Toxicity 101

Bioassay Basics

BACWA Toxicity Workshop, September 18, 2017

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Whole Effluent Toxicity: **Why?**

- EPA says: “Whole Effluent Toxicity (WET) describes the aggregate toxic effect of an aqueous sample … without requiring the identification of the specific pollutants.”*
- Chemists can only find what they are looking for.

How do you find the things you don’t know to look for?

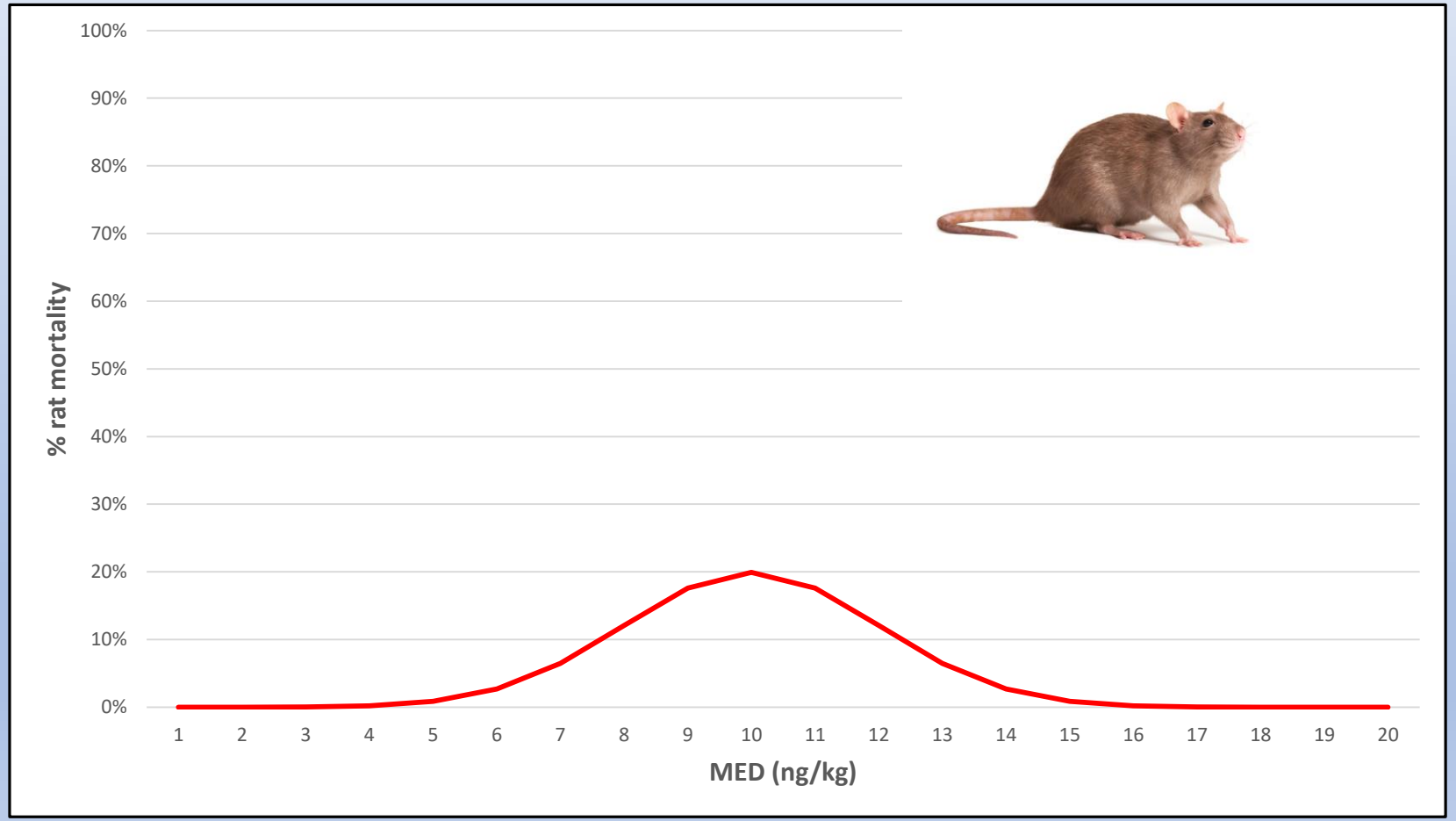
“The unknown unknowns”

*www.epa.gov/npdes/whole-effluent-toxicity-wet

Presentation Outline

- WET test design
- Statistical treatment of data
- Toxicity investigation (TIE/TRE)

Toxicity testing: dose-response

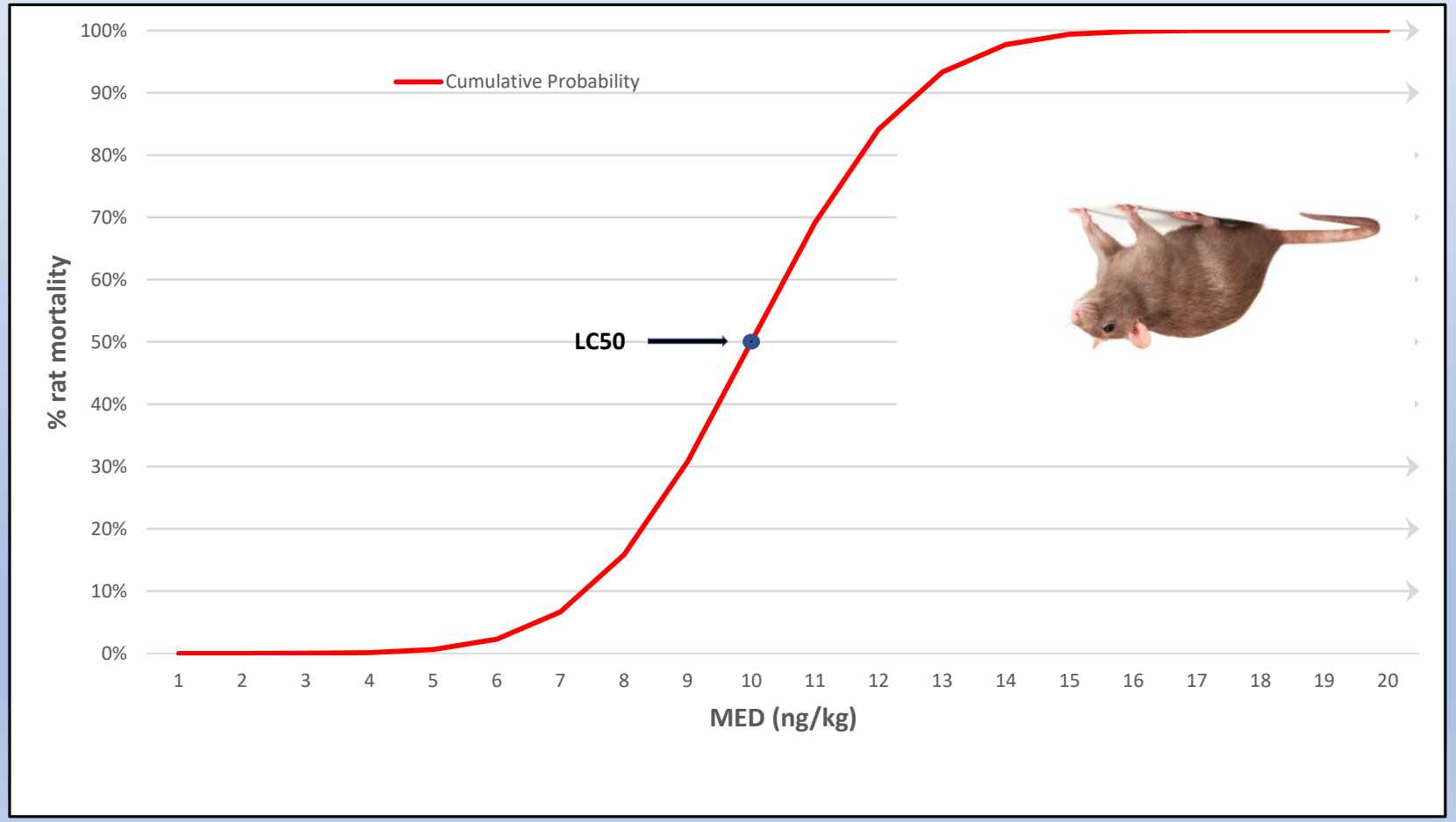


-CH_3

-CH_2-CH_3

Toxicity of Methylethyl-Death (MED) to *R. rattus* in ng/kg

Toxicity testing: dose-response



$-CH_3$

$-CH_2-CH_3$

Toxicity of Methylethyl-Death (MED) to *R. rattus* in ng/kg

Elements of bioassay test design

- What species?
- What endpoint (what to measure during test)
  - Mortality
  - Sub-lethal irreversible effects
- QA/QC

Types of test species

Plants

Selenastrum capricornutum

Invertebrates

Mytilus edulis

Arbacia Punctulata

Ceriodaphnia dubia

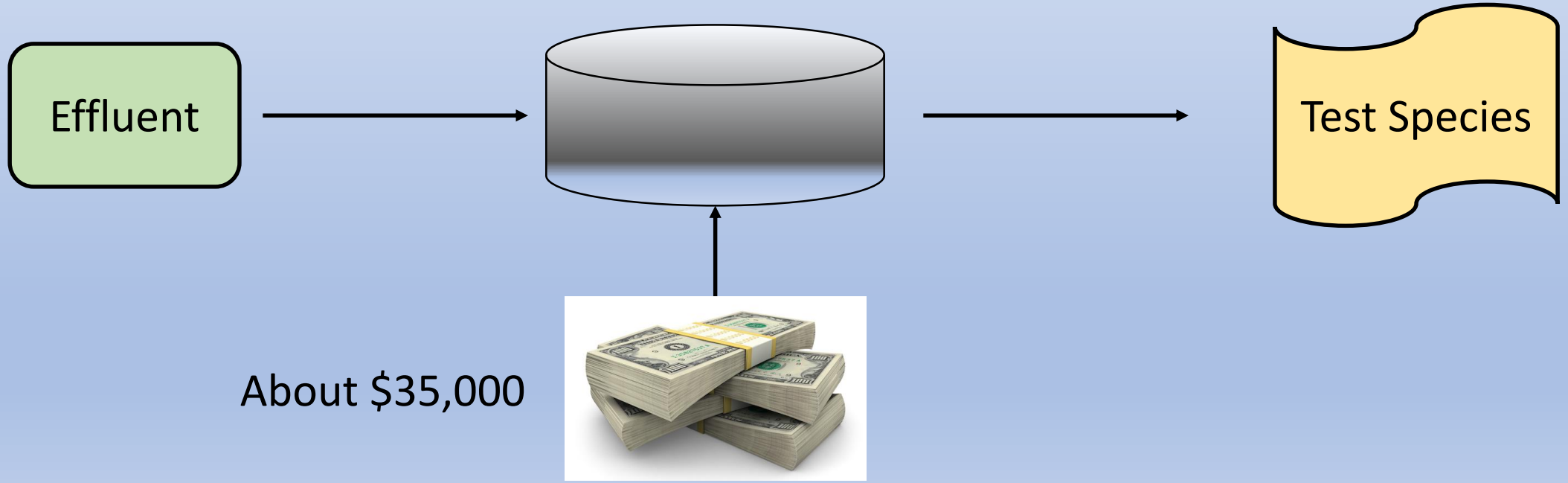
Vertebrates

Pimphales Promelas

Orthorynchus mykiss

Species screening process

- How is your test species selected?
- Successive screening to find most sensitive species



Endpoints: what response do we measure?

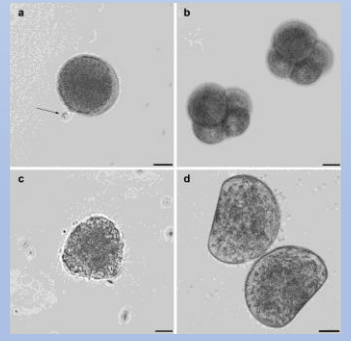
- Mortality (Acute toxicity)
- Non-lethal endpoints (Chronic Toxicity)
  - Growth
  - Normal embryonic development
  - Reproductive success
  - Other responses

Example Chronic Toxicity Test

Mytilus edulis 48-hour embryo development test

1. Spawn adult mussels

2. Expose fertilized eggs to effluent concentration series (48 hours)

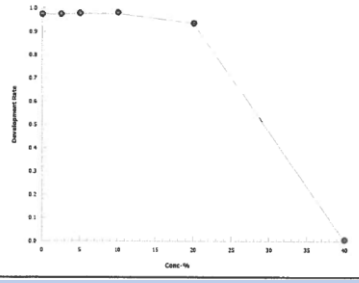


3. Microscopic examination to measure % abnormal embryonic development

4. Analyze data

Development Rate Summary			Calculated Variate(A/B)						
Conc-%	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LW	4	0.974	0.965	1.000	0.009	0.017	1.76%	0.0%
2.5		4	0.975	0.968	0.988	0.005	0.009	0.94%	-0.03%
5		4	0.978	0.967	0.989	0.005	0.009	0.96%	-0.35%
10		4	0.981	0.976	0.986	0.002	0.004	0.44%	-0.64%
20		4	0.935	0.899	0.951	0.012	0.024	2.56%	4.03%
40		4	0.000	0.000	0.000	0.000	0.000		100.0%

Graphics



QA/QC: Test Conditions and Controls

- Apply to both chronic and acute tests
- Goal: prevent non-effluent toxicity (false positives) or interference with toxicity (false negatives).
- Test Conditions
  - Each species has specific test conditions and exposure time
  - Exposure type: static, static renewal or flow-through
  - Temperature, DO, pH, Alkalinity, Hardness
  - Check for Chlorine; often measure Ammonia
  - Measured each day
- Controls
  - Must achieve 90% survival in blank dilution water

QA/QC: Reference Toxicant Tests

- Exposure of test organisms to a known toxicant
- Usually concurrent with effluent bioassay
- Two purposes
  - Demonstration of consistent lab performance
  - Establish that test batch has normal sensitivity

Why run reference toxicant tests?

1) To demonstrate laboratory's on-going capability to produce consistent results with a known toxicant and test species:

4.15.1 Satisfactory laboratory performance is demonstrated by performing at least one acceptable test per month with a reference toxicant for each toxicity test method conducted in the laboratory during that month. (5th Edition p 18)

2) To demonstrate that the batch of test organisms used in toxicity testing responds normally to a known toxicant:

4.15.6 Reference toxicant test results should not be used as a *de facto* criterion for rejection of individual effluent or receiving water tests. Reference toxicant testing is used for evaluating the health and sensitivity of organisms over time and for documenting initial and ongoing laboratory performance. While reference toxicant test results should not be used as a *de facto* criterion for test rejection, effluent and receiving water test results should be reviewed and interpreted in the light of reference toxicant test results. (5th Edition p 19)

Example Bioassay SRT results

Reference Toxicant 96-h Acute Survival Test — City of Benicia

Analysis ID:	00-7148-9021	Endpoint:	Survival Rate	CETIS Version:	CETISv1.8.7		
Analyzed:	03 May-17 8:44	Analysis:	Untrimmed Spearman-Kärber	Official Results:	Yes		

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	0.09361	0.02181	1.241	1.122	1.372

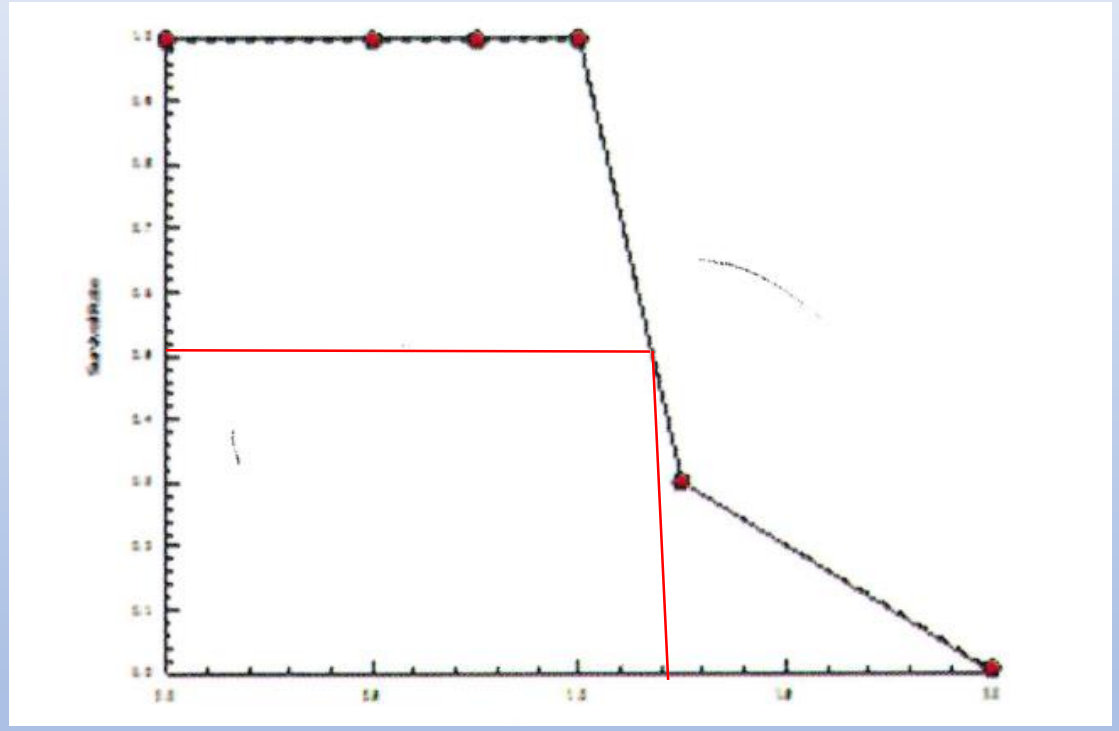
Survival Rate Summary — Calculated Variate(A/B)

C-gm/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	1	1	1	1	0	0	0.0%	0.0%	10	10
0.5		1	1	1	1	0	0	0.0%	0.0%	10	10
0.75		1	1	1	1	0	0	0.0%	0.0%	10	10
1		1	1	1	1	0	0	0.0%	0.0%	10	10
1.25		1	0.3	0.3	0.3	0	0	0.0%	70.0%	3	10
2		1	0	0	0	0	0		100.0%	0	10

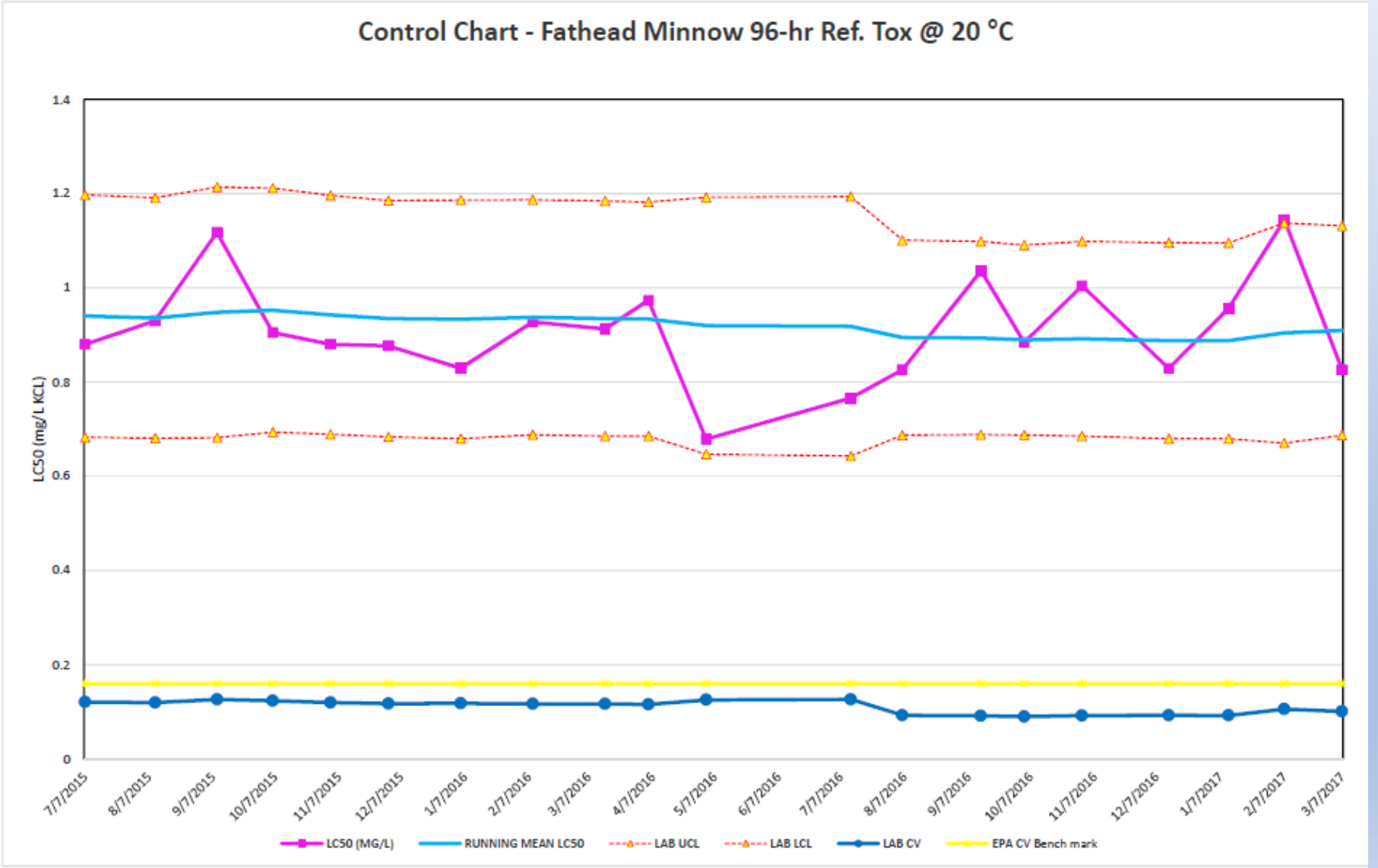
Concentration (Dose)

LC50 (point estimate)

Survival (Response)



Current Ref Tox control chart



Data Analysis

- Numeric Endpoints
  - Use concentration series to establish specific endpoint (LC50, EC25)
- Hypothesis testing
  - Compare toxicity of two samples
  - “Is the effluent significantly more toxic than the control” (or receiving water)

How to measure numeric endpoints

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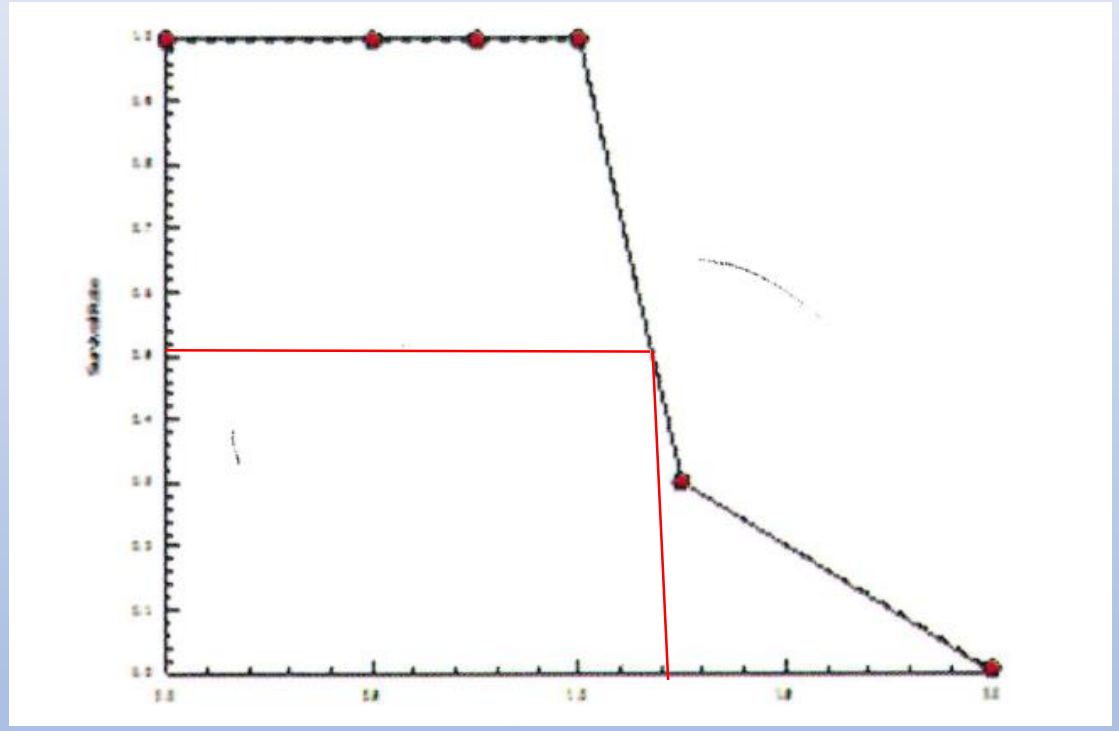


Hypothesis Testing

- Criminal Justice in U.S.
  - Presumed innocent unless proven guilty beyond a reasonable doubt
- Null hypothesis H_0: "Defendant is not guilty"
- Statistical test: jury trial and assessment of uncertainty.

	H_0 is true **Truly not guilty**	**H_1 is true** **Truly guilty**
Accept null hypothesis Acquittal	Right decision	Wrong decision Type II Error
Reject null hypothesis Conviction	Wrong decision Type I Error	Right decision

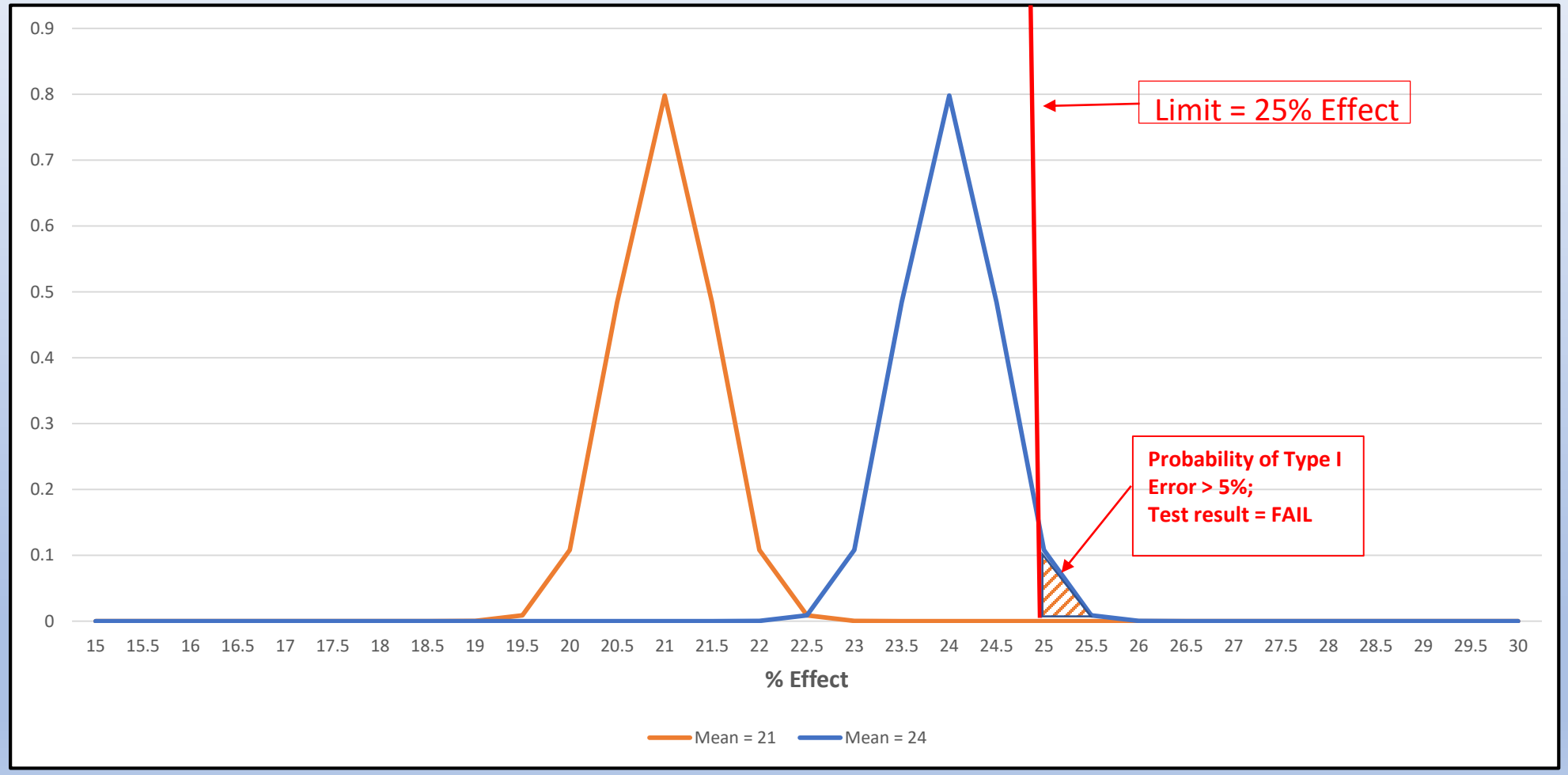
Data Analysis: Endpoints vs. Hypothesis Testing

- Numeric Endpoints
  - Use concentration series to establish specific endpoint (LC50, EC25)
  - Like a normal chemical measurement
  - Can be compared to a limit
- Hypothesis testing
  - Compare toxicity of two samples
  - “Is the effluent significantly more toxic than the control” (or receiving water)
- Different role of dilution
  - Endpoints – dilution applied to effluent limit, like chemical measurement
  - Hypothesis testing – dilution applied to test design

Test of Significant Toxicity (TST)

- Proposed by EPA, but not nationally
- Proposed for future SWRCB Toxicity Policy
- Reverse the null hypothesis:
  - H_0 = “the effluent is toxic”
  - Prove yourself innocent “beyond a reasonable doubt”
  - Goal is to control “false negatives”, where toxicity is not found although actually present
- Test at the In-Stream Waste Concentration (IWC) compared to control

Margin of Error in the TST

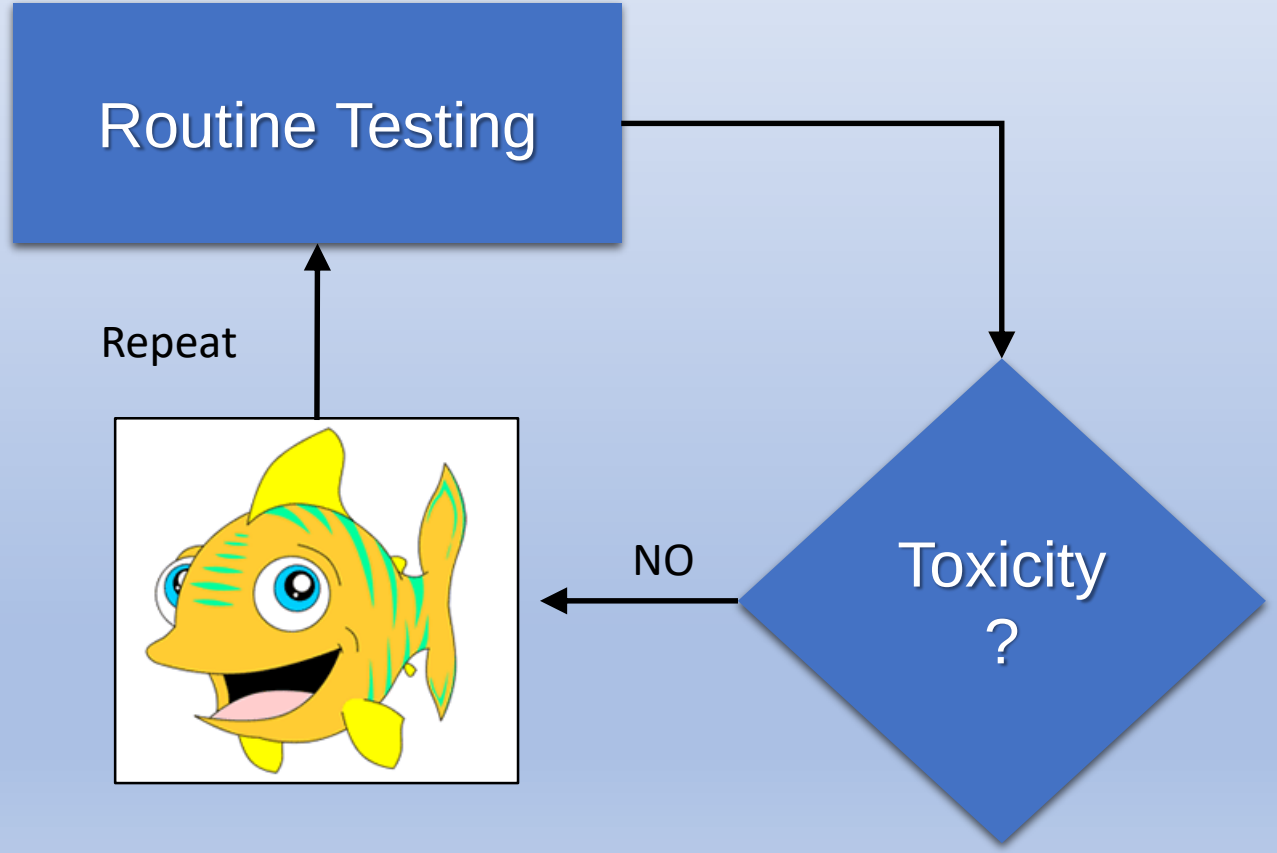


What if you find toxicity?

- Take a vacation …

- Accelerated Monitoring – confirm toxicity
- Toxicity Reduction Evaluation (TRE): stop the toxicity
- Toxicity Identification Evaluation (TIE): find the toxicant

It was an ordinary month of routine toxicity testing…



When suddenly, toxicity was detected…

Routine Testing

Toxicity ?

YES

Accelerated Monitoring

Accelerated Monitoring

Test Weekly

Repeat a few times

YES

Toxicity ?

Confirmed Toxicity: Toxicity Reduction Evaluation (TRE)

TRE Sequence

1. Review recent events
   (Vallejo case: root killer)
2. Start Toxicity Identification Evaluation (TIE)

If toxicant can't be identified try to remove it…

3. Look for correlations
4. Trial and Error
5. Spend $$$ on treatment upgrades

→

I. Characterize
II. Identify
III. Confirm

TIE phase I – toxicity characterization by the book (EPA Guidance)

Static Test	Objective
Aeration + pH adjustment (3, as is, 11)	Toxicity associated with volatile or oxidizable compounds?
Filtration + pH adjustment (3, as is, 11)	Toxicity is in the suspended or soluble fraction?
C18 SPE + pH adjustment (3, as is, 11)	Toxicity due to non-polar organics?
EDTA chelation	Toxicity due to cationic metals?
Oxidant reductin by thiosulfate	Toxicity due to oxidizers?
Graduated pH adjustment (6.0, 6.5, 6.8, 7.0, 7.5)	Toxicity due to pH sensitive compounds, such as ammonia?
Zeolite treatment + ammonia add-back	Toxicity due to ammonia?

Phase III: Confirmation

Hypothesis: Compound X is the toxicant

Removing Compound X should remove toxicity

Adding back Compound X should restore toxicity

Factors that can help TRE/TIE

- If toxicity is always there - consistency
- Strong “signal to noise”
- Able to do testing in-house
- Surrogate test to ease time and resources required
  - Sublethal response that is correlated to mortality
  - Use an earlier lifecycle stage to reduce volume required and increase sensitivity
  - Correlation to rapid toxicity testing (Microtox)