UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

June 24, 2002

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 67712-R / Nature2
DP Barcode: D278673

From: Ian Blackwell, Biologist
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Applicant: Zodiac Pool Care, Inc.

Formulation From Label:

Active Ingredient(s)	% by wt
copper sulfate pentahydrate	6.39
silver	2.96
Inert Ingredient(s)	90.65
Total	100.00

I **BACKGROUND:** Zodiac Pool Care, Inc., has submitted a set of antimicrobial efficacy studies to support the registration of their product "Nature2 G45-VC40". Nature^2 is a swimming pool water disinfectant that comes in the form of a cartridge. These studies are to support this product being registered as a swimming pool water disinfectant. The studies were conducted by ViroMed Biosafety Laboratories, Inc., and MicroBioTest, Inc. The MRID Numbers are 455137-03 through 455137-05. There is also an "Efficacy Discussion", MRID Number 455137-02.

While Nature^2 is sold in the form of a cartridge (please refer to Section II, below), the studies presented in this submission were conducted using a simulated version that one would expect to find from swimming pool water that had been treated using the Nature^2 cartridge. This is acceptable in this submission as the registrant was told in a meeting with AD that it would be acceptable to conduct a study in this method.

Agency guidelines state that this swimming pool water disinfectants are to have laboratory and field tests. This submission only covers the lab studies conducted on Nature^2. Field studies of Nature^2 were reviewed in a 10/2/2000 PSB/AD review.

II **Use Directions**

"The Nature^2 technology is based on the dissolution of silver and copper ions into pool water over a six month period. Disinfection and algicidal effectiveness is based on the maintenance of 0.01 - 0.06 ppm silver and 0.02 - 0.1 ppm copper ions in the circulation water accompanied by 0.5 ppm chlorine. The product consists of a combination of alumina sphere coated with either metallic silver or copper sulfate contained in a cartridge appropriately sized for the pool volume."

Important: Do not install the new cartridge until the pool water is chemically balanced.

1	pH:	7.4-7.8
2	Calcium hardness:	200-400 ppm
3	Total Alkalinity;	80-150 ppm

Installation of Nature^2: Turn off your pool pump. Disconnect the first hose section after your pool's skimmer, or dedicated vacuum line. Connect Nature^2 between the hose sections, turn on the pool pimp and activate the cartridge.

III Agency Standards for Proposed Claims

The Agency Standard for antimicrobial products that bear claims for swimming pool disinfection, were designed to consider numerous factors: numbers of swimmers in the pool, frequency of use, frequency with which water is changed, general weather conditions, and, the types and degree of organic contamination of the water by the swimmers themselves. Therefore, a two-phased study (presumptive laboratory testing and confirmatory field) is required.

1 Laboratory test requirements: Presumptive efficacy of swimming pool water disinfectants may be substantiated with data derived from the A.O.A.C. Method for Water Disinfectants for Swimming Pools or with slight modifications (i.e., pH) thereof, against both *Escherichia coli* and *Streptococcus faecalis*.

2 Performance standard for laboratory test: The lowest concentration of the test germicide providing results equivalent to those of the sodium hypochlorite control is the lowest concentration of the product that can be considered effective.

3 Field test requirements: Confirmatory efficacy data should be derived from in-use tests under an Experimental Use Permit in at least two swimming pools. The test must be conducted for an entire swimming season (4-12 months). Reports must include the following information concerning the test pools:

A The design of the pool
B The recirculation and filter system, and water capacity
C The daily bather load
D The amount and identification of all chemicals added daily to the swimming pool water
E The range of chemical characteristics of the swimming pool water
F The physical characteristics of the swimming pool water
G Meteorological data
H Water samples for bacteriological analysis should be taken on opposite sides of the pool in the shallow area and as remote as possible from the inlets (a minimum of 144 samples should be collected during the test period),
I The concentration of the antimicrobial agent in the swimming pool water monitored daily at the same time-intervals and that the bacteriological assay samples should be obtained at the same time-intervals that the bacteriological samples are obtained
J The method that the product used will employ for monitoring the level of antimicrobial agent contained in the pool water monitored

4 Performance standard for field test: The product, when used as recommended in swimming pool water, should demonstrate that not more than 15% of the samples collected shall fail to meet these bacterial indices:

A The standard plate count at 35°C shall not exceed 200 colonies per 1.0 mL.
B The most probably number of coliform bacteria shall be less than 2.2 organisms per 100 milliliters. When the membrane filter test is used, there shall be no more than 1.0 coliform organisms per 50 mL.
C The most probable number of enterococcal organisms shall be less than 2.2 organisms per 100 mL. When the membrane filter test is used, there shall be no more than 1.0 enterococcal organisms per 50 mL.

IV Comments on the Submitted Efficacy Studies

1 MRID Number 455137-03: "AOAC Disinfectant (Water) for Swimming Pools" by Brad K. Onstad. ViroMed Biosafety Laboratories, Inc. Project Number 10494. Study Completion Date 5/7/1.

This study was conducted to determine the ability of EPA File Symbol 67712-R to kill microorganisms commonly encountered in swimming pools compared to the capacity of NaOCl to kill the same organisms under the same conditions. The two species used in this study were *Enterococcus faecium* (ATCC 6569) and *Escherichia coli* (ATCC 11229). The study was requested (by the registrant) to be conducted with solutions prepared from silver and copper salts to achieve 0.01 - 0.06 ppm silver, and, 0.02 - 0.1 copper ions. The concentration of the chlorine (sodium hypochlorite) in the test substance was determined by the sponsor's provided HACH meter and was adjusted to approximately 0.5 ppm using either 200 ppm chlorine as Cl_2 (to adjust the Cl_2 up), or, H_2O_2 to adjust the Cl_2 down). The test substance was used within 3 hours of preparation.

In each of five 500 mL Erlenmeyer flasks, 200 mL of deionized water was added. To each of the five flasks, aliquots of 200 ± 10 ppm available chlorine prepared from a NaOCl solution was added. Each flask was shaken, and allowed to stand for approximately three minutes. Approximately 1.0 g of crystal potassium iodide and 1.0 mL acetic acid was added and swirled. A 1.0 mL of starch solution was added. A flask showing perceptible blue color indicates that the chlorine demand was satisfied. Flasks 1, 2 and 3 were used for parts of the chlorine control portion of the study. The first flask was assessed for the residual available chlorine. A 1.0 mL aliquot of an *Escherichia coli* suspension was added to the second flask. A 1.0 mL aliquot was removed from flask two and transferred to a neutralizer blank after intervals of 0.5, 1, 2, 3, 4, 5, and 10 minutes. The neutralizer blank was shaken thoroughly immediately after adding the sample. Appropriate serial tenfold dilutions were prepared in Butterfield's buffer and spread plated in duplicate on appropriate agar using standard microbiological techniques.

Following the preparation of the dilution plate counts, five tubes containing 20.0 mL of lactose broth were inoculated with 1.0 mL from each neutralizer blank tube for each time interval for *Escherichia coli*. The above procedure was repeated

using *Enterococcus faecium* with flask number 3. Five tubes containing 20.0 mL of thioglycollate broth were inoculated with 1.0 mL aliquots from each neutralizer blank tube for each time interval for *Enterococcus faecium*.

For the actual test substance assay two flasks were placed in a water bath at the sponsor specified temperature and equilibrated. One flask was inoculated with a 1.0 mL aliquot of a standard test culture suspension of *Escherichia coli* and the other with a 1.0 mL of a standard test culture suspension of *Enterococcus faecium*. The two flasks were subcultured at exactly the same time intervals and in the same manner as the NaCl control.

2 MRID Number 455137-04: "AOAC Official Method Disinfectants for Swimming Pools (Presumptive Efficacy Test)" by Judith B. DeJoseph. MicroBioTest, Inc. Lab Project ID Number: 387-110. Amended Final Report Issued: 9/27/2001.

This study was conducted to determine the ability of EPA File Symbol 67712-R to kill microorganisms commonly encountered in swimming pools compared to the capacity of NaOCl to kill the same organisms under the same conditions. This study will not be reviewed as the registrant has changed it to a non-GLP status study. In MRID Number 455137-02, the registrant cites the following GLP violations:

A Lack of direct and prompt record keeping,
B Lack of direct and prompt recording of test results
C Use of expired reagents,
D Use of untrained personnel and study directors,
E Original final reports did not reflect the actual raw data,
F Frequent mathematical and rounding errors,
G Protocol deviations that were not documented,
H Methodology changes not represented in original final reports,
I Use of incorrect analysis method,
J The study was not conducted according to protocol, and
K Numerous errors were found in the raw data records.

3 MRID Number 455137-05: "AOAC Official Method Disinfectants for Swimming Poole (Presumptive Efficacy Test)" by Nicole L. Braddock. MicroBioTest, Inc. Lab Project ID Number: 387-112. Study Completion Date: 9/28/2001.

This study was conducted to determine the ability of EPA File Symbol 67712-R to kill microorganisms commonly encountered in swimming pools compared to the capacity of NaOCl to kill the same organisms under the same conditions. This study will also not be reviewed due to the registrant having visited the testing facility and assigning the study a non-GLP status. Problems reported (in MRID Number 455137-02) with this study were:

A Lack of direct and prompt record keeping,
B Lack of raw data,
C Lack of direct and prompt recording of test results
D Use of expired reagents,
E Use of unknown/untraceable control material,
F Use of untrained personnel and study directors,
G Frequency of QAU audits were insufficient to assure GLP compliance,
H Use of non-functioning equipment,
I Original final reports did not reflect the actual raw data,
J Frequent mathematical and rounding errors,
K Protocol deviations that were not documented,
L Methodology changes not represented in original final reports,
M Use of incorrect analysis method,
N The study was not conducted according to protocol, and
O Numerous errors were found in the raw data records.

V Results

Table 1 from MRID Number 455137-03:

Calculated 30-Second Log Reductions and Controls				
	Numbers Control (CFU/mL)	Numbers Control Log	Log_{10} After 30-Second Exposure	Log Reduction After A 30 Second Exposure
	Enterococcus faecium			
67712-R; Lot 2-5-01-1	3.4×10^6	6.531	< 0.301	> 6.230
67712-R; Lot 2-5-01-2			< 0.301	> 6.230
Chlorine Control			< 0.301	> 6.230
	Escherichia coli			
67712-R; Lot 740-14	7.0×10^6	6.847	< 0.301	> 6.544
67712-R; Lot 740-17			< 0.301	> 6.544
Chlorine Control			< 0.301	> 6.544

Table 2.

Qualitative Results of Testing with Simulated Nature^2 Pool Water			
Test Material	Number of Subcultures Showing Growth/Number Tested		
	Length of Exposure	*Enterococcus faecium*	*Escherichia coli*
Nature^2 Simulated Pool Water. Lot 2-8-01-1 for *E. faecium*; Lot 740-14 for *E. coli*	30 sec.	0/5	0/5
	1 min.	0/5	0/5
	2 min.	0/5	5/5
	3 min.	0/5	0/5
	4 min.	0/5	0/5
	5 min.	0/5	0/5
	10 min.	0/5	0/5
Nature^2 Simulated Pool Water. Lot 2-5-01-2 for *E. faecium*; Lot 740-17 for *E. coli*	30 sec.	0/5	0/5
	1 min.	0/5	0/5
	2 min.	0/5	0/5
	3 min.	0/5	5/5
	4 min.	0/5	0/5
	5 min.	0/5	0/5
	10 min.	0/5	0/5
Chlorine Control	30 sec.	1/5	0/5
	1 min.	0/5	0/5
	2 min.	2/5	0/5
	3 min.	0/5	0/5
	4 min.	0/5	0/5
	5 min.	0/5	0/5
	10 min.	0/5	0/5

VI Conclusions

In order for the laboratory test to be considered a valid study, the chlorine (Cl) control should show complete kill of the target microorganisms within 0.5 min. Although the Cl control subculture tubes for *Enterococcus faecium* (MRID Number 455137-03) showed one positive tube out of five, the test substance subcultures for that assay were completely negative. The pre-test and post-test levels of available chlorine for the control was 0.60 ppm and 0.54 ppm, respectively. The available chlorine level in the test substance was 0.5 ppm, therefore, the test substance was a more stringent challenge than the chlorine control. In this case, PSB believes that the one positive control tube should not invalidate the test.

In addition, the presumptive laboratory study phase for swimming pool disinfectants is designed to determine the lowest concentration of test substance material which is equivalent to the Cl control and could be expected to provide acceptable disinfecting activity in swimming pool water. This concentration is then confirmed through field-use testing of the proposed product in at least two swimming pools for an entire swimming season (4 to 12 months) .

In the case of the Nature2 product, the field studies were conducted (on 23 in ground pools over a five month period) prior to the presumptive laboratory studies. Additional studies conducted under the ANSI/NSF-50 standard were also submitted to the Agency. These studies were reviewed and found to be acceptable in October 2000.

VII Recommendations

1 The request to add labeling claims that the Nature2 system (used in conjunction with 0.5 ppm available chlorine) can be used to maintain a disinfection level in a a swimming pool is accepted.

2 Field studies were only conducted over a five month period, therefore, instructions to replace the cartridge every six months must be removed.

3 Page 2 of the submitted label states that this product is effective against *Staphylococcus aureus*. No data supporting this claim has been submitted to the EPA/AD. This statement must be removed.