pharmaceutical agent. We show here that when compared to the commercially available disinfectants hydrogen peroxide and sodium hypochlorite (NaOCl), this formulation has improved in vitro antimicrobial activity and therapeutic index. Furthermore, we present data demonstrating an excellent safety profile for NVC-101 in animal toxicology studies. We believe the improved properties of our pure physiologically balanced stabilized form of HOCl may allow for its use in a clinical situation such as in the treatment or prevention of infection in burn or other wounds.

MATERIALS AND METHODS

Preparation of HOCl

Reagent-grade NaOCl was purchased from J. T. Baker. Hypochlorous acid was prepared in 154 mM NaCl by acidifying reagent-grade NaOCl to the pH range of 3.5 to 4.0 with dilute HCl. A Beckman pH meter was used to accurately measure the final pH values. The concentration of active total chlorine species in solution expressed as $[HOCl]_T$ (where $[HOCl]_T = [HOCl] + [Cl_2] + [Cl_3^-] + [OCl^-]$) in 0.9% saline was determined by converting all the active chlorine species to OCl^- with 0.1 M NaOH and measuring the concentration of OCl^-. The concentration of OCl^- was determined spectrophotometrically at 292 nm ($\varepsilon = 362\ M^{-1}\ cm^{-1}$)[15] with an Agilent 8453 UV-visible spectrophotometer.

Microbiological materials

All microorganisms used in these studies were purchased from the American Type Culture Collection (ATCC), grown and propagated according to the recommendations for each strain by ATCC. Bacterial cells were harvested at stationary phase and concentrations were determined by 10-fold dilution as direct colony count. To prepare inoculum, bacteria were diluted in sterile saline before use to minimize the effect of broth on HOCl.

Minimum bactericidal concentration

A modification of the National Committee Consensus on Laboratory standardized protocol "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically" was used in these studies. Sterile 0.9% saline at pH 3.5 to 4.0 (vehicle) was used as the diluent. Use of such a diluent allows for the determination of the intrinsic activity of HOCl in the absence of any interfering molecules. Specifically, each test article is diluted using 2-fold serial dilution in acid-washed glass tubes to give a range of concentrations from approximately 2 to 0.002 mM in a final volume of 1 mL. Each dilution is inoculated with 5×10^5 CFU/mL test bacteria and coincubations were carried out at room temperature for 60 minutes. At 60 minutes posttreatment, 0.1 mL of each reaction was immediately

transferred into prelabeled 1.5-mL microfuge tubes containing 0.9 mL Dey and Engley (D/E) neutralizer broth (Hardy Diagnostic, Santa Maria, CA). Minimum bactericidal concentration (MBC) is determined by plating 0.1 mL of each sample onto an agar plate. Plates were then incubated overnight at 35˚C, and examined for colony growth. The concentration at which there was a complete absence of colony growth is determined to be the MBC. Comparative MBC results provide estimates of the susceptibility of various test articles against test organisms.

Time kill

For time kill studies, 5 mL of test article at an approximate MBC concentration was inoculated with approximately 10^6 CFU/mL of each test organism and incubated for 0, 5, 10, 15, 20, 30, 60, and 90 minutes at room temperature. For each time point, 0.1 mL was transferred into 0.9 mL of D/E neutralizer broth and 0.1 mL of this mixture was plated and incubated as previously described.

Cytotoxicity

L929 (ATCC CCL-1, NCTC clone 929) is a connective-tissue cell line derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H mouse. L929 cells were purchased from ATCC and propagated according to supplier's recommendations. These cells were then seeded at 1.5×10^4 cells per well in 96-well plates and incubated overnight at 37˚C. On the day of testing, growth medium was aspirated from each well, and 30 μL fresh medium was added per well. Test articles were diluted by 2-fold serial dilution using 154 mM saline at the desired pH for each test article. Following that, 170 μL of each dilution was added to each well for a total volume of 200 μL per well. After 60 minutes' exposure at 37˚C, test articles were replaced with 200 μL of fresh tissue culture media and incubated for 24 hours at 37˚C. Cell viability was determined by addition of WST-8 (Dojindo, Japan) reagent and the absorption at 450 nm read spectrophotometrically. Orange-red formazan, which is produced by live cells, is a direct measure of cell viability in this assay.

Therapeutic index

The therapeutic Index of an antimicrobial agent is defined as the ratio of the concentration to achieve 50% cell toxicity (CT_{50}) to MBC.

Animal safety and toxicity studies

Ocular irritation, skin sensitization, and wound toxicology studies were performed. A preliminary (non–good laboratory practice [non-GLP]) study with a development formulation of HOCl (2.5 mM; 0.013% w/v) was carried out at the Brookdale Eye Clinic (K. Najafi, MD, unpublished data). Dutch pigmented rabbits received either 5% ophthalmic povidone-iodine (Betadine) (15 eyes) or the development formulation (15 eyes). Each eye received 0.1 mL of solution every 8 hours for a total of 72 hours and observations were made periodically during this time. The effect of the development formulation was compared to 5% ophthalmic-grade Betadine.

A GLP ocular irritation study (NAMSA, Toledo, OH) was designed to determine the potential for ocular irritation following a single instillation in the rabbit. New Zealand White rabbits (5 per group) were used. Hypochlorous acid (NVC-101) was instilled in the right eye at concentrations of 0.01%, 0.03%, and 0.1% w/v (pH 3.5). The left eyes were used as the controls and were untreated, vehicle (saline) or positive control treated. In all cases, the volume used was 0.1 mL, which was placed into the lower conjunctival sac. Evaluations for irritation were made at 24, 48, and 72 hours. At 24 hours, the cornea was examined using fluorescein stain.

GLP repeat-dose wound toxicity studies (Charles River, Spencerville, OH) were designed to provide maximum exposure to full-thickness wounds in rats and mini-pigs. Wounds were treated with NVC-101 at concentrations of 0.01%, 0.03%, and 0.1% w/v (pH 3.5). The test material was applied to the wounded area directly using soaked gauze. The treated site was covered for approximately 24 hours per day for 28 days. Wounds achieving 75% closure were kept open by abrasion. Parameters used to assess systemic toxicity included clinical signs (including observations of the site), body weights, food intake, clinical chemistries (blood and urine), hematology, organ weights, and gross and microscopic tissue evaluations.

RESULTS

Synthesis of hypochlorous acid

Hypochlorous acid can be synthesized by one of the 3 methods: hydrolysis of chlorine gas (eq 1), electrolysis of salt solution (eqs 2a and 2b), and acidification of hypochlorite (eq 3).

$$Cl_2 + H_2O \rightleftarrows HOCl + H^+ + Cl^- \quad (1)$$

$$2Cl^- + 2e^- \rightarrow Cl_2 \quad (2a)$$

$$Cl_2 + H_2O \rightleftarrows HOCl + H^+ + Cl^- \quad (2b)$$

$$OCl^- + H^+ \rightleftarrows HOCl \quad (3)$$

The limitations of using equation 1 are the inherent hazards of handling chlorine and the difficulty in manipulation. The disadvantage of the electrolysis method (eq 2) is the difficulty in controlling the target concentration of solution. Since hypochlorite is commercially available, the use of the method in equation 3 is the preferred method and is more convenient, safe, and controllable when compared to the other 2 methods.

Distribution of active chlorine species

This section discusses the distribution of active chlorine species as a function of pH in physiologically balanced saline solution. The presence of Cl^- in HOCl solution could result in the formation of Cl_2 and Cl_3^-. The formation of Cl_2 has a significant impact on the stability of HOCl (Fig 2).

Equations 4 to 7 show the equilibria existing in HOCl/NaC1 solution.

$$HOCl \rightleftarrows H^+ + OCl^- \qquad pKa = 7.5 \quad (4)$$

$$HOCl + Cl^- + H^+ \rightleftarrows Cl_2(aq) + H_2O \qquad K_1 = 9.6 \times 10^2\ M^{-2} \quad (5)$$

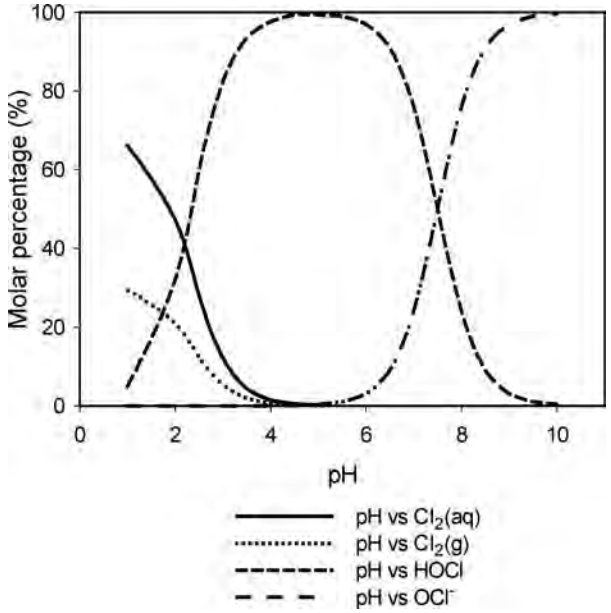

Figure 2. Chlorine speciation profile as a function of pH.

$$Cl_2(aq) + Cl^- \rightleftarrows Cl_3^- \qquad K_2 = 0.18\ M^{-1} \tag{6}$$

$$Cl_2(aq) \rightleftarrows Cl_2(g) \qquad K_3 = 10.87\ atm\ M^{-1} \tag{7}$$

The molar percentage of each species in physiologically balanced HOCl solution is a function of pH (Fig 2). As is shown in equation 5, low pH and high [Cl^-] favors the formation of Cl_2. Once Cl_2 is formed in the aqueous phase, it migrates into the headspace to reach the equilibrium shown in equation 7. The transfer of Cl_2 from the solution to the headspace of the container results in a decrease in active chlorine concentration in solution. Therefore, the degassing of Cl_2 becomes a major path for loss of HOCl in an open system (nonsealed). This is a potential problem for clinical use of HOCl. To stabilize the physiologically balanced HOCl solution, minimizing the formation of Cl_2 is essential. Figure 2 shows the chlorine species distribution as a function of pH in accordance with the equilibria shown in equations 4 to 7. The lines are the calculated values based on the equilibrium constants shown in equations 4 to 7.[16–19]

Microbicidal effect of HOCl

Stabilized HOCl demonstrates broad-spectrum antimicrobial activity at concentrations ranging from 0.1 to 2.8 μg/mL (Table 1).

The exception is *Aspergillus niger*, where a higher concentartion of HOCl (86.6 μg/mL) was required for effective killing of the organism under the same assay conditions. The overall summary of MBC findings against various microorganisms is shown in Table 1. Similarly, differences in HOCl sensitivity among different bacteria have been reported previously.[20–22]

Table 1. *Minimum bactericidal concentration (μg/mL) of HOCl for a broad spectrum of microorganisms tested at room temperature for 60 min*

Pathogen	ATCC	MBC
Escherichia coli	25922	0.7
Pseudomonas aeruginosa	27853	0.35
Staphylococcus aureus	29213	0.173
Staphylococcus epidermidis	12228	0.338
Micrococcus luteus	7468	2.77
Corynebacterium amycolatum	49368	0.169
Haemophilus influenzae	49144	0.338
Proteus mirabilis	14153	0.340
Staphylococcus hominis	27844	1.4
Staphylococcus haemolyticus	29970	0.338
Staphylococcus saprophyticus	35552	0.35
Candida albicans	10231	2.7
Klebsiella pneumoniae	10031	0.7
Serratia marcescens	14756	0.169
Streptococcus pyogenes	49399	0.169
Enterobacter aerogenes	51697	0.676
Candida albicans	10231	0.17
Aspergillus niger	16404	86.6
Methicillin-resistant *Staphylococcus aureus*	33591	0.682
Vancomycin-resistant *Enterococcus faecium*	51559	2.73

ATCC indicates American Type Culture Collection; MBC, minimum bactericidal concentration.

Time kill

Time kill is an in vitro measure of how fast a given antimicrobial can kill test bacteria. The rate of kill by stabilized HOCl was first demonstrated at the MBC values for each microorganism using an inoculum size of 1×10^6 mL^{-1} for each test bacteria (Tables 2 and 3). As it is shown in this table, majority of test organisms were killed (>99.99%) within the first 2 minutes of exposure. Among the bacterial species tested, *Streptococcus pyogenes* 49399 was the only exception, which required approximately 10 minutes of exposure for effective killing, under the same assay conditions. The killing rate of stabilized HOCl with NaOCl and H_2O_2 was then determined against 3 specific test organisms—*E. coli 25922, P. aeruginosa* 27853, and *S. aureus* 29213—at room temperature for a total of 90 miniutes.

It is worth mentioning that all these time kill studies were also performed with an inoculum size of 1×10^6/mL for each test bacteria, and the comparative results are depicted in Table 4.

As the results show, HOCl at its MBC values for different test organisms (5.6–12.5 μM) was able to kill all 3 test bacteria in less than 1 minute, with no significant bacterial killing effect from its excipient, saline at pH 4.0 (data not shown). However, the kill time for NaOCl at MBC values 10 to 50 μM varied from 5 to 15 minutes for the same 3 test organisms. In contrast, $H_2O_2$was only able to kill *P. aeruginosa* 27853 at 7500 μM in about 10 minutes, but did not kill *S. aureus* 29213 at its highest concentration tested (20,000 μM) even up to 90 minutes' exposure time under the same assay conditions (Fig 3).

Table 2. *Time kill for stabilized hypochlorous acid at MBC concentrations against different pathogens at room temperature*

	Time kill (min), recovered CFU/mL × 10^2 (n = 3)								
Pathogen	**0**	**1**	**2**	**5**	**10**	**15**	**30**	**60**	**90**
Escherichia coli 25922	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa 27853	0	0	0	0	0	0	0	0	0
Staphylococcus aureus 29213	>60	0	0	0	0	0	0	0	0
Staphylococcus epidermidis 12228	0	0	0	0	0	0	0	0	0
Micrococcus luteus 7468	0	0	0	0	0	0	0	0	0
Corynebacterium amycolatum 49368	0	0	0	0	0	0	0	0	0
Haemophilus influenzae 49144	0	0	0	0	0	0	0	0	0
Proteus mirabilis 14153[a]	0	0	0	0	0	0	0	0	0
Staphylococcus hominis 27844	0	0	0	0	0	0	0	0	0
Staphylococcus haemolyticus 29970	0	0	0	0	0	0	0	0	0
Staphylococcus saprophyticus 35552	0	0	0	0	0	0	0	0	0
Klebsiella pneumoniae 10031	0	0	0	0	0	0	0	0	0
Serratia marcescens 14756	0	0	0	0	0	0	0	0	0
Streptococcus pyogenes 49399	>300	>300	>300	>300	>300	52	0	0	0
Enterobacter aerogenes 51697	0	0	0	0	0	0	0	0	0
Candida albicans 10231	0	0	0	0	0	0	0	0	0
Asperigillus niger 16404	>300	120	0	0	0	0	0	0	0
Methicillin-resistant *Staphylococcus aureus* 33591	>300	0	0	0	0	0	0	0	0
Vancomycin-resistant *Enterococcus faecium* 51559	>300	0	0	0	0	0	0	0	0
Escherichia coli 25922	0	0	0	0	0	0	0	0	0

Comparative cell toxicity

The relative cell toxicity of HOCl, NaOCl, and H_2O_2 was assessed following a standard method used to examine the cytotoxicity of liquid disinfectants.[23] This toxicity assay utilizes an established adherent cell line, L929, and the end point is relative cell viability measured by addition of WST-8 (Dojindo, Japan) colorimetric reagent. Orange-red formazan, which is produced by live cells, is a direct measure of cell viability in this assay. Cytotoxicity was measured using 2-fold dilutions of HOCl, NaOCl, and H_2O_2 as compared to untreated or vehicle-treated control L929 cells. The CT_{50} was calculated for each test article, the values for which are shown in Figure 4. The CT_{50} values for HOCl (15–25 μg/mL) and NaOCl (38–42 μg/mL) were reproducible and closely matched published results for NaOCl.[16]

Table 3. *Comparative time kill studies of HOCl, NaOCl, and H_2O_2 against 3 test organisms at room temperature for a total of 90 min*

		Time kill (min)		
Pathogen	**ATCC**	**HOCl**	**OCl^-**	**H_2O_2**
Escherichia coli	25922	0	<5	>90
Pseudomonas aeruginosa	27853	<1	<20	<15
Staphylococcus aureus	29213	0	<10	>90

ATCC indicates American Type Culture Collection.

Table 4. *Comparative MBC (μM) of HOCl, NaOCl, and H_2O_2 tested against 3 organisms at room temperature for 60 min*

		MBC (μM)		
Pathogen	**ATCC**	**HOCl**	**OCl^-**	**H_2O_2**
Escherichia coli	25922	5.6	40	7,500
Pseudomonas aeruginosa	27853	6.2	10	>20,000
Staphylococcus aureus	29213	12.5	50	>20,000

ATCC indicates American Type Culture Collection; MBC, minimum bactericidal concentration.

However, the CT_{50} values for H_2O_2 were more variable (5–35 μg/mL), probably due to the chemical instability of H_2O_2 under these assay conditions.

Relative therapeutic index

The therapeutic indices for HOCl, NaOCl, and H_2O_2 were assessed using L929 cells and 3 clinically relevant bacterial strains—*E. coli* 25922, *P. aeruginosa* 27853, and *S. aureus* 29213. The calculated therapeutic index values for all 3 organisms are summarized in Figure 5.

The value for stabilized HOCl is approximately 98-fold higher than that for H_2O_2 for the gram-negative bacterium *E. coli* 25922, and more than 1000-fold higher than H_2O_2 for gram-positive organisms like *S. aureus* 29213.

Animal safety and toxicity

Stabilized HOCl is reactive, and therefore is not persistent. To evaluate its potential toxicity, several well-established animal models were used. Stabilized HOCl was found to be

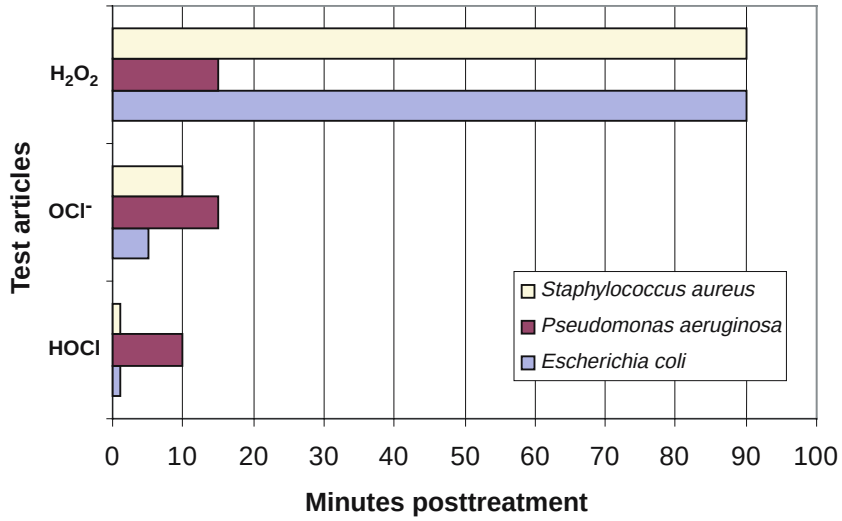

Figure 3. Comparative time kill studies of HOCl, NaOCl, and H_2O_2 against 3 test organisms—*Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, and *Staphylococcus aureus* 29213—at room temperature for total of 90 miniutes.

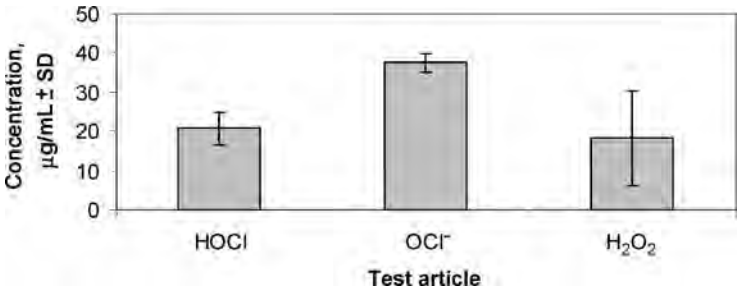

Figure 4. Relative cell toxicity of hypochlorous acid (HOCl; pH 4.0), hypochlorite (OCl^-; pH 10.5), and hydrogen peroxide (H_2O_2; pH 7.0) on L929 cells. Cytotoxicity measured in a cell proliferation assay is expressed as the concentration (μg/mL) that reduces the cell number by 50% of vehicle-treated control. CT_{50} is shown as the average of 7, 3, and 5 independent experiments (consisting of 10 different concentrations of each test article plus/minus the standard deviation), respectively.

nonirritating in (rabbit eye) and nonsensitizing in (guinea pig) animal models (Table 5). No ocular irritation was observed following the instillation of a development formulation (0.013% HOCl) into the eyes of Dutch pigmented rabbits every 8 hours for 72 hours (data not shown). Stabilized HOCl at concentrations of 0.01%, 0.03%, and 0.10% w/v in a standard Buehler-design dermal sensitization study in guinea pigs showed no evidence of dermal

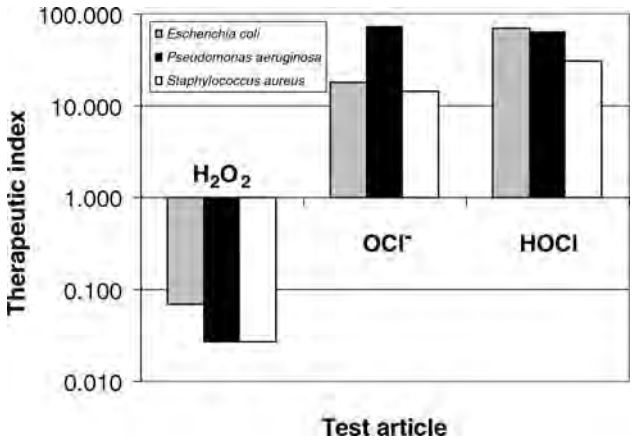

Figure 5. Relative therapeutic index of hypochlorous acid (HOCl; pH 4.0), hypochlorite (OCl^-; pH 10.5), and hydrogen peroxide (H_2O_2; pH 7.0). Therapeutic index is expressed as a ratio of the CT_{50} concentration (μg/mL) on L929 cells divided by the minimum bactericidal concentration (μg/mL) for *Staphylococcus aureus* 29213, *Pseudomonas aeruginosa* 27853 and *Escherichia coli* 2592. The higher the therapeutic index, the safer the test article will be.

Table 5. *Safety studies with control vs stabilized HOCl in 4 different animal species*

Studies	Species	Site applied	NVC-101 (% w/v)	Results
Eye irritation	Rabbits	Eye, Q8 for 72 h	Saline 0.013 Betadine	No irritation No irritation Progressive irritation
Eye irritation	Rabbits	Eye, single instillation	Saline 0.01, 0.03, and 0.1	No irritation No irritation at any dose
Skin sensitization	Hartley-derived albino guinea pig	Skin	Saline 0.01, 0.03, and 0.1	No sensitization No irritation at any dose
28-Day toxicology	Rat	Full-thickness wound	Saline 0.01, 0.03, and 0.1	No systemic toxicity at any dose and histopathology consistent with wound healing
28-Day toxicology	Mini-pig	Full-thickness wound	Saline 0.01, 0.03, and 0.1	No systemic toxicity at any dose and histopathology consistent with wound healing

reaction. Similarly, 28-day toxicity studies in full-thickness wounded rats and mini-pigs with daily application of stabilized HOCl at 0.01%, 0.03%, and 0.1% w/v together with a 24-hour occluded dressing showed no evidence of systemic toxicity. Microscopic examination of the wound area showed the expected signs of wounding and subsequent wound repair. A summary of all toxicological safety results with stabilized HOCl is shown in Table 5.

DISCUSSION

The germicidal properties of HOCl have been well reported.[1,10,13,20,21,24] Hypochlorous acid is widely used as a disinfectant, for example, in sanitizing wash solutions and swimming pools. In these applications, the reactive chemical is formed in solution by the addition of chlorine to water. Similarly, HOCl is used to treat drinking water and is formed following addition of chlorine gas or NaOCl.

Figure 2 shows the relative molar distribution of various chlorine species in a closed saline solution system as a function of pH. Between pH levels of 3 and 6, the predominant species is HOCl. At higher pH, hypochlorite ion (OCl^-) is formed, whereas at lower pH, the solution exists as a mixture of chlorine (Cl_2) in solution, chlorine gas in the headspace, and HOCl. The control of this reaction has been utilized in industrial practices to optimize the availability of the active antimicrobial, HOCl.

In this report, stabilized HOCl is prepared by the addition of NaOCl to a solution of sodium chloride in sterile water, followed by addition of a solution of hydrochloric acid to form the active component, HOCl. Stabilized HOCl (referred to as NVC-101) is a dilute solution of HOCl in 150 mM (0.9%) sodium chloride at an unbuffered pH of 3.5. The solution is stored in inert sealed containers designed for maximum product stability.

As shown in Figure 2, in a pH range of 3 to 6 the predominant species is HOCl. At pH values greater than 5.5, hypochlorite ion (OCl^-) is formed, and around pH 7.5 (the *p*Ka of HOCl of the chlorine species in solution is at 50/50% mixture [$HOCl/OCl^-$]).[12] As the

pH increases from 9.5, the concentration of OCl^- in solution reaches its maximum level, becoming 100% hypochlorite (also referred to as bleach). However, on the acidic side at pH less than 4, the solution exists as a mixture of chlorine (Cl_2) in aqueous phase, chlorine gas in the headspace, trichloride (Cl_3^-), and HOCl. At pH less than 3, an appreciable amount of Cl_2 gas forms, which may cause the rapid loss of all active chlorine in an open container. To keep the solution stable and maintain its desired activity, the pH of the solution should remain between 3.5 and 5 and the solution should be stored in a tightly sealed container. For the first time, we have been able to determine these conditions to stabilize HOCl and to assess its biological properties as a pharmaceutical product.

The biological effect of HOCl on bacteria has been extensively studied.[22,25,26] HOCl has broad-spectrum antimicrobial activity and is able to kill microorganisms very rapidly. Respiratory loss in bacterial cell membrane as a result of an irreversible reaction of HOCl with sulfur- and heme-containing membrane enzymes and structural proteins[12] lead to cell death and nonviability.[21,22,25]

Topical antiseptics with a long history of use, such as NaOCl (Dakins' solution), hydrogen peroxide, acetic acid, and povidone-iodine remain in widespread use today. These antimicrobial agents used at typical concentrations are cytotoxic and impede wound healing, and so are now discouraged by some experts for use on chronic ulcers. NVC-101 is a low-concentration, acidified, unbuffered solution of HOCl in saline. Under the conditions of the formulation, the active ingredient is primarily HOCl in equilibrium with a small amount of dissolved chlorine. The studies presented here have shown that stabilized HOCl exhibits rapid, concentration-dependent activity against a wide variety of gram-negative and gram-positive bacteria, yeast, and fungal pathogens, as long as the narrow effective pH range is maintained. In vivo, HOCl is produced intracellularly in abundance in response to phagocytosis of pathogens by neutrophils and plays an important role in the destruction of pathogens.

HOCl, the active ingredient of stabilized HOCl (NVC-101), has rapid and broad-spectrum antimicrobial activity against clinically relevant microorganisms in vitro and in vivo. Although vegetative bacteria are more susceptible to NVC-101 than endospore-forming bacteria and fungi (Table 1), NVC-101 is fully capable of inactivating all groups of gram-negative and gram-positive bacteria, yeast, and fungi, including *S. aureus*, methicillin-resistant *S. aureus*, vancomycin-resistant *E. faecium* (Table 1), and *Bacillis anthracis* spores (data now shown). NVC-101 has been shown to be nonirritating and nonsensitizing in animal models. There was no evidence of ocular irritation following a single instillation of NVC-101 in the eyes of New Zealand White rabbits at concentrations of 0.01%, 0.03%, and 0.1% w/v. No ocular irritation was observed following the instillation of a development formulation in the eyes of Dutch pigmented rabbits every 8 hours for 72 hours (data not shown). NVC-101 at concentrations of 0.01%, 0.03%, and 0.1% w/v in a standard Buehler-design dermal sensitization study in guinea pigs showed no evidence of dermal reaction.

The active ingredient is reactive, and therefore is not persistent. Its persistence of antimicrobial properties has not yet been tested in the in vivo wound environment. Thus, absorption and systemic toxicity are expected to be insignificant. Therefore, in the 28-day wound toxicity studies in rats and mini-pigs with daily application of NVC-101 at 0.01%, 0.03%, and 0.1% w/v, with 24-hour occluded dressing, there was no evidence of systemic toxicity. Furthermore, microscopic examination of the wound area showed the expected signs of wounding and subsequent wound repair.

Heggers and colleagues[26] have investigated the toxic effects of various concentrations of NaOCl (at pH 7.5, this was actually a 50:50 mixture of NaOCl and HOCl) in vitro and in vivo in the rat incision model. Concentrations used in previous studies were often quite high and although they had antimicrobial properties, they also exhibited some local toxicity that was not desirable. Heggers et al. conducted their experiments in the range of concentrations they expected to be active but not toxic to the cells or detrimental to wound healing. The concentrations evaluated were 0.25%, 0.025%, and 0.0125% w/v in the in vitro studies and 0.25% and 0.025% w/v in the in vivo studies. Ten clinical isolates were used in the in vitro studies (both gram-positive and gram-negative species). The bactericidal potential of the 3 concentrations was determined. All concentrations killed gram-positive bacteria within 30 minutes, but the lowest concentration did not kill gram-negative bacteria. Mouse fibroblasts were exposed to various concentrations of NaOCl for 10-, 20-, or 30-minute intervals. These cells remained viable except at the highest concentration, where cell death by 10 minutes was noted. In the incision rat model, 3 (2.5-cm) full-thickness wounds were created on each animal. The incisions were closed and the covered gauze was saturated every 4 hours with the NaOCl or saline. Subsets of animals were sacrificed on days 3, 7, and 14. Tissue sections were collected. Breaking strength was measured (force required to rupture the scar, in kilograms). The values for the breaking strength were higher as a function of duration, but the treated and control groups were not different. This study concluded that the concentration of 0.025% retains its bactericidal property without causing injury to the fibroblast cells.

Dakins' solution (NaOCl) has been used as an antimicrobial for decades. A study to assess the bactericidal activity and toxicity of 0.5% and 0.1% NaOCl was undertaken.[27] Only the toxicity portion of the study is discussed here. The insult to guinea pig skin was assessed following application of 0.5% solution of NaOCl buffered to a pH of 7.49 for up to 2 weeks (soaked gauze resoaked every 8 hours). The animals were sacrificed on day 1, 4, 7, or 14. The hair was removed from the skin before application but the skin was intact. The application of 0.5% solution resulted in basal cell toxicity (15% decrease in viability after 2 weeks of treatment), and so a lower concentration of 0.1% solution was evaluated (pH 7.4). This lower concentration did not result in toxicity to the basal cells. Control and treated skin sites were similar when the microscopic morphology was evaluated. Epidermal hyperplasia and an inflammatory influx were noted in the treated animals at 2 weeks. The authors concluded that the solutions were therapeutic candidates for thermal injury. It is important to note that at pH 7.4, these solutions will have approximately equimolar quantities of HOCl and NaOCl.

In the present comparative studies, we have demonstrated that H_2O_2 and hypochlorite (NaOCl) are effective against certain bacteria (more effective against gram negatives, but not gram positives). However, those effective antimicrobial concentration ranges begin to correlate with higher cytotoxicity on mammalian cells, as compared to NVC-101. This antiseptic profile of NaOCl resemble some of the over-the-counter antiseptics: silver nitrates or silver ions, Betadine, and acetic acid (data not shown). Moreover, there are other antiseptics that are less toxic to mammalian cells but at the same time have lower antimicrobial activity (ethanol, hydrogen peroxide, and 5% mafenide acetate solution) as compared to NVC-101 (data not shown). The Department of Health and Human Services discourages the use of commonly used antiseptic solutions to treat wound infection in general and chronic nonhealing wounds in particular because, for reasons mentioned above, their uses are contraindicated. Therefore data presented in this study should help in selecting safer

antimicrobial agents for wound disinfection, irrigation, and dressing. This reevaluation of accumulated evidence is intended as a basis to help practitioners make informed decisions for choosing the appropriate topical antimicrobial for wound care management.

As the development of bacterial resistance to antibiotics continues and controversy regarding the use of topical antiseptics persists, the need for research and development of new classes of antimicrobial agents that are safe and broadly effective and have low toxicity and low propensity to induce antimicrobial resistance becomes inevitably critical. Currently, the use of broad-spectrum topical antibiotics to treat wounds that are failing to heal or those at risk for getting infected is not recommended by the Department of Health and Human Services.[28] These recommendations are based on the following reasons: antibiotics may cause allergic reactions; especially when applied topically may have lower tissue distribution; greater effect on endogenous microflora (disturbance of the normal commensal microflora); induce resistance; and eventually have reduced therapeutic efficacy. By the same token, antiseptics are not encouraged because of their higher toxicity, potential development of resistance (like antibiotics), and, more important, direct impact on wound healing process.

Efficacy of topical antimicrobial agents in the management of serious infections, for example, biofilm- and catheter-related wounds, particularly when chronic and nonhealing, is inconclusive. These observations vary greatly because of (*a*) inconsistent in vitro test specifications, (*b*) use of different animal species models, (*c*) use of different organisms for determining the efficacy outcome. Therefore, overall results make direct comparisons less than ideal. While in vitro testing is required to select potential agents for clinical trials, these models will never totally mimic in vivo conditions.

Thus, in light of published results on HOCl and data obtained in the present investigations, there is enough compelling evidence to show that our new formulation of HOCl (NVC-101), which resembles the HOCl molecule made by neutrophils during oxidative burst (a natural defense process against invading microorganisms), could lend itself for safe and effective treatment modalities of infection. Because of NVC-101's broad-spectrum, fast-acting antimicrobial activity, the chances of developing resistance would be minimal and based on its safety profile the potential for collateral damage to infected tissues is also very low. Therefore, in vivo experiments in a chronic infected granulating wound model are planned to determine NVC-101's ability to persist in a hostile environment where the pH range may not be ideal and where inflammation may produce exudates that limit its use as a wound care agent.

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Appendix V: Literature Research about Hypochlorous Acid

f) Hypochlorous Acid as a Potential Wound Care Agent: Part II. Stabilized Hypochlorous Acid: Its Role in Decreasing Tissue Bacterial Bioburden and Overcoming the Inhibition of Infection on Wound Healing.

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Hypochlorous Acid as a Potential Wound Care Agent

Part II. Stabilized Hypochlorous Acid: Its Role in Decreasing Tissue Bacterial Bioburden and Overcoming the Inhibition of Infection on Wound Healing

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Background: A topical antimicrobial that can decrease the bacterial bioburden of chronic wounds without impairing the wound's ability to heal is a therapeutic imperative. A stabilized form of hypochlorous acid (NVC-101) has been demonstrated in vitro and in standard toxicity testing to possess properties that could fulfill these criteria. **Materials and Methods:** Using a standard rodent model of a chronically infected granulating wound, various preparations of NVC-101 and multiple treatment regimens were investigated to evaluate the role of NVC-101 in decreasing tissue bacterial bioburden and overcoming the inhibition of infection on wound healing. Quantitative bacteriology of tissue biopsies and wound healing trajectories were used to compare the various NVC-101 preparations and regimens to saline-treated negative controls and silver sulfadiazine–treated positive controls. **Results:** NVC-101 at 0.01% hypochlorous acid with a pH of 3.5 to 4.0 proved to be an effective topical antimicrobial. It was most effective when used for a brief period (15–30 minutes), and followed with another application. Possibly this was due to its rapid neutralization in the wound bed environment. Although not as effective at decreasing the tissue bacterial bioburden as silver sulfadiazine, NVC-101 was associated with improved wound closure. **Conclusions:** This stabilized form of hypochlorous acid (NVC-101) could have potential application as an antimicrobial wound irrigation and treatment solution if its effective pH range can be maintained in the clinical situation. NVC-101 solution was equally effective at pH 3.5 or 4.0 and more efficient soon after its application. As opposed to other antimicrobials investigated in this animal model, NVC-101 controls the tissue bacterial bioburden without inhibiting the wound healing process.

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Wound healing is the end result of a series of interrelated cellular processes initiated by humoral factors such as cytokine growth factors.[1] These cellular processes are inhibited by a large tissue bacterial bioburden.[2] The cytokines and growth factors are also degraded by bacteria.[3] The level of tissue bacterial bioburden has been shown in multiple studies to be more than 10^5 or at least 1×10^6 bacteria per gram of tissue.[4,5] Such high levels of tissue bacteria can be present without clinical signs of infection, and when present can deleteriously affect wound healing.[6]

Attempts at controlling the tissue bacterial bioburden have been difficult. Systemically administered antibiotics do not effectively decrease the level of bacteria in a chronic granulating wound.[7] Therefore, topical antimicrobials or temporary biologic dressings have been the methods of choice.[4,8] Topical use of antibiotics that are used effectively systemically for purposes other than wound infection is discouraged because of an increased risk for developing allergies or the potential for bacteria to develop resistance to the drug.[9] Antiseptics and nonantibiotic antimicrobials such as povidone-iodine, silver sulfadiazine, or mafenide acetate cream have been demonstrated to be cytotoxic to the cellular components of wound healing.[10–12]

Stabilized hypochlorous acid (NVC-101) prepared by the addition of sodium hypochlorite to a solution of sodium chloride in sterile water followed by addition of a solution of hydrochloric acid and maintained at a pH between 3.5 and 5 has been demonstrated to have excellent in vitro antibacterial properties. Its potential limitation is the requirement to maintain its narrow pH range in the clinical wound environment.

The purpose of the studies reported here was to evaluate various concentrations of stabilized hypochlorous acid (NVC-101) topically administered to an experimental chronic infected granulating wound at different pHs and with different treatment regimens. The effects evaluated were the ability of NVC-101 to control the tissue bacterial bioburden and the ability of the agent to overcome the inhibition of wound healing caused by infection.

MATERIALS AND METHODS

Reagents

The various NVC-101 preparations ranging in concentrations from 0.01% to 0.02% and from pH 3.5 to 4.5 were provided by NovaBay Pharmaceuticals, Emeryville, Calif. In brief, the stabilized HOCl was prepared in 150 mM NaCl by acidifying reagent-grade NaOCl to the pH of 3.5 to 4.5 with dilute HCl. The concentration of active chlorine species ($[HOCl]_T = [HOCl] + [Cl_2] + [Cl_3] + [OCl^-]$) in 0.9% saline was determined by converting all the active chlorine species to OCl^- with 0.1 M NaOH and measuring the concentration of OCl^- spectrophotometrically at 292 nm using a molar absorbtivity of 362 M^{-1} cm^{-1}.[13]

Microbiological methods

Escherichia coli (ATCC 25922) was purchased from the American Type Culture Collection, and grown and propagated according to the ATCC recommendations. Bacteria for use in the animal model were obtained from fresh 18-hour broth culture, and inoculum size was confirmed by back-plating.

Animal model of chronic granulating wound

Chronic granulating wounds were prepared as previously described.[7,14–16] Male Sprague-Dawley rats weighing 300 to 350 g were acclimated in the facility for a week before use. Under intraperitoneal pentobarbital (Nembutal) anesthesia (35 mg/kg), the rat dorsum was shaved and depilated. A full-thickness dorsal burn measuring 30 cm^2 was created by immersing in boiling water. Infected groups were seeded with 5×10^9 CFU of *E. coli* (ATCC 25922) after the rats had been allowed to cool for 15 min.[16]

Animals were individually caged and given food and water ad libitum. Uninfected animals were kept in a physically separate facility. All experiments were conducted in accordance with the American Care and Use Committee at the Department of Veterans Affairs Medical Center, Bay Pines, Fla.

Five days after burning, the eschar was excised from anesthetized animals, resulting in a chronic granulating wound. Histological characterization of this wound with comparison to a human granulating wound has previously been performed.[7]

Treatment groups

Two different experiments using multiple treatment regimens were performed. In experiment 1, 45 rats were divided into 9 groups of 5 animals each. The groups were treated as follows: group I served as uninfected controls and received no inoculation of bacteria. Following escharectomy, these rats were treated with a saline (0.9% NaCl)-soaked gauze dressing, which was changed every 24 hours. Group II was an infected control and was inoculated, as were groups III to VIII. After escharectomy, the rats in group II were treated with daily changes of saline-soaked gauze dressing. Group III animals had their escharectomized infected wounds treated with gauze dressing saturated with 0.01% NVC-101, pH 3.5, changed every 24 hours. Groups IV and IVb were treated identically. Animals in these 2 groups had their escharectomized infected wounds treated with a dressing soaked with 0.01% NVC-101, pH 3.5, which remained in place for 30 minutes and then was replaced with a dressing soaked in 0.9% NaCl for 23.5 hours. This regimen was repeated every 24 hours. Group V received similar treatment as group III except that the 0.01% NVC-101 had a pH of 4.0. The regimen for group VI was similar to groups III and V except that the pH was adjusted to 4.5. Group VII was treated similarly to group III except that the concentration of NVC-101 was increased 0.02%, with the pH at 3.5. Finally, group VIII animals were treated with 1% silver sulfadiazine cream (Silvadene) without dressing and changed every 24 hours. The moist gauze dressings in groups I to VII were covered with one layer of petrolatum-impregnated gauze (Adaptic) and then covered with Coban dressing. A summary of animal treatment groups is depicted in Table 1.

Following evaluation of the results of experiment 1, in vitro modifications of techniques were investigated. It was decided that wiping off the wound following an initial application of stabilized hypochlorous acid and then replacing it may have added benefits (data not presented).

Experiment 2 consisted of 8 groups of 5 animals each. Group I served as the infected control and escharectomized infected wounds were treated with 0.9% NaCl–soaked dressing changed every 24 hours. Group II was treated with a gauze soaked in 0.01% NVC-101, pH 3.5, for 15 minutes, followed by gentle atraumatic wiping of the wound, and then treated by

Table 1. *Summary of treatment groups of rats in experiment 1*

Group*	Treatment
I	Uninfected/normal saline
II	Infected/normal saline
III	Infected/NVC-101, 0.01%, pH 3.5, changed q24 h
IV	Infected/NVC-101, 0.01%, pH 3.5, 30 min/23.5 h normal saline
IVb	Infected/NVC-101, 0.01%, pH 3.5, 30 min/23.5 h normal saline
V	Infected/NVC-101, 0.01%, pH 4.0, changed q24 h
VI	Infected/NVC-101, 0.01%, pH 4.5, changed q24 h
VII	Infected/NVC-101, 0.02%, pH 3.5, changed q24 h
VIII	Infected/Silvadene changed q24 h

*Five animals per group.

another application of 0.01% NVC-101, pH 3.5, for 23.75 hours. This regimen was repeated every 24 hours. Group III was treated the same as group II except that the pH of NVC-101 solution was adjusted to pH 4.0. The regimen for group IV was identical to group III using the pH 4.0 solution as the first application. However, after the gentle wiping, 0.9% NaCl was substituted for the remaining 23.75 hours instead of a repeat application of NVC-101. Group V had normal saline (0.9% NaCl) applied on the first dressing for 15 minutes, followed by wiping, and then another saline-soaked dressing for 23.75 hours. This was repeated every 24 hours. Group VI was treated identical to group II and had a 15-minute application of 0.01% NVC-101, pH 3.5, followed by wiping, but then followed by a gauze dressing soaked with 0.01% NVC-101, pH 3.5, left in place for 47.75 hours. This was repeated every 48 hours. Group VII mimicked group VI except that the second dressing consisted of a saline-soaked sponge for 47.75 hours. Finally, group VIII animals were treated after escharectomy with a 0.9% NaCl–soaked dressing for 30 minutes, followed by 23.5 hours of a second saline-soaked dressing. No gentle wiping was interspersed between dressings in group VIII. A summary of the animal treatment groups in experiment 2 is depicted in Table 2.

Animal procedures

In experiment 1, rats were premedicated with buprinorphine (0.1 mg/kg) and anesthetized with halothane inhalation on postescharectomy days 4, 8, 12, 16, and 20. Any dried exudates that formed were atraumatically removed. Wounds were biopsied for quantitative

Table 2. *Summary of treatment/group of rats in experiment 2*

Group*	Treatment
I	Infected/normal saline
II	Infected/NVC-101, 0.01%, pH 3.5, 15 min, wipe, then q23.75 h NVC-101, pH 3.5
III	Infected/NVC-101, 0.01%, pH 4.0, 15 min, wipe, then q23.75 h NVC-101, pH 4.0
IV	Infected/NVC-101, 0.01%, pH 4.0, 15 min, wipe, then q23.75 h normal saline
V	Infected/normal saline, 15 min, wipe, then q23.75 h normal saline
VI	Infected/NVC-101, 0.01%, pH 3.5, 15 min, wipe, then q47.75 h NVC-101, pH 3.5
VII	Infected/NVC-101, 0.01%, pH 3.5, 15 min, wipe, then q47.75 h normal saline
VIII	Infected/normal saline, 30 min, no wipe, then q23.75 h NVC-101, pH 3.5

*Five animals per group.

bacteriology on the day of escharectomy (day 0) and on each of the days of reanesthesia according to the methods described by Heggers and Robson.[5] The wound surface was cleaned with 70% isopropyl alcohol prior to biopsy to exclude surface contamination. Biopsies were aseptically weighed, homogenized, serially diluted, and back-plated onto nonselective media. Bacterial counts were completed after 48 hours' incubation and expressed as colony-forming units (CFU) per gram of tissue.[5]

While the rats were anesthetized for the wound biopsies, outlines of the wounds were traced onto acetate sheets, and area calculations were performed using computerized digital planimetry (Sigma Scan Jandel Scientific, Corte Madera, CA). Care was taken only to record the perimeter of the wound that represented the advancing full-thickness margin rather than the edge of any advancing epithelium. This avoided the small component of advancement provided by the smooth, pink, translucent, hairless neoepithelium.[16] All animals were weighed at the time of biopsy and wound measurement.

The animals were sacrificed by Nembutal overdose and bilateral thoracotomies when the wound had completely healed or decreased to less than 10% of its original area. Haywood et al demonstrated that measurement of very small wounds by manual tracing introduced significant systematic error and found that wounds followed past this point remained static for prolonged periods of time.[17]

The animals in experiment 2 had the same procedures performed as those in experiment 1 except they were performed at different time points, that is, days 0, 2, 4, 7, 9, 11, and 14, with the final wound size recorded on day 16. The time points were chosen to capture earlier time points and more frequent changes in the wound size and bacteriology.

Statistical analysis

Mean bacterial counts for each group of animals in both experiments were determined and expressed as CFU/g of tissue. These values were compared for each experiment using a one-way analysis of variance. Post hoc analyses of differences between groups were carried out using Tukey's test (all pairs, multiple-comparison test), with $P < .05$ considered significant. Sigma Stat statistical software (Jandel Scientific, Corte Madera, CA) was used for data analysis.

Serial wound area measurements were plotted against time. For each animal's data, a Gompertz equation was fitted (typical $r^2 = 0.85$).[18] Using this approach, a best-fit curve was generated for each group. Comparison between groups was performed using life table analyses and the Wilcoxon rank test. These statistical analyses were performed using SAS[19] and BMDP[20] packages on a personal computer.

RESULTS

Quantitative bacteriology

Quantitative bacteriology of the chronic granulating wounds treated with various formulations of stabilized HOCl (NVC-101) or Silvadene were determined. The mean bacterial counts for each biopsy day in experiment 1 are depicted in Table 3. Plots of mean $\log_{10}$ versus time for the various treated groups in experiment 1 are depicted in Figure 1 with the statistical comparisons.

Table 3. *Summary of mean bacterial counts for each treated group in experiment 1*

	Days of punch biopsy postescharectomy (mean CFU/g)					
Group*	**Day 0**	**Day 4**	**Day 8**	**Day 12**	**Day 16**	**Day 20**
I	8.4×10^2	2.48×10^3	1.49×10^4	1.63×10^4	3.71×10^4	8.04×10^3
II	2.92×10^7	8.96×10^6	4.88×10^5	1.39×10^5	1.68×10^5	2.12×10^4
III	3.12×10^7	1.39×10^5	3.6×10^4	3.24×10^4	5.28×10^4	5.16×10^3
IV	2.42×10^7	1.72×10^5	1.31×10^3	4.92×10^3	8.8×10^2	1.0×10^1
IVb	9.52×10^7	3.36×10^7	1.28×10^5	1.51×10^4	2.05×10^3	1.5×10^2
V	1.56×10^8	1.37×10^5	8.6×10^4	2.05×10^4	6.36×10^3	3.12×10^3
VI	2.45×10^7	1.44×10^5	1.25×10^5	6.14×10^4	3.56×10^4	1.38×10^4
VII	2.01×10^8	1.45×10^5	5.8×10^4	9.75×10^4	3.89×10^4	9.7×10^3
VIII	6.52×10^7	3.75×10^6	2.4×10^4	NG†	NG	NG

*Five animals per group.
†NG indicates no growth.

It is clear that Silvadene was the best topical antimicrobial at decreasing the tissue bacterial burden. 0.01% NVC-101, pH 3.5, applied for 30 minutes and then removed from the wound proved to be the next most effective regimen for decreasing the bacterial load in experiment 1. This regimen was used in both groups IV and IVb and the results were similar (Table 3 and Fig 1). The bacterial data from experiment 2 are also depicted in Table 4. Plots of

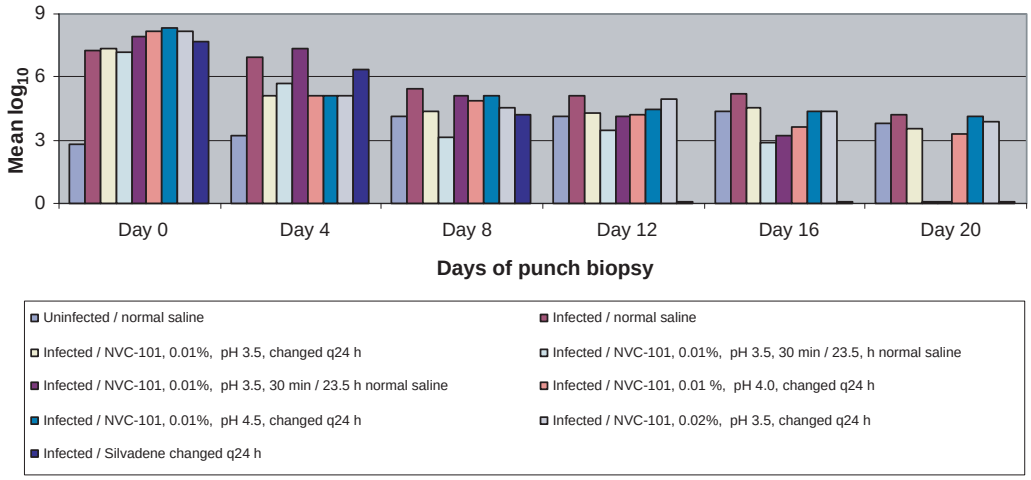


Figure 1. Depiction of the various groups in experiment 1 demonstrating the superiority of 30 minutes of NVC-101 application followed by another dressing for 23.5 hours over other regimens of hypochlorous acid. 1% silver sulfadiazine cream (Silvadene) was the most effective of all agents tested at decreasing the tissue bacterial bioburden.

mean $\log_{10}$ versus time for the various groups in experiment 2 are depicted in Figure 2 with statistical comparisons. Experiment 2 greatly expanded the knowledge of dosing regimen for NVC-101. Experiment 2 looked more carefully at the earlier, more frequent time points. Three regimens in experiment 2 were as good as or better than the best regimen in experiment 1 at decreasing the tissue bacterial burden. Groups II, III, and IV all had counts less than 10^3 CFU/g of tissue by day 14. In groups II and III, which

Table 4. *Summary of mean bacterial counts for each treated group in experiment 2*

	Days of punch biopsy postescharectomy (mean CFU/g)						
Group*	**Day 0**	**Day 2**	**Day 4**	**Day 7**	**Day 9**	**Day 11**	**Day 14**
I	5.0×10^8	5.0×10^8	1.27×10^8	1.4×10^7	1.85×10^6	2.32×10^5	1.41×10^5
II	5.0×10^8	1.59×10^6	8.74×10^5	1.03×10^5	8.8×10^3	3.48×10^3	5.0×10^2
III	5.0×10^8	3.24×10^6	2.51×10^6	3.12×10^4	2.54×10^3	3.48×10^3	6.4×10^2
IV	5.0×10^8	1.12×10^8	2.31×10^7	1.37×10^7	9.16×10^4	7.28×10^3	4.0×10^2
V	5.0×10^8	5.0×10^8	2.78×10^7	4.38×10^6	8.53×10^5	2.53×10^5	4.4×10^4
VI	5.0×10^8	2.01×10^7	2.60×10^7	7.74×10^6	2.24×10^5	3.68×10^4	1.60×10^3
VII	5.0×10^8	2.17×10^8	4.06×10^7	4.06×10^7	3.48×10^6	1.95×10^5	1.56×10^3
VIII	5.0×10^8	4.01×10^8	5.24×10^7	2.72×10^7	1.58×10^6	2.42×10^5	4.04×10^4

*Five animals per group.

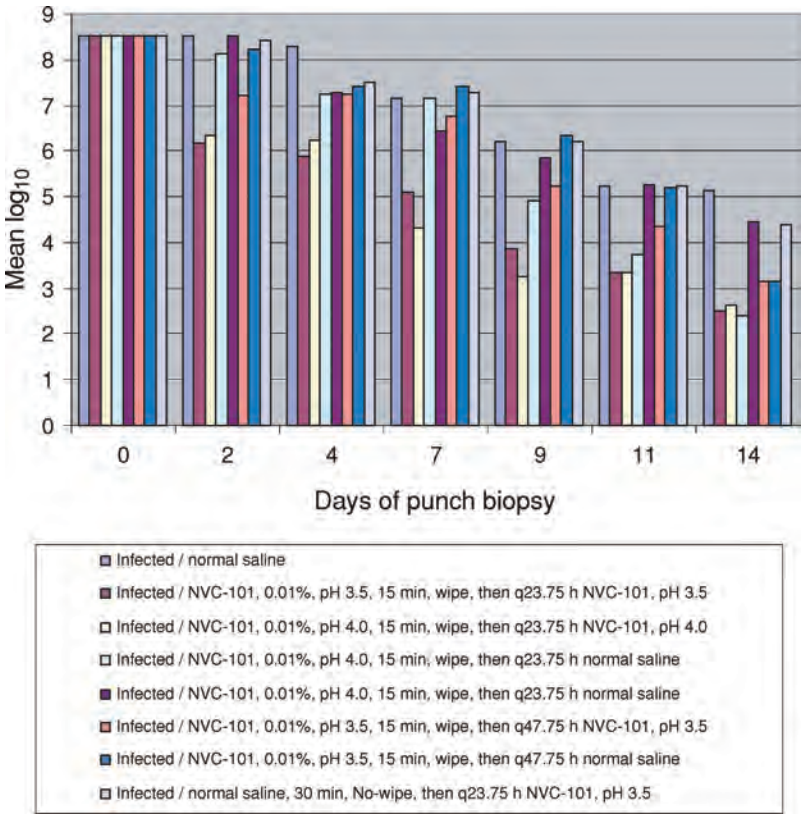


Figure 2. Depiction of the various groups in experiment 2 demonstrating the superiority at decreasing bacterial counts of NVC-101 applied for 15 minutes, followed by gentle atraumatic wiping then 23.75 hours of a second dressing of NVC-101. There was essentially no difference in the effects with pH 3.5 and 4.0.

had essentially the same treatment regimens, the bacterial counts decreased more rapidly than in group IV. For these groups (II and III), the regimen consisted of NVC-101 being placed on the wound for 15 minutes, atraumatically wiped off, and then reapplied for 23.75 hours. The only difference between the treatments for groups II and III was the pH of NVC-101. No significant differences were seen between pH 3.5 and pH 4.0 (Table 4 and Fig 2).

Body weights

There was an equivalent gain in body weight among all groups during the period of study, with no significant variations among the groups in either experiment 1 or experiment 2

Wound area

Best-fit healing curves demonstrated that none of the treatment regimens resulted in the area of the wound increasing in size (Figs 3 and 4).

Infected control animals (group II in experiment 1, group I in experiment 2) retarded healing as compared to the noninfected controls (group I in experiment 1). Healing curves for groups IV and IVb in experiment 1 demonstrated statistically significant increases in reduction in the fraction of open wounds when compared to groups I, III, V, and VIII ($P < .05$) and groups II, V, and VII ($P < .01$) (Fig 3). Groups II, V, and VII demonstrated a slower trajectory than all other groups, also statistically significant ($P < .05$) (Fig 3).

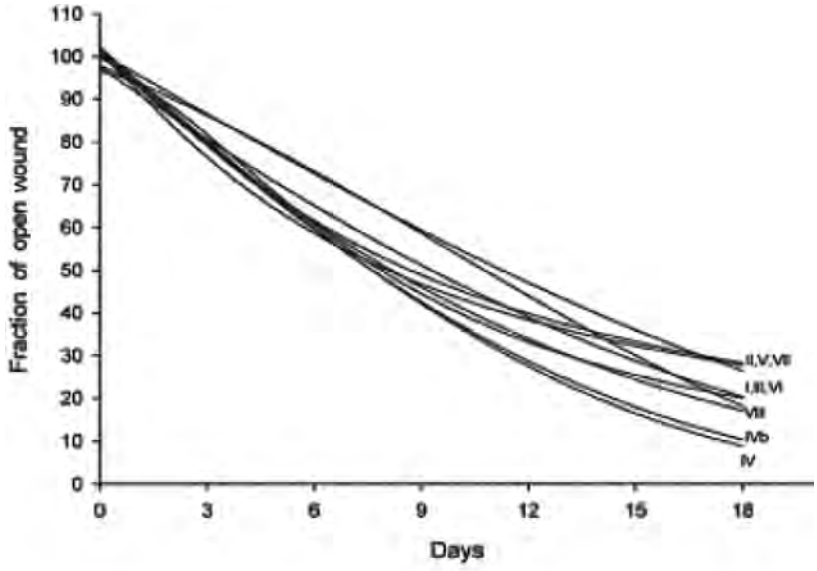
In experiment 2, healing curves for groups II and III demonstrated statistically significant larger reductions in the fraction of open wounds when compared to groups IV, VI, and VII ($P < 0.05$) and groups I, V, and VIII ($P < .01$) (Fig 4). Groups I, V, and VIII demonstrated a slower healing trajectory than all other groups, which was also statistically significant ($P < .05$).

DISCUSSION

Because of the deleterious effect of a high tissue bacterial burden on the process of wound healing, an effectual antimicrobial agent becomes a therapeutic imperative. Such an agent should be effective as a topical preparation, yet not to be cytotoxic to the cells involved in the wound healing process.[21] Stabilized hypochlorous acid, as tested in the 2 experiments reported, may prove to be such an agent. Its in vitro antibacterial properties and tissue safety profile suggest its potential as a wound care agent.[13] However, it is likely rapidly neutralized in the wound environment.

In experiment 1, Silvadene was, as expected, the most effective antibacterial. However, Silvadene was not as effective at promoting wound closure as were two of the NVC-101 regimens (IV and IVb) (Fig 3). The healing that occurred with Silvadene was probably due to elimination of the tissue bacterial load (Table 3). The reason the wounds did not totally heal or exceed that with groups IV and IVb is because of the known cytotoxic properties of Silvadene.[11,12]

From a review of the quantitative bacteriology data from both experiments, it is clear that a brief application of NVC-101, followed by a second dressing change is better than a



I Uninfected control Rx'd with NaCl 0.9% q 24 h
II Infected control Rx'd with NaCl 0.9% q 24 h
III Infected Rx'd with NVC-101 (HOCl) 0.01%, pH 3.5, q 24 h
IV Infected Rx'd with NVC-101 (HOCl) 0.01%, pH 3.5, for 30 min, followed by 23.5 h 0.9% NaCl and repeated q 24 h
IVb Infected Rx'd with NVC-101 (HOCl) 0.01%, pH 3.5, for 30 min, followed by 23.5 h 0.9% NaCl and repeated q 24 h
V Infected Rx'd with NVC (HOCl) 0.01%, pH 4.0, q 24 h
VI Infected Rx'd with NVC (HOCl) 0.01%, pH 4.5, q 24 h
VII Infected Rx'd with NVC (HOCl) 0.02%, pH 3.5, q 24 h
VIII Infected Rx'd with Silvadene cream q 24 h

Figure 3. Wound healing trajectories from experiment 1, demonstrating faster healing for groups IV and IVb treated with a short application of NVC-101 followed by a second dressing of normal saline. These 2 groups had a more favorable wound healing trajectory than did group VIII treated with 1% silver sulfadiazine cream (Silvadene).

single application of NVC-101 left in place for 24 hours (group III, experiment 1) (Tables 3 and 4).[21] When the second dressing is again NVC-101 (groups II and III from experiment 2), the rate of bacterial reduction is faster than when the initial application of NVC-101 is followed by normal saline (groups IV and IVb in experiment 1, or group IV in experiment 2) (Table 3 and 4, and Figs 1 and 2). There was no apparent difference in the wound healing trajectory whether the second dressing contained NVC-101 or saline (Figs 3 and 4).

It is clear that the effect of NVC-101 on bacteria occurs in a short period of time after application. Possibly leaving NVC-101 in place for 24 hours stimulates greater plasma or serum response to inflammatory stimuli and that plasma milieu allowed bacterial growth over time. This is not unlike suggestions from Fleming's classic article of 1919.[21] Therefore, it may be useful to use NVC-101 for a short duration of time. The initial bacterial kill by NVC-101 appeared sufficient not to allow regeneration of bacteria when replaced by saline (groups IV and IVb, experiment 1). The antibacterial effect was obviously due to NVC-101,

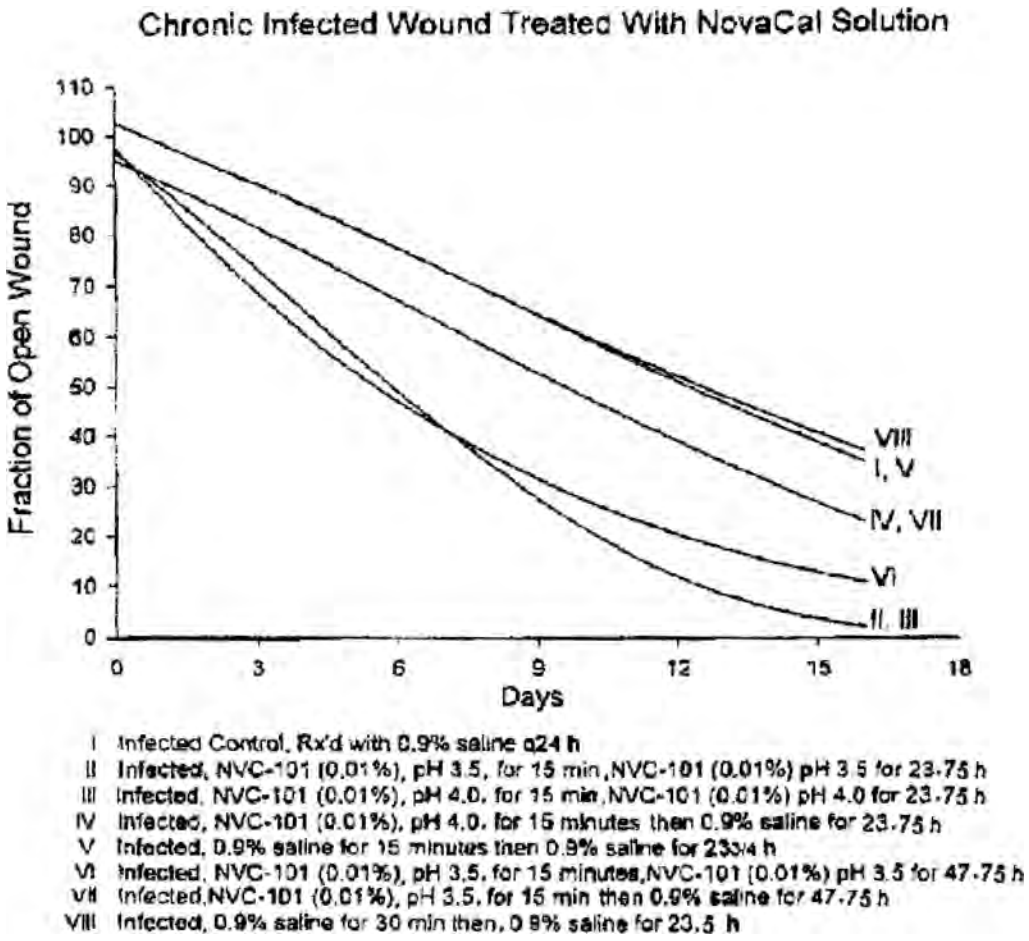


Figure 4. Wound healing trajectories from experiment 2, demonstrating the superiority of groups II and III, which consisted of a brief application of NVC-101, followed by gentle wiping, and a second application of NVC-101 over other regimens. The 2 groups with the fastest healing were also the most effective at controlling the tissue bacterial bioburden.

since when only saline was used in 1 or 2 applications, the bacterial kill was less (group II, experiment 1; groups V and VIII, experiment 2) (Tables 3 and 4, Figs 1 and 2).

The differences between pH 3.5 and 4.0 were not detectable. However, when the pH of NVC-101 was raised to 4.5, the control of the tissue bacterial burden seemed slightly less effective, with a slower wound healing trajectory. Therefore, it appears that a pH of 3.5 or 4.0 may be more useful. However, these differences may not be significant.

The role of the atraumatic wiping between dressing applications is not entirely clear. If NVC-101 kills bacteria immediately or soon after initial contact, then the gentle wiping may remove the devitalized bacteria and any possible debris, allowing the second application of NVC-101 to be in immediate contact with any remaining viable bacteria. This may explain the faster decrease in tissue bacterial levels seen in groups II and III in experiment 2.

In conclusion, the pilot in vivo study results for the 2 experiments indicate that the stabilized form of hypochlorous acid (NVC-101) is equally effective at pH 3.5 or 4.0 and

more effective soon after its application. As opposed to other antimicrobials investigated in this animal model, NVC-101 controls the tissue bacterial bioburden without inhibiting the wound healing process.

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Appendix V: Literature Research about Hypochlorous Acid

g) Hypochlorous Acid: An Ideal Wound Care Agent with Powerful Microbicidal, Antibiofilm, and Wound Healing Potency.

Wounds. 2014 Dec;26(12):342-50.

Hypochlorous Acid: an ideal wound care agent with powerful microbicidal, antibiofilm, and wound healing potency.

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Author information

Abstract

INTRODUCTION: Chronic wounds and the infections associated with them are responsible for a considerable escalation in morbidity and the cost of health care. Infection and cellular activation and the relation between cells are 2 critical factors in wound healing. Since chronic wounds offer ideal conditions for infection and biofilm production, good wound care strategies are critical for wound healing. Topical antiseptics in chronic wounds remain in widespread use today. These antiseptics are successful in microbial eradication, but their cytotoxcity is a controversial issue in wound healing.

OBJECTIVE: The aim of this study was to investigate the effect of stabilized hypochlorous acid solution (HOCl) on killing rate, biofilm formation, antimicrobial activity within biofilm against frequently isolated microorganisms and migration rate of wounded fibroblasts and keratinocytes.

MATERIALS AND METHODS: Minimal bactericidal concentration of stabilized HOCl solution for all standard microorganisms was 1/64 dilution and for clinical isolates it ranged from 1/32 to 1/64 dilutions.

RESULTS: All microorganisms were killed within 0 minutes and accurate killing time was 12 seconds. The effective dose for biofilm impairment for standard microorganisms and clinical isolates ranged from 1/32 to 1/16. Microbicidal effects within the biofilm and antibiofilm concentration was the same for each microorganism.

CONCLUSION: The stabilized HOCl solution had dose-dependent favorable effects on fibroblast and keratinocyte migration compared to povidone iodine and media alone. These features lead to a stabilized HOCl solution as an ideal wound care agent.

PMID: 25785777

[PubMed]

Appendix V: Literature Research about Hypochlorous Acid

h) Hypochlorous acid: Its multiple uses for wound care.

Hypochlorous Acid: Its Multiple Uses for Wound Care

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Reynoldsburg Podiatry Center, Reynoldsburg, OH; and Circleville Foot and Ankle, Circleville, OH

In today's medical environment, it is necessary to get the most out of the products we have at hand. When we can find a tool that can be utilized in many ways and still be cost effective, we need to take advantage of it. One such product is hypochlorous acid (HOCl). Although basically a wound cleanser, we have utilized it in our practice for much broader indications.

Hypochlorous acid is a naturally occurring small molecule generated by white blood cells during the oxidative burst to kill pathogens.[1] It has been shown in independently published *in vitro* studies[2] to be highly effective in killing drug-resistant bacteria and essentially all human pathogens. *In vitro* analysis[3,4] of cell toxicity testing showed no negative effects on keratinocytes or fibroblasts. As such, it should be useful in the treatment of chronic wounds.

The HOCl used in our practice (Vashe Wound Therapy Solution, SteadMed Medical LLC, Ft. Worth, TX) is intended for cleaning, irrigating, and debriding acute and chronic dermal lesions by the mechanical action of removing foreign materials, including micro-organisms and biofilms, from wounds. The solution has been shown to be active against a range of micro-organisms in *in vitro* testing.[2] When used as a soak on wounds followed by gentle wiping with gauze, it has been demonstrated to effect a "soft" debridement.[5] In addition, it is not painful to the patient and tends to remove odor from the wounds.[6,7]

In a large series of cases, HOCl has been used in a wide variety of indications within our wound population. HOCl has been effective as a wound cleanser with both sharp debridement and ultrasonic debriding equipment. It has proven effective at soaking infected wounds to decrease the use of systemic antibiotics and for keeping skin grafts and dermal matrices hydrated. When used on primarily closed surgical incisions, it appears to reduce surgical site infections. Similarly, when used in negative pressure wound therapy (NPWT) systems, it appears to lower the bioburden. All of these benefits are attained while dramatically reducing wound odor and reducing wound discomfort by providing a cooling sensation.

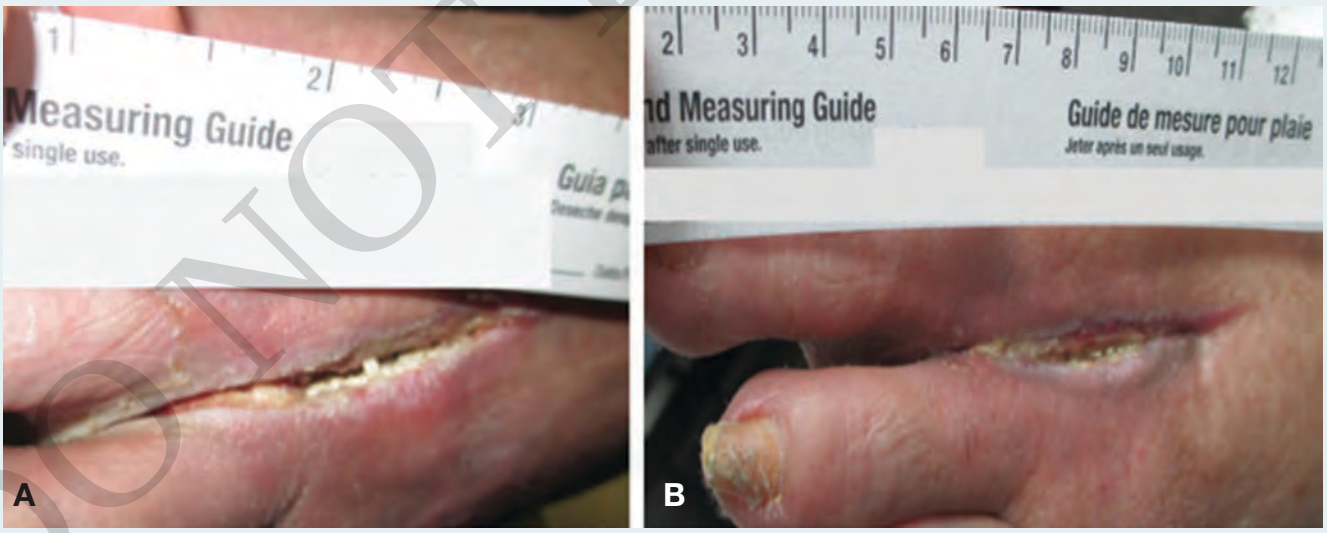

Figure 1. A: Abscess of left foot following incision and debridement and institution of negative pressure wound therapy with hypochlorous acid. B: Four weeks after incision and debridement and 2 weeks after switching from negative pressure wound therapy to dressings with collagen dermal matrix and hypochlorous acid irrigation and soaks.

Pearls for Practice is made possible through the support of SteadMed Medical, LLC, Fort Worth, TX (www.steadmed.com). The opinions and statements of the clinicians providing Pearls for Practice are specific to the respective authors and not necessarily those of SteadMed Medical, LLC, OWM, or HMP Communications. This article was not subject to the Ostomy Wound Management peer-review process.

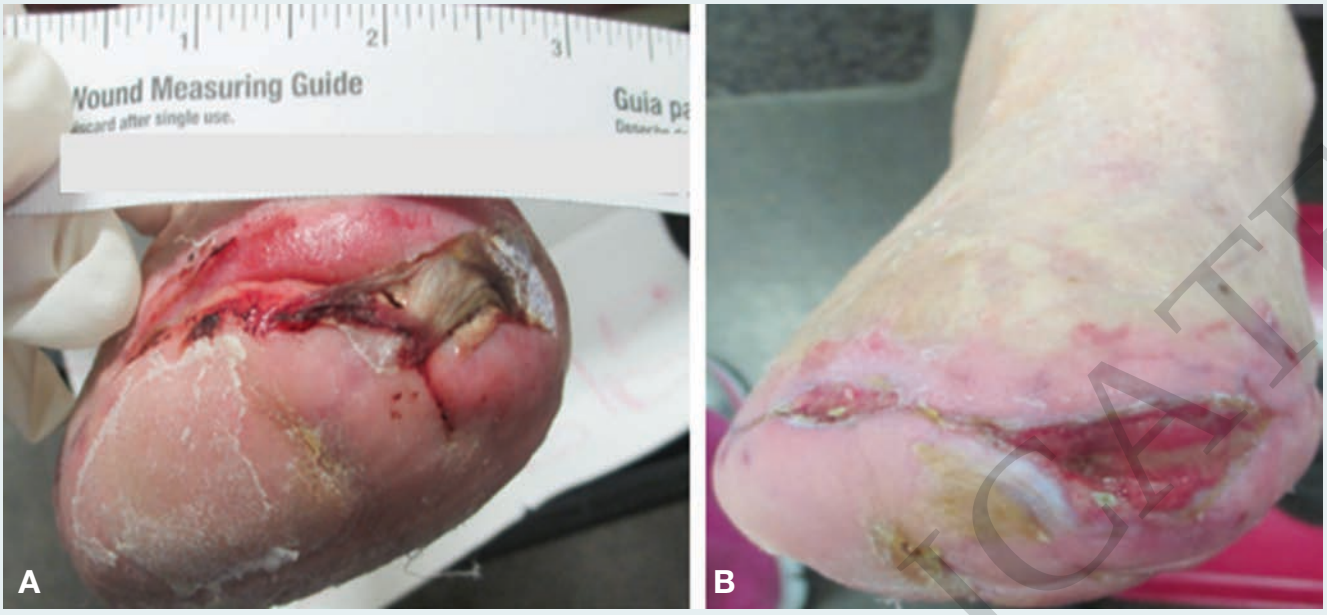

Figure 2. A: Following dehiscence of transmetatarsal amputation stump treated with hypochlorous acid (HOCl) soaks and collagen dermal matrix dressings hydrated with HOCl. B: Three weeks following the treatment regimen, the wound was closing and the exposed tendon was covered with healthy granulation tissue.

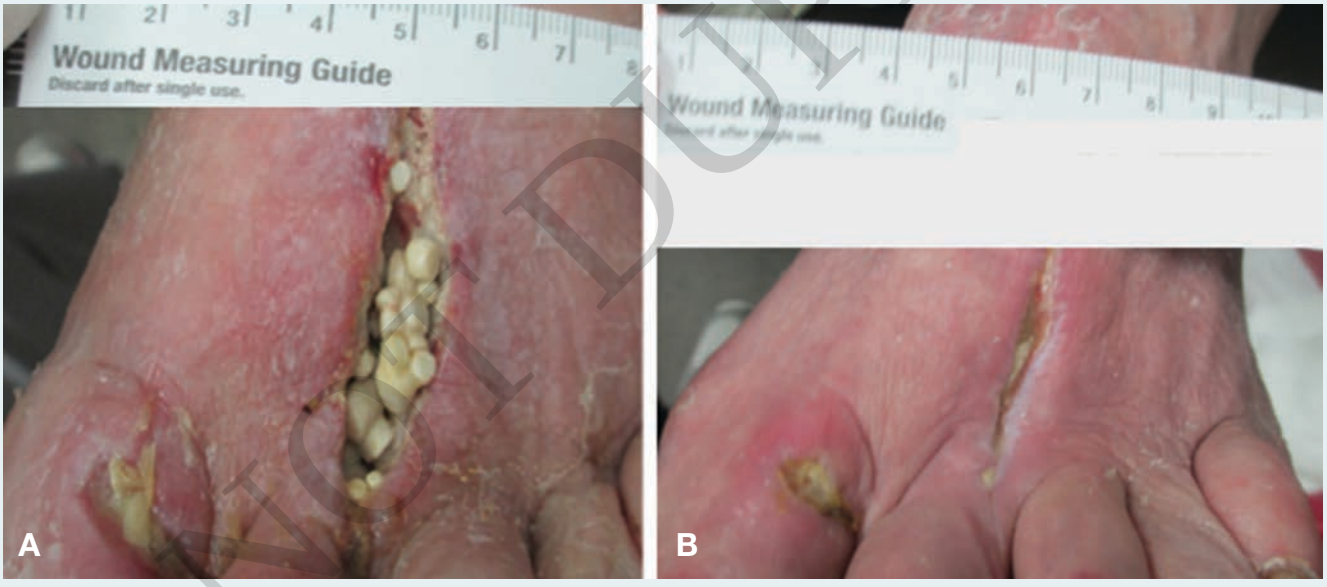

Figure 3. A: Dog bite wound of the foot following incision and debridement. B: Three weeks following irrigation and soaks with hypochlorous acid (HOCl) and dressing changes with collagen dermal matrices hydrated with HOCl.

Examples of cases that demonstrate the effectiveness of HOCL in a wound care practice include a 53-year-old obese man with diabetes mellitus, gout, and peripheral vascular disease who developed an abscess in his left foot at the 3rd to 4th interspace. Following incision and drainage (I&D) of the abscess, he was treated with NPWT with HOCl irrigation (see Figure 1a). Within 2 weeks, he was switched to collagen dermal matrix dressings once a week with HOCl irrigation and soaks at the time of dressing changes. By 4 weeks, following I&D, the wound was on a positive healing trajectory and approaching closure (see Figure 1b).

A second example is a 45-year-old man with a diabetic foot ulcer and peripheral neuropathy who sustained an injury to the right foot requiring a transmetatarsal amputation. Wound dehiscence was treated with HOCl irrigation and soaks and dressing with a collagen dermal matrix dressing (see Figure 2a). In this case, the HOCl also was used to hydrate the dermal matrix dressing. After 3 weeks of this treatment, the wound closed and the exposed tendon was covered with healthy granulation tissue (see Figure 2b).

A third example involves a 68-year-old obese man with diabetes mellitus, congestive heart failure, and asthma who sustained a dog bite to his left foot. Following I&D, the wound was irrigated and soaked with HOCl and dressed with a collagen dermal matrix dressing that was kept hydrated with HOCl (see Figure 3a). Within 3 weeks, the wound was on a positive healing trajectory (see Figure 3b). The treatment was continued, and the wound healed completely without further intervention.

We conclude from our experience that HOCl can be used effectively for many indications in a wound care practice. Expanding use of wound products is prudent in an environment of decreasing resource availability. ■

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Appendix V: Literature Research about Hypochlorous Acid

i) Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers.

J Wound Care. 2006 Jan;15(1):33-7.

Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers.

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Author information

Abstract

OBJECTIVE: Hypochlorous acid (HOCl) is a highly microbiocidal agent active against bacteria, viruses and fungi. Using quantitative microbiology, preliminary studies showed it achieved an appreciable reduction in the bacterial burden in chronic venous leg ulcers. The study aimed to determine whether it has a role as an additional treatment for chronic venous ulcers that have not healed with conventional treatment.

METHOD: On the basis of previous reports we designed a study in which patients acted as their own controls, in that only patients who failed to achieve a 44% reduction in wound size with standard treatment (compression bandaging) received HOCl washes.

RESULTS: Of 30 patients admitted to the study, 10 achieved a 44% ulcer reduction after three weeks of standard treatment. In addition to the standard compression treatment, the remaining 20 patients were given HOCl washes over 12 weeks. Of the 20 ulcers, nine (45%) healed and five (25%) reduced in size by over 60%. All patients became free of pain.

CONCLUSION: These findings confirm the clinical efficacy of treating venous leg ulcers with hypochlorous washes. Use of HOCl washes as an adjunctive therapy for recalcitrant venous leg ulcers appreciably increases healing and rapidly relieves pain.

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[PubMed - indexed for MEDLINE]

Appendix V: Literature Research about Hypochlorous Acid

j) Conquering Chronic Non-Healing Wounds with Pure Hypochlorous Acid.

Seton Medical Center

NovaBay PHARMA

Conquering Chronic Non-Healing Wounds with Pure Hypochlorous Acid

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Abstract

Chronic non-healing wounds, such as venous stasis ulcers, diabetic ulcers, and pressure ulcers are serious unmet medical needs that affect a patient's morbidity and mortality. Systemically administered antibiotics do not effectively decrease the level of bacteria or the associated biofilm in a chronic granulating wound. Topical antiseptics have a long history of use, such as sodium hypochlorite (Dakin's solution), hydrogen peroxide, acetic acid and povidone-iodine that remain in widespread use today. However used at typical concentrations, these antiseptics can actually impede wound healing[1]. We have more than 30 clinical case studies where we used NeutroPhase® with and without negative pressure wound therapy (NPWT) to assist in wound healing. Common pathogens observed in these chronic wounds include *Staphylococcus* including MRSA, *Pseudomonas*, *Enterobacter*, *Stenotrophomonas*, and *Serratia* spp. In our *in vitro* experiment, we also show that 0.01% pure HOCl is more active than sodium hypochlorite against *S. aureus* by a zone of inhibition assay. HOCl has been described as being 80-100 times more potent as a germicide than the hypochlorite anion (OCl^-; high pH). NeutroPhase® is pure 0.01% hypochlorous acid (i.e. >97% relative molar distribution of active chlorine species as HOCl) in a 0.9% saline solution at pH 4-5 and NovaBay has an FDA cleared 510(k) for wound care. Our data show that there is a clear utility for the use of NeutroPhase® as a key agent in wound care and to address this serious unmet medical need.

Introduction

Chronic non-healing wounds have many factors contributing to the impairment of healing such as the presence of foreign bodies, tissue maceration, ischemia, infection, and biofilms. The clinical picture can be further complicated by systemic factors such as diabetes, malnutrition, renal disease, and advanced age. Therefore, chronic non-healing wounds are a clinical problem that for some is a serious unmet medical need. Pure 0.01% HOCl (NeutroPhase®) has been shown in a well-established chronic granulating wound rat model[2] to be an effective topical antimicrobial and to have a potential application as an antimicrobial wound irrigation. NeutroPhase® is a 510(k) cleared self-preserved preparation of HOCl that has been shown to inactivate *P. aeruginosa, E. coli, S. aureus, C. albicons* and *A. niger* in solution. Using Sorbact as the wound mesh dressing in combination with NeutroPhase® as the irrigation solution assists in wound healing. NeutroPhase® is not toxic to living tissue.

Materials & Methods

For the Zone of inhibition assay, 100 uL of the formulations were applied every 15 min and removed before next application. Up to 4 treatments were completed over 1 hr. The clear parts represent "kill zones". A combination of NeutroPhase® (0.01% HOCl) as the irrigation solution and Sorbact (Abigo Medical AB, Askim, Sweden) as the wound mesh dressing was used to treat patients with chronic non-healing wounds. Before treatment, the wound area was cleansed and the wound was debrided, then the skin was dried. Then Sorbact mesh was sized and placed in the wound. A Blake drain was placed on and in the Sorbact mesh. The adhesive drape was attached and placed over the entire area including the Sorbact mesh. The area around the tubing was sealed with Stomadhesive. The tubing was connected to a three-way stopcock and a one-way valve was added. The VAC was then turned on and adjusted from 50 mm to 125 mm suction. The pre-determined amount of NeutroPhase® was injected through the three-way stopcock and allowed to stay in the wound for 15 minutes before it was vacuumed out. This was subsequently changed to a separate inflow tube (IV tubing) and irrigated while the VAC was kept on.

Results

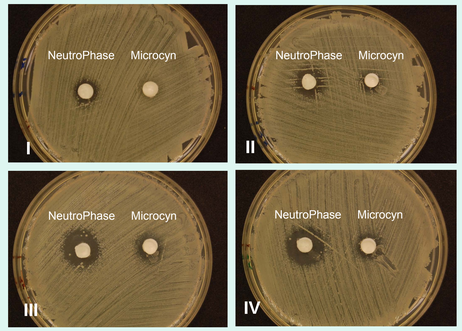
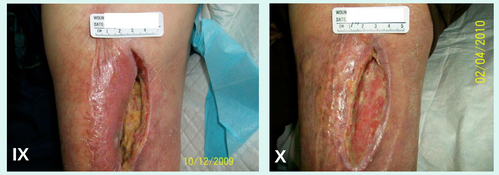


Figure 1. Antimicrobial activity comparison of NeutroPhase® vs Microcyn (sodium hypochlorite) gel formulations in Zone of Inhibition assay against *S. aureus* ATCC 29213. (I) One treatment was applied. (II) Two treatments were applied. (III) Three treatments were applied. (IV) Four treatments were applied. Formulations were applied every 15 min and removed before next application.



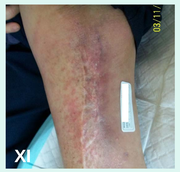


Figure 3. (IX) 67 year old female, with history of liposarcoma on left lateral thigh which underwent radical surgery and radiation therapy, presented to Emergency Department with cellulitis of left thigh. Left thigh was excised and drained; culture taken was positive for MRSA. Open ulcer was difficult to heal due to scar tissue from radiation therapy and MRSA infection. (X) With serial debridement of non-viable tissue and negative pressure wound therapy with HOCl 0.01 % instill, wound is now healing evidenced by granulation tissue formation filling in the cavity.(XI) When adequate granulation was achieved and no sign and symptoms of infection, plastic surgery was consulted for flap closure of the wound.

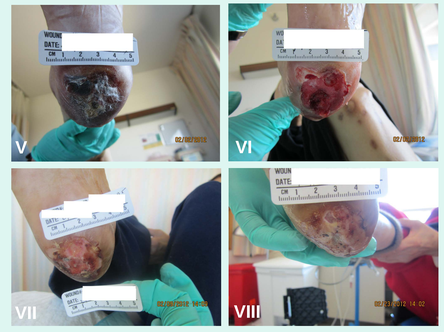


Figure 2. (V)75 year old female with DM II, End-Stage renal disease presents to wound center with chronic ulcer on the right foot. (VI) Wound post-debridement. (VII) 1 week post-debridement. (VIII) Week 2 of treatment: Patient continues to heal and is now ready for biological skin equivalent to be applied.

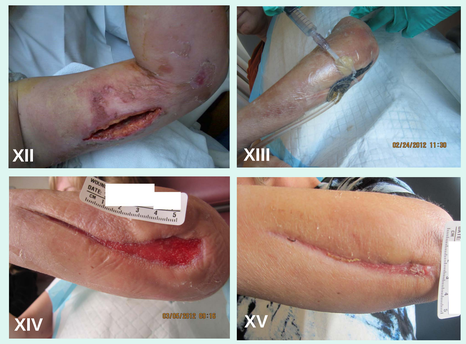


Figure 4. (XII, XIII, XIV, XV) This 51 y/o fell bruising left arm in a parking lot. Three days later arm flared up with streptococcal cellulitis and fasciitis. She went into shock and was admitted to ICU. Incision and Drainage (I and D) one day later followed by repeat lower arm I and D with VAC and instillation of NeutroPhase® three times a day. Repeat extension of surgery in forearm. Discharged on VAC and instillation at home 3 1/2 weeks later, now @ 5 weeks upper arm healed and lower arm @ 90% with biological graft in place. No positive cultures obtained, just gram stain streptococcus found.

Discussion

Chronic non-healing wounds have many factors contributing to the impairment of healing such as the presence of foreign bodies, tissue maceration, ischemia, infection, pressure and biofilm. The clinical picture can be further complicated by systemic factors such as diabetes, malnutrition, renal disease, and advanced age. Therefore chronic non-healing wounds impact the patient's morbidity and are a serious unmet medical need. Our studies using Sorbact as the wound mesh dressing in combination with NeutroPhase® as the self-preserved irrigation solution assisted in wound healing. NeutroPhase® is not toxic to living tissues. Sorbact is a hydrophobic mesh that traps bacteria yet decreases maceration of the adjacent skin next to the wound. Appropriate wound care remains essential with debridement, offloading, antibiotics and appropriate follow up care in the outpatient setting. The treatment can be maximized with the use of negative pressure wound treatment (NPWT) as well by the instillation irrigation and aspiration twice daily with NeutroPhase®. In this treatment with the appropriate instillation procedure we used Sorbact mesh as the sponge which does not leave macerating fluids on the skin. Overall this simplifies the vacuum instillation procedure significantly.

Conclusions

- NeutroPhase® formulation shows greater antimicrobial activity than Microcyn formulation in the Zone of Inhibition assay against *S. aureus*.
- The results demonstrate that NeutroPhase® is an important irrigation solution in treatment of chronic non-healing wounds.
- Sorbact helps reduce tissue maceration.
- NeutroPhase® in combination with Sorbact as the wound mesh dressing utilizing negative pressure wound therapy assists in wound healing.
- These case studies show NeutroPhase® in combination with Sorbact® has the potential to be a very effective wound care product for use in managing difficult to heal wounds.

Disclaimer: NeutroPhase is a 510k cleared product for wound cleansing only and we are claiming only antibacterial activity in solution

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Appendix V: Literature Research about Hypochlorous Acid

k) A Randomized Clinical Trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wound.

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A Randomized Clinical Trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wound.

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Abstract
Background: Hypochlorous acid is a highly microbiocidal active agent against a broad spectrum of microorganisms. It achieved a marked reduction in the bacterial burden in a septic wound. This study **aimed** to evaluate the efficacy of Hypochlorous acid as a wound care agent in a septic traumatic wound. **Materials and Method:** **Design**: The current study used a randomized clinical trial to investigate the effectiveness of Hypochlorous acid as a wound care agent in a septic traumatic wound. **Setting**: trauma unit at Assiut university hospital was the setting of the study. **Subjects:** A random selection of 60 patients was done. Then a random distribution of the subjects to study group and control group, (30 subjects each) was done. **Methods**: The 30 subjects of the study group received Hypochlorous Acid for daily washing a septic wound for 3 to 5 minute, while the 30 subjects of the control group received Povidine Iodine and the results were compared. Bacterial count and culture were done before start washing, after one week, and two weeks in the two groups. **Results:** wound pain, odor, discharge and bacterial count were dramatically reduced by using as a disinfectant agent compared to the use of Povidine Iodine. **Conclusion:** Hypochlorous Acid appears to be inexpensive, easy to perform, painless and effective as a potent wound care dressing against a wide range of microorganisms. Hypochlorous Acid controls the tissue bacterial bio-burden without inhibiting the wound healing process, rapidly relieves pain and the area well prepared to skin flap or graft. **Recommendation:** The study recommended the use of Hypochlorous Acid as a potent wound care dressing. Further research is needed on a larger scale to validate the effectiveness of Hypochlorous acid as a wound care agent in a septic traumatic wound.
Keywords: Efficacy, Hypochlorous acid, Septic wound, Bacterial burden

1.Introduction

Acute wounds are wounds that heal within an expected time frame without any complications (1). Traumatic wounds are one type of acute wounds that account for about 20-25% of emergency department workload (2). According to the nature and mechanism of injury, traumatic wounds can vary from simple abrasion to soft tissue loss (3). Any traumatic wounds should be considered contaminated at presentation (4). And often predispose to infection, due to the presence of devitalized tissue, foreign bodies and bacteria, which may range from cellulites to deep myositis (5). Bio-film formation due to bacterial colonization is recognized to play a significant, detrimental role in the wound-healing process and progress to closure (6, 7). Wound is very rapidly colonized and ninety-five percent of acute wounds are infected with aerobic microorganisms (8). Wound being painful is due to bacterial adverse effects (9, 10).

A critical part of wound-bed preparation includes treating infection (11). And quantitative reduction of bacteria to a level that is treatable by the immune system, also pain and odor due to infection should be eliminated (12). Removal of bacteria and surface contaminants should consist of thorough wound-bed preparation and aggressive wound cleansing with a prepared non-cytotoxic wound cleanser to reduce bacterial bio-burden and infection rate and this allows the wound to move more rapidly from inflammation to proliferation, minimizing the risk of infection (11, 13,14,15).

If the wounds present with local clinical signs and symptoms of infection, topical administration of anti-microbial therapeutic agents is frequently adequate to address bio-burden in these wounds (16). Wound cleansing forms an integral part of the management of traumatic wounds, because bacteria do not typically survive in a clean, healthy wound (8). It applies to the application of f1uid to help removal of exudates, debris, slough and contaminants (17). Many cleansing and topical antimicrobial agents, such as Dakin solution (NaOCl), Hydrogen Peroxide (H2O2), Acetic Acid, and Povidine Iodine are known to be toxic to many of the cells involved in the wound-healing cascade and so impede wound healing (18,19,20,21,22).

Povidine iodine even at low concentrations has been shown to be toxic to granulocyte and monocyt (23) and results in decreased chemotaxis (24). 1t is also capable of suppressing lymphocyte functions (25). Hypochlorous acid (HOCl) is a non-cytotoxic and a highly active microbiocidal agent that is active against all bacterial, viral and fungal human pathogens (26). It has been used in acute wounds and has a positive impact on maintaining the wound environment and subsequently, supporting the healing process. Also, Hypochlorous acid reduces the pain and odor that is often associated with the wounds (27).

The objectives of wound management are to remove the tissue debris and to create optimum local conditions for

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wound healing (28). And to reduce patient's pain, malodour, the frequency of dressing changes required the volume of exudates, the local signs of infection and to prevent deterioration of the wound (29).
Wound care is mainly a nursing job, that depends on observation, recording; and reporting the consistency, color, odor of any drainage, the type, extend and characteristics of the wound (30). The purposes of the wound dressing include cleaning a wound to removing any dirt and debris from the wound bed, preventing infection to prepare the wound for healing and protecting a clean wound from trauma so it can heal normally and notifying any signs of abnormal wound healing (31).
The nurse must follow the infection control protocol for keeping the wound free from infection, and the patient's environment should be free as possible from contamination. Closely monitor sign and symptoms of wound infection is an essential role of the nurse, if an infection develops culture and sensitivity test should be done to determine the organism and the most effective antibiotic and local antiseptic solution for that specific organism (32).When the nurse is changing a dressing, inappropriate facial expressions can alert the patient to problems with the wound or the nurse's ability to care for it. Winking of the noise by the nurse may convey disgust to the patient. A nurse should also be careful not to be focus on the wound to the extent that the patient is not treated as total person (33).

Significance of the study

Recent reports from doctors and nursing staff in the traumatic unit at Assiut university hospital and also from the patients pointed out to an increased incidence of wound infection that lead to long of hospital stay, increased hospital cost and nurses load which interfere with optimal care and increase patient disability. The number of patients with traumatic wound admitted to traumatic unit of Assiut University Hospital in the last year was 5000 case according to the Hospital statistical record (2013).

2.Patients and methods

2.1Aim of the study

This study aimed to evaluate the efficacy of Hypochlorous Acid (HOCl) compared to Povidine Iodine as a wound care agent in a septic traumatic wounds.

2.2Research Hypothesis

Septic traumatic wounds that were cleaned by Hypochlorous Acid (HOCl) will have fewer odors, exudates, heal faster and patients will have less pain than those whose wounds were cleaned with Povidine Iodine as a wound care agent.

2.3 Research Design:

The current study used a randomized clinical trial to investigate the effectiveness of Hypochlorous acid compared to Povidine Iodine as a wash therapy in treating acute infected traumatic wounds.

2.4 Setting:

This study was carried out in trauma unit at Assiut university hospital

2.5 Subjects:

Simple random sample of 60 patients who were admitted in trauma unit at Assiut University Hospital between May and December 2012,and willing to participate in the study were recruited. They were randomly assigned into two equal groups, study and control group, (30 patients each).

2.5.1 Inclusion criteria

The patients had been selected according to the following criteria:

1. Age more than 15 years and less than 45 years
2. Both sex
3. Conscious and alert
4. Acute traumatic wound

2.6 Tools of data collection

2.6.1 Tool 1. An interview questionnaire to illicit information about: patient's age, gender, occupation, size, site and condition of the trauma, medical data as laboratory investigation...

2.6.2 Tool II: **Open wound Assessment using an observation chart:**

It was developed by the researcher for initial wound assessment such as wound bed, surrounding skin exudates, color, exudates' amount, and odor, frequency of wound pain, and condition of the dressing.

2.6.3 Tool III: Evaluation sheet:

2.6.3.1 Part one: It was developed by the researcher for evaluating signs and symptoms of infection such as pus, pyrexia, level and frequency of pain, color, amount and odor of exudates. It was used at the initial assessment of the wound, at every dressing, at the 7^{th} day and at the end of study (14^{th} day).

2.6.3.2 Part Two:. Physiological Measurement was assessed using a sterile swab that was pressed laterally on the wound to express underlying tissue fluid and exudates.It was taken for semi -quantitative microbiology at the start and the end of the treatment regimens.

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3. Methods

3.1 Administrative approval: An official was forwarded from the dean of the Faculty of Nursing, Assiut University explaining the aim of the study, and requesting a permission to conduct the study. A written approval was obtained from the director of trauma department to carry out the study. The study was approved by an institutional ethics committee.

3.2 Tools development: The study tools were developed by the researchers after extensive review of the relevant literature.

3.4 Validity: This tool was tested for content validity by five experts in the field of nursing and surgical specialists. Modifications were done accordingly then the tool was designed in its final format.

3.5 Ethical consideration: Consent: The study was approved by an institutional ethics committee. Informed consent was obtained from patients to participate in the study. The researchers initially introduced themselves to all potential subjects and they were assured that the collected data were absolutely confidential. They were informed that participation is voluntary and they can withdraw at any time of the study.

3.6 Pilot study: A pilot study was conducted before starting data collection on (3) patients who was included in the sample to test the clarity, and applicability of the tool and to estimate the time required to fill the sheet. Modifications were done as needed.

4. Data collection:

The data collection was done in the following phases:

4.1 Assessment phase:

The researcher interviewed the patients individually and gets their oral consent to participate and they answered the questions in the interview questionnaire. Initial assessment of the wound condition was done and recorded.

4.2 Implementation phase:

Hypochlorous acid was used as an antiseptic wash solution for the study group, while Povidine iodine was used for the control group. A standardized sheet recording patient's details included, age, sex, location of lesion, clinical history, and wound assessment, special investigations, clinical response were recorded and reported. Also, the wound condition was recorded by a serial of photographs and special investigations before, during, and after completion of the treatment.

Pain: All wounds were assessed by the researcher daily in accordance with standard of care. At each visit, the degree of pain was assessed using the developed questionnaire, patients were asked to report their pain as it happens in the following forms: none, only during dressing, intermittent, or continuous

Exudates: A sterile swab was taken from the exudates for quantitative microbiology at the start, 7th, and 14th day of the treatment regimens, odor was assessed and recorded as none, only when dressing was removed, or before and after dressing was removed. The nature and amount of exudates were also assessed and recorded as high, when the dressing was soaked with discharge and the patient need twice daily dressing, moderate, when the patient needed once daily dressing , or low exudates, when dressing was changed every other day.

Color: Wound bed was assessed as healthy (red in color and no dead tissue) or non-healthy (white or black color and there is necrotic tissue) granulation tissue . Routine blood samples were taken on admission, 7th, 14th days for a biochemical screen and full blood count

Pyrexia: Systemic antibacterial chemotherapy was routinely restricted to the patients when their temperature more than 38 C and depended on the culture and sensitivity results.

4.3 Procedure: Patients received either conventional therapy (control group) or treatment of the wound with Hypochlorous Acid (study group).

The study group: remove any old dressing from the wound, then gently scrub the wound by using sponge soaked with normal saline (Nacl 0.9%) to remove any debris and excessive wound drainage and, then irrigate the wound with Hypochlorous acid in a concentration by adding Nacl 0.5% to HCL 51.5% at ratio 9 :1 and for 3-5 minutes.

Control group: The same procedure was done for control group except, use of Povidine iodine instead of Hypochlorous acid as a wash therapy.

Changing dressing was done daily, once daily, or once every other day according to the amount of exudates for both groups.

4.4 Follow up: The follow-up of the two groups was done in trauma unit, during each assessment, standard parameters of wound pain, odor level, and color and amount of exudates were evaluated. After clinical improvement of the wound, the target lesion was operatively reconstructed by flap or graft.

5.Data Analysis

The data obtained were reviewed, prepared for computer entry, coded, analyzed and tabulated. Descriptive statistics (i.e., frequencies, percentage, mean standard deviation, etc) was done using computer program SPSS version (17). Chi-square and T-test, test used to compare differences in the distribution of frequencies among different groups.

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6. Results

6.1(Table 1). Illustrated that, this study was performed on 60 patients with traumatic septic wound divided equally to control and study group, their mean ages ranged between 32.47±10 and 32.17±11.2 respectively. There were 47 men and 13 women. Most of the wounds in both groups were at the lower limb.

Wound was ready for surgical reconstruction in 90% of the study group (using Hypochlorous acid HOCl) compared to none in control group within 2 weeks, and the rest of the study group (10 %) were ready for coverage within 3 weeks compared to 6.67% in the control group. However, the majorities of the control group (93.33%) was slow in healing and were ready after more than 4 weeks.

7.2(Table 2) and (figure 8). Showed that, at the beginning of the study, 63% and 67% of the study group and control group successively, complained from continuous pain. However, the pain was decreased among the entire study group after 7 days compared to 33.3% and 20% successively, after 7 days and even after 14 days. The differences were statistically significant (P value 0.004 Fig 8.)

Regarding the wound odor, Table 2 showed that 87% of the study and control group at the beginning of the assessment had offensive odor that the researcher smelled before and after dressing was removed. As for the study group who were treated with HOCl, the odor was reduced to nil within 7 days compared to 70% , 50% of the control group who still had odor at the 7^{th} and 14^{th} day's dressing successively, (P value 0.001)

Concerning exudates, the table illustrated that, at the beginning of the study, the majority of the patients had purulent exudates (80%, 83%) among the study and control group. However, at the 7^{th} day none of the study group had purulent exudates compared to 57% at the 7^{th} day and 50% at the 14^{th} day of the control group. Sanguineous exudates were observed among 97% of study group compared to 10% of the control group. Also, all wounds of the study group were Serous at the 14^{th} day compared to only 10% of the control group. The differences between the study group and control group were statistically significant. (P value 0.004)

There was a significant difference between control and study groups in the volume of exudates where, the amount of exudates was high, among 70% of wounds in both groups at the start of this research. This percentage had changed to zero among the study group compared to 57%, 36.7% for the control group at the 7^{th} and 14^{th} days respectively. The differences were statistically significant (p value 0.005)

There were 5 different types of microorganisms in this study according to the culture results Klebsella, Proteus, NIF, MRSA, and Psydomonus. There were a statistically significance decrease in quantitative reduction of micro-organisms between using Hypochlorous acid and Povidine iodine as a wash therapy in treating infected traumatic wounds (P value 0.0001, Fig 9)

7. Discussion

Despite a growing enthusiasm in the antiseptic products in the last few years, in the field of treatment of infected wounds, there is no universal agreement on what product is the best to wash infected traumatic wounds. Therefore the current study investigated whether washing infected wounds with HOCl would bring an improvement in patients with infected traumatic wounds more than the conventional method of using to Povidine iodine. Infection of traumatic wounds by MRSA, Pseudomonas, Proteus, NIF, and Klebsella is problematic because these pathogens can form a biofilm that can often go unchecked, leading to an unrecognized infection. This infection compromise normal wound healing and becomes a major obstacle for wound closure (34, 35, 36, and 37). Controlling the bacterial bioburden in traumatic wounds has been very difficult. If the physician cannot control the infection in these traumatic wounds, the patient may become further compromised by additional devastating tissue damage, bacteremia, sepsis or deeper wound infections. Topical antiseptics have been the method of choice, and have been used in preference to topical antibiotics because of concerns about the development of bacterial resistance. but still do not fully provide effective alternatives or significant improvement due to, the cytotoxic effects of these agents on the host, dermal and epidermal cells that may affect the wound healing process (38).

The search for a safe and effective topical antiseptic remains the biggest challenge. Povidine iodine is the most commonly used antiseptic dressing of traumatic wounds but the use of it has decreased due to its cytotoxic effect to cells that essential for wound repair, and due to tissue damage of fibroblasts in the wounds, which are required for healing and epithelization (21,37,38,39). However, there have been few controlled studies of the efficacy of the Povidine iodine and other antiseptics, such as ionized silver, alcohol, acetic acid, or hydrogen peroxide (38,39). It is reported that HOCl can kill bacteria without cytotoxic effect to human cells (40,41,42,43,44). So it could be an alternative to Povidine iodine , as it has shown antimicrobial efficacy, against many pathogens and even against antibiotic-resistant bacteria, such as MRSA without inducing toxicity (38,39,44).The result of this study supports the previous studies that, Hypochlorous acid is highly effective against a broad range of microorganisms including MRSA, Pseudomonas, Proteus, NIF, and Klebsella compare to use Povidine iodine as a washing therapy in treating infected traumatic wounds and this is evidenced by no further necrosis, rapid formation of healthy granulation tissue and by quantitative microbacterial results. Randell (2012), reported that

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HOCl is a key agent in promoting healing for a wide variety of wound types (45).The present study revealed that HOCL is highly effective in treating wound infection, and this enables the body's natural healing process. Also, the findings f the current study proved that the traumatic wounds are surgically closed sooner after using of HOCl as a washing therapy when compared to Povidine iodine.

A tender painful wound is an adverse effect of bacterial infection (34,35). Selkon et al.,(2006) & Randell (2012) ,supported the study findings where pain has decreased and totally disappeared after 14 days of using HOCL compared to the control group who were using Povidine iodine(44,45).. Pain was, present in all patients upon starting the study, completely ceased to exist in all patients of Hypochlorous acid group compare to 16.6% of the control group, and also the researcher observed that HOCl has been well tolerated throughout the course of therapy. These findings contribute to HOCl which has an excellent effect in reducing pain of the wound

As for the wound odor, the findings from the present study support a previously accepted hypothesis that, a significant odor was completely eliminated in all patients of HOCl group, even in those who were not ready for coverage completely, compared to patients of control group at the end of study. Similarly, Selkon et al.,(2006) reported that HOCl breaks the structural bonds by reacting with proteins, fatty acids, and DNA and this leads to soften the wound surface eschar, necrotic tissue, and biofilm on a painful granulation tissue. (44). And this concomitant with the current result where, the researcher observed that HOCl softens, cleans, and removes the necrotic tissue and biofilm from acute infected traumatic wounds at the end of this study.

As regard exudates amount, the current findings showed that about three quarters of wounds were infected with high amount of exudates at the beginning of the study which dropped to nil after 14 days of using HOCL among the study group, compared to 36.7% among the control group. This finding was supported by McDonnell G, Russell AD, (1999) and Ovington (2004). Who reported that a clean wound should show signs of healing and improvement within two to four weeks (46,47). Likewise, Selkon and Cameron (2001) reported that Hypochlorous acid reduce the bacterial flora of the ulcers after the two days (48).

Concerning exudates color, the results of the wound observation in the current study revealed that the majority of the wounds in the study group and control group were purulent at the initial assessment of the wound. This color improved to be Serous among almost all the study group after 7 days and among all of them after 14 days, compared to more than half of the control group after 7 days and half of them after 14 days. This finding means that there was marked quantitative reduction of bacteria when using Hypochlorous acid compared to Povidine iodine. This finding was supported by McDonnell G, Russell AD, (1999) and Ovington (2004).

8. Conclusions

Hypochlorous acid (HOCl) is an effective; easy to perform, comfortable, inexpensive and safe in cleansing (treating) infected acute traumatic wounds and allows for earlier surgical closure and hospital discharge. HOCl controls the tissue bacterial bioburden without inhibiting the wound healing process rapidly relieves pain and the area becomes well prepared for skin flap or graft. The photographs were an evidence to support the effectiveness of HOCL in accelerating wound healing process.

9. Recommendations:

Further studies to confirm the effect of HOCl with larger number of patients is needed.

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Table (1): Subject characteristic of socio-demographic characteristics for control and study groups

Variable	Group			
	Control (n=30)		Study (n=30)	
	No.	%	No.	%
1. Age (years):				
▪ 15-25	10	33.33	10	33.3
▪ 26 – 35	11	36.67	10	33.3
▪ 36-45	9	30	10	33.3
Mean + SD	32.47 +10.44		32.17+11.27	
2. Gender:				
▪ Male	24	80.00	23	76.67
▪ Female	6	20.00	7	23.33
3. Wound site:				
▪ Upper limb	1	3.33	4	13.33
▪ Lower limb	25	83.33	18	60.00
▪ Sacral	3	10.00	4	13.33
▪ Abdomen	1	3.33	4	13.33
4.Wound ready for flab or graft (healthy granulation tissue) after				
▪ 2 wks	0	0	27	90
▪ 3 wks	2	6.66	3	10
▪ >4 wks	28	93.33	0	0
4. HGB: **Mean + SD**	10.13+1.67		9.23+1.77	
5. PT **Mean + SD**	13.01+0.55		12.52+0.82	

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Table (2): Frequency and percentage distribution of the sample according to wound assessment among control and study groups

Wound assessment	Control group(n=30)						Study group(n=30)						P.value
	0 day		7th day		14th day		0 day		7th day		14th day		
	No	%	no	%	no	%	No	%	No	%	No	%	
Exudate (colour)													
- Serous	0	0	0	0	3	10	0	0	1	3	30	100	0.004
- Serosanguinous	0	0	3	10	1	3	1	3	29	97	0	0	**
- Sanguinous	5	17	10	33	11	37	5	17	0	0	0	0	
purulent	25	83	17	57	15	50	24	80	0	0	0	0	
Exudate (amount)													
- low	0	0	1	3	9	30	3	10	27	90	30	100	0.005
- moderate	9	30	12	40	10	33.3	6	20	3	10	0	0	**
- high	21	70	17	57	11	36.7	21	70	0	0	0	0	
Odour:													
• none	0	0	0	0	4	13.3	0	0	25	83	30	100	0.001
• -only when dressing removed	4	13	9	30	11	36.6	4	13	5	17	0	0	**
• (before and after dressing removed)	26	87	21	70	15	50	26	87	0	0	0	0	
Wound pain (frequency)													
- None	0	0	3	10	5	16.6	0	0	20	66.6	30	100	0.004
- only at dressing	3	10	7	23.3	7	23.3	5	17	5	16.6	0	0	**
- Intermittent	7	23	10	33.3	12	40	6	20	5	16.6	0	0	
- continuous	20	67	10	33.3	6	20	19	63	0	0	0	0	

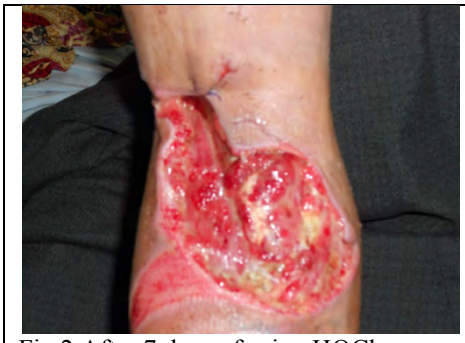

Fig 2.After 7 days of using HOCl

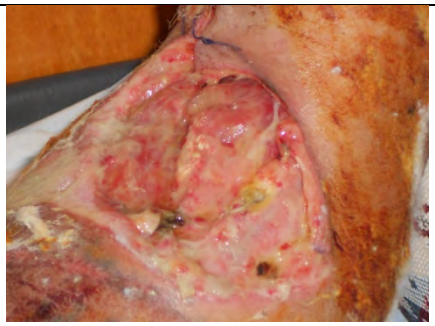

Fig 1 Male patient has unhealthy granulation tissue at Lt.cubital fossa at the start of study.

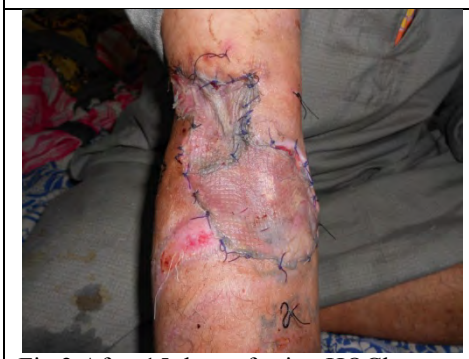

Fig 2.After 15 days of using HOCl

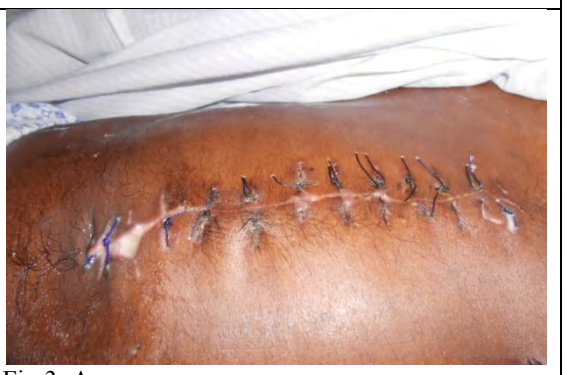

Fig 3 A

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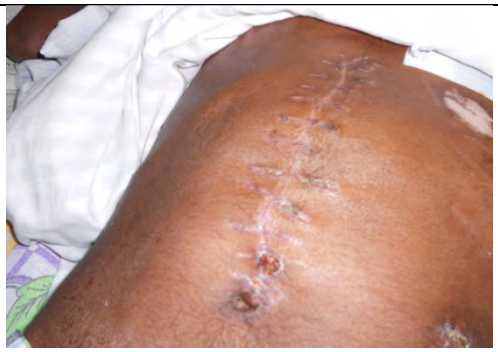


Fig 3 B

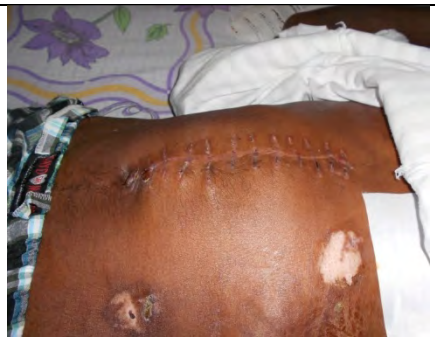


Fig 3 C

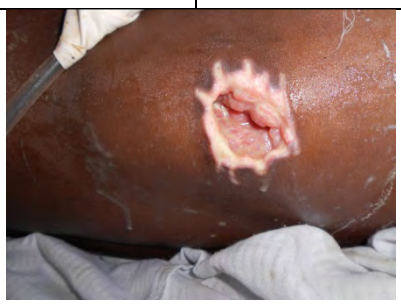


Fig 3 D

Fig 3.A, B, C, & D Male patient 35 years old, show infected traumatic wound at the start of the study

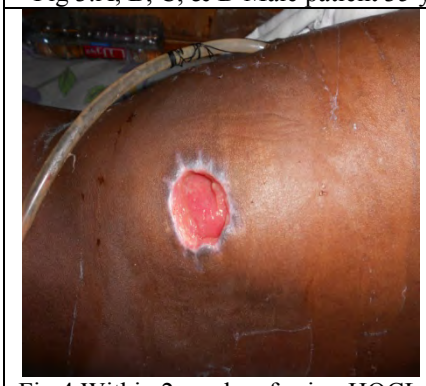
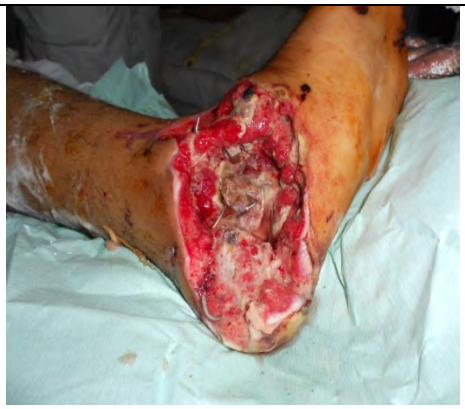
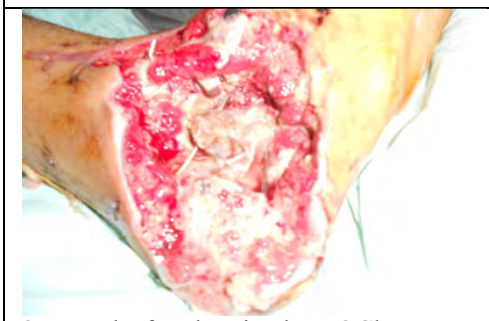


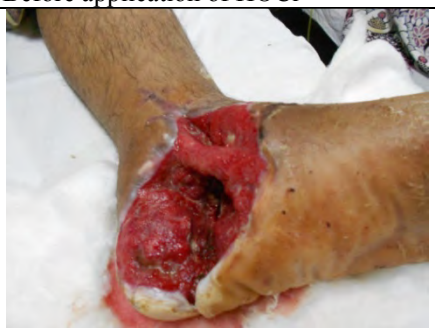
Fig 4.Within 2 weeks of using HOCL



Before application of HOCl



One week after dressing by HOCl



2 weeks after dressing by HOC

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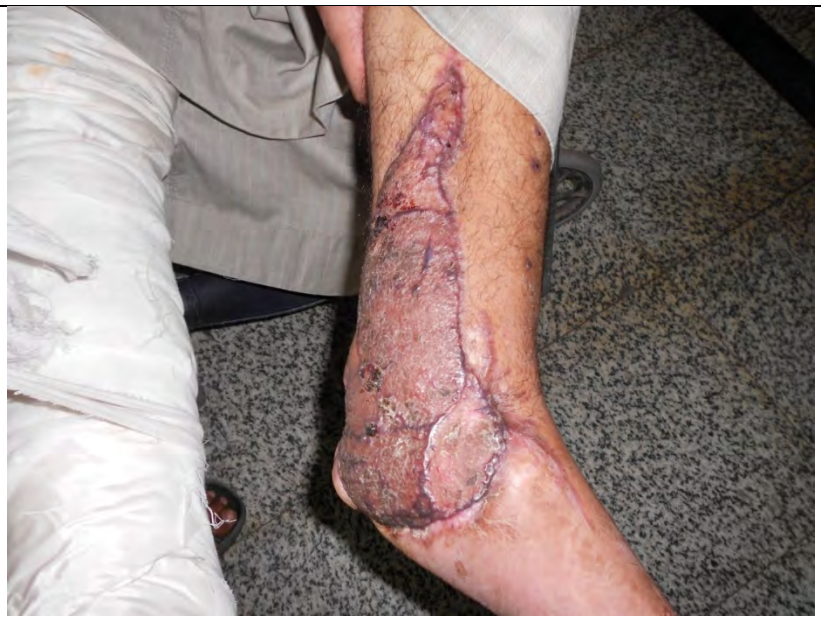

After three weeks,patient flaping

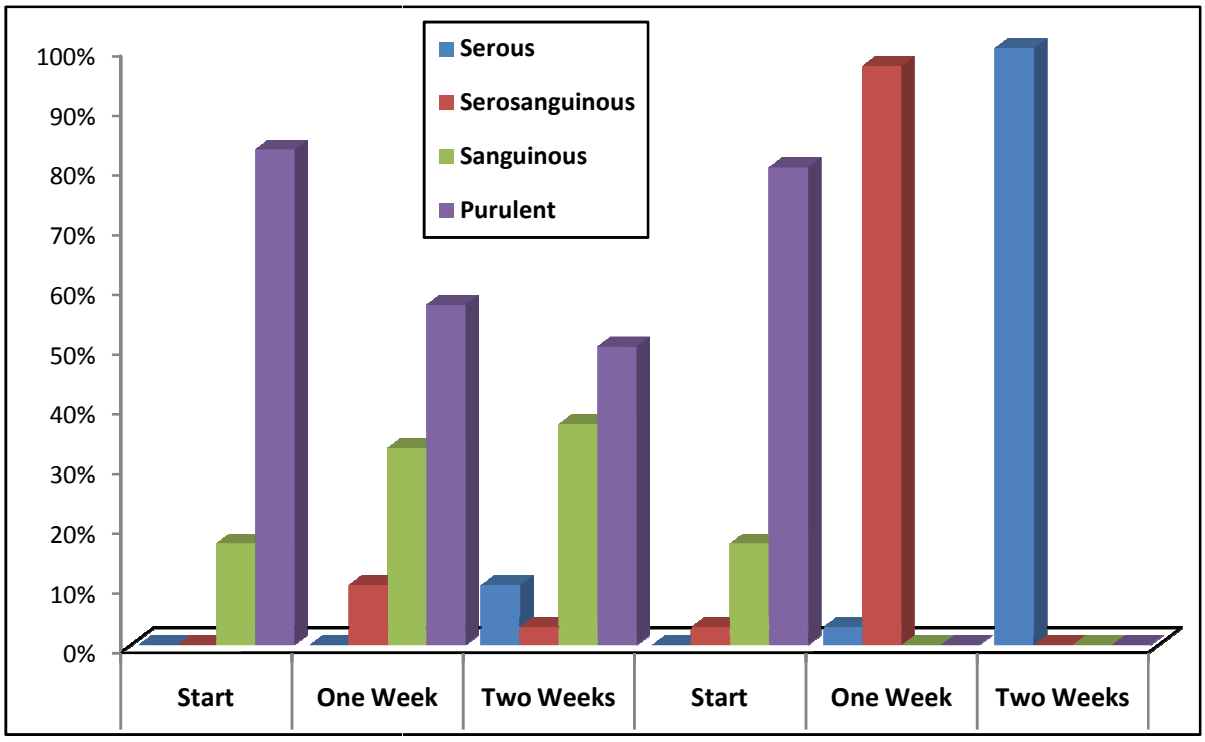

Fig. 5: Distribution of the sample according to the Colour of Exudates

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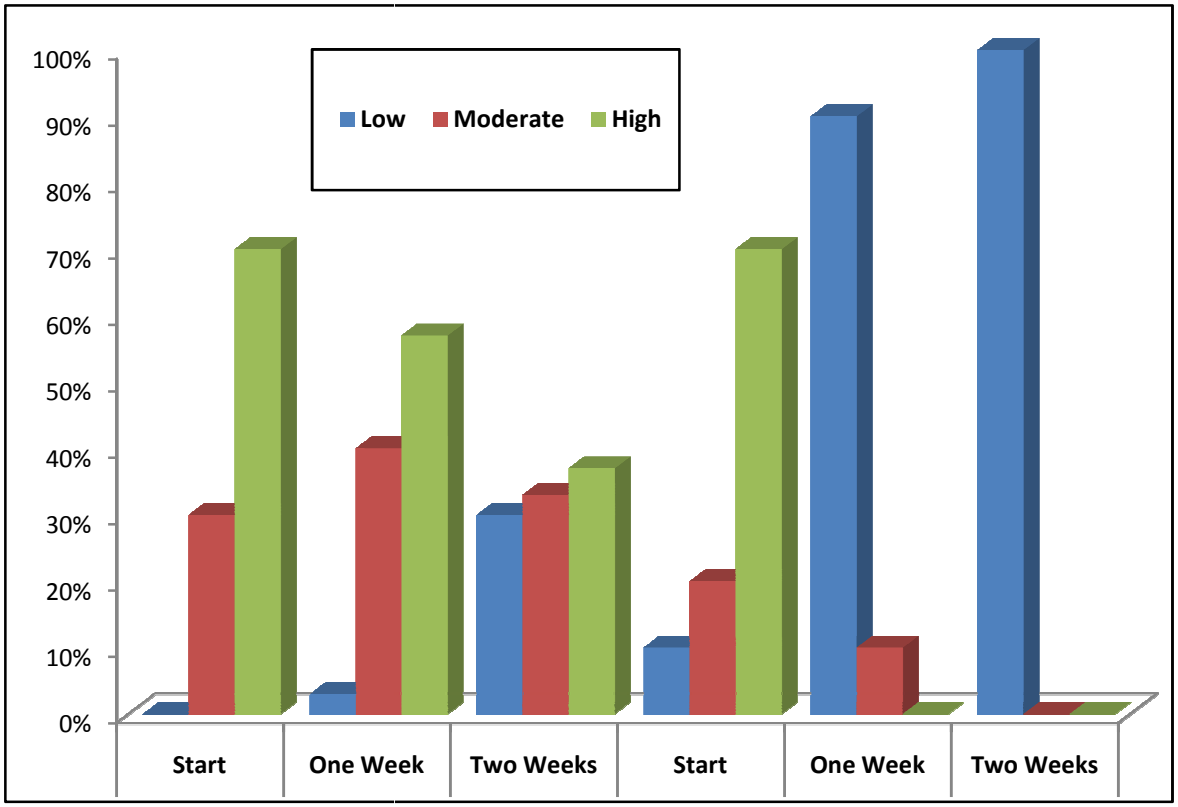

Fig. 6: Distribution of the sample according to the Amount of Exudates

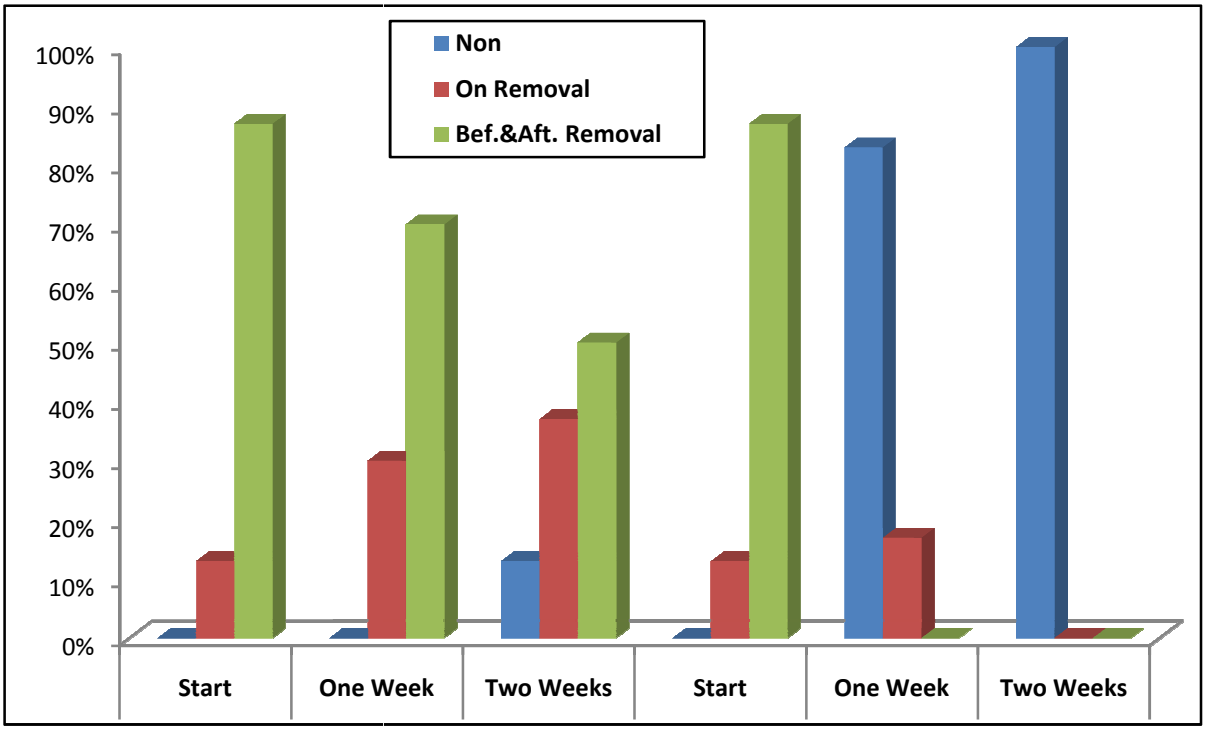

Fig. 7: Distribution of the sample according to Wound Odour

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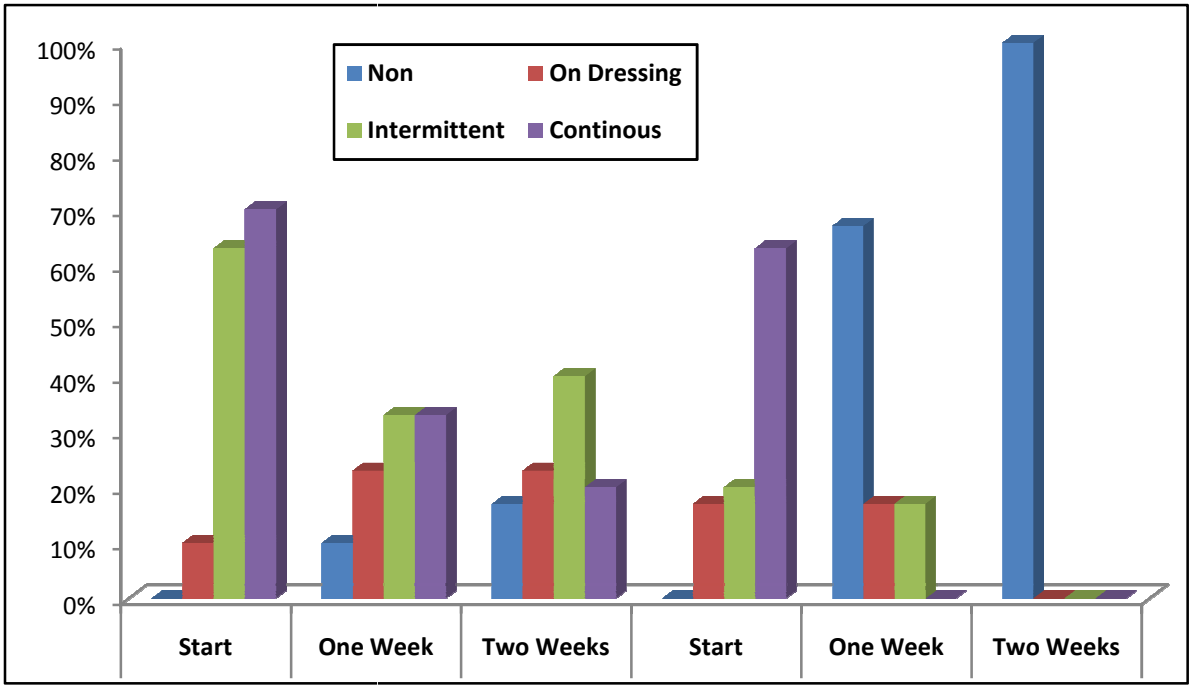

Fig. 8: Distribution of the sample according to Wound Pain

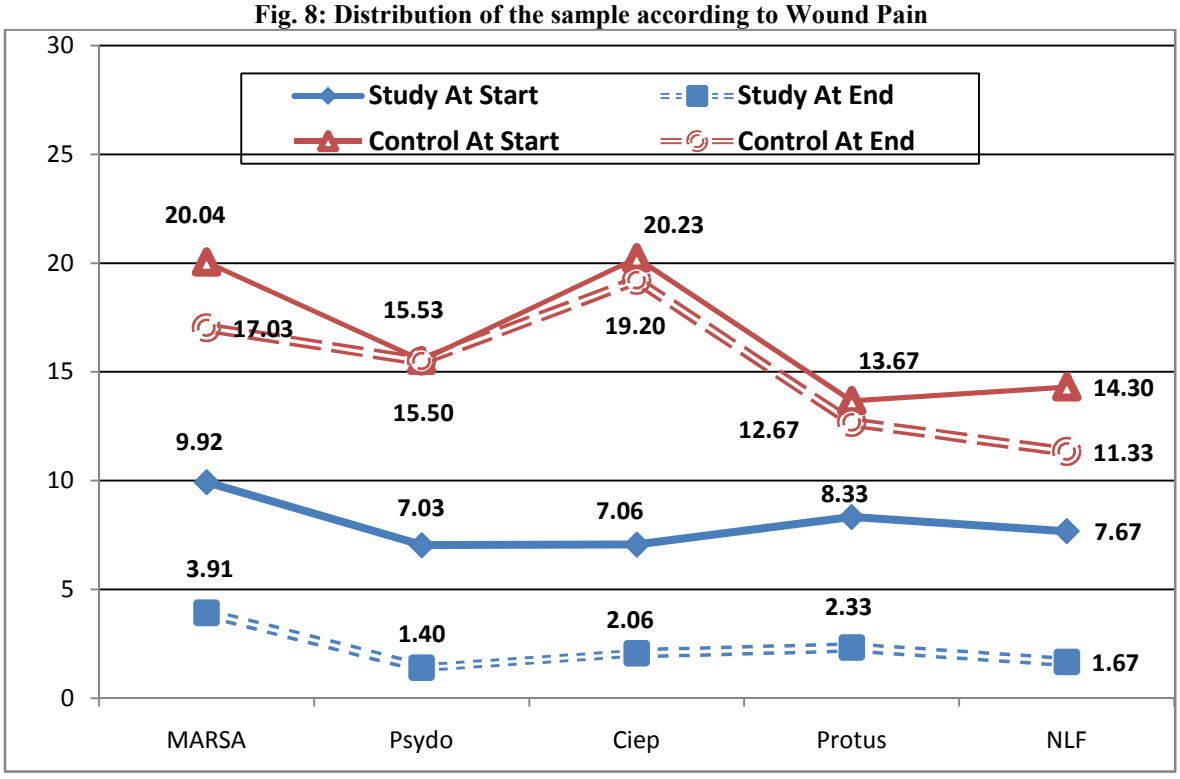

Fig. 9: Microorganism means scores in both groups at the start and end of the study.

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Appendix V: Literature Research about Hypochlorous Acid

I) Use of Hypochlorous Acid Solution as a Disinfectant in Laboratory Animal Facilities.

SciMedCentral

Annals of Clinical and Medical Microbiology

Review Article

Use of Hypochlorous Acid Solution as a Disinfectant in Laboratory Animal Facilities

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Keywords

- Microbiota
- *Pseudomonas aeruginosa*
- Rat
- Weak acid Hypochlorous Solution

Abstract

Hypochlorous acid solution was effective not only in preventing contamination of pathogens including opportunistic pathogens, but also in preventing infection without affecting serum biochemical variables. But bacterial microbiota may have changed due to drinking these solutions.

INTRODUCTION

Recently, hypochlorous acid solution, a weak acid, has been used in a wide variety of settings, including agricultural fields, hospitals, food industry, day care centers, and animal facilities [1]. In this review, we introduce the use of weak hypochlorous acid solutions as disinfectants in laboratory animal facilities.

Hypochlorous acid solution is generated by the electrolysis of a sodium chloride solution. At the positive electrode, the anode, water (H_2O) is transformed into oxygen (O_2) and hydrogen ions (H^+), and chlorite ions (Cl^-) are transformed into chlorine (Cl_2). Chlorine then reacts with water to produce hypochlorous acid (HClO) and hydrochloride (HCl) [2]. Weak acid hypochlorous solution is a chlorine-based disinfectant that is produced by mixing NaClO and HCl in water and adjusting it to a weak acidity of approximately pH 6 [3]. The most effective form of chlorine in weak acid hypochlorous solution is HClO. HClO has been reported to be effective against various microorganisms [4]. Ono *et al.* demonstrated *in vitro* that when *Pseudomonas aeruginosa*, *Acinetobacterbaumannii*, *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *Enterococcus faecium,* and *Enterococcus avium* are treated with HClO solutions in the pH range of 5–8, within 15 seconds, the organisms are not detected in the cultures. In addition to these pathogens, mouse hepatitis virus, Sendai virus, lymphocytic choriomeningitis virus, *Bordetellabronchiseptica*, *Pasteurellapneumotropica*, and *Corynebacteriumkutscheri* could not be detected after treatment with 9 or 99 volumes of weak acid hypochlorous solution for 5 minutes at 25˚C. They concluded that the solution has inactivation activity against laboratory rodent-specific viruses and bacteria when used in a sufficiently large volume or for a longer reaction time [3].

In contrast, the efficacy of HClO in solution is decreased, possibly owing to contact with organic materials in the stomach and intestines. Accordingly, when added to the drinking water provided to the animals, it has not been effective in eliminating pathogens [5]. As shown in Figure 1, when 10 ppm or 5 ppm of weak acid hypochlorous solution was incorporated into the drinking water provided to *P. aeruginosa* infected rats for 32 days, the number of the bacteria in feces was significantly decreased. The infection could not, however, be completely eradicated. Takimoto *et al.* also suggested that incorporating the solution into the drinking water provided to animals is ineffective in preventing mouse norovirus infection and in eliminating mouse norovirus from already infected mice [6].

In an attempt to prevent *P. aeruginosa* infection in rats by incorporating hypochlorous solution into the drinking water, it was observed that the bacteria were not detected in feces from the 6 sentinel rats exposed to infected rats in a group that had received the hypochlorous solution up until 49 days of cohabitation [7]. These results suggest that the solution had sufficient preventative activity against *P. aeruginosa* infection.

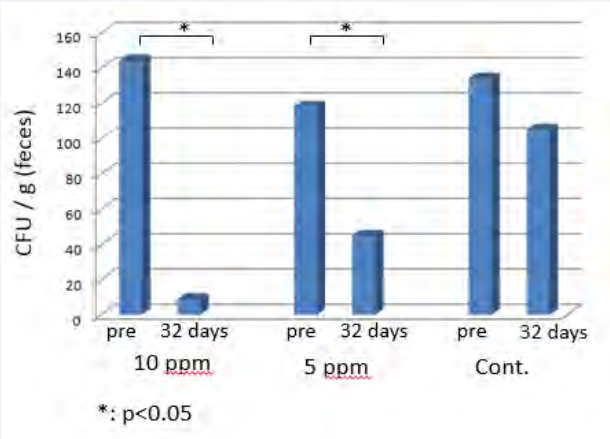

Figure 1 Number of *P. aeruginosa* in rat feces when 10 ppm or 5 ppm of weak acid hypochlorous solution or tap water was provided.

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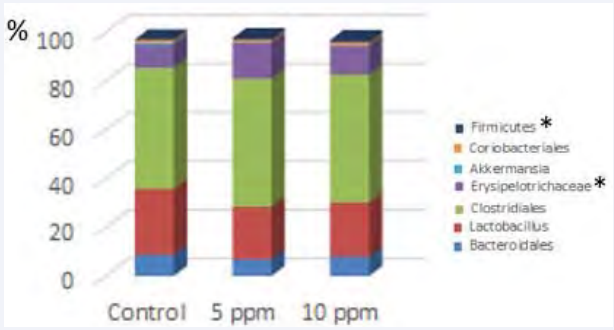

Figure 2 Microbiota of rats was analyzed using polymerase chain reaction amplification and terminal restriction fragment length polymorphism (T-RFLP) analysis. Ratio of T-RFLP peaks for *Erysipelotrichaceae* in rats who received 5 ppm solution was higher (X = 14.67, SD = 0.61) than that of the control group (X = 9.29, SD = 2.01; $p < 0.01$). For *Firmicutes* spp., the ratio of T-RFLP peaks for rats who received 10 ppm solution (X = 0.99, SD = 0.46) was higher than that of the control group (X = 0.18, SD = 0.36; $p < 0.01$). Results for other bacteria were similar across all groups.

The hypochlorous acid solution is an effective tool for cleaning animal facilities but cannot completely eliminate pathogens from infected animals directly. The solution can, however, be used to prevent some bacterial infections such as those caused by *P. aeruginosa*. In these cases, hypochlorous acid solution can be incorporated into the drinking water; however, this may affect the cecum microbiota.

The effect of the solution on cecum microbiota in these rats is shown in Figure 2. The ratio of the terminal restriction fragment length polymorphism (T-RFLP) peaks did not differ across rats administered with 5 or 10 ppm solution as compared to that of the control group for any bacteria except *Erysipelotrichaceae* and *Firmicutes*.

CONFLICT OF INTEREST

These results suggest that the solution had sufficient preventative activity against P. aeruginosa infection when the solution was used as drinking water, however bacterial microbiota may have changed due to weak acid hypochlorous solution administration.

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Appendix V: Literature Research about Hypochlorous Acid

m) Living with a killer: the effects of hypochlorous acid on mammalian cells.

IUBMB *Life*, 50: 259–266, 2000

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Critical Review

Living with a Killer: The Effects of Hypochlorous Acid on Mammalian Cells

Juliet M. Pullar, Margret C. M. Vissers, and Christine C. Winterbourn
Free Radical Research Group, Pathology Department, Christchurch School of Medicine, PO Box 4345, Christchurch, New Zealand

Summary

The production of hypochlorous acid (HOCl) by the myeloperoxidase-H_2O_2-Cl^- system of phagocytes plays a vital role in the ability of these cells to kill a wide range of pathogens. However, the generation of a potent oxidant is not without risk to the host, and there is evidence that HOCl contributes to the tissue injury associated with inflammation. In this review, we discuss the biological reactivity of HOCl, and detail what is known of how it interacts with mammalian cells. The outcome of exposure is dependent on the dose of oxidant, with higher doses causing necrosis, and apoptosis or growth arrest occurring with lower amounts. Glutathione (GSH) and protein thiols are easily oxidized, and are preferred targets with low, sublethal amounts of HOCl. Thiol enzymes vary in their sensitivity to HOCl, with glyceraldehyde-3-phosphate dehydrogenase being most susceptible. Indeed, loss of activity occurred before GSH oxidation. The products of these reactions and the ability of cells to regenerate oxidized thiols are discussed. Recent reports have indicated that HOCl can activate cell signaling pathways, and these studies may provide important information on the role of this oxidant in inflammation.

IUBMB *Life*, 50: 259–266, 2000

Keywords Glutathione, hypochlorous acid, oxidative stress, thiols.

INTRODUCTION

Phagocytic white blood cells, particularly neutrophils, are a major source of oxidants in mammalian systems. These cells ingest and destroy invading pathogens, and are the predominant cell present in the early stages of acute inflammation. Phagocytosis of bacteria is accompanied by the activation of an NADPH oxidase complex in the cell membrane, resulting in generation of superoxide (O_2^-) and the release of cytoplasmic granule proteins into the phagosome [reviewed in (*1*)]. One of the major granule proteins is the enzyme myeloperoxidase, a heme protein that accounts for 5% of the total neutrophil protein. The combination of O_2^- production and myeloperoxidase release gives neutrophils a broad and unique oxidative potential (Fig. 1). Hydrogen peroxide (H_2O_2), generated by dismutation of O_2^-, is utilized by myeloperoxidase to oxidize halides to generate hypohalous acids. At physiological concentrations chloride is the most likely substrate, and hypochlorous acid (HOCl) has been shown to be a major end-product of the neutrophil respiratory burst (*2–5*).

The powerful antimicrobial nature of HOCl and its conjugate base, hypochlorite (OCl^-) has been well documented (*6–8*). It is the active ingredient in household bleach and the species responsible for the microbicidal properties of chlorinated water supplies. The production of HOCl by neutrophils is an integral part of the ability of these cells to kill a wide range of pathogens (*1*). However, the properties that make it such a useful antimicrobial agent also place the host at considerable risk, because HOCl has the potential to damage host tissue through the same processes used in the destruction of invading microorganisms. Neutrophil oxidants have been implicated in the tissue injury associated with inflammatory diseases, including respiratory distress, ischemia-reperfusion injury, acute vasculitis, arthritis, and glomerulonephritis (*9*). Many in vitro studies have shown that HOCl is able to mediate tissue injury (*1*, *10–12*), and recent work has provided direct evidence for the production of HOCl in various pathological disease states. Using an antibody raised against HOCl-modified protein, the generation of HOCl in vivo has been demonstrated in diseased kidney tissue and in human atherosclerotic plaques (*13*, *14*). This material was recently found to colocalize with myeloperoxidase in atherosclerotic lesions (*15*). Elevated levels of 3-chlorotyrosine, a marker of HOCl generation, have also been demonstrated in LDL molecules from human atherosclerotic intima (*16*).

Taken together, these studies confirm that HOCl is released from phagocytic cells in vivo and suggest that HOCl produced by neutrophils and other phagocytic cells could form a significant

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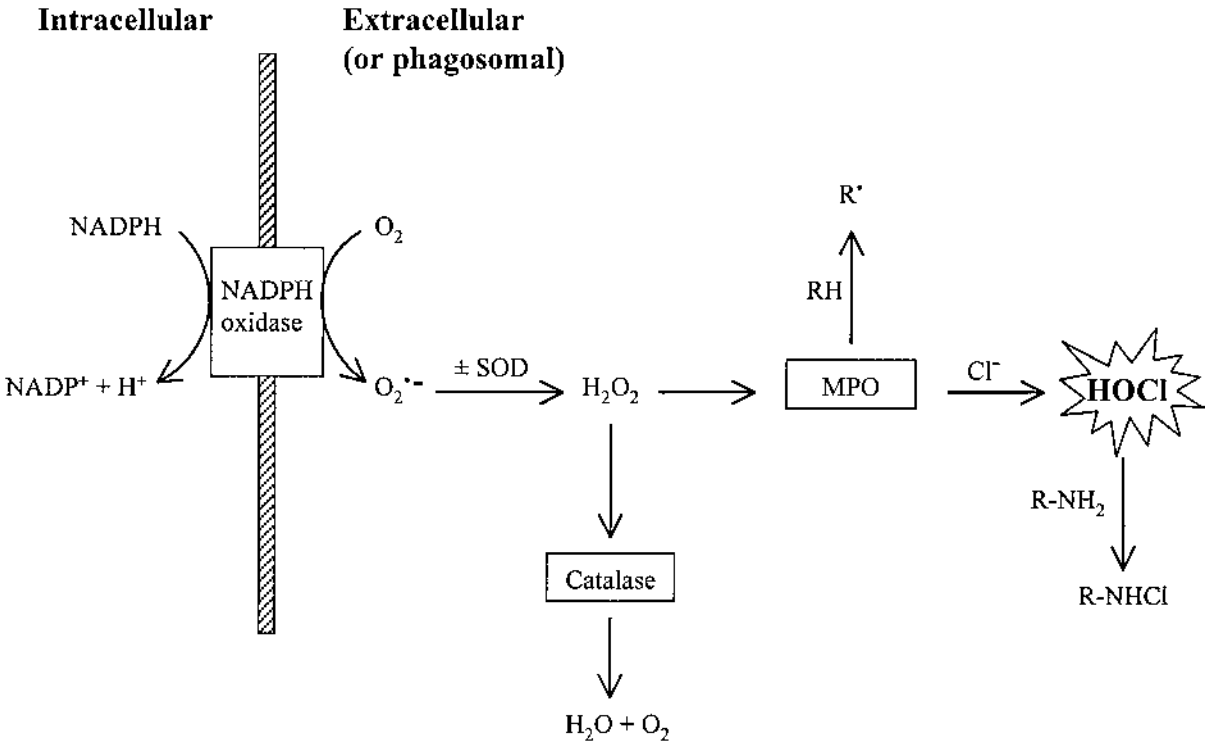

Figure 1. Neutrophil oxidative products.

oxidative stress on host cells and tissues. In this paper, we present background information on the chemical reactivity of HOCl with biological compounds and review what is known about how this translates into the effects of HOCl in cells and tissues.

BIOLOGICAL REACTIONS OF HOCl

HOCl exists in equilibrium with OCl^- and chlorine gas (Cl_2). Because HOCl has a pK_a of 7.5, it is present as an equal mixture of HOCl and OCl^- physiologically, and Cl_2 is present in significant amounts only at much lower pH (*17*). The 'active' chlorine in these species is in a formal oxidation state of +1. It may act as either a one-electron or two-electron oxidizing agent, but reduction potentials favor two-electron oxidation (*18*). HOCl is a powerful oxidising agent that can react with many biological molecules. Thiol groups and thioethers such as methionine are the most readily oxidized, at a rate approximately 100 times that of amine groups (*19*). Rate constants for the reaction of HOCl with reduced glutathione (GSH) and ascorbate are $>10^7\ M^{-1}\ s^{-1}$ and $\sim 6 \times 10^6\ M^{-1}\ s^{-1}$ respectively (*20*). Heme groups and iron–sulfur centers also react readily (*21*).

HOCl can also halogenate cell constituents. The most favored chlorinating reaction of HOCl is with amines to form monochloramines and dichloramines.

$$RNH_2 + HOCl \rightarrow RNHCl + H_2O \quad \text{(Reaction 1)}$$
$$RNHCl + HOCl \rightarrow RNCl_2 + H_2O \quad \text{(Reaction 2)}$$

Chloramines, while longer-lived and less reactive than HOCl, retain the two oxidising equivalents. They can oxidize thiols, thioethers and heme proteins, and thus extend the reactivity of HOCl (*22*, *23*). Chloramines can also be toxic to cells and bacteria, the extent of which depends on their structure and their ability to cross the plasma membrane (*24*, *25*). Additionally, chloramines, particularly those of α-amino acids, are susceptible to breakdown to aldehydes, which themselves are cytotoxic (*26*).

HOCl can react with nucleotides and with DNA. It reacts rapidly with NADH and the NH-groups of pyrimidines, and slow denaturation of DNA has been observed (*27*, *28*). A recent study has demonstrated DNA double-strand breaks and the formation of modified nucleotides in an epithelial cell line exposed to HOCl (*29*).

Chlorination of unsaturated fatty acids and cholesterol to chlorohydrin derivatives has been demonstrated, which suggests that the lipid component of cell membranes could be susceptible to attack by HOCl. However this reaction is relatively slow, and chlorohydrins have only been detected in cells after exposure to cytotoxic concentrations of HOCl (*30–33*). Several studies have shown that HOCl does not cause lipid peroxidation (*34*, *35*).

Cell proteins are likely to be a major target for HOCl and varied products are possible. Cysteine and methionine residues are readily oxidized (*36*). The amino groups of lysine and the N-terminal amines react to form chloramines, and these can

undergo secondary reactions with thiols and thiol-containing proteins (*37*). Another possible consequence of chloramine formation is the generation of radicals that may result in protein fragmentation or lipid peroxidation (*38*, *39*). The formation of carbonyls in proteins exposed to HOCl can also occur via a chloramine intermediate (*40–42*). Irreversible protein crosslinks have been observed in cell membranes exposed to HOCl and are associated with cell lysis (*32*). Although the mechanism for crosslinking is unclear, this reaction is rapid and is seen with low doses of oxidant (*43*). Free tyrosine and the tyrosine residues of protein can also be chlorinated to form the mono- or dichloro derivatives (*44*). This reaction of HOCl is being successfully used as a marker of neutrophil activation in vivo. Other susceptible protein targets include tryptophan, histidine, arginine, and the amide peptide bond (*45*).

EFFECT OF HOCl ON CELL VIABILITY

Studies with stimulated neutrophils and the cell-free myeloperoxidase-H_2O_2-chloride system provided the initial evidence for the toxicity of HOCl to mammalian cells. Inhibition of cytotoxicity with myeloperoxidase inhibitors and by the HOCl scavenger methionine strongly implicated HOCl as the mediator of these reactions (*46–50*). Similarly, neutrophils lacking myeloperoxidase or the membrane oxidase complex were not cytotoxic to target cells unless myeloperoxidase or H_2O_2 levels were restored (*46*). Also, reagent HOCl can kill a wide range of cell types including red cells (*34*, *51*, *52*), endothelial cells (*24*), epithelial cells (*53*, *54*), fibroblasts (*55*), T-cell lines (*48*), and tumor cells (*35*). These studies have used membrane integrity and cell lysis as a measure of cell death and consequently provide evidence for necrotic cell death.

The cellular changes responsible for necrosis have not been fully elucidated. Work investigating the bactericidal effects of HOCl first emphasized the importance of protein components of the bacterial cell wall and cytoplasmic membrane as sensitive targets (*56*, *57*). The mammalian cell membrane is similarly susceptible. In tumor cells, low concentrations of HOCl caused disruption of various plasma membrane transport functions, a decline in cellular K^+ and an increase in cell volume. There was a concurrent loss of membrane thiol groups and a similar loss of membrane functions, caused by a thiol binding agent, led the authors to suggest that membrane thiol oxidation mediated the above changes (*35*). In red cells, membrane protein modification closely correlated with lysis, and the formation of pores due to protein crosslinking was proposed as the mechanism responsible for lysis (*32*).

The ability of HOCl to induce apoptotic cell death has recently been described (*58*). Treatment of cultured endothelial cells with intermediate concentrations of HOCl caused the cells to undergo apoptosis, while higher concentrations induced necrotic cell death (Fig. 2). Apoptosis was characterized by phosphatidylserine exposure, changes in nuclear morphology, DNA fragmentation and caspase activity. In HL-60 cells, apoptosis initiated by H_2O_2 was shown to be dependent on myeloperoxidase activity in the cells, and HOCl was implicated (*59*).

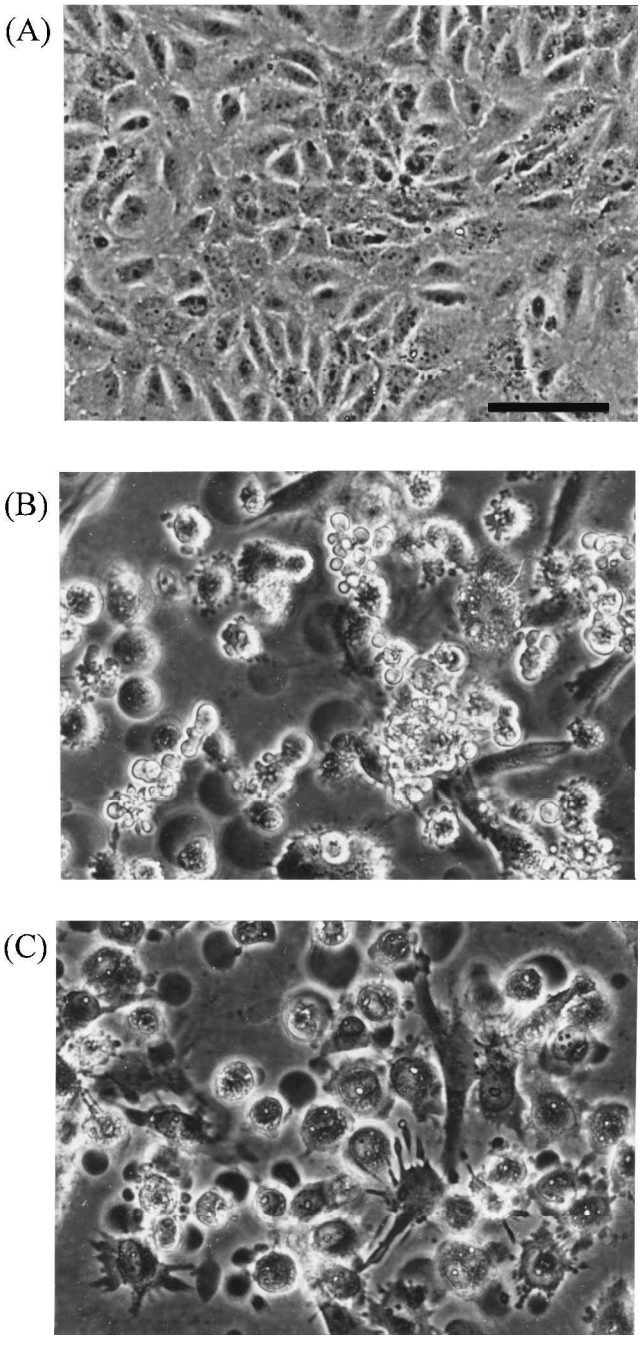

Figure 2. Morphological changes to human endothelial cells exposed to HOCl. (A) Control monolayer of endothelial cells. (B) After exposure to intermediate concentrations of HOCl, many cells detach and exhibit typical apoptotic blebbing. (C) With higher doses of oxidant, cell swelling and necrosis predominate. Bar represents 100 μm. Figure from Vissers, Pullar, and Hampton, 1999 (*58*)].

REACTION OF HOCl WITH GSH AND THIOL PROTEINS

The very fast reaction of HOCl with thiols suggests that these would be major cell targets. Several studies have investigated the relative susceptibilities of different cell constituents to HOCl, and have shown that GSH and protein thiols are preferred targets for oxidation (*60*, *61*). In red cells, when 80% of the GSH was reacted, only about 20% of membrane thiols were lost (*61*). Changes in cell parameters such as cell swelling and formation of membrane cross-links, were only observed once all the GSH was oxidized. Protein thiol loss was also demonstrated in neutrophils (*62*) and in a tumor cell line (*35*). With endothelial cells (*24*), about 30% of cellular GSH and 13% of total protein thiols were lost with a sublethal dose of HOCl. These studies helped establish that HOCl can penetrate the cell membrane and react with intracellular constituents. Previously, it had been thought that HOCl reacted almost exclusively at the membrane and that only derivative lipophilic chloramines reached the interior of the cell (*22*, *63*).

The effectiveness of antioxidant defenses against HOCl will depend on the products formed on reaction with thiols and whether the parent thiols can be regenerated. Several studies have suggested that higher oxidation states are produced (*19*, *27*). The reaction of HOCl with GSH is complex, and in vitro generates two novel products in addition to GSSG (*64*). One has been preliminarily identified as an internal sulfonamide of GSH (Fig. 3), and the other as a further oxidation product of GSSG, glutathione thiolsulfonate. Few studies have investigated the products of the reaction in cells. When red cells were exposed to HOCl the GSH was initially converted to GSSG and at low oxidant concentrations could be regenerated (*61*). In contrast, with neutrophils (*62*) and endothelial cells (Fig. 3 and J. M. Pullar, M. C. M. Vissers, and C. C. Winterbourn, submitted for publication), very little GSSG was formed and only a small fraction of the GSH loss was accounted for as protein mixed disulfides. The remaining GSH products were rapidly exported from the cells, and were detected in the extracellular medium. They appear to include the sulfonamide as well as other yet to be identified products. These findings have major implications for the maintenance of the cell's redox state. The sulfonamide could also serve as a useful marker of neutrophil oxidative injury in inflammation.

Protein thiols may vary in reactivity with HOCl depending on their accessibility and pK_a. In human endothelial cells susceptibility to inactivation by low amounts of HOCl varied between three thiol-containing enzymes (*60*). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was much more susceptible to oxidation than were creatine kinase or lactate dehydrogenase. It was also more readily oxidized than GSH (*60*). GAPDH has an essential cysteine at the active site (Cys 149) that is

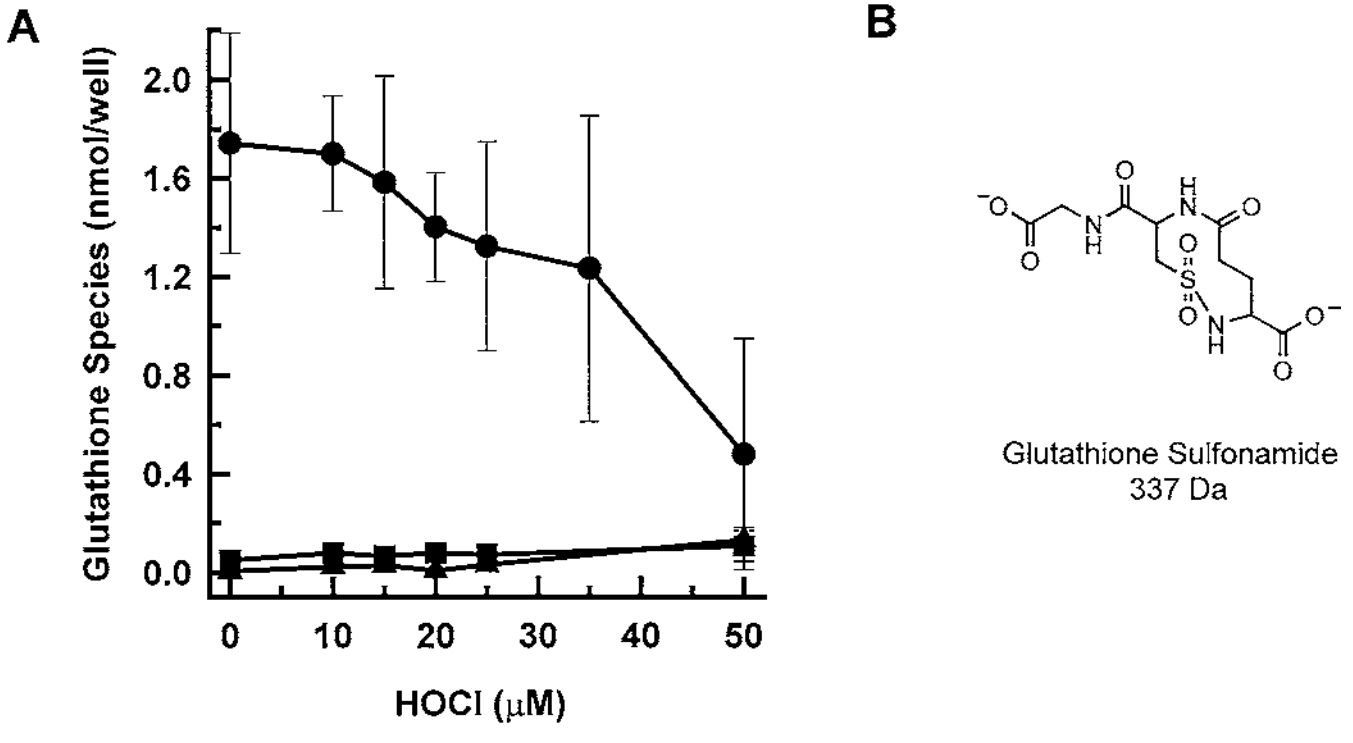

Figure 3. Loss of GSH and formation of products after exposure of endothelial cells to HOCl (A). Total intracellular and extracellular GSH (●), GSSG (■) and glutathione sulfonic acid (▲) were measured in endothelial cells 10 minutes after exposure to HOCl. As shown, there was minimal formation of GSSG or the sulfonic acid. (B) Proposed structure of glutathione sulfonamide, a novel product of the reaction of HOCl with GSH.

present as the thiolate anion at neutral pH and is particularly reactive (*65*). Thiol-labeling of tissue samples from patients with inflammatory bowel disease has provided strong evidence that GAPDH oxidation occurs at sites of inflammation and suggests that this is a particularly sensitive marker for oxidative stress (*66*). In endothelial cells, oxidation of GAPDH was reversible but only at lower concentrations of HOCl (*60*). Higher amounts of oxidant may convert the essential cysteine to a sulfonic acid or some other higher oxidation state that cannot be regenerated.

Isolated rat hearts perfused with HOCl showed impaired contractile function that occurred concurrently with protein thiol loss (*67*). Dithiothreitol could restore both function and protein thiol levels, suggesting that HOCl induced injury through the oxidation of protein thiol groups (*68*). In a follow-up study, the impaired contractile performance was associated with a decline in sarcoplasmic reticulum Ca^{2+}-ATPase activity, Ca^{2+} uptake, and protein thiol levels (*69*). Dithiothreitol significantly reversed the above changes, implicating thiol oxidation of the Ca^{2+}-ATPase. This enzyme plays a major role in the regulation of intracellular Ca^{2+} levels and the generation of force in the cardiomyocytes, and it can be inhibited by HOCl (*70*). Treatment of isolated cardiac myocyte sarcolemmal membranes with HOCl also caused inhibition of Na^+-K^+-ATPase (*71*, *72*), an enzyme that regulates the intracellular Na^+ concentration. Inhibition may alter Ca^{2+} levels via Na^+/Ca^+ exchange reactions, and hence affect myocyte function.

HOCl can cause the release of zinc from zinc finger proteins in which the metal is bound to the sulfydryl group of cysteines by thiolate bonds (*73*). Treatment of cultured cells with HOCl causes the mobilization of intracellular zinc, most likely from these metalloproteins (*74*, *75*). In endothelial cells Zn^{2+} mobilization was accompanied by a loss of cell thiols, shortening of actin microfilaments, retraction of cells, and an increase in endothelial cell permeability that is related to oxidative damage to the cytoskeleton proteins (*24*, *76*).

HOCl has been shown to cause alterations in the levels or activity of a number of cell constituents important in maintaining cellular function. ATP levels are decreased by treatment of cells with HOCl, and this can occur at sublethal doses (*35*, *54*, *60*, *77*). The reactions causing a decline at ATP levels have not been established, but inhibition of GAPDH, mitochondrial respiration and glucose transport have all been shown to occur with HOCl treatment of cells (*35*, *78*). HOCl can also react directly with ATP (*27*). Treatment of tumor cells with HOCl induced a decrease in NAD and an inhibition of cellular respiration (*35*). These changes occurred at reasonably high concentrations of HOCl.

HOCl AS A SIGNALING MOLECULE

The finding that HOCl can react with intracellular components, combined with its high reactivity with thiols, has led to speculation that it can regulate specific cell processes. Several recent studies suggest that this could occur. Exposure to low doses of HOCl initiates a transient growth arrest in endothelial cells (*58*) and human skin fibroblasts (*79*). In fibroblasts this was associated with an increase in the levels of the transcription factor p53, and the p53-dependent gene product WAF1/CIP1 was upregulated, confirming that the increased p53 was transcriptionally active (*79*). HOCl has also been shown to activate the transcription factor NF-κB in a T-lymphocyte cell line (*80*). In this study, the HOCl was added in the presence of whole medium, and this effect is likely to be caused by the action of secondary chloramines.

Apurinic endonuclease (APE) is a DNA repair enzyme that also regulates the redox state of several transcription factors. Pretreatment with a sublethal dose of HOCl reduced the frequency of chromosomal aberrations caused by subsequent exposure to H_2O_2 (*81*). This was attributed to transcription of the APE gene. Thus, HOCl induced an adaptive response in mammalian cells that protected cells from further oxidant-mediated chromosomal aberrations. Many signaling pathways involve the activation of MAP kinases. Our laboratory has recently found that low doses of HOCl can cause selective phosphorylation of components of the MAP kinase cascade, with activation of extracellular signal regulated protein kinase (ERK) and p38 kinase, dependent on the amount of oxidant added (R. Midwinter, C. C. Winterbourn, M. C. M. Vissers, unpublished observations).

CONCLUSIONS AND FUTURE DIRECTIONS

The tendency to refer to oxidants generically as ROS (reactive oxygen species) or RONS (reactive oxygen and nitrogen species) encourages the concept that these oxidants are similar and that they react similarly. Based on known differences in chemical reactivity, this is unlikely to be true (*82*). Other oxidant species, particularly H_2O_2 and peroxynitrite, are known to affect cell viability and functions. However, there are notable differences between the various oxidative systems. In particular, the amount of HOCl required for toxicity is markedly lower than the concentration of H_2O_2. This may reflect the absence of an enzymatic scavenger system for HOCl, or alternatively, its greater ability to damage specific cell targets. Another important consideration is that HOCl causes irreversible loss of intracellular GSH, which can presumably be replaced only by resynthesis. The same may be true for protein thiols, in which case, repair mechanisms will be less effective than for oxidants such as H_2O_2. These effects would have major consequences for the maintenance of the cell's redox state.

There is currently much interest in the role of oxidants in cell signaling. H_2O_2 and peroxynitrite have been shown to activate kinase pathways and to cause transcription factor activation, and there is evidence for the generation of undefined oxidants in many receptor-mediated signaling events. The findings that some signaling processes can also be initiated by low doses of HOCl, together with the profile of targets for this oxidant, may help in the search for the mechanism of oxidant-induced signaling. It could be noted that the growth arrest and apoptosis initiated in response to low and intermediate doses of HOCl are stress responses, and there is currently no evidence that HOCl

causes modulation of function, as has been seen with H_2O_2. This will become of interest as the differences between oxidants and the role they play in inflammation are better defined.

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Appendix V: Literature Research about Hypochlorous Acid

n) White Paper: Evaluation of Vetericyn Plus™ Pinkeye Spray as an aid in corneal healing and reduction of pain and infection of the cornea following experimentally induced Bovine Keratoconjunctivitis, Auburn University College of Veterinary Medicine, Auburn University.

White Paper:

Evaluation of Vetericyn Plus™ Pinkeye Spray as an aid in corneal healing and reduction of pain and infection of the cornea following experimentally induced Bovine Keratoconjunctivitis

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Introduction:

Infectious Bovine Keratoconjunctivitis (IBK), commonly called "pink eye", is a very painful condition affecting beef and dairy cattle worldwide. The bacterium, *Moraxella bovis* is known to be responsible for this condition. It has been estimated that annual losses associated with only decreased weight gain from infected cattle exceeds 150 million dollars (Lane et al., 2006). Infectious Bovine Keratoconjunctivitis has been referred to as the most important ocular disease in cattle worldwide (1 Funk et al 2014). There are a number of pathogens associated with IBK in cattle, such as Bovine Herpes Virus-1 (BHV-1) which is the causative agent of Infectious Bovine Rhinotracheitis (IBR). However, *M. bovis*, a gram negative rod shaped bacteria, has thus far been the only organism demonstrated to cause IBK in cattle (Angelos et al., 2010, Gould et al, 2013). There are other organisms which have the ability to result in severe conjunctivitis and edema of the cornea but they are not known to cause central corneal ulceration (Angelos 2010, Gould et al., 2013). Antibiotic therapy has been primarily utilized for this condition. However, there has been a strong push by the Centers for Disease Control (CDC), the Food and Drug Administration (FDA), the World Health Organization (WHO), and the American Veterinary Medical Association (AVMA) to develop and utilize products that do not predispose to antimicrobial resistance. Hence, the necessity for further evaluation of therapeutics such as Vetericyn™ Pink Eye Spray as an alternative therapy to aid in corneal healing and reduction of pain and infection due to ocular infection in cattle resulting from *M. bovis.*

Objectives:

the objectives of the research performed herein were to: 1) assess the ability of Vetericyn Plus™ Pink Eye Spray to decrease and/or eliminate the growth of *M. bovis* in vivo in cattle experimentally infected with *M. bovis* organisms, 2) assess the ability of Vetericyn Plus™ Pink Eye Spray to aid in corneal healing and reduction of pain of experimentally induced corneal lesions resulting from abrasion of the epithelium of the cornea and concurrent *M. bovis* infection, 3) to assess chlorine and sodium residues in the serum, plasma, and chlorine in liver, fat muscle and urine following twice daily administration Vetericyn Plus™ Pink Eye Spray in calves with corneal lesions, 4) collect and assess chlorine residues in milk in lactating dairy cattle following twice daily administration of Vetericyn Plus™ Pink Eye Spray topically on experimentally induced corneal lesions and epidermal lesions in dairy cows, and 5) perform a cost analysis of Vetericyn Plus™ verses, parenteral administration of Oxytetracycline, Tulathromycin, and Florfenicol.

Hypothesis:

The hypothesis of this research herein was that Vetericyn Plus™ Pinkeye Spray, when utilized in a standard therapeutic protocol, will be found to be an economically advantageous product which promotes corneal healing and aids in the reduction of pain and infection due to experimentally induced IBK and results in no detectable residues in plasma, serum, milk, urine, fat, liver, and muscle.

Materials and Methods:

Calves:

Thirty dairy, 8 Holstein and 22 Jersey, bull calves having determined to have normal ophthalmic examinations and who were culture negative for *M. bovis* were randomly assigned to 3 groups for a single eye block randomized blinded challenge study. Calves were housed in pairs according to their respective group in an approved isolation facility. Following topical corneal administration of proparacaine hydrochloride and a single dose of 1.1 mg/kg of Banamine® intravenously (IV), each calf in Groups 1 and 2 had a 0.6 mm corneal lesion made on the left central corneas utilizing n-heptanol. Immediately following lesion formation, 1.0×10^7 of *Moraxella bovis* (strain Epp63-300; origin: NADC) was administered topically to the left central corneas of Groups 1 and 2. The calves in Group 3 (Control group) received topical corneal administration of *M. bovis* to their left eyes but nothing further. In Group 1, two ml of Vetericyn Plus™ Pinkeye Spray was administered topically to each calves' cornea twice daily for 10 days. In Group 2, two ml of 0.9% Saline was administered topically to each calves' cornea twice daily for 10 days. Each animal was given a pain score twice daily (based on blepharospasm, ocular discharge and tearing) utilizing a scale of one to four. All eyes were cultured on day -7, 0, 1-5, and day 10. Daily fluorescein staining was performed on the eyes of all calves followed by digital photography of the lesion to assess healing of the corneas. The sizes of the lesions were assessed daily utilizing J-image software. Additionally, serum and plasma samples were drawn from all calves on days 0, 1, 10, 11, and 17 and evaluated for changes in sodium and chloride levels. Total chlorine levels were measured in Group 1 on days 0, 11, and 17 on urine, fat, liver, and muscle via DPD chlorometric assay.

Lactating Cows:

An additional group of 10 adult lactating Holstein dairy cows were utilized for residue testing on milk samples. A single eye design was also utilized in this portion of the study. Following topical corneal administration of proparacaine hydrochloride and a single dose of 1.1 mg/kg of Banamine® IV, each cows had a 0.6 mm corneal lesion made on the left central corneas utilizing n-heptanol. The cows did not receive topical corneal administration of *M. bovis* to their left eye. Also two 0.6 mm skin lesions were made in the middle of the left side of each of the cows in the study to maximize absoption of Vetericyn Plus™ Pinkeye Spray. Two ml of Vetericyn Plus™ Pinkeye Spray was administered topically to both corneas of each cow and to one of the skin lesions twice daily for 10 days. Pain scores for each cow were recorded twice daily. Milk samples were collected on day 0, 11, and 17 and analyzed for chlorine via the DPD colorimetric method.

Statistical evaluation was performed utilizing SAS® software. The data were natural log transformed and a Kenwood-Roger correction was utilized. The covariance structure was autoregressive or Toeplitz.

Results:

All lactating cows and all calves in group 1 and 2 developed lesions in the left eye as determined by fluorescein staining. All calves in group 2 developed lesions consistent with IBK in the left eyes. Calves in group 2 only were determined to be culture positive for *M. bovis* during the study period. Between Days 1 and 2, Group 1 had significantly, $P < 0.05$, decreased pain scores when compared to controls. On

average there was a reduction in pain score by 79.1% by day 2 and an 83.7% reduction in pain by day 10 when compared to controls. Group 2 had an average reduction in pain score of 18.3%, and 67.9% by day 2 and by day 10, respectively, when compared to controls. Following 24, 36, 48, and 60 hr of twice daily topical corneal administration of Vetericyn Plus™ Pinkeye Spray to the cows, it was found that 50%, 60%, 70%, and 100% of cows had no signs of pain and upon examination no evidence of corneal lesions. None of the cows developed IBK clinical signs.

The average of the Days to cure for Group 1 was 2.2 days and Group 2 was 5.5 days, respectively. It was found that the Days to cure was significantly different between Group 1 and Group 2 ($P = 0.0161$). As far as lesion sizes: Lesion circumference, treatments were significantly different ($P = 0.0375$), and days were significantly different ($P = <0.0001$) but interaction of treatment and day was not significantly different ($P = 0.329$); Lesion width, treatments were significantly different ($P = 0.0147$) and days were significantly different ($P = <0.0001$) but interaction of treatment and day was not significantly different ($P = 0.329$); Lesion height, treatments were not significantly different ($P = 0.108$) but days were significantly different ($P = <0.0001$) and interaction of treatment and day was not significantly different ($P = 0.244$): Lesion area, treatments trended towards significance ($P = 0.0829$), days were significantly different ($P = <0.0001$) but interaction of treatment and day was not significantly different ($P = 0.158$). All samples of plasma and serum from the cows and calves fell within the normal reference ranges for sodium and chloride and there was no appreciable difference in the amount of sodium and chloride in the plasma and serum among all calves and cows at any of the sampling time points. Additionally, there were no differences in the amount of chlorine in the milk, urine, fat, liver and muscle at any of the time points sampled in any of the cow sampled.

In a cost comparison of Vetericyn Plus™ Pinkeye Spray verses that of Oxytetracycline, Tulathromycin, and Florenfenicol, there was found to be a total drug cost savings of $34.84, $58.13, and $108.02 when utilizing a 40 ml regimen of Vetericyn Plus™ Pinkeye Spray and $32.42, $55.71 and $105.60 when utilizing an 80 ml regimen, respectively. The milk loss would make utilization of Vetericyn Plus™ Pinkeye Spray that much more economically advantageous due to no milk with-drawl with use of this product. There is a savings of income from milk at $100 for a cow that is producing 50 lbs per head per day and a savings of $160 for a cow that is producing 80 lbs of milk per head per day at a milk price of $20/cwt of milk. The total cost savings of Vetericyn Plus™ Pinkeye Spray verses that of Oxytetracycline in a 1200 lb lactating cow is $137.84 for a cow that is producing 50 lbs per head per day and a difference of $197.84 for a cow that is producing 80 lbs of milk per head per day when adding drug, equipment and milk loss. Tulathromycin, and Florenfenicol are not approved for use in lactating dairy cattle. Labor costs are not calculated in this analysis.

Conclusions:

The results of this study indicates that Vetericyn Plus™ Pinkeye Spray can be utilized as an aid in corneal healing and in the reduction of pain and infection due to IBK in cattle with no detectable residues in plasma, serum, milk, urine, liver, fat or muscle. Additionally, results of the drug cost analysis indicate that Vetericyn Plus™ Pinkeye Spray is an economically advantageous therapy when compared to Oxytetracycline, Tulathromycin, and Florenfenicol.

Acknowledgments: Innovacyn

Appendix V: Literature Research about Hypochlorous Acid

o) Study Report: The Effect of Vetericyn® Technology (hypochlorous acid) on the Treatment of Hairy Foot Warts in Dairy Cows, California Polytechnic State University, San Luis Obispo.

Report: The Effect of Vetericyn® Technology (hypochlorous acid) on the Treatment of Hairy Foot Warts in Dairy Cows. 2011*

M. S. Aseltine, and B. L. Golden
California Polytechnic State University, San Luis Obispo, California

ABSTRACT

A method for the treatment of hairy foot warts in lactating dairy cattle was evaluated to determine if it can be effective. Modified hypochlorous acid (Vetericyn® Technology) was sprayed on affected animals twice daily at 220 ppm for a period of 30 days. At the end of 30 days there was no statistical difference between treatment and control animals ($p>.05$). At this time the treatment group was not treated for two weeks. After the two week period the hypochlorous acid solution was increased to 400 ppm and applied to the treatment group in place of the 220 ppm solution. At the end of 30 days of treatment with the 400 ppm solution there was a significant difference between the treatment and control animals ($p< .01$). The area was cleaned with water to remove any organic matter before spraying approximately 10cc of Vetericyn on the affected area. A score from one to five indicating the degree of severity of the infection was assigned to the treated and control animals weekly throughout the trial. Warts treated first with the 220 ppm, and then subsequently with the 400 ppm solution, were reduced based on the hairy foot wart score. The control group had no significant reduction in hairy foot wart score by the end of the trial.

Keywords: Hairy foot warts, Vetericyn® Technology, Vetericyn®, hypochlorous acid.

INTRODUCTION

Papillomatous Digital Dermatitis (PDD), commonly known as hairy foot wart (HFW), has been a condition plaguing the dairy industry throughout the United States. Contagious and expensive to treat, HFW can cause a reduction in milk production ranging from 20% to 50%. Furthermore, PDD can lead to reproductive problems, premature culling and dairy lameness. Brown et al. (2000) concluded that almost 30% of cull cows at slaughterhouses in the U.S. had PDD.

The most effective treatment for the disease has been through the use of topical antibiotics. Formalin and copper sulfate footbaths on dairy farms have been a more common treatment. Currently, there are no recommended disposal methods for these chemicals after they have been used. While studies have been conducted on the acute effects of these chemicals in a controlled

environment, little is known about the potential long term chronic effects of improper disposal of formalin and copper sulfate on the environment.

The objective of this pre-clinical trial was to determine if hypochlorous acid (a weak acid with chemical formula HOCl) has the potential to be an effective treatment of HFW in dairy cattle.

The technology behind the Vetericyn® Technology-based family of products (Vetericyn® in animal healthcare) involves a patented and proprietary solution of electrically charged oxychlorine small molecules designed to treat a wide range of organisms that cause disease. It is a pH neutral solution of hypochlorous acid. A comparable oxychlorine compound is also produced by the neutrophils in the human body's immune system in response to foreign pathogens.

Unlike standard electrochemical processes where the unstable nature of the product makes onsite generation necessary, the company claims that the Vetericyn® Technology has a clinically validated shelf stability of over two years.

MATERIALS AND METHODS

The Cal Poly dairy consists of approximately 200 lactating cows at any one time. All lactating cows were given an HFW Score on the first day of the trial (Table 1). On the first day of the study 13 cows with a HFW Score greater than zero were identified. Of these cows, four had HFW Scores greater than zero on two feet. The remaining nine cows had HFW Scores of greater than zero on only one foot. This resulted in 17 total observable feet with HFW Scores greater than zero.

The 13 cows with at least one foot with an HFW Score greater than zero were randomly assigned to a Treatment group or a Control group on the first day of the trial. The Treatment group had the affected area washed with water to remove any organic matter. Vetericyn® at 220 ppm was administered twice daily in amounts sufficient to cover the foot wart (approximately 10 cc) for the first 30 days of the trial. The product was administered with a spray bottle. After the first 30 days of the trial the Treatment group was not treated for two weeks. After the two-week period the hypochlorous acid solution was increased to 400 ppm and applied to the treatment group in place of the 220 ppm solution for t30 days.

Cows in the Control group were treated for HFW using current protocol which was to treat when a cow was scored as a 3 or greater by placement on a hoof-trimming table, cleaning the wart and applying tetracycline powder, and then wrapping with a Vet-Wrap® bandage. The bandage was removed 72 hours after treatment. Cows in the Treatment group that showed signs of HFW (score of 2) were identified with a leg band and treated with the Vetericyn® product for a

period of no less than 48 hours twice daily. If the treatment with the Vetericyn® product was ineffective (an increase in HFW score to 3+), the cow was then treated using the current protocol.

Animals were not vaccinated for HFW. All animals used a footbath with copper sulfate (8 lbs per 50 gallons of water) once per day after the afternoon milking.

Table 1. Hairy Foot Wart score

0	No visible wart or signs of pain.
1	Small visible wart with no pain or discomfort.
2	Small stages of wart, cow shows signs of discomfort (irritated by spraying water).
3	Small clinical wart. Cow is showing some signs of pain and discomfort.
4	Medium clinical wart. Cow is limping and showing signs of pain and tenderness.
5	Large clinical wart. Cow is in constant discomfort and shows signs of pain in affected area.

Statistical Analysis. Data were analyzed using both the Proc Probit and Proc Mixed procedure of SAS. The model for the underlying scale in the probit analysis fit HFW Score as a dependent variable and the independent class effects of Treatment versus Control (TorC), and animal foot within TorC. Linear week of observation, and week by TorC interaction as a two-degree polynomial (linear and quadratic terms) were fit as continuous fixed effects.

In a subsequent analysis a mixed model was fit to confirm the probit model results and obtain least squares means on the score scale. The mixed models included HFW Score as a continuous dependent variable with the independent class effects of TorC, week of observation, and the week by TorC interaction. The mixed model included the random effects of error and foot observed.

Additionally, subsets of both the probit and the mixed model were analyzed in which the data were divided into the weeks where the 220 ppm Vetericyn® was used and then the 400 ppm Vetericyn® was used.

RESULTS AND DISCUSSION

Data for the HFW scores are shown in Table 2. One cow from the Control group was removed at the end of the first 30 days for treatment with antibiotics because of increasing in severity of HFW Score.

The results from both the probit and mixed models indicated nearly the same levels of significance for the effects. For this report only the results of the mixed model will be presented. The probit model was included to account for the categorical nature of the data. The overall effect of Treatment or Control was significant (P<.05).

The subset analyses showed that there was no significant difference in the effect of the 220 ppm Vetericyn® treatment from the Control in the first 30 days of the trial. The subset analysis that included only the last 30 days of the trial, where 400 ppm level of Vetericyn® was administered, showed a highly significant (P<.01) effect of the Vetericyn® (Figure 1).

Because all cows in the Treatment group were first given the lower dose of Vetericyn®, it is not possible to tell from this study if the response from the higher dose within thirty days was entirely due to the larger dose of Vetericyn® or a combination of the lower dose followed by the higher dose.

Further studies should look at the response rate to the higher dose of Vetericyn® only and also on the effect of Vetericyn® without the combination of the copper sulfate footbath.

Brown C.C., P. D. Kilgo, and K. L. Jacobsen. 2000. Prevalence of papillomatous digital dermatitis among culled adult cattle in the southeastern U.S. *American J. of Veterinary Research.* 61:928

Table 2. Hairy foot wart scores for all animals on the trial

CONTROL		WEEK						
		1	2	3	4	6	7	8
Animal ID	Foot							
2275	LF	4	4	4.5	4			
2307	RR	2	2	2	2	3	2	2
2331	LR	2	2	2	2	2	1	1.5
2331	RR	3	3	3	3	3	2.5	2.5
2360	RR	1	0	0	0	0	0	0
2370	LR	2	2.5	1	1	1	2.5	2
2379	LR	3	1	1	1	2	3	3
2379	RR	3	1	1	1	1	3	3
TREATMENT								
167	RR	3	0	0	0	0	0	0
2267	RR	5	5	2	2	1	0	0
2318	LR	1	1	1	1	0	0	0

2329	LR	2	3.5	3.5	3	3.5	0	0
2329	RR	5	4	4	4	3.5	1	0
2352	RR	5	1	3	3	0.5	0	0
2382	LR	4	3.5	3.5	3	3	1	0
2397	LR	2	1	1	1	0.5	0	0
2397	LR	1	3.5	1	1	0	0	0

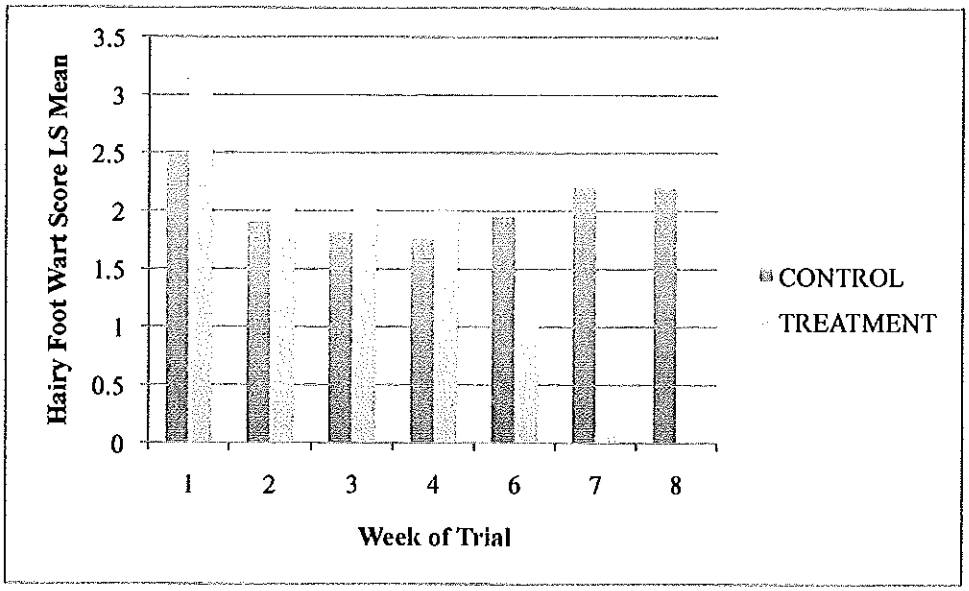

Figure 1. Least squares means for HFW Score for each week by treatment group. At the end of week 3 the Vetericyn® dose was increased from 220 ppm to 400 ppm.

**This paper is a report to Innovacyn, Inc. of a pre-clinical trial of the Vetericyn® product. It is the result of funding received by Cal Poly from the company to perform the pre-clinical trial. The statements made in this paper by the authors do not represent an endorsement of the product or an affirmation that the product will perform as claimed by the manufacturer of Vetericyn®. Results will vary according to many factors including, but not limited to, random error, environment, breed, management, and others. This paper has not been peer reviewed. All public statements related to the information contained in this paper such as press releases, advertisements, promotional material and others must be pre-approved by the corresponding author of this paper. The corresponding author is Dr. B. L. Golden, Cal Poly Dairy Science, San Luis Obispo, CA 93407, bgolden@calpoly.edu.*

Appendix V: Literature Research about Hypochlorous Acid

p) Pilot Study Report: The Effect of Puracyn® Plus on Deep Dermal Infections, Miller School of Medicine, University of Miami.

MILLER
SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

Department of Dermatology and Cutaneous Surgery
Wound Healing Research Laboratory

Pilot Study Report

The Effect of Puracyn® Plus on

Deep Dermal Infections

February 9, 2015

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INSTITUTIONAL POLICIES AND REGULATIONS

The experimental animal protocols used for this study were approved by the University of Miami Institutional Animal Care and Use Committee and all the procedures followed the federal guidelines for the care and use of laboratory animals (U.S. Department of Health and Human Services, U.S. Department of Agriculture). The studies were conducted in compliance with the University of Miami's Department of Dermatology and Cutaneous Surgery Standard Operating Procedure (SOPs). Animals were monitored daily for any observable signs of pain or discomfort. In order to help minimize possible discomfort, analgesics were used during the entire experiment.

OBJECTIVE

The objective of this study was to determine the effect of Puracyn® on Methicillin Resistant *Staphylococcus aureus* (MRSA) infected porcine wounds.

MATERIALS AND METHODS

Experimental Animals

A porcine model was used for our experimental research due to the morphological similarities between swine skin and human skin. One (1) animal was used for this study. The young female's specific pathogen free (SPF: Looper Farms, North Carolina) pig weighing 35-40 kg was kept in house for at least 5 days prior to initiating the experiment. The animal was fed a basal diet *ad libitum* and was housed individually in our animal facilities (meeting American Association for Accreditation of Laboratory Animal Care [AAALAC] accredited) with controlled temperature (19-21^{o}C) and lighting (12h/12h LD).

Wounding Technique

The back of the experimental animal was clipped with standard animal clippers on the day of the experiment. The skin on both sides of each animal was prepared for wounding by washing

with a non-antibiotic soap (Neutrogena Soap Bar; Johnson and Johnson, Los Angeles, CA) and sterile water. Each animal was anesthetized and given analgesics till the end of the study.

Fifteen (15) deep reticular dermal wounds measuring (22 mm x 22 mm x 3 mm deep) were made in the paravertebral and thoracic area with a specialized electrokeratome fitted with a 22 mm blade. The wounds were separated from one another by 5-7 cm of unwounded skin. All wounds were inoculated within 20 minutes after wounding (see Wound Inoculation below).

Wound Inoculation

The pathogenic strain of Methicillin Resistant *Staphylococcus aureus* (USA300) was used in this study. All bacterial inoculum suspensions were made by swabbing a 3-cm diameter area of the overnight growth from a culture plate into 4.5 ml of sterile water. This resulted in a suspension consisting of approximately 10^{10} colony forming units/mL (CFU/mL). One ml of this suspension was diluted into 35 ml of Tryptic Soy Broth (TSB), making the inoculum suspension 10^6 CFU/ml. A sample of this suspension was further diluted and plated onto culture media to enumerate viable CFU/ml of organism prior to the experiment. The inoculum suspension was used directly to inoculate each wound by pipetting a 25 µl aliquot into a sterile glass cylinder (22 mm diameter) in the center of each wound site. All wounds were covered with a polyurethane film dressing (Tegaderm Transparent Dressing; 3M Health Care, St. Paul, MN USA) for 48 hours to allow for biofilm formation. Dressings were secured with surgical tape and wrapped with Coban elastic wrap (3M, St. Paul MN).

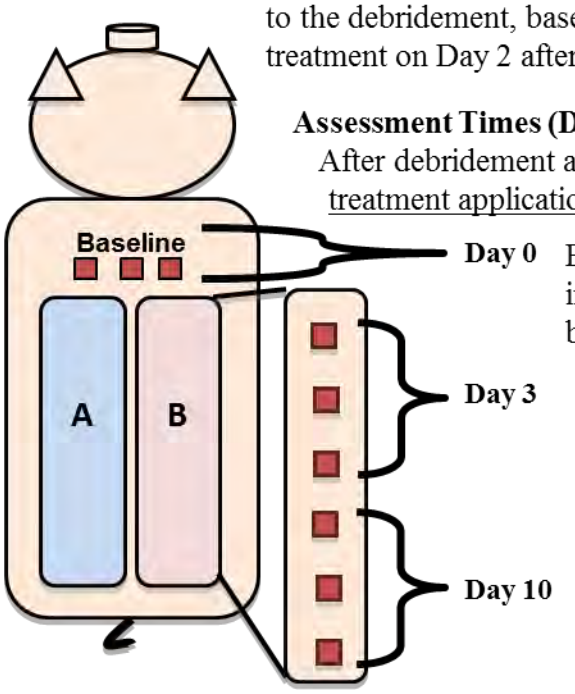
Experimental Design

After 48 hour biofilm formation, three (3) wounds were recovered as described below for baseline counts. Six (6) wounds were randomly assigned to one of two treatment groups according to the experimental design below. All wounds were surgically debrided using a curette prior to treatment.

Experimental Design

Number of Animals	Treatments Groups
1	A. Puracyn Plus B. Saline

Note: 48 hour biofilms were established prior to the debridement, baseline recovery and first treatment on Day 2 after wounding.



Treatment Regimen

Wounds which were assigned to Saline or Puracyn Plus treatment groups followed the below treatment schedule.

1. Wounds were debrided with curette (see Figures 1, 2 and 3).

2. Wounds were rinsed twice (x2) using 10ml syringes with 1.5" long 21 gauge needles held at a 45 degree angle over the wounds. During each treatment, wounds in adjacent wounds were covered with sterile 1 ½" metal caps to prevent the rinse from flowing into the other wounds (Figure 4).

3. Sterile gauze soaked with 2 ml of treatments (Saline or Puracyn Plus) were then applied (Figures 5 and 6).

4. All treatments were cover with polyurethane film to retain moisture (Figures 7 and 8).

5. Wounds were treated once daily.


Figure 1


Figure 2


Figure 3


Figure 4


Figure 5


Figure 6

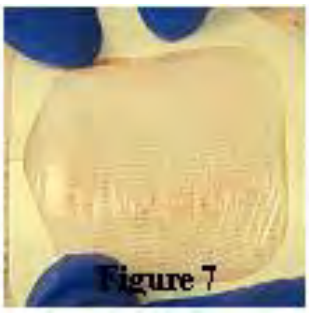


Figure 7

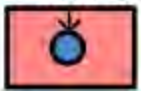

Figure 8

RECOVERY METHODS

Microbiology Assessment

Three (3) wounds were biopsied (6mm punch biopsy) per group and were collected on day 0 post treatment application and on day 3 and 10 post treatment. The punch biopsy was taken from the center of the wound (see Figure 9). Biopsies were weighed and immediately placed in 1 ml of All Purpose Neutralizing Solution. The sample was combined with an additional 4 ml of Neutralizing Solution

Figure 9

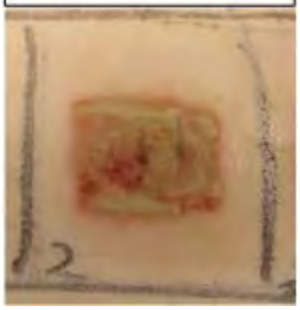


and homogenized in a sterile homogenization tube. Serial dilutions were made and scrub solutions were quantified using the Spiral Plater System which deposits a small defined amount (50µl) of suspension over the surface of a rotating agar plate. ORSAB selective media was used for count of MRSA present in the Neutralizing Solution. After plating, all samples were incubated aerobically for 24 hours at 37° C. After the incubation period, colonies on the plates were counted and the CFU/ml calculated.

OBSERVATIONS

Descriptive terms for swelling and erythema: absent < slight < mild < moderate < marked < exuberant

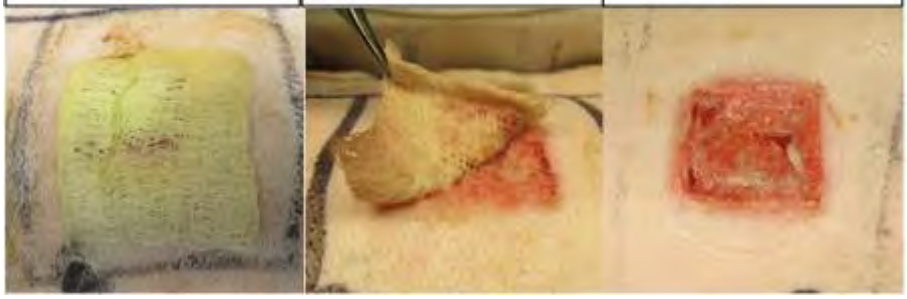
Figure 10



Representative photos of wounds were taken during the study. Observations were made during treatment application and assessment days.

On day 0 before debridement and treatment application all wounds had slight erythema. (See figure 10). No signs of edema or erythema were observed after day 0. From day 1 to day 4 all gauze covering wounds (both treatment groups) were found to remain in place (see figure 11). A slight adherence of the gauzes to wound bed was observed in both treatment groups, however tissue re-injury was only noted in Saline treated wounds on days 1 and 4 (See figure 12 and 13).

Figure 11 | Figure 12 | Figure 13



Beginning on day 5 and until the end of the study no adherence of gauzes was observed.

RESULTS

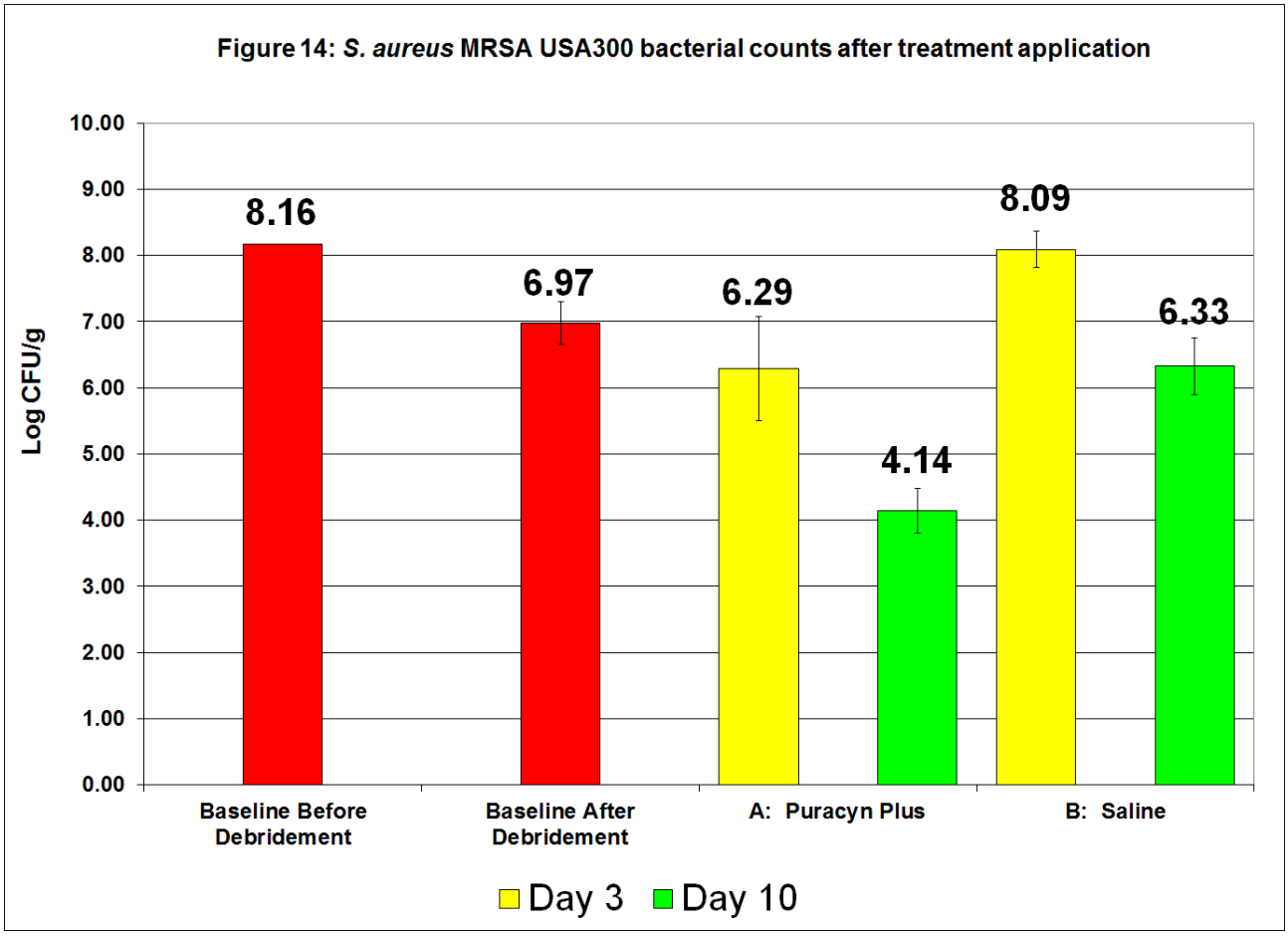
After counting the colonies, the data was tabulated and the Log of colony forming units per gram (Log CFU/g) was determined. The mean of the Log CFU/g and standard deviation were calculated for each treatment. Appendix 1 contains all the raw data.

All wounds were allowed to have 48 hours to develop a biofilm formation. One (1) baseline wound (biopsy 3) was recovered before debridement with a bacterial count of 8.16±0 Log CFU/g. After debridement, two (2) baseline wounds (1 and 2) had a bacterial count of 6.97±0.32 Log CFU/g (Figure 14).

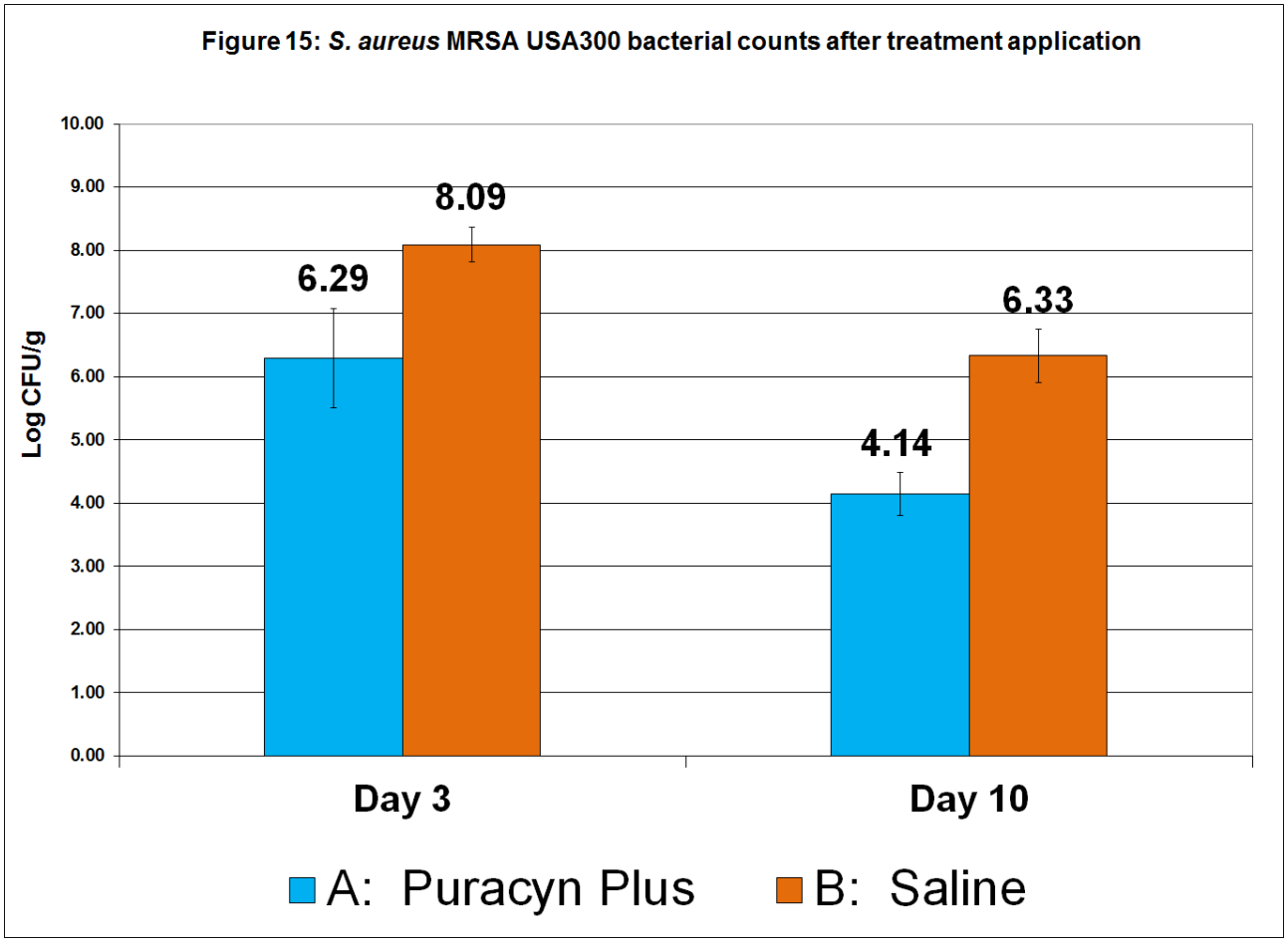
On Day 3 after treatment application, wounds treated with treatment A – Puracyn Plus had a bacterial count of 6.29±0.78 Log CFU/g. While Treatment B – Saline had a bacterial count value of 8.09±0.28 Log CFU/g. Both treatment showed lower bacterial count than shown from the baseline biopsies (both before and after debridement) recovered on day 0. Wounds treated with Puracyn Plus resulted in a decrease by 1.87±0.78 Log CFU/g, resulting in a bacterial reduction percentage of 99.66%. Puracyn Plus treated wounds resulted in a decrease of 0.68±0.46 Log CFU/g, which yields a bacterial reduction percentage of 79.20%. Wounds treated with Saline (treatment B) resulted in similar values than baseline wounds before debridement (8.09±0.28 and 8.16±0 Log CFU/g, respectively). Wounds debrided and treated with treatment B – Saline, resulted in a bacterial count increase of 1.12±0.04 Log CFU/g compared to baseline wound after debridement. See figure 14

On day 10 after treatment application, the remaining three (3) wounds for both treatments were recovered. Wounds treated with treatment A – Puracyn Plus and treatment B – Saline had a bacterial count value of 4.14±0.34 and 6.33±0.43 Log CFU/g, respectively. Both treatments showed lower Log CFU/g values when compared to the before and after debridement values. Treatment A – Puracyn Plus when compared to the baseline before debridement has a difference of 4.02±0.34 Log CFU/g and a bacterial reduction percentage of 99.99%, when treatment A was

compared to the baseline value after debridement the difference value was 2.83±0.02 Log CFU/g and a bacterial reduction percentage of 99.85%. Wounds treated with treatment B – Saline and compared to the baseline before debridement resulted in a decrease of 1.84±0.43 Log CFU/g and a bacterial reduction of 98.54%, but when was compared against baseline after debridement the difference was 0.64±0.11 and a bacterial reduction percentage of 77.33%.



Bacterial counts in both treatments on day 10 were lower than those on day 3. Wounds treated with Treatment A – Puracyn Plus and Treatment B - Saline exhibited reduction amounts of 2.15±0.44 and 1.76±0.15 Log CFU/g of MRSA, respectively, between assessment day 3 and day 10 (Figure 15). These values correspond to a 99.29% and 98.87%, respectively (See Figure 15).



CONCLUSIONS

Overall, the results of the study indicate that the Puracyn Plus treatment formulation was the most efficient at reducing amounts of MRSA USA300. The greatest reduction amounts compared to baseline wounds (before and after debridement) were demonstrated to be the Puracyn Plus on both assessment days. These results demonstrate that the Puracyn Plus was the most efficient at reducing the amounts of MRSA. An alternative option for this study would have been to further the number of treatment applications by extending its timeframe. It is possible that if treatments were applied twice daily a larger increase bacteria reduction may be observed. Additional studies are needed to substantiate these findings.

APPENDIX 1 Raw Data

Pig #1 P15-072/297

Inoculum

Strain	Dilution	Count	CFU/ml	Log CFU/ml
S. aureus MRSA USA300	-4	47	9.39E+06	6.97

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
Baseline before Debridement	3	-5	50	5.00E+08	8.70	STDV
			Mean	5.00E+08	8.70	0.00

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
Baseline before Debridement	3	50	5	100000	0.172	1.45E+08	8.16	STDV
					Mean	1.45E+08	8.16	0.00

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
Baseline After Debridement	1	-4	48	4.80E+07	7.68	
	2	-3	179	1.79E+07	7.25	STDV
			Mean	3.30E+07	7.47	0.30

Number of organism per g

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
Baseline After Debridement	1	48	5	10000	0.152	1.58E+07	7.20	
	2	179	5	1000	0.161	5.56E+06	6.74	STDV
					Mean	1.07E+07	6.97	0.32

ORSAB Bacterial count in wounds Debrided 48 hours Biofilm Formation recovered Day 3

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
A: Puracyn Plus	1	-4	39	3.90E+07	7.59	
	2	-2	94	9.39E+05	6.00	
	3	-3	48	4.80E+06	6.68	STDV
			Mean	1.49E+07	6.76	0.80

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A: Puracyn Plus	1	39	5	10000	0.154	1.27E+07	7.10	
	2	94	5	100	0.135	3.48E+05	5.54	
	3	48	5	1000	0.143	1.68E+06	6.22	STDV
					Mean	4.90E+06	6.29	0.78

ORSAB Bacterial count in wounds Debrided 48 hours Biofilm Formation recovered Day 3

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
B: Saline	1	-5	51	5.10E+08	8.71	
	2	-5	48	4.80E+08	8.68	
	3	-4	197	1.97E+08	8.29	STDV
			Mean	3.96E+08	8.56	0.23

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
B: Saline	1	51	5	100000	0.114	2.24E+08	8.35	
	2	48	5	100000	0.182	1.32E+08	8.12	
	3	197	5	10000	0.157	6.27E+07	7.80	STDV
					Mean	1.39E+08	8.09	0.28

ORSAB Bacterial count in wounds Debrided 48 hours Biofilm Formation recovered Day 10

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
A: Puracyn Plus	1	-1	21	2.10E+04	4.32	
	2	-1	49	4.90E+04	4.69	
	3	-1	101	1.01E+05	5.00	STDV
			Mean	5.70E+04	4.67	0.34

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
A: Puracyn Plus	1	21	5	10	0.177	5.93E+03	3.77	
	2	49	5	10	0.153	1.60E+04	4.20	
	3	101	5	10	0.180	2.81E+04	4.45	STDV
					Mean	1.67E+04	4.14	0.34

ORSAB Bacterial count in wounds Debrided 48 hours Biofilm Formation recovered Day 10

Treatment	Biopsy	Dilution	Count	CFU/ml	Log CFU/ml	
B: Saline	1	-3	57	5.70E+06	6.76	
	2	-4	28	2.80E+07	7.45	
	3	-3	42	4.20E+06	6.62	STDV
			Mean	1.26E+07	6.94	0.44

Treatment	Biopsy	Number of Colonies (N)	Volume of ALL purpose Neutralizer (V)	Dilution Factor (D)	Weight Biopsy(g) X	CFU/g	Log CFU/g	
B: Saline	1	57	5	1000	0.191	1.49E+06	6.17	
	2	28	5	10000	0.216	6.48E+06	6.81	
	3	42	5	1000	0.212	9.91E+05	6.00	STDV
					Mean	2.99E+06	6.33	0.43

PETITION ENDS HERE.