

Eden Research Laboratory

# ASTM E1963 - Conducting Terrestrial Plant Toxicity Tests - [REDACTED]

To: [REDACTED]

Report Number: [REDACTED]

Date: [REDACTED]

# Report

From: Thomas Poth - Eden Research Laboratory

Regarding: 15 days test of [REDACTED] Sample ERL# 34

## RESULTS

Following 40 months of biodegradation as per ASTM D5511, no retardation or mutations are apparent using the residual inoculum of exposed [REDACTED] Plastic Sample 34 when compared to the inoculum alone.

## METHOD

ASTM method E1963 measures the effect of substances on plant growth. The method details several measurements to determine the toxicity of test substances. Depending on the nature of the test substance the method allows for mixing of the test substance directly into the seedling mix or extraction of the substance and addition of this to the mix. The test stipulates that testing be done under controlled illumination and consistent temperature and humidity.

Evaluation of seedling emergence, epicotyl and hypocotyl length as well as epicotyl weight. Among the many species mentioned in the method bean, corn and pea give very good results. Boron positive control is employed to compare results from run to run.

Seeds are screened in advance to achieve a uniform size, then stored in a cool, dry place. Seeds are then planted, 2-4 per cell, depending on size to a depth of 1.5 to 2 times the seed diameter in a mixture of potting soil and perlite. The test is run in duplicate with from 5 to 20 seeds per duplicate. Flats are then placed under fluorescent lights with an illumination of 100-200 uM m<sup>-1</sup> s<sup>-1</sup> for 12 to 16 hours per day. Two weeks later growth is terminated and data is collected. Temperature and humidity are kept within narrow limits and monitored constantly.

## DATA

Four seed groupings are germinated. They are as follows:

GROUPING	DESCRIPTION
BACKGROUND	General Soil
POSITIVE CONTROL	General Soil & 20mM Boric Acid
SAMPLE	3 Parts General Soil & 2 Parts BTE Sample 34 Exposed Inoculum
INOCULUM	3 Parts General Soil & 2 Parts Inoculum

### POSITIVE (in cm and g)

BEAN									
EP LENGTH	0.50	3.00	3.00						
HY LENGTH	0.90	0.41	0.41						
EP+HY Weight	0.3	0.7	0.7						

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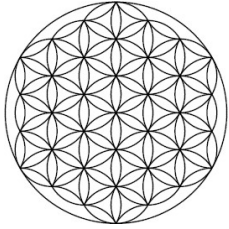
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<b>CORN</b>										
EP LENGTH	3.5	6.0	13.0							
HY LENGTH	0.17	0.24	0.44							
EP+HY Weight	0.6	0.8	1.1							

### INOCULUM (in cm and g)

<b>BEAN</b>										
EP LENGTH	4.5	8.5	4.5	6.0	4.0	5.0				
HY LENGTH	0.2	0.6	0.3	1.3	0.7	0.6				
EP+HY Weight	0.4	1.2	0.5	2.3	1.5	1.2				
<b>CORN</b>										
EP LENGTH	1.65	11.0	18.0	15.0	21.0	10.0	8.0	19.5	12.0	23.0
HY LENGTH	0.40	0.16	0.43	0.4	0.7	0.1	0.1	0.5	0.4	0.6
EP+HY Weight	1.44	0.56	1.40	1.4	2.4	0.4	1.1	1.9	0.9	1.7

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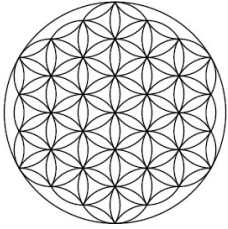
### BioTec (34) - SAMPLE (in cm and g)

<b>BEAN</b>										
EP LENGTH	5.0	2.5	6.0	6.0						
HY LENGTH	0.6	0.1	0.5	0.6						
EP+HY Weight	0.9	0.4	0.8	1.3						
<b>CORN</b>										
EP LENGTH	9.0	8.5	6.0	14.5	20.0	23.5	13.0	14.0	10.5	25.0
HY LENGTH	0.5	0.5	0.1	0.5	0.6	0.8	0.5	0.3	0.2	0.9
EP+HY Weight	1.6	23.4	1.2	1.2	1.1	3.8	1.5	1.2	0.6	2.7

### BACKGROUND (in cm and g)

<b>BEAN</b>										
EP LENGTH	6.0	7.5	5.0	3.0	4.0	5.5				
HY LENGTH	1.2	0.9	1.1	0.5	0.5	0.9				
EP+HY Weight	1.9	1.5	1.9	1.3	0.9	1.4				
<b>CORN</b>										
EP LENGTH	1.9	7.5	20.0	13.0	9.0	10.0	20.0	13.0	24.5	21.5
HY LENGTH	0.6	0.08	0.61	0.39	0.38	0.30	0.61	0.37	0.85	0.56
EP+HY Weight	1.8	0.7	1.7	0.2	0.9	1.1	1.9	1.6	2.1	1.8

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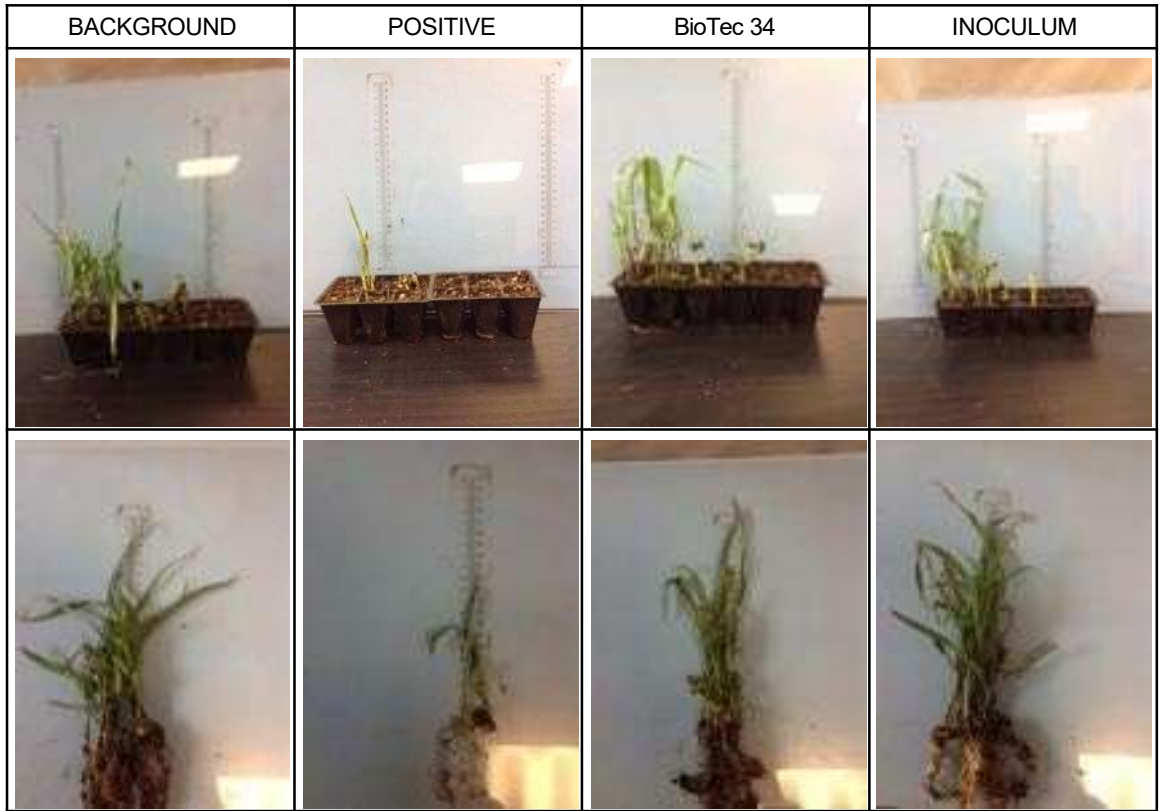
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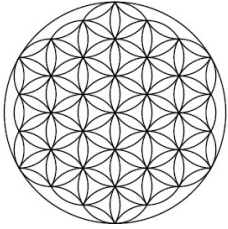
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	AVERAGE (cm)	% GERMINATION	WEIGHT (g)	WEIGHT EACH
<b>POSITIVE BEAN</b>				
EP LENGTH	2.17	25.00		
HY LENGTH			1.72	0.57
EP+HY Weight			1.61	0.54
<b>CORN</b>				
EP LENGTH	7.50	25.00		
HY LENGTH			0.85	0.28
EP+HY Weight			2.44	0.81
<b>INOCULUM BEAN</b>				
EP LENGTH	5.42	50.00		
HY LENGTH			3.76	0.63
EP+HY Weight			7.07	1.18
<b>INOCULUM CORN</b>				
EP LENGTH	13.92	83.33		
HY LENGTH			3.66	0.37
EP+HY Weight			13.10	1.31
<b>BioTec (34) BEAN</b>				
EP LENGTH	6.39	58.33		
HY LENGTH			3.57	0.51
EP+HY Weight			7.91	1.13
<b>BioTec (34) CORN</b>				



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	AVERAGE (cm)	% GERMINATION	WEIGHT (g)	WEIGHT EACH
EP LENGTH	14.40	83.33		
HY LENGTH			4.76	0.48
EP+HY Weight			38.26	3.83
BACKGROUND BEAN				
EP LENGTH	5.17	50.00		
HY LENGTH			5.12	0.85
EP+HY Weight			8.76	1.46
CORN				
EP LENGTH	14.04	83.33		
HY LENGTH			4.70	0.47
EP+HY Weight			13.79	1.38

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### CONCLUSION

Upon consideration of the data, it becomes apparent that there is no effect of biodegraded products of the [REDACTED] Sample 34 when subject to the conditions of the ASTM D5511-11. The beans are a good indicator, since they are very sensitive to soil conditions, that the inoculum is still "hot" from high levels of nutrients out of balance with required distribution.

Almost without fail, the [REDACTED] Sample shows no inhibitory effect from the inoculum after exposure to the plastic and its biodegradation products. Considering noticeable mutations in the positive control it is worth noting there are no indications of mutation in size or shape.

Thomas Poth  
Laboratory Director  
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