When things go wrong

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Overview

- Purpose of bioassays

- Bioassay Test Acceptability Criteria

- Case Study 1: Reference Toxicant Test Failure

- Case Study 2: Control Failure

- Case Study 3: Toxicant in sample?!

Purpose of bioassays

- Assess toxicity of test sample as a whole on live organisms

- Required by discharge permits
  - Frequency
  - Sampling location
  - Specific species
  - Method
  - Additional supporting analysis during course of bioassay

Test Acceptability Criteria

- General Criteria
  - Concurrent reference toxicant test
    - Reference toxicant is of known toxicity
    - Expose organism to concentration series of reference toxicant in control water matrix
    - Determine LC50, concentration of toxicant that kills half of sample population, statistically from concentration series exposure data
    - Determine sensitivity of the test organisms
    - Demonstrates laboratory capability
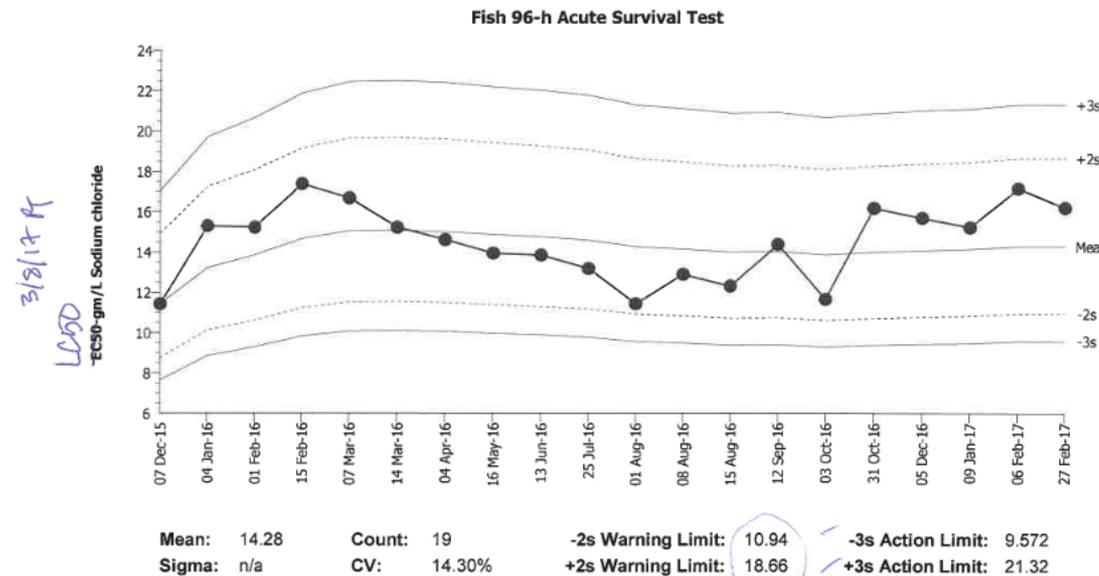   - Assess inter and intra laboratory comparability

- ## General Criteria
  - – Maintain control chart of the most recent 20 LC50 with mean and control limits (defined as ± 2SD from mean of the 20 data points)

Fish 96-h Acute Survival Test				East Bay Municipal Utility District	
Test Type: Survival (96h)		**Organism:** Oncorhynchus mykiss (Rainbow Tro		**Material:**	Sodium chloride
Protocol: EPA/600/4-90/027F (1991)		**Endpoint:** 96h Survival Rate		**Source:**	Reference Toxicant-REF

Fish 96-h Acute Survival Test

Mean:	14.28	Count:	19	-2s Warning Limit:	10.94	-3s Action Limit:	9.572
Sigma:	n/a	CV:	14.30%	+2s Warning Limit:	18.66	+3s Action Limit:	21.32

Test Acceptability Criteria

- Test Specific Criteria

  - Acceptable survival/ growth in Control

  - Precision among replicates
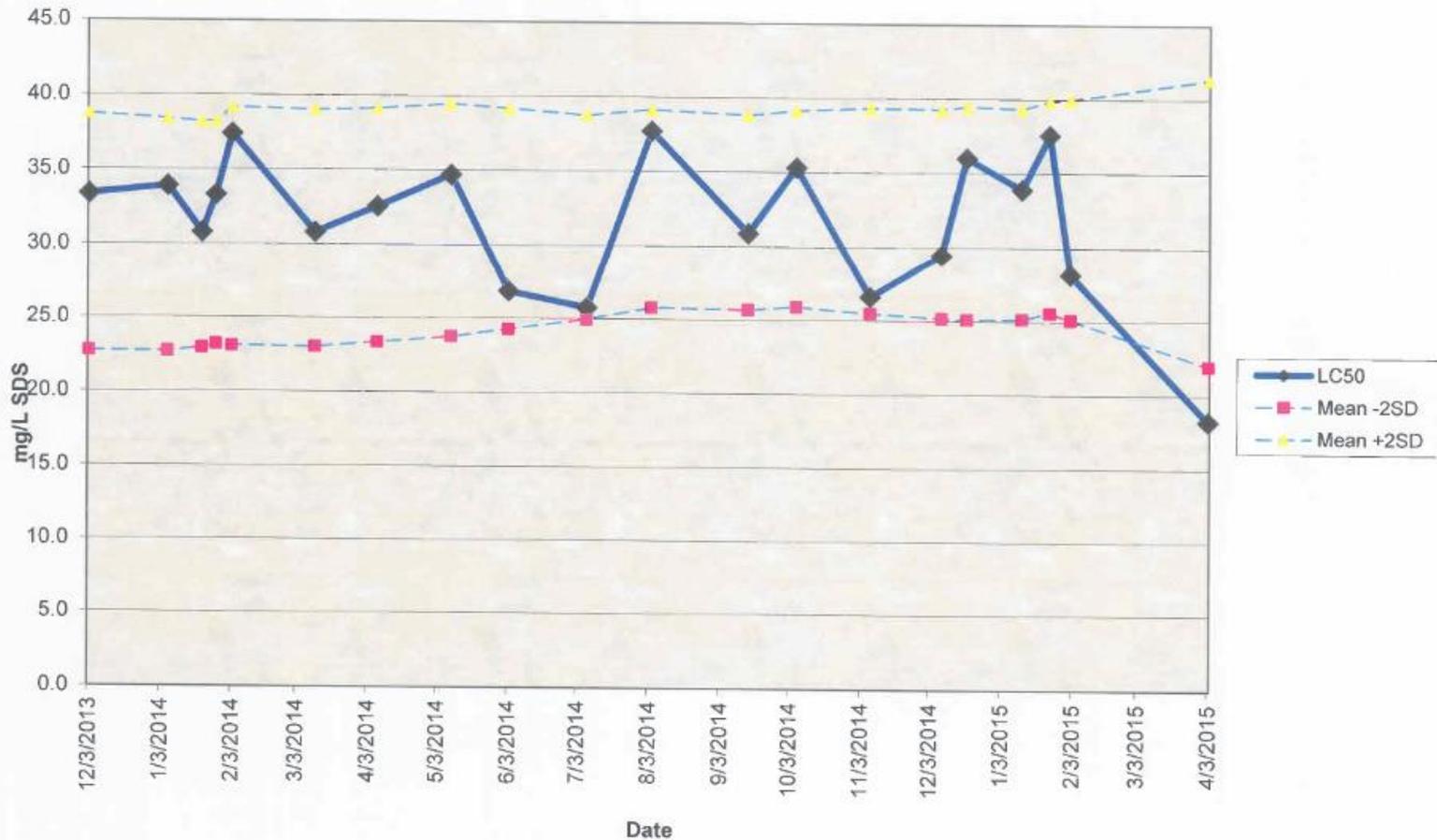
    - Percent Minimum Significant Difference (PMSD)

Acute Toxicity Bioassay using Rainbow Trout

- Issue -

  – Reference Toxicant test resulted in LC50 below lower control limit

  – Test organism exhibited hyper sensitivity, poor health?

  – Control demonstrated 100% survival!

  – Samples also demonstrated 100% survival!!

96 Hour Control Chart -- Rainbow Trout
Toxicant: Sodium Dodecylsulfate

Case Study 1: Ref. Tox. Test Failure

REFERENCE TOXICANT TEST FOR FISH CULTURES

Growth surrounding the gill is evident

Turbidity observed in ALL tanks
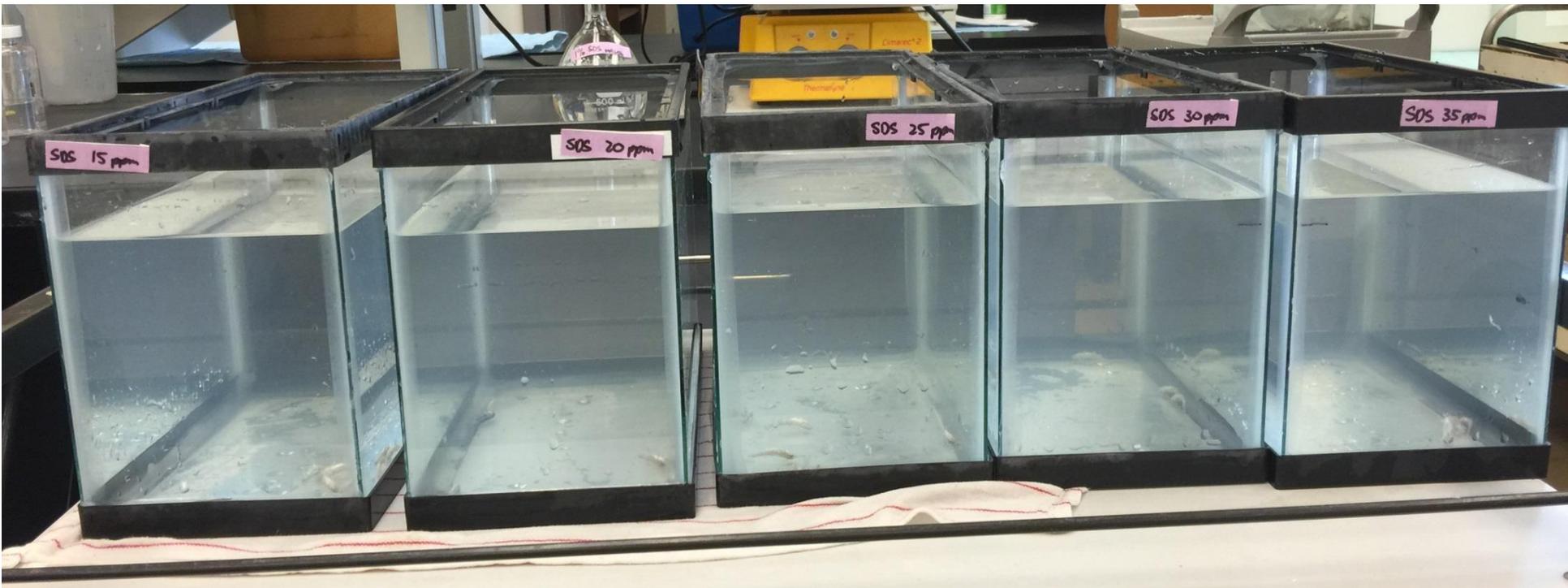
- ## Identifying the bacteria

  – Took aliquots of 35 ppm SDS fish tank

  – Filtered through 0.45μm filter grow on mEndo

  – Picked colonies of different morphology for identification by Analytical Profile Index

Case Study 1: Ref. Tox. Test Failure

- All identified to be Aeromonas hydrophila

  – Can be eliminated using sodium hypochlorite

- Unable to completely eradicate Aeromonas hydrophila as it is commonly found in fish

- Use of alternative toxicant

  – NaCl

  – Concentration series: 10, 12.5, 15, 17.5 & 20 g/L

Case Study 1: Ref. Tox. Test Failure

- Turbidity and DO-drop not observed in NaCl Ref Tox Test
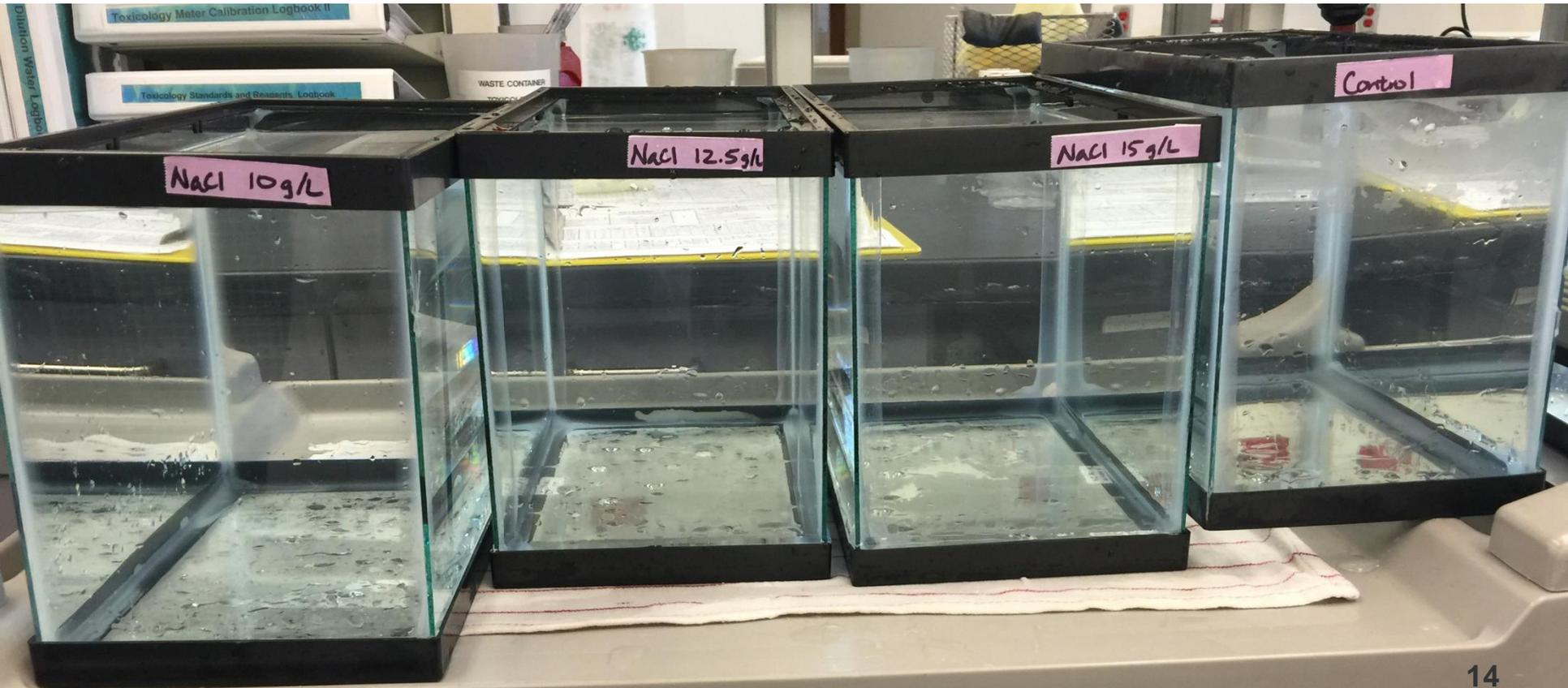
Case Study 1: Ref. Tox. Test Failure

- Control chart established

96 Hour Control Chart -- Rainbow Trout
Toxicant: NaCl
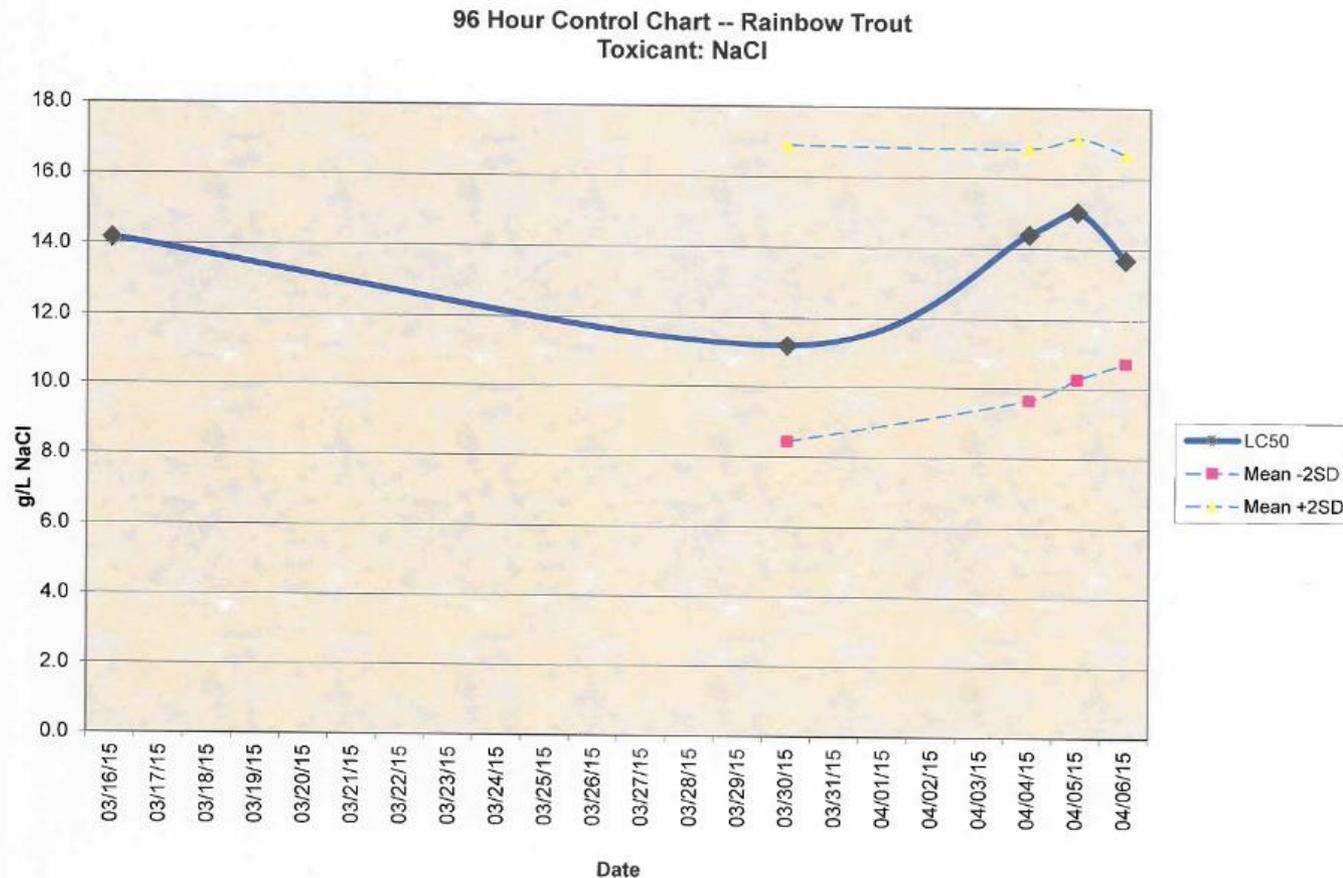
Case Study 1: Ref. Tox. Test Failure

- ## What we learned
  - ### Review data
    - Keep notes/ record observations
  - ### Reach out to those with relevant expertise
  - ### Prepare for changes

Chronic Toxicity Bioassay using Mytilus mussels

Chronic Toxicity Bioassay using Mytilus mussels

- Induce spawning of Mytilus mussels

Chronic Toxicity Bioassay using Mytilus mussels

- Fertilization

Chronic Toxicity Bioassay using Mytilus mussels

- Endpoint: survival and shell development
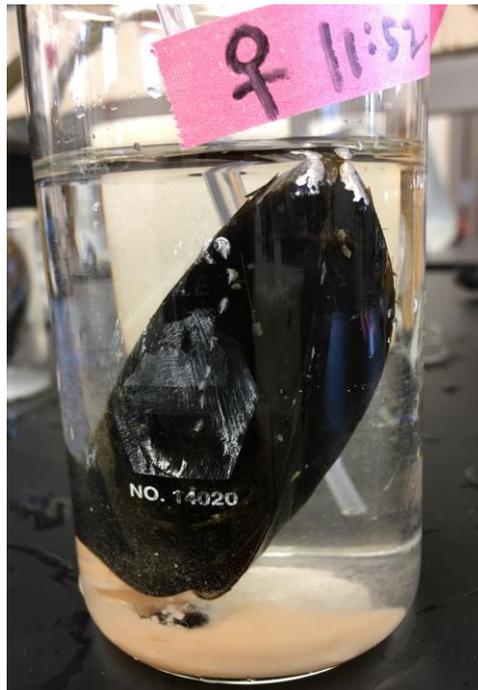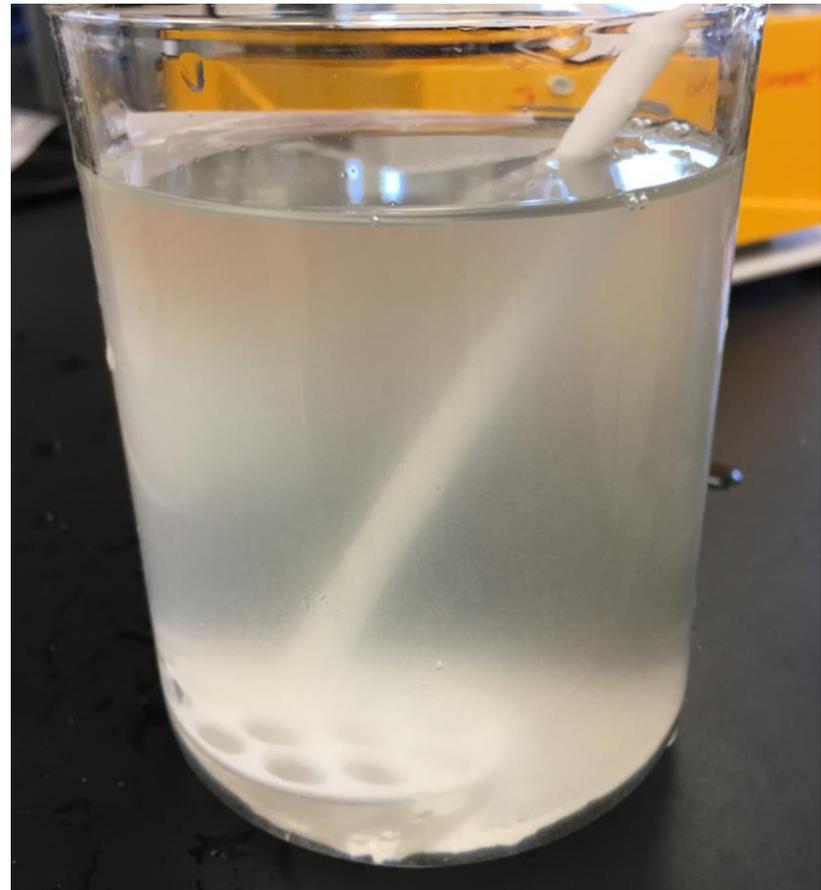
Case Study 2: When control failed
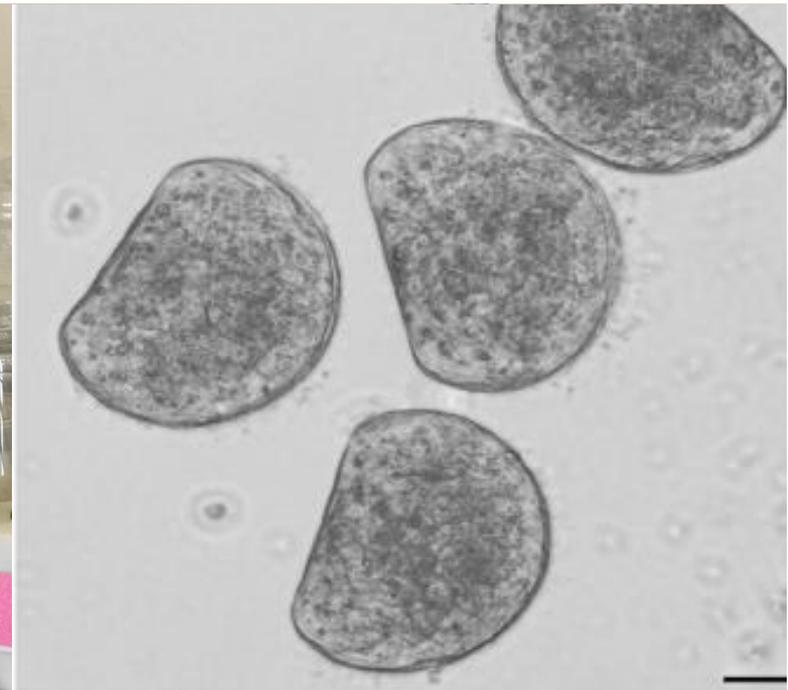
Chronic Toxicity Bioassay using Mytilus mussels

- Issue -

  - Reference toxicant test yielded EC50 within control limits ✓

  - Control failed to meet acceptance criteria of 90% normal shell development in surviving organisms ✗

All effort wasted!!
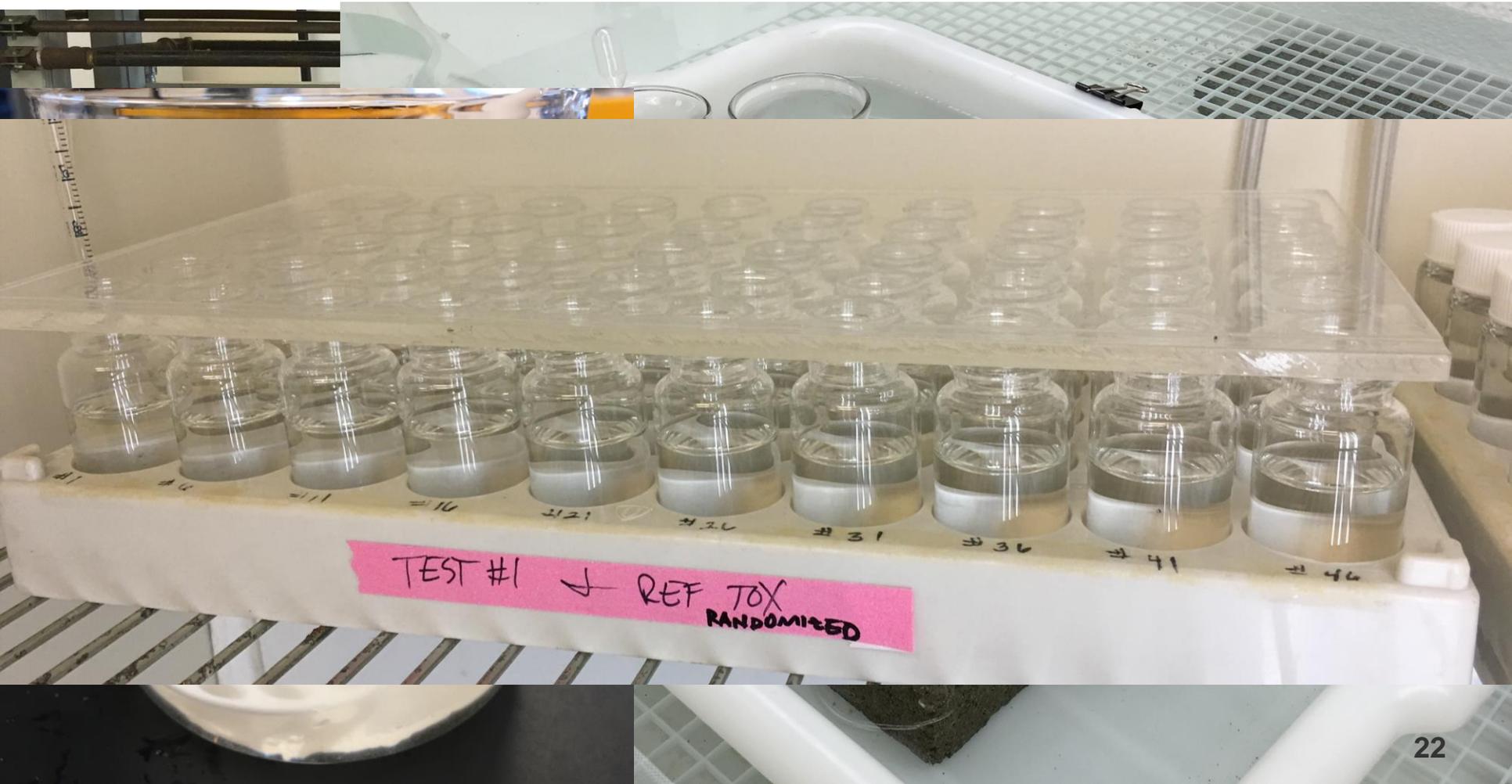
Case Study 2: When control failed

Root Cause Analysis -

– Embryos condition?

- • Reference toxicant test yielded EC50 within control limits

– Sea water?

- • Met annual control water quality criteria
  - – No detectable organics
    - » organochlorine pesticides & PCBs
  - – No detectable metals
    - » Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Pb, Ni & Zn

Root Cause Analysis -

– Filter?

- Charcoal filter and a 1.0 μm filter
- Compare before and after filter for all the parameters monitored in annual water check

- Unfiltered seawater from Bodega Marine Laboratory

Sample Comments: Unfiltered seawater from Bodega Marine Laboratory.

Method Reference Parameter	Qualifier	Result	Units	Dilution	MDL
Method: EPA 200.7 - Rev. 4.4, ICP Scan					
TARGET ANALYTES					
SILVER	U	27.5	ug/L	5.5	27.5
ALUMINUM	U	71.5	ug/L	5.5	71.5
CADMIUM	U	11.0	ug/L	5.5	11
COBALT	U	3.30	ug/L	5.5	3.3
CHROMIUM	U	16.5	ug/L	5.5	16.5
COPPER	U	38.5	ug/L	5.5	38.5
IRON	U	22.0	ug/L	5.5	22
NICKEL	U	16.5	ug/L	5.5	16.5
LEAD	U	27.5	ug/L	5.5	27.5
ZINC	U	16.5	ug/L	5.5	16.5

Run ID: R255808 / Work Group No.: WG195305

Case Study 2: When control failed

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COBALT	U	3.30	ug/L	5.5	3.3
CHROMIUM	U	16.5	ug/L	5.5	16.5
COPPER	U	38.5	ug/L	5.5	38.5
IRON		**65.5**	ug/L	5.5	22
NICKEL	U	16.5	ug/L	5.5	16.5
LEAD	U	27.5	ug/L	5.5	27.5
ZINC	U	16.5	ug/L	5.5	16.5

Run ID: R255808 / Work Group No.: WG195305

- ## What we learned
  - – Filtration apparatus contributed to detectable amount of iron to filtered seawater
  - – Switched to PALL Maxi Capsule for filtered seawater preparation
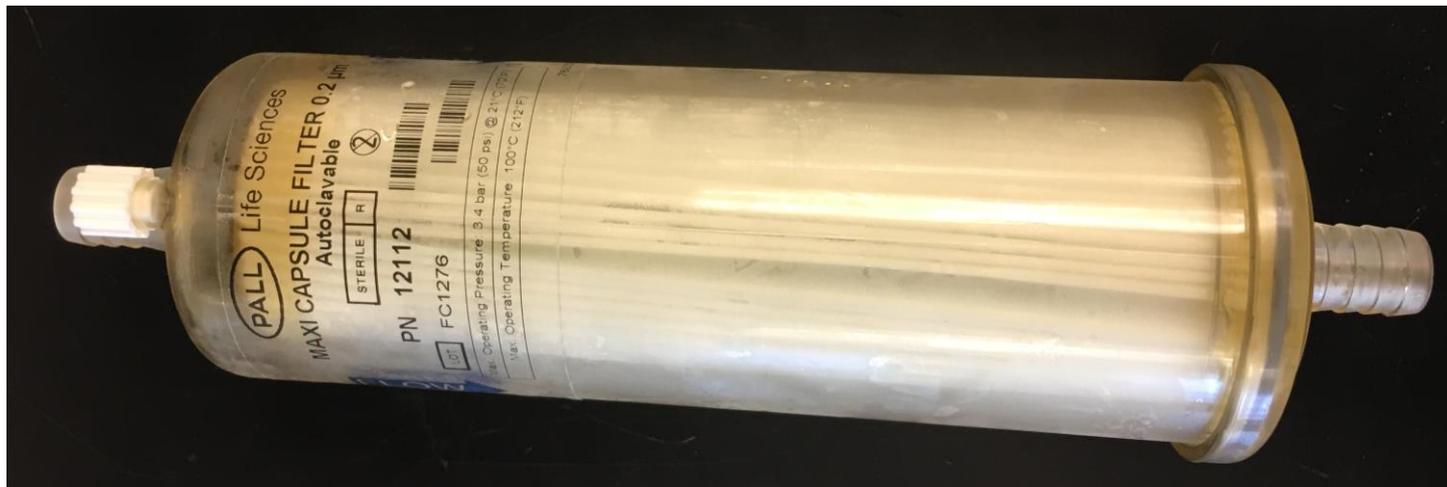
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- Corrective Action

  – Monitor quality of control water in addition to the source of control water

- With *new* filtration apparatus, control of subsequent tests consistently met Test Acceptance Criteria of 90% normal development:

  93.5%, 94.5% and 91.3% in subsequent tests

Case 3: Toxicant in Sample?

Acute Toxicity Test with Rainbow Trout

- Issue

  - Reference Toxicant Test exhibited acceptable LC50 within control limits ✓

  - Control 100% survival ✓

  - Sample 22% survival ✗

What went wrong?   Review data

Daily Measurements
Test Effluent Tanks

What went wrong?   Review data

Daily Measurements
Effluent Mixing Tank - Dissolved CO2

	Time	Alkalinity (mg/L)	pH (Mix Tank)	DO (mg/L)	Flow Rate (mL/min)	pH Set Point	pH (pH Adjust Chamber)	Initials
Day 0	1300	132	6.95	10.27	44	6.005	6.05	CET
Comments								
Day 1	0815	148	7.00	10.68	44	6.005	6.08	CET
	1512	157	6.97	10.51	44	6.005	6.06	CET
Comments								
Day 2	0820	145	6.79	10.44	43/47 *	6.005	6.04	CET
	1520	154	6.76	10.17	47	6.005	6.06	CET
Comments	* increased slightly to 47 @ 0825 cet							
Day 3	1030	165	6.97	10.06	47/45	6.005	6.00	lw
	1510	110	6.84	10.59	44	5.800	5.81	lw
Comments	lowered pH set to 5.800 to enhance CO2 removal. lw 2/4/15 @ 1115 / lowered pump rate to effluent. lw 2/4/16							
Day 4	0828	115	6.82	11.02	44	5.800	5.82	lw
Comments								

Case 3: Toxicant in Sample?

- Dissolved CO_2 resulting in mortality?

Appendix C

Table 2: Dissolved carbon dioxide concentration (mg/L) as a function of pH and alkalinity (mg/L as CaCO3) at 12 °C

pH \ ALK	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150	155	160
6.00	182	194	206	218	230	242	254	267	279	291	303	315	327	339	351	364	376	388
6.05	162	173	184	194	205	216	227	238	248	259	270	281	292	302	313	324	335	346
6.10	144	154	164	173	183	193	202	212	221	231	241	250	260	270	279	289	298	308
6.15	129	137	146	154	163	172	180	189	197	206	214	223	232	240	249	257	266	275
6.20	115	122	130	138	145	153	161	168	176	184	191	199	206	214	222	229	237	245
6.25	102	109	116	123	129	136	143	150	157	164	170	177	184	191	198	204	211	218
6.30	91	97	103	109	115	121	128	134	140	146	152	158	164	170	176	182	188	194
6.35	81	87	92	97	103	108	114	119	125	130	135	141	146	152	157	162	168	173
6.40	72	77	82	87	92	96	101	106	111	116	121	125	130	135	140	145	150	154
6.45	64	69	73	77	82	86	90	95	99	103	107	112	116	120	125	129	133	138
6.50	57	61	65	69	73	77	80	84	88	92	96	100	103	107	111	115	119	123
6.55	51	55	58	61	65	68	72	75	79	82	85	89	92	96	99	102	106	109
6.60	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97
6.65	41	43	46	49	52	54	57	60	62	65	68	71	73	76	79	81	84	87
6.70	36	39	41	44	46	48	51	53	56	58	60	63	65	68	70	73	75	77
6.75	32	34	37	39	41	43	45	47	50	52	54	56	58	60	62	65	67	69
6.80	29	31	33	35	36	38	40	42	44	46	48	50	52	54	56	58	60	61
6.85	26	27	29	31	33	34	36	38	39	41	43	45	46	48	50	51	53	55
6.90	23	24	26	27	29	31	32	34	35	37	38	40	41	43	44	46	47	49
6.95	20	22	23	24	26	27	29	30	31	33	34	35	37	38	39	41	42	44
7.00	18	19	21	22	23	24	25	27	28	29	30	32	33	34	35	36	38	39
7.05	16	17	18	19	21	22	23	24	25	26	27	28	29	30	31	32	33	35
7.10	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
7.15	13	14	15	15	16	17	18	19	20	21	21	22	23	24	25	26	27	27
7.20	11	12	13	14	15	15	16	17	18	18	19	20	21	21	22	23	24	24
7.25	10	11	12	12	13	14	14	15	16	16	17	18	18	19	20	20	21	22
7.30	9	10	10	11	12	12	13	13	14	15	15	16	16	17	18	18	19	19
7.35	8	9	9	10	10	11	11	12	12	13	14	14	15	15	15	16	17	19
7.40	7	8	8	9	9	10	10	11	11	12	12	13	13	14	14	14	15	15
7.45	6	7	7	8	8	9	9	9	10	10	11	11	12	12	12	13	13	14
7.50	6	6	7	7	7	8	8	8	9	9	10	10	10	11	11	11		12

Case 3: Toxicant in Sample?

Collected on Day 2, before mortality
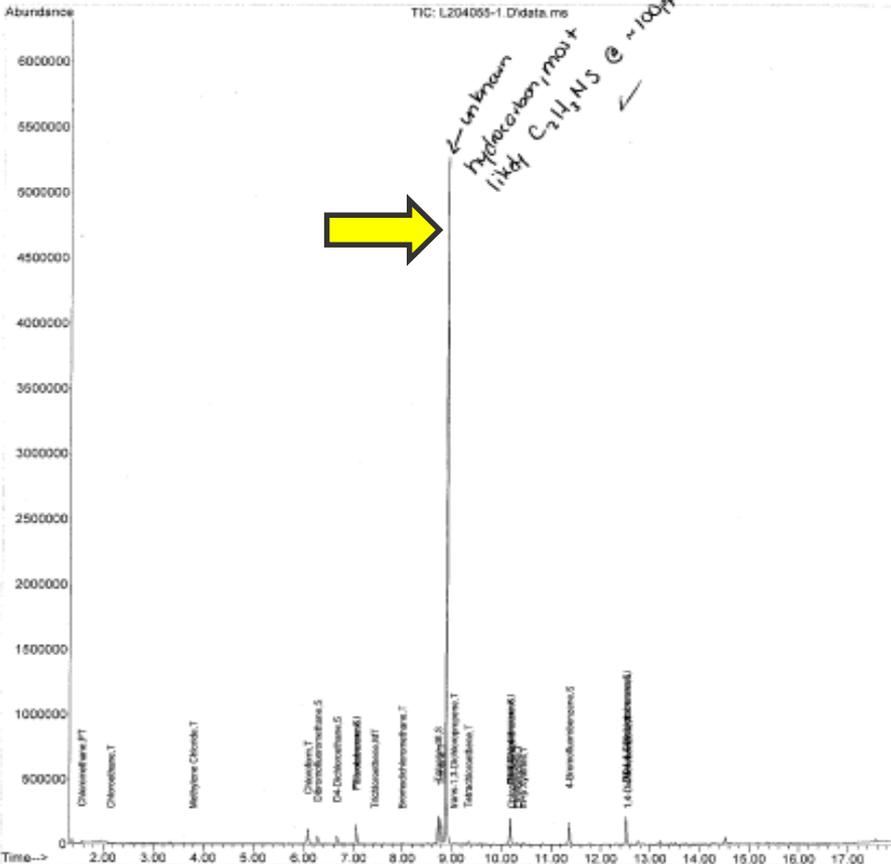
Typical effluent chromatogram
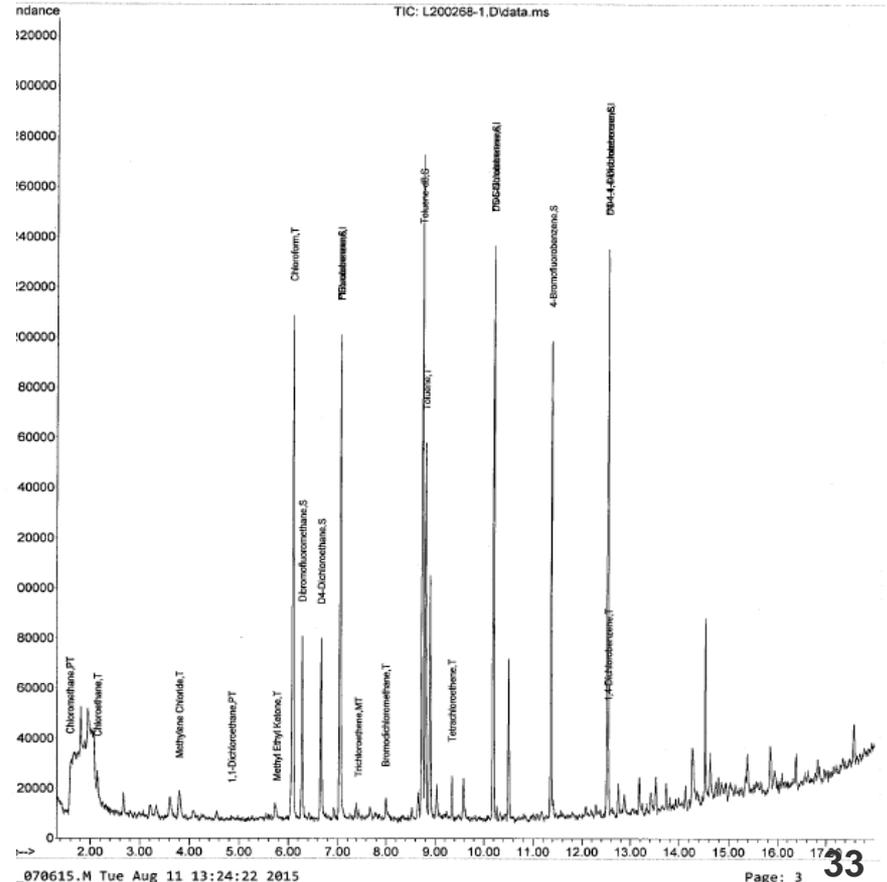
Collected on Day 2, before mortality

Methyl Isothiocyanate (MITC)

- Identified by library spectrum search
- Breakdown product of metam sodium, a common soil fumigant used for fungi and weeds
- SDS listed it as "very toxic to aquatic organisms"
- Rainbow trout: LC50 = 0.37 mg/L
- After obtaining standards, the *estimated* concentrated of this sample is *330 ug/L*
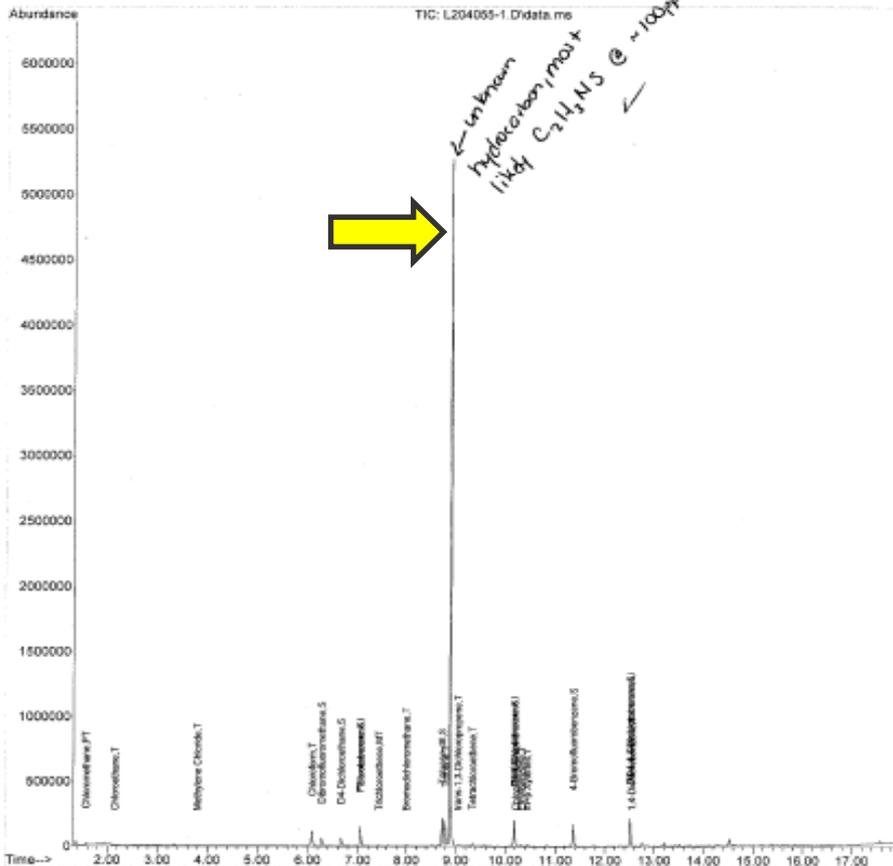
- Investigation continued with archived samples collected during acute toxicity bioassay

- Detection of MITC confirmed from Day 2, Day 3 and Day 4 samples

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Confirmed Methylisothiocyanate (MITC) values:

Rainbow trout LC50 of 370 ug/L

Semi-Annual Organics sample collected ahead of bioassay mortality event:

Sample #      Collect Date      Locator          Result
L204055-1    2/3/2016 12:10 EFF EPS 04    MITC concentration 330 ug/L

Bioassay Archive Samples From the Test Initiated 2/1/2016 (22% Survival)
Sample collected durring mortality event

Sample #      Collect Date      Locator          Result
L204118-1   2/4/2016 2:00    EFF EPS 04    MITC concentration 44 ug/L
```

Case 3: Toxicant in Sample?

- Bioassay repeated with 100% survival

- MITC was not detected throughout the duration of bioassay

Bioassay Archive Samples From the Test Initiated 2/15/2016 (100% Survival)

Sample #	Collect Date	Locator	Result
L204282-1	2/15/2016 20:00	EFF EPS 04	MITC concentration < 1 ug/L
L204284-1	2/16/2016 8:00	EFF EPS 04	MITC concentration < 1 ug/L
L204315-1	2/17/2016 2:00	EFF EPS 04	MITC concentration < 1 ug/L
L204383-1	2/18/2016 14:03	EFF EPS 04	MITC concentration < 1 ug/L

Case 3: Toxicant in Sample?

- What we learned
  - Lab collaboration is crucial
  - Keep up with archive sampling
    - Volatile organics
    - Semi-volatile organics
    - No headspace sampling without preservation
  - Bioassay is a useful tool to detect presence of toxicant

Summary

- Root Cause Analysis

- Identify Corrective Action

- Toxicology requires analytical support

  – Microbiological analysis

  – Metal analysis

  – Wet chemistry analysis: alkalinity, hardness, etc.

  – Organic analysis

Acknowledgements

- EBMUD Laboratory Team:
  - Biology, Inorganics, Organics, QAO, Project Managers, sample control staff

- Experts from environmental laboratory community:
  - who generously shared their knowledge

Questions?