

## **Appendix C**

### **Phase 2A Toxicity Test Data**

## **Appendix C1**

### **Data Tables**

**Sample ID:****Lab control**

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	8	10	10	9	9	9	10	8	10	10	10
Percent Survival - Day 28	100	80	100	100	90	90	90	100	80	100	100	100
Tare Wt (g)	1.03248	1.03593	1.03978	1.01434								
Tin + Dry amphipods (g)	1.03603	1.04125	1.04465	1.01992								
Dry Wt per Amphipod (mg)	0.355	0.665	0.487	0.558								
# Amphipods Alive - Day 42					9	9	8	10	8	10	10	10
Percent Survival - Day 42					90	90	80	100	80	100	100	100
# Female Amphipods					5	5	4	5	4	7	5	6
# Male Amphipods					4	4	4	4	5	3	5	4
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					6	0	0	0	0	0	0	0
# Offspring - Day 35					16	31	44	40	13	48	18	27
# Offspring - Day 42					17	12	7	14	10	22	25	24
# Offspring/Female					7.8	8.6	12.75	10.8	5.75	10	8.6	8.5

**Station:****OT-6 (Reference area)****Sample ID:****TX0001**

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	10	9	10	10	9	9	9	10	10	7	10
Percent Survival - Day 28	100	100	90	100	100	90	90	90	100	100	70	100
Tare Wt (g)	1.05297	1.04878	1.04469	1.01662								
Tin + Dry amphipods (g)	1.05764	1.05363	1.04863	1.02064								
Dry Wt per Amphipod (mg)	0.467	0.485	0.43778	0.402								
# Amphipods Alive - Day 42					9	8	9	9	9	10	7	10
Percent Survival - Day 42					90	80	90	90	90	100	70	100
# Female Amphipods					2	4	5	4	5	4	4	6
# Male Amphipods					7	4	4	5	4	6	3	4
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	1	0	0	0	0	0	0
# Offspring - Day 35					10	6	19	16	5	16	9	10
# Offspring - Day 42					2	9	29	1	24	9	7	10
# Offspring/Female					6	4	9.6	4.25	5.8	6.25	4	3.333

**Station:** S344  
**Sample ID:** TX0002

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	4	5	1	3	2	4	1	5	1	2	4	1
Percent Survival - Day 28	40	50	10	30	20	40	10	50	10	20	40	10
Tare Wt (g)	1.06614	1.04898	1.03235	1.02762								
Tin + Dry amphipods (g)	1.0682	1.0504	1.03248	1.02849								
Dry Wt per Amphipod (mg)	0.515	0.284	0.13	0.29								
# Amphipods Alive - Day 42					2	3	1	3	0	2	3	1
Percent Survival - Day 42					20	30	10	30	0	20	30	10
# Female Amphipods					1	1	0	2	0	1	1	1
# Male Amphipods					0	1	1	1	0	1	2	0
# Immature Amphipods					1	1	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					0	0	0	0	0	0	0	0
# Offspring - Day 42					0	0	0	5	0	8	0	0
# Offspring/Female					0	0		2.5		8	0	0

**Station:** S342  
**Sample ID:** TX0003

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	2	1	1	2	1	2	2	1	0	1	1	2
Percent Survival - Day 28	20	10	10	20	10	20	20	10	0	10	10	20
Tare Wt (g)	1.03252	1.03419	1.05801	1.06426								
Tin + Dry amphipods (g)	1.03382	1.03502	1.05853	1.06562								
Dry Wt per Amphipod (mg)	0.65	0.83	0.52	0.68								
# Amphipods Alive - Day 42					0	2	2	0	0	1	0	2
Percent Survival - Day 42					0	20	20	0	0	10	0	20
# Female Amphipods					0	1	0	0		0	0	1
# Male Amphipods					0	1	2	0		1	0	1
# Immature Amphipods					0	0	0	0		0	0	0
# Offspring - Day 28					0	0	0	0		0	0	0
# Offspring - Day 35					0	0	0	0		0	0	0
# Offspring - Day 42					0	12	0	0		0	0	0
# Offspring/Female						12						0

**Station:** S355  
**Sample ID:** TX0004

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	8	6	8	10	5	9	6	10	10	9	10	9
Percent Survival - Day 28	80	60	80	100	50	90	60	100	100	90	100	90
Tare Wt (g)	1.02415	1.03523	1.04002	1.0298								
Tin + Dry amphipods (g)	1.03152	1.0418	1.04715	1.03718								
Dry Wt per Amphipod (mg)	0.92125	1.095	0.89125	0.738								
# Amphipods Alive - Day 42					5	7	2	9	10	6	10	8
Percent Survival - Day 42					50	70	20	90	100	60	100	80
# Female Amphipods					4	2	1	3	4	2	7	1
# Male Amphipods					1	5	1	6	6	3	3	7
# Immature Amphipods					0	0	0	0	0	1	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					37	8	1	0	16	5	9	11
# Offspring - Day 42					44	3	10	10	31	20	38	0
# Offspring/Female					20.25	5.5	11	3.333	11.75	12.5	6.714	11

**Station:** S354  
**Sample ID:** TX0005

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	10	9	10	10	10	9	10	9	8	9	10
Percent Survival - Day 28	100	100	90	100	100	100	90	100	90	80	90	100
Tare Wt (g)	1.0639	1.03169	1.04393	1.04058								
Tin + Dry amphipods (g)	1.0708	1.03985	1.05203	1.05052								
Dry Wt per Amphipod (mg)	0.69	0.816	0.9	0.994								
# Amphipods Alive - Day 42					9	9	9	10	7	8	9	10
Percent Survival - Day 42					90	90	90	100	70	80	90	100
# Female Amphipods					4	6	6	5	2	4	3	3
# Male Amphipods					5	3	3	5	5	4	6	7
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					27	3	15	35	8	34	5	2
# Offspring - Day 42					9	66	64	26	5	23	4	18
# Offspring/Female					9	11.5	13.17	12.2	6.5	14.25	3	6.667

**Station:** S337  
**Sample ID:** TX0006

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	5	6	7	6	6	7	10	7	7	5	7	5
Percent Survival - Day 28	50	60	70	60	60	70	100	70	70	50	70	50
Tare Wt (g)	1.05311	1.06148	1.0649	1.05236								
Tin + Dry amphipods (g)	1.0549	1.06317	1.06798	1.05486								
Dry Wt per Amphipod (mg)	0.358	0.28167	0.44	0.41667								
# Amphipods Alive - Day 42					4	6	8	6	7	5	6	5
Percent Survival - Day 42					40	60	80	60	70	50	60	50
# Female Amphipods					2	4	5	3	3	2	3	0
# Male Amphipods					2	2	3	3	4	3	3	5
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					0	0	7	0	0	0	0	0
# Offspring - Day 42					6	31	4	13	2	2	11	0
# Offspring/Female					3	7.75	2.2	4.333	0.667	1	3.667	

**Station:** S332  
**Sample ID:** TX0007

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	4	2	3	0	4	5	2	2	3	2	5	2
Percent Survival - Day 28	40	20	30	0	40	50	20	20	30	20	50	20
Tare Wt (g)	1.04534	1.0448	1.02086	1.04164								
Tin + Dry amphipods (g)	1.04697	1.04588	1.0219									
Dry Wt per Amphipod (mg)	0.4075	0.54	0.34667									
# Amphipods Alive - Day 42					2	5	2	2	1	2	5	2
Percent Survival - Day 42					20	50	20	20	10	20	50	20
# Female Amphipods					0	4	2	0	1	1	3	0
# Male Amphipods					2	1	0	2	0	1	2	2
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					0	5	0	0	0	0	0	0
# Offspring - Day 42					0	30	0	0	5	8	21	0
# Offspring/Female						8.75	0		5	8	7	

**Station:** S315  
**Sample ID:** TX0008

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	9	10	8	8	10	10	8	10	9	7	8
Percent Survival - Day 28	100	90	100	80	80	100	100	80	100	90	70	80
Tare Wt (g)	1.03133	1.01494	1.01705	1.02219								
Tin + Dry amphipods (g)	1.03783	1.02142	1.02424	1.02937								
Dry Wt per Amphipod (mg)	0.65	0.72	0.719	0.8975								
# Amphipods Alive - Day 42					5	10	10	8	10	8	7	8
Percent Survival - Day 42					50	100	100	80	100	80	70	80
# Female Amphipods					1	4	7	2	5	5	5	5
# Male Amphipods					4	6	3	6	5	3	2	3
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					2	9	11	14	2	17	3	0
# Offspring - Day 42					4	43	99	0	52	57	78	74
# Offspring/Female					6	13	15.71	7	10.8	14.8	16.2	14.8

**Station:** S317  
**Sample ID:** TX0009

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	7	8	9	10	6	8	7	6	7	9	7	7
Percent Survival - Day 28	70	80	90	100	60	80	70	60	70	90	70	70
Tare Wt (g)	1.03871	1.03968	1.06433	1.04161								
Tin + Dry amphipods (g)	1.04181	1.04284	1.06775	1.04515								
Dry Wt per Amphipod (mg)	0.44286	0.395	0.38	0.354								
# Amphipods Alive - Day 42					6	8	6	6	7	9	7	7
Percent Survival - Day 42					60	80	60	60	70	90	70	70
# Female Amphipods					4	5	2	2	4	4	6	1
# Male Amphipods					2	3	4	4	3	5	1	6
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					0	7	1	0	0	0	6	0
# Offspring - Day 42					27	1	1	8	7	11	37	0
# Offspring/Female					6.75	1.6	1	4	1.75	2.75	7.167	0

**Station:** S372  
**Sample ID:** TX0010

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	9	9	10	10	10	10	10	8	10	9	10	10
Percent Survival - Day 28	90	90	100	100	100	100	100	80	100	90	100	100
Tare Wt (g)	1.06356	1.04245	1.047	1.01677								
Tin + Dry amphipods (g)	1.07051	1.05052	1.05625	1.02449								
Dry Wt per Amphipod (mg)	0.77222	0.89667	0.925	0.772								
# Amphipods Alive - Day 42					10	10	9	7	9	9	10	8
Percent Survival - Day 42					100	100	90	70	90	90	100	80
# Female Amphipods					1	7	5	3	3	4	3	2
# Male Amphipods					9	3	4	4	6	5	6	6
# Immature Amphipods					0	0	0	0	0	0	1	0
# Offspring - Day 28					0	0	29	16	0	13	5	2
# Offspring - Day 35					0	0	38	2	14	8	29	0
# Offspring - Day 42					0	89	12	31	14	41	8	14
# Offspring/Female					0	12.71	15.8	16.33	9.333	15.5	14	8

**Station:** S305  
**Sample ID:** TX0011

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	9	10	10	6	9	10	9	10	9	9	8
Percent Survival - Day 28	100	90	100	100	60	90	100	90	100	90	90	80
Tare Wt (g)	1.02858	1.07081	1.0631	1.06488								
Tin + Dry amphipods (g)	1.03248	1.07422	1.0671	1.06958								
Dry Wt per Amphipod (mg)	0.39	0.37889	0.4	0.47								
# Amphipods Alive - Day 42					5	9	8	9	10	9	9	8
Percent Survival - Day 42					50	90	80	90	100	90	90	80
# Female Amphipods					2	3	4	4	4	5	5	4
# Male Amphipods					3	6	4	5	6	4	4	4
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					5	12	8	1	12	10	24	5
# Offspring - Day 42					9	4	5	14	4	11	7	13
# Offspring/Female					7	5.333	3.25	3.75	4	4.2	6.2	4.5



**Station:** S302  
**Sample ID:** TX0012

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	9	9	9	10	10	10	8	10	8	9	7
Percent Survival - Day 28	100	90	90	90	100	100	100	80	100	80	90	70
Tare Wt (g)	1.03934	1.0393	1.0284	1.02275								
Tin + Dry amphipods (g)	1.0454	1.04413	1.03205	1.0276								
Dry Wt per Amphipod (mg)	0.606	0.53667	0.40556	0.53889								
# Amphipods Alive - Day 42					10	8	10	7	9	8	9	7
Percent Survival - Day 42					100	80	100	70	90	80	90	70
# Female Amphipods					5	3	6	2	5	4	5	5
# Male Amphipods					5	5	4	5	4	4	4	2
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					22	3	15	13	15	13	7	15
# Offspring - Day 42					15	20	28	0	24	15	22	30
# Offspring/Female					7.4	7.667	7.167	6.5	7.8	7	5.8	9

**Station:** S303  
**Sample ID:** TX0013

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	10	10	10	9	8	9	10	10	10	10	9
Percent Survival - Day 28	100	100	100	100	90	80	90	100	100	100	100	90
Tare Wt (g)	1.04784	1.03531	1.06301	1.05525								
Tin + Dry amphipods (g)	1.05444	1.04287	1.07003	1.0626								
Dry Wt per Amphipod (mg)	0.66	0.756	0.702	0.735								
# Amphipods Alive - Day 42					9	8	8	9	10	10	9	9
Percent Survival - Day 42					90	80	80	90	100	100	90	90
# Female Amphipods					4	5	4	2	5	3	3	3
# Male Amphipods					5	3	4	6	5	6	6	6
# Immature Amphipods					0	0	0	0	0	1	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					18	14	16	0	14	28	6	15
# Offspring - Day 42					25	22	27	23	22	22	36	15
# Offspring/Female					10.75	7.2	10.75	11.5	7.2	16.67	14	10

**Station:** S323  
**Sample ID:** TX0014

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	8	9	7	9	9	8	9	9	9	10	8	10
Percent Survival - Day 28	80	90	70	90	90	80	90	90	90	100	80	100
Tare Wt (g)	1.05459	1.04103	1.03845	1.03838								
Tin + Dry amphipods (g)	1.05701	1.04331	1.04043	1.04165								
Dry Wt per Amphipod (mg)	0.3025	0.25333	0.28286	0.36333								
# Amphipods Alive - Day 42					9	8	9	9	9	10	8	10
Percent Survival - Day 42					90	80	90	90	90	100	80	100
# Female Amphipods					5	3	6	4	5	5	4	7
# Male Amphipods					0	5	1	5	4	5	4	3
# Immature Amphipods					1	0	1	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					7	2	1	2	2	0	15	3
# Offspring - Day 42					28	0	18	19	8	15	0	14
# Offspring/Female					7	0.667	3.167	5.25	2	3	3.75	2.429

**Station:** S320  
**Sample ID:** TX0015

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	8	8	10	10	9	10	8	7	9	9	10
Percent Survival - Day 28	100	80	80	100	100	90	100	80	70	90	90	100
Tare Wt (g)	1.03319	1.04865	1.02636	1.02785								
Tin + Dry amphipods (g)	1.0359	1.05217	1.02909	1.03073								
Dry Wt per Amphipod (mg)	0.271	0.44	0.34125	0.288								
# Amphipods Alive - Day 42					10	9	10	8	7	9	9	10
Percent Survival - Day 42					100	90	100	80	70	90	90	100
# Female Amphipods					6	5	6	5	5	8	6	5
# Male Amphipods					4	4	4	3	2	3	3	3
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					3	11	3	2	13	19	6	1
# Offspring - Day 42					23	31	20	15	6	40	17	14
# Offspring/Female					4.333	8.4	3.833	3.4	3.8	7.375	3.833	3

**Station:** S365  
**Sample ID:** TX0016

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	3	4	2	2	3	1	9	8	7	4	4	5
Percent Survival - Day 28	30	40	20	20	30	10	90	80	70	40	40	50
Tare Wt (g)	1.05734	1.06283	1.05842	1.06071								
Tin + Dry amphipods (g)	1.05838	1.06363	1.05906	1.06187								
Dry Wt per Amphipod (mg)	0.34667	0.2	0.32	0.58								
# Amphipods Alive - Day 42					2	1	7	7	7	3	3	5
Percent Survival - Day 42					20	10	70	70	70	30	30	50
# Female Amphipods					0	1	3	5	0	0	0	2
# Male Amphipods					2	0	4	2	7	3	3	2
# Immature Amphipods					0	0	0	0	0	0	0	0
# Offspring - Day 28					0	0	0	0	0	0	0	0
# Offspring - Day 35					0	0	7	2	0	0	0	2
# Offspring - Day 42					0	0	0	8	0	0	0	6
# Offspring/Female							2.333	2				4

**Station:** OT-7 (Reference area)  
**Sample ID:** TX0017

Test Endpoint	Replicate Test Vessel											
	C	G	K	L	A	B	D	E	F	H	I	J
# Amphipods Alive - Day 28	10	10	10	10	10	10	10	10	10	10	8	10
Percent Survival - Day 28	100	100	100	100	100	100	100	100	100	100	80	100
Tare Wt (g)	1.04455	1.04838	1.04444	1.04479								
Tin + Dry amphipods (g)	1.04895	1.05095	1.0487	1.04927								
Dry Wt per Amphipod (mg)	0.44	0.257	0.426	0.448								
# Amphipods Alive - Day 42					10	10	10	3	9	9	8	10
Percent Survival - Day 42					100	100	100	30	90	90	80	100
# Female Amphipods					5	6	7	3	4	5	5	7
# Male Amphipods					5	4	3	0	4	4	2	3
# Immature Amphipods					0	0	0	0	1	0	0	0
# Offspring - Day 28					10	0	0	7	0	0	0	0
# Offspring - Day 35					22	14	1	7	2	9	22	42
# Offspring - Day 42					17	38	4	0	0	9	13	17
# Offspring/Female					9.8	8.667	0.714	4.667	0.5	3.6	7	8.429

# Day 20: Chironomid Survival and Ash-Free Dry Weight

Station	Sample ID	Rep.	Number of		Number of	Percent	Tin +	Tin +	Ash-Free
			Chironomid	Chironomid			Chironomid Dry	Chironomid	
			Larvae	Pupae	Chironomid	Survival	Weight (g)	Ashed Weight (g)	Dry Weight (mg)
Lab Control	Lab Control	b	9	1	0	83	0.96024	0.95556	0.5200
		d	12	0	0	100	0.93266	0.92721	0.4542
		h	8	2	2	100	1.05193	1.04761	0.5400
		I	7	3	2	100	1.09003	1.08647	0.5086
OT-6	TX0001	b	9	0	0	75	0.93765	0.93191	0.6378
		d	10	0	0	83	0.94135	0.93532	0.6030
		h	2	7	2	92	0.92837	0.9267	0.8350
		I	6	1	0	58	0.94595	0.93988	1.0117
S344	TX0002	b	0	0	0	0	--	--	--
		d	0	0	0	0	--	--	--
		h	0	0	0	0	--	--	--
		I	0	0	0	0	--	--	--
S342	TX0003	b	0	0	0	0	--	--	--
		d	0	0	0	0	--	--	--
		h	0	0	0	0	--	--	--
		I	0	0	0	0	--	--	--
S355	TX0004	b	0	3	5	67	--	--	--
		d	2	0	6	67	0.94188	0.9398	1.0400
		h	0	5	2	58	--	--	--
		I	7	4	0	92	0.95667	0.9498	0.9814
S354	TX0005	b	6	1	2	75	0.9458	0.9398	1.0000
		d	3	2	4	75	0.94074	0.93931	0.4767
		h	4	1	7	100	0.94884	0.94511	0.9325
		I	8	1	2	92	0.95563	0.95205	0.4475
S337	TX0006	b	0	0	0	0	--	--	--
		d	0	0	0	0	--	--	--
		h	0	0	0	0	--	--	--
		I	0	0	0	0	--	--	--
S332	TX0007	b	0	0	0	0	--	--	--
		d	0	0	0	0	--	--	--
		h	0	0	0	0	--	--	--
		I	0	0	0	0	--	--	--
S315	TX0008	b	1	1	4	50	0.94575	0.94522	0.5300
		d	1	1	0	17	0.9385	0.93801	0.4900
		h	9	1	1	92	0.95843	0.95539	0.3378
		I	7	1	1	75	0.9554	0.95009	0.7586
S317	TX0009	b	4	0	0	33	--	--	--
		d	8	1	0	75	0.95885	0.95556	0.4113
		h	4	0	0	33	0.92616	0.92441	0.4375
		I	12	0	0	100	0.93802	0.93524	0.2317
S372	TX0010	b	5	1	2	67	1.07318	1.07109	0.4180
		d	8	0	3	92	1.03838	1.03488	0.4375
		h	5	3	0	67	1.05739	1.05453	0.5720
		I	9	0	1	83	1.06231	1.06023	0.2311
S305	TX0011	b	10	0	0	83	1.06424	1.0556	0.8640
		d	6	2	0	67	1.05253	1.04639	1.0233
		h	5	2	0	58	1.07273	1.06791	0.9640
		I	12	0	0	100	1.06903	1.05779	0.9367

## Day 20: Chironomid Survival and Ash-Free Dry Weight

Station	Sample ID	Rep.	Number of		Number of	Percent	Tin +	Tin +	Ash-Free
			Chironomid	Chironomid			Chironomid Dry	Chironomid	
			Larvae	Pupae	Chironomid	Survival	Weight	Ashed Weight	Dry Weight
							(g)	(g)	(mg)
S302	TX0012	b	10	0	0	83	1.07448	1.06878	0.5700
		d	7	0	0	58	1.06171	1.05779	0.5600
		h	9	0	1	83	1.07272	1.06466	0.8956
		I	4	1	0	42	1.02933	1.02624	0.7725
S303	TX0013	b	2	2	3	58	1.04873	1.04621	1.2600
		d	1	3	3	58	1.05612	1.05562	0.5000
		h	4	3	0	58	1.064	1.0599	1.0250
		I	5	2	2	75	1.0694	1.06439	1.0020
S323	TX0014	b	0	1	0	8	--	--	--
		d	1	0	0	8	1.05735	1.05724	0.1100
		h	3	1	0	33	1.06032	1.05997	0.1167
		I	1	1	0	17	1.0224	1.02201	0.3900
S320	TX0015	b	11	0	0	92	1.08182	1.07524	0.5982
		d	3	0	0	25	1.09675	1.09448	0.7567
		h	8	1	0	75	1.07541	1.07131	0.5125
		I	2	3	0	42	1.06125	1.0598	0.7250
S365	TX0016	b	8	0	0	67	1.04205	1.04009	0.2450
		d	6	0	0	50	1.07323	1.07288	0.0583
		h	4	0	0	33	1.05787	1.05692	0.2375
		I	4	0	0	33	1.07595	1.07429	0.4150
OT-7	TX0017	b	7	3	0	83	1.05101	1.04535	0.8086
		d	12	0	0	100	1.05331	1.04538	0.6608
		h	11	0	0	92	1.05706	1.05068	0.5800
		I	9	1	0	83	1.06744	1.06151	0.6589

Sample ID: Lab Control

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	2	1	1	0	0	1	0	0	38	19	19	0	0	19	0	0
20	0	0	1	2	0	0	1	0	0	0	20	40	0	0	20	0
21	0	1	1	0	0	1	0	0	0	21	21	0	0	21	0	0
22	0	0	1	1	1	0	0	0	0	0	22	22	22	0	0	0
23	3	1	1	0	0	0	1	0	69	23	23	0	0	0	23	0
24	2	0	0	0	0	1	0	0	48	0	0	0	0	24	0	0
25	0	1	0	0	1	2	0	0	0	25	0	0	25	50	0	0
26	1	1	0	0	0	1	0	1	26	26	0	0	0	26	0	26
27	0	1	1	1	2	1	1	1	0	27	27	27	54	27	27	27
28	2	1	2	0	1	2	0	0	56	28	56	0	28	56	0	0
29	0	0	1	0	0	2	1	2	0	0	29	0	0	58	29	58
30	0	1	0	1	1	0	0	1	0	30	0	30	30	0	0	30
31	0	1	0	1	0	0	1	1	0	31	0	31	0	0	31	31
32	0	0	0	0	1	0	1	0	0	0	0	0	32	0	32	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	1	0	0	0	0	0	0	0	34	0
35	0	0	0	0	1	0	1	0	0	0	0	0	35	0	35	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	38
39	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	78
40	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	40
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	10	9	9	6	8	11	8	10	Weighted Mean Time							
% emergence:	83	75	75	50	67	92	67	83	to Emergence:							
Mean % Emergence:	74								Mean time to Emergence:							
									27							

Station: OT-6 (Reference area)  
Sample ID: TX0001

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	3	0	1	0	0	0	0	0	60	0	20	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	1	1	3	2	2	0	0	0	22	22	66	44	44	0	0	0
23	0	1	1	0	1	0	0	0	0	23	23	0	23	0	0	0
24	0	0	0	0	1	1	0	1	0	0	0	0	24	24	0	24
25	0	0	0	0	0	2	0	0	0	0	0	0	0	50	0	0
26	2	0	1	1	0	1	0	1	52	0	26	26	0	26	0	26
27	0	2	0	1	0	0	0	1	0	54	0	27	0	0	0	27
28	0	1	0	0	0	0	0	0	0	28	0	0	0	0	0	0
29	0	1	0	2	2	1	0	1	0	29	0	58	58	29	0	29
30	0	0	0	0	0	0	1	0	0	0	0	0	0	0	30	0
31	0	0	0	0	0	1	1	1	0	0	0	0	0	31	31	31
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	1	0	0	0	0	0	0	0	33	0	0	0	0	0	0
34	0	1	0	0	0	0	0	0	0	34	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	1	0	0	0	0	0	0	0	37	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	1	0	0	0	0	0	0	0	40	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	3	8	8	6	7	6	4	5	Weighted Mean Time							
% emergence:	25	67	67	50	58	50	33	42	to Emergence:							
Mean % Emergence:	49								Mean time to Emergence:							
									27							

Station: S355  
Sample ID: TX0004

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	2	2	2	0	0	0	0	0	38	38	38
20	4	3	6	0	2	1	0	1	80	60	120	0	40	20	0	20
21	2	2	2	6	3	0	1	0	42	42	42	126	63	0	21	0
22	0	1	0	0	0	1	1	3	0	22	0	0	0	22	22	66
23	0	0	0	0	0	1	0	1	0	0	0	0	0	23	0	23
24	0	0	0	1	0	0	0	0	0	0	0	24	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	1	1	0	1	0	0	0	0	26	26	0	26
27	0	0	0	0	0	0	1	0	0	0	0	0	0	0	27	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	1	1	0	0	0	0	0	0	29	29	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	31
32	0	0	0	0	0	0	1	0	0	0	0	0	0	0	32	0
33	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	33
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	6	6	9	8	6	6	6	10	Weighted Mean Time							
% emergence:	50	50	75	67	50	50	50	83	to Emergence:							
Mean % Emergence:	59								Mean time to Emergence:							
									22							

Station: S354  
Sample ID: TX0005

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	1	1	1	1	1	2	0	2	19	19	19	19	19	38	0	38
20	1	2	1	1	1	2	0	0	20	40	20	20	20	40	0	0
21	0	0	0	1	0	1	2	1	0	0	0	21	0	21	42	21
22	1	0	3	0	1	2	0	1	22	0	66	0	22	44	0	22
23	1	2	0	0	0	2	0	1	23	46	0	0	0	46	0	23
24	1	1	1	1	0	0	1	1	24	24	24	24	0	0	24	24
25	0	0	0	0	1	1	1	0	0	0	0	0	25	25	25	0
26	0	3	1	0	1	1	0	2	0	78	26	0	26	26	0	52
27	0	0	1	0	1	1	0	0	0	0	27	0	27	27	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	1	1	1	0	0	0	0	0	29	29	29	0	0	0
30	3	0	0	3	0	0	0	0	90	0	0	90	0	0	0	0
31	0	0	0	0	1	1	0	0	0	0	0	0	31	31	0	0
32	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	32
33	0	0	0	0	0	1	0	0	0	0	0	0	0	33	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	8	9	9	8	8	14	4	9	Weighted Mean Time							
% emergence:	67	75	75	67	67	100	33	75	to Emergence:							
Mean % Emergence:	70								Mean time to Emergence:							
									24							

Station: S315  
Sample ID: TX0008

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	2	1	0	0	0	0	1	1	40	20	0	0	0	0	20	20
21	1	1	0	1	0	2	1	1	21	21	0	21	0	42	21	21
22	1	0	0	2	2	3	2	1	22	0	0	44	44	66	44	22
23	0	1	1	0	0	1	0	0	0	23	23	0	0	23	0	0
24	0	0	1	0	1	1	0	1	0	0	24	0	24	24	0	24
25	0	0	0	0	1	0	0	0	0	0	0	0	25	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	2	1	0	0	0	0	0	0	54	27
28	0	0	1	0	0	0	0	1	0	0	28	0	0	0	0	28
29	0	0	0	0	0	0	0	2	0	0	0	0	0	0	58	58
30	0	0	0	0	0	1	2	0	0	0	0	0	0	30	60	0
31	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	31
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	1	0	0	0	0	0	0	0	34	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	1	0	0	0	0	0	0	0	39	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	4	3	3	3	6	8	10	9	Weighted Mean Time							
% emergence:	33	25	25	25	50	67	83	75	to Emergence:							
Mean %									Mean time to							
Emergence:	48								Emergence:							
									20.8 21.3 25 21.7 27.7 23.1 25.7 25.7							

Station: S317  
Sample ID: TX0009

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel								
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
20	0	0	0	1	0	0	0	0	0	0	0	20	0	0	0	0	
21	1	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0	
22	1	0	0	0	0	0	0	1	22	0	0	0	0	0	0	22	
23	0	0	1	0	0	0	0	1	0	0	23	0	0	0	0	23	
24	1	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
26	1	0	0	2	0	0	0	0	26	0	0	52	0	0	0	0	
27	0	1	0	0	0	0	0	0	0	27	0	0	0	0	0	0	
28	0	0	1	1	1	2	0	0	0	0	28	28	28	56	0	0	
29	0	0	1	0	1	1	0	0	0	0	29	0	29	29	0	0	
30	0	0	1	0	0	0	0	0	0	0	30	0	0	0	0	0	
31	1	1	0	0	0	0	0	0	31	31	0	0	0	0	0	0	
32	0	0	1	0	0	0	0	0	0	0	32	0	0	0	0	0	
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
34	0	0	0	0	1	0	0	0	0	0	0	0	34	0	0	0	
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
36	0	0	0	0	0	0	1	0	0	0	0	0	0	0	36	0	
37	0	1	0	0	0	0	0	0	0	37	0	0	0	0	0	0	
38	0	0	1	1	0	0	0	0	0	0	38	38	0	0	0	0	
39	0	0	0	1	0	0	0	1	0	0	0	39	0	0	0	39	
40	0	2	0	1	0	0	0	0	0	80	0	40	0	0	0	0	
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total emerged:	5	5	6	7	3	3	1	3	Weighted Mean Time								
% emergence:	42	42	50	58	25	25	8	25	to Emergence:								
Mean %									Mean time to								
Emergence:	34									Emergence:							
									24.8	35	30	31	30.3	28.3	36	28	



Station: S372  
Sample ID: TX0010

Test Day	Number of Chironomid Emerged per Vessel									Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l		a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
19	0	0	1	0	1	2	1	0		0	0	19	0	19	38	19	0
20	0	0	0	0	2	1	0	0		0	0	0	0	40	20	0	0
21	3	1	1	1	0	0	0	0		63	21	21	21	0	0	0	0
22	1	1	1	0	2	0	0	1		22	22	22	0	44	0	0	22
23	1	1	1	0	0	0	1	1		23	23	23	0	0	0	23	23
24	2	0	1	1	0	1	0	1		48	0	24	24	0	24	0	24
25	0	0	1	0	0	0	0	0		0	0	25	0	0	0	0	0
26	0	0	1	0	2	0	0	0		0	0	26	0	52	0	0	0
27	1	0	1	0	0	0	0	0		27	0	27	0	0	0	0	0
28	0	0	1	0	0	0	0	0		0	0	28	0	0	0	0	0
29	0	1	2	0	3	2	0	1		0	29	58	0	87	58	0	29
30	0	1	0	0	0	1	0	1		0	30	0	0	0	30	0	30
31	0	0	0	0	0	0	1	0		0	0	0	0	0	0	31	0
32	1	0	0	0	1	0	0	0		32	0	0	0	32	0	0	0
33	0	1	0	0	0	0	1	0		0	33	0	0	0	0	33	0
34	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
35	0	0	0	0	1	0	0	0		0	0	0	0	35	0	0	0
36	0	0	0	1	0	1	2	0		0	0	0	36	0	36	72	0
37	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
39	0	0	0	1	0	0	0	0		0	0	0	39	0	0	0	0
40	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0
Total emerged:	9	6	11	4	12	8	6	5	Weighted Mean Time to Emergence:	23.9	26.3	24.8	30	25.8	25.8	29.7	25.6
% emergence:	75	50	92	33	100	67	50	42									
Mean % Emergence:	64								Mean time to Emergence:	26							

Station: S305  
Sample ID: TX0011

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	1	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0
22	0	0	1	0	0	0	1	0	0	0	22	0	0	0	22	0
23	0	0	0	0	1	1	1	0	0	0	0	0	23	23	23	0
24	3	2	0	0	1	0	0	0	72	48	0	0	24	0	0	0
25	1	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0
26	0	0	0	0	1	0	1	0	0	0	0	0	26	0	26	0
27	1	0	0	0	0	0	1	1	27	0	0	0	0	0	27	27
28	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	56
29	0	1	1	0	0	1	1	2	0	29	29	0	0	29	29	58
30	0	2	0	2	0	1	0	0	0	60	0	60	0	30	0	0
31	0	0	1	1	0	1	0	0	0	0	31	31	0	31	0	0
32	1	0	0	0	1	2	0	0	32	0	0	0	32	64	0	0
33	0	2	0	1	0	1	0	0	0	66	0	33	0	33	0	0
34	0	0	2	2	0	1	0	0	0	0	68	68	0	34	0	0
35	0	0	0	1	0	0	0	0	0	0	0	35	0	0	0	0
36	0	0	1	0	0	0	0	0	0	0	36	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	1	1	0	0	0	0	0	0	39	39	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	7	8	7	7	4	8	5	5	Weighted Mean Time to Emergence:							
% emergence:	58	67	58	58	33	67	42	42		25.3	30.3	32.1	32.4	26.3	30.5	25.4
Mean % Emergence:	53								Mean time to Emergence:	29						

Station: S302  
Sample ID: TX0012

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	4	0	0	0	0	0	0	0	80	0	0	0	0
21	3	0	2	0	0	1	1	0	63	0	42	0	0	21	21	0
22	0	0	2	2	0	0	1	0	0	0	44	44	0	0	22	0
23	0	1	0	2	1	0	0	1	0	23	0	46	23	0	0	23
24	0	0	0	0	1	2	1	1	0	0	0	0	24	48	24	24
25	0	0	1	1	0	2	0	0	0	0	25	25	0	50	0	0
26	0	0	0	0	0	1	0	0	0	0	0	0	0	26	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	1	0	2	0	0	0	0	0	28	0	56
29	0	0	0	0	2	0	2	0	0	0	0	0	58	0	58	0
30	0	0	0	0	0	1	0	1	0	0	0	0	0	30	0	30
31	1	0	0	0	0	0	0	1	31	0	0	0	0	0	0	31
32	0	0	0	0	0	0	1	0	0	0	0	0	0	0	32	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	1	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0
35	1	0	0	0	0	0	0	0	35	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	36
37	0	0	0	0	0	0	1	0	0	0	0	0	0	0	37	0
38	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	38
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	1	0	0	0	0	0	0	0	42	0	0	0	0	0	0
Total emerged:	6	2	5	9	4	8	7	8	Weighted Mean Time							
% emergence:	50	17	42	75	33	67	58	67	to Emergence: 27.2 32.5 22.2 21.7 26.3 25.4 27.7 29.8							
Mean %									Mean time to							
Emergence:	51								Emergence: 27							

Station: S303  
Sample ID: TX0013

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	2	0	2	2	2	1	5	0	38	0	38	38	38	19	95	0
20	2	0	1	2	1	2	1	2	40	0	20	40	20	40	20	40
21	2	1	1	1	0	0	0	0	42	21	21	21	0	0	0	0
22	1	1	0	0	0	0	0	0	22	22	0	0	0	0	0	0
23	0	1	0	0	1	0	0	0	0	23	0	0	23	0	0	0
24	0	3	1	0	0	0	0	0	0	72	24	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	1	1	0	0	0	0	0	0	26	26	0	0
27	1	0	0	0	0	0	0	0	27	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	3	0	0	0	0	0	0	0	87	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	1	0	0	0	0	0	0	0	42	0	0	0
Total emerged:	8	6	5	5	6	7	6	2	Weighted Mean Time							
% emergence:	67	50	42	42	50	58	50	17	to Emergence: 21.1 23 20.6 19.8 24.8 24.6 19.2 20							
Mean %									Mean time to							
Emergence:	47								Emergence: 22							

Station: S323  
Sample ID: TX0014

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	1	0	0	0	0	0	0	0	23	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	1	0	0	0	0	0	0	0	31	0	0	0	0	0
32	0	0	0	1	1	0	0	0	0	0	0	32	32	0	0	0
33	0	0	1	0	0	0	0	0	0	0	33	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	1	0	0	0	0	0	0	0	38	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	0	0	3	2	1	0	0	0	Weighted Mean Time							
% emergence:	0	0	25	17	8	0	0	0	to Emergence:							
Mean % Emergence:	6								Mean time to Emergence:							
									32							

Station: S320  
Sample ID: TX0015

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel							
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	1	0	0	0	1	0	0	0	22	0	0	0	22	0	0
23	0	0	0	1	0	0	0	1	0	0	0	23	0	0	0	23
24	0	0	0	1	0	1	0	0	0	0	0	24	0	24	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0	1	0	1	0	0	1	0	0	26	0	26	0	0	26	0
27	0	0	0	1	1	0	0	0	0	0	0	27	27	0	0	0
28	0	0	0	0	0	1	0	0	0	0	0	0	0	28	0	0
29	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	58
30	0	0	0	0	1	0	0	0	0	0	0	0	30	0	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	32
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	1	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	1	0	0	0	0	0	0	0	38	0	0
39	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	39
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total emerged:	1	2	0	4	2	4	1	5	Weighted Mean Time							
% emergence:	8	17	0	33	17	33	8	42	to Emergence:							
Mean % Emergence:	20								Mean time to Emergence:							
									28							

Station: S365  
Sample ID: TX0016

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel									
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l		
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
22	0	0	0	0	1	0	0	0	0	0	0	0	22	0	0	0		
23	0	0	0	0	1	0	0	0	0	0	0	0	23	0	0	0		
24	0	0	1	0	0	0	0	0	0	0	24	0	0	0	0	0		
25	0	0	0	0	1	0	0	0	0	0	0	0	25	0	0	0		
26	0	0	0	1	0	0	0	0	0	0	0	26	0	0	0	0		
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
28	2	2	1	0	0	0	1	0	56	56	28	0	0	0	28	0		
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
30	0	0	1	0	0	0	0	0	0	0	30	0	0	0	0	0		
31	0	0	1	0	0	0	0	0	0	0	31	0	0	0	0	0		
32	0	0	0	0	0	0	1	0	0	0	0	0	0	0	32	0		
33	0	1	0	0	0	0	0	1	0	33	0	0	0	0	0	33		
34	0	0	0	0	0	2	0	0	0	0	0	0	0	68	0	0		
35	0	0	0	1	0	0	0	0	0	0	0	35	0	0	0	0		
36	1	0	0	0	0	1	0	0	36	0	0	0	0	36	0	0		
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
39	0	0	0	1	1	0	0	0	0	0	0	39	39	0	0	0		
40	0	1	0	0	0	0	0	0	0	40	0	0	0	0	0	0		
41	0	0	1	0	0	0	0	0	0	0	41	0	0	0	0	0		
42	0	1	1	0	0	0	0	0	0	42	42	0	0	0	0	0		
Total emerged:	3	5	6	3	4	3	2	1	Weighted Mean Time to Emergence:	30.7	34.2	32.7	33.3	27.3	34.7	30	33	
% emergence:	25	42	50	25	33	25	17	8		32	36	34	33	28	35	30	33	
Mean % Emergence:	28									Mean time to Emergence:	32							

Station: OT-7 (Reference area)  
Sample ID: TX0017

Test Day	Number of Chironomid Emerged per Vessel								Time to Emergence per Vessel									
	a	c	e	f	g	j	k	l	a	c	e	f	g	j	k	l		
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
23	0	0	0	3	0	1	0	0	0	0	0	69	0	23	0	0		
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
25	0	0	0	0	0	0	1	0	0	0	0	0	0	0	25	0		
26	1	2	0	0	0	1	1	1	26	52	0	0	0	26	26	26		
27	1	2	1	1	0	1	0	0	27	54	27	27	0	27	0	0		
28	1	2	0	0	0	2	1	0	28	56	0	0	0	56	28	0		
29	1	0	3	0	0	0	1	1	29	0	87	0	0	0	29	29		
30	0	0	0	0	0	0	1	2	0	0	0	0	0	0	30	60		
31	1	0	0	0	0	0	1	0	31	0	0	0	0	0	31	0		
32	0	2	0	1	0	0	0	1	0	64	0	32	0	0	0	32		
33	0	0	0	1	1	0	0	0	0	0	0	33	33	0	0	0		
34	1	2	0	0	0	0	0	0	34	68	0	0	0	0	0	0		
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
36	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	36		
37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
40	0	0	0	0	2	0	0	0	0	0	0	0	80	0	0	0		
41	0	0	0	0	2	0	0	1	0	0	0	0	82	0	0	41		
42	0	0	0	0	1	0	0	0	0	0	0	0	42	0	0	0		
Total emerged:	6	10	4	6	6	5	6	7	Weighted Mean Time to Emergence:	29.2	29.4	28.5	26.8	39.5	26.4	28.2	32	
% emergence:	50	83	33	50	50	42	50	58		29.2	29.4	28.5	26.8	39.5	26.4	28.2	32	
Mean % Emergence:	52									Mean time to Emergence:	30							

## **Appendix C2**

### **QA/QC Evaluation of Onondaga Lake 2000 Toxicity Test Data**

## Introduction

This report documents the results of the quality assurance and quality control (QA/QC) review of the data generated for the 42-day amphipod and 42-day chironomid toxicity tests that were performed in 2000 on sediment from 15 stations at Onondaga Lake and from two stations at Otisco Lake (reference area). These tests were conducted by Springborn Laboratories in Wareham, Massachusetts. The quality assurance review was conducted by Exponent to ensure that the toxicity testing was consistent with the specifications of the statement of work and that the data are acceptable for use in future stages of the study.

The quality assurance review consisted of an evaluation of the following major elements for each of the two toxicity tests:

- **Field Methods** – Were the major specifications of the field sampling procedures followed, as described in the work plan (Exponent, 2000)?
- **Laboratory System and Testing Methods** – Were the major specifications of the laboratory testing procedures followed, as described in the statement of work? Were the specified methods (i.e., USEPA, 2000) followed, and were any modifications adequately justified and documented?
- **Sediment Holding Time** – Was each sediment sample analyzed within the specified holding time after collection?
- **Water Quality Conditions** – Were water quality conditions monitored adequately during testing, and were the measured conditions within the specified ranges for each test chamber?
- **Negative Control Responses** – Were the responses in the negative controls (i.e., clean seawater) within specified limits?
- **Positive Control Responses** – Did the positive controls (i.e., reference toxicant) indicate that the test organisms were suitably responsive for testing?

Throughout this report, the term “sample” refers to the whole sediment sample collected from each station in the field for each kind of toxicity test. The term “replicate” refers to one of the eight subsamples of each sediment sample collected in the field that was subjected to toxicity testing in the laboratory. In the data tables in this appendix, the eight replicates for each sample are distinguished by the letters A–L.

The following section of this report presents the results of the QA/QC evaluation for the toxicity tests. QA/QC considerations are then summarized, and conclusions are presented in the final section.

# Quality Assurance and Quality Control Evaluation

## Field Methods

From August 9 to 14, 2000, 15 composite sediment samples were collected from Onondaga Lake and two composite sediment samples were collected at Otisco Lake (reference area). Sediment sampling was conducted according to the procedures and plans described in the work plan (Exponent, 2000), with one exception. After repeated attempts, due to a hard layer of sand encountered at approximately 13 cm, the full penetration of 0–15 cm could not be attained at Station S372, so the 0–13 cm interval was collected.

## Laboratory System

Water from a 100-m bedrock well was pumped to a concrete reservoir, where it was supplemented on demand with untreated, unchlorinated city well-water. The water was characterized with a normal pH range of 6.9–7.7, a total hardness of 40–60 mg/L, and a specific conductance of 110–160  $\mu\text{mhos/cm}$ . The pH, total hardness, alkalinity, and specific conductance of this water was monitored weekly at a central location in the laboratory. The quality of the water was judged by periodic analyses of representative samples conducted to ensure the absence of potential toxicants, including pesticides, polychlorinated biphenyls, and selected metals.

All testing was conducted according to the good laboratory practices described in ASTM (1995).

## 42-Day Amphipod Toxicity Test Using *Hyaella azteca*

The amphipod toxicity test using *Hyaella azteca* determines percent survival, growth, and reproduction in adult amphipods exposed for 42 days to test sediment. Amphipods are exposed to the sediment sample to assess survival and growth on test day 28 and reproduction on test day 42 (i.e., test termination).

## Test Organism Culturing, Holding, and Acclimation

*H. azteca* were cultured at Springborn Laboratories. Culture conditions were maintained according to suggested USEPA methods (USEPA, 2000). Amphipods were cultured in 20-L glass aquaria (containing approximately 10-L of culture water) under flow-through conditions. Water used to culture the amphipods was similar to the overlying water used during the 42-day test. The culture-water temperature was maintained at  $23 \pm 1^\circ\text{C}$ . While being maintained in the culture, prior to test initiation, the amphipods were fed once a day. The amphipods were fed a mixture of yeast, Cerophyl®, flaked fish-food suspension (YCT), and a unicellular green algae (*Ankistrodesmus falcatus*).

To obtain amphipods of a suitable age for testing, approximately seven to eight days prior to test initiation, adult amphipods were removed from the culture tanks and placed into 9.5-L aquaria with approximately 8-L of water. Young produced by these isolated adults were then removed and pipetted into a holding container until test initiation. Amphipods were not used if greater than 5 percent mortality was observed

in the holding containers during the 48 hours prior to test initiation. Test organisms were seven to eight days old at test initiation.

### ***Test Methods***

Overall, the recommended protocols were followed closely during testing. All biological testing was in compliance with USEPA (2000). Samples were collected and stored properly. The 42-day amphipod toxicity test was initiated on August 22, 2000, which was within the specified 14-day holding time.

Juvenile organisms seven days old were used for the test. All organisms used in the test were from the same source (see above discussion). The day before test initiation, thoroughly homogenized test sediment, reference sediment, and laboratory control sediment (i.e., negative control) were added to the replicate test vessels and the overlying water was added.

For each toxicity test replicate, 10 amphipods were exposed to 100 mL of bedded test sediment in a 300-mL chamber filled with 175 mL of acceptable overlying water from Day 0 to Day 28 (175 to 275 mL in the water-only exposure from Day 28 to Day 42). Twelve replicate analyses were conducted for each sediment sample. Four replicates were for the 28-day survival and growth endpoint and eight replicates were for the 35- and 42-day survival, growth, and reproduction endpoint. Overlying water was renewed at a rate of two volume additions per day (continuous or intermittent) throughout the exposure period. The amphipods were fed daily.

On Day 28, the surviving amphipods in four of the test chambers were sieved from the sediment and counted and weighed. Percent survival was determined relative to the total of 10 individuals added to each chamber at the beginning of the test. On Day 28, the remaining eight beakers/sediment were also sieved, and the surviving amphipods in each sediment beaker were placed in 300-mL water-only beakers containing 275 mL of overlying water and a 3-cm × 3-cm piece of Nitex screen used in culturing amphipods. Reproduction of amphipods was measured on Day 35 and Day 42 in the water-only beakers by removing and counting the adults and young in each beaker. On Day 35, the adults were then returned to the same water-only beakers. Adult amphipods surviving on Day 42 were preserved in sugar formalin. The number of adult females was determined by counting the adult males (mature male amphipods have an enlarged second gnathopod) and assuming all other adults were females. The number of females was used to determine number of young/female/beaker from Day 28 to Day 42.

Endpoints measured in the 42-day *H. azteca* test were survival (Days 28, 35, and 42), growth (as dry weight on Days 28 and 42), and reproduction (number of young/female produced from Day 28 to 42).

### ***Water Quality Measurements***

Water-quality monitoring was conducted during the amphipod toxicity test. Using a pipette, measurements of the overlying water were taken just prior to the introduction of the test organisms to the test chambers, then at the same time each day (just prior to water renewal), until the conclusion of the test. The pipette was checked to make sure no organisms were removed during sampling of overlying water. Water quality was



measured for each batch of water prepared for the test. This monitoring consisted of the following measurements:

- Temperature was measured daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber was continuously monitored. The daily mean test temperature must be  $23 \pm 1^{\circ}\text{C}$ . The instantaneous temperature must always be  $23 \pm 3^{\circ}\text{C}$ . Temperatures measured during the testing period ranged from  $22^{\circ}\text{C}$  to  $24^{\circ}\text{C}$ .
- Dissolved oxygen was measured in all treatments at the beginning and at the end of the sediment-exposure portion of the test, and three times per week during the test. In addition, dissolved oxygen was measured at the beginning and end of the reproductive phase (Day 29 to Day 42). Concentrations of dissolved oxygen were measured more often if dissolved oxygen had declined by more than 1 mg/L since the previous measurement. Dissolved oxygen was measured directly in the overlying water with a probe. The probe for dissolved oxygen measurements was equilibrated in the overlying water. The probe was inspected between samples to make sure that organisms were not attached, and was rinsed between samples to minimize cross-contamination. As recommended, dissolved oxygen was maintained above 2.5 mg/L in the overlying water. Dissolved oxygen concentrations were greater than or equal to 4.0 mg/L throughout the study in all control and test sediment replicates. The lowest dissolved oxygen concentration was 4.0 mg/L. The dissolved oxygen levels ranged from 4.0 to 9.8 mg/L.
- The pH of all treatments was measured at the beginning and at the end of the sediment-exposure portion of the test, and was also measured three times per week during the test. In addition, pH was measured at the beginning and end of the reproductive phase (Day 29 to Day 42). The pH was measured directly in the overlying water with a probe. The probe was inspected between samples to make sure that organisms were not attached, and was rinsed between samples to minimize cross-contamination. Values for pH ranged from 6.5 to 8.5, which is slightly outside the recommended range of 7.0 to 9.0.
- Ammonia was measured in all treatments at the beginning and at the end of the sediment-exposure portion of the test. Ammonia was also measured at the beginning and end of the reproductive phase (Day 29 to Day 42). Ammonia and sulfide were measured in the overlying water, and in the porewater (collected by centrifugation) of one replicate of each sediment sample on Days 0, 20, and 42 (i.e., at test termination). Three additional replicate vessels were established during the amphipod test to monitor ammonia and sulfide in the overlying water and ammonia in porewater. The concentration of ammonia nitrogen (ammonia-N) in the overlying water during the testing period ranged from less than 0.1 mg/L (detection limit) to 2.16 mg/L. The concentration of ammonia-N in the porewater ranged from less than 0.42 to 18.93 mg/L in the laboratory control on Day 28.

The average concentration of ammonia-N in the porewater was 4.72 mg/L. The concentration of total sulfide in the overlying water and in the porewater was less than 0.02 mg/L (detection limit).

- Conductivity, hardness, and alkalinity were measured in all treatments at the beginning and at the end of the sediment-exposure portion of the test. Conductivity, hardness, and alkalinity were also measured at the beginning and end of the reproductive phase (Day 29 to Day 42). In addition, conductivity was measured weekly during the test. Conductivity values ranged from 160 to 280  $\mu$ mhos/cm. Hardness values ranged from 48 to 100 mg/L as CaCO<sub>3</sub>. Alkalinity values ranged from 30 to 60 mg/L as CaCO<sub>3</sub>. As recommended, conductivity, hardness, and alkalinity in the overlying water did not vary by more than 50 percent during the sediment-exposure portion of the test.

## Controls

A reference sediment test (i.e., sediment from Otisco Lake, New York), conducted with 12 replicates, was used to evaluate the survival, growth, and reproduction potential of the test organisms in a non-contaminated sediment. In addition, a laboratory control sediment (i.e., negative control), prepared by the testing laboratory, was used to evaluate the survival, growth, and reproduction potential of the test organisms. The laboratory control sediment was prepared by mixing together 10 percent sphagnum peat, 20 percent kaolin clay, and 70 percent industrial sand (with greater than 50 percent of the particles between 50 and 200 microns). The negative control test was also conducted with 12 replicates. All reference sediment and negative control testing was in compliance with USEPA methods (USEPA, 2000). Performance standards developed for this test are provided in Table C2-1 and in USEPA (2000).

**Table C2-1. Performance Standards for Control and Reference Sediments Used in Amphipod Toxicity Test**

Toxicity Test	Laboratory Control Sediment Performance Standard	Reference Sediment Performance Standard <sup>a</sup>
<i>Hyalella azteca</i> (42-day)		
Survival endpoint	≤20 percent absolute mean mortality (on Day 28)	≤30 percent absolute mean mortality
Growth endpoint	≥0.15 mg per individual (dry weight)	
Reproduction endpoint	≥2 young/female	

<sup>a</sup> Reference sediment performance standards are intended to be a measure of reference sediment acceptability for regulatory decision-making (i.e., was the reference sediment toxicity sufficiently low for valid comparisons with the test sediments to support decisions regarding the possible need for remediation?). Laboratory performance and test acceptability were evaluated solely on the results of the toxicity tests using the control sediment.

Mean survival in the amphipod survival and growth test for the laboratory control sediment (i.e., negative control) was 94 percent and the mean dry weight of amphipods was 0.516 mg per amphipod. Mean survival in the amphipod survival and reproduction (number of offspring per female) test for the laboratory control sediment (i.e., negative control) was 93 percent and the mean number of offspring per female was 9.1. Mean survival for sediment from the two reference area samples was 94 and 98 percent, respectively. These results are above the performance standards set for the test and suggest that the test organisms were sufficiently healthy for testing.

A positive control was tested using potassium chloride as the reference toxicant. The positive control exhibited a 96-hour LC50 value of 370 mg KCl/L, which is within the testing laboratory's control chart warning limits (175 to 430 mg KCl/L) for this test. The observed LC50 value suggests that the test organisms were suitably sensitive for testing.

### **Chironomid Toxicity Test Using *Chironomus tentans***

The chironomid toxicity test using *Chironomus tentans* measures percent survival, growth, and emergence of larval chironomids exposed to test sediment during a 42-day exposure period.

#### ***Test Organism Culturing, Holding, and Acclimation***

*C. tentans* were cultured at Springborn Laboratories. Culture conditions were maintained according to suggested USEPA methods (USEPA, 2000). The culture temperature was maintained at  $23 \pm 1^\circ\text{C}$ . Water used to culture the chironomids was similar to the overlying water used during the 42-day test. While being maintained in the culture, prior to test initiation, the chironomids were fed once a day. The chironomids were fed a 1.5-mL suspension of finely ground, flaked fish food (4.0 mg/mL). The newly hatched larvae were collected for the toxicity test from egg masses less than 24 hours after hatching.

#### ***Test Methods***

Overall, the recommended protocols were followed closely during testing. All biological testing was in compliance with USEPA (2000). Samples were collected and stored properly. The 42-day chironomid toxicity test was initiated on August 18, 2000, which was within the specified 14-day holding time.

Less than 24-hour-old larvae were used for the test. All organisms in a test were from the same source. For each toxicity-test replicate, 12 chironomids were exposed to 100 mL of bedded test sediment in a 300-mL chamber filled with 175 mL of acceptable overlying water. Twelve replicate analyses were conducted for each sediment sample (12 replicates at Day 1, and 4 replicates for auxiliary males on Day 10). Overlying water was renewed at a rate of two volume additions per day (continuous or intermittent) throughout the 42-day exposure period. The chironomids were fed daily.

On Day 20, percent survival was determined relative to the total of 12 individuals added to each chamber at the beginning of the test for four replicates. For each of the four replicates, the biomass (i.e., ash-free dry weight) of the pooled survivors was determined to the nearest 0.01 mg, after drying to constant weight

at 60 to 90°C for 24 to 48 hours and ashing at 550°C for two hours. Pupae or adult organisms were not included in the sample to estimate dry weight. All emerged test organisms were counted at test termination.

### ***Water Quality***

Water-quality monitoring was conducted during the chironomid toxicity test. Using a pipette, measurements of the overlying water were taken just prior to the introduction of the test organisms to the test chambers, then at the same time each day (just prior to water renewal), until the conclusion of the test. The pipette was checked to make sure no organisms were removed during sampling of overlying water. Water quality was measured on each batch of water prepared for the test. This monitoring consisted of the following measurements:

- Temperature was measured daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber was continuously monitored. The daily mean test temperature must be  $23 \pm 1^\circ\text{C}$ . The instantaneous temperature must always be  $23 \pm 3^\circ\text{C}$ . Temperatures measured during the testing period ranged from  $22^\circ\text{C}$  to  $24^\circ\text{C}$ , except in samples TX009 and TX0010 on Day 39. Temperature in these two samples was measured at  $21^\circ\text{C}$  on a single occasion.
- Dissolved oxygen was measured in all treatments at the beginning and at the end of the test and three times per week during the test. Concentrations of dissolved oxygen were measured more often if dissolved oxygen had declined by more than 1 mg/L since the previous measurement. Dissolved oxygen was measured directly in the overlying water with a probe. The probe for dissolved oxygen measurements was equilibrated in the overlying water. The probe was inspected between samples to make sure that organisms were not attached, and was rinsed between samples to minimize cross-contamination. It is recommended that dissolved oxygen be maintained above 2.5 mg/L in the overlying water in each replicate during testing. Dissolved oxygen levels declined during the first seven days of testing. Water renewal rates were increased from two to four turnovers per day. On Day 20, the dissolved oxygen concentration in one replicate (replicate D, sample TX0002) was 1.8 mg/L. The dissolved oxygen levels (not including replicate D, sample TX0002) ranged from 3.6 to 9.5 mg/L. With this one exception, dissolved oxygen concentrations in all control and test replicates were above the minimum 2.5 mg/L throughout the study.
- The pH of all treatments was measured at the beginning and at the end of the test, and was also measured three times per week during the test. The pH was measured directly in the overlying water with a probe. The probe was inspected between samples to make sure that organisms were not attached, and was rinsed between samples to minimize cross-contamination. Values for pH ranged from 6.5 to 8.4, which is slightly outside the recommended range of 7.0 to 9.0.

- Ammonia and sulfide were measured in the overlying water of one replicate of each sediment sample on Days 0, 20, and 42 (i.e., at test termination). Three additional replicate vessels were established during the amphipod test to monitor ammonia and sulfide in the overlying water. The concentration of ammonia nitrogen (ammonia-N) in the overlying water during the testing period ranged from less than 0.1 mg/L (detection limit) to 1.91 mg/L. The concentration of total sulfide was less than 0.02 mg/L (detection limit).
- Conductivity, hardness, and alkalinity were measured in all treatments at the beginning and at the end of the test. Conductivity, hardness, and alkalinity were also measured on Day 20. In addition, conductivity was measured weekly during the test. Conductivity values ranged from 150 to 330  $\mu$ mhos/cm. Hardness values ranged from 36 to 88 mg/L as CaCO<sub>3</sub>. Alkalinity values ranged from 30 to 70 mg/L as CaCO<sub>3</sub>. As recommended, conductivity, hardness, and alkalinity in the overlying water did not vary by more than 50 percent during the test.

### Controls

A reference sediment test (i.e., sediment from Otisco Lake, New York), conducted with 12 replicates, was used to evaluate the survival, growth, and emergence potential of the test organisms in a non-contaminated sediment. In addition, a laboratory control sediment (i.e., negative control), prepared by the testing laboratory, was used to evaluate the survival, growth, and reproduction potential of the test organisms. The negative control test was also conducted with 12 replicates. All reference sediment and negative control testing was in compliance with USEPA methods (USEPA, 2000). Site-specific performance standards developed for this test are provided in Table C2-2 and in USEPA (2000).

**Table C2-2. Performance Standards for Control and Reference Sediments Used in Chironomid Toxicity Test**

Toxicity Test	Laboratory Control Sediment Performance Standard	Reference Sediment Performance Standard <sup>a</sup>
<i>Chironomus tentans</i> (42-day)		
Survival endpoint	≤30 percent absolute mean mortality on Day 20 and ≤35 percent absolute mean mortality at test end	≤35 percent absolute mean mortality
Growth endpoint	0.48 mg mean weight per surviving organism (ash-free dry weight)	
Emergence endpoint	≥50 percent emergence	

<sup>a</sup> Reference sediment performance standards are intended to be a measure of reference sediment acceptability for regulatory decision-making (i.e., was the reference sediment toxicity sufficiently low for valid comparisons with the test sediments to support decisions regarding the possible need for remediation?). Laboratory performance and test acceptability were evaluated solely on the results of the toxicity tests using the control sediment.



Mean survival for the laboratory control sediment (i.e., negative control) was 96 percent, and the mean ash-free dry weight of chironomids was 0.51 mg per midge larvae. The mean emergence in the laboratory control sediment (i.e., negative control) was 74 percent, and the mean time to emergence was 26.7 days. None of the test organisms exhibited partial emergence from the control or test sediment. Mean survival for sediment from the reference area samples was 77 and 90 percent, respectively. These results are above the performance standards set for the test and suggest that the test organisms were sufficiently healthy for testing.

A positive control was tested using potassium chloride as the reference toxicant. The positive control exhibited a 96-hour LC50 value of 6,700 mg KCl/L, which is within the database range for this test (4,750 to 8,600 mg KCl/L). The observed LC50 value suggests that the test organisms were suitably sensitive for testing.

## **Summary of Quality Assurance and Quality Control Considerations**

### **42-Day Amphipod Test**

Mean survival in the amphipod survival and growth test for the laboratory control sediment (i.e., negative control) was 94 percent and the mean dry weight of amphipods in the negative control was 0.516 mg per amphipod. Mean survival in the amphipod survival and reproduction (number of offspring per female) test for the negative control was 93 percent and the mean number of offspring per female was 9.1. These results are above the performance standards set for the 42-day amphipod test. The reference toxicant test (i.e., positive control) results were within the laboratory's control chart limits for the test.

During the amphipod test, concentrations of dissolved oxygen were slightly less than the recommended level of 5.0 mg/L. However, dissolved oxygen concentrations were greater than or equal to 4.0 mg/L throughout the study in all control and test sediment replicates, and safely above the minimum 2.5 mg/L that is required for this type of test. The measured levels of ammonia in porewater in the amphipod test approached the reported 4-day LC50 value for *H. azteca* and ammonia (i.e., 20 mg/L) in soft water. However, no correlation was observed between amphipod mortality and porewater ammonia concentration. Values for pH ranged from 6.5 to 8.5 during the amphipod test, which is slightly outside the recommended range of 7.0 to 9.0. However, there was no correlation between amphipod mortality and this slight pH deviation (i.e., 0.5).

The data are determined to be acceptable for use in the remedial investigation and feasibility study.

### **42-Day Chironomid Test**

Mean survival for the laboratory control sediment (i.e., negative control) was 96 percent and the mean ash-free dry weight of chironomids was 0.51 mg per midge larvae. The mean emergence in the laboratory control sediment was 74 percent, and the mean time to emergence was 26.7 days. These results are above the performance standards set for the 42-day chironomid test. The reference toxicant test (i.e., positive control) results were within the laboratory's control chart limits for the test.

There was a decline in the dissolved oxygen concentration during the first seven days of the test. To prevent the dissolved oxygen from dropping to unacceptable levels (i.e., less than 2.5 mg/L), the water renewal rate was increased from two to four turnovers per day. This increase in the water renewal rate allowed the dissolved oxygen to remain stable and above acceptable levels. Dissolved oxygen concentrations were greater than or equal to 3.6 mg/L throughout the study, with one exception. The dissolved oxygen concentration in sample TX0002, replicate D, on Day 20 was 1.8 mg/L. All other dissolved oxygen measurements in other replicates of this sample (TX0002) were greater than 4.4 mg/L. Because no midge larvae survived in any of the replicates for this sample, the low dissolved oxygen concentration measured on Day 20 probably had no impact on the results of the study. Water temperature in two samples (TX0009 and TX0010) on Day 39 was measured at 21°C. This is slightly outside the temperature criteria stipulated for the test (22 to 24°C). Values for pH ranged from 6.5 to 8.5 during the chironomid test, which is slightly outside the recommended range of 7.0 to 9.0. However, there was no correlation between chironomid mortality and this slight pH deviation (i.e., 0.5).

The data are determined to be acceptable for use in the remedial investigation and feasibility study.

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