UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 27, 2006

MEMORANDUM

Subject: Efficacy Review for Nature^2 Spa; EPA Reg. No. 67712-RL;
DP Barcode: D323851

From: Marcie Wawzysko Tidd, Microbiologist Marcie Tidd 1/27/06
Product Science Branch
Antimicrobials Division (7510C)

Thru: Nancy Whyte, Acting Team Leader
Product Science Branch
Antimicrobials Division (7510C)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

To: Marshall Swindell PM 33 / Martha Terry
Regulatory Management Branch I
Antimicrobials Division (7510C)

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

Formulation from the Label:

Active Ingredient(s)	% by wt.
Metallic Silver*	0.92%
Other Ingredients	99.08%
Total	100.00%

*From Silver Nitrate

I. BACKGROUND

The product, Nature^2 Spa, is a new product. It is intended for use as a sanitizer and disinfectant for hot tubs. The applicant is re-submitting laboratory efficacy data in response to deficiencies outlined in a July 8, 2004 efficacy review by The Agency. The study was conducted by ATS Labs located at 1285 Corporate Center Drive, Suite 110 in Eagan, Minnesota 55121.

The data package contained two letters from The Agency to the applicant (dated July 16, 2004 and July 28, 2004), a letter from the applicant to The Agency (dated August 29, 2005), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-35 (Data Matrix), a report (labeled MRID 466340-02) entitled "Efficacy Discussion", one study (MRID No. 466340-03) with Statements of No Data Confidentiality and Good Laboratory Practice, the proposed label and owners manual.

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 dichloro (sodium dichloroisocyanurate). 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. SUMMARY OF SUBMITTED STUDY

MRID 466340-03 "AOAC Disinfectant (Water) for Swimming Pools" in support of Nature^2 Mineral Sanitizer for Spas by David Rottjakob. Study conducted by ATS Labs, Project Number A02520. Study completed April 29, 2005.
This study was conducted against *Escherichia coli* (ATCC 11229). Six lots of the product (Lot Nos. 041105-A, 041105-B, 041208, 050216A, 050216B, and 050103) were tested following AOAC Official Method 965.13 Disinfectants (Water) for Swimming Pools, Official Methods of Analysis of the AOAC, 17th Edition 2000 (ATS Protocol No. SRC19082004.SWM.2). Due to product or testing deficiencies, only 3 lots (Lot Nos. 041105-A, 050216A, and 050216B) of the product were evaluated in what were considered to be valid tests. In the case of lots 041105-A, 041105-B, 041208, and 050103, the test agent was prepared by adding oxidizer and silver to balanced spa water prior to shipment to the test facility and adjusted prior to analysis. The remaining two lots were prepared on-site the day of testing by adding oxidizer and silver to 37°C balanced spa water. 18-24 hour old *E. coli* cultures were standardized with PBDW liquid to give a turbidity of 0.5 (McFarland Standard). It was determined that the chlorine demand of 200 mL of the deionized test water was 0.1 mL of 200 ppm chlorine. For the NaOCl control, exactly 2.25 mL KH_2PO_4 buffer and 0.5 mL KH_2PO_4 were added to a 1 L flask and diluted to 900 mL with sterile deionized water. 3.245 mL (11/11/04) and 3.40 mL (2/18/05) of NaOCl were added to satisfy the chlorine demand and provide a 0.6 ppm residual available chlorine level. The volume was made up to 1 L. 199 mL of the test solution was added to each of three 500 mL Erlenmeyer flasks which were held at 37±1°C. The first flask was used to determine the residual available chlorine level. To the second flask a 1 mL aliquot of the bacterial suspension was added with centrifugal motion to prevent pooling at the point of addition. A 1 mL aliquot was then removed and transferred to 9 mL Phosphate Buffered Dilution Water (PBDW) with 0.1% sodium thioglycollate and 0.1% sodium thiosulfate neutralizer after intervals of 0.5, 1,2,3,4,5, and 10 minutes and mixed. Serial dilutions were made in Butterfield's buffer and

plated in duplicate. In addition, 1.0 mL aliquots of each neutralizer tube were added to five tubes with 20 mL of lactose broth. The third flask was evaluated for residual chlorine after the 10 minute exposure period. The test substance assay was conducted in the same manner as the NaOCl control. All dilution plates and subcultures were incubated at 35-37°C for 48±4 hours. Controls included those for purity, sterility, viability, neutralization confirmation, numbers, and initial suspension control.

V. RESULTS

MRID 466340-03
Escherichia coli (ATCC 11229)
Laboratory Test

Test Date	Test Solution	Numbers Control CFU/mL	Contact Time	Average CFU/ mL Recovered	Log_{10} Reduction	% Red.	#+/ # of Tubes
11/11/04	041105-A*	1.2×10^6	30 sec	$<1 \times 10^2$	>4.1	>99.9917	1/5
			1 min	<1	>6.08	>99.9999	0/5
			2 min	<1	>6.08	>99.9999	0/5
			3 min	<1	>6.08	>99.9999	0/5
			4 min	<1	>6.08	>99.9999	0/5
			5 min	<1	>6.08	>99.9999	0/5
			10 min	<1	>6.08	>99.9999	0/5
11/11/04	041105-B	1.2×10^6	30 sec	<1	>6.08	>99.9917	0/5
			1 min	<1	>6.08	>99.9999	0/5
			2 min	<1	>6.08	>99.9999	0/5
			3 min	<1	>6.08	>99.9999	0/5
			4 min	<1	>6.08	>99.9999	0/5
			5 min	<1	>6.08	>99.9999	0/5
			10 min	<1	>6.08	>99.9999	0/5
12/15/04	041208*	1.10×10^6	30 sec	7.1×10^5	0.190	35.5	5/5
			1 min	5.7×10^5	0.280	48.2	5/5
			2 min	4.6×10^5	0.380	58.2	5/5
			3 min	1.2×10^5	0.960	89.1	5/5
			4 min	2.5×10^3	2.64	99.8	5/5
			5 min	2×10^2	3.7	99.9818	5/5
			10 min	$<1 \times 10^2$	>4.0	>99.9909	1/5
2/18/05	050216-A†	1.1×10^6	30 sec	<1	>6.04	>99.9999	0/5
			1 min	<1	>6.04	>99.9999	0/5
			2 min	<1	>6.04	>99.9999	0/5

			3 min	<1	>6.04	>99.9999	0/5
			4 min	<1	>6.04	>99.9999	0/5
			5 min	<1	>6.04	>99.9999	0/5
			10 min	<1	>6.04	>99.9999	0/5
2/18/05	050216-B†	1.1×10^6	30 sec	<1	>6.04	>99.9999	0/5
			1 min	<1	>6.04	>99.9999	0/5
			2 min	<1	>6.04	>99.9999	0/5
			3 min	<1	>6.04	>99.9999	0/5
			4 min	<1	>6.04	>99.9999	0/5
			5 min	<1	>6.04	>99.9999	0/5
			10 min	<1	>6.04	>99.9999	0/5
11/11/04	Chlorine Control	1.2×10^6	30 sec	<1	>6.08	>99.9999	0/5
			1 min	<1	>6.08	>99.9999	0/5
			2 min	<1	>6.08	>99.9999	0/5
			3 min	<1	>6.08	>99.9999	0/5
			4 min	<1	>6.08	>99.9999	0/5
			5 min	<1	>6.08	>99.9999	0/5
			10 min	<1	>6.08	>99.9999	0/5
12/15/04	Chlorine Control	1.10×10^6	30 sec	<1	>6.04	>99.9999	0/5
			1 min	<1	>6.04	>99.9999	0/5
			2 min	<1	>6.04	>99.9999	0/5
			3 min	<1	>6.04	>99.9999	0/5
			4 min	<1	>6.04	>99.9999	0/5
			5 min	<1	>6.04	>99.9999	0/5
			10 min	<1	>6.04	>99.9999	0/5
2/18/05	Chlorine Control†	1.10×10^6	30 sec	<1	>6.04	>99.9999	0/5
			1 min	<1	>6.04	>99.9999	0/5
			2 min	<1	>6.04	>99.9999	0/5
			3 min	<1	>6.04	>99.9999	0/5
			4 min	<1	>6.04	>99.9999	0/5
			5 min	<1	>6.04	>99.9999	0/5
			10 min	<1	>6.04	>99.9999	0/5

*Lot was later determined to be expired

† Initial bacterial suspension was below the 2.0×10^8 CFU/mL designated in the AOAC test

MRID 462311-04 (From previous submission)
Enterococcus faecium (ATCC 6569)

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

VI. CONCLUSIONS

1. The submitted data (MRID No. 466340-03, Lot #s 041105-B, 050216-A, and 050216-B) partially supports the use of the product, Nature^2 Spa Sanitizer, as a spa disinfectant when used in combination with potassium peroxymonosulfate (MPS), an oxidizer as recommended by the label. Initial *E. coli* counts on dates 11/11/04 and 12/15/04 were above the AOAC minimal suggested count of 2.0×10^8, however the count was slightly lower on 2/18/05 (1.4×10^8 CFU/mL). Numbers control counts were within the acceptable range for all three dates. Available chlorine levels were standardized to 0.6 ppm at zero time. Residual available chlorine present at the 10-minute exposure interval was >0.4 ppm, as required, for all three test dates. NaOCl control test results demonstrated complete kill of the organism within 0.5 minutes. Reductions of greater than 6.08 log (Lot# 041105-B) and 6.04 log (Lot# 050216-A, and 050216-B) were shown after 30 second exposure to Nature^2 Spa Mineral Sanitizer. Controls were acceptable for a valid test.

2. The submitted data (MRID No. 466340-03, Lot #s 041105-A and 041208) does not support the use of the product, Nature^2 Spa Sanitizer, as a spa disinfectant when used in combination with potassium peroxymonosulfate (MPS), an oxidizer as recommended by the

label. Post-testing, it was determined that these test substances were expired at the time of the test.

3. The cited laboratory data (MRID No. 462311-04, previously submitted) partially supports the use of the product, Nature^2 Spa Sanitizer, as a spa disinfectant when used in combination with potassium peroxymonosulfate (MPS), an oxidizer as recommended by the label. In this study, results for *E. faecium* were acceptable; however those for *E. coli* were not.

4. The previously submitted field efficacy data (MRID 462311-06) was found to be in support of use of the product, Nature^2 Spa Sanitizer, as a spa disinfectant when used in combination with potassium peroxymonosulfate (MPS), an oxidizer as recommended by the label.

VII. RECOMMENDATIONS

1. The proposed label claims that the product is effective as a spa disinfectant. This claim is acceptable as it is supported by previously submitted field efficacy data and laboratory data against both *Escherichia coli* (resubmitted) and *Enterococcus faecium* (previously submitted).

2. DIS/TSS-12 and the AOAC test used are applicable to products which are to be used as disinfectants for swimming pools, spas, and hot tubs. The proposed label uses the terms "Disinfectant" and "Sanitizer" interchangeably. These two terms convey different levels of efficacy. Since the product was evaluated as a disinfectant, all instances of the term "Sanitizer" should be removed from the product label. (This was previously stated in a 7/16/2004 letter from The Agency to the applicant's representative, however the language still appears on the label)