



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

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

April 29, 2002

**MEMORANDUM**

**Subject:** Efficacy Review for EPA Reg. No. 70060-EE / Aseptrol SE Water Purifier  
DP Barcode: D280770  
Case No.: 070737

**From:** Ian Blackwell, Biologist  
Efficacy Evaluation Team  
Product Science Branch  
Antimicrobials Division (7510C)

**Through:** Emily Mitchell, Team Leader  
Efficacy Evaluation Team  
Product Science Branch  
Antimicrobials Division (7510C)

**To:** Robert Brennis, PM 32 / Wanda Mitchell  
Regulatory Management Branch I  
Antimicrobials Division (7510C)

**Applicant:** Engelhard Corp.

**Formulation From Label:**

<u>Active Ingredient(s)</u>	<u>% by wt</u>
Sodium chlorite	6.4
Sodium dichloroisocyanurate dihydrate	1.0
<u>Inert Ingredient(s)</u>	<u>92.6</u>
Total	100.0

- I. BACKGROUND: The registrant has resubmitted two antimicrobial efficacy studies to replace studies that were rejected in a 10/31/2001 AD/PSB review. Both of the replacement studies were conducted by MicroBioTest, Inc. The MRID Numbers are 455889-04 and -05. The subject of this submission is to prove the antimicrobial effectiveness of Engelhard Corporation's water purifier tablets.

In the previous review, studies conducted testing Aseptrol SE against bacteria and viruses failed. However, the study conducted against cysts from *Giardia muris* and *Cryptosporidium parvum* was acceptable.

II. Use Directions:

Aseptrol-SE Water Purifier Tablets are only for the emergency disinfection of drinking water. Aseptrol-SE tablets are for use by campers, hikers, militaries, emergency organizations and anyone who needs to drink water of questionable microbiological quality.

For control of bacteria, viruses and cysts: Aseptrol-SE Water Purifier Tablets kill bacteria, viruses and cysts in non-turbid waters. To Use: Remove the 250 mg Aseptrol-SE Water Purifier Tablet from its foil envelope and quickly insert the tablet into ½ liter (or ½ quart) of contaminated water. Allow to react for 30 minutes, in an area away from sunlight, in order to generate a use-solution of 4 ppm chlorine dioxide. The treated water is now ready for drinking.

III. Agency Standards for Proposed Claims

Water purifiers are designed to decontaminate water. The intention is to remove, kill or inactivate all types of disease-causing microorganisms from water to yield water that is safe for drinking. Typical water contaminating microbes include bacteria, viruses and protozoan cysts.

Concerning the testing of water purifiers, Subdivision G of Series 91: Product Performance/Efficacy Test Guidelines states:

In order to bear the label claim "microbiological water purifier" units must be tested and demonstrated to meet the following microbiological reduction requirements.

- (A) Bacteria: A 6-log (99.9999%) reduction of an influent challenge of  $10^7$  organisms per 100 ml of the bacterial challenge organism, *Klebsiella terrigena* (ATCC 33257).

- (B) Viruses: A 4-log (99.99%) reduction of a mixed influent challenge of 10<sup>7</sup> organisms per liter of each of the following viruses: Poliovirus (LSc) (ATCC-VR-59) and Rotavirus (Wa or SA-11) (ATCC-VR-899 or VR-2018). [Virus types are to be mixed in roughly equal 1 x 10<sup>7</sup>/L concentrations and a joint 4 log reduction will be acceptable.]
- (C) Cysts (Protozoan): A 3 log (99.9%) reduction of an influent challenge of 10<sup>6</sup> organisms per liter of *Giardia muris* or *Giardia lamblia*; OR a 3 log reduction of an influent challenge of 10<sup>7</sup> particles or spheres of 4 to 6 micron diameter (as an option for units or components based on occlusion filtration, where disinfection or other active processes are not involved).

#### IV. Comments on the Submitted Efficacy Studies

- 1 MRID Number 455889-04: "EPA Water Purifier Challenge Using *Klebsiella terrigena*. Aseptrol SE Water Purifier Tablets" by Shiva D. Rajaram. Laboratory Project Identification Number 414-114.

This study was conducted to assess the ability of Aseptrol SE Water Purifier Tablets (EPA File Symbol 70060-EE) to kill/eliminate the coliform *Klebsiella terrigena* in inoculated or contaminated water. This assay used two types of test water as per the April, 1986, EPA document "Guide Standard and Protocol for Testing Microbiological Water Purifiers":

- A Type 1 is normal, "non-stressed", non-challenge water:
  - a. Free of chlorine or other disinfectant residuals
  - b. pH is 6.5 - 8.5
  - c. Total Organic Carbon (TOC) 0.1-5.0 mg/L
  - d. Turbidity 0.1-5 NTU
  - e. Temperature 20°C
  - f. Total Dissolved Solids (TDS) 50-500 mg/L.
- B Type 2 is for the stressed, challenge phase of the study:
  - a. Free of chlorine or other disinfectant residual
  - b. pH 9.0 ± 0.2 °C.
  - c. TOC not less than 10 mg/L
  - d. Turbidity not less than 30 NTU
  - e. Temperature 4°C ± 1°C
  - f. TDS 1,500 mg/L ± 150 mg/L

The product was tested using contact times of either 15 or 30 minutes. The contact temperatures were 20°C for Water Type 1 and 4°C for Water Type 2. The test material solution was made by dissolving 1 tablet in 625 mL of water.

The test waters were made to contain loads of at least  $10^5$  CFU/mL. After the contact periods, 10 mL of each *Klesiella*/Aseptrol solution was filtered through a 0.45  $\mu$ m membrane filter. The filter was then flushed with 20 mL of sterile 0.85% Sterile NaCl containing  $\text{Na}_2\text{S}_2\text{O}_3$  to inactivate the antimicrobial. The filter was then placed on a TSA plate.

- 2 MRID Number 455889-05: "EPA Water Purifier Challenge Using Viruses. Aseptrol SE Water Purifier Tablets" by Shiva D. Rajaram. Laboratory Project Number 414-115. Study Completion Date 1/16/2002.

This study was conducted to assess the ability of Aseptrol SE Water Purifier Tablets (EPA File Symbol 70060-EE) to eliminate/inactivate viruses in inoculated or contaminated water. The viruses used in this study were Rotavirus Strain SA-11, ATCC VR-899, and, Poliovirus type 1, ATCC VR-59. This assay used the same two types of test water that were used in MRID 455889-04. The product was added to the test waters at a rate of 1 tablet per 625 mL. Each of the two test water types will contain  $10^7$  viruses per mL of water. The viral inoculums were each produced so that they contained at least 5% organic soil. The viral solutions were exposed to the test product for 15 minutes at 20°C for EPA Test Water No. 1, and at 4°C for EPA Test Water No. 2. After the end of the 15 minute exposure, the test material/virus solution was added to a dilution tube containing 1 mL of 0.85% saline solution + 0.3%  $\text{Na}_2\text{S}_2\text{O}_3$  to neutralize the water purifier solution. A 0.5 mL portion of that mixture was then passed through a Sephadex column. After the mixtures were passed through the column, the neutralized virus was diluted and used to infect host cell monolayers. The host cell-containing plates will be incubated at 37°C in air containing 5%  $\text{CO}_2$  for 60-90 minutes to allow the viruses to adsorb onto the cells. After the adsorption, the fluids were with drawn, the monolayers washed and the cultures were re-fed with culture medium. The cultures were incubated and observed for plaque-forming units.

V. Results.

MRID Number 455889-04.

Test Water	Test Material Lot No.	Contact Time (minutes)	Log Reduction (CFU/100 mL)
EPA Water Purifier Test Water Type 1	18922-27-1	15	6.58
		30	6.58
	18922-27-2	15	6.58
		30	6.58
	18922-20-2TA	15	6.58
		30	6.58
EPA Water Purifier Test Water Type 2	18922-27-1	15	6.18
		30	6.18
	18922-27-2	15	6.18
		30	6.18
	18922-20-2TA	15	6.18
		30	6.18

Average CFU/100 mL

EPA Water #1 =  $3.8 \times 10^7$

EPA Water #2 =  $1.5 \times 10^7$

Table 2.  
MRID Number 455889-05

Dilution	Poliovirus/Rotavirus			
	Test Water No. 1		Test Water No. 2	
	Polio/Rotavirus	Neutralized Control	Polio/Rotavirus	Neutralized Control
10 <sup>-2</sup>	----	++++	----	++++
10 <sup>-3</sup>	----	++++	----	++++
10 <sup>-4</sup>	----	++++	----	++++
10 <sup>-5</sup>	----	not tested	----	not tested
10 <sup>-6</sup>	----	not tested	----	not tested
10 <sup>-7</sup>	----	not tested	----	not tested
PFUD <sub>50</sub> /mL	≤ 10 <sup>1.50</sup>	N/a	≤ 10 <sup>1.50</sup>	N/a

+ = growth  
- = no growth

## VI. Conclusions

- 1 MRID Number 455889-04: The submitted efficacy data supports the use of the product, Aseptrol SE, as a water purifier disinfectant of bacterially contaminated water when tested with EPA Test Water Types 1 and 2 with exposures of 15 or 30 minutes.
- 2 MRID Number 455889-05: The submitted efficacy data supports the use of the product, Aseptrol SE, as a water purifier of virally contaminated water when tested with an exposure of 15 minutes.

## VII Recommendations

The request to add labeling claims that Aseptrol SE is an effective water purifier that is effective against bacteria, viruses and cysts is denied. PSB/AD cannot accept the four hour length of time that it takes to treat cyst infected water. Also, the four hour period is not acceptable in conjunction with the treatment period of only 15 minutes for bacteria and viruses.