October 31, 2001

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No.: 70060-EE / Aseptrol SE
DP Barcode: D276350
Case No.: 070737

From: Ian Blackwell, Biologist
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Antimicrobials Division (7510C)

Through: Emily Mitchell, Team Leader 11/1/01
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To: Bobert Brennis, PM 32 / *Wanda Mitchell*
Regulatory Management Branch I
Antimicrobials Division (7510C)

Applicant: Engelhard Corp.

Formulation From Label:

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

I. **BACKGROUND:** Engelhard Corporation has submitted a set of three antimicrobial efficacy studies to support the registration of their new "Aseptrol-SE Water Purifier Tablets". The studies were conducted by MicroBioTest Inc. The MRID Numbers are 454510-03 through -05.

Two of the submitted studies were conducted on products other than Aseptrol SE Tablets. MRID Numbers 454510-03 was conducted using Aseptrol WTSF 2.5. MRID Number 454510-04 was conducted using Aseptrol WTSF 2.5, Aseptrol WBT60F2.5-4g and Carnebon 200. A 7/5/2001 letter from Lewis & Harrison states that these products are chemically similar and are designed to generate free chlorine dioxide. However, PSB/AD notes that neither the EPA File Symbols nor the CSFs for either of these three products was available for this review.

II Use Directions

These are designed for the emergency purification of drinking water. The tablets are for use by campers, hikers, militaries, emergency organizations, and anyone needing to drink water of questionable bacteriological quality. Aseptrol-SE is not for use in turbid water or for use on a continuous basis.

For the control of bacteria and viruses: "Remove the 250 mg Aseptrol-SE Water Purifier Table from its foil envelope and quickly insert the tablet into the contaminated water. Allow to react for 15 minutes in an area away from sunlight, to generate a use-solution of approximately 3 ppm of chlorine dioxide."

For the control of bacteria, viruses and cysts: "Remove the 250 mg Aseptrol-SE Water Purifier Table from its foil envelope and quickly insert the tablet into the contaminated water. Allow to react for 30 minutes in an area away from sunlight, to generate a use-solution of approximately 4 ppm of chlorine dioxide."

III Comments on the Submitted Efficacy Studies

1. MRID Number 454510-03: "EPA Water Purifier Challenge Using a Bacterium" by Cynthia Kay Osborne. MicroBioTest, Inc. Lab Project ID 414-105. Study Completion Date 10/4/99. This study was conducted to determine the ability of this product to remove/inactivate bacteria from contaminated water. According to the EPA's April, 1986, Guide Standard and Protocol for Testing Microbiological Water Purifiers, the standard test organism for this is *Klebsiella terrigena* (ATCC 33257). *Klebsiella terrigena* is considered to be a common coliform bacteria. This study was conducted using **Aseptrol WTSF 2.5**, not Aseptrol SE Tablets.

This study was conducted by mixing test water with enough *Klebsiella terrigena* to provide a solution of at least 10^5 of the bacteria. There are two types of test water used in this study (1) sterile test water free of chlorine at a neutral pH and (2) water at pH 9 at 4°C with a turbidity ≥ 30 NTU, Total Dissolved Solids ~ 1500 mg/L and Total Organic Carbon ≥ 10 mg/L. The inoculated solutions were treated with Aseptrol Se for ten (10) minutes. After the exposure period, the water suspension is filtered through a 0.45 µm membrane filter and the filters were placed on Trypticase Soy Agar plates. The plates were incubated for 48 hours and the colonies were counted. Under the conditions of this study, **Aseptrol WTSF 2.5** met the Agency's requirements for a water purifier when challenged with EPA water (1) and *Klebsiella terrigena* (ATCC 33257). This study **did not demonstrate efficacy** against *Klebsiella terrigena* (ATCC 33257) when the diluent was EPA test water (2). However, the product calls for treatment times of at least **fifteen (15)** minutes, but the study only used an exposure time of ten minutes.

2. MRID Number 454510-04: "EPA Water Purifier Challenge Using Viruses" by Erica L. Eaton. MicroBioTest, Inc. Lab Project ID 414-106. Study Completion Date 12/16/99. This study was designed to test the ability of Aseptrol SE to remove/inactivate viruses that have been introduced into samples of test water. According to the EPA's April, 1986, Guide Standard and Protocol for Testing Microbiological Water Purifiers, the standard test viruses for this assay are Poliovirus1 (ATCC VR-59) and Rotavirus Strain SA-11 (ATCC VR-899). This study was conducted using AseptrolWTSF 2.5, Aseptrol WBT60F2.5-4g and Carnebon 200.

   This study was conducted by adding enough mixed Poliovirus1 (ATCC VR-59) and Rotavirus Strain SA-11 (ATCC VR-899) to provide solutions of 10^7 virus/Liter of test water. There are two types of test water used in this study (1) sterile test water free of chlorine at a neutral pH and (2) water at pH 9 at 4°C with a turbidity ≥ 30 NTU, Total Dissolved Solids ~ 1500 mg/L and Total Organic Carbon ≥ 10 mg/L. The test solutions were made as follows:

   a. An Aseptrol WBT60F2.5-4g sachet was dissolved in 1000 mL of sterile DI water.
   b. An Aseptrol WTFS2.5 sachet was dissolved in 250 mL of sterile DI water.

      The stock Aseptrol solutions were then diluted together in EPA test water to yield the final concentration of free-chlorine dioxide 3-ppm in the test water.

   c. Seven drops of Carnebon solution and seven drops of phosphoric acid solution were mixed into 595 mL of test water. As prepared, the solution yields 2 ppm of free-chlorine dioxide.

The inoculum (1 mL of mixed Rotavirus and Poliovirus) was added to 594.5 mL of the test water, and mixed by swirling. The test product was added to the flasks (refer to a, b and c above) of the virus mixture. The exposure of the test product to the inoculated waters lasted for 15 minutes. After contact, a 1 mL sample of each test water mixture was neutralized with an equal amount of sodium thiosulfate in saline. The neutralized water sample (0.5 mL) was loaded into pre-spun Sephacryl columns. The columns were spun for 4 minutes at 1000 rpm. The samples were removed from the columns and dispensed into tubes containing Earle's Balanced Salt Solution (EBSS). Ten-fold serial dilutions were then prepared as per SOP. 1007.2. The diluted sampled were plaque assayed for the presence of infectious viruses in MA-104 cells. No viruses were recovered after exposure to either test agent. Under the conditions of this study, **both Carnebon 200 and the AseptrolWTSF 2.5/Aseptrol WBT60F2.5-4g mixture provided effective antiviral action to EPA type 1 and type 2 test waters inoculated with a mixture of both Poliovirus1 (ATCC VR-59) and Rotavirus Strain SA-11 (ATCC VR-899).**

3. MRID Number 454510-05: "EPA Water Purifier Challenge Using (Oo)Cysts" by Donna B. Suchmann. MicroBioTest, Inc. Lab Project Identification Number 414-108. Study Completion Date 5/1/2001. This study was conducted to determine the ability of Aseptrol SE Tablets to purify water contaminated with *Giardia muris* and *Cryptosporidium parvum* oocysts. *Giardia muris* is considered to be a widespread disease vector.

   The test water used in this study was EPA water purifier test water type #2 water at pH 9 at 4°C with a turbidity ≥ 30 NTU, Total Dissolved Solids ~ 1500 mg/L and Total Organic Carbon ≥ 10 mg/L. For each of the three different exposure concentrations (of chlorine dioxide), 625 g of EPA test water #2. Oocysts at a final concentration of 10^5/mL were added. The inoculated test materials to be used in this study were then prepared as follows:

   a. One tablet per 625 g of EPA water #2 for 4 ppm chlorine dioxide.
   b. One tablet per 500 g of EPA water #2 for 5 ppm chlorine dioxide.
   c. One tablet per 313 g of EPA water #2 for 8 ppm chlorine dioxide.

   Contact times were 4 and 8 hours for 4 and 5ppm, 30 and 60 minutes for 8 ppm. Exposures were conducted at 4°C. This study used neonatal mice, SPF-CD1 strain, from Charles River Laboratories. After the exposure period, the test waters were neutralized using EBSS. Groups of test mice were gavaged (volume not reported) with treated and control test waters. The intestines were harvested, ground in sintered-glass grinders and stained using the Merifluor system. Slides of the ground guts were then observed for the presence of cysts

from *Giardia muris* and *Cryptosporidium parvum* oocysts. Under the conditions of this study, with exposures of 4 or 8 hours at 4°C, Aseptrol SE Tablets used to treat EPA purification test water type #2, completely eliminated the infectivity of *Giardia muris* cysts and *Cryptosporidium parvum* oocysts in neonatal mice.

V Results

Table 1 on test of Lot 18922-27-1 from the MRID 454510-05 Report:

Group	Concentration	Exposure Time	Total Number of Mice Treated	No. Positive/ Total Number Surviving
1	4 ppm	4 hours	10	0/9
2	4 ppm	8 hours	10	0/9
3	5 ppm	4 hours	10	0/10
4	5 ppm	8 hours	10	0/8
5	8 ppm	30 minutes	10	10/10
6	8 ppm	60 minutes	10	7/7
7	8 ppm Neutralizer Effectiveness	60 minutes	10	6/6
8	Positive Control	NA	10	8/8
9	Negative Control	NA	10	0/10
10	*C. parvum* oocysts	NA	10	9/9
11	*G. muris* cysts	NA	10	10/10
12	Uninoculated control	NA	10	0/8

VI Conclusions

1. Under the conditions of this study, Aseptrol WTSF 2.5 **did not demonstrate efficacy** against *Klebsiella terrigena* (ATCC 33257) as a water purifier.

2. Under the conditions of this study, both **Carnebon 200 and the AseptrolWTSF 2.5/Aseptrol WBT60F2.5-4g mixture** provided effective antiviral action to EPA

type 1 and type 2 test waters inoculated with a mixture of both Poliovirus1 (ATCC VR-59) and Rotavirus Strain SA-11 (ATCC VR-899). However, as the lab tested unregistered products and did not even supply the CSFs of these products, this data is currently not acceptable to support the registration of 70060-EE.

3. Under the conditions of this study, with exposures of 4 or 8 hours at 4°C, Aseptrol SE Tablets used to treat EPA purification test water type #2, completely eliminated the infectivity of *Giardia muris* cysts and *Cryptosporidium parvum* oocysts in neonatal mice.

VII Recommendations

1. This study was not conducted using Aseptrol SE Tablets (EPA File Symbol 70060-EE), but used Aseptrol WTSF2.5 and Aseptrol WBT60F2.5-4g. In addition, the test product was not able to remove or inactivate EPA water purification test water type #2 (please refer to III, 1 above). The registrant needs to submit another water purifier study against *Klebsiella terrigena* conducted on 70060-EE to support the registration of this product.

2. This study was also not conducted using Aseptrol SE Tablets, but used Carnebon 200 and a mixture of AseptrolWTSF 2.5 and Aseptrol WBT60F2.5-4g. Under the conditions of this study, both Carnebon 200 and the AseptrolWTSF 2.5/Aseptrol WBT60F2.5-4g mixture provided effective antiviral action to EPA type 1 and type 2 test waters inoculated with a mixture of both Poliovirus1 (ATCC VR-59) and Rotavirus Strain SA-11 (ATCC VR-899). **At this time, the request to add labeling stating that Aseptrol SE is effective against water contaminated with viruses cannot be supported.** However, once CSFs for Carnebon 200 and a mixture of Aseptrol WTSF 2.5 and Aseptrol WBT60F2.5-4g can be obtained, the PM Team may need to meet with PSB to determine whether the data obtained in this study may be used to support claims of Aseptrol SE Tablets as a purifier of virus contaminated water.

3. The submitted data supports the claims of this product as water treatment against *Giardia muris* cysts and *Cryptosporidium parvum*; however, this product does not meet the current Agency guide standards for water purifiers.