Minncare® Cold Sterilant **Research Data**

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EFFECTIVENESS

In Vitro Aqueous Testing

To show that Minncare is effective against other microorganisms, the following in vitro tests were performed using a .5% solution of Minncare. All tests were done on aqueous test solutions at 20°C.

In Vitro Aqueous Test at 20°C

Minncare Concentration .5%		
Species	Count per ml	Time for 100% kill in minutes
Bac. subtilis	6 x10^6	2.5
Bac: stearothermophilus	6 x 10^6	2.5
Bac. subtiis NCTC 3610	2.4 x 10^9	5.0
Bac. mesentericus	1.6 x 10^9	5.0
Clostr. perfringens	1 x 10^7	10.0
Clastr. tyrobutyricum	1 x 10^7	5.0
Sacchar. cereisiae	6 x 10^7	0.5
Cand. mycoderma	1.4 x 10^8	0.5
Hansenula anomala	6.4 x 10^8	0.5
Pichia membronaefaciens	4.8 x 10^8	0.5
Pen. camerunense	1.7 x 10^8	2.5
Mucor plumbeus	3 x 10^6	2.5
Geotrichum candidum	2 x 10^7	0.5
Byssochlamys nivea	6 x 10^7	0.5
Staph. aureus	2.6 x 10^9	0.5
Strept. faecalis	4.6 x 10^9	0.5
Kleb. aerogenes	2.3 x 10^9	0.5
Ps. fluorescens	4.6 x 10^9	0.5
Ps. aeruginosa	2 x 10^9	0.5
Salm. thyphimurium	2.8 x 10^9	0.5
Coryneb. rubrum	1 x 10^7	1.0
Leuconostoc spec.	5.3 x 10^8	0.5

AOAC Sporicidal Testing

To show that Minncare is sporicidal, the test method of the American Organization of Analytical Chemist (AOAC) was performed. This test is accepted by the E.P.A. to demonstrate the efficacy of a chemical as a sterilant.

Cultures of Bacillis subtilis and Clostridium sporogenes were grown. Both silk suture and loops and ceramic cylinders were contaminated with the cultures and dried for 24 hours in a vacuum oven. Tests were then run to show that after dehydration the spores are still viable and that resistance is within tolerance.

Five loops or cylinders were then placed into 10ml of the test solution, in this case diluted Minncare solution. The test time was set for 11 hours.

Following the 11 hour contact time, the carriers were removed from the test solution and individually placed into test tubes containing a subculture medium. The test samples were then transferred to a fresh tube of thioglycolate and incubated for 21 days at 37°C.

A total of 720 tests were performed on three lots of the test chemical. Results of the test are shown on the chart below.

AOAC Sporicidal Testing

Lot/Organism	Carrier Type	Positives/Total
1 Bacillus subtilis	loop	0/60
1 Bacillus subtilis	cylinder	0/60
1 Clostridium sporogenes	loop	0/60
1 Clostridium sporogenes	cylinder	0/60
2 Bacillus subtilis	loop	0/60
2 Bacillus subtilis	cylinder	0/60
2 Clostridium sporogenes	loop	0/60
2 Clostridium sporogenes	cylinder	0/60
3 Bacillus subtilis	loop	0/60
3 Bacillus subtilis	cylinder	0/60
3 Clostridium sporogenes	loop	0/60
3 Clostridium sporogenes	cylinder	0/60

NO POSITIVE CULTURES WERE OBTAINED, THUS MINNCARE PASSED THE AOAC SPORICIDAL TEST.

The performance standards are as follows:

- For sporicidal claims, no more than 2 failures can be tolerated.
- For sterilizing claims, no failures can be tolerated.
- Growth must be observed in tubes with carriers exposed for 2 minutes to 2.5N HC (per the dehydration/ resistance test).
- Controls must show growth.

All of these performance standards were met by an 11 hour exposure to Minncare solution.

Vapor Kill Testing

The ability of Minncare vapor to kill Bacillus subtilis spores was tested in vitro.

An aqueous solution of Bisubtilis spores was made by grinding spore strips with sterile water in a sterile container. The solution was then filtered through .45 µm membrane filters. A specific amount of the solution was filtered through each membrane to give a 103, 104, 105, 106 spores respectively. Each of the filters supported 1" above a 1% solution of Minncare. Controls had the filters supported 1" above sterile water. All jars were then covered lightly with plastic caps. The jars were allowed to stand for 21 hours at ambient temperature.

The filters were then removed and transferred to petri dishes containing plate count agar. The dishes were incubated for 48 hours at 35°C. The results are recorded in the chart below.

Deactivation of Organisms By Minncare Vapor

Estimated spore concentration of filter suspended 1 inch above	B. subtilis spores/filter
Control: water	loop
10^3 spores	9.6 x 103 CFU*
10^6 spores	> 3 x 103 CFU
Minncare solution (1%)	
10^3 spores	<1 CFU
10^4 spores	<1 CFU
10^5 spores	<1 CFU
10^6 spores	<1 CFU
* Colony forming unit	

TOXICITY ASSESSMENT SUMMARY

AOAC Sporicidal Testing			
Toxicity Summary	Specimen	Results	Reference
Oral toxicity	Male Rats	LD_{50} = 2.43 (2.04-2.88) g/kg	Litchfield & Wilcoxon
	Female Rats	LD_{50} = 2.10 (1.92-2.30) g/kg	
Inhalation toxicity	Male Rats	Established lethal concentration	
	Female Rats	LC = 13,439 mg/cubic meter	
Skin sensitivity	Mice	No reaction	Burkhard's Test
	Humans	No visiable effects	
Mucos membranes	New Zealand Rabbits	Effects completely gone within 7 days	HH Draiz
Dermatological sensitivity qualities	White Guinea Pigs	No difference between control and test group	Klugman & Magnusson
Intravenous toxicity	Male Rats	LD_{50} = 212 mg/kg	Litchfield & Wilcoxon

Acute Oral Toxicity Testing

The test for acute oral toxicity of Minncare solution was conducted utilizing male wistar rats weighing 197 grams and female wistar rats weighing 157 grams. Ten rats were used per dosage. The Minncare was diluted with water and administered to the animals by means of force feeding hose. The solution was administered at a constant 20 ml/kg of body weight. The animals were observed after the treatment for period of 8 days. As symptoms of poisoning, the following variables were observed: skin rashes, decreased mobility, difficulties in breathing, cramps, and inability to stand up. The LD50 value was statistically, it was shown to be:

Male rats: LD_{50} = 2.43 (2.04 - 2.88) g/kg
Female rats: LD_{50} = 2.10 (1.92 - 2.30) g/kg

The dosage that was survived by all rats was 1.25 g/kg for male rats and 1.58 g/kg for female rats. The difference is due to the weight difference between males and females. After testing was completed, all animals were dissected. An analysis revealed etching of internal organs, but indicated that there were no specific toxic effects.

Inhalation Toxicity Testing

Because the possibility exists that persons exposed to Minncare for even a short period of time could inhale vapors, we have tested Minncare solution for acute inhalation toxicity with animals. Ten male and ten female rats were placed in an inhalation room and given a 5% solution of Minncare in the form of a fine mist. This concentration was inhaled by the rats for a period of 4 continuous hours without any toxic symptoms.

The test was then repeated with another 20 rats. This time, undiluted Minncare was sprayed into the inhalation chamber. Over a 4 hour test period, 38.2 grams of diluted solution and 28.8 grams of undiluted solution were administered to the animals. These products were mixed with 2123 and 2023 liters of air, respectively. The capacity of the inhalation chamber was 120 liters; therefore, 1.91 grams of undiluted Minncare from the diluted solution and 28.8 grams of undiluted Minncare were sprayed into the test chamber during the two tests. This resulted in concentrations of 851 mg/m^3 of Minncare in the test using 5% (diluted) concentration of Minncare and 13,429 mg/m^3 of Minncare in the test using undiluted.

Neither group of rats that were exposed to the Minncare solution showed any symptoms of poisoning. The rats that were sprayed with the undiluted product exhibited the following symptoms: scratching their noses, inducement to sneeze, wet skin, general discomfort (which was expressed by crawling together in a corner of the room), and bent backs. These symptoms remained for 1 hour after the test ended. All 40 rats survived the chamber for the observed period of 1 week.

The lethal concentration of Minncare is therefore established at greater than 13,439 milligrams per cubic meter.

We can conclude from these tests that Minncare in the vapor form, when used according to the instructions, does not create any health hazard for personnel.

Testing of Skin Sensitivity

Repeated Application on the Skin of Hairless Mice

A group of 10 hairless mice was tested for skin sensitivity to Minncare. Minncare concentration of 2% was applied to a skin patch the size of a silver dollar twice a day for 2 weeks. It was rubbed into the skin and left there. Two mice showed a slight reddening of the skin after the fourth treatment. After the sixth treatment, this was still the case with 1 mouse. These were the only animals who displayed any such symptoms. The other animals did not show any symptoms during or after the treatment. After the eighth and through the twelfth treatment, none of the mice exhibited any skin reaction.

Burckhardt - Test with Volunteers (Humans)

The testing of skin sensitivity with repeated applications to 5 human volunteers was conducted according to the W. Burckhardt-Test as described in the professional Dermatology Magazine (p 179-188, 1970). Using a glass stick, a 3% Minncare solution was applied to a skin patch the size of a silver dollar located on the inside of each test subject's underarm. The treatment was repeated at 30 second intervals for a period of 30 minutes.

Neither during nor after this test, were there any visible effects of the product on the skin

Mucous Membranes Sensitivity Test

Two groups of four white male New Zealand Rabbits were used. Both groups were given 0.1 ml of a 3% water solution of Minncare. The solution was applied to the tear duct of the right eye. The left eye of the rabbits was left untreated to serve as a control. Group one's treated eyes were thoroughly rinsed 10 seconds after the Minncare solution was applied. Group two's eyes were not rinsed after the application.

Per the time schedule developed by the Draize method, the assessment of the effect on the cornea, iris, and the white of the eye followed 2, 6, 24, 48, 72 and 144 hours after the treatment (H.H. Draize, "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics," Assn. of Food and Drug Officials of the U.S., pp. 49-52, 1959). No effects on the cornea or iris and no infectious symptoms were observed. A severe reddening of the white of the eye occurred with both groups. The reddening experienced by groups one and two was 57.5% and 77.5% of the maximum possible reaction, respectively. This reddening decreased greatly within 3 days after the treatment, and was completely gone 7 days after the treatment.

This research indicates that the contact with mucous membranes in the eye with both undiluted or slightly diluted product must be avoided. Contact of the eye with diluted Minncare can result in reddening of the eye. If such contact does occur, a fast, intensive rinsing of the eye should be performed, and an ophthalmologist should be consulted. Safety glasses should always be worn when working with Minncare solution

Dermatological Sensitivity Test

This test was conducted according to the method of Kligman and Magnusson (Journal of Investigation Dermatology, Volume 52, pp. 268-276, 1979). Two groups of pure white guinea pigs were used. Each guinea pig was between 300-400 grams. A spot 5 x 6 cm was carefully shaved behind their shoulder blades.

The test animals in group one were given three intracutaneous injections on each side of the back bone. The injections were as follows: 1) 0.1 ml Freund' Schem adjuvans placebo; 2) 0.1 ml solution of 1% Minncare; and 3) .1ml of mixture of .05 ml placebo and .05 ml 1% Minncare solution.

The control group two were given two intracutaneous injections on each side of the backbone. The injections were as follows: 1) 0.1 ml placebo; and 2) 0.1 ml distilled water. Eight days later, a 1 % solution of Minncare and Vaseline® was applied to the shaved patch with gauze and held in place with tape. This gauze was removed 48 hours later.

After a 14 day interval in treatment, an area 2 x 2 cm was shaved on the right flank of all two groups of animals. Vaseline® mixed with a 1% Minncare solution was applied to the area and covered with gauze. The gauze was taped in place for 24 hours. After the removal of the gauze, all animals in both group one and group two showed equally strong reddening of the skin, which must be given a Kligman and Magnusson value of 1. Twenty-four hours later, this was only the case with 4 animals. Another 24 hours later, none of the 20 animals showed any reddening of the skin.

Because we cannot observe a difference between the test animals and the control animals, it was concluded that Minncare does not have any sensitivity effect on the skin. In this test, only primary skin reactions which were caused by the 1% Minncare solution were observed.

Environmental Effects

The effects of Minncare on the environment were evaluated according to standard testing methods. (USEPA Guideline No. 72-1) The tests included the following:

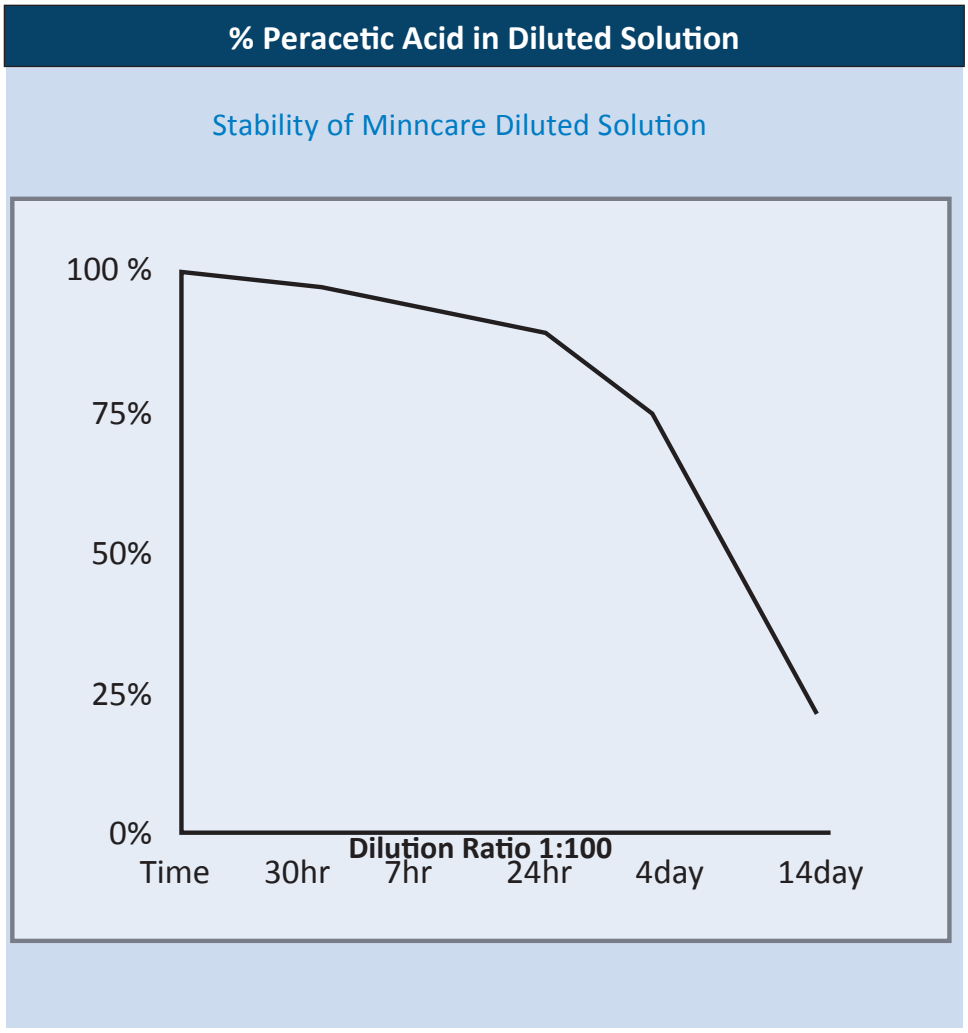
1. Minncare as H_20_2 : Trimetric Analytical Method Validation in Freshwater. Laboratory project #J9207003d.

2. Minncare Acute Toxicity to Rainbow trout, Oncurhynchus mykiss, Under Static Test Conditions. Laboratory project #J9207003b.

3. Minncare: Acute Toxicity to Bluegill, Lepomis macrochirus, Under Static Test Conditions. Laboratory project #J9207003c.

4. Minncare: Acute Toxicity to the Water Flea, Daphnia magna, Under Static Test Conditions. Laboratory project #J9207003a.

The results of this testing are available upon request.



Stability Over Time

Although Minncare will remain stable in its concentrated form for over 1 year, once it is diluted, a decay process begins to take place. The decay is such that when diluted with AAMI quality water, 50% of the peracetic acid will remain after 7 days. The graph that follows shows the decay curve of Minncare diluted 1:100 over a 14 day period. The ratio of dilution effects the rate of decay of peracetic acid contained in the Minncare solution. Thus, if the dilution ratio is less than 1:100, the rate of decay will be less.

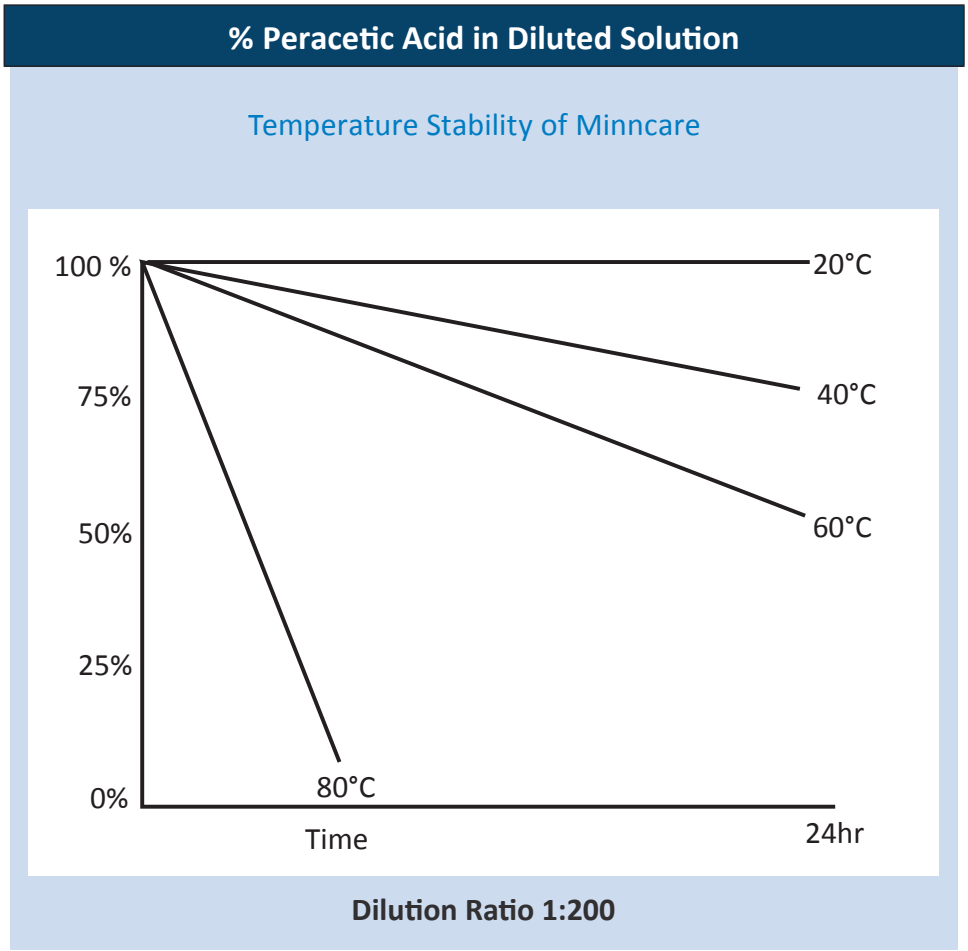
Temperature Stability

Concentrated Minncare remains stable at temperatures up to 30°C (86°F) for a period of 1 year. However, once Minncare is diluted, the rate of decay of the peracetic acid is greatly increased as the temperature increases.

%Peracetic Acid in Diluted Solution

Minncare Stability:

Once the product is diluted, it must be used within 7 days, and storage and use temperature must be maintained below 30°C (86°F).



Storage Conditions

A test was conducted on a .5% solution of Minncare to demonstrate the stability of peracetic acid in various storage conditions. The storage conditions researched consisted of translucent containers at room temperature, dark containers at room temperature and dark containers at 37°C. The selection of these test conditions was based on the knowledge that both temperature and light can affect the level of peracetic acid. The results of this test are shown in the table below:

Stability of Peracetic Acid with Storage Conditions

Minncare Concentration .5%			
Storage Condition	**% of Decrease in 7 Days**	**% of Decrease in 14 Days**	**% of Decrease in 112 Days**
Translucent Container Room Temperature	43%	58%	83%
Dark Container Room Temperature	36%	43%	80%
Dark Container 37°C	51%	78%	78%

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