IMPACT DIVERSITY SOLUTIONS: 2022 WHITEPAPER

CoveXall™

THE FIRST WATERBASED HAND SANITIZER THAT KILLS PATHOGENS ON CONTACT FOR **UP TO 4 HOURS.**

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I. ABOUT US

IMPACT DIVERSITY SOLUTIONS

IMPACT Diversity Solutions, Inc. is a Qualified Small Business (QSB) under the definition and regulations that govern the Small business Program administered by the Small Business Administration (SBA). IMPACT Diversity Solutions has received SBA Certification which pertains to both Veteran and Service-Disabled Veteran Classifications. The healthcare market is exploding with new technology and innovative new programs for the treatment of disease and together with our manufacturing partners, IMPACT is bringing these amazing new products to both the public and government markets. Improving heath with cost-effective, non-invasive solutions is a major objective of IMPACT.

OUR MANUFACTURING PARTNERS

Our manufacturing partners are selected for their creativity, reliability, and history of cutting-edge innovation. Product uniqueness is important in any consumer-based landscape. Our partners excel at helping us develop products that are as compelling as they are efficacious. Our partners have manufacturing facilities both domestically and internationally for many of our product lines to help ease the import/export burden.

OUR PRINCIPLE FOUNDER

Charles D Brown, President and CEO

Charles has substantial expertise relating to compliance with the diversity program administered by the Small Business Administration and is an expert relative to Compliance with the SBA. Charles served first as MRI Recruiting Account Manager and then Director of Staffing for the Southwest Region of Physiotherapy Associates, the 2nd largest physical therapy company in the nation. Charles served for more than 15 years in the United States Army and is classified by the U.S. Department of Veteran Affairs as a Service-Disabled Veteran.

II. MISSION
OUR MISSION AND GOALS

As the world is locked in a cycle of uncertainty due to the novel COVID-19 virus, personal preventative measures are key to returning our societies and economies to a greater degree of normalcy. While social distancing and face coverings are chief among these measures, unique products are needed to fill the gaps and help reduce the risk of transmission.

Though COVID-19 remains at the forefront of global concerns, the climbing rate of hospitalizations has brought to light the need to combat may other harmful and infectious pathogens. Bacteria such as *Clostridium difficile* (*C. diff*), E. *Coli, Burkholderia cepacia*, Methicillin-resistant *Staphylococcus aureus* (MRSA) and many other pathogens have proven to be formidable adversaries in their own right.

Our mission is to supply a suite of products that are clinically proven to kill these pathogens and help protect front line workers and essential personnel while they perform their jobs. The average citizen can also greatly benefit from having access to technology that can help them reduce the risk of incidental transmission. The need for these products extends far beyond the end of this current pandemic. They will always be needed to help mitigate the risk of infection and transmission.

COVID-19

A scientifically proven, continuous 4+ hour kill rate with a single application of the Hand Sanitizer gives us a powerful new weapon against the transmission of this novel virus.

C. DIFFICILE & SPORES

C. diff transmission and infection is a serious concern in the medical arena. This harmful bacteria and its spores are very difficult to kill. Our products are scientifically proven to kill both with a 5.2+ log reduction over 30 seconds.

OTHER PATHOGENS

Our products do not just kill COVID-19 and *C. diff*. They are effective in killing many other harmful bacteria and viruses that may plague us now or in the fututre.

III. THE TECHNOLOGY

COPPER INFUSED MAGNESIUM HYDROXIDE MICROPLATELET TECHNOLOGY.

This technology represents a novel material science comprising discs or wafers of Magnesium Hydroxide molecules arranged in 'sheets' or 'layers'. This results in extremely large surface area with potentially reactive hydroxyl groups studding the surface.

Microplatelets (MP's) require contact between themselves and the target microorganism or virus. MP action is focused and direct. Our typical MP configuration is a disc of 200 nm x 100 nm x 10 nm. For comparison is about one tenth of the length of an E.coli bacterium (1,000nm), and about 2/5 it's width (500 nm).

For further reference, the COVID-19 Coronavirus is a sphere of about 125 nm in diameter. These size relations indicate that Microplatelets are in the size range of a number of pathogens and the intimate contact that occurs between the surface of MicroPlatelets and target microorganisms is key to MP antimicrobial potency.

Copper, long known for its anti-microbial properties, is then infused onto the surface of these Magnesium Hydroxide Microplatelets. This combined with the reactive hydroxyl effects of the platelet itself and aided by the addition of Benzalkonium Chloride work in unison to destroy the target micro-organisms.

III. THE TECHNOLOGY continued

PATENT PENDING TECHNOLOGIES ARE THE ESSENCE OF THESE PRODUCTS.

Magnesium Hydroxide Microplatelets

Mg(OH)2

Magnesium has very unique properties that make the perfect material for MP's.

Infused Copper

CU

Copper has been exploited for its health benefits since ancient times

Benzalkonium Chloride

BZK

Benzalkonium Chloride is recommended by the FDA for sanitizing purposes.

Surface Area is the key

The key to our technological advantage is in the microplatelet itself. Our partner has developed a cutting edge material science manufacturing method that produces flat plates rather than nodules. Nodules, while possessing a large roughly spherical surface area, have the disadvantage of a very low potential contact area with regards to viruses, bacteria, and other pathogens. Since our kill methodology requires surface contact, it is essential that we have as large a surface area for the pathogens to interact with as possible.

Infused copper adds to the kill rate

Copper has been exploited for health purposes since ancient times. The process involves the release of copper ions (electrically charged particles) when microbes, transferred by touching, sneezing or vomiting, land on the copper surface. The ions prevent cell respiration, punch holes in the bacterial cell membrane or disrupt the viral coat and destroy the DNA and RNA inside.

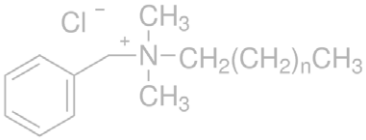
These technologies have U.S. patents and patents pending status which is shown in the Documentation section of this white paper.

III. THE TECHNOLOGY continued

BENZALKONIUM CHLORIDE

BZK

Benzalkonium Chloride has a 99.9% bacterial kill rate.



U.S. FDA recommends Benzalkonium Chloride as an effective sanitizer.

Has recently shown a marked reduction in colony forming units over a several hour period after an extensive antibacterial study.

Studied for virucidal properties against influenza, Newcastle disease, and avian infectious bronchitis.

The FDA has recently indicated support for one of our key ingredients, Benzalkonium Chloride (BZK). BZK is thought to work by cation (positive ion) donation or surfactant activity, either of which have the effect of disrupting the bacterial membrane or viral envelope. In recent clinical studies to demonstrate persistent antibacterial efficacy of a hand sanitizer, BZK produced a marked reduction in colony-forming units at each time points tested at one hour, two hours, and three hours of (3.75-4.16-logl0 reductions).

This active ingredient also actively assists by disrupting the cell membranes of the target organisms and is active at relatively low concentrations (0.12%-0.13%). Benzalkonium chloride has also been studied for virucidal activity against influenza, Newcastle disease, and avian infectious bronchitis viruses.

III. THE TECHNOLOGY continued

THE KILLING MECHANISM: KEY FACTS AND HIGHLIGHTS

Proven Results

Arizona State University's Bio Design institute and the Southwest College of Naturopathic Medicine & Health Science conducted our SARS-COV-2 Testing. Outcomes yielded a continuous kill rate of 99% at four hours for the virus.

How It works

Viruses and micro-organisms such as bacteria exist within a gel like capsid envelope which protects them from the normal environment. This biofilm surrounds the virus or bacteria and is largely responsible for keeping it viable between hosts. Bacteria within these biofilms are over 1,000 times more resistant to antibiotics. Essentially, the antibiotics can not penetrate the biofilm layer to work against the pathogen contained within.

MicroPlatelets kill from the outside. Unlike other approaches, MicroPlatelets are not ingested by the the bacteria or fungi. MP's are not taken up by cells. Their surface area render them too large for this concern. They interact with the biofilm directly.

Our MicroPlatelet technology is designed to destroy this biofilm by a chemical/mechanical means, destroying the capsid envelope and ultimately killing the virus, fungi or bacteria hidden inside. The MicroPlatelet is unaffected by this interaction and can survive the encounter to continue killing destroying any biofilm it comes in contact with. Thus, the prolonged and sustained killing effect is realized.

IV. WATER-BASED ADVANTAGES

NON-ALCOHOL BASED AND NATURALLY MOISTURIZING

Drawbacks of Alcohol Based Hand sanitizers

Alcohol based hand sanitizers have several drawbacks versus water based sanitizers. Alcohol based gels or foaming sanitizers tend to dry out the hands by effectively flushing the natural oils from the skin. These oils act as both a skin moisturizer and as part of the body's anti-microbial defense system.

By flushing these oils from the skin, there is a greater chance of hands drying and cracking. Dry hands lead to tiny fissures in the skin that can run deep into the epidermis. These fissures allow additional entry points for harmful bacteria and viruses to enter the body. A moisturizing, water based sanitizer keeps the hands from drying out thereby reducing this risk.

Additionally, alcohol based sanitizers typically use either alcohol or isopropanol. Both are highly flammable substances. The FDA recommends concentrations between 60-90% for maximum efficiency in killing germs. At these high concentrations, these sanitizers become fire hazards.

Hospitals and other medical facilities are required to consult with local fire authorities and adhere to strict regulations and codes regarding flammable substances. This can result in the alcohol based sanitizer being placed in awkward and inefficient locations for routine staff access when placing dispenser stations or storing large quantities of the sanitizer.

IV. WATER-BASED ADVANTAGES continued

BENEFITS OF OUR WATER BASED HAND SANITIZER

Moisturizes the skin while effectively killing 99.9% of harmful bacteria and viruses on contact.

Continues to kill for a period of time longer than that of an alcohol based sanitizer after the solution has dried on the skin.

Hypoallergenic Formulation for less skin irritation.

Uses a **Federally approved** effectiveness protocol.

Protects **against germs and fungus**.

Painless application for those with cuts, scrapes, or other wounds on the hands.

Delivered as a **pleasant smelling lotion** and drys within 30 seconds leaving the hands feeling soft and clean.

Nontoxic formula is safer for children if accidentally ingested.

Is **non-flammable** and will not stain surfaces.

Will not dry out and crack the skin.

V. COMPREHENSIVE TESTING

BACTERIA KILL RATE TEST RESULTS

MICROCONSULT INC.
Premium Quality Results

Covexall® was tested in a Kill Rate Study using eleven bacterial species by a leading microbiological testing facility. The exposure times were 30 and 60 seconds. The 30 second exposure killed all of the organisms from (> 105 du/ml) from nine of the species and greater that three log10 from the other two. The 60 second exposure killed all of the organisms from all eleven species.

A second Kill Rate Study was performed on the spore stage of *C. difficile* using the same exposure times.). Both the 30 second and 60 second exposers showed complete kill of the test organisms. These data show a very high degree of efficacy suggesting that this hand sanitizer could have a strong impact on bacterial transmission. The action against the spores of c. difficile is particularly remarkable.

Microrganism Kill Rate Test Results	30 sec. Log Reduction	Inoculum Level (cfu/ml)	Growth Average	60 sec log reduction
E. Coli	**5.93**	8.59 X 105	No Growth	5.93
MRSA	**5.88**	7.55 X 105	No Growth	5.88
P. aeruginosa	**5.75**	5.56 X 105	No Growth	5.75
B. cepacia	**3.30**	6.24 X 105	310 / No Growth	5.58
S. enterica	**5.77**	5.91 X 105	No Growth	5.77
E. faecalis	**5.95**	8.84 X 105	No Growth	5.95
K. pneumoniae	**4.40**	3.81 X 105	15 / No Growth	5.58
S. pyogenes	**5.41**	2.25 X 105	No Growth	5.41
L. monocytogenes	**5.78**	5.98 X 105	No Growth	5.78
C. jejuni	**5.38**	2.42 X 105	No Growth	5.38
C. difficile	**5.38**	2.40 X 105	No Growth	5.38
C. difficile (Spore Form)	**5.22**	1.67 X 105	No Growth	5.22

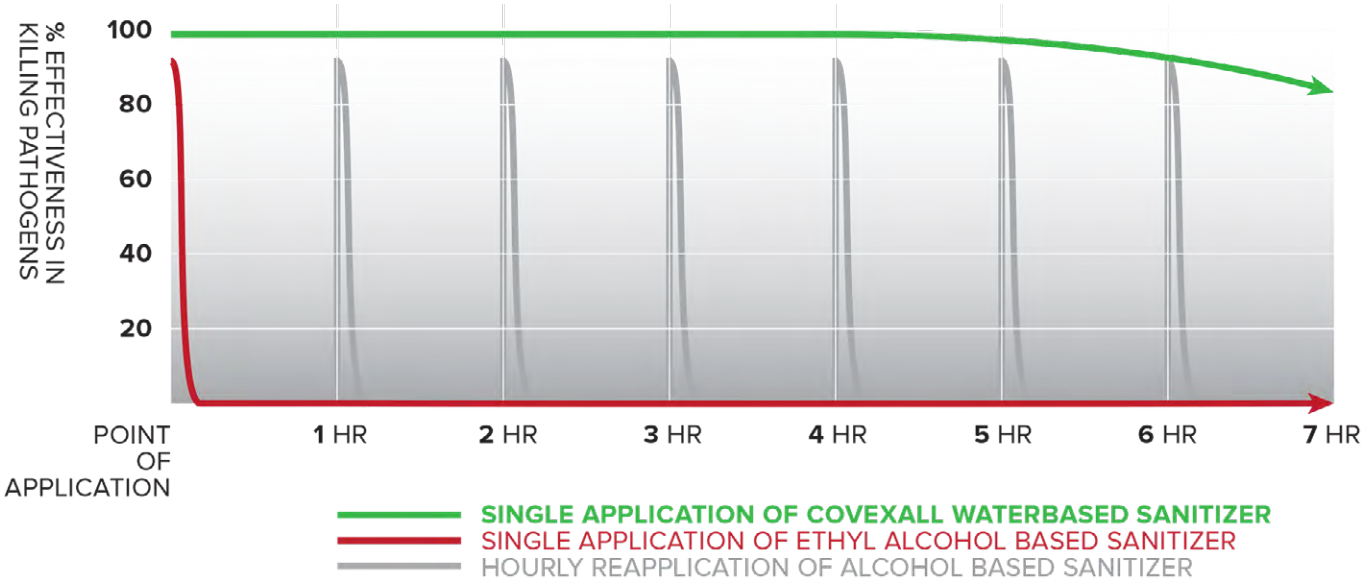
VI. DURATION TESTING

CONFIRMED 4+ HOUR KILL TIME CLAIM

ASU Biodesign Institute
Arizona State University

The results of this study, conducted in a certified Biosafety Level 3 facility, support that the 2 in 1 Invisible Glove (Hand Sanitizer) and Mask Spray products are all able to kill the SARS-CoV-2 virus even after drying on a surface for 1 hour or 4 hours.

COVEXALL VS. ALCOHOL-BASED HAND SANITIZER



In a side by side comparison with Ethyl Alcohol (70% or higher) based hand sanitizers, Covexall outperformed the competition both in effectiveness against more pathogens as well as duration of effectiveness. Alcohol based sanitizers are effective at the moment they are applied and last only until evaporated. Any new contact thereafter causes new contamination thus requiring additional applications, which can be painful and dangerous.

VII. MANUFACTURING

CAPABILITIES & SHIPPING

Packaging

A wide range of packaging are available. Typically, the 2 in 1 Invisible Glove is packaged in 1oz airless pumps, 4 oz squeeze bottles, and various automatic dispenser bladders. However, any size from 1/2 gallon to sachets can be accommodated.

Bulk Shipments

Bulk shipments of product can be be delivered in 5 gallon pales, 25 gallon totes, 50 gallon totes, or 250 gallon totes. These can be shipped domestically or internationally as needed.

Production capacity

Our manufacturing partner has several production facilities in the U.S. and abroad. As of this writing, the production capacity is over 200,000 gallons per day, per shift. New productions facilities have been purchased and are in the construction phase. Once complete, the production capacity will be doubled. Greater production capacity will be addressed as needed.

VIII. INDEX OF RESOURCES

Links

The following items are linked to resources within this document as well as sourced from external sites.

Product Sales Sheets >>
Product Descriptions

ASU BSL3 Lab Results >>
Dr. Jeff Langland

Kill Rate Results >>
Microconsult

Product Safety Report >>
Dr. John Harbell

Benzalkonium Chloride study >>
Dr. John Harbell

Summary of Antimicrobial Effects >>
Dr. John Harbell

Platelet Technology White Paper >>

IX. CONCLUSIONS

The Hand Sanitizing Product Line Summary

Our revolutionary product line features cutting edge material science to achieve unparalleled killing power against a wide range of harmful microorganisms. Our MicroPlatelet technology coupled with copper and benzalkonium chloride provide for a highly effective product. Our water based solution removes the harmful side effects of alcohol based solutions while remaining a much safer product. Recent scientific studies conducted at highly reputable labs have shown products to kill the novel SARS-CoV-2 (COVID-19) virus with a 99.98% kill rate over for more than 4 hours and a the ability to kill *C. difficile* active cultures and spores as well as many other dangerous pathogens, all with a single application. We hope you now share our enthusiasm for these products and can help us work them into a more common usage. Together, we can help make a positive change possibly help reduce the spread of dangerous pathogens.

Charles Brown
CEO / Founder

Tim Johnson
COO / Founder

Product Sales Sheet

Reduce The Risk

YOUR SAFETY IS OUR PRIORITY

KILLS COVID19 GUARANTEED ∞

WHAT IS INVISIBLE GLOVE?

THE PROBLEM - Bacteria and viruses can survive on surfaces for many days infecting people without discrimination. The CDC recommends regular and thorough hand washing to help prevent the spread of disease, but hand washing only kills viruses already on your hands. You can become infected immediately after washing!

THE SOLUTION – A revolutionary bacteria and viral defense system developed by Impact Diversity Solutions provides protection against viruses in a one-step application that is alcohol-free.

INVISIBILE GLOVE uses a unique, proprietary, and patented mode of action that penetrates the epidermis, to provide protection against viruses. The copper and magnesium platelets utilized are safe for humans. These platelets disable viruses and bacteria by destroying their capsid envelope and disrupting their genetic code.

Organism	Inoculum Level (cfu/mL)	Growth Average (cfu/g)	Log^{10} Reduction
E. Coli	8.59×10^5	No Growth	5.93
MRSA	7.55×10^5	No Growth	5.88
P. aeruginosa	5.56×10^5	No Growth	5.75
B. cepacia	6.24×10^5	310	3.30
S. enterica	5.91×10^5	No Growth	5.77
E. faecalis	8.84×10^5	No Growth	5.95

Organism	Inoculum Level (cfu/mL)	Growth Average (cfu/g)	Log^{10} Reduction
K. pneumoniae	3.81×10^5	15	4.40
S. pyogenes	2.25×10^5	No Growth	5.41
L. monocytogenes	5.98×10^5	No Growth	5.78
C. jejuni	2.42×10^5	No Growth	5.38
C. difficile	2.40×10^5	No Growth	5.38
C. difficile (spores)	1.67×10^5	No Growth	5.22

* Kill Rate results after 30 seconds of exposure

PROVEN TO KILL
• COVID-19 • E. COLI • SALMONELLA
• MRSA • C. DIFFICILE • ... & MORE

IMPACT DIVERSITY SOLUTIONS

Contact Us :
http://www.impactdiversity.com
NDC: 77238-221-24

ASU BSL3 Lab Results

- Arizona State University Biodesign Institute
- Southwest College of Naturopathic Medicine & Health Sciences
- Dr. Jeffrey Langland, Research Director
- February 23, 2021

Project Name	Anti-SARS-CoV-2 properties of novel hand sanitizer solutions
Project Description	Characterization of the long-term anti-SAR-CoV-2 properties of novel hand sanitizer solutions
Project Lead	Southwest College of Naturopathic Medicine, Ric Scalzo Institute for Botanical Research. Dr. Jeffrey Langland, Research Director
Start Date	February 2021
Summary Date	February 23, 2021

Purpose
Characterize the long-term antimicrobial properties of Impact Diversity Solutions 2 in 1 Invisible Glove and Mask Spray against SARS-CoV-2

SARS-CoV2 Long-term killing assay:

Materials:

SARS-CoV-2 strain USA-WA1/2020 (BEI Resources)
Vero E6 cells (ATCC)
BSL3 facility (contract service with Arizona State University, Biodesign Institute)
D-MEM media with 10% fetal bovine serum (COMPLETE MEDIA)
PBS (phosphate buffered saline)

Stocks:

Vero cells were maintained in D-MEM media with 10% fetal bovine serum
All cells were used under limited passage conditions

SARS-CoV-2 virus stocks were grown in Vero cells under standard protocols. Viral titers were determined by plaque assay in Vero cells. Final stock titer was $3x10^7$ PFU/ml

Experimental procedure 1:

1. In the BSL2 tissue culture room, treat four 6-well tissue culture plates with 50 ul of each of the following solutions. Spread solution evenly with the large end of a sterile pipet tip.
    a. PBS
    b. 0.95 Glycerin solution

c. 2 in 1 Invisible Glove
d. Mask Spray

2. Remove the lid and air dry in the hood for 50 min. Cover and immediately bring into the BSL3 facility
3. Dilute SARS-CoV-2 virus stock to 10^5 PFU diluted into 100 ul with PBS
4. For TWO of the six-well plates that have dried for 1 hour, immediately add 100 ul virus solution per well and spread over the surface by rocking. Rock every 5 min.
5. After 25 minutes, add 0.4 ml complete media to each well, pipet/wash over the well 5-times, and transfer the solution to a sterile tube for subsequent titering.
6. For the remaining TWO plates, at 4 hours (1 hour drying + 3 hours dish sitting in the hood), repeat steps 4-5.
7. For the 24 samples total (6 in duplicate at 1 hr, and 6 in duplicate at 4 hr), perform serial dilutions (undiluted, 1:10, 1:100, 1:1000 in complete media). Titer each virus sample by plaque assay on Vero cells by standard protocols

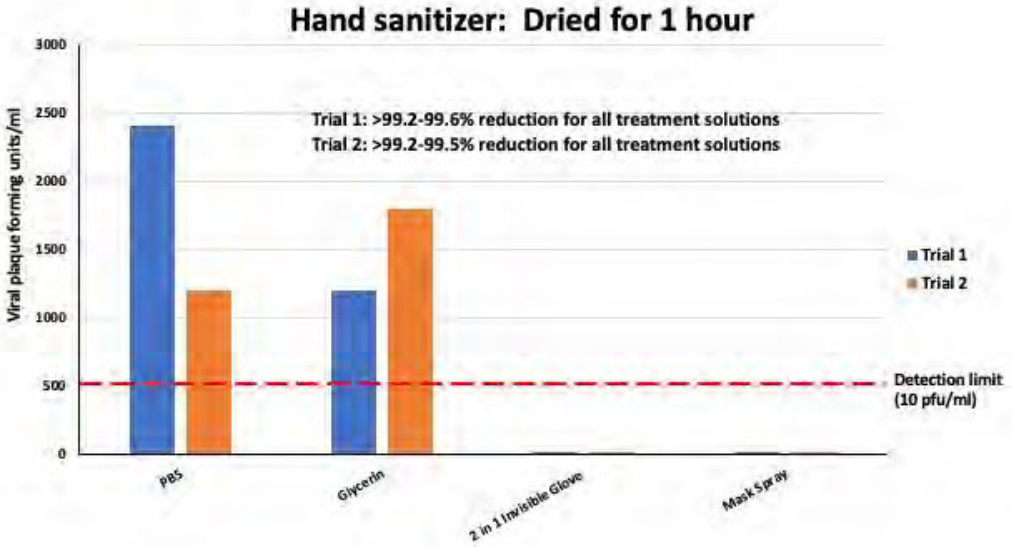
Experimental procedure 2:

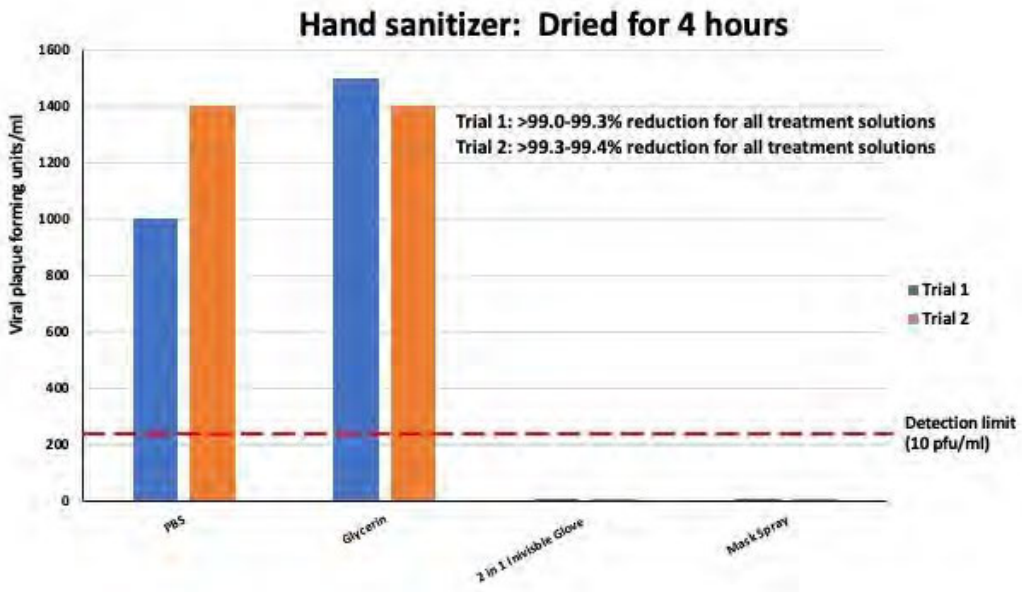
1. In the BSL2 tissue culture room, treat two sets of 6-well tissue culture plates with 50 ul of each of the following solutions. Spread solution evenly with the large end of a sterile pipet tip.
    a. PBS
    b. 0.95 Glycerin solution
    c. 2 in 1 Invisible Glove
    d. Mask spray
    e. 2 in 1 Invisible Glove (diluted 1:5 in PBS)
    f. Mask Spray (diluted 1:5 in PBS)

2. Remove the lid and air dry in the hood for 50 min. Cover and immediately bring into the BSL3 facility
3. Dilute SARS-CoV-2 virus stock to 10^5 PFU diluted into 100 ul with PBS
4. For TWO of the six-well plates that have dried for 1 hour, immediately add 100 ul virus solution per well and spread over the surface by rocking. Rock every 5 min.
5. After 25 minutes, add 0.4 ml complete media to each well, pipet/wash over the well 5-times, and transfer the solution to a sterile tube for subsequent titering.
6. For the remaining TWO plates, at 4 hours (1 hour drying + 3 hours dish sitting in the hood), repeat steps 4-5.
7. For the 20 samples total (10 for the 1 hr, and 10 for the 4 hr), perform serial dilutions (undiluted, 1:10, 1:100, 1:1000 in complete media). Titer each virus sample by plaque assay on Vero cells by standard protocols

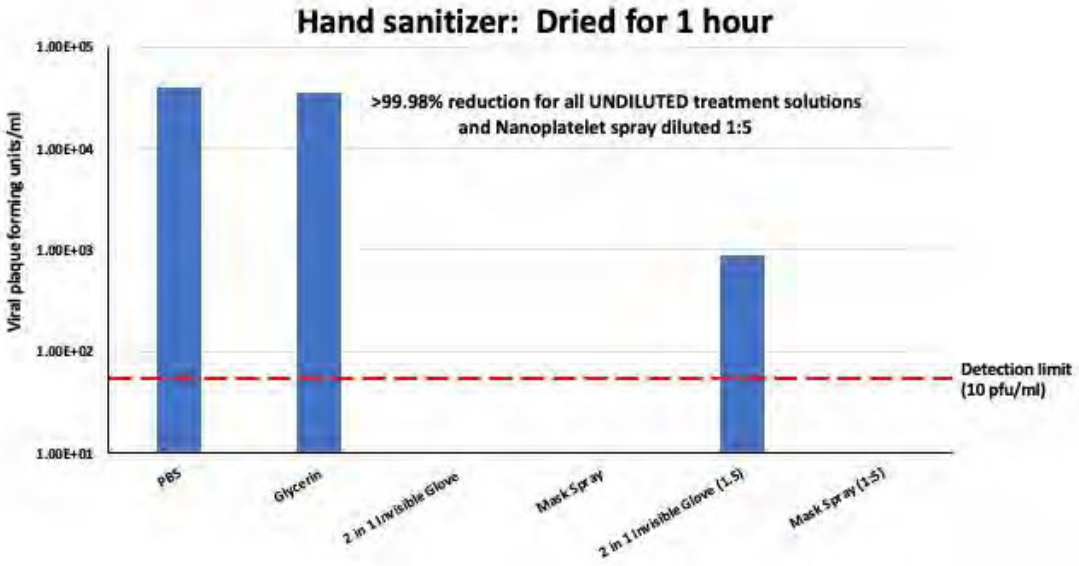
Project results:

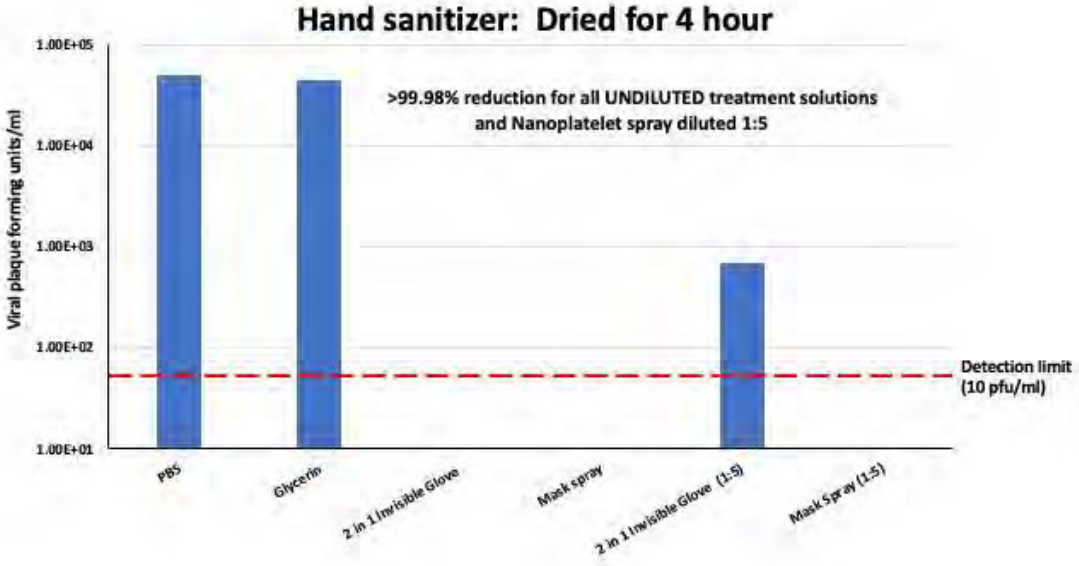
Experimental study 1:





Experimental study 2:


Hand sanitizer: Dried for 1 hour
Viral plaque forming units/ml
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
>99.98% reduction for all UNDILUTED treatment solutions and Nanoplatelet spray diluted 1:5
Detection limit (10 pfu/ml)
PBS
Glycerin
2 in 1 Invisible Glove
Mask Spray
2 in 1 Invisible Glove (1:5)
Mask Spray (1:5)


Hand sanitizer: Dried for 4 hour
Viral plaque forming units/ml
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
>99.98% reduction for all UNDILUTED treatment solutions and Nanoplatelet spray diluted 1:5
Detection limit (10 pfu/ml)
PBS
Glycerin
2 in 1 Invisible Glove
Mask spray
2 in 1 Invisible Glove (1:5)
Mask Spray (1:5)

Results summary:

The results of this study support that the hand sanitizer solutions containing 2 in 1 Invisible Glove and Mask Spray are all able to kill the SARS-CoV-2 virus even after drying on a surface for 1 hour or 4 hours.

In the first study, negative control samples (surface treated with PBS or glycerin) had a non-inhibitory effect on the virus with the presence of approximately 1000-2500 virus/ml. When this amount of virus was applied to surfaces treated with any of the hand sanitizer solutions, no virus could be detected in the samples. The detection limit of this assay was 10 virus/ml. Similar results were observed when the hand sanitizer treatments were left on the surface for 1 hour or 4 hours, indicating no loss of virus killing activity over this time period. The results support that these hand sanitizer solutions remain active related to anti-SARS-CoV-2 activity for up to 4 hours on a surface and provide over a 99.0% virus killing response.

In the second study, negative control samples (surface treated with PBS or glycerin) had a non-inhibitory effect on the virus with the presence of approximately 30,000 virus/ml. When this amount of virus was applied to surfaces treated with any of the undiluted hand sanitizer solutions, no virus could be detected in the samples. The detection limit of this assay was 10 virus/ml. Similar results were observed when the hand sanitizer treatments were left on the surface for 1 hour or 4 hours, indicating no loss of virus killing activity over this time period. The results support that these hand sanitizer solutions remain active related to anti-SARS-CoV-2 activity for up to 4 hours on a surface and provide over a 99.98% virus killing response.

In the second study, when the hand sanitizer solutions were diluted 1:5 in PBS and then applied to the surface, virus killing was approximately 90-95% for the hand sanitizer solution containing 2 in 1 Invisible Glove. The Mask Spray diluted 1:5 in PBS was still able to kill with over a 99.98% virus killing response.

Certification:

Experimental design and analysis were conducted at the Southwest College of Naturopathic Medicine, Ric Scalzo Institute for Botanical Research under the guidance and supervision of Dr. Jeffrey Langland, Research Director. Experimental procedures were performed at the Arizona State University Biodesign Institute, Biosafety Level 3 facility.

Results are certified as valid based on experimental procedures performed

Jeffrey Langland
Research Director
Southwest College of Naturopathic Medicine
Ric Scalzo Institute for Botanical Research

SCNM
SOUTHWEST COLLEGE OF NATUROPATHIC MEDICINE & HEALTH SCIENCES

Microconsult Testing

- Microbiological & Analytical Testing Laboratory
- Covexall Kill Rate Test for 11 Microorganisms
- Microconsult, Inc., Carrolton, TX
- September 15, 2020

Clostridium difficile *

Introduction

Infection of the colon with the Gram-positive bacterium *Clostridium difficile* is potentially life-threatening infection. *C. difficile* is the leading cause of health-care-associated infective diarrhea. The factors responsible for the epidemic of some *C. difficile* strains are poorly understood. Recurrent infections are common and can be debilitating. Infections are commonly treated with specific antimicrobial agents, but prevention is key to reducing the significant cost, morbidity, and mortality of these infections.

It was not until the 1970s that a detailed characterization of the bacterium, then called *C. difficile*, revealed its involvement in human disease. This disease became widely known as *C. difficile*-associated diarrhea, or *C. difficile* Infection (CDI). In the early 2000s, an increase in severe cases of CDI was noted in Canada, the United States and Europe, which was attributed to the emergence of certain epidemic types of *C. difficile*. *C. difficile* is now recognized as the leading cause of health-care-associated infective diarrhea and is increasingly being linked to community-acquired cases of colitis. *C. difficile* can be found in the intestinal tracts of both humans and animals, but its spores are also ubiquitous in the environment and can be isolated from food. When *C. difficile* germs are outside the body, they become spores. These spores are an inactive form of the germ and have a protective coating allowing them to live for months or sometimes years on surfaces and in the soil. The germs become active again when these spores are swallowed and reach the intestines.

The US Centers for Disease Control and Prevention (CDC) estimated that almost 500,000 patients had CDI, with 29,000 attributable deaths in the United States in 2011. Between 2001 and 2010, the incidence of CDI among hospitalized adults in the United States approximately doubled according to International Classification of Diseases (ICD)-9 discharge diagnoses. The all-cause mortality associated with CDI due to non-epidemic PCR ribotypes has been reported to be ~15–20% within a month of diagnosis. Given the multiple comorbidities (such as respiratory disease and renal failure) typically present in patients with CDI, mortality is often related to one or more of these conditions.

C. difficile lifecycle

C. difficile is transmitted via the oral–fecal route. Spores are dormant cells that are highly resistant to environmental conditions, including some disinfectants and many antimicrobials, which generally target metabolically active cells. Spores are thought to be the infectious vehicle given that vegetative (metabolically active) cells of obligate anaerobic bacteria are unlikely to survive the oxygenated environment outside the host or the acidic environment of the

stomach. Indeed, an asporogenic strain of *C. difficile* is unable to persist in the environment or transmit between hosts.
Most antimicrobial compounds target metabolically active cells and have limited or no activity against dormant cells, such as spores. This intrinsic resistance of spores ensures that Clostridium difficile can persist in the presence of antibiotics or in the host immune system. *C. difficile* also demonstrates extensive acquired antimicrobial resistance.

Symptoms and risk factors

The clinical symptoms associated with CDI range from mild, self-limiting diarrhea to fulminant colitis, and can include pseudomembranous colitis, toxic megacolon (severe dilatation of the colon), bowel perforation and sepsis, and/or multiple organ dysfunction syndrome. Toxic megacolon is considered the most serious disease entity and is characterized by systemic toxicity and high mortality. Known risk factors for CDI are previous hospitalization, underlying disease, advanced age (>65 years) and, most importantly, the use of antibiotics.

In a health-care setting, transmission of *C. difficile* spores occurs primarily via the contaminated hands of health-care workers, but contact with a contaminated environment, contaminated utensils or medical devices has also been implicated; *C. difficile* spores have been identified in rooms of patients who have tested negative. Environmental decontamination of clinical areas, ideally using chlorine-releasing agents or a sporicidal product, is recommended; however, in practice, compliance with cleaning protocols is often suboptimal. Newer alternatives for environmental decontamination have been introduced, notably gaseous hydrogen peroxide and, more recently, UV decontamination. The former is effective at killing *C. difficile* spores, but the cost-effectiveness of these approaches is unclear.

Economic burden of CDI

The burden of health-care-associated CDI can be expressed in terms of mortality, recurrence, (additional) length of hospital stay or economic cost. Economic analyses of healthcare-associated CDI have shown that direct health-care cost, and costs due to increased length of stay, were the main cost drivers. An integrative review showed a wide variation in the difference in length of stay between people with and those without CDI (2.8–16.1 days), which was attributed to differences in design and data collection. Overall, people with CDI stay longer in hospital than people without CDI despite this variation. A recent meta-analysis identified a total of 45 studies (mostly from North America) that measured the economic impact of CDI. For hospitalized patients, attributable mean CDI costs ranged from $8,911 to $30,049. Estimates for the economic burden of CDI in the United States and Canada are more than US $1 billion and CAN $280 million, respectively. These figures do not include the indirect socioeconomic costs.

Prevention

According to the CDC:

> "Because alcohol does not kill *C. diff* spores, use of soap and water is more effective than alcohol-based hand rubs. However, early experimental data suggest that, even using soap and water, the removal of *C. diff* spores is more challenging than the removal or inactivation of other common pathogens."

Alcohol-based sanitizers are ineffective against *C. difficile* spores either when wet or dry. Furthermore, soap and water actually don't destroy them either but do help decrease the number on your hands by dilution and physically removing them down the drain. The CDC recommends soap and water currently after removing gloves when working with CDI patients, but cautions that more complex hand sanitization protocols may result in poorer compliance. The CDC currently estimates hospital staff have about a 50% failure rate at hand cleaning protocol compliance.

Impact Diversity Solutions products, however, provide a simple and effective alternative. These products do show actual killing of the spore-form via novel technology that works differently than regular antibiotics, so resistance is as unlikely as with alcohol-based sanitizers and surface disinfection. However, they work without any toxicity to humans or animals unlike current surface cleaners used for disinfection. Unlike other hand sanitizers, they are glycerin based and spread easily and completely with a 1ml application, even on medium to large hands and destroy the spore-form along with all other pathogens that alcohol-based sanitizers also cover. They have also been tested at 4 hours after application and shown to still destroy Covid-19 and *S. aureus*, with further pathogens awaiting testing at the 4-hour time frame.

*The preceding was a brief summary of the following two publicly-available articles and they should be read for more details and references. **Clostridium difficile infection**: Nat Rev Dis Primers. ; 2: 16020. doi:10.1038/nrdp.2016.20, Clostridium difficile infection (nih.gov) and **FAQs for Clinicians about *C. diff*,** FAQs for Clinicians about C. diff | CDC

Kill Rate Test – Hand Sanitizer Lot # 1152

Summary:

DGH Hand sanitizer was tested in a Kill Rate Study using eleven bacterial species (Microconsult Report 1 September 2020). The exposure times were 30 and 60 seconds. The 30 second exposure killed all of the organisms (> 10^5 cfu/mL) from nine of the species and greater that three $\log_{10}$ from the other two. The 60 second exposure killed all of the organisms from all eleven species. A second Kill Rate Study was performed on the spore stage of C. difficile using the same exposure times (Microconsult Report 15 September 2020). Both the 30 second and 60 second exposers showed complete kill of the test organisms. These data show a very high degree of efficacy suggesting that this hand sanitizer could have a strong impact on bacterial transmission. The action against the spores of C. difficile is particularly remarkable.

This summary report was compiled from two Kill Rate Study reports issued by Microconsult, Inc. 1 September 2020 (11 organisms) and 15 September 2020 (C. difficile in the spore stage).

Objective:
To demonstrate the antibacterial properties of the test product against a selection of gram positive and gran negative bacteria.

References:

A. 21 CFR 333. Topical antibacterial products for over-the-counter human use.
B. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics: Topical antimicrobial drug products for over-the-counter human use; proposed amendment of the tentative final monograph. Section V. Comments on the Proposed Rule and FDA Response Subsection C. Comments on Effectiveness and FDA Response [list of test organisms for *in vitro* efficacy testing]
C. Microconsult, Inc. Test Method 011_00 Kill Rate Testing

Test Article: Labeled as Hand Sanitizer, lot # 1152 [redacted]

Test Organisms
The list of test organisms, their American Type Culture Collection (ATCC) numbers, source and short names (see Table 2) are provided in Table 1. Active cultures of the test organisms were maintained by the laboratory and renewed from the reference stock after five passages. *Campylobacter jejuni* and *Clostridium difficile* were maintained under anaerobic culture conditions. To kill the C. difficile vegetative organisms, the 24-hour growth plate was treated quickly with 70% isopropyl alcohol to yield the spore form cells for the second study.

Table 1 List of organisms tested

Organism	ATCC Number	Source	Short Name
Escherichia coli	8739	Microbiologics	*E. coli*
Methicillin-resistant (MRSA) Staphylococcus aureus	33591	Microbiologics	*MRSA*
Pseudomonas aeruginosa	24853	Microbiologics	*P. aeruginosa*
Burkholderia cepacia	25416	Microbiologics	*B. cepacia*
Salmonella enterica	14028	Microbiologics	*S. enterica*
Enterococcus faecalis	51575	Microbiologics	*E. faecalis*
Klebsiella pneumoniae	700603	Microbiologics	*K. pneumoniae*
Streptococcus pyogenes	19615	Microbiologics	*S. pyogenes*
Listeria monocytogenes	SLR2249	Microbiologics	*L. monocytogenes*
*Campylobacter jejuni**	49943	Microbiologics	*C. jejuni*
*Clostridium difficile**	9689	Microbiologics	*C. difficile*

*Anaerobes

Reagents:
Tryptic Soy Agar with Lecithin and Tween 80
Sterile Phosphate Buffered Saline (for diluting)
DE Neutralizing Broth: Dey-Engley Neutralizing Broth is intended to stop the action of the antimicrobial preparation at the end of the exposure period. It is formulated to neutralize several types of antibacterial active ingredients including benzalkonium chloride.

Procedure:

1. Prepare each bacterial culture, inoculate the growth medium (broth) with the actively growing bacteria and allow to grow at 30-35ºC for 24-48 hours. These suspension cultures will be used to determine the antibacterial activity of the test article against the specific bacterium. Eleven such suspension cultures were prepared, one for each organism. These cultures were incubated for 24 to 48 hours to obtain the desired bacterial titers. At this point the number of organisms per mL (cfu/mL) was determined and the same cultures were used to challenge the test article. It should be understood that the exact number of organisms in the inoculum will not be known until step 2 is completed.

2. To obtain the number of viable microorganisms (colony forming units per mL [cfu/mL]), a sample was removed and diluted in sterile phosphate buffered saline. Subsequent serial

dilutions were prepared from this sample to seed test plates with dilutions of 10^{-6} and 10^{-7} of the original suspension. Each test plate was then filled with 20 mL of 45°C Tryptic Soy Agar, swirled to mix and then allowed to harden. The plates were incubated for 24 – 48 hours to allow the viable bacteria to form colonies in the agar. The bacterial colonies were counted and the number of colony forming units per mL in the original inoculum determined. The number of cfu/mL in the inoculum was then calculated to determine the number in the test samples using the formula below:

$$\frac{(\text{cfu/mL inoculum}) \times (\text{volume added to the test article sample})}{\text{Weight of test article (g)}} = \text{cfu/g product}$$

$$\frac{(\text{cfu/mL inoculum}) \times (0.1 \text{ mL})}{9.9 \text{ (g)}} = \text{cfu/g of test article}$$

3. Samples of test article were prepared for inoculation with each bacterium. A volume of 9.9 mL was measured out into properly labeled test tube. These tubes were held at room temperature until the bacteria were added.
4. Inoculation of the test article with the bacterial inoculum was performed by adding 0.1 mL of the bacterial inoculum to the tube holding 9.9 mL of the test article. The tube was mixed and then allowed to stand for the time of the first incubation period (30 seconds). At that point one mL of the test article-inoculum mixture was removed a placed immediately into 9.0 mL of DE Neutralizing solution to stop the action of the test article. After the second incubation period (60 seconds) a second sample of one mL was taken and added to a second tube containing 9.0 mL of DE Neutralizing solution. This process was repeated for each of the bacteria tested.
5. Each suspension of bacteria in the DE Neutralizing solution was serially diluted (1:10) in duplicate in phosphate buffer to prepare dilutions of 10^{-1} to 10^{-5}.
6. One mL of each dilution was transferred to a prelabeled 100 x 15 mm petri plate.
7. Each plate was overlaid with 20 mL of melted (45°C) Tryptic Soy Agar and the plate gently swirled to mix the bacteria with the agar. The plates were then allowed to harden,
8. The inoculated plates were placed into an incubator at 30-35°C for 48 to 72 hours. Again, the *C. jejuni* and *C. difficile* plates were incubated under anaerobic conditions.
9. At the end of the incubation period, the number of colonies in each plate was counted. From the count value and the dilution of the original sample, the number of colony forming units remaining in the treated samples was calculated
10. The $\log_{10}$ reduction was calculated from ratio $\log_{10}$ of the inoculum to the log10 of the remaining colony forming units after treatment. For example:

For the E. coli sample treated for 30 seconds, the $\log_{10}$ inoculum of bacteria was 5.93/mL and the number of colony forming units after treatment was zero. The zero value is converted to one

which has a $\log_{10}$ of zero. The $\log_{10}$ reduction is 5.93-0 = 5.93. A second example shows the case where there was some survival at 30 seconds of exposure. *B. cepacia* had an initial inoculum of $6.24x10^5$ cfu/ml ($\log_{10}$ is 5.80). At 30 seconds of exposure, 310 cfu/mL ($\log_{10}$ 310 is 2.49) remained viable. The $\log_{10}$ reduction was 5.80-2.49 = 3.30.

The $\log_{10}$ reduction for each bacterium at each of the two exposure times is shown in Table 2.

Table 2 Log reduction of viable cfu/mL

Organism (Exposure Time)	Inoculum Level (cfu/mL)	Growth Average (cfu/g)	Log_{10} Reduction
E. coli (30 seconds)	8.59×10^5	No Growth	5.93
E. coli (60 seconds)	8.59×10^5	No Growth	5.93
MRSA (30 seconds)	7.55×10^5	No Growth	5.88
MRSA (60 seconds)	7.55×10^5	No Growth	5.88
P. aeruginosa (30 seconds)	5.56×10^5	No Growth	5.75
P. aeruginosa (60 seconds)	5.56×10^5	No Growth	5.75
B. cepacia (30 seconds)	6.24×10^5	310	3.30
B. cepacia (60 seconds)	6.24×10^5	No Growth	5.8
S. enterica (30 seconds)	5.91×10^5	No Growth	5.77
S. enterica (60 seconds)	5.91×10^5	No Growth	5.77
E. faecalis (30 seconds)	8.84×10^5	No Growth	5.95
E. faecalis (60 seconds)	8.84×10^5	No Growth	5.95
K. pneumoniae (30 seconds)	3.81×10^5	15	4.40
K. pneumoniae (60 seconds)	3.81×10^5	No Growth	5.58
S. pyogenes (30 seconds)	2.25×10^5	No Growth	5.41
S. pyogenes (60 seconds)	2.25×10^5	No Growth	5.41
L. monocytogenes (30 seconds)	5.98×10^5	No Growth	5.78
L. monocytogenes (60 seconds)	5.98×10^5	No Growth	5.78
C. jejuni (30 seconds)	2.42×10^5	No Growth	5.38
C. jejuni (60 seconds)	2.42×10^5	No Growth	5.38
C. difficile (30 seconds)	2.40×10^5	No Growth	5.38
C. difficile (60 seconds)	2.40×10^5	No Growth	5.38
C. difficile (Spore form) (30 seconds)	1.67×10^5	No Growth	5.22
C. difficile (Spore form) (60 seconds)	1.67×10^5	No Growth	5.22

Discussion:

As shown in Table 2, most of the bacterial species tested were completely killed with the 30 second exposure and all were completely killed with a 60 second exposure. 21 CFR 333 Topical antibacterial products for over-the-counter human use calls for a two $\log_{10}$ reduction in viability for a product to be considered antibacterial. This regulation applied to topical antiseptics. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics calls for a three $\log_{10}$ reduction in viability for a hand rub (hand sanitizer) to be considered to have antibacterial efficacy. This hand sanitizer achieved a three $\log_{10}$ kill with a 30 second exposure and complete kill with a sixty second exposure for all eleven species tested. Of particular interest was the activity against C. difficile spores. Complete kill of the 1.67×10^5 cfu/mL inoculum was achieved with a 30 second exposure.

This study was performed at Microconsult, Inc. Carrollton, TX under the direction of Alix Paulson, Microbiology Technician II September 2020.

Summary prepared by John W. Harbell, Ph.D.

Product Safety Report

- Covexall
- Hand Sanitizer
- March 29, 2020

Product Safety Report

Product: Antibacterial Hand Sanitizer Glove – [redacted]

Formula number 32011.9

[redacted] **INC**

[redacted]

Responsible Person:
Greg Pilant: [redacted]

Date:

July 14, 2020

Prepared by:

John W. Harbell, Ph.D.

Summary

An ingredient safety review has been completed on [redacted] Antibacterial Hand Sanitizer Glove – [redacted] Inactive Ingredients

Part A-Product safety information

1. Quantitative and qualitative composition of the product [redacted] Antibacterial Hand Sanitizer Glove – [redacted]

   The individual ingredients, their concentrations and reported functions for [redacted] Antibacterial Hand Sanitizer Glove – [redacted] are provided in Table 1.1.

Table 1 Composition of [redacted] Antibacterial Hand Sanitizer Glove – [redacted]

2. Physical/chemical characteristic and stability of the cosmetic product

Table 2.1 provides a listing of the ingredients in [redacted] Antibacterial Hand Sanitizer Glove – [redacted] by Trade name and Supplier as well as physical state (liquid or solid).

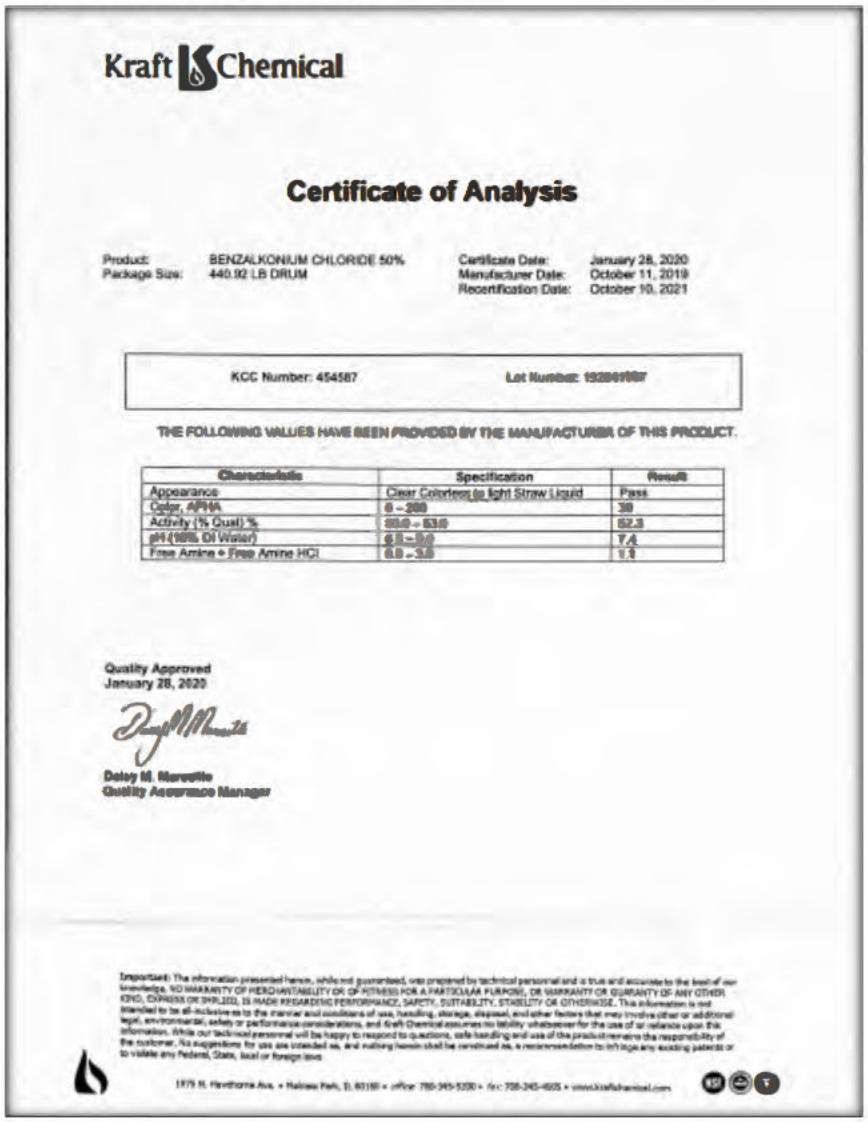
Table 2.1 Ingredients, by descending order of concentration, showing trade names, suppliers, physical state and ingredient source material for [redacted] Antibacterial Hand Sanitizer Glove – [redacted]

Number	Description	%(w/w)	Trade Name	Source	Physical State	Ingredient Source
	Active ingredient					
1	Benzalkonium Chloride, 50%	0.10%	Benzalkonium Chloride, 50%	Kraft Chemicals	Liquid	Synthetic
	Inactive Ingredients					
1	Purified Water	94.3357%	Water	In House	Liquid	Natural
2	Polyethylene Glycol 4000	1.5960%	Polyethylene Glycol (PEG) 4000	Dow	Solid	Synthtic
3	Polyethylene Glycol 400	1.1970%	CARBOWAX™ SENTRY™	Dow	Liquid	Synthetic
4	Glycerin, 99.5%	1.1970%	Glycerin USP (95.5%)	VVF Illinois Services	Liquid	Synthetic
5	Hydroxyethylcellulose	0.6500%	Natrosol 25HHR-CS	Kraft Chemicals	Powder	Synthetic
6	Phenoxyethanol	0.4000%	Phenoxyethanol	Acme-Hardesty Co	Liquid	Synthetic
7	Polysorbate 20	0.1500%	Polysorbate 20	MakingCosmetics	Liquid	Synthetic
8	Micro Magnesium Hydroxide, 10%	0.1343%	Magnesium Hydroxide Slurry	Aqua Resources	Suspension	Synthetic
9	Citrus Sinensis (Orange) Fruit Oil	0.1200%	Orange Oil 1-fold	MCI Miritz	Liquid	Natural
10	Copper (II) Chloride, Dihydrate	0.1200%	Cupric chloride dehydrate USP	Spectrum Chemical	Powder	Synthetic

Stability data will be added when they become available.

Table 2.2 Details the specifications for [redacted] Antibacterial Hand Sanitizer Glove – [redacted]

Benzalkonium Chloride, 50%



Kraft Chemical

Certificate of Analysis

Product: BENZALKONIUM CHLORIDE 50%
Package Size: 440.92 LB DRUM

Certificate Date: January 28, 2020
Manufacturer Date: October 11, 2019
Recertification Date: October 10, 2021

KCC Number: 454587 Lot Number: [redacted]

THE FOLLOWING VALUES HAVE BEEN PROVIDED BY THE MANUFACTURER OF THIS PRODUCT.

Characteristic	Specification	Result
Appearance	Clear Colorless to light Straw Liquid	Pass
Color, APHA	0 – 200	30
Activity (% Quat) %	49.0 – 53.0	52.3
pH (10% DI Water)	6.0 – 9.0	7.4
Free Amine + Free Amine HCl	0.0 – 3.0	1.9

Quality Approved
January 28, 2020

Daisy M. Marcotte
Quality Assurance Manager

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1-Water-In House

RAW MATERIAL SPECIFICATION / TESTING STANDARD **RM Lot: _____**

Item: Purified Water, USP

Stock Code: C1125 **RM Spec: 1125-01** Date: 2-1-20

INCI: Water

Description: Water produced by further processing Memphis City drinking water with carbon filtration and mixed bed ion-exchange resins so that it meets the requirements for USP Purified Water

Approval requirements:

Approved Sources: Purchase water meeting the requirements or produce on site using suitable means.

Storage Conditions: When packaged, preserve in unreactive storage containers that are designed to prevent microbial entry.

Retest Interval: Retest conductivity and aerobic plate count after 7 days

Attribute	Specification	Method(s)	Observation	Result (Pass/Fail)	By / Date
Appearance	Clear, Colorless Liquid	Organoleptic			
Color	Colorless	Organoleptic			
Odor	Odorless	Organoleptic			
TOC	< 0.10 mg/L	TOC Instrument			
Conductivity	Meets <643> for Stage I, II or III	Conductivity Meter			
Bacteria	<100 cfu/g	Aerobic Plate Count			
Objectionable Bacteria	Absence of S. Aureus and P. Aeruginosa	Type-specific agar – 1 g or ml sample			
Combined Yeast & Mold	<10 cfu/g	Sabouraud-Dex Agar			

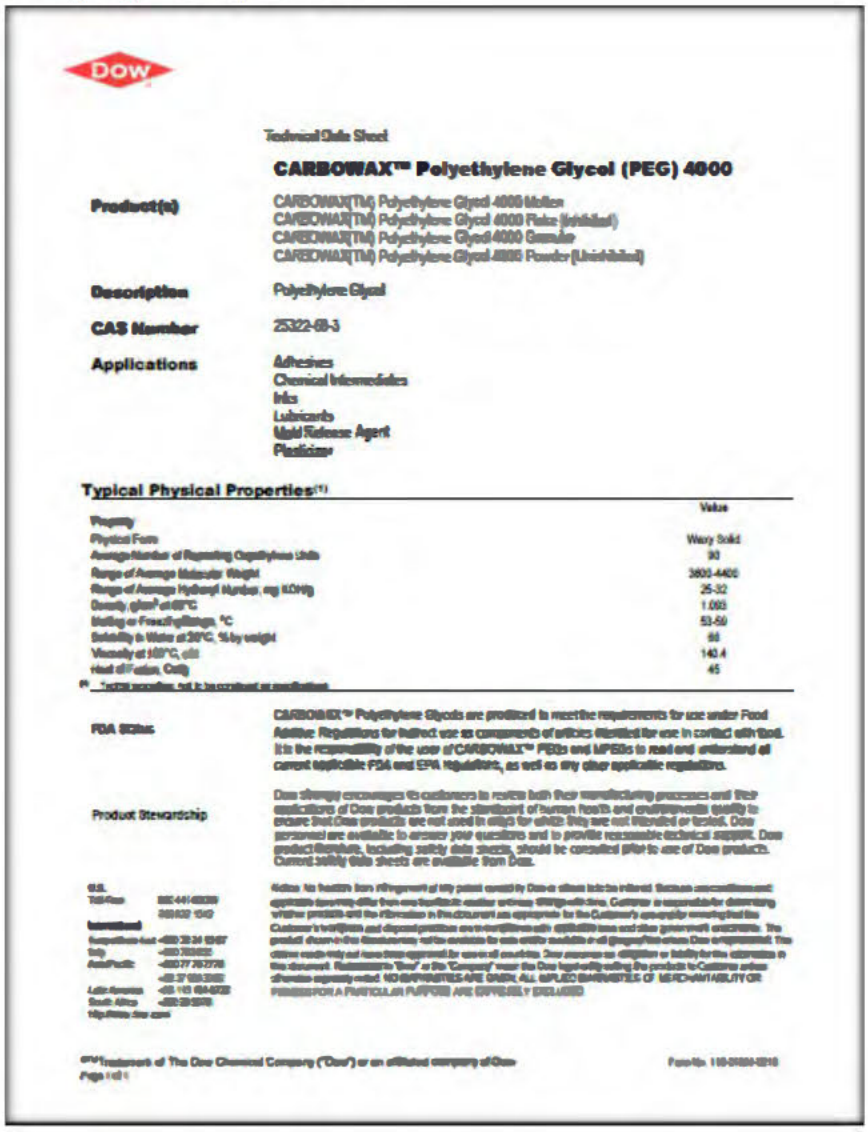
Raw Material Mfg: _____ Mfg Lot Number: _____

Date Received: _____ Quantity Received: _____

Disposition: _____ Approved for Use _____ Not Approved for Use Retain Location: _____

By: _____ Date: _____

2-Polyethylene Glycol 4000



Technical Data Sheet

CARBOWAX™ Polyethylene Glycol (PEG) 4000

Product(s)

CARBOWAX(TM) Polyethylene Glycol 4000 Molten
CARBOWAX(TM) Polyethylene Glycol 4000 Flake (Inhibited)
CARBOWAX(TM) Polyethylene Glycol 4000 Granular
CARBOWAX(TM) Polyethylene Glycol 4000 Powder (Uninhibited)

Description

Polyethylene Glycol

CAS Number

25322-68-3

Applications

Adhesives
Chemical Intermediates
Inks
Lubricants
Mold Release Agent
Plasticizer

Typical Physical Properties[1]

Property	Value
Physical Form	Waxy Solid
Average Number of Repeating Oxyethylene Units	90
Range of Average Molecular Weight	3600-4400
Range of Average Hydroxyl Number, mg KOH/g	25-32
Density, g/cm³ at 60°C	1.060
Melting or Freezing Range, °C	53-59
Solubility in Water at 20°C, % by weight	60
Viscosity at 100°C, cSt	140.4
Heat of Fusion, Cal/g	45

[1] Typical properties; not to be construed as specifications

FDA Status

CARBOWAX™ Polyethylene Glycols are produced to meet the requirements for use under Food Additive Regulations for indirect use as components of articles intended for use in contact with food. It is the responsibility of the user of CARBOWAX™ PEGs and MPEGs to read and understand all current applicable FDA and EPA regulations, as well as any other applicable regulations.

Product Stewardship

Dow strongly encourages its customers to review both their manufacturing processes and their applications of Dow products from the standpoint of human health and environmental quality to ensure that Dow products are not used in ways for which they are not intended or tested. Dow personnel are available to answer your questions and to provide reasonable technical support. Dow product literature, including safety data sheets, should be consulted prior to use of Dow products. Current safety data sheets are available from Dow.

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International
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Italy - [redacted]
Asia Pacific - [redacted]
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Form No. 110-01830-1210

3- Polyethylene Glycol 400

DOW

Technical Data Sheet

CARBOWAX™ SENTRY™ Polyethylene Glycol (PEG) 400

Product	CARBOWAX™ SENTRY™ Polyethylene Glycol 400 NF; Macrogol 400 Ph. Eur.
Description	Polyethylene Glycol
CAS Number	25322-68-3
Applications	Adhesives Antistatic Agent and Humectant Chemical Intermediates Inks and Dye Carrier Lubricants Mold Release Agent Plasticizer

Typical Physical Properties – CARBOWAX™ PEG 400[1]

Property	Value
Physical Form	Liquid
Average Number of Repeating Oxyethylene Units	8.7
Range of Average Molecular Weight	380-420
Range of Average Hydroxyl Number, mg KOH/g	264-300
Density, g/cm³ at 20°C	1.125
Melting or Freezing Range, °C	4-8
Solubility in Water at 20°C, % by weight	Complete
Viscosity at 100°C, cSt	7.3
Heat of Fusion, Cal/g	38

(1) Typical properties, not to be construed as specifications

FDA Status	CARBOWAX™ Polyethylene Glycols are produced to meet the requirements for use under Food Additive Regulations for indirect use as components of articles intended for use in contact with food. It is the responsibility of the user of CARBOWAX™ PEGs and MPEGs to read and understand all current applicable FDA and EPA regulations, as well as any other applicable regulations.
Product Stewardship	Dow strongly encourages its customers to review both their manufacturing processes and their applications of Dow products from the standpoint of human health and environmental quality to ensure that Dow products are not used in ways for which they are not intended or tested. Dow personnel are available to answer your questions and to provide reasonable technical support. Dow product literature, including safety data sheets, should be consulted prior to use of Dow products. Current safety data sheets are available from Dow.

[illegible]

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Page 1 of 1

4- Glycerin, 99.5%

VVF Biofuels Services, LLC

Industrial Chemicals
Product Data Sheet

Glycerin, USP, 99.50 %

Generic Name: Glycerin, Glycerol **CAS No.** 56-81-5

Chemical Name: 1,2,3-Propanetriol

Formula: $C_3H_5(OH)_3$ **Molecular Wt.:** 92.09

Description: A clear, virtually water white, viscous hygroscopic liquid, concentrated and purified from crude glycerin recovered from natural fats and oils splitting processing. Meets or exceeds the stringent quality standards established in the USP (United States Pharmacopeia - USP 32, 2009) monograph, Glycerin.

Product Specifications:

Property	Specification
Identification A: Infrared Absorption	To Match Standard
Identification B: Gas Chromatography	To Match Standard
Identification C: Reinsch Test	To Match Standard
Specific Gravity (SG) @ 25°/25°C	1.26073 Min.
Glycerin, %	99.50 Min.
Color, APHA (Spectrocolorimeter)	20 Max.
Residue on Ignition, %	0.01 Max.
Chloride, %	0.001 Max.
Sulfate, %	0.002 Max.
Heavy Metals, ppm	5.0 Max.
Chlorinated Compounds,%	0.005 Max.
Related Compounds	Meet U.S.P.
Fatty Acids and Esters (FAsE)	1.0 ml 0.5N NaOH/50 gms Max.
Water, % (Karl Fischer)	1.50 Max.

Note: Results on individual shipments will vary within the specifications. Specifications are subject to change. Please contact us to confirm current information.

5- Hydroxyethylcellulose

Kraft Chemical

Certificate of Analysis

Product: NATROSOL 250 HHR
Package Size: 50 LB BAG

Certificate Date: April 9, 2020

KCC Number: 200940 Lot Number: 19585

THE FOLLOWING VALUES HAVE BEEN PROVIDED BY THE MANUFACTURER OF THIS PRODUCT.

Characteristic	Specification	Result
Loss on Drying, as packaged, %	0.0 – 5.0	3.6
Aqualon 8.0 pH Hydration, min	6 – 25	15
Viscosity, 1% Water, 4@30, 25C, cps	3400 – 5000	4630
pH of Viscosity Solution	6.0 – 8.5	6.9
Ash, as Na2SO4, Electrode, %	0.0 – 5.5	3.0
Aqueous Grit Test, Count		14

Quality Approved
April 9, 2020

Daisy M. Marcelle
Quality Assurance Manager

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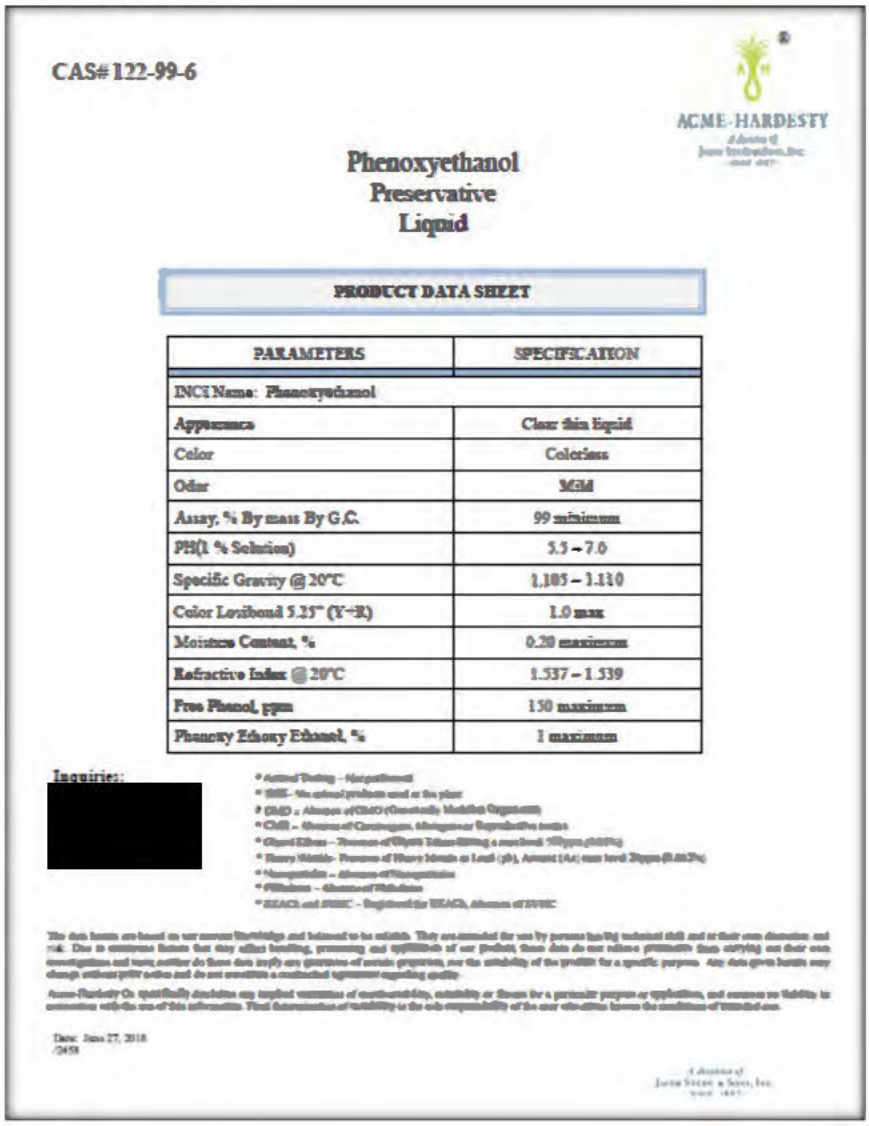
6- Phenoxyethanol



CAS#122-99-6

ACME-HARDESTY

Phenoxyethanol
Preservative
Liquid

PRODUCT DATA SHEET

PARAMETERS	SPECIFICATION
INCI Name: Phenoxyethanol	
Appearance	Clear thin liquid
Color	Colorless
Odor	Mild
Assay, % By mass By G.C.	99 minimum
PH(1 % Solution)	5.5 – 7.0
Specific Gravity @ 20°C	1.105 – 1.110
Color Lovibond 5.25" (Y+R)	1.0 max
Moisture Content, %	0.20 maximum
Refractive Index @ 20°C	1.537 – 1.539
Free Phenol, ppm	150 maximum
Phenoxy Ethoxy Ethanol, %	1 maximum

Inquiries:

[redacted]

7- Polysorbate 20

MAKINGCOSMETICS

Certificate of Analysis

(Representative Sample Certificate)

Product Name: Polysorbate 20
INCI Name: Polysorbate 20
CAS Number: 9005-64-5
Lot Number: Not available (data may vary slightly with different lots or batches)
Expiration Date: 24 months from production date

Characteristic	Specifications	Laboratory Values	Results
*Color Gardner	3 max	1	pass
Hydroxyl Value (mg KOH/1g sample)	96-108	97.72	pass
Water	3.0% max	2.43	pass
Saponification Value (mg KOH/1g sample)	40-50	45.30	pass
IR (NEAT)	TNS	pass	pass

The above data was obtained using the test indicated and is subject to the deviation inherent in the test method. Results may vary under other test methods or conditions.

All product characteristic test methods conforms to USP/NF unless noted with asterisk (*).

This product does not contain bovine, ovine, or caprine materials of any type.

The above data were obtained using the test indicated and is subject to the deviation inherent in the test method. Results may vary under other test methods or conditions.

This report is not to be signed.

Disclaimer: This information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any other process. Such information is to the best of the company's knowledge and believed accurate and reliable as of the date hereof. However, no representation, warranty or guarantee of any kind, express or implied, is made as to its accuracy, reliability or completeness and we assume no responsibility for any loss, damage or expense incurred consequential, arising out of use. It is buyer's responsibility to satisfy himself as to the suitability & complete scope of such information for his own particular use.

MakingCosmetics.com Inc.
3518 SE Carter Street, Snoqualmie, WA 98065
Phone 425-292-9602 Fax 425-283-9801 www.makingcosmetics.com

8- [redacted] Magnesium Hydroxide, 10%

RAW MATERIAL SPECIFICATION / TESTING STANDARD **RM Lot:** _____

Item: Magnesium Hydroxide Slurry

Stock Code: C1263 **RM Spec: 1263-01** Date: 9-15-09

INCI: Magnesium Aluminum Silicate

Description: A slurry of magnesium hydroxide micro-platelets of approximately 300 nm x 300 nm x 35 nm produced by electrolytic means. Supernatant solution contains soluble magnesium and sodium chlorides.

Approval requirements:

Approved Sources: Aqua Resources

Storage Conditions: Warehouse conditions

Retest Interval: 24 Months – verify appearance and loss on drying

Attribute	Specification	Method(s)	Observation	Result (Pass/Fail)	By / Date
Appearance	White liquid that will settle upon standing	Organoleptic			
Color	White	Organoleptic			
Odor	Odorless	Organoleptic			
pH	10.2 – 10.8	pH - Neat			
Solids	11% – 14%	Loss on Drying – 2 g / 103C			
Assay	9.0% – 10.5%	Back Titration -			
Bacteria	<100 cfu/g no objectionables	Aerobic Plate Count			
Yeast & Mold	<100 cfu/g	Sabouraud-Dex Agar			

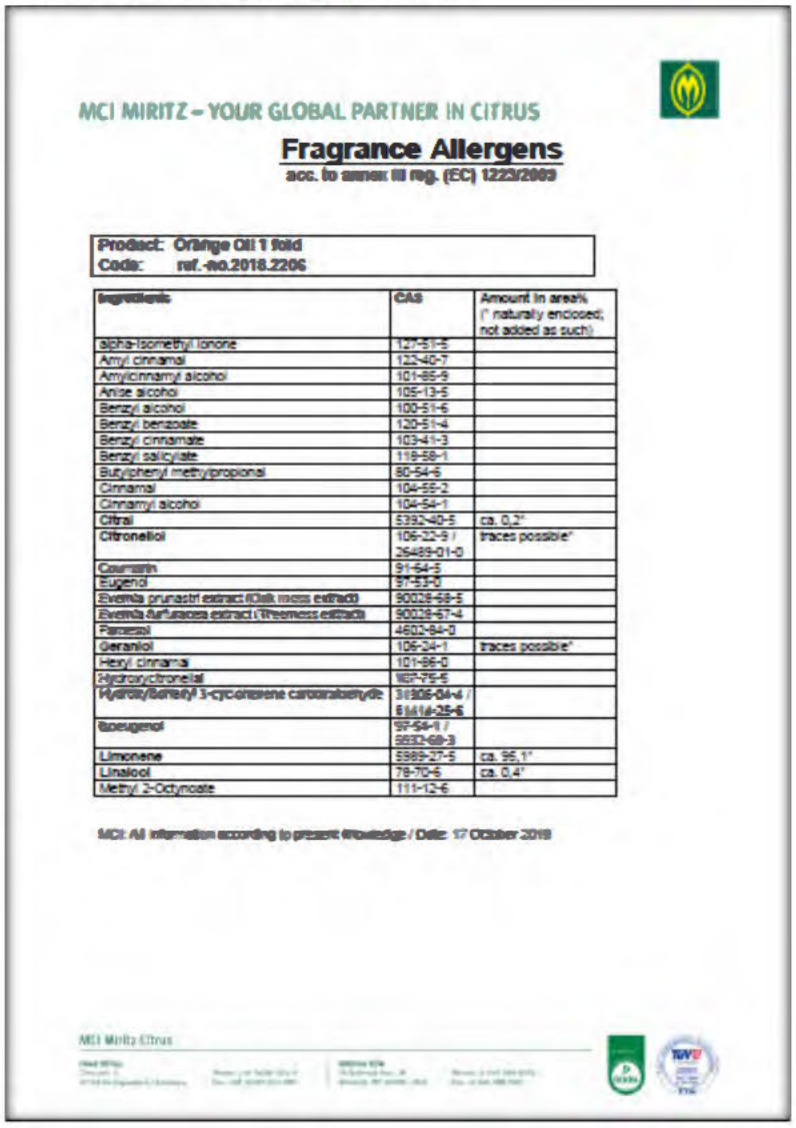
Raw Material Mfg: _____ Mfg Lot Number: _____

Date Received: _____ Quantity Received: _____

Disposition: _____ Approved for Use _____ Not Approved for Use Retain Location: _____

By: _____ Date: _____

9- Citrus Sinensis (Orange) Fruit Oil



MCI MIRITZ – YOUR GLOBAL PARTNER IN CITRUS

Fragrance Allergens

acc. to annex III reg. (EC) 1223/2009

Product: Orange Oil 1 fold
Code: ref.-no.2016.2206

Ingredients	CAS	Amount in area% (* naturally enclosed; not added as such)
alpha-Isomethyl Ionone	127-51-5	
Amyl cinnamal	122-40-7	
Amylcinnamyl alcohol	101-85-9	
Anise alcohol	105-13-5	
Benzyl alcohol	100-51-6	
Benzyl benzoate	120-51-4	
Benzyl cinnamate	103-41-3	
Benzyl salicylate	118-58-1	
Butylphenyl methylpropional	80-54-6	
Cinnamal	104-55-2	
Cinnamyl alcohol	104-54-1	
Citral	5392-40-5	ca. 0,2*
Citronellol	106-22-9 / 26489-01-0	traces possible*
Coumarin	91-64-5	
Eugenol	97-53-0	
Evernia prunastri extract (Oak moss extract)	90028-68-5	
Evernia furfuracea extract (Treemoss extract)	90028-67-4	
Farnesol	4602-84-0	
Geraniol	106-24-1	traces possible*
Hexyl cinnamal	101-86-0	
Hydroxycitronellal	107-75-5	
Hydroxybenzyl 3-cyclohexene carboxaldehyde	31906-04-4 / 51414-25-6	
Isoeugenol	97-54-1 / 5932-68-3	
Limonene	5989-27-5	ca. 95,1*
Linalool	78-70-6	ca. 0,4*
Methyl 2-Octynoate	111-12-6	

MCI: All information according to present Knowledge / Date: 17 October 2019

MCI Miritz Citrus

10- Copper (II) Chloride, Dihydrate

spectrum CHEMICAL MFG CORP

Specification for Cupric Chloride, Dihydrate, USP (C1018)

[redacted]	C1018
[redacted]	Cupric Chloride, Dihydrate, USP
CAS Number	10125-13-0
Molecular Formula	$CuCl_2 \cdot 2H_2O$
Molecular Weight	170.48
MDL Number	
[redacted]	Copper (II) Chloride

Test	Specification	
	Min	Max
ASSAY ($CuCl_2$, DRIED BASIS)	99.0	100.5 %
LOSS ON DRYING	20.9	21.4 %
SODIUM		0.02 %
POTASSIUM		0.01 %
CALCIUM		0.005 %
IRON		0.005 %
NICKEL		0.01 %
INSOLUBLE MATTER		0.01 %
SULFATE		0.005 %
IDENTIFICATION	TO PASS TEST	
RETEST DATE		
MANUFACTURE DATE		
APPEARANCE		
RESIDUAL SOLVENTS	TO PASS TEST	

Spectrum Chemical Mfg Corp

[redacted]

Table 2.3 Showing the Stability Testing results for [redacted] Antibacterial Hand Sanitizer Glove – [redacted]

Data to be added once the studies are complete.

3. Microbiology Quality

Data to be added once the studies are complete.

4. Impurities, traces, information about the packaging material

4.1 Impurities and Traces

Impurities and traces, including heavy metals are listed for each ingredient in its safety assessment. The levels are below the threshold of concern for this formulation.

5. Normal and reasonably foreseeable use

[redacted] Antibacterial Hand Sanitizer Glove – [redacted]

For optimal results, apply approximately 0.5 mL of the product to your hands and massage over the skin using a circular motion. Apply twice a day (i.e., morning and noon)

Warnings: For external use only. Avoid contact with eyes. Keep out of reach of children.

6. Exposure to the cosmetic product

- Site and area of application: The hands (~860 cm^2)
- The amount of product applied: Apply 0.5 mL twice a day
- The duration and frequency of use: This is a leave on product but it is expected that the product will be removed as the end of the day (for example at home) in the normal course of hand washing.
- The normal and reasonably foreseeable exposure routes: The product is applied to the hands It is not applied to the lips, eye, or urogenital areas so dermal exposure is the primary route of exposure.
- The targeted population: The product is intended for adults.

7. Exposure to the substances

Table 7.1 is a copy of the spreadsheet (in three parts) used to perform the Systemic Exposure, Margin of Safety, and Dose Per Unit Area for each ingredient in Antibacterial Hand Sanitizer Glove – [redacted]. These calculations were performed using the maximum application rate for the product.

Table 7.1 Margin of Safety Calculations

Number	Description	CAS#	Wt%	Ingredient %	Ingredient Concentration (mg/mg product)
	Active ingredient				
1	Benzalkonium Chloride, 50%	8001-54-5	0.2000%	0.10%	0.00100
	Inactive Ingredients				
1	Purified Water	7732-18-5	93.0274%	94.3357%	0.94336
2	Polyethylene Glycol 4000	none	1.5960%	1.5960%	0.01596
3	Polyethylene Glycol 400	5117-19-1	1.1970%	1.1970%	0.01197
4	Glycerin, 99.5%	56-81-5	1.1970%	1.1970%	0.01197
5	Hydroxyethylcellulose	9004-62-0	0.6500%	0.6500%	0.00650
6	Phenoxyethanol	122-99-6	0.4000%	0.4000%	0.00400
7	Polysorbate 20	9005-64-5	0.1500%	0.1500%	0.00150
8	Micro Magnesium Hydroxide, 10%	1309-42-8	1.3426%	0.1343%	0.00134
9	Citrus Sinensis (Orange) Fruit Oil	8008-57-9	0.1200%	0.1200%	0.00120
10	Copper (II) Chloride, Dihydrate	10125-13-0	0.1200%	0.1200%	0.00120

Total Product (mg)/Application	Area of Application (cm^2)	Product (mg)/cm^2	Applications per Day	Product Application per day (mg/day)	Product Applications (mg/cm^2/day)	Ingredient Applied (mg/day)	Ingredient Applied (60 Kg) (mg/Kg(bw)/day)
500	860	0.58	2	1000	1.16	1.000	0.0167
500	860	0.58	2	1000	1.16	943.357	15.72261667
500	860	0.58	2	1000	1.16	15.960	0.2660
500	860	0.58	2	1000	1.16	11.970	0.1995
500	860	0.58	2	1000	1.16	11.970	0.1995
500	860	0.58	2	1000	1.16	6.500	0.1083
500	860	0 58	2	1000	1.16	4.000	0.0667
500	860	0 58	2	1000	1.16	1.500	0.0250
500	860	0.58	2	1000	1.16	1.343	0.0224
500	860	0.58	2	1000	1.16	1.200	0.0200
500	860	0.58	2	1000	1.16	1.200	0.0200

Exposure Factor (leave on = 1, rinse off = 0.01)	Ingredient Penetration Factor	Effective Ingredient Systemic Exposure (mg/Kg(bw)/day)	NOAEL (mg/Kg(bw)/day)	Basis for NOAEL	Safety factor	Margin of Safety	Pass	Exposure per unit area (mg/cm^2/day)
1	1	0.017	50	animal oral	100	3.00E+03	Yes	1.16E-03
1	1	15.72261667	NA					
0.5	0.5	0.0665	1600	animal oral	100	2.41E+04	Yes	1.86E-02
0.5	0.5	0.049875	1,100	animal oral	100	2.21E+04	Yes	1.39E-02
1	1	0.1995	10000	animal oral	100	5.01E+04	Yes	1.39E-02
0.1	0.5	0.005416667	5000	animal oral	100	9.23E+05	Yes	7.56E-03
1	1	0.067	500	animal dermal	100	7.50E+03	Yes	4.65E-03
1	1	0.03	2000	animal oral	100	8.00E+04	Yes	1.74E-03
1	1	0.022	1000	animal repro	1000	4.47E+04	Yes	1.56E-03
1	1	0.02	5	animal oral	100	2.50E+02	Yes	1.40E-03
1	0.01	0.00020	16.7	animal oral	100	8.35E+04	Yes	1.40E-03

8. Toxicological profile of the substances

The ingredient reviews are presented in the order in which they appear in Table 7.1.

1-Ingredient Safety Assessment - Benzalkonium Chloride (Active)

Trade Name: Kraft Chemicals
INCI Name: Benzalkonium Chloride 50%
CAS No.: 8001-54-5
EINECS/ELINCS No: 264-151-6
Date: July 9, 2020
Summary Assessment: Approved as used

Data Source(s): 1) GRAS Notification, Burdock Group, September 6, 2013, submitted to the Food and Drug Administration
2) CIR Final report of the safety assessment of Benzalkonium Chloride, Journal of the American Journal of Toxicology 8(4):589-625, 1989

Ingredient function: Antimicrobial cationic surfactant. In cosmetic formulations, it is used as foaming and cleansing agent, conditioner, and bactericide.

Systemic Toxicity: The acute oral toxicity of benzalkonium chloride in rats was measured in two studies. The LD_{50} values ranged from 400 to 525 mg/Kg (Ref 2)

The subchronic oral toxicity was measured in rats that were treated by way of a stomach tube. Ten animals (males) were used for each treatment group. Doses were 50 mg/Kg (bw)/day or 100 mg/Kg (bw)/day. Doses were prepared in water or milk. Water or milk alone was used as the controls. Animals were treated for 12 weeks. Hematological samples were taken at 4 weeks for the rats in the higher treatment group. Animals were sacrificed at 12 weeks. The animals in the treatment groups 50 mg/Kg/day prepared in water or mild and the 100 mg/Kg/day prepared in milk showed similar growth rate as their respective control groups. The rats treated with 100 mg/Kg/day showed a 29% decrease in growth compared to the water controls. The hematological parameters and overall pathology was the same in all treatment groups. Use 50 mg/Kg (bw)day was the NOAEL from this study for the purposes of the calculation of Margin of Safety (Ref 2).

Dermal Toxicity: Several studies were performed to measure acute skin irritation. In the first study, 0.5 mL of a 0.5% benzalkonium chloride solutions were applied to the slipped skin of nine female rabbits. The test material was applied under occluded patch conditions for 24 hours. Sites were scored at 2 and 24 hours after patch removal. Only one of the animals showed a barely perceptible erythema at both the 2 and 24 hour post-exposure reading. This gave a PII of 0.17

where is very mild. A similar study was performed using a 0.3% and no positive reactions were observed (Ref 2). Another study used the same test article as used in the acute ocular irritation study below. This was a moisturizing cream containing 0.13% benzalkonium chloride. Six albino rabbits were patched onto skin with the hair clipped off. Nature of the patch was not given. The application remained in place of 24 hours. At this end of this period, the sites were scored and a repeat patch was applied. This cycle was repeated for a total of three 24-hour applications. No positive reactions were observed (Ref 2).

A human repeat insult patch test study was performed using a cream containing 0.1% benzalkonium chloride (n=101 mixed male and female). A dose of 0.1 mL was applied under semi-occlusive patch conditions. For the first three weeks, the patches were applied for 24 hours on Monday, Wednesday and Friday. The skin reactions were scored after patch removal. A final induction patch was applied on the Monday of the final week for 24 hours. The panelists were rested until the 6th week when a challenge patch was applied to a naïve site and removed 48 hours later. The sites were scored for sensitization at 48 and 72 hours after application of the challenge. No indications of skin irritation or sensitization were reported (Ref 2).

Skin Sensitization: See above

Ocular Irritation: Several acute ocular irritancy studies were reported where benzalkonium was tested at concentration germane to this product. A solution of 0.3% benzalkonium chloride was tested following the basic Draize protocol (0.1 mL instilled into the lower conjunctival sac or one eye with the other eye acting as the control) (n=6) and the animals followed until the eyes clears. There was a very slight irritation on day 2 post instillation (Draize score of 2) which resolved by day three (Ref 2). A skin moisturizing cream was prepared with 0.13% benzalkonium chloride and tested according the standard Draize protocol (as above) in six animals. No irritation was reported in any of the animals (Ref 2). Finally, a solution of 0.1% benzalkonium chloride was instilled directly onto the cornea (low volume eye test protocol) at 0.01, 0.03 and 0.1 mL onto one eye with the other eye as the control (n=6). No irritation was detected at any of the volumes tested (Ref 2).

Genetic Toxicity: Mutagenic potential of benzalkonium chloride was assessed using the bacterial reverse mutation assay (Ames) with four strains of S. typhimurium (TA 98, 100, 1537, and 1538). The plate incorporation method was used. The test article was presented to the test system in the presence and absence of S9 metabolic activation. Treatment concentrations ranged from 10 to 100 µg/plate. No evidence of mutagenic activity was observed (Ref 2).

Phototoxicity: Benzalkonium Chloride does not absorb UV light above 300 nm (Ref 2).

Other endpoints as required:

Traces (as required): No traces were reported.

Assessment:

Risk assessment analysis:

Benzalkonium Chloride will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 0.10%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^3$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, Benzalkonium Chloride is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

1-Ingredient Safety Assessment-Water

INCI Name: Water/Aqua (In house product)
CAS No.: 7732-18-5
EINECS/ELINCS No: 231-791-2
Date:
Summary Assessment: **Approved as used**

Data Source(s): Ingredient Specifications Document

RAW MATERIAL SPECIFICATION / TESTING STANDARD **RM Lot:** _____

Item: Purified Water, USP
Stock Code: C1125 **RM Spec: 1125-01** Date: 2-1-21
INCI: Water

Description: Water produced by further processing Memphis City drinking water with carbon filtration and mixed bed ion-exchange resins so that it meets the requirements for USP Purified Water

Approval requirements:

Approved Sources: Purchase water meeting the requirements or produce on site using suitable means.

Storage Conditions: where packaged, preserve in unreactive storage containers that are designed to prevent microbial entry.

Retest Interval: Retest conductivity and aerobic plate count after 7 days

Attribute	Specification	Method(s)	Observation	Result (Pass/Fail)	By / Date
Appearance	Clear, Colorless Liquid	Organoleptic			
Color	Colorless	Organoleptic			
Odor	Odorless	Organoleptic			
TOC	< 0.10 mg/L	TOC Instrument			
Conductivity	Meets <643> for Stage I, II or III	Conductivity Meter			
Bacteria	<100 cfu/g	Aerobic Plate Count			
Objectionable Bacteria	Absence of S. Aureus and P. Aeruginosa	Type-specific agar – 1 g or ml sample			
Combined Yeast & Mold	<10 cfu/g	Sabouraud-Dex Agar			

Raw Material Mfg: _____ Mfg Lot Number: _____
Date Received: _____ Quantity Received: _____
Disposition: _____ Approved for Use _____ Not Approved for Use Retain Location: _____
By: _____ Date: _____

2-Ingredient Safety Assessment-Polyethylene Glycol 4000

Trade Name: **CARBOWAX™ Polyethylene Glycol (PEG) 4000**
INCI Name: PEG-90
CAS No.: -
EINECS/ELINCS No:
Date: July 9, 2020
Summary Assessment: Approved as used

Data Source(s): 1) Final Report of the Cosmetic Ingredient Review Expert Panel Amended Safety Assessment of Triethylene Glycol and Polyethylene Glycols (PEGs)-4, -6, -7, -8, -9, -10, -12, -14, -16, -18, -20, -32, -33, -40, -45, -55, -60, -75, -80, -90, -100, -135, -150, -180, -200, -220, -240, -350, -400, -450, -500, -800, -2M, -5M, -7M, -9M, -14M, -20M, -23M, -25M, -45M, -65M, -90M, -115M, -160M and -180M and any PEGs ≥ 4 as used in Cosmetics, June 29, 2010 2) CIR Final report on the safety assessment of polyethylene glycols (PEGs) -6, -8, -32, -75, -150, -14M, -20M. Journal of the American College of Toxicology 12(5): 429-457, 1993.

Ingredient function: PEG-90 has a molecular weight average of ~4068 D. The number 90 refers to the average number of ethyl oxide monomers in the chain. This ingredient is used primarily as a humectant and binder in formulations. The neat ingredient is a solid at room temperature (Ref 1).

The skin penetration of PEG-4 (~200 Da MW) was measured in vitro using Bronaugh chambers holding human cadaver skin samples. Using a "leave-on" formulation containing PEG-4, the overall absorption was listed as 8.42% (of the applied dose). Of that percentage 4.5% was found in the stratum corneum and only 1.44% passed through to the receiver fluid (Ref 1). Use 10% as the factor for PEG-90 ingredient passage into the skin.

Data on PEG-90 were not available for endpoints required. Therefore, data from PEG-8 and PEG-75 were used.

Systemic Toxicity: PEG-75 was not absorbed by the intestinal track of rodents and therefore no oral toxicity was observed (Shaffer and Critchfield, 1947) (Ref 2). An oral LD_{50} in the rabbit was reported as 76 g/Kg by Smyth et al (1950) but this result is rather meaningless in the cosmetic setting.

A subchronic feeding (in drinking water) study was performed on rats using five rats per group and treatment concentrations of 0 to 16%. (Equivalent to 0.4 to 19 g/Kg (bw)/day).

Growth, blood picture and organ histology were performed. The NOAEL was 1.6 g/Kg/day (Smyth, 1950) (Ref 2). Use 1,600 mg/Kg (bw)/day for the calculation of the Margin of Safety.

Dermal Toxicity: A subchronic toxicity by the dermal route (and concurrent skin irritation) study was performed in rabbits (10 per treatment group). Animals were treated with neat PEG-6, PEG-8, PEG-75, or PEG-150 at 10mL/Kg (-6 and -8) or 10 g/Kg (-75 and -150) on the shaved abdominal skin. Animals were treated five days per week for 12-13 weeks. No skin irritation or evidence of systemic toxicity were reported (Ref 1).

Skin Sensitization: Carpenter et al (1971) used a modified Landsteiner intradermal sensitization test (0.1 mL of 0.1% PEG-75) (7 doses over three weeks and then rested for 3 weeks). Upon intradermal challenge, no evidence of sensitization was observed (Ref 1).

Ocular Irritation: Acute ocular irritation of PEG-8 (neat?) was measured using the French Method (Journal Officiel de la Republique, Francaise 1973). The eyes were followed until day 7. The authors concluded that PEG-8 is not an eye irritant (Ref 1).

The acute ocular irritancy of a 10% solution of PEG-75 (solvent not given) was no more than reference solution of 2% glycerol (Ref 1).

Genetic Toxicity: Genetic toxicity studies of the PEGs have focused on the lower molecular weight materials (PEG-4 and PEG-8). PEG-4 was tested in the bacterial reverse mutation assay (Ames) in the presence and absence of metabolic activation at concentrations of up to 10,000 µg/plate. No evidence of mutagenic activity was observed. An *in vivo* bone marrow chromosome aberration assay was performed on PEG-4 at doses up to 5,000 mg/Kg (bw). No dose-dependent toxicity or statistically significant increase in chromosome aberrations was detected (Ref 1).

The clastogenic activity of PEG-8 was measured in an *in vitro* CHO chromosome aberration assay in the presence of metabolic activation. No concentration-dependent increase in chromosome aberrations was observed (Ref 1).

Phototoxicity: Not expected with this chemistry.

Other endpoints as required:

Carcinogenicity: PEG-8 is used as a test article solvent in chronic studies. No evidence of carcinogenic activity was evident from these studies (Ref 1).

Traces (as required): none listed

Assessment:

Risk assessment analysis:

PEG-90 will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 1.596%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^4$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, PEG-90 is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

3-Ingredient Safety Assessment- Polyethylene Glycol 400

Trade Name: CARBOWAX™ SENTRY™ Polyethylene Glycol (PEG) 400
NCI Name: PEG-8
CAS No.: 5117-19-1
EINECS/ELINCS No: 225-856-4
Date: 10 July 2020
Summary Assessment: Approved as used

Data Source(s): 1) Final Report of the Cosmetic Ingredient Review Expert Panel Amended Safety Assessment of Triethylene Glycol and Polyethylene Glycols (PEGs)-4, -6, -7, -8, -9, -10, -12, -14, -16, -18, -20, -32, -33, -40, -45, -55, -60, -75, -80, -90, -100, -135, -150, -180, -200, -220, -240, -350, -400, -450, -500, -800, -2M, -5M, -7M, -9M, -14M, -20M, -23M, -25M, -45M, -65M, -90M, -115M, -160M and -180M and any PEGs ≥ 4 as used in Cosmetics, June 29, 2010

Ingredient function: PEG-8 has a molecular weight average of ~380-420 Da. The number 8 refers to the average number of ethyl oxide monomers in the chain. This ingredient is used primarily as a humectant and binder in formulations. The neat ingredient is a liquid at room temperature (Ref 1).

The skin penetration of PEG-4 (~200 Da MW) was measured in vitro using Bronaugh chambers holding human cadaver skin samples. Using a "leave-on" formulation containing PEG-4, the overall absorption was listed as 8.42% (of the applied dose). Of that percentage 4.5% was found in the stratum corneum and only 1.44% passed through to the receiver fluid (Ref 1). Use 10% as the factor for PEG-8 ingredient passage into the skin.

Systemic Toxicity: The acute oral toxicity of PEG-8 in rats is 32.8 g (Ref 1).

Repeat dose toxicity: A 13 week gavage feeding study was performed in Fischer 344 rats (10 per sex per dose). Treatments were performed five days per week for 65 doses. Dose groups were 1.1, 2.8, and 5.6 grams/Kg and controls were treated with 5 g/Kg water. Some changes in the higher dose groups were attributed to the bulk effects of the PEG-8. Slight changes in the kidneys and urinary bladder were seen in the two higher dose groups. The NAOEL was 1.1 g/Kg(BW)/day (Ref 1). Use this figure for the calculation of margin of safety.

Dermal Toxicity: Human Repeat Insult Patch Testing (HRIPT) was performed on several formulations containing 1% PEG-8. Some minimal to mild skin irritation was observed during the induction periods but these were not consistent across the studies (Ref 1).

Skin Sensitization: In the same HRIPT studies, occasional minimal erythema was observed in some small number of panelists but the erythema did not persist to the 48 hour reading. Therefore the reaction was probably an irritant rather than an allergic reaction. PEG-8 would be considered a non-sensitizer (Ref 1).

Ocular Irritation: Acute ocular irritation of PEG-8 (neat?) was measured using the French Method (Journal Officiel de la Republique, Francaise 1973). The eyes were followed until day 7. The authors concluded that PEG-8 is not an eye irritant (Ref 1).

Genetic Toxicity: Genetic toxicity studies of the PEGs have focused on the lower molecular weight materials (PEG-4 and PEG-8). PEG-4 was tested in the bacterial reverse mutation assay (Ames) in the presence and absence of metabolic activation at concentrations of up to 10,000 µg/plate. No evidence of mutagenic activity was observed. An *in vivo* bone marrow chromosome aberration assay was performed on PEG-4 at doses up to 5,000 mg/Kg (bw). No dose-dependent toxicity or statistically significant increase in chromosome aberrations was detected (Ref 1).

The clastogenic activity of PEG-8 was measured in an in vitro CHO chromosome aberration assay in the presence of metabolic activation. No concentration-dependent increase in chromosome aberrations was observed (Ref 1).

Phototoxicity: Not expected with this chemistry

Other endpoints as required:

Carcinogenicity: PEG-8 is used as a test article solvent in chronic studies. No evidence of carcinogenic activity was evident from these studies (Ref 1).
Traces (as required): None listed

Assessment:

Risk assessment analysis:

PEG-8 will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 1.1970%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^4$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of

other toxicological concerns identified in this review, PEG-8 is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

4-Ingredient Safety Assessment- Glycerin, 99.5%

Trade Name: Glycerin USP (95.5%)/VVF Illinois Services
INCI Name: Glycerin (USP)
CAS No.: 56-81-5
EINECS/ELINCS No: 200-289-5
Date: July 9, 2020
Summary Assessment: Approved as used

Data Source(s): 1) CIR Final Report Safety Assessment of Glycerin as Used in Cosmetics January 2015 (used as the source of data for specific endpoints)
2) ECETOC Technical Report No. 48 Eye Irritation: Reference Chemicals Data Bank, 1972
Ingredient must meet the requirements for USP glycerin. (See Certificate of Analysis)

Ingredient function: skin conditioning agent-humectant to increase moisturization of the skin.

Systemic Toxicity: Glycerin is Generally Recognized as Safe (GRAS) (USFDA 21 CFR172.866 and 21 CFR182.1320). Glycerin was fed to humans for 50 days at up to 2,200 mg/Kg(bw)/day without effect (NOAEL >2,200 mg/Kg(bw)/day. A two year carcinogenesis reported the NOAEL in rats to be ~10,000 mg/Kg(bw)/day (Ref 1). Use 10,000 mg/Kg(bw)/day for the calculation of Margin of Safety.

Dermal Toxicity: Neat glycerin was administered to 30% of the body surface of rabbits for 45 weeks without negative effect (0.5 to 4 mL/rabbit/day) (Ref 1).

Skin Sensitization: Glycerin is not sensitizing when tested in the guinea pig maximization test (Ref 1).

Ocular Irritation: Glycerin is not an ocular irritant when tested in the Draize eye irritation test (ECETOC) (Ref 2).

Genetic Toxicity: Glycerin in negative in the Ames, chromosome aberration, CHO HGPRT and sister chromatid exchange assays (Ref 1).

Phototoxicity: Not applicable for this chemical structure.

Reproductive and Developmental Toxicity: was evaluated in a 2-generation reproductive study in rats (male and female) which received 0 or 2,000 mg/Kg (bw)/day in drinking water for 8 weeks before mating. The treatment was continued until the pups were weaned. No negative

effects were reported for the parental generation or for the growth, fertility or reproductive performance of the pups. The F1 and F2 generation were evaluated for histological change in selected organs and none was found. Endocrine function was normal (Ref 1).

Traces (as required): None reported

Assessment:

Risk assessment analysis:

Glycerin will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 1.197%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^4$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, Glycerin is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

5-Ingredient Safety Assessment- Hydroxyethylcellulose

Trade Name: Natrosol 25HHR-CS/Kraft Chemicals
INCI Name: hydroxyethylcellulose
CAS No.: 9004-62-0
EINECS/ELINCS No: -
Date: 10 July 2020
Summary Assessment: Approved as used

Data Source(s): 1) CIR Final report on the safety assessment of hydroxyethylcellulose, hydroxypropylcellulose, methyl cellulose, hydroxypropyl methylcellulose, and cellulose gum, Journal of the American College of Toxicology 5(3):1-59, 1986
2) CIR, Amended safety assessment of cellulose and related polymers as used in cosmetics, March 2009.

Ingredient function: Hydroxyethylcellulose is used to increase viscosity in cosmetic formulations. It is also an approved direct food additive as a binder for pharmaceutical tablets (Ref 1).

Systemic Toxicity: Acute oral systemic toxicity was investigated using a 50% solution of hydroxyethylcellulose in corn oil at doses of 6,834, 10,250, 15,380, and 23,070 mg/kg. Rats were followed for two weeks after dosing and no toxicity was observed (Ref 2).

Subchronic oral toxicity was measured in rats. Diets containing 0.2, 1.0, and 5.0% Hydroxyethylcellulose were fed to the animals for 90 days. No test article-related toxicity was observed (Ref 2). 5.0% is 50,000 parts per million. Using the method of Auletta[1], this dose would be 5,000 mg/Kg/day. Use this value for the subchronic oral NOAEL.

Dermal Toxicity: Dermal irritation potential was measured in a series of clinical studies with repeat application protocols. A human RIPT study was performed on 50 panelists using a 5% solution of hydroxyethylcellulose (details of the study not given). Under the conditions of this test, the test article was neither a skin irritant nor skin sensitizer (Ref 2).

Skin Sensitization: see above

Ocular Irritation: Acute ocular toxicity was measured using the standard Draize method (100 mg instilled into the lower conjunctiva sac in one eye). Eight rabbits were used and four of

[1] Auletta, C.S., *General Toxicology*, in *Handbook of Toxicology*, M.J. Derelanko and C.S. Auletta, Editors. 2013, Taylor & Francis Group: Boca Raton. p. 57-94.

these had their eyes rinsed one minute after instillation while the other four were not rinsed. At one hour, the eyes showed slight conjunctival irritation and that resolved by 24 hours. A subsequent test using a 2% solution in water showed essentially no irritation (Ref 2).

Genetic Toxicity: Methyl cellulose has been extensively evaluated for mutagenic potential using the bacterial reverse mutation assay (Ames) and cytogenetic test systems. No indication of mutagenic or clastogenic activity were reported (Ref 1).

Phototoxicity: Phototoxic potential was evaluated using a mascara containing 0.4% hydroxyethylcellulose. Shaved sites received 0.25 mL of the test material. Some sites were irradiated with UV light. 8-methoxypsoralen was used as the positive control. No indication of phototoxic activity was reported for the mascara (ref 2).

Other endpoints as required:

Traces (as required):

Assessment:

Risk assessment analysis:

Hydroxyethylcellulose will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 0.650%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^5$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, hydroxyethylcellulose is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]

Providing Safety Assessment to Industry

6-Ingredient Safety Assessment- Phenoxyethanol

Trade Name: Phenoxyethanol/Acme-Hardesty Co
INCI Name: Phenoxyethanol
CAS No.: 122-99-6
EINECS/ELINCS No: 204-589-7
Date: July 9, 2020
Summary Assessment: Approved as used

Data Source(s): 1) CIR, Final Report on the Safety Assessment of Phenoxyethanol. Journal of the American College of Toxicology 9(2):259-277, 1990
2) European Cosmetics Regulation Annex V [1]: Line 29 lists phenoxyethanol as an approved preservative in the EU at concentrations up to 1%.
3) FEBEA – 2011, From Afssaps - Saisine 2009BTC0058bis – French, Risk assessment on the use of phenoxyethanol in cosmetic products.

Ingredient function: Preservative

Phenoxyethanol is used as a preservative in cosmetic formulations to retard the growth of microorganisms. It can also serve to stabilize fragrance components. It is approved for use at up to 1% by US and EU preservative regulations.

Systemic Toxicity: The oral LD_{50} in rats is 1.4 g/Kg(bw). NOAEL calculations will be based on the approved use concentration. Subchronic oral NOAEL was 80 mg/Kg(bw)/day in rats (Ref 1).

Dermal Toxicity: Dermal toxicity was assessed by applying 2 mL/Kg(bw) to the shaved skin of rabbits under occlusive patch conditions (10% of the surface area) for 24 hours. Animals were followed for 14 days. No signs of toxicity were observed. Skin irritation potential was evaluated using the standard Draize skin irritation test. Two mL of cosmetic grade phenoxyethanol were applied to shave and unabraded/ abraded skin under occlusive conditions for 24 hours. Animals were followed for 14 days. Slight irritation was observed which resolved in all but one rabbit which showed some desquamation which persisted for 14 days (Ref 1).

Subchronic systemic toxicity by dermal exposure was evaluated by application of phenoxyethanol to rabbit skin for 6 hours per day/five days per week for 90 days. Doses were 0, 50, 150, and 500 mg/Kg(bw)/day. The authors concluded that the dermal NOAEL was 500 mg/Kg(bw)/day (Ref 3). Use this value for cosmetic applications for determining the Margin of Safety.

Skin Sensitization: Guinea pigs were tested for sensitization using ten repeated applications of the phenoxyethanol to the shaved back. No increased redness was observed (Ref 1). An HRIPT was performed on a 10% solution of phenoxyethanol using 51 panelists to whom 0.3 mL was applied three times per week for three weeks. After a two week rest, the subjects were challenged on naïve sites. Challenge patches were read at 24 and 72 hours. No indication of sensitization was observed (Ref 1).

Ocular Irritation: Phenoxyethanol was assessed for eye irritation potential using the standard Draize eye irritation test protocol (0.1 mL, unwashed and washed). All animals showed some degree of irritation but all recovered by day 14. Neat phenoxyethanol is a mild to moderate eye irritant (Ref 1).

Genetic Toxicity: Phenoxyethanol was found not mutagenic in a five strain Ames test (with and without S9) at concentrations up to 5000 µg/plate. It was also negative in a mouse micronucleus assay at doses up to 1,600 mg/Kg(bw) (Ref 1).

Phototoxicity: A human phototoxicity study was performed with 28 panelists. An application of 0.3 mL of neat phenoxyethanol was applied to the volar forearm for 24 hours. After removal of the patch, test and control sites received 16-20 J/cm2 of UVA light. Sites were scored at 24, 48, 72 hours after irradiation. Some mixes weak positives were observed (on test and control sites) but the test material was judged to not be a phototoxin (Ref 1).

Other endpoints as required: The impact of phenoxyethanol on reproductive capacity was measured in CD-1 mice by the National Toxicology Program. The study was carried through the F0, F1 and F2 generations. The test article was presented in the feed at 0, .025, 1.25 and 2.5 percent. In the first phase, the test article was presented seven days before mating and then throughout the gestation (both sexes). The F1 generation produced in this part of the study was used for the subsequent parts of the study. The high does group showed a decrease in the number of liters and the number of pups in each litter. The second part of the study measured the impact of treating the sexes separately by pairing treated males and with untreated females and the reverse (plus controls). Only the treated females showed an impact of phenoxyethanol treatment (high does only) with reduced pup weight indicting some degree of fetal toxicity. F1 pups taken from the first phase of the study were paired to measure their reproductive capacity. Only a limited number of the high dose group were available (three) and so the high does group was not included in the final statistical analysis. Comparing the control, 0.25 and 1.25% does groups for the F2 generation, seminal vessel weight were reduced in the 1.25% treatment group males. Body weights and reproductive capacity were not affected in the 0.25 and 1.25% dose groups (Ref 1).

Teratology induction was measured in New Zealand rabbits (n=10 per dose group) with dermal exposure of 300, 600, 1,000 mg/Kg(bw)/day. Treatment ran from days 6 to 18 of gestation. Nine of the ten rabbits in the high dose group died or were sacrificed as moribund. Five in the mid dose group also died or were sacrificed. The survivors in each dose group produced health liters and the conclusion of the study was that 600 mg/Kg (bw)/day of dermal exposure did not produce teratogenic effects in the face of some maternal toxicity (Ref 1).

Traces (as required): phenol ≤ 150 ppm; heavy metals ≤ 20 ppm

Assessment:

Risk assessment analysis:

Phenoxyethanol will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 0.40%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was FF relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, $>7x10^3$ is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

7-Ingredient Safety Assessment- Polysorbate 20

Trade Name: Polysorbate 20/MakingCosmetics
INCI Name: Polysorbate 20
CAS No.: 9005-64-5
EINECS/ELINCS No: -
Date: July 9, 2020
Summary Assessment: Approved as used

Data Source(s): 1) CIR Journal of the American College of Toxicology 3(5):1-82, 1984;
2) US FDA Food additives permitted for direct addition to food for human consumption, Flavoring agents and related substances, Synthetic flavoring substances and adjuvants (21CFR172.515);
3) Evaluation of food additives, Polysorbates, Food Safety Commission (Japan), 2007.

Ingredient function: Surfactant (liquid)

Systemic Toxicity: Polysorbate 20 is approved as a direct food additive (ref 2). The acute oral toxicity (LD_{50}) values range up to 37 g/Kg in rats (ref 1). A lifespan feeding study in rats was conducted at concentrations of 0.5 to 2%. No significant gross, hematological or histopathological findings were observed. Use NOAEL of 20,000 ppm/day or 2000 mg/Kg(bw)/day[1].

Dermal Toxicity: Acute dermal toxicity studies in guinea pigs showed that the dermal LD_{50} value was greater than 3 g/Kg. The polysorbate 20 was applied to both intact and abraded skin and there was no observable gross pathology (ref 1).

Skin Sensitization: A Magnusson-Kligman guinea pig maximization test was performed on several lots of polysorbate 20. The induction what included subcutaneous injects of the test substance with complete Freund's adjuvant and subsequent topical application. After a rest period, the challenge phase was performed by topical application. Under the conditions of this study, moderate sensitization was observed. HRIPT studies on formulas containing 2 to 2.4% polysorbate 20 showed minimal irritation and no evidence of sensitization.

Ocular Irritation: Acute eye irritation studies (Draize) using an instillation volume of 0.1 mL and standard scoring showed only minimal eye irritation (ref 1).

Genetic Toxicity: Mutation data on polysorbate 20 was not available but data on polysorbate 60 showed no mutagenic potential based on bacterial reverse mutation (Ames) and CHO chromosome aberration studies (ref 3).

Phototoxicity: Not phototoxic (ref 1).

Other endpoints as required:

Traces (as required): None

Assessment:

Risk assessment analysis:

Polysorbate 20 will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 0.150%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^4$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, Polysorbate 20 is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

1. Auletta, C.S., *Acute, subchronic and chronic toxicology*, in *Handbook of Toxicology Second Edition*, M.J. Derelanko and M.A. Hollinger, Editors. 2002, CRC Press: Boca Raton. p. 69-126.

8-Ingredient Safety Assessment- MicroMagnesium Hydroxide

Trade Name: Magnesium Hydroxide Slurry/Aqua Resources
INCI Name: Magnesium Hydroxide
CAS No.: 1309-42-8
EINECS/ELINCS No: 215-170-3
Date: 10 July 2020
Summary Assessment: Approved as used

Data Source(s): 1) CIR, Safety Assessment of Inorganic Hydroxides as Used in Cosmetics, January 25, 2016
2) Raw Material Specification: slurry of magnesium hydroxide micro-platelets - Aqua Resources, Corp.
3) PART 184 -- DIRECT FOOD SUBSTANCES AFFIRMED AS GENERALLY RECOGNIZED AS SAFE, Subpart B--Listing of Specific Substances Affirmed as GRAS, Sec. 184.1428 Magnesium hydroxide, April 1, 2019
4) European Chemicals Agency, REACH summary, Magnesium Hydroxide.
Ingredient function: The magnesium hydroxide is supplied in the form of "microplatelets" with dimensions of 300 x 300 x 35 nm (ovoid shape) (Ref 2). According to the manufacturer, they solid phase is maintained in the suspension at a pH of ~10. The toxicological assessment from the CIR source would be based on testing of either solutions or solid (powder) (not nano) magnesium hydroxide. Magnesium hydroxide is water soluble at 0.0117 g/L at 25^{o}C.

Systemic Toxicity: The acute oral toxicity in rats is listed as >2,000 mg/Kg (Ref 1).

Dermal Toxicity: Skin corrosion potential was measured using OECD test guide 431 (reconstructed human skin corrosion assay). The test material was applied to replicated tissues for 3 and 60 minutes and the viability relative to the water treated control tissues was measured using the vital dye MTT. The relative viability of the test article-treated cultures was greater than 50% with both exposures and so Magnesium hydroxide was determined to be non-corrosive according the this protocol (Ref 4).

Since the viability of the tissue treated for one hour in the above assay was 95% of that of the control tissue, the test article was also judged to be non-irritating (Ref 4).

Skin Sensitization: Skin sensitization was assessed using the local lymph node assay (LLNA) OECD test guide line 429. Test solutions were magnesium hydroxide at 10%, 25% and 50% in propylene glycol. The solutions were applied to the ears of the dose groups for five days. The change in cell replication in the draining lymph nodes was measured by the uptake of 3H-

thymidine. The decays per minute for the lymph nodes of the treated mice were compared to those of the negative control-treated mice. The resulting values were 2.0, 3.6 and 5.9 fold increases over the negative control values for the 10, 25, and 50% treatment groups respectively. The 25 and 50% values are greater than the 3.0 fold cutoff for a positive prediction of skin sensitization (Ref 4).

Ocular Irritation: The acute ocular irritancy of magnesium hydroxide was measured using OECD test guideline 437. The solids protocol was employed and so the test material was applied as a 20% suspension in water to the corneas and the exposure time was 240 minutes. The overall In vitro score was ~5.1 which is essentially non-irritating (Ref 4).

Genetic Toxicity: The mutagenic potential of magnesium hydroxide was evaluated in the bacterial reverse mutation assay (Ames), OECD test guideline 471. S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 and E. coli WP2 uvr A were the test system. The test article was exposed to the test system in the presence and absence of S9 metabolic activation. The highest test concentration was 5,000 µg/plate. No mutagenic activity was observed in any of the tester strains in the presence or absence of S9 activation (Ref 4)

Reproductive Toxicity: Reproductive toxicity was assessed following OECD test guideline 422 using Wistar rats. Dosing of the magnesium hydroxide was performed in the drinking water. The males were treated with 0, 100, 330, or 1,000 mg/Kg (bw)/day for 29 days (two weeks before mating, during mating up to the treatment end). The females were exposed for 41-45 days (two weeks before mating, during mating, during gestation and up to four days of lactation). Clinical signs were monitored (weight gain, food/water consumption) and hematology, blood chemistry, organ weights and histology. Slight changes in blood chemistry were observed but these were within the normal range for the strain. No pathology was observed in the parental or the F1 generation. The NOAEL was given as 1,000 mg/Kg (bw)/day for parental systemic, reproductive effects and F1 effects (Ref 1). Use 1,000 mg/Kg (bw)/day as the NOAEL for Margin of Safety Calculations and use 1,000 as the required margin.

Phototoxicity: Not expected with this chemistry

Other endpoints as required:

Traces (as required): USP specifies Pb ≤ 2 ppm, heavy metals ≤20 ppm

Assessment:

Risk assessment analysis:

Magnesium Hydroxide will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 0.1343%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^4$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 1000 is considered sufficient based on the derivation of this NOAEL (based on the reproductive toxicity endpoint). Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, Magnesium Hydroxide is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

9-Ingredient Safety Assessment- Citrus Sinensis (Orange) Fruit Oil

Trade Name: Orange Oil 1-fold/MCI Miritz
INCI Name: Citrus Sinensis (Orange) Fruit Oil
CAS No.: 8008-57-9
EINECS/ELINCS No: 232-433-8
Date: 10 June 2020
Summary Assessment: Approved as used

Data Source(s): 1) CIR Safety assessment of citrus-derived peel oils as used in cosmetics. September 30 2014
2) CIR, Safety Assessment of Citrus Fruit-Derived Ingredients as Used in Cosmetics, February 8, 2016.
3) Dosoky, N.S., Setzer, W.N. (2018) Biological activity and safety of citrus spp. Essential oils. International Journal of Molecular Sciences 9:1966+
4) World Health Organization, Concise International Chemical Assessment Document 5-Limonene, 1998.

Citrus Sinensis (Orange) Fruit Oil is produced by the cold pressing of peel to release the oil. It can be used as part of the fragrance mixture for the formulation and/or as a skin-conditioning agent. Citrus oils are potential sources of 5-methoxypsoralen (a phototoxin) and 7-methoxycoumarin (a sensitizer). Thus purity standards have been established by IFRA which will be discussed below (Ref 1). The oil consists primarily of d-limonene (~95%) (as provided in the product specifications from the supplier).
Where the citrus oil data are not available for a toxicological endpoint, the corresponding results from d-limonene will be reported.

Citrus-derived ingredients must contain less than 0.0015% (15 ppm) 5-methoxypsoralen (5-MOP). Citrus Sinensis (Orange) Fruit Oil is not expected to contain 5-MOP.

Systemic Toxicity: Citrus Sinensis (Orange) Fruit Oil is a common component of the human diet and the exposure from food sources is expected to far eclipse any exposure from cosmetic use. No margin of safety calculation will be made for the oil. For d-limonene, a subchronic (90 day) study was conducted in rat with oral doses of 0, 2, 5, 10, 30 and 75 mg/Kg (bw)/day. Kidney toxicity was the limiting factor in determining the NOAEL. This NOAEL was 5 mg/Kg (bw)/day (Ref 4). Use this value for the calculation of the Margin of Safety for the oil.

Dermal Toxicity: Acute dermal toxicity (LD_{50}) in rats was measured for lemon, mandarin, and orange oil using limit doses. The limit dose tests showed acute dermal toxicity to be greater than 5 or 10 grams per Kg (bw) depending on the study performed (Ref 1).

Skin Sensitization: A Human Repeat Insult Patch Test (HRIPT) was performed using a night moisturizer containing 1.2% Citrus aurantium dulcis (Orange) Fruit Extract. One hundred panelists were used. The product was tested neat (volume not given) and was not occluded. Nine repeat patches (induction phase) were applied over a three week period. Sites were scored after each patch. After a two week rest, the challenge phase sample was applied to a neat site. The challenge sites were read at 24 and 48 hours post-application. No dermal irritation was reported (Ref 2).

In the HRIPT above, no signs of sensitization were reported from the challenge phase of the study (Ref 2).

Ocular Irritation: This ingredient will be highly diluted in the formulation.

Genetic Toxicity: While specific genetic toxicity studies on Citrus Sinensis (Orange) Fruit Oil were not reported, studies on C. limon (lemon) peel oil were available (38 to 96% limonene). C. limon (lemon) peel oil was tested in the bacterial reverse mutation assay (limits of toxicity) in the presence and absence of S9 activation. No increase in mutant frequency was reported (Ref 1). The Mouse lymphoma assay (OECD test guideline 476) was used to test the forward mutation induction. Again the test material was exposed to the test system in the presence and absence of metabolic activation (S9). No increase in mutant frequency was reported (Ref 1). Final, a chromosome aberration study in vitro (OECD test guideline 473) was performed and showed no increase in chromosome aberrations (Ref 1).

Phototoxicity: Citrus grandis (Grapefruit) Peel Oil (94% limonene) was tested for phototoxic potential on hairless mice and miniature swine. A 20 µL sample was applied to the skin as the neat oil. No phototoxic reaction was observed (Ref 1).

Other endpoints as required:

This ingredient is 95% Limonene and may also contain 0.4% Linalool.

The following values were published for d-limonene by RIFM [1]

Compound	NESIL ($\mu g/cm^2$)	SAF (factor)	Max Allowed ($\mu g/cm^2$)
Limonene	10,000	300	33.33
Linalool	15,000	300	50.0

NESIL No expected sensitization induction level

Actual daily exposure for Citrus Sinensis (Orange) Fruit Oil is 1.4 $\mu g/cm^2$ so the limonene and linalool exposures are within acceptable limits.
Traces (as required): None

Assessment:

Risk assessment analysis:

Citrus Sinensis (Orange) Fruit Oil will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 0.120%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was 250 relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, Citrus Sinensis (Orange) Fruit Oil is not expected to pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]

Providing Safety Assessment to Industry

1. Api, A.M., D. Basketter, and J. Lalko, *Correlation between experimental human and murine skin sensitization induction thresholds.* Cutaneous and Ocular Toxicology, 2014. **34**(4): p. 298-302.

10-Ingredient Safety Assessment- Copper (II) Chloride, Dihydrate

Trade Name: Cupric chloride dehydrate USP/Spectrum Chemical
INCI Name: Copper (+2) Dichloride
CAS No.: 7447-39-4
EINECS/ELINCS No: 231-210-2
Date: July 9, 2020
Summary Assessment: Approved as used

Data Source(s): 1) ECHA registration dossier, Copper Dichloride accessed 6/12/2020. This database is linked to the REACH summary data from which the actual values were obtained.
2) Hostynek, J.J. and Maibach, H.I. 2006 Skin penetration of metal compounds with special reference to copper. Toxicology Mechanisms and Methods 16(5):245-265.
3) Rosmarie A. Faust, Ph.D., Chemical Hazard Evaluation and Communication Group, Biomedical and Environmental Information Analysis Section, Health and Safety Research Division, *, Oak Ridge, Tennessee 1992 **Toxicity Profiles Formal Toxicity Summary for COPPER.**

Density = 3.4 g/cc; Molecular weight = 170.48 g/mole

Ingredient function: Under review

Systemic Toxicity: Acute oral systemic toxicity in rats was determined to be 584 mg/Kg (Ref 1).
Oral sub-chronic (90 day) dose feed study was performed using rats with doses of 0 (control), 500, 1000, 2000, 4000 or 8000 ppm which were administered (providing estimated intakes of 0, 8, 17, 34, 67 or 138 mg Cu/kg (bw)/day). The test article was Cu^{++} as copper sulphate pentahydrate. Animals were treated 7 days per week for 92 days. NOAEL was 1,000 ppm for both sexes and all tissues (16.7 mg/Kg/day) (Ref 1).

Dermal Toxicity: Acute dermal systemic toxicity was listed as 1224 to 2000 mg/Kg (Ref 1).

Skin Sensitization: A guinea pig maximization test was performed on CuCl which is more inherently toxic than the divalent $CuCl_2$. (Ref 1). Twenty percent of the treated animals showed a reaction (degree not reported) at 24 hours after patching but the reactions were gone at 48 hours. Based on these data, the authors concluded that Cu is not a generalized sensitizer (Ref 1).

Ocular Irritation: In the industrial setting, copper dust has inducted ocular irritation but no data were available for ionic copper (Ref 3).

Genetic Toxicity: The mutagenic and clastogenic potential of Cu^{++} (as copper sulphate pentahydrate) was measured in three assays: bacterial reverse mutation assay (Ames), rat liver unscheduled DNA synthesis and mouse bone marrow micronucleus assays (in vivo).

The bacterial reverse mutation assay (plate incorporation assay) was performed with five strains of Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA102. The test article was exposed to the test systems in the presence and absence of S9 metabolic activation. Test article concentrations ranged up to 1,000 µg/plate. No evidence of mutagenic activity was observed in either of the two replicate assays.

The hepatocyte unscheduled (OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells) was performed in rats treated with doses of 632.5 or 2,000 mg/Kg by oral gavage. No increase in unscheduled DNA synthesis was observed.

The mouse micronucleus assay was performed in CD-1 mice. A single dose of 447 mg/Kg was employed. No increase in micronucleated RBCs was observed (Ref 1).

Phototoxicity: No expected with this chemistry

Other endpoints as required: Skin penetrations: Penetration of copper ions through human cadaver skin was measured in vitro using Franz chamber. Multiple skin donors were used. The Kp calculated was between 10^{-5} and 10^{-6} cm/hr. This range indicated that copper ions poorly penetrate intact human skin. Use an ingredient penetration factor of 0.01 in the calculation of Margin of Safety. This value is still a large overestimation of the actual copper ion presentation to the systemic circulation (Ref 2).

Traces (as required): Ni ≤ 0.01% (USP specification)

Assessment:

Risk assessment analysis:

Copper (+2) Dichloride will be used in the [redacted] Antibacterial Hand Sanitizer Glove – [redacted] formulation at 0.120%.

The use instructions call for the product to be used at approximately 500 mg per application twice times per day (total of 1.000 mg/day). The product will be applied to the hands (approximately 860 cm^2). Exposure calculations are provided in the Margin of Safety Calculations table for this product. The Margin of Safety was $>10^4$ relative to the NOAEL for systemic toxicity endpoint listed above. A value of 100 is considered sufficient based on the derivation of this NOAEL. Based on these calculations for the margin of safety and absence of other toxicological concerns identified in this review, Copper (+2) Dichloride is not expected to

pose a risk to the consumer under these conditions of use. Therefore, this ingredient is approved for use in this formula at the concentration indicated.

Assessment performed by:

John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry

9. Undesirable effects and serious undesirable effects

Preclinical and Clinical safety studies

To be added as completed

Part B-Cosmetic product safety assessment

1. Assessment conclusion

CoveXall™

Benzalkonium Chloride Study

- American Journal of Infection Control
- Benzalkonium Chloride Persistent Efficacy after 1, 2, & 4 hours.
- Dr. John W. Harbell

American Journal of Infection Control 47 (2019) 928 932

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Major Article

Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application

Sidney W. Bondurant MD [a], Collette M. Duley BS [b], John W. Harbell PhD [c,*]

[a] University of Mississippi Medical Center, Jackson, MS
[b] BioSciences Laboratories, Inc., Bozeman, MN
[c] EAS Consulting Group, Dallas, TX

Key Words:
Antibacterial
Persistence
Ethanol
Staphylococcus aureus
ASTM E2752-10
Nosocomial infection

Background: Use of hand sanitizers has become a cornerstone in clinical practice for the prevention of dis ease transmission between practitioners and patients. Traditionally, these preparations have relied on etha nol (60% 70%) for bactericidal action.

Methods: This study was conducted to measure the persistence of antibacterial activity of 2 preparations. One was a non alcohol based formulation using benzalkonium chloride (BK) (0.12%) and the other was an ethanol based formulation (63%) (comparator product). The persistence of antibacterial activity was mea sured against *Staphylococcus aureus* using a technique modification prescribed in American Society for Test ing and Materials protocol E2752 10 at up to 4 hours after application.

Results: The test product (BK) produced a marked reduction in colony forming units at each of the 3 time points tested (3.75 4.16 $\log_{10}$ reductions), whereas the comparator produced less than 1 $\log_{10}$ reduction over the same time. The differences were highly significant.

Discussion: In the course of patient care or examination, there are instances where opportunities exist for the practitioner's hands to become contaminated (eg, key boards and tables). Persistent antibacterial activity would reduce the chances of transfer to the patient.

Conclusions: These results show a major improvement in persistent antibacterial activity for the BK formula tion compared to the comparator ethanol based formulation.

The prevention of nosocomial infections has been a goal for the medical community since the elucidation of the germ theory of dis ease. Modern approaches include extensive facilities sanitation pro grams and multiple personal hygiene practices.[1] Of the latter, regular hand washing and the use of hand sanitizer products are now rou tine.[2] Hand sanitizer formulations have traditionally contained etha nol or other short chained alcohols (60% 70%) as the active ingredient responsible for the antibacterial action. Ethanol provides its antimicrobial action through desiccation of the target organisms. Applied to the skin, the ethanol based sanitizers are effective in reducing the bioburden of many types of microbes.[3] However, alcohols are volatile and can evaporate from the skin's surface, so the residual antibacterial activity may be limited.[4] The importance of per sistent antimicrobial activity has been increasingly recognized in the medical/surgical setting.[2,5] Recent reports have also shown that cer tain pathogen populations are becoming more tolerant to ethanol exposure.[6] These data suggest that the use of alternative antibacterial actives might be a benefit in the clinical setting.

Alcohol free formulations have been developed, with the surfac tant benzalkonium chloride (BK) as the active antibacterial agent. This active ingredient acts by disrupting the cell membranes of the target organisms and is active at relatively low concentrations (0.12% 0.13%).[7] Since this surfactant is not volatile, it is expected to remain on the skin as the product dries. Although this report focuses only on the antibacterial action of BK against *Staphylococcus aureus*, this surfactant has also been studied for virucidal activity against influenza, Newcastle disease, and avian infectious bronchitis viruses.[8]

* Address correspondence to John W. Harbell, PhD, Toxicology Consultant, EAS Consulting Group, 16334 Sunset Valley Dr, Dallas, TX 75248
E-mail address: jharbell@easconsultinggroup.com (J.W. Harbell).
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Conflicts of interest: There are none.

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This study was performed to measure the residual antibacterial activity of 2 hand sanitizer products using the standard method pre scribed in the American Society for Testing and Materials protocol E2752 10.[9] The test product was a surfactant based product using BK (0.12%) as its active antibacterial agent, and the second product was a standard commercial ethanol based formulation (with 63% ethanol but no other antibacterial actives), which served as the comparator product. The comparator product's ethanol concentration falls within the recognized effective concentration range for effective immediate contact antimicrobial activity.[3] Persistence of antibacterial activity was measured as a function of $\log_{10}$ kill of reference bacteria versus time after application of the hand sanitizer. The antibacterial activity was measured from 1 4 hours after application of the products. The test product was evaluated at 1, 2, and 4 hours after application, whereas the comparator product was evaluated at 1 and 4 hours after application.

METHODS

For this study of residual antibacterial activity on the skin, 2 prod ucts were compared. The commercial brand DAB hand sanitizer (active ingredient 0.12% BK) and a comparator hand sanitizer, con taining 63% ethyl alcohol), were provided by Best Sanitizers (Walton, KY) to the testing laboratory, Biosciences Laboratories, Inc. (Bozeman, MN).[10] The DAB brand is produced by Best Sanitizer under contract to Three Kings Inc. (Corinth, MS). The study was conducted in compli ance with good laboratory practices for nonclinical studies (21CFR58). As stated in the study protocol, "The purpose of this study was to evaluate the residual antibacterial efficacy of 1 test product verses a comparator ethanol based product, as determined by the dif ference between the number of challenge bacteria species recovered following exposure to the test materials and the number recovered from the untreated (negative control) test sites."

Panelists and skin preparation

The study was performed on 24 subjects (19 63 years old) with healthy skin (16 men and 8 women). The study protocol and informed consent form were approved by the Gallatin Internal Review Board. The volar forearms were used, and the test sites were marked for the test product, comparator product, and negative con trol. The volar forearm was chosen to provide multiple replicate test sites on each arm, which would not be possible using the hands. The sites and arms were randomized among the treatment groups to pre vent anatomical bias. The arms were washed with nonmedicated soap to remove surface dirt and oil, dried, and finally decontaminated with 70% isopropyl alcohol and allowed to air dry. The test sites and control sites were marked with a surgical marker as rectangles (2 $\times$ 6 inch [5.08 $\times$ 15.24 cm]) for the test product on 1 arm and as rectan gles (2 $\times$ 4 inch [5.08 $\times$ 10.16 cm]) for the comparator product on the other arm. An area for the untreated control skin (no further treat ment) was also marked. The areas for the test and comparator prod ucts were randomized between arms across the test panel. Within the test sites, 3 circles (2 cm in diameter) were marked with a surgical marker. Only 2 circles were marked in the 2 $\times$ 4 inch box for the comparator product, as only 2 time points were to be assessed. These were the sites to which the bacteria were to be applied.

Challenge bacteria

The challenge bacterial strain for this study was *S aureus* (ATCC 6538). *S aureus* is a common skin contaminant and therefore provides an appropriate test organism.[11] Fresh, active stocks were prepared in broth medium daily. The day before testing, a sample of the broth cul ture was applied to and spread over the surface of a tryptic soy agar plate and incubated for 24 hours. Just before beginning the study, a portion of the bacteria on the surface of the agar plate was transferred to phosphate buffered saline. After mixing the bacteria into the saline to form a uniform suspension, the turbidity of the suspension was measured and the sample diluted to approximately 1.0×10^8 colony forming units (CFU) per mL of suspension. Ten microliters of this sus pension (approximately 10^6 CFU) were applied to and spread over the 2 cm circles at the appropriate times.

Product neutralizer

It is essential that once the bacteria are removed from the treated skin that residual skin sanitizer not continue to act on the bacteria as they are being prepared (diluted and plated). To this end, a product neutralizer was prepared and added to the dilution liquids. For this study, the same product neutralizer was selected for both the test and comparator products. Before the study began, the effectiveness of the product neutralizer was confirmed using American Society for Testing and Materials E1054 (2013), Standard Test Method for Evalu ation of Inactivators of Antibacterial Agents.[12] Four replicate samples for each of the 2 exposure periods (1 and 30 minutes) were tested for each treatment condition: untreated control, test product, compara tor product, Butterfield's Phosphate Buffer (BPB++), and Stripping Suspension Fluid (SSF++). The "++" refers to the presence of the prod uct neutralizer. In addition, the antibacterial efficacy of the test and comparator products without neutralization were verified.

Evaluation of antibacterial efficacy

Application of the test and comparator products

Each product was applied to the skin at a rate of 0.25 mL per square inch (0.039 mL/cm^2) (3 mL for the 2 $\times$ 6 inch test rectangle and 2 mL for the 2 $\times$ 4 inch comparator product rectangle). In both cases, the liquid was applied in stages, spread over the whole area, and allowed to dry for 1 2 minutes between each application. Once all of the applications were made, the subjects were sequestered and monitored at the test facility to ensure test site integrity.

The persistent efficacy of the test product was evaluated at 1, 2, and 4 hours after application of the product to the skin. The compara tor product was evaluated at only 1 and 4 hours after application. At each time point, 10 μL of the bacterial suspension were applied to 1 of the 2 cm circles in the test product treatment area and spread over the surface with a sterile glass rod. The procedure was repeated on the comparator product treatment area (except for the 2 hour time point) and on the negative control area. Each inoculation was allowed to dry in place for at least 20 but not for more than 25 minutes. At the end of this exposure period, a 2 step procedure known as the cup scrub technique was used to remove the bacteria for determina tion of viability. A sterile stainless steel cylinder with an interior area of 3.46 cm^2 was held against the skin within the 2 cm circle. A vol ume of 2.5 mL of sterile SSF was dispensed into the cylinder. The fluid contained the specific product neutralizer (SFF++) to stop the action of the test and comparator products. A sterile rod was used to mas sage the skin for 1 minute to lift the bacteria from the skin into the fluid. This fluid was transferred to a sterile tube, and a second 2.5 mL volume of SSF++ was dispensed into the cylinder. Again, the skin was massaged for 1 minute, and the second fluid sample was combined with the first. This process was repeated for each exposure condition at that time point. For example, at the 1 hour postexposure time point, 3 bacterial suspensions were collected from each of the 24 sub jects; 1 from the test product treated skin, 1 from the comparator product treated skin, and 1 from the negative control treated skin. To determine the number of viable bacteria (number of CFU) in each sample, serial 10 fold dilutions of each bacterial suspension sample were prepared in BPB solution again containing the product neutral izer (BPB++). Samples from each dilution were spread onto 2

Table 1
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 1 hour following application of the test product or comparator product

		Test product 1 h after application		Comparator product 1 h after application	
Measure	Untreated log_{10} microbial recovery	Treated log_{10} microbial recovery	Log_{10} difference	Treated log_{10} microbial recovery	Log_{10} difference
Median	5.23	0.86	4.22	4.81	0.51
Mean	5.20	1.08	4.12	4.50	0.70
SD	0.189	0.395	0.359	0.727	0.703
		P value (1 tailed)	*P* <.001		

individual mannitol salt agar plates, which were incubated at 35±2°C for 48 hours. On mannitol salt agar, *S aureus* produce golden yellow colonies, and only those colonies were counted.

Calculation of the recovery of viable CFU of bacteria

By definition, a CFU is 1 bacterium that is capable of continued replication to produce a large number of bacteria to form a colony. Each inoculum to the skin contained approximately 10^6 CFU. Each sample from the skin was serially diluted and samples plated. Know ing the area of the skin sampled (3.46 cm^2), the volume of SSF (5 mL), the dilution of the sample producing the counted plate, and volume of the sample added to the plate, the number of CFU per unit area on the skin could be calculated.

The number of CFU from each site at each postapplication time was converted to a log_{10} value. The residual antibacterial activity was calculated by comparing the log_{10} value from the negative control site (time matched) to the log_{10} value from the test and comparator product treated sites to determine the log_{10} difference (antibacterial effectiveness) for each treatment. The relative values were internally controlled for each subject. For the 1 and 4 hour postexposure times, the statistical significance between the log_{10} difference for the test and comparator values for the 24 subjects was evaluated using a paired Student t test (Excel).

RESULTS

The results of the product neutralizer testing showed the efficacy of the neutralization formulation. In all cases, there was no significant difference between the mean untreated control log_{10} colony counts (n = 4) and the mean treated log_{10} colony counts (n = 4), indicating that there was no significant residual antibacterial activity.

The results of the study are expressed as log_{10} mean recovery of CFU of *S aureus* from the untreated control site, the test product, and the comparator product sites for each postapplication time point. The mean values from the individual postapplication time point values for the test and the comparator products are provided (Tables 1 3).

DISCUSSION

This study was performed to measure the antibacterial efficacy of a benzalkonium based test product in comparison with a comparator product containing 63% ethanol as a function of time after application of the individual products to human skin. *S aureus* was used as the test organism since it is a known skin pathogen.[11] The test and com parator products were applied to defined areas of opposing forearms at 0.039 mL/cm^2. Within those areas, 2 cm diameter circles were marked, to which the bacterial suspension would be applied at the specific times after application of the products. For the test product treatment, bacteria were applied at 1, 2, and 4 hours after product application and for the comparator product treatment, bacteria were applied at 1 and 4 hours after product application. Bacteria were applied to untreated skin at each time point to provide the baseline bacterial recovery. The difference in the recovery between the test and comparator products was striking. Although the test product reduced bacterial viability by 3 4 log_{10} at each time point, the com parator product did not reduce bacterial viability by even 1 log_{10}. The differences in efficacy were statistically significant at $P < .001$. These data suggest that the active ingredient BK (0.12%) can provide a marked improvement in persistent antibacterial activity over the 63% ethanol based product.

Table 2
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 2 hours following application of the test product

Sample	Sample size	Mean (log_{10})	SD
Untreated log_{10} microbial recovery (2 h)	23*	5.17	0.20
Treated log_{10} microbial recovery (2 h)	24	1.01	0.37
Log_{10} difference (2 h)	23	4.16	0.35

*One untreated control sample lost.

The effectiveness of BK as an antibacterial agent on skin has been evaluated in the past. Dyer et al (1998) compared the efficacy of 3 hand sanitizer preparations containing either ethanol (63% or 70%) or BK (0.13%) against *Serratia marcescens* applied to the hands.[7] In this study, the hands were contaminated with 5 mL of *S marcescens*, spread over the hands, and allowed to dry for 45 seconds. Five grams of test product were used to “wash” the hands, and then the remaining bacteria were recovered using the “glove juice sampling method.” Polyethylene gloves with 50 mL of recovery fluid were placed, and the hands and the fluid massaged for 1 minute to recover the bacteria. The bacterial suspension was diluted and plated to obtain the number of CFU recovered. This process was

Table 3
Mean log_{10} microbial recoveries and reductions from the untreated control of *Staphylococcus aureus* (ATCC 6538), 4 hours following application of the test product or the comparator product

		Test product 4 h after application		Comparator product 4 h after application	
Measure	Untreated log_{10} microbial recovery	Treated log_{10} microbial recovery	Log_{10} difference	Treated log_{10} microbial recovery	Log_{10} difference
Median	5.08	0.86	3.96	4.58	0.17
Mean	4.92	1.17	3.75	4.59	0.32
SD	0.420	0.503	0.602	0.649	0.597
		P value (1-tailed)	*P* <.001		

repeated 10 times for each treatment condition, and the reduction factors were calculated. The process took approximately 10 minutes per cycle. Only the BK formulation produced a progressive increase in effectiveness (increased reduction factor) over the 10 cycles. The ethanol formulations showed declines in effectiveness relative to the first cycle for each.

The concentration of ethanol in the hand sanitizer formulation can have a marked impact on antibacterial activity. Kampf (2008) com pared 4 ethanol based formulations (85%, 62%, 61%, and 60%) and 2 application volumes of 2.4 and 3.6 mL (total both hands) were evalu ated.[13] Again, *S marcescens* was used as the test bacterium. Approxi mately 5 mL of bacterial suspension were rubbed over the hands and allowed to dry. The viable bacteria were recovered using the glove juice sampling method described in the preceding text. The bacterial suspension was diluted and plated to obtain the number of CFU recovered. The untreated recovery values were compared to the treated conditions where either 2.4 or 3.6 mL were provided to rub over the hands (covering all skin). Both volumes were sufficient to cover the hands of most of the 16 subjects in each test group. The mean $\log_{10}$ reductions for each treatment were statistically compared by an analysis of variance analysis. Although all of the preparations reduced the number of viable bacteria, the larger volume was more effective at all ethanol concentrations and the 85% ethanol formula tion was statistically more effective than the other 3 concentrations. For the 3.6 mL application volume, the mean $\log_{10}$ reduction for the treatment groups were 3.04 ± 0.81 (85%), 2.85 ± 0.51 (62%), 2.63 ± 0.59 (61%), and 2.53 ± 0.60 (60%). However, 85% ethanol is much higher than what is normally contained in current commercial hand sanitizer formulations.

Although *S aureus* accounts for a large fraction of the hospital acquired infections, other bacteria are a concern. *Enterococcus faecium* is a gram positive bacterium, which has become a leading antibiotic resistant pathogen (bloodstream, urinary tract, and surgical wounds).[14] Hospital strains can be resistant to multiple antibiotics, which make them particularly difficult to treat once the infection is established.[15] The rise in incidents of nosocomial infections has raised concerns that preventive measures, such as the use of ethanol based hand sanitizers, have applied selection pressure on the populations to select for more tolerant strains. Pidot et al (2018) have examined the resistance to isopropyl alcohol in 139 strains of hospital associ ated *E faecium* isolated from 2 major Australian hospitals over 17 years.[6] These hospitals have active hand sanitation programs based on alcohol based hand disinfectants. To measure resistance, bacterial suspensions were exposed to 23% isopropyl alcohol for 5 minutes and the number of remaining CFU determined. The concen tration of isopropanol and time of exposure were selected to maxi mize resolution among the strains. Breaking the isolates into groups by date of isolation (1997 2003, 2004 2009, and 2010 2015), there was a high statistically significant decrease in mean sensitivity (based on mean $\log_{10}$ reduction) for the 2010 2015 isolates compared to the 1997 2003 and to the 2004 2009 isolates. These data suggest that there has been a population selection, which has reduced the overall sensitivity to the alcohol based infection control measures.

Selection for increased tolerance to other disinfectants as a func tion of repeated use/exposure has been examined under various envi ronmental exposure conditions. Holah et al (2002)[16] compared *Listeria monocytogenes* and *Escherichia coli* populations found in can nery processing lines where quaternary anomia disinfectants were routinely used. These isolates were compared to isolates from sites not routinely subjected to disinfectant use. They concluded that the persistent populations on the cannery lines were not inherently more tolerant to the disinfectant but that other factors (ie, surface attach ment, biofilm formation, and growth rate) were likely responsible for their ability to persist in the disinfectant treated environment. Kim et al (2018)[17] examined the impact of continuous exposure to BK on bacterial populations isolated from contaminated river sludge. The sediment samples were maintained for extended periods (3 years) in bioreactors containing nutrient medium and increasing concentra tions of BK or nutrient medium alone. Changes in benzalkonium tol erance were measured using the minimal inhibitory concentration assay on nutrient agar. Certain species (ie, *Pseudomonas aeruginosa*) showed increased tolerance to BK (200 vs 50 mg/L), whereas others did not (ie, *Klebsiella michiganensis*). The basis for the difference in the selected strains with increased tolerance was a small change in the antibiotic efflux gene sequence.

It is not surprising that disinfectants can provide some selective pressure on bacterial populations. This pressure is most effective at sublethal concentrations of the disinfectant, which allow the more tolerant subpopulations to thrive and predominate. Lethal concentra tions are less likely to select for tolerant clones where the surviving fraction of the population is very low.[18,19] The current study was not designed to measure selection pressure on the *S aureus* population. It was designed to measure persistence of antibacterial efficacy. The persistence of high antibacterial efficacy from the BK containing test product may reduce the chances for selection of more tolerant clones.

Normal clinical infection control protocols specify use of hand sanitizers between patients to prevent patient to patient microbial transfer. That is not expected to change with the use of a persistent antimicrobial agent. However, in the course of patient care or exami nation, there are instances where there are opportunities for the practitioner's hands to become contaminated. Various surfaces such as key boards, tables, chairs, bed frames and other fixtures will need to be touched or handled. Use of a persistent antimicrobial hand sani tizer would be expected to reduce the opportunity for microbial transfer to the patient.

This study was undertaken to measure the absolute and relative persistence of antibacterial activity under very controlled test condi tions. Having demonstrated persistent activity, the logical next step would be a clinical use study. As a first evaluation, a study is planned that will compare a 70% ethanol product and the test product from this study. Subjects will be medical clinic personnel, who will use both products in a cross over study design.

In the United States, hand sanitizers (both medical professional and consumer) fall under the purview of the U.S. Food and Drug Administration, the 1994 tentative final monograph or proposed rule (the 1994 TFM) for over the counter antiseptic drug products (Fed eral Register of June 17, 1994 [59 FR 31402]). These rules are in the process of being revised to separate the professional and consumer products, and the agency is seeking additional data on active ingre dients, including ethanol and BK. One factor to consider is the persis tence of the antibacterial activity on the skin. This study provides quantitative data on the persistence of BK induced antibacterial action, which could be a marked benefit in the prevention of nosoco mial infections.

CONCLUSIONS

These results show a major improvement in persistent antibacte rial activity for the BK formulation compared to the comparator etha nol based formulation. Persistent antibacterial activity may be beneficial in the patient care setting to reduce the chances of inciden tal contamination of the hands and subsequent transfer to the patient.

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Antimicrobial Summary

- Explanation of Kill Rate Testing Results
- Ingredient Safety Assessment
- Dr. John W. Harbell

JHarbell Consulting LLC
[redacted]
Providing Safety Assessment to Industry
John W. Harbell, Ph.D.
President
Direct: [redacted]

December 1, 2020

RE: Antimicrobial efficacy data and ingredient safety assessment on [redacted] formula (32015.0)

To Whom It May Concern:

The following provides a summary of the antimicrobial efficacy study performed by Microconsult, Inc. and a summary of the ingredient safety assessment of the individual ingredients for this formulation.

Antimicrobial Study:

Summary:

DGH Hand sanitizer was tested in a Kill Rate Study using eleven bacterial species (Microconsult Report 1 September 2020). The exposure times were 30 and 60 seconds. The 30 second exposure killed all of the organisms ($> 10^5$ cfu/mL) from nine of the species and greater that three $\log_{10}$ from the other two. The 60 second exposure killed all of the organisms from all eleven species. A second Kill Rate Study was performed on the spore stage of C. difficile using the same exposure times (Microconsult Report 15 September 2020). Both the 30 second and 60 second exposers showed complete kill of the test organisms. These data suggest that this hand sanitizer could have a strong impact on bacterial transmission.

This summary report was compiled from two Kill Rate Study reports issued by Microconsult, Inc. 1 September 2020 (11 organisms) and 15 September 2020 (C. difficile in the spore stage).

Objective:
To demonstrate the antibacterial properties of the test product against a selection of gram positive and gran negative bacteria.

References:

A. 21 CFR 333. Topical antibacterial products for over-the-counter human use.

B. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics: Topical antimicrobial drug products for over-the-counter human use; proposed amendment of the tentative final monograph. Section V. Comments on the Proposed Rule and FDA Response Subsection C. Comments on Effectiveness and FDA Response [list of test organisms for *in vitro* efficacy testing]
C. Microconsult, Inc. Test Method 011_00 Kill Rate Testing

Test Article: Labeled as Hand Sanitizer, lot # 1152 ([redacted]

Test Organisms
The list of test organisms, their American Type Culture Collection (ATCC) numbers, source and short names (see Table 2) are provided in Table 1. Active cultures of the test organisms were maintained by the laboratory and renewed from the reference stock after five passages. *Campylobacter jejuni* and *Clostridium difficile* were maintained under anaerobic culture conditions. To kill the C. difficile vegetative organisms, the 24-hour growth plate was treated quickly with 70% isopropyl alcohol to yield the spore form cells for the second study.

Table 1 List of organisms tested

Organism	ATCC Number	Source	Short Name
Escherichia coli	8739	Microbiologics	*E. coli*
Methicillin-resistant (MRSA) Staphylococcus aureus	33591	Microbiologics	*MRSA*
Pseudomonas aeruginosa	24853	Microbiologics	*P. aeruginosa*
Burkholderia cepacia	25416	Microbiologics	*B. cepacia*
Salmonella enterica	14028	Microbiologics	*S. enterica*
Enterococcus faecalis	51575	Microbiologics	*E. faecalis*
Klebsiella pneumoniae	700603	Microbiologics	*K. pneumoniae*
Streptococcus pyogenes	19615	Microbiologics	*S. pyogenes*
Listeria monocytogenes	SLR2249	Microbiologics	*L. monocytogenes*
*Campylobacter jejuni**	49943	Microbiologics	*C. jejuni*
*Clostridium difficile**	9689	Microbiologics	*C. difficile*

*Anaerobes

Reagents:
Tryptic Soy Agar with Lecithin and Tween 80

Sterile Phosphate Buffered Saline (for diluting)
DE Neutralizing Broth: Dey-Engley Neutralizing Broth is intended to stop the action of the antimicrobial preparation at the end of the exposure period. It is formulated to neutralize several types of antibacterial active ingredients including benzalkonium chloride.

Procedure:

1. Prepare each bacterial culture, inoculate the growth medium (broth) with the actively growing bacteria and allow to grow at 30-35^{o}C for 24-48 hours. These suspension cultures will be used to determine the antibacterial activity of the test article against the specific bacterium. Eleven such suspension cultures were prepared, one for each organism. These cultures were incubated for 24 to 48 hours to obtain the desired bacterial titers. At this point the number of organisms per mL (cfu/mL) was determined and the same cultures were used to challenge the test article. It should be understood that the exact number of organisms in the inoculum will not be known until step 2 is completed.

2. To obtain the number of viable microorganisms (colony forming units per mL [cfu/mL]), a sample was removed and diluted in sterile phosphate buffered saline. Subsequent serial dilutions were prepared from this sample to seed test plates with dilutions of 10^{-6} and 10^{-7} of the original suspension. Each test plate was then filled with 20 mL of 45^{o}C Tryptic Soy Agar, swirled to mix and then allowed to harden. The plates were incubated for 24 – 48 hours to allow the viable bacteria to form colonies in the agar. The bacterial colonies were counted and the number of colony forming units per mL in the original inoculum determined. The number of cfu/mL in the inoculum was then calculated to determine the number in the test samples using the formula below:

$$\frac{\text{(cfu/mL inoculum)x(volume added to the test article sample)}}{\text{Weight of test article (g)}} = \text{cfu/g product}$$

$$\frac{\text{(cfu/mL inoculum)x(0.1 mL)}}{\text{9.9 (g)}} = \text{cfu/g of test article}$$

3. Samples of test article were prepared for inoculation with each bacterium. A volume of 9.9 mL was measured out into properly labeled test tube. These tubes were held at room temperature until the bacteria were added.
4. Inoculation of the test article with the bacterial inoculum was performed by adding 0.1 mL of the bacterial inoculum to the tube holding 9.9 mL of the test article. The tube was mixed and then allowed to stand for the time of the first incubation period (30 seconds). At that point one mL of the test article-inoculum mixture was removed a placed immediately into 9.0 mL of DE Neutralizing solution to stop the action of the test article.

After the second incubation period (60 seconds) a second sample of one mL was taken and added to a second tube containing 9.0 mL of DE Neutralizing solution. This process was repeated for each of the bacteria tested.

5. Each suspension of bacteria in the DE Neutralizing solution was serially diluted (1:10) in duplicate in phosphate buffer to prepare dilutions of 10^{-1} to 10^{-5}.
6. One mL of each dilution was transferred to a prelabeled 100 x 15 mm petri plate.
7. Each plate was overlaid with 20 mL of melted (45°C) Tryptic Soy Agar and the plate gently swirled to mix the bacteria with the agar. The plates were then allowed to harden,
8. The inoculated plates were placed into an incubator at 30-35°C for 48 to 72 hours. Again, the *C. jejuni* and *C. difficile* plates were incubated under anaerobic conditions.
9. At the end of the incubation period, the number of colonies in each plate was counted. From the count value and the dilution of the original sample, the number of colony forming units remaining in the treated samples was calculated
10. The $\log_{10}$ reduction was calculated from ratio $\log_{10}$ of the inoculum to the log10 of the remaining colony forming units after treatment. For example:

For the E. coli sample treated for 30 seconds, the $\log_{10}$ inoculum of bacteria was 5.93/mL and the number of colony forming units after treatment was zero. The zero value is converted to one which has a $\log_{10}$ of zero. The $\log_{10}$ reduction is 5.93-0 = 5.93. A second example shows the case where there was some survival at 30 seconds of exposure. ***B. cepacia*** had an initial inoculum of 6.24×10^5 cfu/ml ($\log_{10}$ is 5.80). At 30 seconds of exposure, 310 cfu/mL ($\log_{10}$ 310 is 2.49) remained viable. The $\log_{10}$ reduction was 5.80-2.49 = 3.30.

The $\log_{10}$ reduction for each bacterium at each of the two exposure times is shown in Table 2.

Table 2 Log reduction of viable cfu/mL

Organism (Exposure Time)	Inoculum Level (cfu/mL)	Growth Average (cfu/g)	Log_{10} Reduction
E. coli (30 seconds)	8.59×10^5	No Growth	5.93
E. coli (60 seconds)	8.59×10^5	No Growth	5.93
MRSA (30 seconds)	7.55×10^5	No Growth	5.88
MRSA (60 seconds)	7.55×10^5	No Growth	5.88
P. aeruginosa (30 seconds)	5.56×10^5	No Growth	5.75
P. aeruginosa (60 seconds)	5.56×10^5	No Growth	5.75
B. cepacia (30 seconds)	6.24×10^5	310	3.30
B. cepacia (60 seconds)	6.24×10^5	No Growth	5.8
S. enterica (30	5.91×10^5	No Growth	5.77

Organism (Exposure Time)	Inoculum Level (cfu/mL)	Growth Average (cfu/g)	Log_{10} Reduction
seconds)			
S. enterica (60 seconds)	5.91×10^5	No Growth	5.77
E. faecalis (30 seconds)	8.84×10^5	No Growth	5.95
E. faecalis (60 seconds)	8.84×10^5	No Growth	5.95
K. pneumoniae (30 seconds)	3.81×10^5	15	4.40
K. pneumoniae (60 seconds)	3.81×10^5	No Growth	5.58
S. pyogenes (30 seconds)	2.25×10^5	No Growth	5.41
S. pyogenes (60 seconds)	2.25×10^5	No Growth	5.41
L. monocytogenes (30 seconds)	5.98×10^5	No Growth	5.78
L. monocytogenes (60 seconds)	5.98×10^5	No Growth	5.78
C. jejuni (30 seconds)	2.42×10^5	No Growth	5.38
C. jejuni (60 seconds)	2.42×10^5	No Growth	5.38
C. difficile (30 seconds)	2.40×10^5	No Growth	5.38
C. difficile (60 seconds)	2.40×10^5	No Growth	5.38
C. difficile (Spore form) (30 seconds)	1.67×10^5	No Growth	5.22
C. difficile (Spore form) (60 seconds)	1.67×10^5	No Growth	5.22

Discussion:

As shown in Table 2, most of the bacterial species tested were completely killed with the 30 second exposure and all were completely killed with a 60 second exposure. 21 CFR 333 Topical antibacterial products for over-the-counter human use calls for a two log_{10} reduction in viability for a product to be considered antibacterial. This regulation applied to topical antiseptics. 21 CFR 310 Safety and Efficacy of Consumer Antiseptics calls for a three log_{10} reduction in viability for a hand rub (hand sanitizer) to be considered to have antibacterial efficacy. This hand sanitizer achieved a three log_{10} kill with a 30 second exposure and complete kill with a sixty second exposure for all eleven species tested. Of particular interest was the activity against C. difficile spores. Complete kill of the 1.67×10^5 cfu/mL inoculum was achieved with a 30 second exposure.

This study was performed at Microconsult, Inc. Carrollton, TX under the direction of Alix Paulson, Microbiology Technician II September 2020.

Ingredient safety assessment:

The first step in assessing the potential toxicity of a formulation is a complete review of the toxicological hazard of each of the ingredients. This review is based accepted measures of potential toxicity by oral ingestion, absorption through the skin, irritation to the skin and eyes, sensitization of the skin (delayed contact hypersensitivity), genetic toxicity, phototoxicity (enhancing sunburn potential) and, where appropriate, developmental toxicity and carcinogenesis potential. This review includes the assessment of hazard (independent of the concentration used in the formulation) as well as the risk from the ingredient at the concentration employed in the formulation and the amount applied to the skin on a daily basis.

The first issue is oral toxicity. We use this as the basic measure of toxicity of the formulation and it is assessed in two ways. Even though this product is going onto the skin, we use oral toxicity to model the maximum exposure and toxicity. First what is the toxicity of a onetime exposure and second what is the toxicity of repeated exposure over months. The first is measured by the "Acute Toxicity Classification for Mixtures" proposed by the Globally Harmonized System (GHS) for toxicological assessment (https://www.chemsafetypro.com/Topics/GHS/GHS_classification_mixture.html). While this is more of an EPA program, the results can be instructive. The GHS has five classes of acute oral toxicity with Category 5 being the least toxic. **The [redacted] formula is projected to be even less toxic than a Category 5 by these calculations!** The second consideration is the repeat systemic exposure over weeks and months of using the product. For this measure, we calculate a Margin of Safety for each ingredient [1]. The Margin of Safety compares the maximum potential systemic exposure (if any) from using the product with the published no effect exposures from 3 month studies. Here we are looking to see how much less our potential exposure is compared to the published data for no effect. A good figure is 100 fold less. **Our values are 5,000 or more less so our Margins of Safety are excellent.** The full spreadsheet of the calculations is available as client confidential data since it contains the detailed formula.

The absence of skin irritation is important for any product used on a daily basis. At the concentrations used, none of our ingredients are expected to show any skin irritation potential. A review of the formulation (Table 1) shows that in fact many of the ingredients would also be found in cosmetic formulation to provide esthetics for the product.

The lack of skin sensitization potential is also important. Skin sensitization is an immune-mediated action and a minimum dose to the skin is required to begin the process. The weaker the sensitization potential, the more that is required. For example, d-limonene is listed as a sensitizer

by some but in fact, the amount of d-limonene required to produce this action is far greater than could be achieved with this formulation[2]. Thus, skin sensitization is not an issue with this formulation,

Even though [redacted] is intended to be applied to the hands and not the face, it is important that the formulation not be an eye irritant just in case of accidental eye exposure. At the concentrations employed none of the ingredients are eye irritants and so we do not expect that the formulation will have any eye irritation potential.

Genetic toxicity is damage to the genetic material (DNA) of the cell and is something one wishes to avoid completely. All the ingredients have been tested in one or more assays and found not to induce genetic damage. Depending on the ingredient, genetic toxicity was assessed using the bacterial reverse mutation assay (with and without S9 metabolic activation), in vitro chromosome aberration assay (with and without S9 metabolic activation), and in vivo mouse micronucleus assay.

Phototoxicity can be induced when a chemical absorbs ultraviolet light and releases that energy in a way that activates surrounding chemicals that can act to damage the surrounding cells. Certain drugs and some natural products are known to cause this problem. If the ingredient absorbs UV light, then it should be tested. All of the ingredients in this formulation do not absorb UV light or have been tested and found negative for phototoxic activity.

Developmental toxicity and carcinogenesis: Many of the ingredients in this formulation are so nontoxic they have no potential to cause these issues. Others have been used extensively in cosmetic, drugs and other products so that testing has been performed. In all cases, they were not toxic.

This document is just a summary of the review of the ingredients. Ultimately, the final formulation will be subjected to confirmatory tests in both the laboratory and clinic for final mildness assessment.

Table 1 [redacted] (35015.0)

Number	Description	CAS#
	Active ingredient	
1	Benzalkonium Chloride	8001-54-5
	Inactive Ingredients	
1	Purified Water	7732-18-5
2	Polyethylene Glycol 4000	none
3	Polyethylene Glycol 400	5117-19-1
4	Glycerin, 99.5%	56-81-5
5	Hydroxyethylcellulose	9004-62-0
6	Trisodium Citrate	68-04-2
7	Polysorbate 20	9005-64-5
8	Phenoxyethanol	122-99-6
9	Potassium sorbate	24634-61-5
10	Copper (II) Chloride, Dihydrate	10125-13-0
11	d-Limonene	5989-27-5
12	Magnesium Hydroxide	1309-42-8

Prepared by:
John W. Harbell, Ph.D.
JHarbell Consulting LLC
[redacted]

References:

1. SCCS, *The SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation, 10th Revision, SCCS/1602/18.* 2018.
2. Basketter, D., et al., *Categorization of chemicals according to their relative human skin sensitization potential.* Dermatitis, 2014. **25**(1): p. 11-21.

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Platelet Technology

- Breakthrough Material Science
- Benefits of Platelets over spheres and other shapes

PROPRIETARY INFORMATION

White Paper

An Innovative Platform of Technology

[redacted] focuses on microtechnology research and development to develop technology which is safe, highly efficacious and patentable while being affordable for practical use in products referred to as microparticles. Simply stated – the technology is produced in nano and micron forms providing formulas configurations along an x,y,z axis. This configuration results in exceptional microparticle efficacy and safety.

[redacted] has successfully conducted studies in independent labs to validate safety and proof-of-concept for a variety of uses with its proprietary disc-wafer-like microparticles. The objectives of this White Paper are to describe key points about our new microparticles structure, efficacy against target microbes, and safety to non-target organisms.

Structure:

Recent manufacturing and structural breakthroughs enable new shapes and sizes that have surpassed performance and dispelled many of the inherent concerns attributed to use of previous microtechnologies. Unlike any known previous microtechnology, the disc-wafer-like microparticles have been shown to be highly effective for control of prokaryotes and viruses. At sizes in the micro-range (10^{-9}m, one billionth of a meter), metal compounds exhibit properties not observed for larger particles of the same chemical composition. Notable is the ability to kill a broad range of bacteria, fungi and viruses. Almost all previous microparticles exist as some form of spheres, rods, belts and other variations of shapes and sizes. Many of which are metal salts and are toxic upon prolonged exposure (Lewinski, et al 2008).

[redacted] has primarily concentrated on is micro-copper and magnesium hydroxide and/or a magnesium oxide lattice of unique disc-wafer-like morphology. This arrangement is like the micelle structure of clay particles in that both contain a prodigious surface/volume ratio. Magnesium hydroxide ($Mg(OH)_2$) occurs naturally as the mineral brucite. Magnesium oxide (MgO) is the oxide salt of magnesium.

[redacted] research team and partner companies have developed an electrolytic process that uses $MgCl_2$ to gradually deliver Mg^{2+} ions from one side of a conduction cell and –OH ions from the other, as shown by the reaction equation [$Mg^{2+} + 2\ –OH\ Mg(OH)_2$]. This reaction proceeds with a slow, gradual growth of crystals that arrange into platelets only a few molecular layers thick. These $Mg(OH)_2$ microtechnology are synthesized to great specificity to achieve a consistent size and shape uniformity with mass production cost efficiencies.

Anhydrous microparticles of $Mg(OH)_2$ and MgO are individual crystallites, approximately 200–200 nm in a highly specific, narrow width range (Figure 1 photo). This dimensional feature of the microparticles has a distinct advantage over traditional microparticles where most of the molecules are embedded within the microparticle rather than on the surface (Pal, et al. 2007, Ruparelia, et al. 2008).

Particles this thin appear to interact as 2-dimensional structures on the micro scale. However, these microparticles are edged with free $Mg(OH)_2$ groups available for attachment to a broad range of ester-, acetal-, and ether-forming reagents. This disc-wafer like structure enables functional substituents at the edge surfaces to act as connectors to modify the physicochemical properties of the microparticles. When the microparticles are integrated into a polymeric material, then further significant modifications can occur (Makhluf, et al. 2005, Thill, et al. 2006).

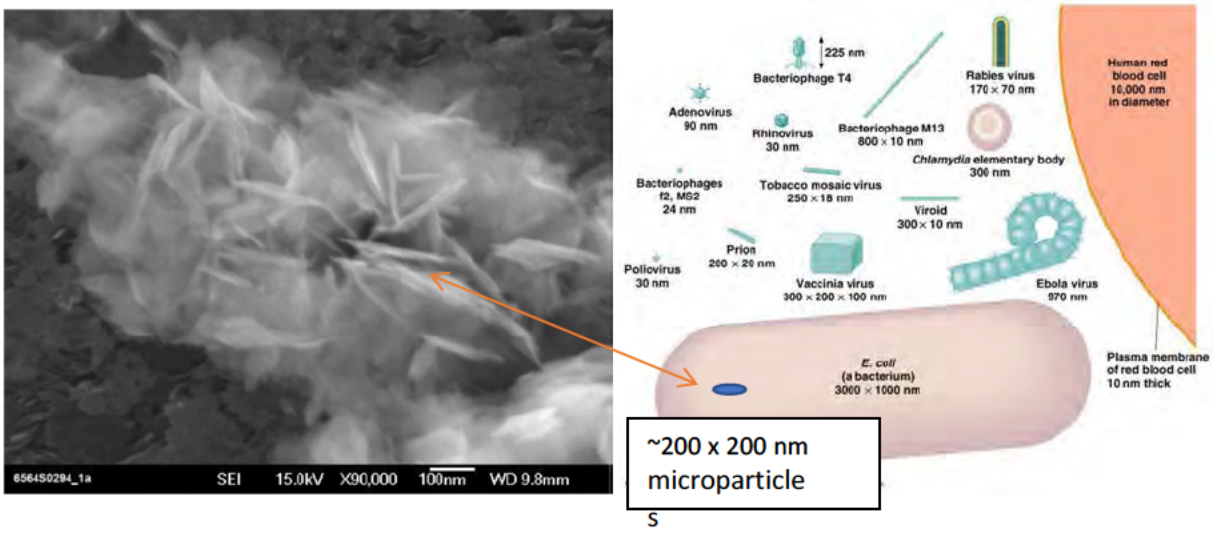

Figure 1.
Left: Magnesium Hydroxide Microparticles.
Right: A diagram showing approximate size ranges of cells, viruses and other infective particles relative to ARC's approx. 200 x 200 nanometer wafer-like-disc microtechnology.

The microparticle production process conducted is entirely safe, green, and efficient with byproducts of chlorine and hydrogen that can be recycled for use in other industries (Gao, et al. 2009).

Efficacy:

The antimicrobial properties of microparticle technology have been tested and proven to be effective for use in medical facilities for control of human pathogens including against Multiple Drug Resistance Organisms (MDRO's). MIcroparticle technologies has been used to effectively mitigate infections from wounds and burns. The Technology is also very effective on surfaces to sanitize. (Adams, et al. 2004, Auffan, et al. 2008, Morones, et al. 2005, Stoimenov, et al. 2002, Thill, et al. 2006, Zhang, et al. 2007)

Independent tests of microparticle technologies were done against challenging human pathogens such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Carbapenem-Resistant *Enterobacteriacea E. coli* (CRE). Our microparticles demonstrated sustained efficacy with no pathogen rebounding (i.e., no resurgence) of pathogens after treatment even from spore-forming pathogens (Fabrega, et al. 2009, Li, et al. 2005, Lok, et al. 2007).

[redacted] research team along with our research partner companies also developed micron-sized agglomerates composed of safe, effective microtechnology intercalated with MgO, termed Microparticle

Antimicrobial Spheres (MAS). MAS exhibit extremely strong biocidal activity against a broad-range of bacteria species that are resistant to current antibiotics and generally recalcitrant to other biocides. Independent lab assay tests, demonstrated MgO comprised MAS has high efficacy against Gram-positive and Gram-negative pathogens, including spore formers. Tests have shown effectiveness against: *Bacillus anthracis (Ames), Bacillus anthracis (Sterne), Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa, Salmonella typhimurium*, *Staphylococcus aureus*, and industrial isolates of Microbacteriaceae, Propionibacteria species and several environmental Fungal isolates. A subset example of results, taken from lab assays, is summarized in Table 1. This illustrates log kill efficacy of MAS against both ATCC and clinical isolates.

This lab test demonstrated that our MAS MgO material at various levels of concentration provided complete kill of this MRSA strain. Concentrations of 5mg/mL and 10mg/mL provided complete kill within 3 hr, while 1mg/mL achieved complete kill after 6 hr. Levoflaxin provided a reduction of MRSA population but did not achieve complete kill after 24 hr. Note that there is little to no difference in the concentration levels of 5mg/mL and 10mg/mL, with the lower concentration of 1mg/mL still achieving the same efficacy but at a slower rate to register log 7 kill. In all MAS MgO treatment cases, zero growth was observed at 24hr. By contrast the antibiotic Levoflaxin, administered at a current clinical dose rate, reduced the population by 4 logs, thereafter its activity plateaus – to leave log 2 cfu/mL at 24hr.

SUSTAINED EFFICACY – Kill with No Rebounding

Bacterial populations can exhibit resistance when continually challenged by antimicrobial agents. Selection pressures for survival lead to development of subsequent generations of 'Persisters', also called small colony variants (SCVs) and spore formation. These subpopulations of SCVs are genetically identical to the parent population but have altered metabolism that allows them to combat or evade the effects of antibiotics/biocides (Cohen, et al 2013). While the majority of the vegetative bacterial population may be killed by a biocide, the few SCVs that persist gradually multiply when conditions permit and return population levels to previous, or even greater numbers (e.g. *Colistin* rebound in *Figure 3 below*). The consequence of this 'rebound' is that patients who appeared clear of infection and in recovery, are afflicted a second time when SCVs that were not cleared from their system, resurge once the course of antibiotics is concluded.

MAS doses provided identical "Time-Kill Kinetics". The Colistin antibiotic applied at a clinical dose rate provided apparent kill at 3 hours. However, the Colistin treatment resulted in incomplete sustained killing, then 'rebounding' occurred when the assay was extended to 24hr. This rebound CRE population resurgence over time elevated to levels that exceeded the initial inoculum starting point. By contrast MAS MgO clearly mitigated the CRE bacteria, as the bacteria were completely killed with no observed rebound effects.

Multiple Modes-of-Action

MPs kill pathogenic microbes by several mechanisms, thus resistance is less likely to arise in bacterial populations. Mechanisms of cytotoxicity that have been described include production of reactive oxygen species (ROS), penetration of cells via ionic uptake, and influence on electrostatic charges of cell membranes. Physical contact between a microparticle and a bacterial cell has been shown to be necessary to cause mortality (Kang, et al 2007, Thill, et al 2006, Stoimenov, et al 2002, Zhang, et al 2007, ARC unpublished results). The physical interaction appears to damage the membrane (Gorgoi, et al 2006; Makhluf, et al 2005, Stoimenov, et al 2002). Smaller microparticles appear to be more cytotoxic

than larger particles (Lok, et al 2007; Zhang, et al 2007;) and in a mixture, the small sized microparticles may be responsible for most of the toxicity (ARC unpublished results).

MPs trigger initiation of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals that are responsible for membrane damage (Zhang, et al 2007; Dawson, et al 2009). This claim is given support by correlations between degree of ROS production and degree of membrane damage measured by bacterial mortality.

UNIVERSALLY SAFE, NON-TOXIC BIOMEDICAL DECONTAMINATION TECHNOLOGY

- [redacted] has developed two biological decontamination formulations based on magnesium hydroxide microplatelet (MP) and MP antimicrobial spheres (MAS) technology

  -- Universally safe and non-toxic for people, food, water and the environment

  -- Deployable in wet or dry formats

  -- Effective against spore forming and non-spore forming bacteria, mold/fungi

    --- Dramatically outperforms frontline antibiotics

    --- Efficacy verified and documented by independent labs

    --- Multiple kill mechanisms confer broad-based target efficacy, restricting microbial response

    --- Kills antibiotic-resistant bacteria (MRSA, CRE, etc)

    --- Sustained efficacy; MP/MAS capable of multiple cycles of killing, protects against re-emergence

  Long shelf life (maintains potency over 5 years and counting)

    --- Heat resistant (185°C/365°F), cold resistant (-85°C/-121°F)

    --- Functional at temperature extremes (i.e., should be effective against extremophile pathogens)

- **Microplatelet Technology vs Frontline Antibiotics**

  -- In every head-to-head lab test, Microplatelet Antibacterial Spheres (MAS) dramatically outperformed frontline antibiotics (see Table 1) [Tests performed by Micromyx, Kalamazoo, MI, except *B. anthracis* Ames which was performed by MRI Global, Kansas City, MO]

-- No internal testing in humans or animals have yet been conducted

Table 1. MAS vs Antibiotic Resistant Pathogens, Compared with Efficacy of Frontline Antibiotics

Independent Lab Tested	Pathogen	MAS			Antibiotic Parallel Tests		
		Agent Used	Log Kill	Time to Zero	**Antibiotic Used**	Log Kill	Time to Zero
Yes	MRSA (S.*aureus*, MMX 5999)	**MAS**	Log 7	3 hr	Levoflaxin	Log 4.5	Not Reached
Yes	CRE (K. pneumoniae, MMX 4691, CDC)	**MAS**	Log 8	3 hr	Tigecycline	Log 8	24 hr
Yes	CRE (E.coli MMX 5980) (CRE, NDM-1; ATCC 14579)	**MAS**	Log 8	3 hr	Colistine	See Note	Not Maintained
Yes	MRSA (S.*aureus*, MMX 2123) (V1SA)	**MAS**	Log 7	3 hr	Linezolid	Log 0.5	Not Reached
Yes	B. *cereus* MMX 2006 (ATCC 14579)	**MAS**	Log 8	1 hr	Ciprofloxacine	Log 0.5	Not Reached
Yes	B. *anthracis* (Ames) (B.E.I strain NR-411)	**MAS**	Log 6	6 hr	Not Tested	-	-

Note: Colistine appeared to achieve Log 8 kill in 3 hours, then pathogen rebounded back to Log 9

- **Safety:** Magnesium hydroxide affirmed as "Generally Recognized As Safe (GRAS)"

-- Title 21, Food and Drug Admin, Part 184, Direct Food Substances Affirmed As "Generally Recognized As Safe" (GRAS) Sec. 184.1428 Magnesium hydroxide

-- "(c) IAW 184.1(b)(1), the ingredient is used in food with no limitation other than current good manufacturing practice."

-- "(d) Prior sanctions for this ingredient different from the uses established in the section do not exist or have been waived."

-- [redacted]'s patented magnesium hydroxide MP is commercially used in meat processing industry to increase shelf life and lower/prevent bacterial growth

- Technology Readiness Level (TRL) by Pathogen Types

Table 2. TRL Status of R&D

Tasks	Pathogen Type	Examples	Deployment Type	Lab Status	Next Step
RD-1	Bacteria (Non-Sporulating)	E.coli, MRSA, Plague	Wet or Dry Kill	Formulation and kill testing complete	
RD-2	Bacteria (Sporulating)	Anthrax, C.diff, Bacillus thuringiensis	Wet Kill	Formulation and kill testing complete	
			Dry Kill	Formulation 50% complete (additional R&D)	
RD-3	Fungi/Mold	T. rubrum, Candida albins, rust, blight	Wet Kill	Formulation and kill testing complete	
			Dry Kill	Formulation 50% complete (additional R&D)	
RD-4	Viral	COVID 19, Smallpox, Ebola, Marburg, Lassa	Wet Kill	Formulation and kill testing complete	Other viral tests complete, COVID 19 complete by 3-22-20

All diseases/threats in table are Category A (Centers for Disease Control and Prevention)

-- Wet or dry formulation is for spaces without sensitive equipment issues (office spaces, public venues, public transportation), though dry is preferred option

-- Dry formulation is for spaces *with* sensitive equipment issues (e.g., aircraft, ships, spacecraft)

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IMPACT Diversity Solutions, Inc. is a Qualified Small Business (QSB) under the definition and regulations that govern the Small Busines Program administered by the Small Business Administration (SBA). IMPACT Diversity Solutions has received CVE Certification which pertains to both Veteran and Service-Disabled Veteran Classifications. The healthcare market is exploding with new technology and innovative new programs for the treatment of disease and together with our manufacturing partners, IMPACT is bringing these amazing new products to both the public and government markets. Improving heath with cost-effective, non-invasive solutions is a major objective of IMPACT.

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