EPA's Test of Significant Toxicity: Impact on the Permit Compliance of Honolulu's Wastewater Treatment Plants

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Abstract -The U.S. Environmental Agency has developed an alternative statistical approach, based on bioequivalence, to address the limitations associated with the use of No Observed Effect Concentration (NOEC) for interpreting whole effluent toxicity (WET) data. The traditional hypothesis testing may deem small effects with no environmental consequence as significant or fail to detect unacceptable toxicity when data precision is low. The test of significant toxicity (TST) corrects these anomalous conclusions by determining whether there is a biologically relevant difference between the effluent at the critical concentration and the control and by integrating both Type I and Type II error rates in the statistics. This new approach is under consideration by the State regulatory agency for inclusion in permits issued to Hawaii dischargers. Such change in the permits could affect the City & County of Honolulu's regulatory compliance. The City operates four wastewater treatment facilities that are required to monitor effluent toxicity with EPA chronic protocols using *Ceriodaphnia dubia* and a local sea urchin, *Tripneustes gratilla*. Data sets from 820 tests meeting acceptability criteria, collected over a five year period of WET monitoring of the City's treated discharges, were analyzed using the NOEC method and the TST approach. The NOEC method, even after subjecting to a percent minimum significant difference limits-based review, declared a remarkably greater number of toxic incidences particularly with the more sensitive urchin fertilization method. On the other hand, the TST approach found toxic effects in C. *dubia* reproduction data from some effluent samples that the NOEC method declared acceptable. Conclusions derived using these two statistical approaches on WET data from wastewater facilities with different levels of treatment are compared.

Keywords - Whole Effluent Toxicity   Hypothesis Testing   No observed Effect Concentration

INTRODUCTION

The NPDES permitting requirements under the Clean Water Act include the routine performance of Whole Effluent Toxicity Tests (WET) to determine compliance with set limits. Such compliance is hinged on statistical concepts to determine significant effects of an effluent treatment compared to a control [1,2]. Determination of effects are based on either a point estimate relative to a predetermined value such as 25% or the No Observed Effect Concentration (NOEC), an inference drawn from the hypothesis testing of WET data. The traditional NOEC test design seeks to falsify the null hypothesis that the sample is as good as the control. To ensure certainty in concluding toxic effects, an α error rate of 0.05 is applied. However, a decision criterion for false negative conclusions is not defined by EPA for WET data analysis. This test design has given rise to the following limitations: small differences of no environmental consequence may be found significant when data variability is low; and, there is insufficient protection against concluding the absence of toxicity due to poor test sensitivity arising from high data variability [3].

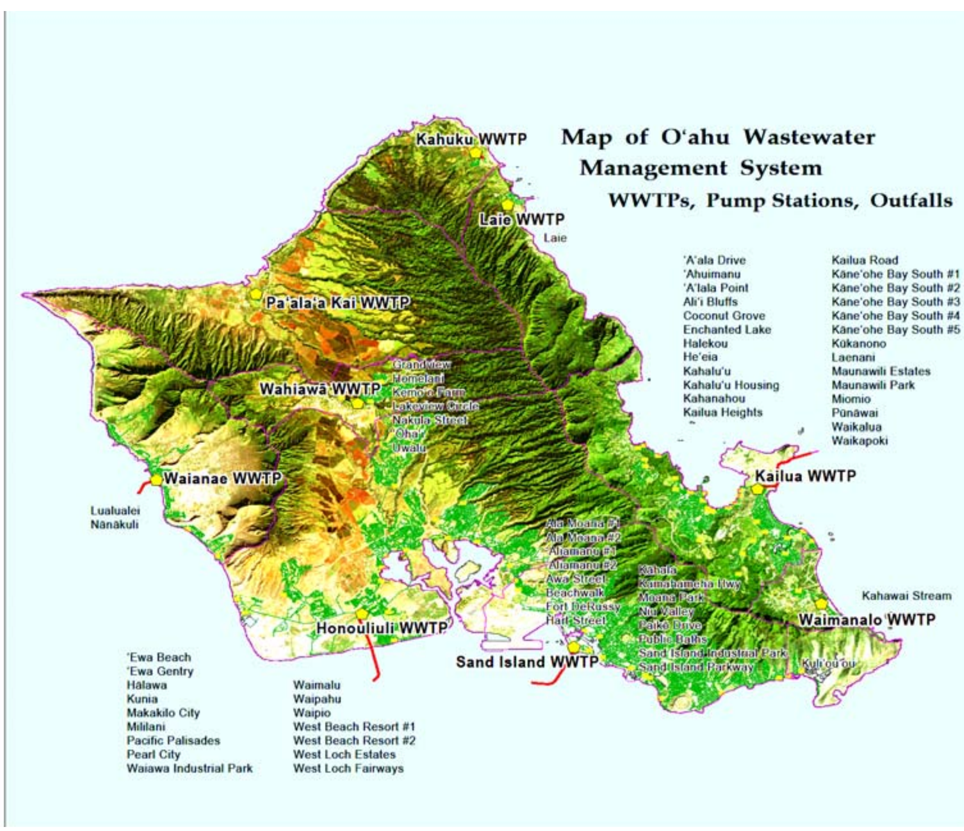
USEPA has very recently reshaped the philosophy and approach for interpreting WET test results in an attempt to minimize the errors inherent in the traditional NOEC approach. The Test of Significant Toxicity (TST), which is structured to test for an effect size of practical significance, is another option for the analysis of WET data [4]. These data should be generated by tests performed in accordance with EPA methods and verified to meet test acceptability criteria such as control performance and a valid dose-response pattern [5]. TST is then applied as a two-concentration data analysis that seeks to determine whether the sample at the critical concentration such as the Instream Waste Concentration (IWC) is not substantially inferior to the control. Toxicity is concluded when the sample mean at the critical concentration is lower than the control mean by a biologically relevant difference. This difference, a risk management decision, is defined to be 20 % for acute tests and 25% for chronic tests.

The values for α, the negative error rates, vary from 0.05 to 0.25 depending on routine control precision such that at least 75% of toxic samples are declared as toxic. False positive error rate is set at 0.05 when the sample at the critical concentration displays an effect of 10%.

The State Department of Health, the permitting authority for wastewater dischargers in Hawaii, is in the process of incorporating the TST approach in the Hawaii Administrative rules for permit compliance determinations. The objective of this paper is to determine how such change in the permit could impact the compliance of four Wastewater Treatment plants operated by the City and County of Honolulu.

MATERIALS and METHODS

The City & County of Honolulu collects between 125-130 million gallons daily (mgd) of wastewater from homes, workplaces, and industrial users on Oahu. The wastewater is delivered to nine wastewater treatment plants spread over the island (Fig. 1). Effluent treated to tertiary level at Wahiawa WWTP goes to the Wahiawa Reservoir. Effluents from Waimanalo, Kahuku, Laie, and Paalaa Kai treatment plants are injected into the ground. Sand Island, Honouliuli, Kailua, and Waianae treatment plants discharge treated wastewater to deep ocean outfalls. Sand Island WWTP, the largest wastewater treatment plant on Oahu, is a primary treatment facility. It processes an average of approximately 78 mgd of wastewater. The Honouliuli WWTP provides both primary and secondary treatment. The plant currently processes approximately 26 mgd daily, with 13 mgd going to secondary treatment. Both the Waianae WWTP and Kailua Regional WWTP are secondary treatment facilities with the former handling an average of 3.6 mgd of wastewater and the latter about 12 mgd.



These four WWTP's operate under NPDES Permits that authorize the City and County of Honolulu to discharge treated wastewater to receiving waters of the Pacific Ocean through deep ocean outfalls in accordance with the effluent limitations and other monitoring requirements. Compliance with WET limits is determined by monthly short-term chronic toxicity tests using *Ceriodaphnia dubia*, EPA method 1000.2 [1] and a Hawaiian sea urchin species, *Trypneustes gratilla* [2,6] . Chronic Toxicity discharge limitation is expressed in TUc which is equivalent to the reciprocal of NOEC. Violation of the discharge limitation increases the monitoring frequency to either weekly or biweekly until six consecutive compliance events are achieved.

NOEC endpoints of toxicity tests are calculated using macros in Excel, according to flowcharts for statistical analyses as described in EPA manuals, with Dunnett's procedure for normal and homogeneous data or with Steel's Many-one Rank test for non-parametric data. Statistical outcomes are accepted, rejected, or modified after reviewing the control coefficient of variation (CV), percent minimum significant difference (PMSD) and dose-response [5,7]. Raw WET data, calculations and statistical results are stored in WET Excel spreadsheets.

Two Excel programs were prepared to automate the TST statistical procedures for analyzing reproduction data from the 7-day *C. dubia* protocol and fertilization data from the *T.gratilla* bioassay. Replicate results corresponding to the control and to the IWC are copied from the WET Excel spreadsheets and pasted on the TST Excel workbook to obtain pass or fail conclusions.

Data collected over five and a half years from chronic WET tests performed on effluent samples from the above four Wastewater treatment plants were analyzed using the alternative TST procedure. Results from this statistics were then compared with the outcomes using the traditional NOEC method.

RESULTS AND DISCUSSION

Ceriodaphnia dubia 7- day reproduction data

There were 255 valid chronic tests evaluated from the four treatment plants (Figs 2-5). Statistical conclusions drawn from the NOEC approach were reviewed to determine whether results displayed excessive within-test variability To minimize the effects of wide variability and increase test sensitivity, an upper limit criterion of 37% PMSD was applied based on EPA's guidance for the *C. dubia* method 1002.0 [7]. In addition, control CV must be less than 40%. None of these tests displayed toxicity by the traditional NOEC approach.

Of the 255 data sets, 15 samples showed effects greater than 10 % but less than 25 %. The variabilities in the control and in the sample at the IWC of these 15 sets were examined. The control CV's in the four sets that showed toxic effects are within the 50th to 80th percentile range for the *C.dubia* method found by EPA using data pooled across laboratories (Fig. 6). There is a greater likelihood of failures declared by TST the closer the effects are to 25% and with increasing dispersion in the control replicates and sample replicates at the IWC.

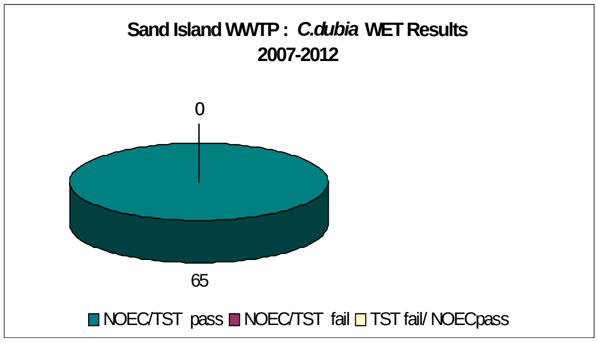


Fig. 2. WET *C. dubia* reproduction data from primary treated effluent.

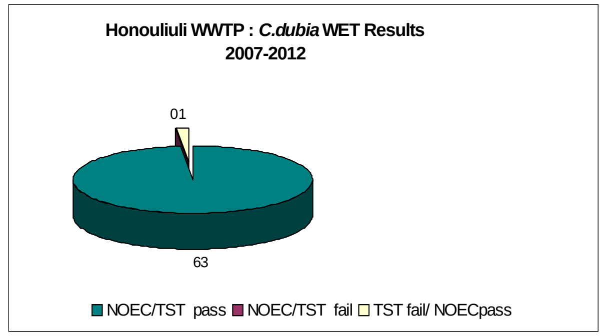


Fig. 3. WET *C. dubia* reproduction data from a mixed primary & secondary treated effluent.

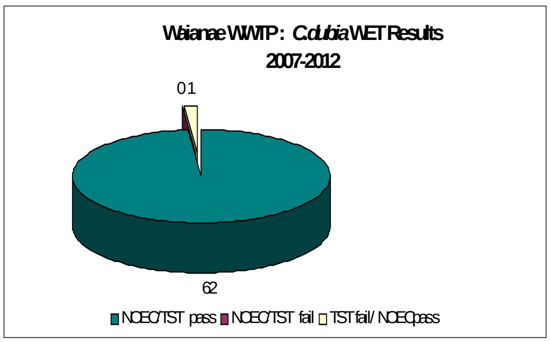


Fig. 4. WET *C. dubia* reproduction data from a secondary-treated effluent.

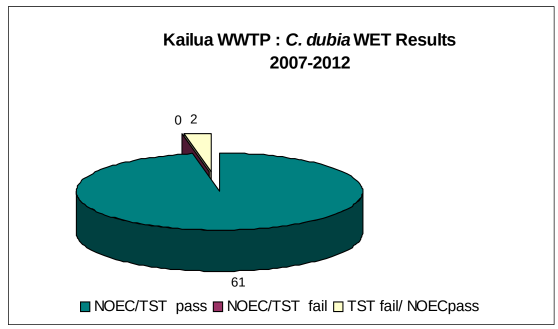


Fig. 5. WET *C. dubia* reproduction data from a secondary- treated effluent.

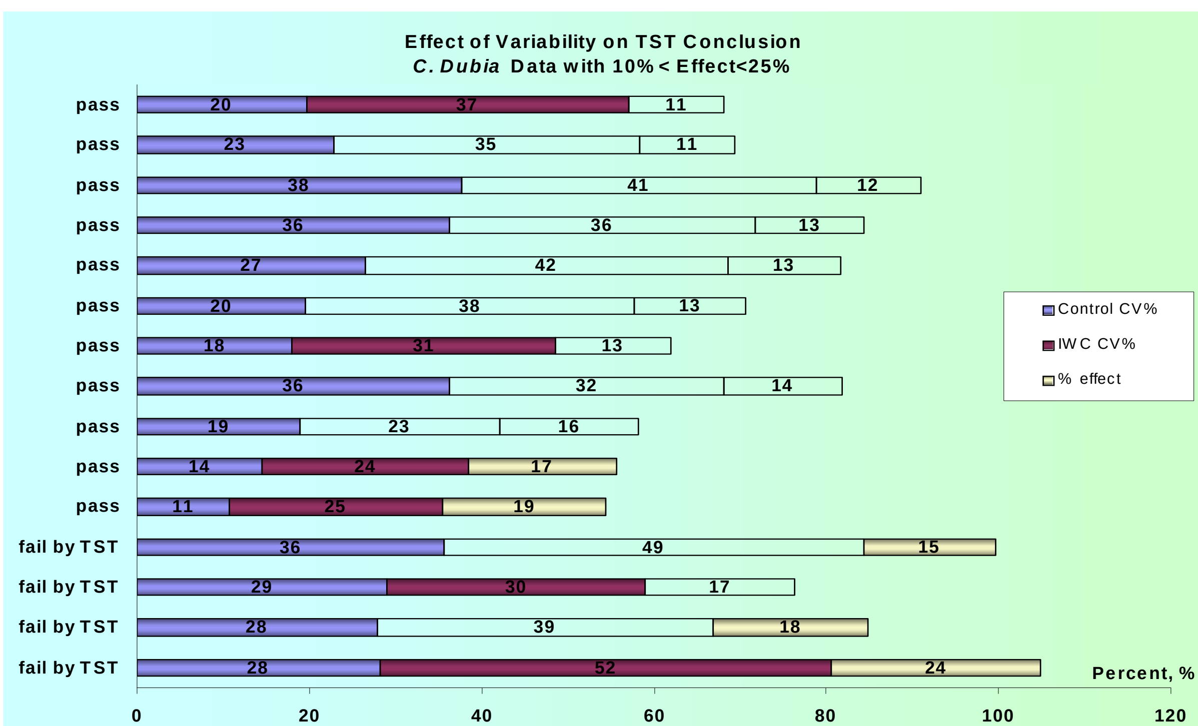


Fig. 6. Variability in Ceriodaphnia dubia reproduction data showing effects between 10 % and 25%.

Tripneustes gratilla Sperm Fertilization Test

There were 563 valid echinoderm chronic tests evaluated from the four wastewater treatment plants. For the NOEC approach, an upper limit variability criterion of 18 % PMSD and a lower limit of 3% PMSD were applied for all tests. These values are the 90th and 10% percentile, respectively, of PMSD results obtained in the City's laboratory for the *T. gratilla* bioassay.

Both approaches agree in declaring no toxicity in 295 tests and toxicity in 200 tests. The NOEC approach declared 73 tests as failures which the TST approach declared as acceptable (Figs. 7-10).

Toxicity conclusions drawn from the NOEC method and from the new approach have a high degree of agreement on effluents that went through primary treatment that is when effects are significantly toxic (Fig. 11). Effluents with low toxicity like those subjected to secondary treatment have high passing rates for both approaches. In this case about 50% of samples declared toxic by NOEC were deemed acceptable by TST. For effluents with borderline toxicity, such as those coming from mixed primary and secondary levels of treatment, about 58% of samples declared toxic by NOEC were found acceptable by TST.

With the TST approach, samples with effects exceeding the 25% RMD were deemed unacceptable. When effects are below 25% but greater than 10%, high variability in control replicates and in sample replicates at the IWC increases the chance of failures. Effects less than 10% are declared acceptable (Fig. 12).

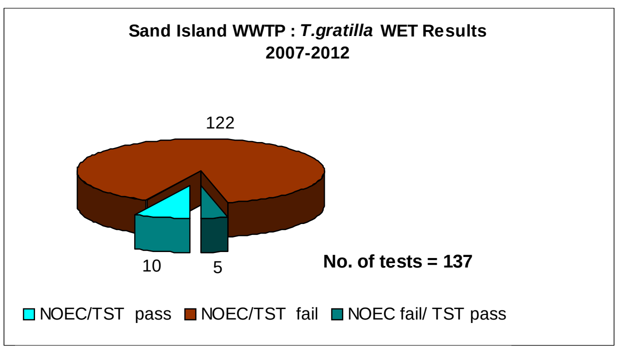


Fig. 7. WET data from sperm fertilization bioassay of primary treated effluent.

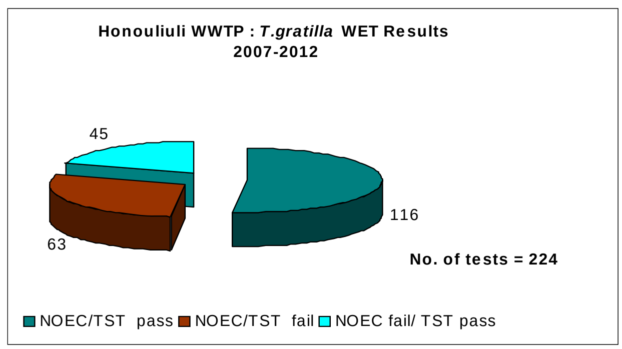


Fig. 8. WET data from sperm fertilization bioassay of mixed primary and secondary effluent.

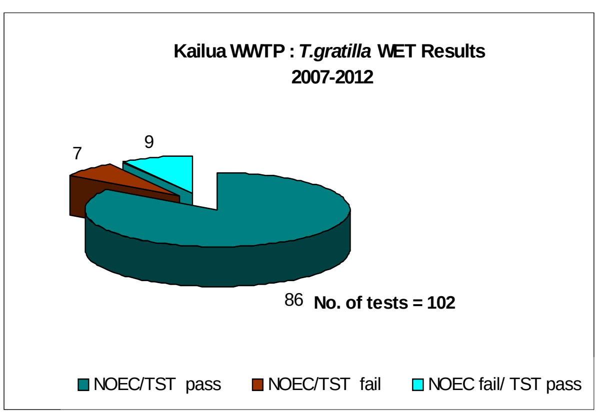


Fig. 9. WET data from sperm fertilization bioassay of secondary treated effluent.

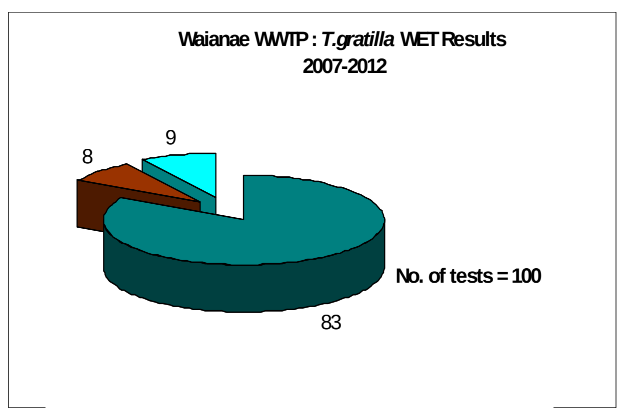


Fig. 10. WET data from sperm fertilization bioassay of secondary treated effluent.

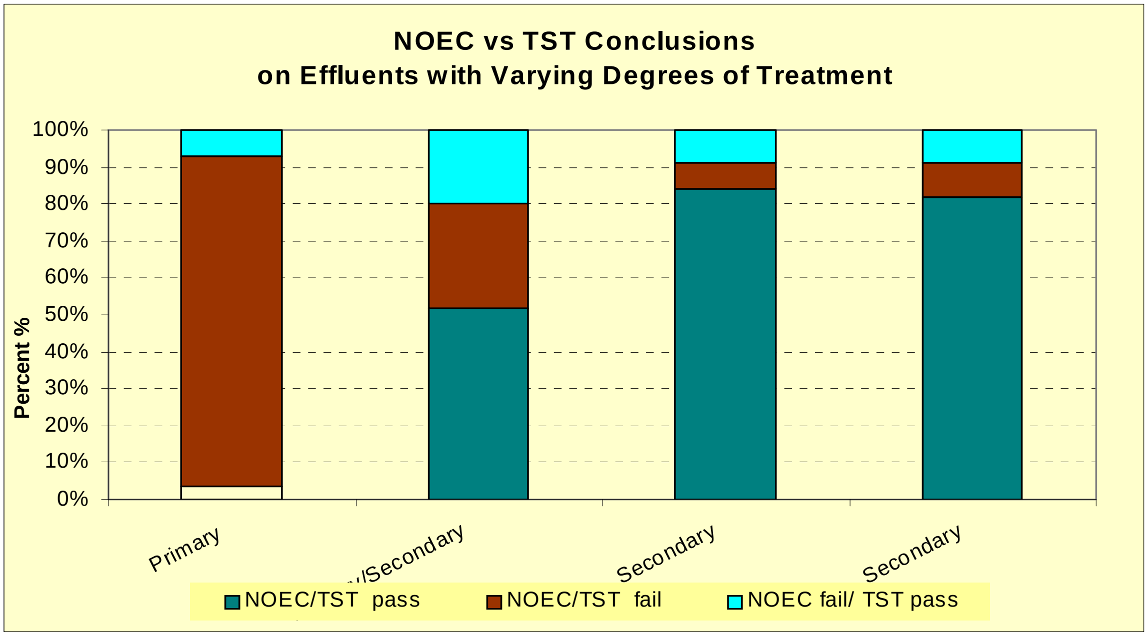


Fig. 11 . Comparison of conclusions drawn from NOEC and TST when applied on *T. gratilla* WET data obtained from effluents subjected to different treatment levels.

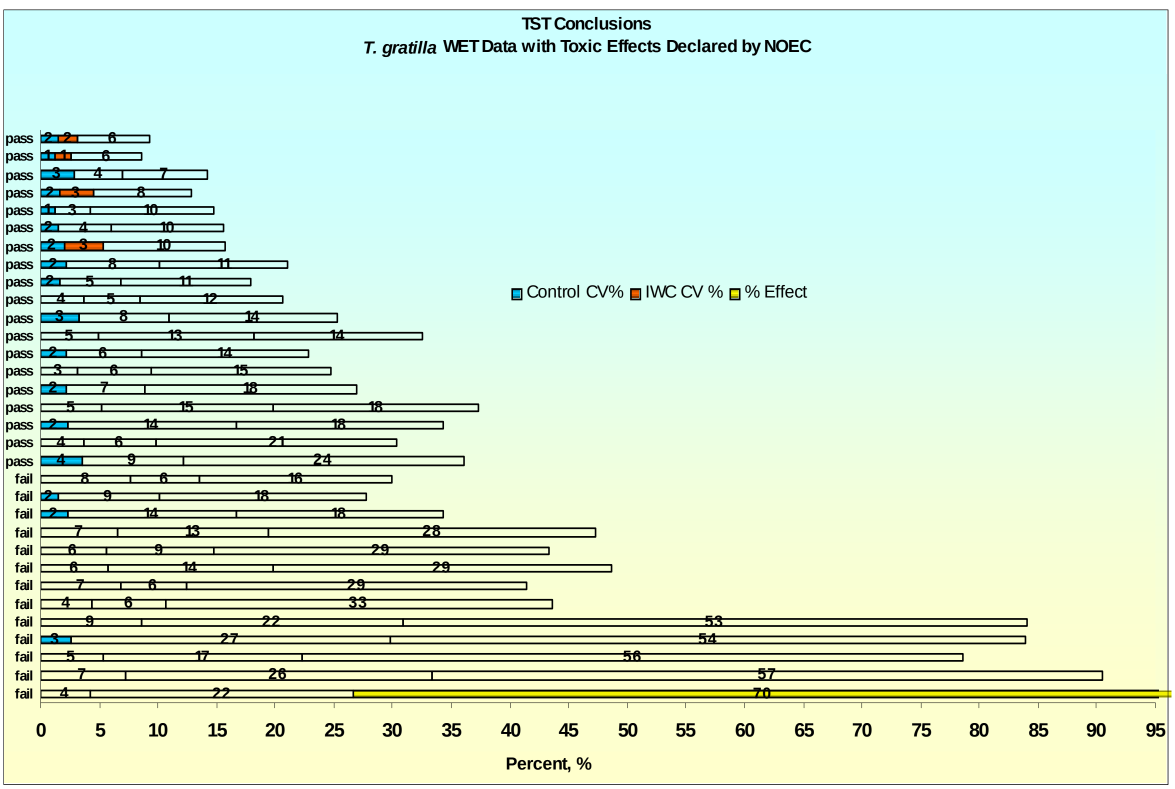


Fig. 12 . Effect of data variability on TST conclusions when applied on WET data from secondary- treated effluents deemed toxic by NOEC.

CONCLUSION

This paper has shown that TST analysis of WET data generated from tests conducted on discharges by the C&C of Honolulu's WWTP's modulates the more significant flaws of the NOEC approach.

Toxic effects of effluents on *C. dubia* reproduction are difficult to detect with the NOEC approach because of the inherent within test variability of this chronic WET test. The alternative TST procedure controls false negatives and identifies toxicity that may have potential adverse environmental effects.

The failures declared by TST in this study were very rare excursions caused by an episode of unusually poor *C. dubia* culture performance. While blocking by parentage minimizes within-test variability, the effect of limited fecundity or mortality of even a single organism may be remarkable. For this reason, there must be an extremely thorough oversight of laboratory protocols to ensure consistent organism vigor. In addition, increased replication in the control and in the sample at the IWC may be adopted to decrease variance.

Compliance with WET monitoring permit values using the *T. gratilla* sperm fertilization method can be tough because very small differences from the control are often deemed as significant effects by the NOEC approach. TST significantly reduces false positives conclusions.

Consequently, with this new statistical alternative, the City will have a significant decrease in the number of WET permit non-compliance events , unnecessary accelerated monitoring, and toxicity reduction investigations

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