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OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

MEMORANDUM

**SUBJECT:** SACB review of biochemical/morphological data and antibiotic sensitivity profiles for the active bacterial ingredients of Foil, Cutlass, and Condor (ID No. 55638-10; MRID Nos. 415396-01 and 415399-01; HED Project No. 0-1515; Caswell No. 066).

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**FROM:** Roy D. Sjoblad, Ph.D., Microbiologist  
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**THROUGH:** Reto Engler, Ph.D., Chief  
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**Background:** Ecogen, Inc. has submitted data on the biochemical/morphological characterization of the active bacterial ingredients of Foil (i.e., strain EG2424), Cutlass (i.e., strain EG2371) and Condor (i.e., strain 2348). In addition, antibiotic sensitivity profiles for these three strains were submitted.

**SACB Conclusion:** The antibiotic susceptibility profiles are adequate for the purposes of Registration of the three strains. The biochemical/nutritional data and description of the bacteria also are sufficient to allow characterization of the strains as varieties of Bacillus thuringensis.

Information/data provided:

1. NCCLS Standard Disk Susceptibility Test of Bacillus thuringensis Strains (by P.W. Barbera of IITRI; IITRI Project No. L08245; February 8, 1990; sponsored by Ecogen, Inc.; Ecogen, Inc. Study No. EC-02; EPA MRID No. 415396-01).

The National Committee for Clinical Standards (NCCLS) disk susceptibility test was used. Cultures were grown in trypticase soy broth and were inoculated onto Mueller Hinton II Agar plates containing disks impregnated with the antibiotics. Test controls included Staphylococcus aureus (ATCC 25923) with erythromycin (BBL 30793; lot 805606) and penicillin G (BBL 35003; lot DOJBPM). The other antibiotics employed were all from Becton Dickinson (BBL) Cockeysville, MD. The results were as follows:

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Zone of inhibition (mm) for the B. thuringiensis strains<sup>a</sup>

<u>Antibiotic</u>	<u>Strain EG2424</u>	<u>Strain EG2371</u>	<u>Strain EG2348</u>
Erythromycin	21 (S) <sup>b</sup>	21(S)	26(S)
Penicillin G	0 (R) <sup>b</sup>	0(R)	0(R)
Tetracycline	17 (I) <sup>b</sup>	17(I)	17(I)
Chloramphenicol	22 (S)	23(S)	23(S)
Streptomycin	17 (S)	18(S)	20(S)
Clindamycin	19 (S)	21(S)	24(S)

<sup>a</sup> Zone size represents average of two plates

<sup>b</sup> (S) means sensitive; (R) means resistant; (I) means intermediate.

There were no significant differences between strains. The controls were in the expected range set by NCCLS (i.e., 22-30 mm for S. aureus and erythromycin, and 26-37 mm for penicillin G).

2. Biochemical and Morphological Characterization of Bacillus thuringiensis Strains (by P.W. Barbera; from IITRI; February 8, 1990; IITRI Project No. L08245; sponsored by Ecogen, Inc.; Ecogen Study No. EC-01; EPA MRID No. 415399).

Standard biochemical testing was done on strains EG2424, EG2371, and EG2348, as specified in Bergey's Manual of Determinative Bacteriology (Vol. 2). The results for all three strains were as follows:

Aerobic, motile, Gram-positive rods; 1.3 to 1.6 x 3.0-4.5 um; opaque cream-colored, irregular colonies, with a filamentous margin of a butyrous texture under aerobic conditriions within 24h on nutrient agar. There was one ellipsoidal spore/cell with a central to subterminal location. The sporangium was not swollen by the spore.

The following tests were negative for all three strains:

Gas production from glucose; acid from L-arabinose, D-xylose and D-mannitol; degradation of tyrosine; growth in 7% and 10% NaCl.

The following were positive for all three strains:

Catalase production; acid from D-glucose; citrate as sole C source; starch hydrolysis; Voges Proskauer reaction (acetyl methyl carbinol production); casein hydrolysis; lecithinase production; growth in 2% and 5% NaCl; growth at pH 5.7 and 6.8; anaerobic growth.

The only discrepancy between Bergey's Manual and the IITRI results was that Bergey's indicates positive growth in 7% NaCl. This result is not not significant with respect to proper characterization of the three strains.