Test of Significant Toxicity (TST) Approach

A Statistical Approach

for Analyzing WET Data

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1 Take the sample

2 Run the test

3 Record the biological response (data)

4 Analyze the data and make a decision

Toxic vs. Non-Toxic

TST

5 Determine reasonable potential

6 Decide permit requirements

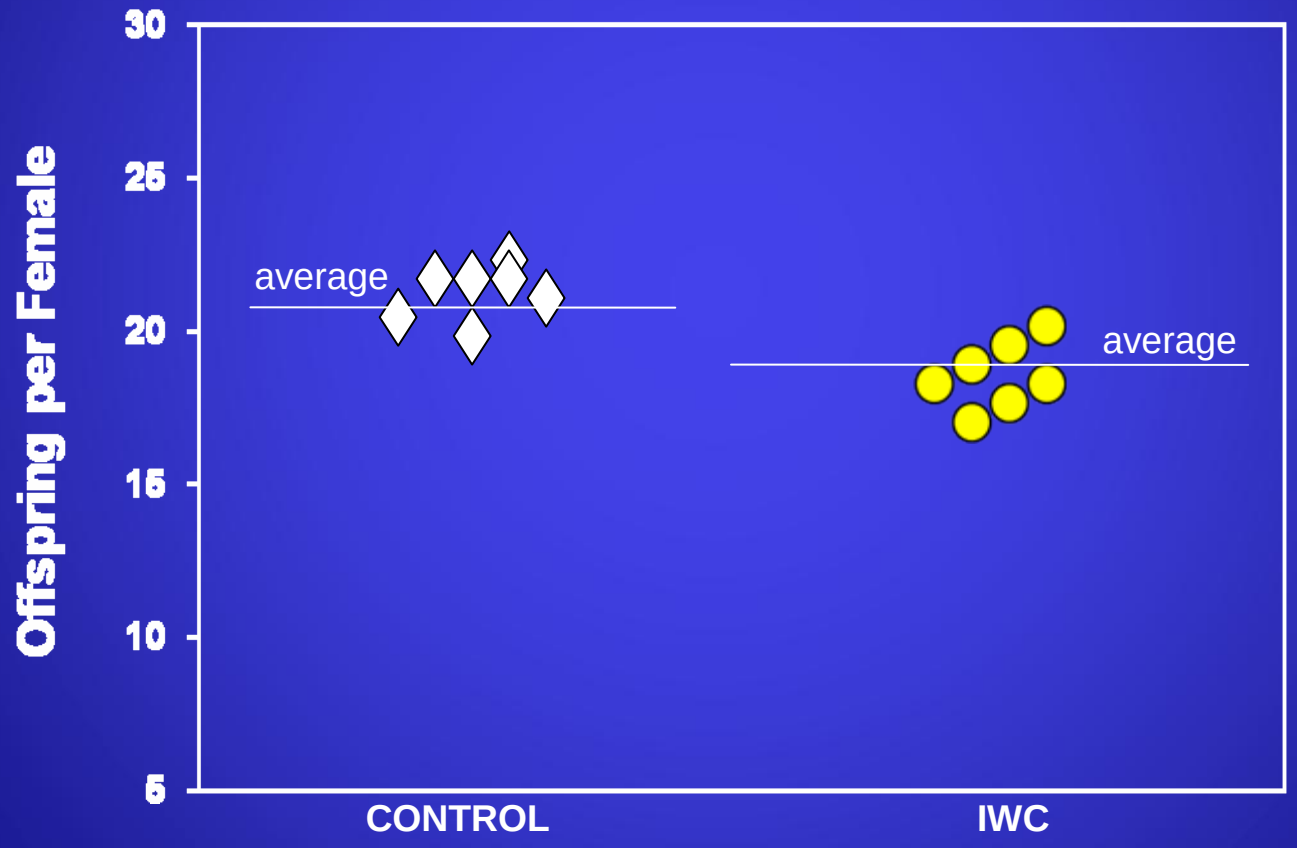
Effluent Limit or Monitoring Trigger

Why new TST approach?

- **To appropriately protect aquatic resources**
  - Declare sample “toxic” when biologically unacceptable
  - Declare sample “not toxic” when biologically acceptable
- **To appropriately respond to stakeholders**
  - Utilize a streamlined statistical approach
  - Address both types of statistical errors
  - Incentive to generate high quality toxicity data
  - Improve confidence in decision making process
- **Technically sound approach**
  - Based on external peer review and internal EPA review

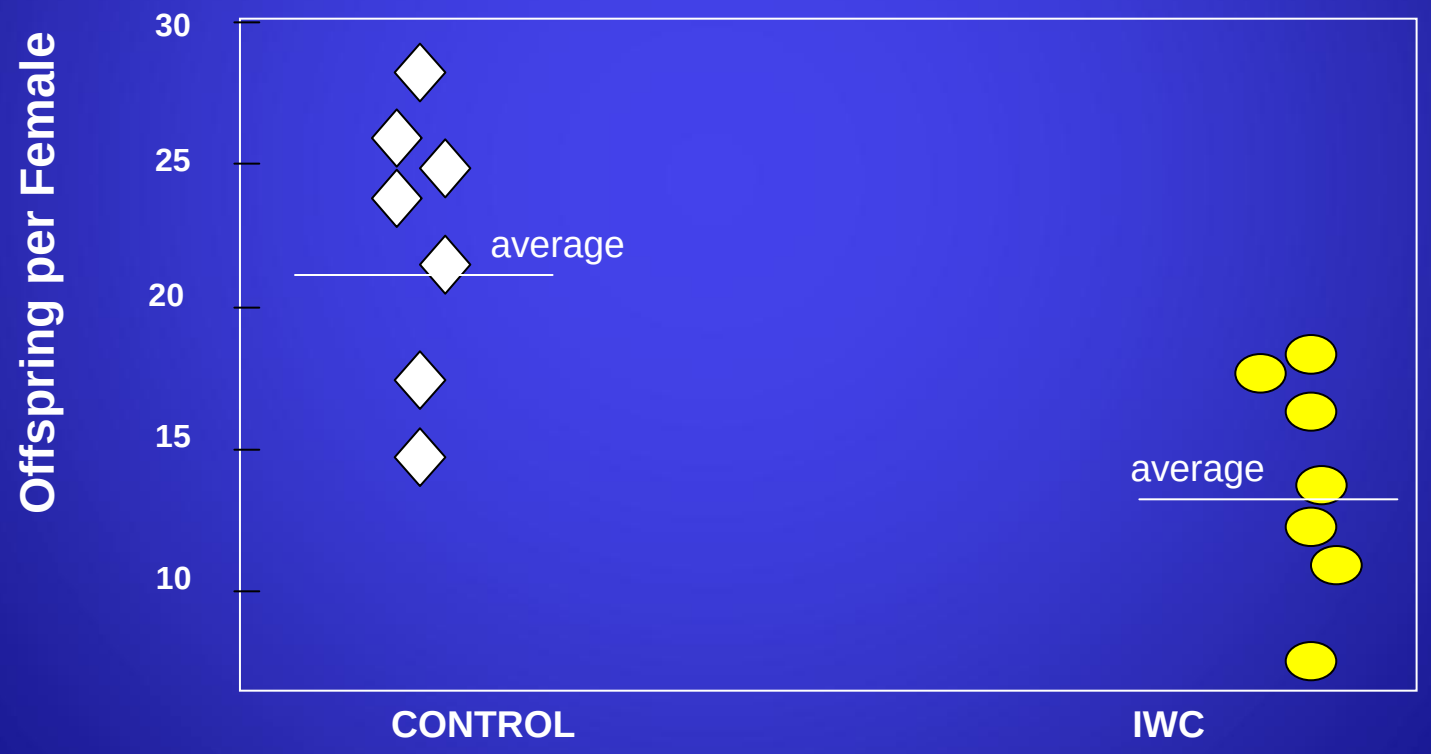
Current analysis approach does not reward high quality test data:

Effluent declared toxic



Current analysis approach rewards poor quality test data:

Effluent declared <u>not</u> toxic



What is TST approach?

- **Another tool - Alternative statistical approach**
  - Used by FDA
  - Builds on previous EPA work
  - Based on research and actual data

- **TST represents 3 paradigm changes**
  - Two concentration analysis - Determines if IWC sample is toxic
  - Uses Bioequivalence - IWC response needs to be within a specified amount of control response to be not toxic
  - Both statistical error rates are defined

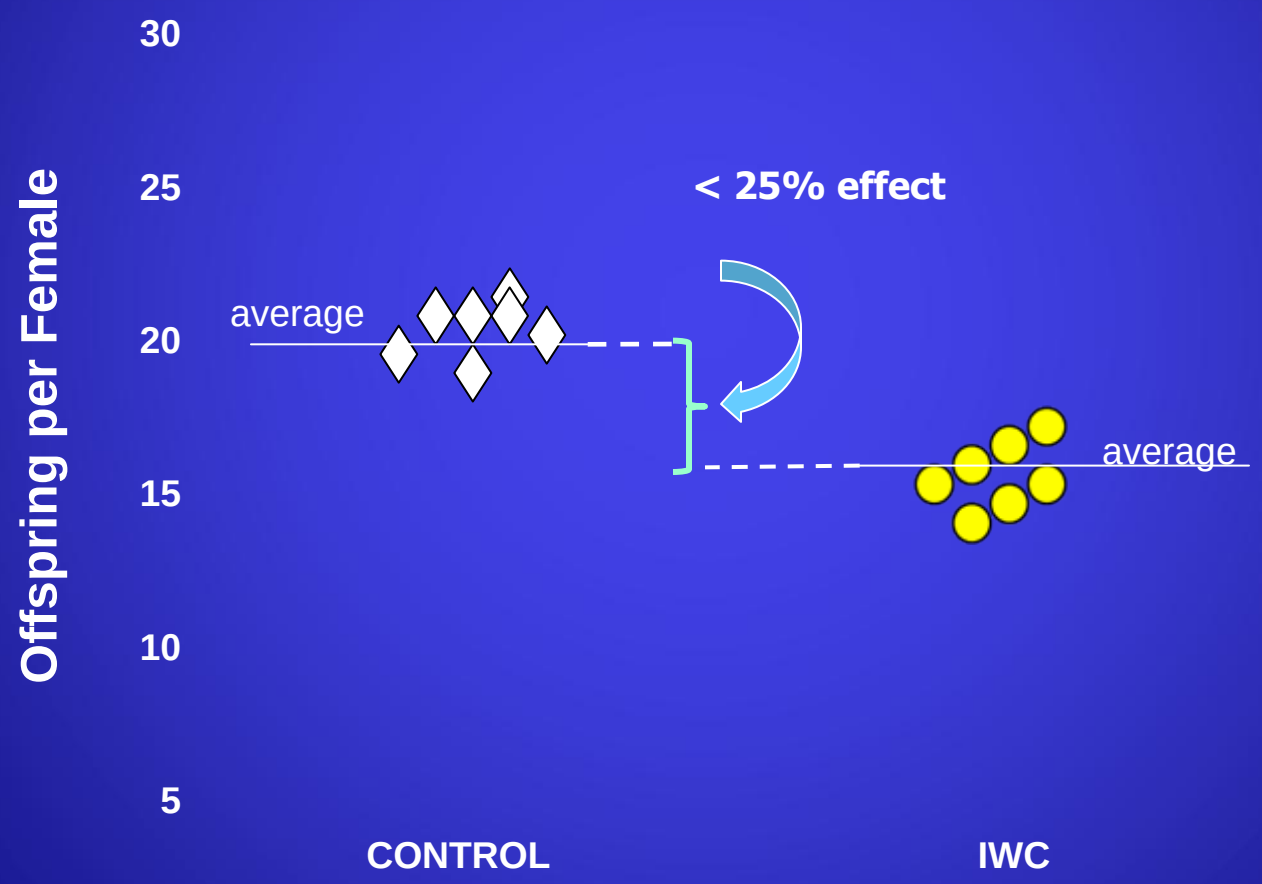
What is unacceptable toxicity using TST approach?

H_0: IWC mean ≤ b · control mean

- b = 0.75 for chronic tests
- b = 0.80 for acute tests

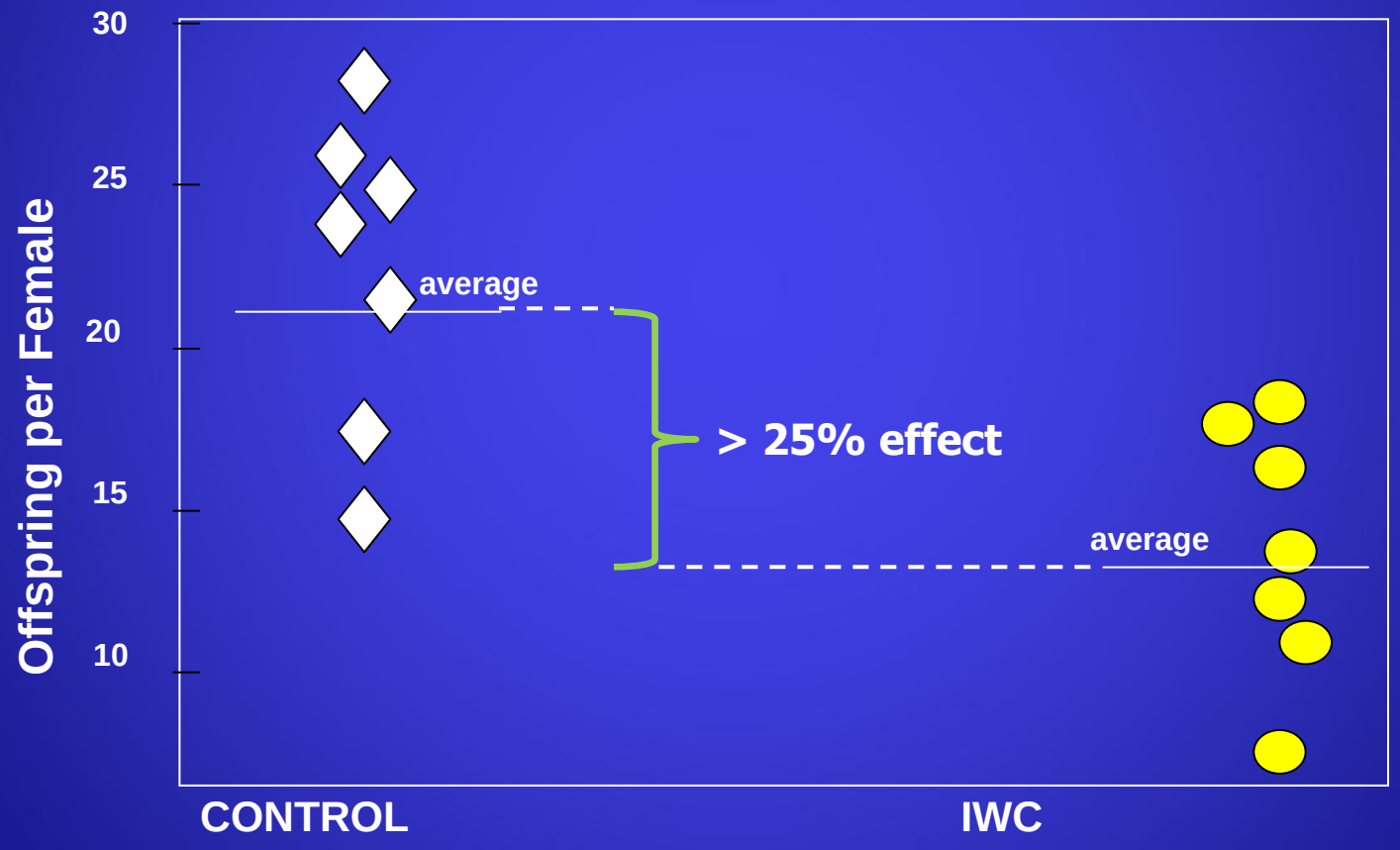
TST rewards high quality data:

Effect < 25%, effluent declared <u>not</u> toxic



TST does not reward poor quality data:

Effect ≥ 25%, effluent declared toxic



Flowchart is Simple

Conduct WET test

↓

Transform percent data

↓

Calculate *t* value

↓

Calculated *t* value > critical *t* value?

↓ YES ↓ NO

NEW TST Calculator_20100907.xls [Compatibility Mode] - Microsoft Excel

Home Insert Page Layout Formulas Data Review View

V36

Test ID	
Test Date	
Test Duration	

Control		Effluent	
Number of Organisms Exposed per Replicate	Response (Final Count, Weight, Length, etc.)	Number of Organisms Exposed per Replicate	Response (Final Count, Weight, Length, etc.)
	0.554		0.351
	0.625		0.415
	0.517		0.48
	0.411		0.469
	0.531		0.463
	0.525		0.346

Arcsine SqRt Transformation	
Control	Effluent

Test Species?	P. promelas (fathead minnow)
Chronic or Acute?	Chronic
Endpoint?	Growth
Percent Data?	No
b=	0.75
α=	0.25

Chronic methods	
b=0.75	
α=0.2	for *C. dubia*
α=0.25	for *P. promelas, C. variegatus, M. berryllina, A. affinis,* and *S.*
α=0.15	for A. bahia
α=0.05	for *A. punctulata, C. gigas, D. excentricus, H. refuscans, M. pyrifera, Mytilus sp., and S.*

Acute method
b=0.8
α=0.1

Note: For acute tests with optional 2 or 4 replicates (e.g., fathead minnow), TST requires 4 replicates.

Conduct WET Test
↓
Apply arcsine square root transformation for percent data
↓
Calculate t value using TST Welch's test
↓
Calculated t-value > table t-value?

↓	↓
YES	NO
"PASS"	"FAIL"
IWC is NOT Toxic	IWC is Toxic

Statistic	Control	Effluent
Mean of Raw Data	0.52717	0.42067
Mean used in Calculation	0.52717	0.42067
Variance of Raw Data	0.00478	0.00362
Variance used in Calculation	0.00478	0.00362
Standard Deviation of Raw Data	0.06915	0.06018
CV of Raw Data	0.13117	0.14306
n	6	6

If no variance, then use the percent difference. For chronic, a % difference <25 is considered "Passing". For acute, a % difference <20 is "Passing".

% Effect at IWC	20.20233955

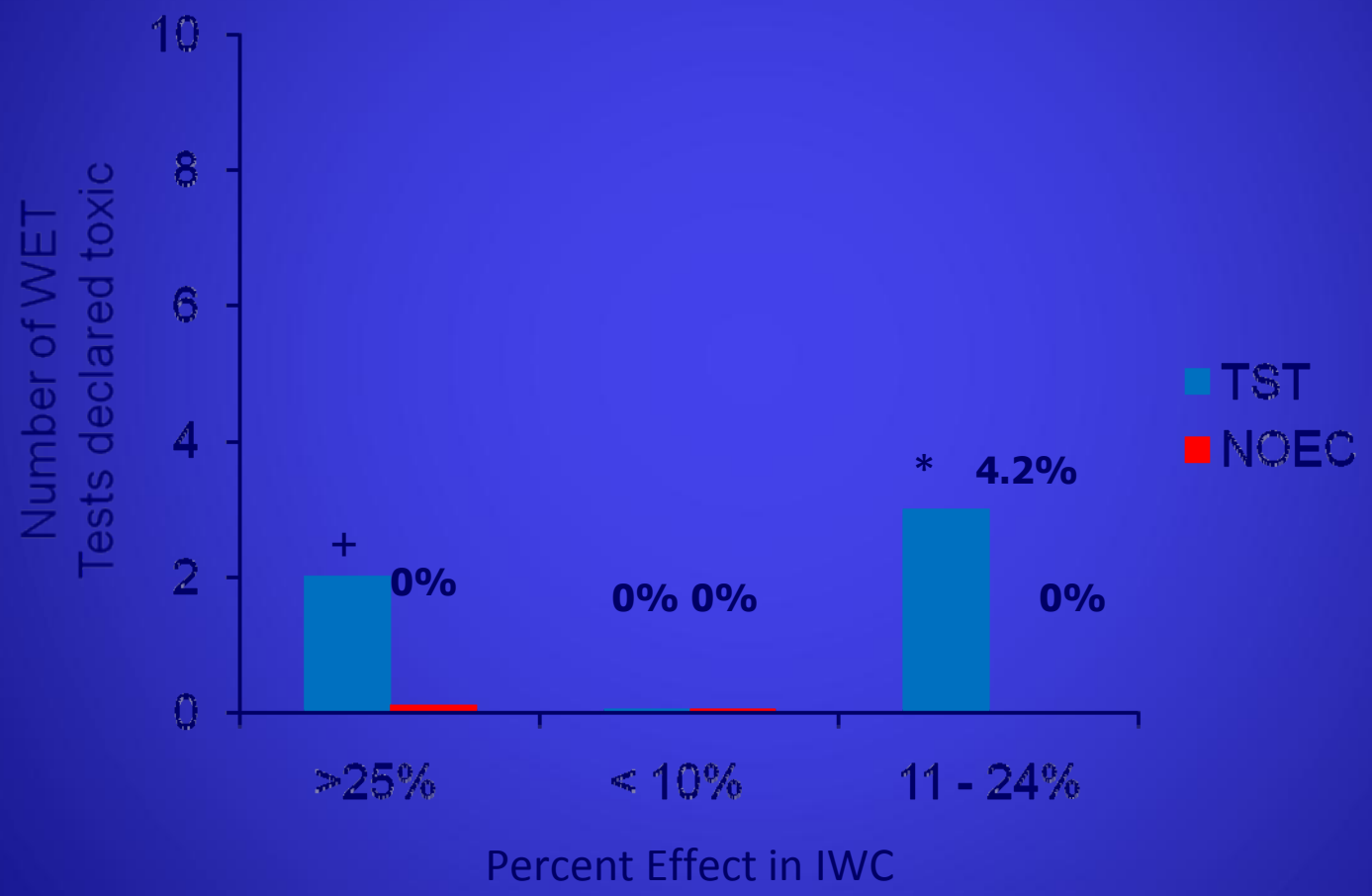
Calculated t-value=	0.7798
v=	10
Table t-value=	0.6998

Click here to save data to "Statistics" worksheet

TST Benefits

- Two concentration test – more cost effective
- Improved transparency of regulatory decisions
- Both error rates are incorporated
- Positive incentives to generate higher quality WET data
- Streamlines the data and interpretation process

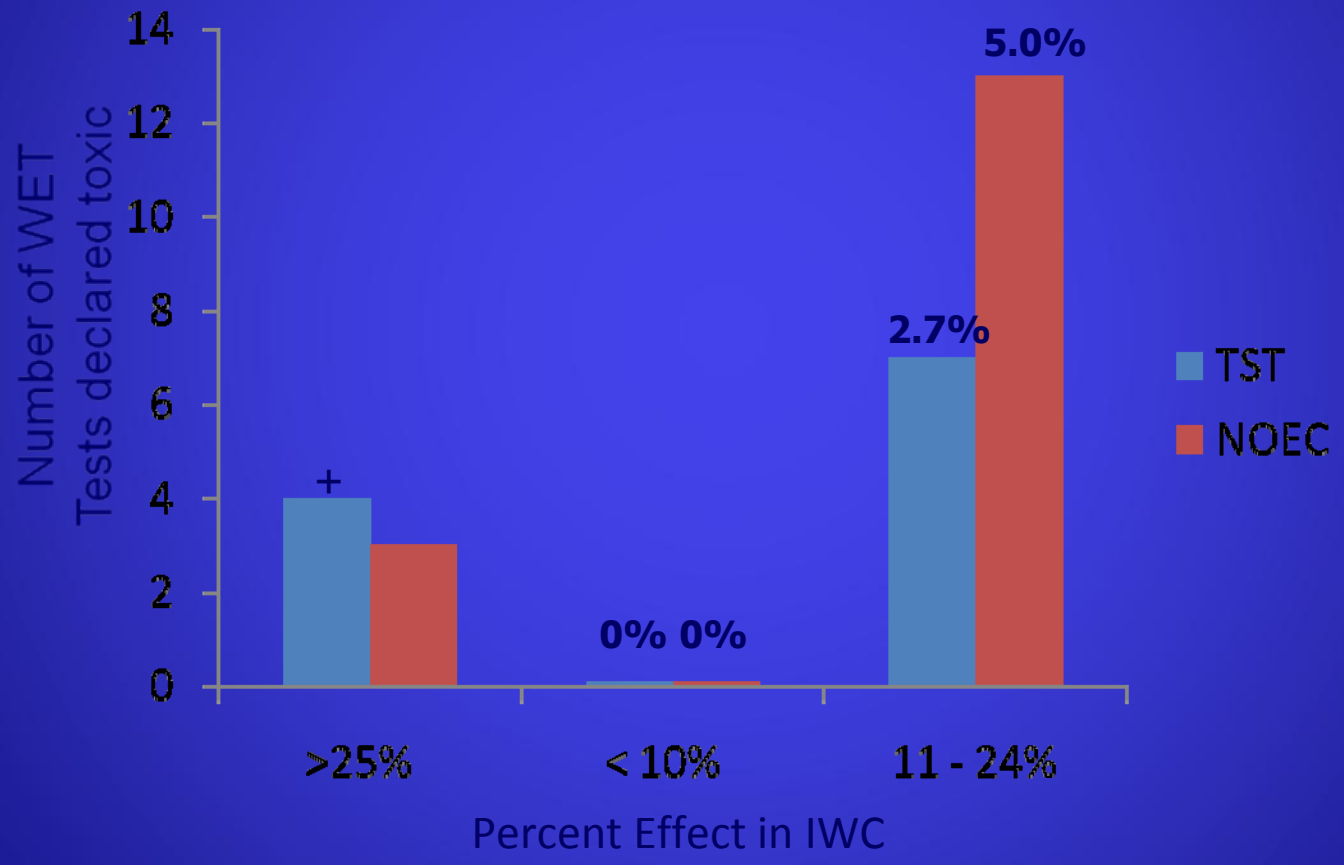
LACSD Ceriodaphnia reproduction N = 72 tests



* All 3 tests had within-test effluent and/or control coefficients of variation exceeding the 75th percentile for this test endpoint

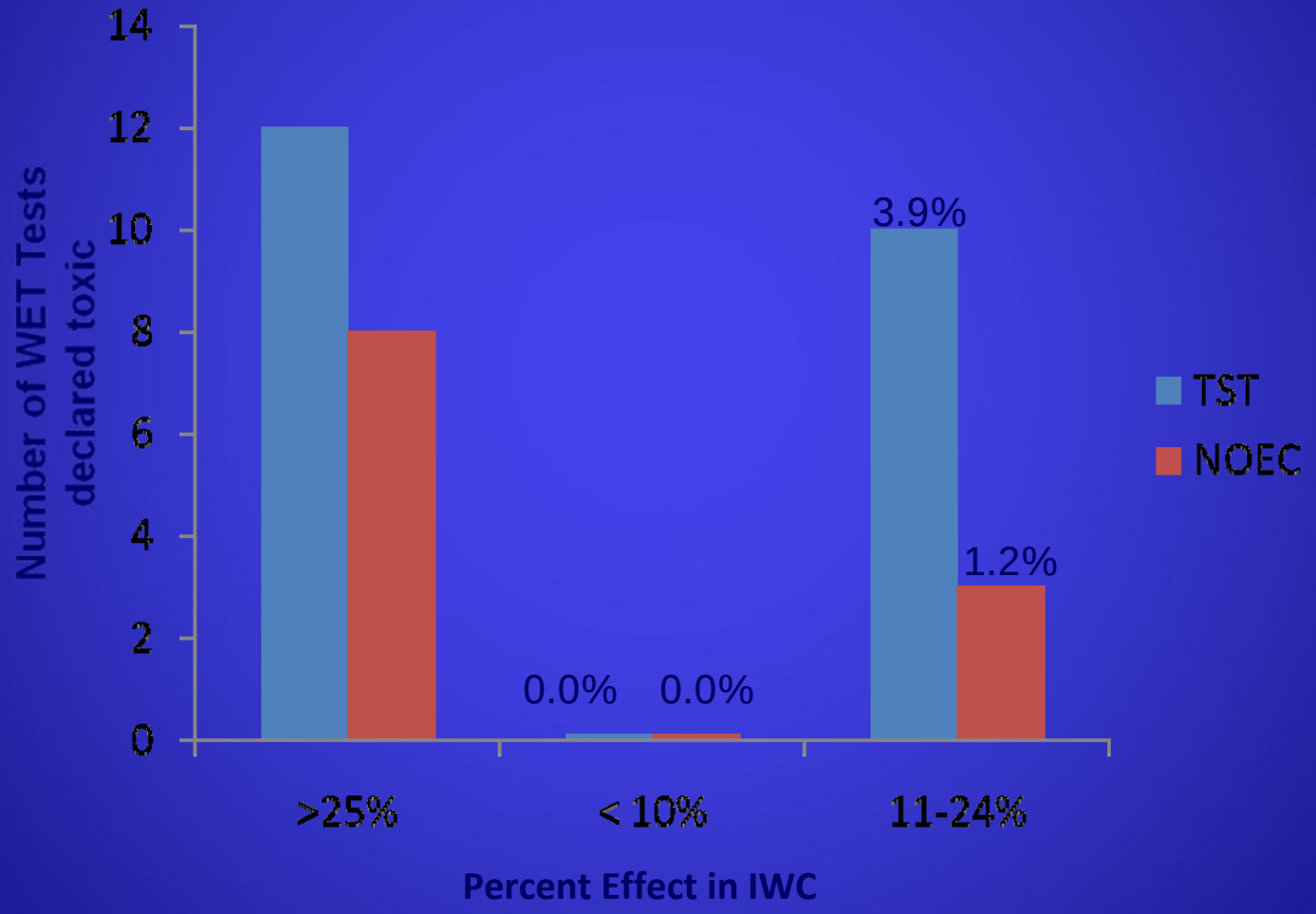
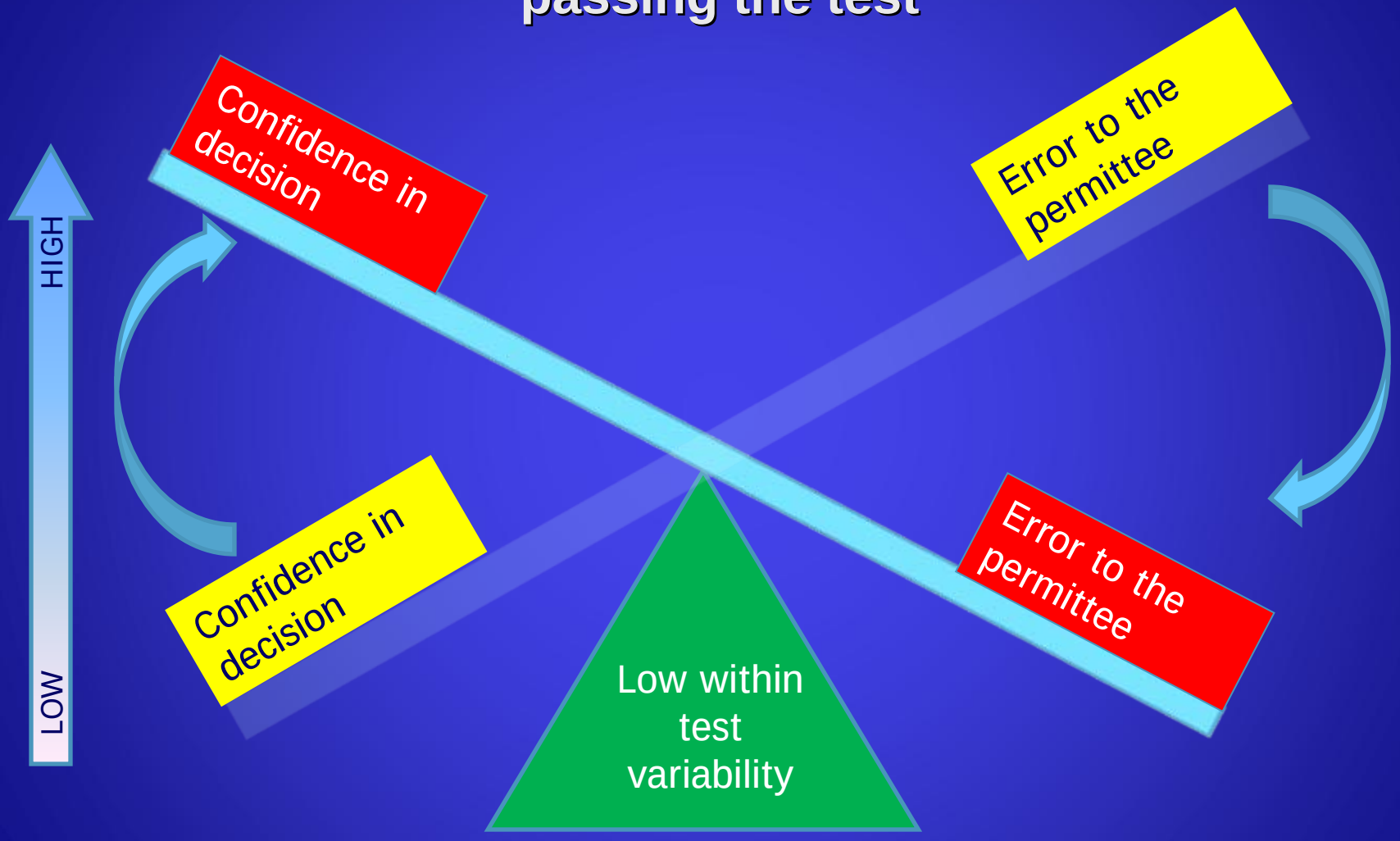
\+ All tests with > 25% effect were declared toxic using TST in this dataset

LACSD fathead minnow growth
N = 257 tests



+ All 4 tests with > 25% effect were declared toxic using TST in this dataset. 3 of the 4 tests were declared toxic using NOEC

LACSD fathead minnow survival N = 257 tests



By increasing test data quality, error to the permittee is reduced and there is a much higher probability of passing the test