Silver Dihydrogen Citrate, A Responsible Antimicrobial Solution

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The most significant of several important milestones for PURE's Silver Dihydrogen Citrate (SDC) technology over the past 5 years was the issuance of an exemption from the requirement of a tolerance published in the June 10, 2009 Federal Register (Vol. 74, No. 110, page27447). The regulation established SDC as a particular form of silver ion technology for use as an active ingredient in a pesticide product for sanitizing food contact surfaces.

SDC is eligible for the small business award. In addition, we submit this nomination for the EPA award focus area #3 -Design of Greener Chemicals. SDC is manufactured in the United States by PURE Bioscience, Inc., a Delaware corporation, and is registered with the US EPA through PURE Bioscience Inc.'s wholly owned subsidiary, ETI H2O. The company's headquarters are located in El Cajon, California.

Abstract

Microbial contamination is a primary concern in almost every aspect of our lives. Antimicrobials are intended to eliminate or control microbial contamination; however, traditional products are typically toxic to the environment and human health.

PURE Bioscience, Inc. has developed Silver Dihydrogen Citrate (SDC), a novel silver-based antimicrobial technology consisting of ionic silver stabilized with food grade citric acid, to address safety and environmental concerns across a platform of markets. In its concentrated form, SDC shows minimal toxicity to human health and remains stable for at least 10 years. At considerably lower concentrations, SDC meets or exceeds industry performance standards held by legacy chemistries. During manufacturing of SDC, no hazardous byproducts or wastes are produced.

SDC is registered under the Federal Insecticide, Fungicide and Rodenticide Act and marketed for use as a ready-to-use surface disinfectant and food contact surface sanitizer. This unique product meets the requirements for EPA's hazard Category IV for acute effects, yet performs as well as or more quickly than competing products in higher hazard categories. Combining rapid kill times with low toxicity, SDC allows for users to implement a practical solution to surface disinfection and sanitization.

SDC is also used as a broad spectrum preservative in personal care products worldwide, replacing more toxic products like triclosan, parabens and formaldehydes and as a deodorant active in Europe and other areas of the world, eliminating the need for aluminum-based actives. Unlike many paraben-containing preservatives, it will not migrate into the oil phase of emulsions and thus maintains strong efficacy against microorganisms in the water phase.

SDC likely has application against the contamination that leads to serious problems in the oil and gas industry from "soured" products and corrosion of drilling, extraction, storage and distribution infrastructure caused by microbial byproducts. SDC is being investigated for use in fracking and spoilage control to replace the more toxic chemicals currently used.

In addition to these primary industry solutions, other areas of research with SDC include pharmaceutical products, food processing aids and water treatment as well as biofilm control in medical, industrial and food processing systems.

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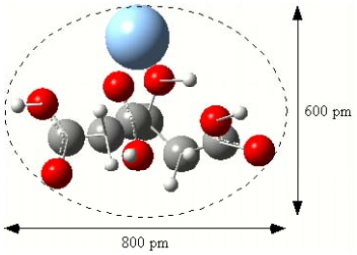
Silver Dihydrogen Citrate



Silver Dihydrogen Citrate;
1,2,3-propanetricarboxylic acid, 2-hydroxy-, silver(1+) salt, monohydrate;
($AgC_6H_7O_7$)

Silver Dihydrogen Citrate (SDC) is a highly soluble form of ionic silver stabilized in citric acid. Silver has been used for centuries for its antimicrobial properties. The antimicrobial action of silver or silver compounds is proportional to the bioactive silver ion released. Due to the stable nature of silver ions in SDC, a much lower concentration of the active substance compared to other forms of silver provides a comparable and often superior, broad spectrum, antimicrobial effect when compared to traditional silver and nonsilver chemical biocides.

SDC utilizes a multiple pronged attack against the cell wall and DNA of microorganisms. The high affinity of silver ions to sulfur-containing thiol groups found in metabolic and structural proteins, allows SDC to target these proteins in an organism's cell wall. This disruption of the organism's membrane function and integrity lyses the membrane and the organism dies. In addition, citric acid is recognized as a food source to some microorganisms. This allows SDC to easily enter the microorganism through membrane transport proteins. Inside the organism, SDC binds to DNA and intracellular proteins causing irreversible damage to the DNA and protein structure. Metabolic and reproductive functions halt, and the organism dies as a result of this "Trojan Horse" attack. Viruses are much smaller than bacteria and present fewer targets sites on which a biocide can act. SDC's efficacy against enveloped and non-enveloped viruses stems from its ability to target both the viral envelope and the viral nucleic acid. Silver destroys the viral envelope, preventing the virus from attaching to a host cell and it destroys the infectious component of the virus, the nucleic acid.

SDC is manufactured by electrolytically ionizing pure elemental silver in an aqueous citric acid solution and not through a chemical stream reaction. There are no other ingredients added to the concentrated solution. The proprietary process yields a very stable and reproducible end product and produces little waste and no toxic byproducts. Food grade/USP citric acid is produced by a natural (microbial) fermentation process that converts corn syrup to citric acid using *Aspergillus niger*. The process water used to manufacture SDC is deionized (DI) onsite allowing for 100% yield and no waste of water during treatment. Unlike common industrial water treatment systems, PURE's DI treatment system requires no electricity to produce the end product. While this practice is slightly more expensive than a combination reverse osmosis/deionization treatment system, it produces far less waste and results in a reduced environmental impact.
SDC is a colorless, odorless, non-flammable liquid that is compatible with a wide range of materials. At its most concentrated form, SDC is not classified as a hazardous chemical and is effective in hard surface applications at merely 0.003% w/w ionic silver. SDC has a low

potential for ecotoxicity because of the environmental fate and transport of ionic silver in natural waters. Ionic silver has a high affinity for reduced sulfur (primarily sulfide ions) and binds tightly with most forms of reduced sulfur, forming insoluble compounds such as silver sulfide.[1] Most wastewaters contain concentrations of sulfide ions far in excess of silver ions where solid metastable precipitates form, such as insoluble silver sulfide. Often, these precipitates are associated with wastewater sludge – which is removed during water treatment processes and absent from the "finished" discharged water. Only small amounts of all silver species (including ionic silver) are released to the post-treatment environment and, by some estimates, as much as 99% removal of silver is typical[2].

While other silver biocides (silver colloids, silver salts, silver zeolites or silver nanoparticles) also rely upon the antimicrobial action of silver ions, the release rate and available concentration of ionized silver varies. Colloidal silver is a suspension of submicroscopic metallic silver particles in a colloidal base. The release of silver ions from colloidal silver is dependent on environmental conditions, making the concentration of ionic silver released unpredictable. Zeolite silver ions strongly interact with the ceramic matrix and are minimally released from the matrix in deionized water, therefore silver zeolites tend to only offer bacteriostatic efficacy. Silver nanoparticles are small colloidal silver particles which also rely upon environmental conditions for release of silver ions, leaving the potential to deliver much higher concentrations of silver ions to a target site than is necessary. Silver nanoparticles are not well understood and may contain residual chemicals and reaction byproducts which may pose negative health and environmental impact. In contrast, SDC is a unique silver technology which provides stabilized ionic silver produced through an electrolytic process. Unlike these other silver technologies, SDC provides predictable concentrations of ionic silver and does not contain colloids or particles of any kind. SDC has the unique ability to deliver stabilized ionic silver in a predictable concentration which is not dependent on environmental conditions. Much lower concentration of silver is necessary when using SDC to achieve its broad spectrum antimicrobial effect.

Traditional chemical biocides include halogenated compounds (e.g., sodium hypochlorite, chlorine dioxide), quaternary ammonium chlorides, alcohols, phenols, gluteraldehyde, , hydrogen peroxide, parabens, formaldehyde and triclosan, many of which are produced through chemical or synthetic manufacturing processes. These biocides not only present hazards to humans during manufacturing and end use but also present hazards to the environment associated with release of toxic manufacturing byproducts. The toxicity of elemental and ionic silver is well characterized by multiple lines of evidence including both animal and human epidemiological studies, documented information on the metabolism of silver in mammalian species[3] (FR Vol. 74, No. 110 at 27448), and a safe history of use across many decades.

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[1] US Silver Task Force. Position paper on environmental fate issues for silver as related to the EPA Work Plan for Silver and Compounds. Pp. 1-170. Dated April 13, 2010.

[2] Eisler, R. Silver hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report 32. U.S. National Biological Service, Laurel, Maryland, Pp. 1-63. 1996.

[3] In fact, the toxicity of ionic silver is so well understood that it was concluded that additional animal data would not be useful in establishing a health endpoint more protective or more relevant than argyria in humans, a well

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PURE has been granted several patents for SDC in the US (6,197,814; 6,583,176; 6,890,953; 7,261,905; 7,435,438; 7,601,755; 7,732,486; 7,763,297; 7,803,407) and internationally for the composition, method of making and various use applications. In addition to the granted patents, there are several patents pending worldwide.

Surface Disinfection and Sanitization

Silver Dihydrogen Citrate is the basis for several products registered with the US EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) for indoor food and non-food uses. All of the registered products are liquids and are not applied as aerosols.

Product	EPA Reg. No.	Description
Axenohl	72977-1	Formulation Intermediate/Concentrate (0.24% Ag+/ 20.66% Citric Acid in water)
Axen	72977-2	Ready-to-Use, disinfectant
Axen30	72977-3	Ready-to-Use, disinfectant
Axen50	72977-4	Ready-to-Use, food contact surface sanitizer
SDC3A (PURE Hard Surface)	**72977-5**	**Ready-to-Use, disinfectant/ food contact sanitizer**
SDC0240CP	72977-6	Ready-to-Use, humidifier bacteriostat

A primary market focus for SDC is in surface disinfection and sanitization. PURE Bioscience markets SDC3A under the brand name PURE Hard Surface. In 2009, the Code of Federal Regulations, Title 40, Part 180.940 was amended in response to PURE's petition for the exemption from the requirement of a tolerance to allow silver, *specifically in the form of SDC*, to be used as an active in food contact surface sanitizers (Vol. 74, No. 110, page 27447).

PURE Hard Surface harnesses the broad-spectrum power of SDC for use on surfaces and equipment in dozens of settings including food processing plants, farms, restaurants, industrial settings, office buildings, schools, hospitals and other institutions as well as in homes. PURE Hard Surface helps prevent cross-contamination and eliminates odors. In addition, the odorless formula contains no VOC's and does not create irritating fumes, which is a particular benefit for restaurants, hospitals, schools and childcare settings.

Unlike traditional surface disinfectants and sanitizers, PURE Hard Surface requires no acute hazard or warning statements. A Signal Word is required based on the overall acute toxicity category assigned to a pesticide by the EPA. Category IV products require no Signal Word and no first aid statements. The Signal Word is determined by the most severe toxicity category assigned to the six acute toxicity endpoints[4]. PURE Hard Surface meets the EPA's requirements for category IV and the concentrate meets toxicity category III requirements. Many of the competing antimicrobial concentrates on the market fall within the highly toxic category I rating while most ready to use solutions fall within category II.

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characterized and understood cosmetic reaction that is not considered to be of any toxicological relevance. See the Antimicrobial Division white paper "Ionic Silver: Toxicity and Weight of the Evidence," dated May 11, 2009.

[4] The US EPA granted a waiver from Acute Inhalation testing and assigned SDC to Category IV.

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SDC Toxicology Studies Performed		
Design	**Test Article**	**Results**
Dermal Sensitization (Buehler Method)	SDC Concentrate	Not a contact sensitizer – **Category IV**
Primary Eye Irritation	SDC Concentrate	Mildly irritating to the eye – **Category III**
Primary Eye Irritation	SDC Ready to Use	Practically non-irritating to eye – **Category IV**
Primary Skin Irritation	SDC Concentrate	Slightly irritating to the skin – **Category IV**
Acute Dermal Toxicity	SDC Concentrate	Acute dermal LD_{50} >5,000 mg/kg – **Category IV**
Acute Oral Toxicity	SDC Concentrate	Acute oral LD_{50} >5,000 mg/kg - **Category IV**

PURE Hard Surface provides powerful protection against a broad range of microbes, including those of particular concern in food processing and kitchen (e.g. *E. coli*, *Salmonella*, *Listeria* and *Campylobacter jejuni*) and to the educational and healthcare setting (e.g., resistant pathogens such as MRSA, Hepatitis B and C viruses, Influenza A (H1N1), etc.). PURE Hard Surface has documented bacterial kill times as quick as 30 seconds, compared with 10 minutes for competing products, and may continue to kill bacteria for up to 24 hours after usage. PURE Hard Surface is also labeled for use in sensitive areas such as those used by children.

In 2011, the Child Safety Network (CSN) named PURE Bioscience the winner of its 2011 National Child Safety Award and granted PURE Hard Surface disinfectant its Safe Family Seal of Approval. CSN, a non-profit children's safety advocacy organization, challenged the major chemical companies to develop an effective but non-toxic disinfectant to combat the growing threat of bacteria, viruses and fungi. Children are unnecessarily exposed to outdated and toxic disinfection products that can put them at risk; however, at the same time, we must move aggressively to protect children from increasingly prevalent and dangerous pathogens. SDC-based PURE Hard Surface solves this dilemma by providing superior efficacy along with residual protection without the threat of accidental poisoning.

The PURE Hard Surface label was amended in August 2011 to expand efficacy claims and reduce kill times, giving it a significant competitive advantage in the surface disinfection marketplace.

Efficacy Data Results to Support Registered Claims	
30 second Contact Time	
Pseudomonas aeruginosa	Influenza A (H1N1)
Salmonella enterica	Swine Influenza A (H1N1)
HIV type 1	Respiratory Syncytial Virus
Rotavirus	Adenovirus Type 2
Human Coronavirus	
60 second Contact Time	
Herpes Simplex Type 1	Avian Influenza A
Hepatitis B Virus (HBV)	Influenza A
Hepatitis C Virus (HCV)	Rhinovirus

Murine Norovirus	Polio Type 2
Norovirus	
2 minute Contact Time	
Staphylococcus aureus	*Escherichia coli* O157:H7
Listeria monocytogenes	*Acinetobacter baumannii*
Vancomycin Resistant *Enterococcus faecium (VRE)*	*Campylobacter jejuni*
Methicillin Resistant *Staphylococcus aureus (MRSA)*	Carbapenem Resistant *Escherichia coli*
Community Associated Methicillin Resistant *Staphylococcus aureus (CA-MRSA)*	Carbapenem Resistant *Klebsiella pneumoniae*
Community Associated Methicillin Resistant *Staphylococcus aureus (CA-MRSA-PVL)*	Carbapenem Resistant *Klebsiella pneumoniae, NDM-1*
5 minute Contact Time	
Trichophyton mentagrophytes (Athlete's Foot Fungus)	
Food Contact Surface Sanitizer	
Staphylococcus aureus, E. coli (1 minute)[1]	

1 -EPA requirement for food contact surface sanitization is 99.999% reduction in 30 seconds for a 1 minute claim.

Also in 2011, PURE Bioscience completed the first Generally Recognized as Safe (GRAS) self-affirmation for SDC when used on food processing equipment, machinery and utensils. A committee of independent experts critically reviewed efficacy and toxicity data for SDC and the SDC-based disinfectant and food contact surface sanitizer. Consistent with the EPA's 2009 Exemption from Tolerance, the committee found no evidence that SDC demonstrates a hazard to the public when used as a contact biocide on food contact surfaces and food-use utensils and therefore concluded this use as Generally Recognized as Safe. PURE Hard Surface meets demands across multiple industries for a surface antimicrobial that reduces human exposures to toxic chemicals yet still provides broad spectrum efficacy at practical kill times.

Personal Care Preservative and Microbial Active

Silver Dihydrogen Citrate is marketed as a natural preservative for personal care products and as a deodorant active. Previously sold under the brand name Tinosan® SDC, the product is now marketed under the brand name, SILVÉRION 2400.

Chemical preservatives are found in almost all personal care products. Commonly used preservatives include parabens, formaldehyde or formaldehyde donors, polychloro phenoxy phenol (Triclosan®), phenoxyethanol and isothiazolinones. Together, these chemicals are linked to cancer (carcinogens), allergic reactions, skin, lung and eye irritation, immunotoxicity, neurotoxicity, developmental problems, endocrine disruption, aquatic toxicity and toxicity to marine life at levels found in the environment. Aluminum-based compounds are found in many deodorant products as an active ingredient and have been shown to build up in the brain and contribute to Alzheimer's and other brain disorders and are thought to contribute to development of breast cancer. SDC is a beneficial alternative because silver does not cause these types of effects.

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The European Commission Directorate for Health & Consumer Protection issued an opinion on SDC which was adopted on October 13, 2009[5] The Committee evaluated SDC as a preservative for aqueous leave-on and rinse-off cosmetic products and as an active ingredient for deodorant products. In addition to chemistry and public literature on safety of silver, the Committee also evaluated acute toxicity data, mucous membrane irritation, dermal absorption, repeated dose toxicity, mutagenicity and genotoxicity, teratogenicity and photo-induced toxicity. Based on the cumulative information, the Committee concluded that the use of SDC in cosmetic and deodorant products at a concentration up to 0.2% as a preservative or deodorant active does not pose a risk to the health of the consumer.

Preservation of personal care products is a necessary practice for consumer safety and SDC is a natural alternative to these highly hazardous chemicals. It may be used alone at low levels and in combination with traditional chemicals to reduce consumer and environmental exposure.

Oil/Natural Gas

Controlling microbial contamination from sulfate reducing bacteria (SRB's) and associated biofilms in the oil and gas industry costs millions of dollars annually in the US. The petroleum industry treats the SRB-containing fluids and infrastructure with high concentrations of biocides or metabolic inhibitors that often have major health risks to humans and all animals in the food chain. Based upon SDCs broad efficacy profile and results demonstrating SDC's effectiveness against biofilm in tests conducted by the University of Medicine & Dentistry of New Jersey, PURE believes the impact of SDC in the oil and gas industry could be substantial. SDC has demonstrated the ability to penetrate the biofilm and kill bacteria within minutes at very low concentrations but also to inhibit the formation of biofilm at levels as low as 1.5 ppm in a citrate-containing medium. Further commercialization of SDC in these applications has the potential to replace the vast quantities of hazardous chemicals pumped into the earth each year.

Conclusion

It is estimated that, in the US alone, the household, industrial, and institutional cleaning products industry consumed approximately 39 million pounds of biocides in 2008. It is anticipated with so many emerging and resistant pathogens and concerns over food safety, these statistics will likely continue to rise. SDC products meet the growing demand of multiple industries for natural, non toxic biocides without sacrificing broad spectrum efficacy. Implementation of SDC based products not only has the potential to drastically reduce the quantities of hazardous biocides used and frequency in which they are applied, but also to increase worker and plant safety, reduce cost associated with handling and transporting hazardous chemicals, and minimize the overall industry reliance on highly toxic biocides. In addition, it is very unlikely that ionic silver released by SDC products will have a negative environmental impact. Overall, compared to existing actives, SDC has a more attractive hazard profile throughout its life cycle, requires much smaller amounts of active ingredient to be effective in use applications and works more quickly against a wide variety of pest organisms.

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[5] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_004.pdf