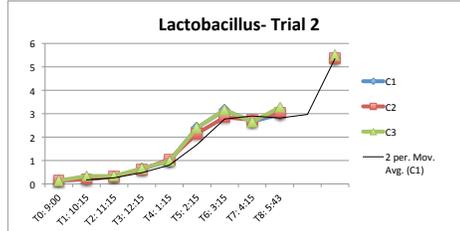
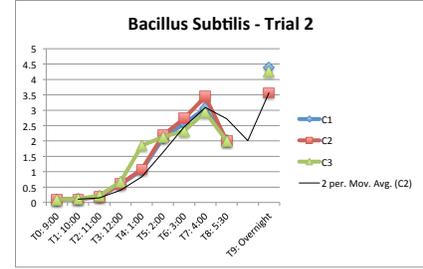


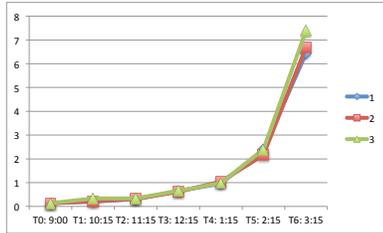
# Trail 2 Growth Curves

With 1:4 dilution at T6				
Time	Lactobacillus			
	C1	C2	C3	
T0: 9:00	0.134	0.136	0.136	
T1: 10:15	0.208	0.209	0.335	
T2: 11:15	0.302	0.315	0.335	
T3: 12:15	0.648	0.618	0.659	
T4: 1:15	0.96	1.04	0.98 (1:4)	
T5: 2:15	2.39	2.152	2.393 (1:4)	
T6: 3:15	3.152	2.852	3.168 (1:4)	
T7: 4:15	2.66	2.74	2.644 (1:4)	
T8: 5:43	2.97	3.048	3.276 (1:4)	
T9: Overnight	5.344	5.368	5.488	

Time	Bacillus Subtilis			
	C1	C2	C3	
T0: 9:00	0.1	0.1	0.1	
T1: 10:00	0.113	0.11	0.113	
T2: 11:00	0.175	0.185	0.226	
T3: 12:00	0.59	0.604	0.67	
T4: 1:00	1.04	1.072	1.856 (1:4)	
T5: 2:00	2.088	2.188	2.124 (1:4)	
T6: 3:00	2.568	2.732	2.328 (1:4)	
T7: 4:00	3.056	3.44	2.936 (1:4)	
T8: 5:30	2.02	2.012	2 (1:4)	
T9: Overnight	4.396	3.568	4.26	

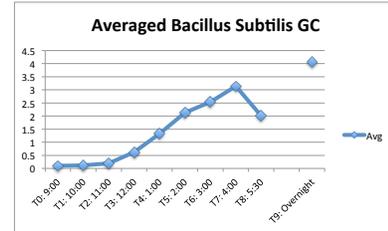
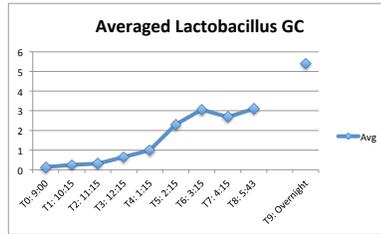


With 1:10 dilution at T6				
Time	Lactobacillus			
	1	2	3	Weird Data- Don't Use
T0: 9:00	0.134	0.136	0.136	
T1: 10:15	0.208	0.209	0.335	
T2: 11:15	0.302	0.315	0.335	
T3: 12:15	0.648	0.618	0.659	
T4: 1:15	0.96	1.04	0.98 (1:4)	
T5: 2:15	2.39	2.152	2.393 (1:4)	
T6: 3:15	6.43	6.69	7.38 (1:10)	



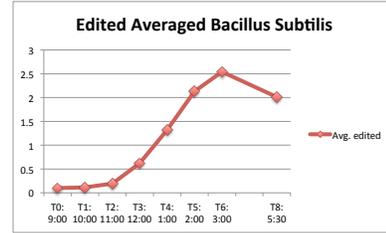
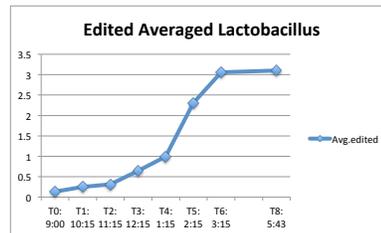
Time	Averages of Lactobacillus Avg
T0: 9:00	0.1353
T1: 10:15	0.251
T2: 11:15	0.317
T3: 12:15	0.642
T4: 1:15	0.993
T5: 2:15	2.31
T6: 3:15	3.057
T7: 4:15	2.68
T8: 5:43	3.098
T9: Overnight	5.4

Time	Average of Bacillus Subtilis Avg
T0: 9:00	0.1
T1: 10:00	0.112
T2: 11:00	0.195
T3: 12:00	0.62
T4: 1:00	1.32
T5: 2:00	2.13
T6: 3:00	2.54
T7: 4:00	3.14
T8: 5:30	2.01
T9: Overnight	4.07



Time	Deleted Time point T7 & Overnight Avg.edited
T0: 9:00	0.1353
T1: 10:15	0.251
T2: 11:15	0.317
T3: 12:15	0.642
T4: 1:15	0.993
T5: 2:15	2.31
T6: 3:15	3.057
T8: 5:43	3.098

Time	Deleted Time point T7 & overnight Avg. edited
T0: 9:00	0.1
T1: 10:00	0.112
T2: 11:00	0.195
T3: 12:00	0.62
T4: 1:00	1.32
T5: 2:00	2.13
T6: 3:00	2.54
T8: 5:30	2.01





	1	2	3	4	5	6	7	8	9	10	11	12
C	0.073	0.075	0.072	0.075	0.064							
D	0.075	0.073	0.066	0.077	0.060							
E												
F												
G												
H												

10.11

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.092	0.091	0.085	0.088	0.08							
B	0.089	0.088	0.083	0.086	0.081							
C	0.091	0.089	0.091	0.091	0.082							
D	0.094	0.09	0.078	0.086	0.082							
E												
F												
G												
H												

0.084 0.08075 0.086 0.091 0.08125

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.074	0.074	0.068	0.072	0.062							
B	0.07	0.073	0.075	0.074	0.064							
C	0.073	0.073	0.072	0.074	0.063							
D	0.076	0.074	0.068	0.077	0.063							
E												
F												
G												
H												

0.07375 0.07425 0.0735 0.07425 0.063

11.10

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.092	0.092	0.085	0.089	0.08							
B	0.089	0.088	0.083	0.086	0.081							
C	0.091	0.089	0.091	0.091	0.082							
D	0.095	0.091	0.077	0.086	0.082							
E												
F												
G												
H												

0.09425 0.0915 0.08425 0.09225 0.0815

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.074	0.075	0.069	0.073	0.062							
B	0.07	0.073	0.074	0.073	0.064							
C	0.074	0.073	0.072	0.074	0.063							
D	0.076	0.074	0.068	0.077	0.063							
E												
F												
G												
H												

0.076 0.0765 0.068 0.075 0.063

10.09

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.092	0.091	0.085	0.088	0.08							
B	0.089	0.088	0.083	0.086	0.081							
C	0.091	0.089	0.091	0.091	0.082							
D	0.095	0.091	0.077	0.086	0.082							
E												
F												
G												
H												

0.0945 0.09175 0.0845 0.09225 0.0815

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.075	0.075	0.069	0.073	0.062							
B	0.07	0.077	0.074	0.075	0.064							
C	0.074	0.074	0.074	0.076	0.063							
D	0.077	0.075	0.069	0.078	0.063							
E												
F												
G												
H												

0.0765 0.07525 0.06825 0.0765 0.063

12.40

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.092	0.091	0.085	0.088	0.08							
B	0.089	0.088	0.083	0.086	0.081							
C	0.091	0.089	0.091	0.091	0.082							
D	0.095	0.091	0.077	0.086	0.082							
E												
F												
G												
H												

0.09475 0.09225 0.0845 0.0915 0.0815

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.075	0.075	0.069	0.073	0.062							
B	0.07	0.077	0.074	0.075	0.064							
C	0.075	0.074	0.074	0.077	0.063							
D	0.077	0.075	0.069	0.078	0.063							
E												
F												
G												
H												

0.07675 0.0755 0.0685 0.0765 0.06275

13.25

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.093	0.091	0.085	0.091	0.08							
B	0.089	0.088	0.083	0.091	0.082							
C	0.093	0.09	0.082	0.085	0.082							
D	0.095	0.091	0.076	0.086	0.082							
E												
F												
G												
H												

0.095 0.0925 0.0845 0.09275 0.0815

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.075	0.076	0.069	0.074	0.062							
B	0.07	0.077	0.074	0.076	0.064							
C	0.075	0.075	0.075	0.076	0.063							
D	0.077	0.075	0.069	0.078	0.063							
E												
F												
G												
H												

0.07675 0.07625 0.0685 0.0765 0.063

14.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.093	0.091	0.085	0.091	0.081							
B	0.089	0.088	0.083	0.084	0.082							
C	0.093	0.091	0.082	0.086	0.082							
D	0.095	0.091	0.076	0.086	0.082							
E												
F												
G												
H												

0.095 0.091 0.0845 0.09425 0.08175

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.075	0.077	0.069	0.075	0.062							
B	0.07	0.076	0.075	0.077	0.063							
C	0.075	0.075	0.075	0.076	0.063							
D	0.078	0.075	0.069	0.078	0.063							
E												
F												
G												
H												

0.0775 0.07625 0.06875 0.077 0.06275









H													
A							0.072	0.07	0.072	0.082	0.065		
B							0.077	0.073	0.071	0.077	0.069		
C							0.077	0.073	0.073	0.081	0.075		
D							0.073	0.081	0.068	0.087	0.075		
E													
F													
G													
H													

GSH mini  
dilution:570

GSH mini  
dilution:595 0.07475 0.07425 0.071 0.08175 0.071  
GSH mini  
dilution:595  
GSH mini  
dilution:595

5:30

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.093	0.09	0.091	0.103	0.088	
B							0.102	0.093	0.093	0.096	0.094	
C							0.099	0.093	0.095	0.103	0.099	
D							0.095	0.103	0.087	0.114	0.099	
E												
F												
G												
H												

GSH mini  
dilution:570 0.09725 0.09475 0.0915 0.104 0.095  
GSH mini  
dilution:570  
GSH mini  
dilution:570

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.073	0.071	0.072	0.083	0.065	
B							0.078	0.074	0.072	0.079	0.07	
C							0.077	0.074	0.075	0.084	0.075	
D							0.074	0.081	0.069	0.09	0.074	
E												
F												
G												
H												

GSH mini  
dilution:595 0.0755 0.075 0.072 0.084 0.071  
GSH mini  
dilution:595  
GSH mini  
dilution:595

6:35

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.095	0.091	0.091	0.104	0.089	
B							0.1	0.093	0.094	0.097	0.095	
C							0.1	0.095	0.095	0.106	0.1	
D							0.095	0.104	0.089	0.118	0.099	
E												
F												
G												
H												

GSH mini  
dilution:570 0.0975 0.09575 0.09225 0.10625 0.09575  
GSH mini  
dilution:570  
GSH mini  
dilution:570

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.075	0.072	0.072	0.084	0.066	
B							0.078	0.075	0.073	0.08	0.071	
C							0.078	0.076	0.075	0.086	0.075	
D							0.075	0.082	0.071	0.094	0.074	
E												
F												
G												
H												

GSH mini  
dilution:595 0.0765 0.07625 0.07275 0.086 0.0715  
GSH mini  
dilution:595  
GSH mini  
dilution:595

7:30

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.096	0.091	0.091	0.104	0.09	
B							0.101	0.094	0.095	0.095	0.096	
C							0.101	0.097	0.096	0.106	0.101	
D							0.096	0.104	0.09	0.12	0.099	
E												
F												
G												
H												

GSH mini  
dilution:570 0.0985 0.0965 0.093 0.10625 0.0965  
GSH mini  
dilution:570  
GSH mini  
dilution:570

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.075	0.072	0.072	0.085	0.067	
B							0.078	0.075	0.074	0.079	0.071	
C							0.08	0.078	0.077	0.087	0.076	
D							0.076	0.083	0.072	0.096	0.075	
E												
F												

GSH mini  
dilution:595 0.07725 0.077 0.07375 0.08675 0.07225  
GSH mini  
dilution:595  
GSH mini  
dilution:595

G												
H												

GSH mini  
dilution:595  
GSH mini  
dilution:595

8:30

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.097	0.092	0.091	0.104	0.091	
B							0.103	0.095	0.096	0.095	0.096	
C							0.102	0.098	0.097	0.106	0.102	
D							0.097	0.105	0.092	0.122	0.099	
E												
F												
G												
H												

GSH mini  
dilution:570  
GSH mini  
dilution:570

0.09975 0.0975 0.094 0.10675 0.097

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.077	0.073	0.073	0.086	0.068	
B							0.081	0.076	0.075	0.079	0.072	
C							0.081	0.079	0.077	0.087	0.076	
D							0.077	0.084	0.073	0.098	0.075	
E												
F												
G												
H												

GSH mini  
dilution:595  
GSH mini  
dilution:595

0.079 0.078 0.0745 0.0875 0.07275

9:30

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.099	0.092	0.092	0.104	0.092	
B							0.104	0.095	0.097	0.096	0.097	
C							0.103	0.1	0.098	0.108	0.101	
D							0.099	0.105	0.093	0.122	0.099	
E												
F												
G												
H												

GSH mini  
dilution:570  
GSH mini  
dilution:570

0.10125 0.098 0.095 0.1075 0.09725

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.079	0.074	0.073	0.086	0.068	
B							0.082	0.076	0.076	0.08	0.072	
C							0.082	0.08	0.079	0.089	0.076	
D							0.079	0.085	0.075	0.099	0.076	
E												
F												
G												
H												

GSH mini  
dilution:595  
GSH mini  
dilution:595

0.0805 0.07875 0.07575 0.0885 0.073

10:30

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.101	0.092	0.092	0.105	0.092	
B							0.104	0.095	0.097	0.098	0.097	
C							0.104	0.102	0.098	0.111	0.102	
D							0.101	0.107	0.095	0.123	0.101	
E												
F												
G												
H												

GSH mini  
dilution:570  
GSH mini  
dilution:570

0.1025 0.099 0.0955 0.10925 0.098

	1	2	3	4	5	6	7	8	9	10	11	12
A							0.08	0.074	0.074	0.087	0.068	
B							0.082	0.077	0.076	0.081	0.073	
C							0.083	0.083	0.079	0.092	0.076	
D							0.08	0.086	0.076	0.1	0.077	
E												
F												
G												
H												

GSH mini  
dilution:595  
GSH mini  
dilution:595

0.08125 0.08 0.07625 0.09 0.0735

Unit Scaling Factors:

OD600/Ab600  
uM Fluorescein/μ.u.

These are imported from the prior two sheets  
4.45  
5.45x107

Enter fluorescence and Abs600 measurements into blue cells on "Raw Plate Reader Measurements"  
They will be copied into the green cells on this sheet.  
Cells are calculated.  
If you have more replicates, update the extra columns

Experimental Values:

Sample set:  
Blank media  
Blank mean:

Raw Abs600

Replicate 1 Replicate 2 Replicate 3 Replicate 4  
0.0391217 0.045427 0.0351252 0.046527

Raw Fluorescence

Replicate 1 Replicate 2 Replicate 3 Replicate 4  
163.48 152.17 171.47 164.97

OD - Background

Replicate 1 Replicate 2 Replicate 3 Replicate 4  
163.24 25

Fluorescence - Background

Replicate 1 Replicate 2 Replicate 3 Replicate 4

uM Fluorescein / OD600

Replicate 1 Replicate 2 Replicate 3 Replicate 4

Summary Statistics

Arith. Mean Arith. Std.Dev Geo. Mean Geo. Std.Dev.

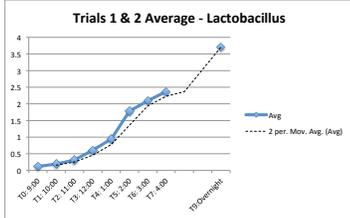
Hour #	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Arith. Mean	Arith. Std.Dev	Geo. Mean	Geo. Std.Dev.
Negative Control (Colony 1)	0.0406399	0.0421468	0.0427705	0.0428139	58366	59996	62627	58566	42113.79	43743.79	46374.79	42313.79	0.54	0.71	0.69	0.68
Negative Control (Colony 2)	0.0406399	0.0405224	0.0432471	0.0437868	62628	60142	62512	70493	44313.79	43889.79	46079.79	44209.79	0.59	0.89	0.63	0.81
Positive Control (Colony 1)	0.0413128	0.043117	0.0431338	0.0452124	71900	75788	75198	79351	55647.79	59315.79	58845.79	63098.79	1.01	0.83	0.95	0.78
Positive Control (Colony 2)	0.0436307	0.0447529	0.0456571	0.0452171	73950	73484	82028	66071	59973.79	57213.79	68676.79	49818.79	0.80	0.69	0.82	0.70
Test Dev 1: J23101.BC2D.E0040.B0015 (Colony 1)	0.0413998	0.0426658	0.0438148	0.0433854	32317	13979	13498	13478	10734.79	11412.79	118745.79	118353.79	1.87	1.72	1.57	1.85
Test Dev 1: J23101.BC2D.E0040.B0015 (Colony 2)	0.0438031	0.0448458	0.0435933	0.0454545	122829	124172	123418	129507	106376.79	107915.79	107165.79	113254.79	1.41	1.29	1.45	1.33
Test Dev 2: J23106.BC2D.E0040.B0015 (Colony 1)	0.0414694	0.0423729	0.0449491	0.0446646	74261	58986	82806	80496	58803.79	42843.79	66533.79	74241.79	1.00	0.67	0.96	0.90
Test Dev 2: J23106.BC2D.E0040.B0015 (Colony 2)	0.0409792	0.0402154	0.0449771	0.0391143	98816	97798	98813	97976	82513.79	81545.79	79800.79	43912.79	1.07	1.06	0.99	1.12
Test Dev 3: J23117.BC2D.E0040.B0015 (Colony 1)	0.0396089	0.0391133	0.0394971	0.0397127	65077	62038	62778	89728	48824.79	45789.79	46025.79	43475.79	1.31	1.23	1.34	1.07
Test Dev 3: J23117.BC2D.E0040.B0015 (Colony 2)	0.0405313	0.0418329	0.0421121	0.0434548	61669	63020	66721	61661	45027.79	46557.79	50468.79	47783.79	0.83	0.67	0.71	0.64
Test Dev 4: J23101.H13504 (Colony 1)	0.0379055	0.0380499	0.0383992	0.0387192	92739	95481	94594	97038	76426.79	79430.79	78341.79	60195.79	2.30	2.76	1.74	1.92
Test Dev 4: J23101.H13504 (Colony 2)	0.0406754	0.0408211	0.0408111	0.0420207	90529	90414	92515	92579	74276.79	74411.79	76662.79	76326.79	1.49	1.43	1.50	1.30
Test Dev 5: J23106.H13504 (Colony 1)	0.0398188	0.0407165	0.0409221	0.0371481	63484	64648	66916	56762	47211.79	50211.79	50683.79	20091.79	1.18	1.05	1.06	0.80
Test Dev 5: J23106.H13504 (Colony 2)	0.0406342	0.0404729	0.0425296	0.0451082	699238	70478	74649	75411	51075.79	54223.79	58396.79	59102.79	0.67	0.65	0.74	0.81
Test Dev 6: J23117.H13504 (Colony 1)	0.0413383	0.0424027	0.0424751	0.0440892	64259	64159	66424	60910	48006.79	47906.79	50171.79	44857.79	0.67	0.60	0.60	0.61
Test Dev 6: J23117.H13504 (Colony 2)	0.0405516	0.0404468	0.0419316	0.0438177	61301	60920	70120	70868	43183.79	50647.79	53856.79	54438.79	0.53	0.61	0.71	0.72
Hour 2																
Negative Control (Colony 1)	0.0406399	0.0421468	0.0427705	0.0428139	61577	64121	67329	66851	48334.79	47868.79	51076.79	50599.79	0.50	0.53	0.55	0.53
Negative Control (Colony 2)	0.0659449	0.0653536	0.0624771	0.0618681	64521	66652	59406	81491	48268.79	50399.79	43153.79	65238.79	0.50	0.20	0.19	0.20
Positive Control (Colony 1)	0.0735566	0.0681317	0.0673984	0.0652789	202444	185112	192771	185193	136193.79	168895.79	176254.79	168940.79	0.58	0.62	0.63	0.67
Positive Control (Colony 2)	0.0651163	0.0650821	0.0627174	0.0674578	202363	201206	198466	29280	136193.79	168940.79	176254.79	168940.79	0.66	0.66	0.68	0.68
Test Dev 1: J23101.BC2D.E0040.B0015 (Colony 1)	0.0440913	0.0448168	0.0435398	0.0435454	171997	174446	174284	185304	155744.79	158211.79	158031.79	160051.79	2.14	2.65	2.16	2.30
Test Dev 1: J23101.BC2D.E0040.B0015 (Colony 2)	0.0411001	0.0414138	0.0430931	0.0413544	142441	148914	144445	144622	120388.79	119304.79	127892.79	128383.79	2.38	2.38	2.16	2.38
Test Dev 2: J23106.BC2D.E0040.B0015 (Colony 1)	0.0378399	0.0384292	0.0386499	0.0370064	178052	175556	178997	200934	133835.79	139823.79	160644.79	148663.79	0.57	0.58	0.58	0.60
Test Dev 2: J23106.BC2D.E0040.B0015 (Colony 2)	0.0756255	0.0645414	0.07361	0.072254	273568	214272	243622	312779	257315.79	196219.79	227369.79	286236.79	2.77	2.78	0.71	0.69
Test Dev 3: J23117.BC2D.E0040.B0015 (Colony 1)	0.0682968	0.0726652	0.0811801	0.0819643	65588	78278	79741	76721	49175.79	56625.79	64848.79	60468.79	0.80	0.88	0.97	0.91
Test Dev 3: J23117.BC2D.E0040.B0015 (Colony 2)	0.0745151	0.0735161	0.0744971	0.0743158	60114	60940	60930	67963	43813.79	41149.79	51377.79	46607.79	0.51	0.58	0.51	0.58
Test Dev 4: J23101.H13504 (Colony 1)	0.0396367	0.0394064	0.0395461	0.0397477	111177	121161	117114	118370	802075.79	800488.79	810631.79	800488.79	2.29	2.28	2.49	3.08
Test Dev 4: J23101.H13504 (Colony 2)	0.0396367	0.0394064	0.0395461	0.0397477	84749	106169	100083	101023	709697.79	703013.79	718297.79	718297.79	1.68	1.19	1.69	1.97
Test Dev 5: J23106.H13504 (Colony 1)	0.0749538	0.0628249	0.0643161	0.0630510	101189	105381	108406	110444	809136.79	89128.79	92153.79	90191.79	0.26	0.39	0.38	0.38
Test Dev 5: J23106.H13504 (Colony 2)	0.0717864	0.0703021	0.0665654	0.0747727	126773	120891	124601	130619	110120.79	106488.79	107814.79	114086.79	0.38	0.38	0.41	0.33
Test Dev 6: J23117.H13504 (Colony 1)	0.0870651	0.0864309	0.0730771	0.0806461	79042	81402	79561	74181	58709.79	60479.79	58801.79	57945.79	0.14	0.27	0.18	0.13
Test Dev 6: J23117.H13504 (Colony 2)	0.0753424	0.0794258	0.0825286	0.0843582	81441	79003	76256	71755	65188.79	62792.79	60003.79	55502.79	0.20	0.17	0.15	0.14
Hour 4																
Negative Control (Colony 1)	0.7379137	0.2605426	0.2368821	0.3013151	76773	73896	37222	50870	0.291524	0.226184	0.2024208	0.266759	0.020	0.021	0.021	0.027
Negative Control (Colony 2)	0.1907169	0.1495898	0.2449051	0.2790602	38675	28748	79192	37394	0.156157	0.1150285	0.1104442	0.242489	0.020	0.021	0.021	0.024
Positive Control (Colony 1)	0.2468874	0.2278204	0.2225007	0.2648562	1270165	1310223	1320683	1514585	0.2104265	0.2881262	0.1873386	0.2031289	0.73	0.76	0.73	0.83
Positive Control (Colony 2)	0.2976348	0.3448997	0.3012324	0.3886357	2234341	3012762	1840445	1407871	0.4630736	0.3203115	0.2660712	0.3349253	2.108	1.15	0.88	0.74
Test Dev 1: J23101.BC2D.E0040.B0015 (Colony 1)	0.0467777	0.0449999	0.0466278	0.051724	280184	298488	320878	349212	0.0212165	0.0214378	0.0146663	0.0171628	2.65	2.78	2.65	2.38
Test Dev 1: J23101.BC2D.E0040.B0015 (Colony 2)	0.0417179	0.0407709	0.0400001	0.0413155	39749	106169	100083	101023	0.0994707	0.0987909	0.0134008	0.0091047	2.94	2.11	1.68	1.89
Test Dev 2: J23106.BC2D.E0040.B0015 (Colony 1)	0.2735831	0.27389	0.2901510	0.322364	2084490	2475145	2831188	3038788	0.2802121	0.2927778	0.2557547	0.2887552	0.04	0.05	0.035	0.131
Test Dev 2: J23106.BC2D.E0040.B0015 (Colony 2)	0.3402878	0.3212916	0.3089249	0.324853	3450250	3204778	2807032	3182767	0.3063265	0.2783523	0.2748183	0.2822177	1.38	1.40	1.25	1.33
Test Dev 3: J23117.BC2D.E0040.B0015 (Colony 1)	0.0431093	0.0445728	0.0471677	0.0412424	98389	109469	106496	131076	0.2895464	0.3193544	0.2650312	0.2813877	0.04	0.05	0.04	0.04
Test Dev 3: J23117.BC2D.E0040.B0015 (Colony 2)	0.3246603	0.3131047	0.3292691	0.3607431	126303	129700	116116	126061	0.280009	0.2864858	0.2947081	0.3261819	0.05	0.05	0.04	0.04
Test Dev 4: J23101.H13504 (Colony 1)	0.0317869	0.0318256	0.0329201	0.0318719	110889	117940	122609	127999	0.0309756	0.0308864	0.0404297	0.0411380	1.779	1.22	0.76	1.09
Test Dev 4: J23101.H13504 (Colony 2)	0.0602868	0.0532208	0.0480519	0.0495413	92189	105381	94109	99023	0.050729	0.0506691	0.0644924	0.0596911	1.62	1.59	1.47	1.09
Test Dev 5: J23106.H13504 (Colony 1)	0.2289202	0.1996371	0.2174171	0.2346462	156001	436488	662007	577131	0.193931	0.1650718	0.2128757	0.1998949	0.38	0.31	0.37	0.34
Test Dev 5: J23106.H13504 (Colony 2)	0.3505674	0.3738245	0.3737877	0.3584069	1182724	1169592	1072532	1017670	0.1247653	0.1317623	0.1411458	0.1238469	0.44			

# Trial 3 - Growth Curves

Average of Trial 1 and Trial 2

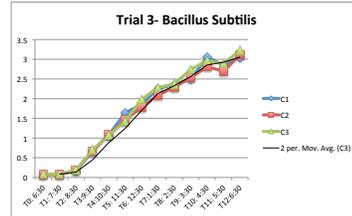
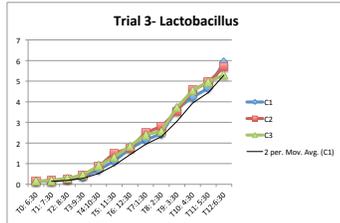
Lactobacillus	Trial 1	Trial 2	Avg
T0: 9:00	0.1	0.1353	0.118
T1: 10:00	0.138	0.251	0.195
T2: 11:00	0.307	0.317	0.312
T3: 12:00	0.57	0.642	0.61
T4: 1:00	0.89	0.993	0.942
T5: 2:00	1.24	2.31	1.78
T6: 3:00	1.5	2.68	2.09
T7: 4:00	1.65	3.098	2.37

T8: Overnight 2.012 T9: Overnight 5.4 T9: Overnight 3.706

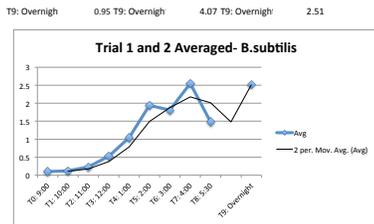


Trial 3 Data	Lactobacillus	C1	C2	C3
T0: 6:30	0.14	0.15	0.15	0.15
T1: 7:30	0.146	0.174	0.177	0.177
T2: 8:30	0.229	0.272	0.288	0.288
T3: 9:30	0.349	0.427	0.45	0.45
T4: 10:30	0.696	0.868	0.896	0.896
T5: 11:30	1.132	1.512	1.324	1.324
T6: 12:30	1.732	1.748	1.852	1.852
T7: 1:30	2.148	2.504	2.404	2.404
T8: 2:30	2.452	2.8	2.568	2.568
T9: 3:30	3.652	3.524	3.728	3.728
T10: 4:30	4.23	4.56	4.55	4.55
T11: 5:30	4.66	4.94	4.95	4.95
T12: 6:30	5.91	5.69	5.3	5.3

Time	B.subtilis	C1	C2	C3
T0: 6:30	0.07	0.07	0.07	0.08
T1: 7:30	0.08	0.075	0.085	0.085
T2: 8:30	0.17	0.18	0.185	0.185
T3: 9:30	0.668	0.66	0.7	0.7
T4: 10:30	1.076	1.08	1.06	1.06
T5: 11:30	1.636	1.456	1.42	1.42
T6: 12:30	1.86	1.78	1.972	1.972
T7: 1:30	2.248	2.088	2.284	2.284
T8: 2:30	2.356	2.288	2.376	2.376
T9: 3:30	2.5	2.528	2.74	2.74
T10: 4:30	3.056	2.808	2.968	2.968
T11: 5:30	2.856	2.696	2.884	2.884
T12: 6:30	3.044	3.108	3.228	3.228

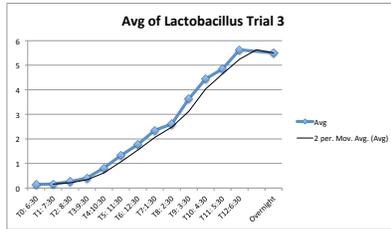
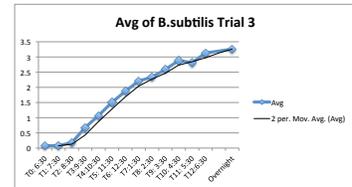


B.subtilis	Trial 1	Trial 2	Avg
T0: 9:00	0.1	0.1	0.1
T1: 10:00	0.124	0.112	0.119
T2: 11:00	0.261	0.195	0.228
T3: 12:00	0.423	0.62	0.527
T4: 1:00	0.761	1.32	1.04
T5: 2:00	1.74	2.13	1.94
T6: 3:00	1.088	2.54	1.81
T7: 4:00	1.56	3.14	2.55
T8: 5:30	0.95	2.01	1.48



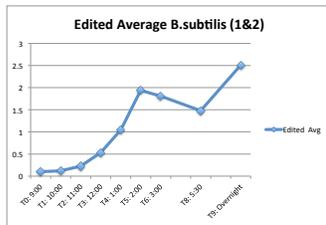
Trial 3 Lactobacillus Average	Avg
T0: 6:30	0.145
T1: 7:30	0.166
T2: 8:30	0.263
T3: 9:30	0.41
T4: 10:30	0.82
T5: 11:30	1.32
T6: 12:30	1.77
T7: 1:30	2.35
T8: 2:30	2.61
T9: 3:30	3.63
T10: 4:30	4.45
T11: 5:30	4.85
T12: 6:30	5.633
Overnight	5.5

Trial 3 B.subtilis Average	Avg
T0: 6:30	0.075
T1: 7:30	0.08
T2: 8:30	0.178
T3: 9:30	0.676
T4: 10:30	1.072
T5: 11:30	1.504
T6: 12:30	1.871
T7: 1:30	2.21
T8: 2:30	2.34
T9: 3:30	2.593
T10: 4:30	2.88
T11: 5:30	2.812
T12: 6:30	3.13
Overnight	3.26

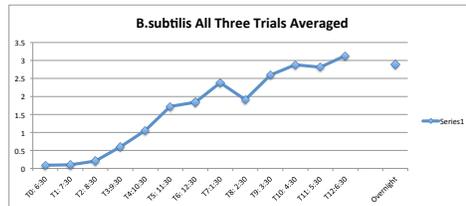


Edited

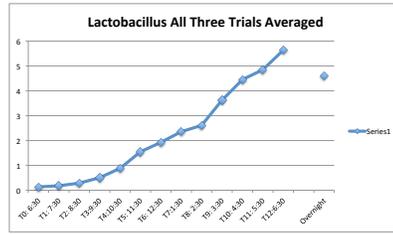
Time	Avg
T0: 9:00	0.1
T1: 10:00	0.119
T2: 11:00	0.228
T3: 12:00	0.527
T4: 1:00	1.04
T5: 2:00	1.94
T6: 3:00	1.81
T8: 5:30	1.48
T9: Overnight	2.51



B.subtilis	Trial 1 & 2 Avg	Trial 3	AVG
T0: 9:00	0.1	0.1	0.075
T1: 10:00	0.119	0.119	0.08
T2: 11:00	0.228	0.228	0.178
T3: 12:00	0.527	0.527	0.676
T4: 1:00	1.04	1.04	1.072
T5: 2:00	1.94	1.94	1.504
T6: 3:00	1.81	1.81	1.871
T7: 4:00	2.55	2.55	2.21
T8: 5:30	1.48	1.48	2.34
T9: 3:30	2.593	2.593	2.593
T10: 4:30	2.88	2.88	2.88
T11: 5:30	2.812	2.812	2.812
T12: 6:30	3.13	3.13	3.13
Overnight	3.26	3.26	2.89



Lactobacillus	Trial 3	Avg	Standard Error
T0: 9:00	0.118	0.145	0.132
T1: 10:00	0.195	0.166	0.181
T2: 11:00	0.312	0.263	0.288
T3: 12:00	0.61	0.41	0.51
T4: 1:00	0.942	0.82	0.881
T5: 2:00	1.78	1.32	1.55
T6: 3:00	2.09	1.77	1.93
T7: 4:00	2.37	2.35	2.36
T8: 2:30	2.61	2.61	2.61
T9: 3:30	3.63	3.63	3.63
T10: 4:30	4.45	4.45	4.45
T11: 5:30	4.85	4.85	4.85
T12: 6:30	5.633	5.633	5.633
Overnight	5.5	5.5	4.603





S	0100	0101	0110	0111	1000	1001	1010	1011				
A	0100	0101	0110	0111	1000	1001	1010	1011				

01001 01000 01000 01000 01000 01000 01000 01000 01000 01000 01000 01000 01000



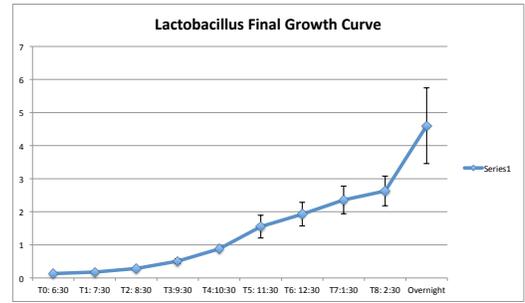
	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	0.11	0.13	0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33
<b>B</b>	0.12	0.14	0.16	0.18	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.34
<b>C</b>	0.13	0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.35
<b>D</b>	0.14	0.16	0.18	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.34	0.36
<b>E</b>	0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.35	0.37
<b>F</b>	0.16	0.18	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.34	0.36	0.38
<b>G</b>	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.35	0.37	0.39
<b>H</b>	0.18	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.40
<b>I</b>	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.35	0.37	0.39	0.41
<b>J</b>	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42
<b>K</b>	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.35	0.37	0.39	0.41	0.43
<b>L</b>	0.22	0.24	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.44
<b>M</b>	0.23	0.25	0.27	0.29	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.45
<b>N</b>	0.24	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.44	0.46
<b>O</b>	0.25	0.27	0.29	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.45	0.47
<b>P</b>	0.26	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.44	0.46	0.48
<b>Q</b>	0.27	0.29	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.45	0.47	0.49
<b>R</b>	0.28	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.44	0.46	0.48	0.50
<b>S</b>	0.29	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.45	0.47	0.49	0.51
<b>T</b>	0.30	0.32	0.34	0.36	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52
<b>U</b>	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.45	0.47	0.49	0.51	0.53
<b>V</b>	0.32	0.34	0.36	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54
<b>W</b>	0.33	0.35	0.37	0.39	0.41	0.43	0.45	0.47	0.49	0.51	0.53	0.55
<b>X</b>	0.34	0.36	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56
<b>Y</b>	0.35	0.37	0.39	0.41	0.43	0.45	0.47	0.49	0.51	0.53	0.55	0.57
<b>Z</b>	0.36	0.38	0.40	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58

0.12% 0.1545% 0.18% 0.15 0.1487% 0.15 0.1485%  
0.15 0.1485% 0.15 0.1485% 0.15 0.1485% 0.15 0.1485%  
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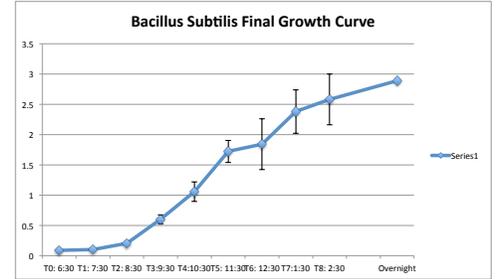
# Final Growth Curve Analysis

Lactobacillus	Trial 2	Trial 3	Average of All	Standard error
Trial 1				
T0: 9:00	0.1 T0: 9:00	0.1353 T0: 6:30	0.145 T0: 6:30	0.132
T1: 10:00	0.138 T1: 10:00	0.251 T1: 7:30	0.166 T1: 7:30	0.181
T2: 11:00	0.307 T2: 11:00	0.317 T2: 8:30	0.263 T2: 8:30	0.288
T3: 12:00	0.57 T3: 12:00	0.642 T3: 9:30	0.41 T3: 9:30	0.51
T4: 1:00	0.89 T4: 1:00	0.993 T4: 10:30	0.82 T4: 10:30	0.881
T5: 2:00	1.24 T5: 2:00	2.31 T5: 11:30	1.32 T5: 11:30	1.55
T6: 3:00	1.5 T6: 3:00	2.68 T6: 12:30	1.77 T6: 12:30	1.93
T7: 4:00	1.65 T7: 4:00	3.098 T7: 1:30	2.35 T7: 1:30	2.36
T8: 5:00	1.87 T8: 5:00	3.42 T8: 2:30	2.61 T8: 2:30	2.63
		T9: 3:30	3.63 T9: 3:30	3.63
T8: Overnight	2.012 T9: Overnight	5.4 T10: 4:30	4.45 T10: 4:30	4.45
		T11: 5:30	4.85 T11: 5:30	4.85
		T12: 6:30	5.633	
		Overnight	5.5 Overnight	4.603

Standard Error  
STDEV (/ SQRT(3))



B. subtilis	Trial 2	Trial 3	Average of All	Standard Error
Trial 1				
T0: 9:00	0.1 T0: 9:00	0.1 T0: 6:30	0.075 T0: 6:30	0.0875
T1: 10:00	0.126 T1: 10:00	0.112 T1: 7:30	0.08 T1: 7:30	0.0995
T2: 11:00	0.263 T2: 11:00	0.195 T2: 8:30	0.178 T2: 8:30	0.203
T3: 12:00	0.433 T3: 12:00	0.62 T3: 9:30	0.676 T3: 9:30	0.602
T4: 1:00	0.761 T4: 1:00	1.32 T4: 10:30	1.072 T4: 10:30	1.056
T5: 2:00	1.74 T5: 2:00	2.13 T5: 11:30	1.504 T5: 11:30	1.722
T6: 3:00	1.088 T6: 3:00	2.54 T6: 12:30	1.871 T6: 12:30	1.84
T7: 4:00	1.94 T7: 4:00	3.14 T7: 1:30	2.21 T7: 1:30	2.38
T8: 5:30	0.95 T8: 5:30	2.01 T8: 2:30	2.34 T8: 2:30	1.91
		T9: 3:30	2.593 T9: 3:30	2.593
T9: Overnight	0.95 T9: Overnight	4.07 T10: 4:30	2.88 T10: 4:30	2.88
		T11: 5:30	2.812 T11: 5:30	2.812
		T12: 6:30	3.13 T12: 6:30	3.13
		Overnight	3.26 Overnight	2.89



## PROBIOTICS and LEAD REMEDIATION

1. Significant removal was observed, and it was found to be metal and bacterial strain specific. Removal was a fast, metabolism-independent surface process. It was also strongly influenced by pH, indicating that ion exchange mechanisms could be involved. The most effective metal removers were *Bifidobacterium longum* 46, *Lactobacillus fermentum* ME3 and *Bifidobacterium lactis* Bb12. The highest maximum cadmium and lead removal capacities of 54.7 mg metal/g and 175.7 mg/g dry biomass, respectively, were obtained with *B. longum* 46. (<http://www.sciencedirect.com/science/article/pii/S0168160506005952>)
2. I suggest a careful study of this research. There are many parallels to yours.
3. I suggest avoiding the use of streptococcus species for bio-safety puposes.
4. Developing the selective stain by evolution is an excellent an excellent idea. Please give consideration to bio-toxicity, bio-acclimation, and toxicity to exoenzymes. Follow the work of the researches in item #1 since genome manipulation is a lengthy process. Probiotics developed by evolution can be freeze dried and stored for future use.
5. Harvest probiotics in the middle phase of "S" growth—first derivative change equal zero.
6. If successful in producing an evolutionary species and mass culturing with appropriate media, you will then engage in animal experimentation with the chromo-proteins for quantification.
7. Any further development (clinical trials) will necessitate FDA involvement.
8. Be aware that only 20 % of lead in absorbed via drinking water.
9. Consideration must be given to the pH of the gut and how this might change with diet.
10. Consideration must be given to competing metals that can reverse metal enzymatic binding by virtue of being stronger oxidizing agents. Say a person taking iron tablets, which is common, could reverse lead binding.
11. The current practice of the MassDEP Drinking and Program in collaboration with EPA is to abate lead, but the long term goal is its ultimate removal everywhere in drinking water facilities and all appurtenants.
12. The work developed here has more relevance in situation where the consumer may be uncertain of the lead content of water; places where are no environmental regulations and caution need to exercised; hence, a prophylactic or emergency resort.

**Conclusion:** Rather than using humans or animal species as portals for lead elimination, the current goal of the Department is to eliminate lead before consumption, but we recognize the potential for emergency intervention is this promising research.

necessary to meet this goal. EPA has adopted the blood lead level of concern of 10  $\mu\text{g}/\text{dL}$  as a benchmark to assist the Agency in evaluating progress in reducing lead exposures. However, EPA does not consider this level to be a threshold below which there are no risks of adverse effects. In establishing MCLGs, the Agency seeks to ascertain the level at which there are no known or anticipated adverse effects on the health of persons and which includes an adequate margin of safety. Section 1412(b)(4). Given the growing body of scientific evidence that risks of adverse effects are present at increasingly lower levels of exposure, and the uncertainty that any blood lead level is free from risk of incurring adverse effects among the sensitive populations, EPA concludes that it would be difficult to identify an adequate margin of safety, and an associated water lead concentration, that would adequately achieve the health goal contained in § 1412(b)(4) of the SDWA.

Based on the available data, EPA believes there are no clearly discernible thresholds for some of the non-carcinogenic adverse health effects associated with lead (EPA, 1990a). Because of the possibility that adverse health effects may occur at blood lead levels below 10  $\mu\text{g}/\text{dL}$ , the Agency believes that an MCLG of zero for lead in drinking water complies with the intent of the SDWA.

In addition, comments that average blood lead levels could be maintained below levels of concern with a higher MCLG ignore the distinction between individual blood lead concentrations at the level of concern (i.e.,  $\text{PbB} \geq 10 \mu\text{g}/\text{dl}$ ) and population average levels expected when this rule takes effect. There is a wide range of lead levels not only in water but also in house dusts, soils, diets, etc. In addition, there is tremendous variability, especially among children, in behavior patterns (including consumption), physiological sensitivity, and nutritional states. Because of these factors, there is a wide distribution of blood lead levels in the population.

Analysis in the Air Quality Criteria Document (EPA, 1986a) of blood lead distributions measured in the Second National Health and Nutrition Survey (NHANES II), the most recently completed nationwide survey of U.S. blood lead levels, indicates that among a population of U.S. children with an average blood lead level of 5  $\mu\text{g}/\text{dl}$ , for example, approximately 2.5 percent would have blood lead levels above 10  $\mu\text{g}/\text{dl}$ . It is estimated that several million children have blood lead levels

above 10  $\mu\text{g}/\text{dL}$ , mainly from lead paint or from old contaminated soils in urban areas (ATSDR, 1988).

Because many children now have blood lead levels above the level of concern, EPA's policy goal continues to be that drinking water should contribute minimal additional lead to existing body burdens of lead. This policy is consistent with the statutory mandate to set MCLGs at a level that provides an "adequate margin of safety," which, as discussed in the legislative history of the SDWA, must consider exposure to contaminants from sources other than drinking water and adverse effects that may be experienced by sensitive sub-populations. For this additional reason, setting a health-based goal of zero for lead in drinking water is consistent with the statutory standard.

## 2. Contribution of Water Lead to Blood Lead Levels

Several commenters believed that EPA could establish an MCLG above zero and still protect public health because the contribution to blood lead levels from drinking water is minimal. These commenters raised two points: (1) the correlation between blood lead and water lead is questionable; and (2) drinking water comprises only a small proportion of total human lead intake.

*a. Blood Lead to Water Lead Relationship.* At the time of proposal, EPA used a correlation coefficient of 0.20  $\mu\text{g}/\text{dL}$  lead in blood per  $\mu\text{g}/\text{L}$  lead in water, derived from duplicate diet studies by Ryu et al. (1983) and Lacey et al. (1985) (EPA, 1988b). Ryu et al. studied infants in Iowa fed a controlled diet of canned formula or cow's milk. Drinking water was not the source of lead, and use of these data assumes that lead absorption from water is equal to that from formula or diet. The Lacey et al. study collected data in Glasgow on infants' blood lead levels, and lead in a duplicate diet sample, in first-draw, random daytime tap water and in typical water use samples (from tea kettles). Several commenters stated that EPA had not established a clear correlation between water lead and blood lead. Other commenters claimed that the studies used to correlate water lead and blood lead had been improperly evaluated by EPA. One of these commenters stated that EPA had underestimated the blood lead response in the Ryu study because the study did not allow infant blood leads to reach a steady state. This commenter suggested that if the nonequilibrium conditions that existed in the Ryu et al. study are considered, a correlation coefficient of 0.48  $\mu\text{g}/\text{dL}$  lead in blood per  $\mu\text{g}/\text{L}$  lead in water is derived. Another commenter

stated that the Ryu study was not a water study but a dietary study involving no drinking water lead impact.

Several studies have examined the contribution that lead levels in drinking water makes to blood lead in children and adults (e.g., Thomas et al., 1979; Worth et al., 1981; Moore, 1977; Moore et al., 1979; Sherlock and Quinn, 1986; Lacey et al., 1985; Raab et al., 1987; Laxen et al., 1987; Maes et al., 1991). These studies have correlated blood lead levels with water lead levels in first-draw water, in random or partially flushed water samples, or in composite samples from first, partially, or fully flushed water. Based on these studies, it is difficult to identify the single measure of water lead that best predicts blood lead (EPA, 1986a).

In response to comments, EPA has reanalyzed the Ryu and Lacey studies, along with a study by Laxen et al. (1987) on school children in Edinburgh in which tap water was sampled after a 5-minute flush and a 30-minute stagnation time. These analyses, summarized in Marcus (1989a; 1989b; 1990b; 1990c), found a nonlinear relationship between children's blood lead and water lead levels and best fit a piece-wise dose-response function with different water lead: blood lead coefficients at different water lead concentrations. This is consistent with the non-linear kinetics of lead transfer from the red blood cell and an apparent saturable transfer mechanism in the gut (EPA, 1986a).

EPA agrees that it is better to rely on studies where drinking water was the source of lead and believes the Lacey study, rather than the Ryu study, is the best study for indicating blood lead responses among infants to lead in drinking water. The Lacey study measured drinking water exposures of children from zero to 6 months of age. Regression analyses of the Lacey study found a slope of 0.26  $\mu\text{g}/\text{dL}$  blood per  $\mu\text{g}/\text{L}$  water at water lead levels below 0.015  $\text{mg}/\text{L}$  and 0.04  $\mu\text{g}/\text{dL}$  blood per  $\mu\text{g}/\text{L}$  water at water lead levels above 0.015  $\text{mg}/\text{L}$ . While EPA believes the Lacey study, because of its reliance on exposure through drinking water, is the best available study for estimating water lead: blood lead relationships for infants, the Agency notes that the Ryu study yielded results similar to the Lacey study (0.24  $\mu\text{g}/\text{dL}$  blood lead per  $\mu\text{g}/\text{L}$  water lead assuming a water lead intake of 1 liter per day).

For older children, EPA is relying on a recent study by (Maes et al., 1991) of Hawaiians exposed to lead in drinking water across a wide range of levels. Again, a piece-wise linear relationship was found to provide the best fit to the

data with a slope of 0.12  $\mu\text{g}/\text{dL}$  blood per  $\mu\text{g}/\text{L}$  water at water lead levels below 0.015 mg/L and 0.06  $\mu\text{g}/\text{dL}$  blood per  $\mu\text{g}/\text{L}$  water at water lead levels above 0.015 mg/L. Because this study controlled for many different variables, including house dust and food, EPA concludes that it provides the most reliable estimate of blood lead:water lead relationships for children.

For adults, the 1986 Criteria Document identified Pocock et al. (1983) as the most useful study; regression analyses yielded a slope of 0.06  $\mu\text{g}/\text{dL}$  blood per  $\mu\text{g}/\text{L}$  water lead.

In conclusion, EPA disagrees with commenters suggesting that the Agency has not established a clear correlation between blood lead and water lead levels and that additional research is needed to substantiate this relationship. EPA recognizes that differences exist in the correlation coefficients derived from the available studies on water lead/blood lead relationships. These differences can be attributed to such factors as differences in study populations, analytical methods, and potential confounders (e.g., other lead sources, including diet, dust, and air). EPA believes, nonetheless, that the studies reviewed and analyzed in the Air Quality Criteria Document (1986a) and the additional analyses cited above have established a quantitatively consistent relationship between blood lead and lead in drinking water for infants, children, and adults.

While the degree to which lead causes increases in blood lead levels is important for evaluating the degree of health effects associated with various water lead levels, this issue is not directly relevant to the Agency's bases for establishing an MCLG of zero. The first basis (lack of clear threshold for adverse effects) is based upon extensive studies of various health endpoints, and does not depend specifically on any water lead-blood lead relationship. The second basis for the zero MCLG is based on the empirically observed fact that a large number of children have blood leads above the level of concern. Even if there is a disagreement regarding the degree of change in blood lead levels that would be caused by water lead levels, it would always be the case that consumption of lead in water would contribute to some increase in blood lead levels, thereby causing an increased risk of adverse effects for the sensitive sub-population of children with blood lead levels already above 10  $\mu\text{g}/\text{dL}$ . The third basis for the MCLG (carcinogenic effects), like the first basis, depends upon the non-threshold nature of lead's health effects,

and not upon any particular correlation between water lead and blood lead levels.

b. *Contribution of Drinking Water to Total Lead Intake.* EPA also disagrees with the assertion that drinking water comprises a small proportion of lead intake. EPA estimated in the proposal that the typical drinking water contribution to total lead exposure for an average 2-year-old child is about 20 percent (EPA, 1988c). The proportion of exposure due to lead, however, will vary with different levels of lead in the water and with variations in other lead exposures. For children with high levels of lead exposure from lead paint, contaminated soils and dusts near roadways or lead smelters, or other point sources of airborne lead, drinking water contributes a much lower, although still relevant, proportion of total exposure. For residents of houses and buildings with relatively new lead solder or lead service lines, drinking water can be the primary source of exposure, especially if the water is corrosive. As such, the total drinking water contribution to overall lead levels may range from as little as 5 percent to more than 50 percent of children's total lead exposure. Infants dependent on formula may receive more than 85 percent of their lead from drinking water. As exposures decline to sources of lead other than drinking water, such as gasoline and soldered food cans, drinking water will account for a larger proportion of total intake. The estimate of the relative contribution of drinking water to blood lead levels is not used in any risk assessments for the final rule. As discussed previously, blood lead impacts from different water lead scenarios have been estimated through application of empirical relationships between water lead and blood lead.

### 3. Carcinogenicity of Lead

As discussed above, the Agency has adopted a carcinogenic classification scheme for chemicals that considers the weight of evidence of carcinogenicity in humans, using bioassays in animals and human epidemiological studies, as well as information that provides indirect evidence (i.e., mutagenicity and other short-term test results). Carcinogens are classified as either Group A, B1, B2, C, D, or E. For known or probably human carcinogens (categories A, B1, or B2), EPA's established policy is to set MCLGs for such contaminants at zero.

EPA determined in the proposal that lead was a Group B2 (probable) human carcinogen. Several commenters disagreed, believing that the data were not adequate to make such a determination. They asked EPA's

Science Advisory Board (SAB) to review the data.

In March and April 1989, an ad hoc SAB committee reviewed the data and basis for EPA's classification of lead as a B2 carcinogen. The findings of the committee, consisting of members of the SAB Executive Committee, the SAB Environmental Health Committee, and the Clean Air Scientific Advisory Committee, were presented in a final report submitted to the EPA Administrator on November 21, 1989 (EPA, 1989b). The final report noted that there was limited understanding of the mechanisms of lead-induced tumorigenesis and that limitations in the available data made it inappropriate to develop a potency factor to perform a quantitative risk assessment for lead at this time. The committee, however, agreed with EPA's conclusion that it had been sufficiently established that lead is a probable human carcinogen, appropriately classified as a B2 carcinogen according to EPA's cancer assessment guidelines. Based on the SAB recommendation, a potency factor for lead has not been developed by EPA. If a potency factor for lead is developed, it will be reviewed by the SAB.

When establishing MCLGs, the Agency usually classifies B2 carcinogens as a Category I contaminant unless there is compelling evidence (e.g., exposure, pharmacokinetics) to place the contaminant into a different category. EPA believes the evidence warrants classifying lead as a Category I contaminant. This determination is based on data from over 20 separate ingestion studies that showed an elevated incidence of kidney tumors in rats and mice (EPA, 1988m; EPA, 1989g). In studies where animals were exposed via drinking water, positive results were reported in one experiment with rats exposed to lead acetate (Koller et al., 1985) but not another (Kanisawa and Schroeder, 1989). Possible induction of lymphocytic leukemia occurred in mice dosed with as little as 0.1 g of lead via drinking water (Blakley, 1987). As noted in EPA's evaluation of the data and reiterated in SAB's 1989 report, there is uncertainty regarding lead's mechanism of action on inducing tumors, but these uncertainties do not provide a basis to alter the weight of evidence for human carcinogenicity. It is known that a significant proportion of ingested lead is absorbed; in adults, the absorption of ingested lead has been estimated to range from 10 to 15 percent, with rates as high as 21-63 percent under fasting conditions, which may be more representative of between-meal absorption (EPA, 1986a; EPA, 1989g).