UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

July 1, 2004

MEMORANDUM

Subject: Efficacy Review for EPA Establishment No. 67712-RL, Nature^2 Spa Mineral Sanitizer for Spas; DP Barcode: 303270

From: Tajah L. Blackburn, Ph.D., Microbiologist
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Thru: Nancy Whyte, Acting Team Leader
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To: Marshall Swindell PM 33/Tony Kish
Regulatory Management Branch I
Antimicrobials Division (7510C)

Applicant: Zodiac Pool Care, Inc.
2028 NW 25th Avenue
Pompano Beach, FL 33069

Formulation from Label

Active Ingredient(s)	% by wt.
Metallic Silver**	3.51%
Other Ingredient(s)	88.26%
Total	100.00%

** From Silver Nitrate

Note– The additive value of ingredients from the proposed label (% by wt)is not equivalent to 100%.

I BACKGROUND

The product, Nature^2 Spa Mineral Sanitizer for Spas (EPA Establishment No. 67712-RL), is a new product that contains silver dispersed onto an inert microporous substrate within a cartridge. The cartridge containing the silver media is placed into a filter unit of a spa. As water flows through the cartridge, silver is dissolved into the spa water at low levels . The applicant requested to register the product as a spa disinfectant/sanitizer based on this patented technology. Per the advise and direction of Tony Kish, the applicant has selected SilSpa Disinfectant (EPA Reg. No. 3432-71) as a substantially similar product. The submitted studies were conducted at AppTec Laboratory Services, located at 2540 Executive Drive, St Paul, MN 55120; MicroBioTest, Inc., located at 105B Carpenter Drive, Sterling, VA 20164; and Hill Top Research, Inc., located at 900 Osceola Drive, West Palm Beach, FL 33409.

The submitted data package contains three efficacy studies (MRID 4623311-04, -05, and -06), summary of efficacy studies, EPA Form 8570-6, No Data Confidentiality Claims for all three studies, and the proposed label.

II USE DIRECTIONS

The product is described as both a sanitizer and a disinfectant for spas. Directions on the proposed label provided the following information regarding preparation and use of the product:

Start-up– Before starting up a new Nature^2 Spa drain and clean debris out of the spa and spa equipment. Refill and balance water according to operating instructions. Install the Nature^2 Spa. Superoxidize spa water with dichlor. Run spa.

Daily Maintenance– Before each use test the water with Nature^2 Spa Test Strip. If the potassium peroxymonopersulfate (MPS) levels is low add 1 tablespoon of MPS to spa per 250 gallons (approx. 1000 liters). Use the Nature^2 Spa Test Strip to test the spa water after each addition of MPS. If the test strip fails to indicate MPS levels in the OK range, add 1 tablespoon of MPS to spa per 250 gallons and re-test. Enter spa only after test strip indicates a sufficient level of non-chlorine oxidizer.

After Each Use– Add tablespoon of MPS to spa per 250 gallons (approx 1000 liters)

Weekly Maintenance– Adjust the pH, total alkalinity, and hardness until the Nature^2 Spa Test Strip indicates that the parameters are within the OK range.

Quarterly Maintenance– Drain and refill your spa. Replace Nature^2 Spa, repeat sanitizer start-up.

As Needed– Shock treat with 1.5 tablespoons of dichlor per 250 gallons (approx 1000 liters) to remedy problems which may occur when bathing loads are high, when successive oxidizer test strip readings indicate high demand, when water appears hazy or dull, when unpleasant odors or eye irritation occur, after heavy winds and rainstorms

or if foam develops.

A cartridge lasts for 4 months. The cartridge should not be used longer than four continuous months.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Water in Swimming Pools, Spa, Hot Tubs, Whirlpools, and Jacuzzis

Swimming pool (and spa) water disinfection presents a unique combination of variables, including the number of swimmers/bathers, the frequency of use, the frequency with which the water is changed, general environmental conditions, and the type/degree of organic contamination of the water by the swimmers/bathers (e.g., suntan lotions and oils) and by various debris. As a result, both laboratory testing and confirmatory field testing are required.

The effectiveness of swimming pool and spa additives may be substantiated with data derived from the AOAC Disinfectants (Water) for Swimming Pools Method, 17th Edition, 2000, against both *Escherichia coli* (ATCC 11229) and *Enterococcus faecium* (ATCC 6569). The method may be modified, such as for pH. An initial bacterial suspension count of 2×10^8 is desired. Time zero bactericidal concentrations must be in the range of 9.9×10^5 to 1.5×10^6. Available chlorine at time zero in the NaOCl test control must be within ≥ 0.58 to ≤ 0.62. Results in the NaOCl control test must show complete kill of *E. coli* within 0.5 minutes, and *E. faecium* in 2 minutes. Test results must show the absence of colony growth on dilution plates and the absence of growth in all 5 lactose or thioglycolate tubes to demonstrate complete kill of the test organisms. Product test results must be equivalent to those of the NaOCl control. These Agency standards are presented in DIS/TSS-12 and the AOAC test method Disinfectants (Water) for Swimming Pools.

Confirmatory field testing must take place in at least two swimming pools (or spas), under Experimental Use Permit, lasting for an entire swimming season (4 to 12 months). Reports must include at least the following data regarding the test pools:

(i) The design of the pool, the re-circulation and filter systems, and the water capacity
(ii) The daily bather load
(iii) The amount and identification of all chemicals added daily (specifying time, site and method)
(iv) The range of chemical characteristics of the water, such as pH, nitrogenous substances, metal and hardness
(v) The physical characteristic of the water, including temperature and clarity, determined at least daily
(vi) Daily meteorological data, including air temperature, rainfall, and number of hours of sunlight for outdoor pools
(vii) Bacteriological monitoring should be conducted daily, in accordance with the suggested Ordinance and Regulations Covering Public Swimming Pool of the American Public Health Association. Water samples for bacteriological analysis should be taken on opposite sides of the pool in the shallow area and as remotely as possible from the inlets, preferably at the midpoints between inlets. A minimum of 144 samples should be taken during the test period. Samples should be taken just below the surface of the

water, and preferably at such times when the number of persons using the pool during the preceding hour has been at least 50% of the maximum bather load of the pool, and the number of persons in the pool water at the time the samples are collected is at least equal to 25% of the maximum bather load of the pool. Pertinent chemical characteristics of the pool water at the sampling site should be determined at the time of sampling.
(viii) The concentration of the antimicrobial agent in the water monitored daily at the same time-intervals that the bacteriological assay samples are obtained.
(ix) The method that the product user will employ for monitoring the level (ppm) of antimicrobial agent in the water.

Field test results must show that 85% of the samples collected meets the following indices (i.e., or that not more than 15% of the samples collected fail the following indices): The standard plate count at 35°C shall exceed 200 colonies/1.0 mL; (2) The most probable number of coliform bacteria shall be less than 2.2 organisms/100.0mL. When the membrane filter test is used, there shall be no more than 1.0 coliform organism/50mL; and (3) The most probable number of enterococcal organisms shall be less than 2.2/organisms/100.0 mL. When the membrane filter test is used, there shall be no more than 1.0 enterococcal organism/50mL. These Agency standards are also presented in DIS/TSS-12.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 462311-04 "AOAC Disinfectant (Water) for Swimming Pools" in support of Nature^2 Spa Mineral Sanitizer, by Karen Ramm. Study conducted at AppTec Laboratory Services. Study completion date– June 20, 2001.

This study was conducted against *Escherichia coli* (ATCC 11229) and *Entercoccus faecium* (ATCC 6569). Two lots of the test substances per organism (Lot Nos. 2-6-01-1 and 2/08/01-2 [for *E. coli*], 740-15 and 750-16 [*E. faecium*]) were tested using the AOAC Disinfectants (Water) for Swimming Pools Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The numbers control demonstrated 7.0 x 10^5 CFU/mL for *E. coli* and 2.5 x 10^6 CFU/mL for *E. faecium*. Approximately 1.5-3.0 mL K_2HPO_4 buffer and 0.5 mL KH_2PO_4 were added to the flasks and diluted to 900 mL. From a standard stock solution 3.83 mL NaOCl was added to satisfy the chlorine demand on 1 L of test water and to provide approximately 0.6 ppm residual available chlorine, then diluted to chlorine. To each of four 500 mL Erlenmeyer flasks, 199 mL of the test solution was transferred and placed in a water bath at the specified exposure temperature. The residual available chlorine was recorded to be 0.615 and 0.61, within the required range of ≥0.58 but ≤0.62 ppm chlorine. A 1.0 mL aliquot of *E. coli* suspension was added midway between center and edge of liquid surface, immersing pipette tip slightly below the surface of the water. Subsequently, 1.0 mL of the mixture was removed and transferred to neutralizer blank after intervals of 0.5, 1, 2, 3, 4, 5, and 10 minutes. The neutralizer blank was shaken thoroughly after adding sample. Appropriate serial tenfold solutions were prepared in Butterfield's buffer and spread plated in duplicates on appropriate agar using standard microbiological techniques. This procedure was repeated using *E. faecium*. Following preparation of the dilution plate counts, five tubes containing 20 mL of lactose broth were inoculated with 1.0 mL aliquots from each neutralizer blank tube for each time interval for *E. coli*. While 5 tubes containing 20 mL of thioglycolate broth were inoculated

with 1.0 mL aliquots from each neutralizer blank tube for each time interval for *E. faecium*.

For test substance efficacy, two flasks were placed in water bath at the 37±1°C and equilibrated. One flask was inoculated with a 1.0 mL aliquot of a standard test culture suspension of *E. coli* and the other with a 1.0 mL aliquot of a standard test culture suspension of *E. faecium*. Each flask was subcultured at exactly the same time intervals and in the same manner used with NaOCl control. Following incubation, the subcultures were examined for the presence or absence of visible growth. Representative neutralized subculture growth was subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

Note– This protocol was amended per Sponsor's request to change substance lot number s for repeat assay. There was insufficient volume of test substance left for the repeat assay. Sponsor provided lots 740-15 and 750-16 for the repeat assay.

Note– Protocol was amended per Sponsor's request to add a nitric acid was for the test flask for the repeat assay. Sponsor provided correspondence detailing the method to be employed (dated Wednesday 02/14/2001, 1:38 PM)

Note– Protocol was amended per Sponsor's request to change the neutralizer from PBDW with 1% (10% thioglycolic acid + 14% Na_2SO_4) as indicated in the protocol, to PBDW with 1% (10% thioglycolic acid + 10% Na_2SO_4).

2. MRID 462311-05 "AOAC Official Method Disinfectants (Water) for Swimming Pools" in support of Nature 2 Spa Mineral Sanitizer, by Shiva Rajaram. Study conducted at MicroBioTest, Inc. Study completion date– December 3, 2001.

This study was conducted against *Escherichia coli* (ATCC 11229) and *Entercoccus faecium* (ATCC 6569). Two lots of the test substances (Lot Nos. 703-46-1 and 703-46-2) were tested using the AOAC Disinfectants (Water) for Swimming Pools Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995 and DIS/TSS-12. Two lots (Lot Nos. 703-35-1 and 703-37-1) of silver (10 ppm) concentrate was included in the test protocol. In this study, *Entercoccus faecium* (ATCC 6569) and *Escherichia coli* (ATCC 11229) were prepared from stock cultures, centrifuged and suspended in phosphate buffered dilution water. The average concentrations 5.8 x 10^5 and 1.6 x 10^6 CFU/mL for *E.coli*, and 1.1x 10^5, 5.9 x 10^5, 1.3 x 10^6, and 1.7 x 10^6 CFU/mL for E. *faecium*. A 199 mL aliquot of test sample was then inoculated with 1 mL of either *E. faecium* or *E. coli* suspension. Following contact times of 0.5 and 1 minute, samples of the microorganism/test sample mixture were neutralized and surviving microorganisms quantitated. Five broth tunes were also inoculated from each neutralized tube. Controls were treated in the same manner. Following incubation at 37°C, the plates were enumerated and broth tubes scored visually for growth. As defined by the AOAC, absence of colony growth on dilution plates and absence of growth in all 5 broth tubes is necessary to show complete kill of the test organism.

Note– Per the enclosed statement, this study does not meet the requirements for GLP in all provisions.

Note– Due to the lack of recorded volumes during the neutralization effectiveness control, it was not possible to calculate the number of colony forming units in the inoculum.

Note– Per the AOAC, the studies require contact times for both control and test solutions, 0.5, 1, 2, 3, 4, 5, and 10 minutes. However the submitted studies 0.5, 1, and 10 minute contact times for control and 0.5 and 1 minute contact times for test substances.

Note– The laboratory phase of this test was performed at MicroBioTest, Inc., from 09/01/99 to 01/02/00. Testing initiated on 09/01/99 for *E. coli* and *E. faecium*, resulted in the NaOCl controls failing to meet the acceptance criteria for complete kill of *E. faecium* within 0.5 minutes. At the request of the study sponsor, testing for *E. faecium* was repeated on 09/29/99. After a complete review of the data, initial counts were found to be below the test acceptance criteria. Testing was repeated for both *E. coli* and *E. faecium* on 12/14/99. Per the sponsor's request, testing was repeated for *E. faecium* on 12/30/99.

Note– The study was conducted on four separate test dates (9/01/99, 9/29/99, 12/14/99, 12/30/99). As a result of the failure of the inoculum to reach target levels on both 9/01/99 and 9/29/99, the study was repeated. Additionally, the NaOCl control did not pass the performance criteria against *E. faecium* on 9/01/99 and 9/29/99. On 9/29/99, the centrifuge could not be confirmed as fully functional. The control data for 12/14/99 was based upon the use of an untraceable source of chlorine.

Note– Numerous GLP transgressions occurred during the conduct of the study, which further compromised the scientific integrity of the study, in addition to GLP compliance status (Appendix I Project Sheet 8). The following GLP violations were discovered:

- Lack of direct and prompt recording of test results
- Lack of raw data
- Use of untraceable control material
- lack of direct and prompt recording of test results
- frequency of QA audits were insufficient to assure GLP compliance
- use of malfunctioning equipment
- use of expired reagents
- use of inexperienced personnel and study directors
- original final reports did not reflect the actual raw data
- frequent mathematical and rounding errors
- protocol deviations that were incorrect analysis method
- the study was not conducted according to protocol
- numerous errors were found in the raw data records

As a result of the numerous GLP violations, this study was changed to a non-GLP status.

Note– The intended concentration was 50 ppb silver and 25 ppm Potassium Peroxymonopersulfate (MPS) compound, with and without the addition of 10 ppm chlorite. However, the inductively coupled plasma analysis of the silver levels of the test materials following the test demonstrated values ranging from 13 ppb to 47 ppb. Likewise, although the target levels of the Potassium Peroxymonopersulfate (MPS) compound were 25 ppm for both test materials, the actual values determined by titration varied from 16 ppm to 29 ppm.

Note– The protocol stipulated that the test solutions were to be prepared with balanced water,

(i.e. water that is neither corrosive nor scale forming with a pH between 7.2 -7.8 and calcium hardness of about 200 ppm). No record of the balancing of the water was found in the raw data. Also there are no records that indicate how the glassware was cleaned prior to the study. Sponsor testing shows the lack of control hardness problems with the silver test solutions. Although the glassware was sterile, residual organic debris in the flask may consume MPS from the test material.

3. MRID 462311-06 "Field Study to Test the Efficacy of a Spa Disinfectant" in support of Nature2 Sap Sanitizer, by Angela Staples. Study conducted at Hill Top Research, Inc. Study completion date– October 8, 2003.

This study was conducted in compliance with GCPs to ensure safety of human subjects. Three outdoor, residential, portable spas were maintained for 29-30 weeks. Bathers were recruited by the Test Facility to utilize the spas based on a rigorous, high frequency schedule. Thirty generally healthy subjects participated in this study. Bather selection criteria is detailed in the study protocol. Bathers entered Spa #1 266 times over 29 weeks. Bathers entered Spa 2 246 times over 30 weeks. Bathers entered Spa #3 254 times over 30 weeks. Each bathing session lasted at least 15 minutes. Before and after each bathing session, subjects were evaluated for changes in health and skin. Two spas were treated with Nature^2 Spa Mineral Sanitizer, which uses low levels of silver to disinfect, and potassium peroxymonopersulfate (MPS, potassium peroxymonopersulfate or the active ingredient in Oxone) as the oxidizer. The third spa was treated with traditional chlorination, and maintained at 3-5 ppm chlorine using an EPA registered chlorine-based spa disinfectant. Each spa was a Reflections Spa, Model Envida L-260 with 500-gallon capacity and 5 person seating capacity. Spa 1 and 3 were manufactured with a blue interior, while Spa 2 with a white interior. Test strips were utilized for spa water maintenance. The study included at least one draining and refilling of each spa. The source water was analyzed before starting the study, during the initial start, and at periods throughout the study. All source water was run through an ion exchange resin bed unit as the spas were filled. The source water was typically collected after sanitization of the tap with 70% isopropanol followed by a 10-minute flush. The following observations were performed: bacteriologic testing of treated and source water; laboratory analysis of treated and source water including pH, total and free oxidizer and total alkalinity, and other physical observations. Meteorological data including air temperature, rainfall, and the number of hours of sunlight (length of day) were obtained from www.wunderground.com. A photographic record of spa water clarity was also maintained. For analysis of hardness, silver, copper and total dissolved solids and anions was outsourced to Flowers Chemical Laboratory, Inc., (Altamonte Springs, Florida) and the Sponsor.

More specifically, on the scheduled sampling days, water samples were collected upon opening of the spa prior to maintenance and 40±10 minutes following bathing. Water samples were taken approximately 12 inches below the surface of the water. Samples were collected from a location that was as far from the intake as possible. Samples were assigned unique identification numbers. For bacterial analysis, water samples were taken by removing 750 mL of water from each spa, then adding it to individual sterile sample bottles containing 1.5 mL of 10% sodium thioglycolate and 1.5 of 14% sodium thiosulfate (neutralizer). These samples were shaken for approximately 10 seconds to allow mixing of the neutralizer. For the determination

of total plate count, duplicate 1 mL and 50 mL aliquots of water were plated on R2A agar using the membrane filtration technique. Plates were inverted and incubated at 35-38°C under aerobic conditions for 96±4 hours. For the determination of coliform bacteria, duplicate 100 mL aliquots were plated on m-Endo LES agar using the membrane filtration technique. Plates were inverted and incubated at 35-38°C under aerobic conditions for 22-24 hours. For the determination of entercoccal bacteria, duplicate 100 mL aliquots were plated on mEndo agar using the membrane filtration technique. Plates were inverted and incubated at 40-43°C under aerobic conditions for 48-72 hours. If growth was present, transfers were made onto EIA agar and incubated at 40-43°C under aerobic conditions for 20 minutes. The presence of a black precipitate confirmed the *Entercoccus* species. Further confirmation and species identification was performed on all suspected *Enterococcus* species using the Remel RapID system. Samples were tested within 1 hour of sampling or stored at 2-8°C for no longer than 4 hours prior to performance of bacteriological analysis at the Test Facility. Consistent with bacteriological sampling, additional water samples were removed for quantitative chemical analysis at the Test Facility. Water samples were also collected in a sample bottle containing a fixative agent, as appropriate, for quantitative chemical analysis.

Note– Spa 2 arrived at the Test Facility with a heavily mold-contaminated filter. The manufacturer of the spas stated that the mold was most likely due to their failure to properly and completely drain the spa following their standard quality control leak test. The filter was replaced prior to the initiation of the study. However the condition of the filter may be reflective of heavy fungal contamination within the Spa 2 plumbing. As Spa 2 demonstrated a higher oxidizer demand throughout the study and performed differently in comparison to Spa 1, the presence of initial mold contamination may have contributed to the stringency of the study for Spa 2.

Note– The study was initially started on August 21, 2002. Due to recurring electrical problems with all three spa, causing each to stagnate unfiltered for unknown periods of time, the study was halted. According to spa manufacturer, the bather load in this study design created an excessive demand for these residential spas. The spas were outfitted with new 250 volt/30 amp fuses that provided increase capacity. The bather load necessitated lengthy operation of the spa circulation system on a daily basis, thus stressing the initial electrical configuration of the spas. Upon installation of the new fuses, the study was restarted on September 17, 2003. Although start-up procedures for both products required an initial 10 ppm chlorine shock, the impacts of the periods of stagnation, due to loss of electrical power, are unknown.

Spa	#1	#2	#3
Initial Start	Nature2 Lot# TSA020708-01	Nature2 Lot# TSB020708-01	Iso Chlor-56 (Chlorine) Lot# Z873A
Full Study	Nature2 Lot# TSB020708-02	Nature2 Lot# TSA020708-03	

Silver Results (ppb)					
Spa 1		Spa 2		Overall	
Mean	Range	Mean	Range	Mean	Range
14.2	1.3 – 20.9	14.2	1.5 – 24.0	14.1	1.3 – 24.0

Note– During weeks with holidays and maintenance issues, in order to maintain the required weekly and overall study bather load and water sampling objectives, the spas were subjected to multiple uses/bather load in a single day.

Note– Over the course of the field study we had to contend with two issues that are atypical for the normal spa consumer– municipal water that did not meet EPA's potable water standards, which is <500 CFU/mL, intermittently throughout the study and a spa that was delivered with fungal contaminated plumbing. Furthermore, the spas underwent long periods of stagnation due to electrical problems in the beginning phase of the study.

Note– The source water bacterial levels exceeded the EPA National Drinking water Standard for potable water. The source water may have been above potable water standards throughout the entire study. However, this cannot be confirmed due to the infrequency of the source water sampling employed throughout the study.

Analysis of Bacterial Content for Source Water

Description	Number of Tests Exceeding Microbial Recovery / Total Tests		
	Total Plate Counts	Total Coliform	*Entercoccus* sp.
Source Water	14/26 = 54%	5/26 = 19%	NA
EPA Drinking Water Criteria	Fail	Fail	Fail
Source Water	21/26 = 81%	5/26 = 19%	0/26 = 0.0%
EPA Pool Disinfection Criteria (<15% Samples exceed requirement)	Fail	Fail	Pass

Note– Although initially no oxidative shocks (e.g. 10 ppm chlorine doses) were employed, spa drain/re-fill and shock combinations were used over the course of the study to respond to the water quality and contaminated plumbing issues. Initially, the spas were drained, refilled and dosed with a 10 ppm chlorine shock in response to increasing bacterial counts, increasing oxidizer demand, or other water quality problems. Spa 3 was initially treated to weekly 10 ppm

chlorine shock treatments per manufacturer's label instructions. However due to increasing bacterial counts, additional shocking was initiated in Week 7 as the product label directs for heavy usage.

Note– The West Palm Beach, Florida Water Authority confirmed that the residual chlorine levels near the Test Facility were unacceptably low.

Note– The protocol states that silver/trace metal analysis and hardness will be performed monthly on source water. Instead these analyses will be performed on the source water at the initiation of the study, at the mid study refill of the spas and approximately every 7 week interval.

Note– In order to allow for quick turn around of results, effective November 20, 2002, Flowers Chemical Laboratories, Inc., will perform chemical analyses instead of the Sponsor on an as needed basis.

Note– Instead of incubation period of 48 hours for entercoccal bacteria, plates will be incubated between 48-72 hours.

Note– Page 15 of the protocol reads, "For the determination of standard plate count . . .incubated 35-38°C under aerobic conditions for 96±2 hours." It is doubtful that additional colony growth will occur after such a prolonged period of incubation; therefore, the range of incubation will be changed to ±4 hours.

V RESULTS

Calculated 30 Second Log Reductions for *Enterococcus faecium* 2/13/01

Sample ID	MRID No.	# Survivors/ mL in 30 Seconds	Log of # Survivors/mL at 30 Seconds	Numbers Control (CFU/mL)	Log_{10} of Numbers of Control	Log_{10} Reduction
Nature[2] Simulated Spa Water Lot 2-06-01-1	462311-04	<1	0	2.5×10^6	6.40	> 6.40
Nature[2] simulated Spa Water Lot 2-08-02-2		<1	0			>6.40
Chlorine Control		<1	0			>6.40

Calculated 30 second Log Reductions for *Escherichia coli* 3/8/01 (MRID No. 462311-04)

Sample ID	MRID No.	# Survivors/ mL in 30 Seconds	Log of # Survivors/mL at 30 Seconds	Numbers Control (CFU/mL)	Log_{10} of Numbers of Control	Log_{10} Reductions
Nature[2] Simulated Spa Water Lot 740-15		<1	0	7.0×10^5	5.85	> 5.85
Nature[2] simulated Spa Water Lot 750-16		<1	0			>5.85
Chlorine Control		<1	0			>5.85

Qualitative Test Results *E. faecium* (MRID No. 462311-04)

Sample I.D.	Date Performed	Number of subcultures	
		Tested	Showing Growth*
Nature[2] Simulated Spa Water Lot 2-6-01-1	2/13/01	5	30 sec= 0 1 min= 0 2 min= 0 3 min=0 4 min=0 5 min=0
Nature[2] Simulated Spa Water Lot 2/08/01-2		5	30 sec= 0 1 min= 0 2 min= 0 3 min=0 4 min=0 5 min=0
Chlorine Control		5	30 sec= 0 1 min= 0 2 min= 0 3 min=0 4 min=0 5 min=0

* Numbers of subcultures showing growth of the test organism.

Qualitative Test results for *E. coli* (MRID No. 462311-04)

Sample I.D.	Date Performed	Number of subcultures	
		Tested	Showing Growth*
Nature^2 Simulated Spa Water Lot 740-15	3/8/01	5	30 sec= 0 1 min= 0 2 min= 0 3 min=0 4 min=0 5 min=0
Nature^2 Simulated Spa Water Lot 750-16		5	30 sec= 0 1 min= 0 2 min= 0 3 min=0 4 min=0 5 min=0
Chlorine Control		5	30 sec= 0 1 min= 0 2 min= 0 3 min=0 4 min=0 5 min=0

* Number of subcultures showing growth of the test organism

Challenge Microorganism: *E. coli*

Test Results Expressed as Average Colony Forming Units (CFU/mL) Recovered, Percent Reduction, Log_{10} Reduction, and Number of Positive Tubes per Total Number

MRID No.	Test Date	Test Solution	Initial CFU/ mL Recovered	Contact Time	Average CFU/ mL Recovered	Log_{10} Reduction	% Red.	#+/ # of Tubes
462311-05	09/01/99	1	5.8×10^5 *	30 sec	$<1.0 \times 10^1$	5.763**	>99.9999%	0/5
				1 min	$<1.0 \times 10^1$	5.763		0/5
				30 sec	$<1.0 \times 10^1$	5.763		0/5
				1 min	$<1.0 \times 10^1$	5.763		0/5
	12/14/99 ***	2	1.6×10^6	30 sec	$<1.0 \times 10^1$	6.204**		0/5
				1 min	$<1.0 \times 10^1$	6.204**		0/5
				30 sec	$<1.0 \times 10^1$	6.204**		0/5
				1 min	$<1.0 \times 10^1$	6.204**		0/5

$< 1.0 \times 10^1$ indicates no recovery at the lowest dilution (10^{-1})

* Initial counts were below test acceptance criteria

** Log reduction calculated based on complete kill as defined by AOAC

***An untraceable solution bleach was used for this test date. Raw data is not available to support the second replicate of the chlorine analysis.

Challenge Microorganism: *E. faecium*

Test Results Expressed as Average Colony Forming Units (CFU)/mL Recovered, Percent Reduction, Log_{10} Reduction, and Number of Positive Tubes per Total Number of Tubes

MRID No.	Test Date	Test Solution	Initial CFU/ mL Recovered	Contact Time	Average CFU/ mL Recovered	Log_{10} Reduction	% Red.	#+/ # of Tubes
462311-05	09/01/99 *** ****	1	1.1×10^5	30 sec	8.0×10^4	0.138*	27.2727%	5/5
				1 min	2.8×10^4	0.594	74.5455%	5/5
		2		30 sec	7.5×10^4	0.166	31.8182%	5/5
				1 min	2.8×10^4	0.594	74.5455%	5/5
	09/29/99 *** ****	1	5.9×10^5	30 sec	$<1.0 \times 10^1$	5.771*	>99.9999%	0/5 *****
				1 min	$<1.0 \times 10^1$	5.771*		0/5
		2		30 sec	$<1.0 \times 10^1$	5.771*		0/5
				1 min	$<1.0 \times 10^1$	5.771*		0/5
	12/14/99 ******	1	1.3×10^6	30 sec	8.8×10^4	1.169	93.2308%	5/5
				1 min	9.6×10^3	2.132	99.2615%	5/5
		2		30 sec	7.8×10^4	1.222	94.0000%	5/5
				1 min	1.5×10^4	1.938	98.8462%	5/5
	12/30/99	1	1.7×10^6	30 sec	$<1.0 \times 10^1$	6.230*	>99.9999%	0/5
				1 min	$<1.0 \times 10^1$	6.230*		0/5
		2		30 sec	$<1.0 \times 10^1$	6.230*		0/5
				1 min	$<1.0 \times 10^1$	6.230*		0/5

$< 1.0 \times 10^1$ indicates no recovery at the lowest dilution (10^{-1})
* Log reduction calculated based on complete kill as defined by AOAC
** Mechanical problems were noted with the centrifuge on this test date **(Not detailed on the table per Agency)**
***Chlorine control fell below AOAC performance criteria
****Initial counts fell below test acceptance criteria
*****Data was not recorded contemporaneously with the study for one of the five tubes
******An untraceable solution of bleach was used for this test date. Raw data is not available to support the second replicate of the chlorine analysis.

NaOCl Results Expressed as Average Colony Forming Units (CFU)/mL Recovered, Percent Reduction, Log_{10} Reduction, and Number of Positive Tubes per Total Number of Tubes

Challenge Organisms and Test Date	Contact Time	Average CFU/ mL Recovered	Percent Reduction	Log_{10} Reduction	Number +/Total Number of Tubes
E. coli 09/01/99****	30 sec	$< 1.0 \times 10^1$	>99.9999	5.763*	0/5
	1 min	$< 1.0 \times 10^1$			
	10 min	$< 1.0 \times 10^1$			
E. faecium 09/01/99****	30 sec	8.3×10^4	24.5455	0.122	5/5
	1min	2.9×10^4	73.6364	0.579	
	10 min	2.5×10^4	77.2727	0.644	
E. faecium 09/29/99 *** ****	30 sec	$< 1.0 \times 10^1$	>99.9999	5.771*	0/5
	1 min	$< 1.0 \times 10^1$			
	10 min	$< 1.0 \times 10^1$			
E. coli 12/14/99**	30 sec	$< 1.0 \times 10^1$	>99.9999	6.204*	0/5
	1 min	$< 1.0 \times 10^1$			
	10 min	$< 1.0 \times 10^1$			
E. faecium 12/14/99**	30 sec	$< 1.0 \times 10^1$	>99.9999	6.114*	0/5
	1 min	$< 1.0 \times 10^1$			
	10 min	$< 1.0 \times 10^1$			
E. faecium 12/30/99	30 sec	$< 1.0 \times 10^1$	>99.9999	6.230*	0/5
	1 min	$< 1.0 \times 10^1$			
	10 min	$< 1.0 \times 10^1$			

$< 1.0 \times 10^1$ indicates no recovery at the lowest dilution (10^{-1}).
* Log reduction calculated based on complete kill as defined by AOAC.
**An untraceable solution of bleach was used for this test date. Raw data is not available to support the second replicate of the chlorine analysis.
*** Mechanical problems were noted with the centrifuge on this test date.
****Initial counts fell below test acceptance criteria.
*****Chlorine control fell below AOAC performance criteria.

Bacteriological Analysis of Spas 1, 2, and 3

MRID No.	Description	Number of Tests Exceeding Microbial Recovery / Total Tests		
		Total Plate Counts	Total Coliform	*Entercoccus* sp
462311-06	Spa 1	9/149=6.0% Pass	2/149= 1.3% Pass	0/149= 0% Pass
	Spa 2	16/150 = 10.7% Pass	3/151 = 2.0% Pass	0/151 = 0.0% Pass
	Spa 3	43/148 = 29.1% Fail	1/148 = 0.7% Pass	0/148 = 0.0% Pass

Note– Spa 2 (Bathing Day 2-2), Subjects remained in the spa for 17 minutes instead of the standard 15 minutes. This may potential affect microbial counts for Spa 2 bathing.

Note– On a few occasions samples were collected outside of the 40±5 minute window (46-49 minutes, Spa 1: 10/17, 10/24, and 11/05; Spa 2: 11/05 and 11/12; and Spa 3: 8/30 and 10/30). The effect upon the study is unknown.

Note– On the week of 11/08/2002, there were only 4 bather loads for Spa 3 due to the shocking of the spa. On the week of 11/25/2002, there were only 3 bather loads for Spas 2 and 3 due to delay in confirmation of water hardness from the refill of these spas.

Note– Before December 4, 2002, there are no records to confirm that the filters removed from each spa were allowed to dry prior to re-use. The effect upon the study is unknown.

Note– Documentation to show the shocking of Spa 3 on 10/11/2002, per Note to File signed 10/08/2002, was not made promptly per CFR 160.130 (e) Quantitative results.

Note– Due to continuous failures of Spa 3, shock level chlorine was added to the spa after bathers exited the spa on November 5, 2002. Bathers were not able to re-enter the spa until November 8th due to high levels of chlorine.

Note– A tap water sample was collected for bacteria analysis on January 6th . Spas 2 and 3 were refilled on January 9th. A preliminary reading of the tap water samples on January 9th showed bacterial counts well above the failing level for spa water (>200 CFU/mL). For this reason shock level chlorine was added to each spa after filing, prior to letting the spas come to temperature overnight per the protocol. The impact upon the study is unknown; however, allowing the contaminated water to stand in the spas without treatment would have provided an unfair challenge to the spas.

Note– The mEndo Plates were incubated on 01/01/2003 and 01/10/2003 were incubated at 38-39°C instead of the 40-43°C mandated by the protocol. The impact upon the study is unknown as no growth of any kind was observed on these plates.

Note– This study does not meet 40 CFR Part 160 in all provisions. Numerous GLP transgressions occurred during the study which compromised the GLP compliance status. These violations were magnified due to the field setting, duration of the study, and the volume of data. As a result, this study was converted to non-GLP status.

VI CONCLUSION

1. The submitted efficacy study (MRID No. 462311-04) does not support the use of the product, Nature^2 Spa Sanitizer, due to lower numbers control for *E.coli*. Initial suspension of *E. faecium* 5.3 x 10^8 CFU/mL, while initial suspension of *E. coli* was 1.28 x 10^8, AOAC recommends a initial count 2.0 x 10^8 so that 1 mL test culture suspension + 199 mL test solution will provide solution containing 0.9- 1.5 x 10^6 in order to achieve the required test inoculum. The number control for *E. coli* was 7.0 x 10^5, a value less than the required test inoculum. However the test parameters and condition were acceptable. Available chlorine at time zero in the NaOCl test control was within the required range of ≥0.58 but ≤0.62. Residual available chlorine present at the 10-minute exposure interval was >0.4 ppm, as required. NaOCl control test results showed complete kill of the organisms with 0.5 minutes, with *E.coli* demonstrating a greater than 5.85 log reduction, and *E. faecium* demonstrating a greater than 6.40 log reduction when exposed to Nature^2 Spa Mineral Sanitizer. Neutralizer effectiveness testing demonstrated positive growth of the test organisms.

2. The submitted efficacy studies (MRID No. 462311-05) do not support the use of the product Nature^2 Spa Sanitizer for several reasons, namely (1) numerous GLP transgressions occurred during the study thus rendering the data non-GLP status [a concise listing of each transgression is listed under Section IV. Comments on the Submitted Efficacy Studies]; (2) the inoculum of the *E.coli* test culture was 5.8 x 10^5 CFU/mL , when an inoculum at 0.9-1.5 x 10^6 CFU/mL is required; (3) the inoculum for *E. faecium* test culture was 1.1 x 10^5 CFU/mL (09/01/99) and *5.9 x 10^5* CFU/mL (09/29/99), both are below the required inoculum of 0.9-1.5 x 10^6 CFU/mL; and the Test Lab's observations of (4) low levels of silver coupled with the varying levels of MPS and sporadic failures of chlorine control resulting in increased variability and lack efficacious data. The study controls consisting of viability controls, sterility controls, neutralizer effectiveness and microorganism control/confirmation were included.

3. The submitted field efficacy studies (MRID No. 462311-06) does support the use of the product Nature^2 Spa Sanitizer as a spa disinfectant when used in conjunction with potassium peroxymonopersulfate (MPS), an oxidizer, as recommended by the label. The additional requirements as set forth by DIS/TSS-12 were addressed, such as least two spas were used, with data collection consisting of bacteriologic analysis testing (≥144 samples), water analysis (including pH, nitrogenous substances, metals, hardness), daily bather load, amount and identification of all chemicals added, meteorological data, methodology for chemical analysis, and design of the spas (i.e. re-circulation and filter system). The performance standards as outlined by DIS/TSS-12, were met for each test spa, and any additional anomalies were attributed to filter contamination and source water (table included).

It should be noted that numerous GLP deficiencies were present, namely, (1) lack of direct and prompt record keeping; (2) lack of direct and prompt recording of test results; (3) raw data omissions and errors; (4) inconsistent unique identification and control of water samples; (5) inconsistent adherence to standard operating procedures; (6) use of inexperienced and insufficiently trained personnel and Quality Assurance Unit; and (7) extensive protocol deviations.

VII RECOMMENDATIONS

1. Due to inadequacies with both laboratory studies (MRID No. 462311-04 and 462311-05) as detailed in the Conclusion section, the proposed label claims do not support the use of the

product Nature^2 Spa Sanitizer as a disinfectant for spa water. The field studies (MRID No. 462311-06) were considered acceptable. However per DIS/TSS-12, both laboratory and field test must be accepted for Agency registration. Upon re-submission of acceptable laboratory studies, the product may be granted registration.

2. On the proposed label, the list of ingredients is not equal to 100.00%. Please reconfigure and correct.

Active Ingredients:
Metallic Silver** 3.51%
Other Ingredients 88.26%
Total 91.77% ≠100.00%

3. DIS/TSS-12 provides standards for products that are to be used in disinfecting water contained in swimming pools, spas, hot tubs, whirlpools, and jacuzzis. The proposed label and applicant's letter uses sanitizer and disinfectant interchangeably. For clarity, disinfectant (in accordance with DIS/TSS-12) should be used.