# Effect of Microtox® Reagent Reconstitution Age on the Variability of Analytical Results from the Microtox® Assay

# Final Report

to

Western Canada Microtox Users Committee

by

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# EXECUTIVE SUMMARY

This report presents the results of a study into the effect of reconstitution age of Microtox® reagent on the sensitivity and reproducibility of the Microtox assay. Additionally, the study dealt with the effect of lot to lot variation on the sensitivity and reproducibility of the Microtox assay. Four different samples (zinc sulphate, copper sulphate, phenol, and sodium dodecyl sulphate) were evaluated at seven different reconstitution times from 0.5 to 24 hours (0.5, 0.75, 1.0, 2.0, 4.0, 8.0, 24.0) using the same vial of reagent. Each sample was analyzed in triplicate with three different lots of reagent. Statistical analysis of results focused on 15 minute $EC_{50}$ data only.

Several conclusions were drawn from this study. Firstly, it was found that there were differences in toxicity over time with different compounds. With the four compounds tested, all three possibilities of an increase, a decrease, and no change in toxicity with reagent age were observed. Secondly, it was observed that due to the increase or decrease in toxicity with aged reagent, data obtained from reagent which is older that 4 hours should be regarded cautiously, if at all. Finally, it was observed that there is lot to lot variation in toxicity results for some of the compounds tested.

Several recommendations were made based on the results of this study.

1. Each lot of reagent should have quality control testing performed using standard reference toxicant(s).
2. Bench recording sheets or computer recording programs should include the time of reconstitution of reagent as well as the start time of the assay.
3. Microtox® test reagent should generally not be used for testing after 4 hours from time of reconstitution. Any assay performed after this time may yield unpredictable results due to reagent age and not the toxicity of the sample being tested. Results obtained from reagent used after 4 hours needs to be evaluated cautiously, with the use of an appropriate control toxicant run in parallel.
4. WCMUC adopt these points within their standard operating procedure and as part of their ongoing inter-laboratory quality control program.

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# A. INTRODUCTION

The Microtox® basic test assay has been used to determine the toxicity of such diverse samples as oil sands tailings water (MacKinnon and Retallack, 1982; Baddaloo, 1986), landfill leachates (Cameron *et al.*, 1982; Baker, 1985;), pulp and paper effluent (Blaise *et al*., 1987), drilling fluids (Strosher, 1984) and several industrial effluents (Bulich *et al*., 1981; Vasseur *et al*., 1984; Coleman and Qureshi, 1985). It has been generally accepted that there is no universal bioassay which can be used in every situation for toxicity testing and that a battery of screening tests should be used. Due to the ease of use, low cost, sensitivity, and reproducibility, the Microtox test system has often been used as a pre-screening tool.

Several refinements to the Microtox basic test assay have been made since its inception. One of the first was the use of colour correction to determine the toxicity of highly coloured samples (Beckman, 1982). In a study on the refinement of the Microtox assay, Qureshi *et al*. (1984) made several recommendations for the assay. First, the sample pH should be considered part of the assay since adjustments cause changes within the sample. Secondly, the 15 minute $EC_{50}$ should be accepted as the standard endpoint for assaying samples, particularly those containing metallic ions. These two recommendations have found general acceptance. The third major recommendation, that freshly reconstituted reagent be used for testing, does not seem to have been generally accepted. This may be due to the expense of the reagent, time constraints within a testing laboratory, or other unknown factors. A recent publication lists the following bacterial factors as causes of variability of the toxicity test results: age of freeze-dried bacteria, bacterial storage and conservation, reconstitution procedure, age of the reconstituted reagent, and temperature equilibration time (Ribo, 1997). However, Ribo did not present any results which would address these issues.

To address the question of the effect of reconstitution age, this study investigated the effect that the reconstitution age of the Microtox® test reagent has on the sensitivity of the Microtox assay. This study investigated the effect of reconstitution age of the Microtox® test reagent on the sensitivity of the Microtox assay when tested against an array of compounds and the effect that lot to lot variation may have on the effect of reconstitution age of the Microtox® test reagent.

# B. OBJECTIVES & SCOPE

## 1. Objectives.

The objectives of this study were to:

1. investigate the effect of reconstitution age of Microtox® test reagent on the results obtained with the assay,

2. investigate the effect of lot to lot variation of Microtox® test reagent, and
3. provide a possible recommendation as to the maximum time after reconstitution for use of the Microtox® test reagent.

### 2. Scope

The scope of the work plan for each of the components was as follows. This study evaluated four different samples at seven different reconstitution times between 0.5 to 24 hours (0.5, 0.75, 1.0, 2.0, 4.0, 8.0, 24.0) using the same vial of reagent. The samples consisted of two metallic salts, copper sulphate and zinc sulphate and two organics, phenol and sodium dodecyl sulphate (SDS) which is also called sodium lauryl sulphate. Each sample was analyzed in triplicate. This series was repeated with three different reagent lots, AVC009-2, AVC009-3, and AVC010-3. Quality control data of each lot of reagent as well as quality control charts for phenol and zinc sulphate as supplied by AZUR Environmental are presented in Appendix 1. Statistical analysis of results focused on 15 minute $EC_{50}$ data only since this is the time point most commonly used for reporting of Microtox testing results.

## C. PROCEDURES

### 1. Microtox® Analysis

The analytical procedure for using the Model 500 analyzer for Microtox® testing has been described previously (Gaudet, 1994). The assays were performed using the standard 82% assay (AEC variation) (Gaudet, 1994). Timing for the assays was determined as follows. Reagent was reconstituted, the clock was started and reagent was allowed to sit in the reagent well for 15 minutes. Reagent was then diluted into the testing wells and allowed to equilibrate for an additional 15 minutes. Initial light readings were taken and, at 30 minutes from the time of reconstitution, the toxicant was added to the testing wells. Time was determined as the total elapsed time from reconstitution to the addition of the toxicant, allowing for temperature equilibration. To run additional assays from the same reagent vial, the reagent was again diluted into testing wells and allowed to equilibrate to run analysis at the additional time points. Reagent was added to diluent 15 minutes prior to running an assay to allow for temperature equilibration.

### 2. Statistical Analysis

For the statistical analysis, two questions were asked. Firstly, "is there a difference between the toxicity values at the various time points?" In order determine this statistical comparison, a standard time of 1 hour was chosen and

all other results were compared to this standard time. The second question was "is there a difference between the reagent lots at each time point?"

**Differences in $EC_{50}$ values at various time points against the time point '1 hour'**. The statistical model for each reagent was the One-way Analysis of Variances. The general form of the model is given by

$$[EC_{50}]_{ijk} = \mu + \beta_{ij}*[\text{Time(Hour)}]_{ijk} + \varepsilon_{ijk},$$

where

$\mu$ = overall mean,

$\beta_{ij}$, j = 0.5, 0.75, 1, 2, 4, 8, 24 = main effect of Time for each
i = ACV009-2, ACV009-3, ACV010-3,

$\varepsilon_{ijk} \sim$ iid $N(0,\sigma^2)$ with k = 1, 2, 3.

For each reagent i, the mean values of $EC_{50}$ of various Time points were compared against the mean $EC_{50}$ of normal time '1 hour' using Dunnett's comparison method in GLM procedure in SAS/STAT software (SAS Institute Inc. 1990a, 1990b, 1994).

**Differences of $EC_{50}$ values between reagent lots at each time point**. The objective of this statistical analysis was to examine whether $EC_{50}$ values of different reagents are significantly different at each time point. The statistical model for this analysis was the One-way Analysis of Covariance. The general form of the model is given by

$$[EC_{50}]_{ijk} = \mu + \alpha_i + \beta_j*[\text{Time(Hour)}]_{ijk} + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where

$\mu$ = overall mean,

$\alpha_i$, i = ACV009-2, ACV009-3, ACV010-3 = main effect of Reagent,

$\beta_j$, j = 0.5, 0.75, 1, 2, 4, 8, 24 = main effect of Time,

$(\alpha\beta)_{ij}$ = interaction between Reagent and Time,

$\varepsilon_{ijk} \sim$ iid $N(0,\sigma^2)$ with k = 1, 2, 3.

After the model was fitted using GLM procedure in SAS/STAT software from SAS institute (SAS Institute Inc. 1990a, 1990b, 1994), the adjusted means of $EC_{50}$ for each reagent within each time point were compared against each other using Tukey's method.

## D. RESULTS AND DISCUSSION

The results of Microtox testing of each sample will be reported and discussed separately. However, for better understanding, general results are described first. Results of each series of analyses is presented in Appendix 2 – Tables and Figures. The first table in each series lists the $EC_{20}$ and $EC_{50}$ at 5 and 15 minutes for all reagent lots, all replicates, and all reconstitution times tested. The second table

contains information that recalculates the 15 minute $EC_{50}$ results and gives arithmetic mean values for each reconstitution time and reagent lot as well as overall means for each. For phenol, the 5 minute data was also recalculated, since AZUR Environmental quality control data for phenol is given for 5 minute assays. Following the reworked data is a graphical representation of these results. Since some compounds showed a sharp increase or decrease in toxicity at 24 hours and occasionally at 8 hours, there are additional graphs presented with these results removed for zinc (24 hour data removed), copper (24 hour data removed, and 8 and 24 hour data removed), and SDS (24 hour data removed). Next is the statistical summary for each compound indicating the arithmetic means, standard error for each reagent lot and each reconstitution time for the $EC_{50}$ data only. Finally, each section concludes with a table which compares the $EC_{50}$ values at various reconstitution times for different lots to see if there is a difference between the lots. Results presented are adjusted means and standard error of those means and a multiple comparison for each time point between lots.

## 1. Zinc

Table 1 summarizes the $EC_{50}$ and $EC_{20}$ data at 5 and 15 minutes for all zinc sulphate assays. $EC_{50}$ 15 minute results are summarized in Table 2. A graphical representation of 15 minute $EC_{50}$ results is presented in Figures 1 and 2. Statistical results are presented in Tables 3 and 4.

The overall mean $EC_{50}$ 15 minutes for zinc sulphate was 1.291 mg/L $Zn^{+2}$ for all reconstitution times (Figure 1). However, a sharp increase in toxicity was observed at 24 h and these assays had an overall $EC_{50}$ of 0.518 mg/L $Zn^{+2}$. When the 24 hour data were removed, the mean $EC_{50}$ was 1.420 mg/L $Zn^{+2}$ (Figure 2).

AZUR certificates of performance list zinc sulphate $EC_{50}$ 15 minutes at 0.6 to 2.2 mg/L $Zn^{+2}$ (Appendix 1). All assays, except for the majority of those performed at 24 hours, fall into these general specifications. In addition, most assays results were similar to the AZUR quality assurance results which list the zinc sulphate $EC_{50}$ 15 minutes (mg/l $Zn^{+2}$) at 1.3 for lot ACV009-2, 1.0 for lot ACV009-3, and 1.2 for lot ACV010-3. $EC_{50}$ results for this study ranged from a low of 0.835 to a high of 2.123 (Table 2).

Statistical analysis of $EC_{50}$ results compared to the chosen standard time of 1 hour found that for all lots, the 24 hour data was significantly different (Table 3). Therefore, the rejection of 24 hour data in Figure 2 was statistically reinforced. In addition, for lot ACV009-2 the $EC_{50}$ results for 0.5, 0.75 and 8 hours were significantly different from the 1 hour results (Table 3). However,

when adjusted means of $EC_{50}$ results were compared, there was no difference between the 3 reagent lots at all time points tested (Table 4).

## 2. Copper

Table 5 summarizes the $EC_{50}$ and $EC_{20}$ data at 5 and 15 minutes for all copper sulphate assays. $EC_{50}$ 15 minute results are summarized in Table 6. A graphical representation of 15 minute $EC_{50}$ results is presented in Figures 3, 4 and 5. Statistical results are presented in Tables 7 and 8.

Copper sulphate had an overall mean $EC_{50}$ at 15 minutes of 0.308 mg/L $Cu^{+2}$ for all reconstitution times (Figure 3). However, a sharp decrease in toxicity was observed at 24 h (overall $EC_{50}$ of 0.461 mg/L $Cu^{+2}$). When the 24 hour data were removed, the mean $EC_{50}$ was 0.282 mg/L $Cu^{+2}$ (Figure 4). The increase in toxicity was still observed at 8 hours (overall $EC_{50}$ of 0.417 mg/L $Cu^{+2}$). When the 24 and 8 hour data were removed, the mean $EC_{50}$ was 0.256 mg/L $Cu^{+2}$ (Figure 5) ranging from a low of 0.156 to a high of 0.406.

Statistical analysis of $EC_{50}$ results compared to the chosen standard time of 1 hour found that, for all lots, the 8 and 24 hour data were significantly different (Table 7). Thus, the rejection of these time points as presented in Figure 5 was statistically reinforced. In addition, the comparisons of adjusted means of $EC_{50}$ results found that there was a difference between the 3 lots at the 4 hour and 8 hour time points (Table 8).

## 3. Phenol

Table 9 summarizes the $EC_{50}$ and $EC_{20}$ data at 5 and 15 minutes for all phenol assays. $EC_{50}$ 5 minute results are summarized in Table 10. A graphical representation of 5 minute $EC_{50}$ results is presented in Figure 6. $EC_{50}$ 15 minute results are summarized in Table 11. A graphical representation of 15 minute $EC_{50}$ results is presented in Figure 7. Statistical results are presented in Tables 12 and 13.

Phenol had an overall mean $EC_{50}$ 5 minutes of 20.199 mg/L for all reconstitution times (Table 10) ranging from a low of 15.916 to a high of 25.228. AZUR quality control data lists phenol $EC_{50}$ 5 minutes at 13 to 26 mg/L. All assays at all time points fit into this specification, however, all are well above the AZUR quality assurance results for each lot which list the phenol $EC_{50}$ 5 minutes at 14.7 for lot ACV009-2, 14.3 for lot ACV009-3, and 13.3 for lot ACV010-3.

The $EC_{50}$ 15 minutes had an overall mean of 21.366 and ranged from a low of 16.601 to a high of 27.061 (Table 11). The graphical plot of these results (Figure 7) shows no particular trend in toxicity values, however, there does appear to be lot to lot variation with results from lot ACV009-2 being consistently high and results from lot ACV010-3 being consistently low. Statistical analysis of $EC_{50}$ results compared to the chosen standard time of 1 hour found that significantly different results were observed at 2 and 24 hours for reagent lot AVC009-2 and at 0.75 and 2 hours for reagent lot ACV010-3 (Table 12). In addition, adjusted means of $EC_{50}$ results comparison finds that there was a difference between the 3 lots at all time points except for 24 hours (Table 13). This reaffirms the observation of high/low distribution with particular reagent lots seen in Figure 7.

### 4. Sodium Dodecyl Sulphate

Table 14 summarizes the $EC_{50}$ and $EC_{20}$ data at 5 and 15 minutes for all sodium dodecyl sulphate assays. $EC_{50}$ 15 minute results are summarized in Table 15. A graphical representation of 15 minute $EC_{50}$ results is presented in Figures 8 and 9. Statistical results are presented in Tables 16 and 17.

SDS had an overall mean $EC_{50}$ at 15 minutes of 0.893 mg/L for all reconstitution times (Figure 8). However, a sharp decrease in toxicity was observed at 24 h (overall $EC_{50}$ of 1.708 mg/L). When the 24 hour data were removed, the mean $EC_{50}$ was 0.757 mg/L (Figure 9) ranging from a low of 0.503 to a high of 1.003 (Table 15).

Statistical analysis of $EC_{50}$ results compared to the chosen standard time of 1 hour found that for all lots, the $EC_{50}$ at 24 was significantly different from that at 1 hour (Table 16). Therefore, the rejection of 24 hour data as presented in Figure 9 is statistically reinforced. In addition, adjusted means of $EC_{50}$ comparison, finds that there was a difference between the 3 lots at 8 and 24 hours (Table 17).

## E. CONCLUSIONS

The first and most obvious conclusion to be drawn from this testing was that there were differences in toxicity over time for the compounds tested. Zinc exhibited a sharp increase in toxicity at 24 hours, however $EC_{50}$ results were relatively stable prior to that time. $EC_{50}$ ranged from a low of 0.835 to a high of 2.123 with the mean value excluding 24 hour data being 1.420. SDS also exhibited an increase in toxicity at 24 hours. Results prior to this time point were fairly stable as well. $EC_{50}$ ranged from a low of 0.503 to a high of 1.003 with the mean value excluding 24

hour data being 0.757. Copper, on the other hand, exhibited a decrease in toxicity starting at 4 hours which continued at the 8 and 24 hour testing points. Prior to this, however, toxicity results were very stable. $EC_{50}$ ranged from a low of 0.156 to a high of 0.406 with the mean value excluding 8 and 24 hour data being 0.256. Phenol produced no toxicity trend over time, however, throughout the 24 hour period, the results were erratic. $EC_{50}$ ranged from a low of 16.601 to a high of 27.061 with the mean value being 21.366. Thus, based on the results of all samples, a prediction of change in toxicity of an unknown compound or sample with time cannot be made. With the four compounds tested, all three possibilities of increase, decrease, or no change in toxicity were observed.

The second conclusion was that Microtox® test reagent should definitely not be used at 24 hours, and probably not at 8 hours. Except for phenol, the 24 hour data were rejected for all compounds tested and the 8 hour data were rejected for copper as well. Thus, data obtained from reagent which is older than 4 hours should be regarded cautiously, if at all.

When statistical analysis of data between lots was performed, it was found that there was variation in toxicity results between lots for copper, phenol, and SDS. With phenol, for example, the results for lot ACV009-2 are consistently higher than those for the other two lots except at 24 hours (Figure 7). Phenol shows a definite trend for lot to lot variation with $EC_{50}$ results being lowest for lot ACV010-3 and highest for lot ACV009-2. Statistical analysis for lot to lot variation for phenol indicated there was variation at all time points except for 24 hours (Table 13).

## F. RECOMMENDATIONS

There are several recommendations which arise from this study. Many of these are part of general practice of most laboratories performing the Microtox test. These recommendations are as follows.

1. Each lot of reagent should have quality control testing performed using standard reference toxicant(s). This long term testing would then indicate lot to lot variation and might indicate if there is a problem with a new lot of reagent. Testing should be performed on each new lot of reagent and at regular intervals during the shelf life of that lot.
2. Bench recording sheets or computer recording programs should include the time of reconstitution of reagent as well as the start time of the assay. In this manner, potential problems in interpretation of data caused by use of aged reagent can be avoided.
3. Microtox® test reagent should generally not be used for testing after 4 hours from time of reconstitution. Any assay performed after this time may yield unpredictable results due to reagent age and not toxicity of the sample being

tested. Results obtained from reagent used after 4 hours needs to be evaluated cautiously, perhaps with the use of an appropriate control toxicant run in parallel.

4. It is further recommended that WCMUC adopt these points within their standard operating procedure and as part of their ongoing inter-laboratory quality control program.

## G. ACKNOWLEDGEMENTS

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# I. APPENDICES

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Certificate of Performance, Lot ACV010-3

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## Phenol Quality Control Chart

## Zinc Sulphate Quality Control Chart

## Certificate of Analysis Lot ACV009-2

## Certificate of Analysis Lot ACV009-3

## Certificate of Analysis Lot ACV010-3

## Table 1: Results of Zinc Sulphate Assays

| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L $Zn^{+2}$) | $EC_{50}$ 5 MIN (mg/L $Zn^{+2}$) | $EC_{20}$ 15 MIN (mg/L $Zn^{+2}$) | $EC_{50}$ 15 MIN (mg/L $Zn^{+2}$) |
|---|---|---|---|---|---|---|
| ACV009-2 | 1 | 0.50 | 3.515 | 15.843 | 0.382 | 1.243 |
| ACV009-2 | 1 | 0.75 | 1.335 | 12.671 | 0.995 | 1.791 |
| ACV009-2 | 1 | 1.00 | 4.969 | 13.562 | 0.485 | 1.452 |
| ACV009-2 | 1 | 2.00 | 3.127 | 15.607 | 0.822 | 2.123 |
| ACV009-2 | 1 | 4.00 | 2.819 | 15.420 | 0.734 | 1.875 |
| ACV009-2 | 1 | 8.00 | 1.624 | 15.021 | 0.367 | 1.204 |
| ACV009-2 | 1 | 24.00 | 0.560 | 12.513 | 0.257 | 0.692 |
| ACV009-2 | 2 | 0.50 | 2.511 | 11.441 | 0.379 | 1.151 |
| ACV009-2 | 2 | 0.75 | 2.336 | 12.700 | 0.300 | 1.106 |
| ACV009-2 | 2 | 1.00 | 5.580 | 13.844 | 0.488 | 1.502 |
| ACV009-2 | 2 | 2.00 | 3.928 | 13.553 | 0.599 | 1.633 |
| ACV009-2 | 2 | 4.00 | 2.506 | 11.008 | 0.477 | 1.377 |
| ACV009-2 | 2 | 8.00 | 1.137 | 10.537 | 0.265 | 1.000 |
| ACV009-2 | 2 | 24.00 | 0.990 | 3.384 | 0.163 | 0.498 |
| ACV009-2 | 3 | 0.50 | 0.373 | 11.401 | 0.333 | 1.108 |
| ACV009-2 | 3 | 0.75 | 2.998 | 12.933 | 0.426 | 1.382 |
| ACV009-2 | 3 | 1.00 | 3.064 | 12.426 | 0.551 | 1.540 |
| ACV009-2 | 3 | 2.00 | 3.252 | 13.666 | 0.625 | 1.711 |
| ACV009-2 | 3 | 4.00 | 2.444 | 14.017 | 0.558 | 1.621 |
| ACV009-2 | 3 | 8.00 | 1.854 | 11.709 | 0.362 | 1.182 |
| ACV009-2 | 3 | 24.00 | 1.054 | 3.807 | 0.237 | 0.640 |
| ACV009-3 | 1 | 0.50 | 4.007 | 13.043 | 0.419 | 1.367 |
| ACV009-3 | 1 | 0.75 | 3.274 | 14.299 | 0.294 | 1.101 |
| ACV009-3 | 1 | 1.00 | 2.924 | 12.545 | 0.252 | 1.584 |
| ACV009-3 | 1 | 2.00 | 3.569 | 15.121 | 0.679 | 1.970 |
| ACV009-3 | 1 | 4.00 | 4.130 | 13.741 | 0.421 | 1.497 |
| ACV009-3 | 1 | 8.00 | 1.709 | 11.611 | 0.272 | 1.027 |
| ACV009-3 | 1 | 24.00 | 1.197 | 3.466 | 0.171 | 0.545 |
| ACV009-3 | 2 | 0.50 | 1.097 | 11.657 | 0.243 | 0.838 |
| ACV009-3 | 2 | 0.75 | 2.915 | 14.051 | 0.252 | 1.015 |
| ACV009-3 | 2 | 1.00 | 4.647 | 13.963 | 0.555 | 1.709 |
| ACV009-3 | 2 | 2.00 | 1.059 | 17.109 | 0.338 | 1.229 |
| ACV009-3 | 2 | 4.00 | 3.382 | 14.816 | 0.370 | 1.278 |
| ACV009-3 | 2 | 8.00 | 1.933 | 10.646 | 0.238 | 0.920 |
| ACV009-3 | 2 | 24.00 | 1.023 | 2.287 | 0.211 | 0.548 |
| ACV009-3 | 3 | 0.50 | 4.304 | 13.990 | 0.292 | 1.099 |
| ACV009-3 | 3 | 0.75 | 3.613 | 15.836 | 0.200 | 0.835 |
| ACV009-3 | 3 | 1.00 | 4.540 | 13.868 | 0.676 | 1.887 |
| ACV009-3 | 3 | 2.00 | 3.643 | 13.278 | 0.585 | 1.653 |
| ACV009-3 | 3 | 4.00 | 2.017 | 10.803 | 0.461 | 1.420 |
| ACV009-3 | 3 | 8.00 | 1.632 | 11.993 | 0.214 | 0.896 |
| ACV009-3 | 3 | 24.00 | 0.976 | 2.814 | 0.159 | 0.469 |
| ACV010-3 | 1 | 0.50 | 4.916 | 30.314 | 0.163 | 1.123 |
| ACV010-3 | 1 | 0.75 | 4.318 | 16.521 | 0.561 | 1.761 |
| ACV010-3 | 1 | 1.00 | 7.225 | 25.235 | 0.508 | 1.893 |
| ACV010-3 | 1 | 2.00 | 3.106 | 24.445 | 0.346 | 1.407 |
| ACV010-3 | 1 | 4.00 | 3.666 | 39.099 | 0.393 | 1.592 |
| ACV010-3 | 1 | 8.00 | 4.048 | 29.766 | 0.429 | 1.607 |

| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L $Zn^{+2}$) | $EC_{50}$ 5 MIN (mg/L $Zn^{+2}$) | $EC_{20}$ 15 MIN (mg/L $Zn^{+2}$) | $EC_{50}$ 15 MIN (mg/L $Zn^{+2}$) |
|---|---|---|---|---|---|---|
| ACV010-3 | 1 | 24.00 | 0.936 | 2.698 | 0.110 | 0.383 |
| ACV010-3 | 2 | 0.50 | 8.898 | 33.495 | 0.373 | 1.706 |
| ACV010-3 | 2 | 0.75 | 7.000 | 25.200 | 0.308 | 1.494 |
| ACV010-3 | 2 | 1.00 | 7.164 | 26.968 | 0.295 | 1.489 |
| ACV010-3 | 2 | 2.00 | 3.234 | 35.945 | 0.239 | 1.333 |
| ACV010-3 | 2 | 4.00 | 4.344 | 24.101 | 0.527 | 1.883 |
| ACV010-3 | 2 | 8.00 | 3.596 | 8.863 | 0.336 | 1.456 |
| ACV010-3 | 2 | 24.00 | 1.292 | 3.412 | 0.158 | 0.519 |
| ACV010-3 | 3 | 0.50 | 4.370 | 34.014 | 0.315 | 1.625 |
| ACV010-3 | 3 | 0.75 | 5.903 | 26.135 | 0.243 | 1.173 |
| ACV010-3 | 3 | 1.00 | 6.643 | 22.430 | 0.370 | 1.534 |
| ACV010-3 | 3 | 2.00 | 5.636 | 25.826 | 0.487 | 1.754 |
| ACV010-3 | 3 | 4.00 | 3.423 | 23.910 | 0.295 | 1.309 |
| ACV010-3 | 3 | 8.00 | 3.969 | 43.350 | 0.264 | 1.201 |
| ACV010-3 | 3 | 24.00 | 0.571 | 3.016 | 0.110 | 0.368 |

**Table 2: Zinc Sulphate $EC_{50}$ 15 minute Data Summary (mg/L $Zn^{+2}$)**

| | | TIME (H) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| REAGENT LOT | REPLICATE | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 | Mean |
| ACV009-2 | 1 | 1.243 | 1.791 | 1.452 | 2.123 | 1.875 | 1.204 | 0.692 | 1.483 |
| | 2 | 1.151 | 1.106 | 1.502 | 1.633 | 1.377 | 1.000 | 0.498 | 1.181 |
| | 3 | 1.108 | 1.382 | 1.540 | 1.711 | 1.621 | 1.182 | 0.640 | 1.312 |
| ACV009-2 Mean | | 1.167 | 1.426 | 1.498 | 1.822 | 1.624 | 1.129 | 0.610 | 1.325 |
| ACV009-3 | 1 | 1.367 | 1.101 | 1.584 | 1.970 | 1.497 | 1.027 | 0.545 | 1.299 |
| | 2 | 0.838 | 1.015 | 1.709 | 1.229 | 1.278 | 0.920 | 0.548 | 1.077 |
| | 3 | 1.099 | 0.835 | 1.887 | 1.653 | 1.420 | 0.896 | 0.469 | 1.180 |
| ACV009-3 Mean | | 1.101 | 0.984 | 1.727 | 1.617 | 1.398 | 0.948 | 0.521 | 1.185 |
| ACV010-3 | 1 | 1.123 | 1.761 | 1.893 | 1.407 | 1.592 | 1.607 | 0.383 | 1.395 |
| | 2 | 1.706 | 1.494 | 1.489 | 1.333 | 1.883 | 1.456 | 0.519 | 1.411 |
| | 3 | 1.625 | 1.173 | 1.534 | 1.754 | 1.309 | 1.201 | 0.368 | 1.281 |
| ACV010-3 Mean | | 1.485 | 1.476 | 1.639 | 1.498 | 1.595 | 1.421 | 0.423 | 1.362 |
| Overall Mean | | 1.251 | 1.295 | 1.621 | 1.646 | 1.539 | 1.166 | 0.518 | 1.291 |

**Figure 1: Zinc Sulphate $EC_{50}$ 15 Minute Data**

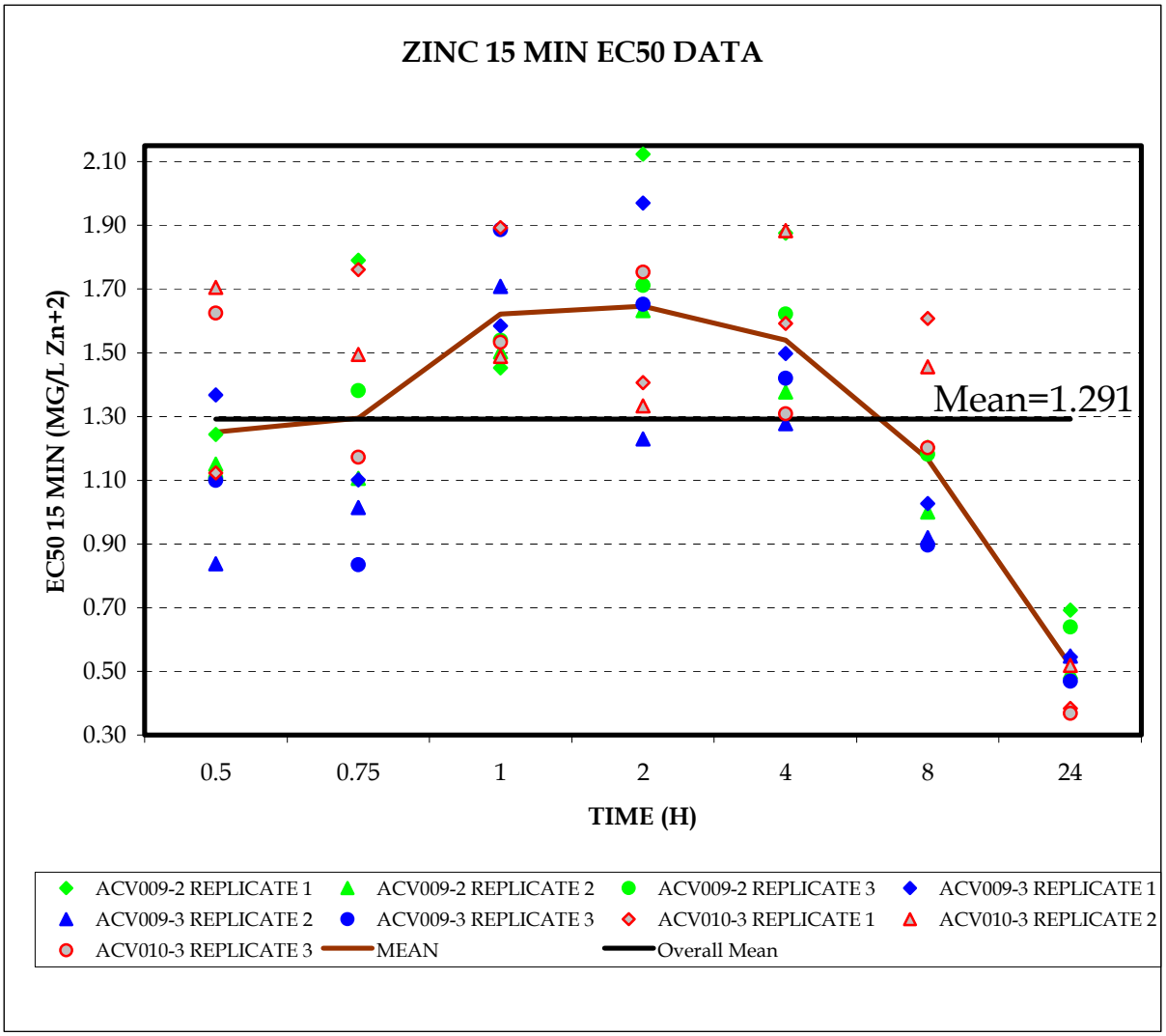


**Figure 2: Zinc Sulphate $EC_{50}$ 15 Minute Data (24 hour data removed)**

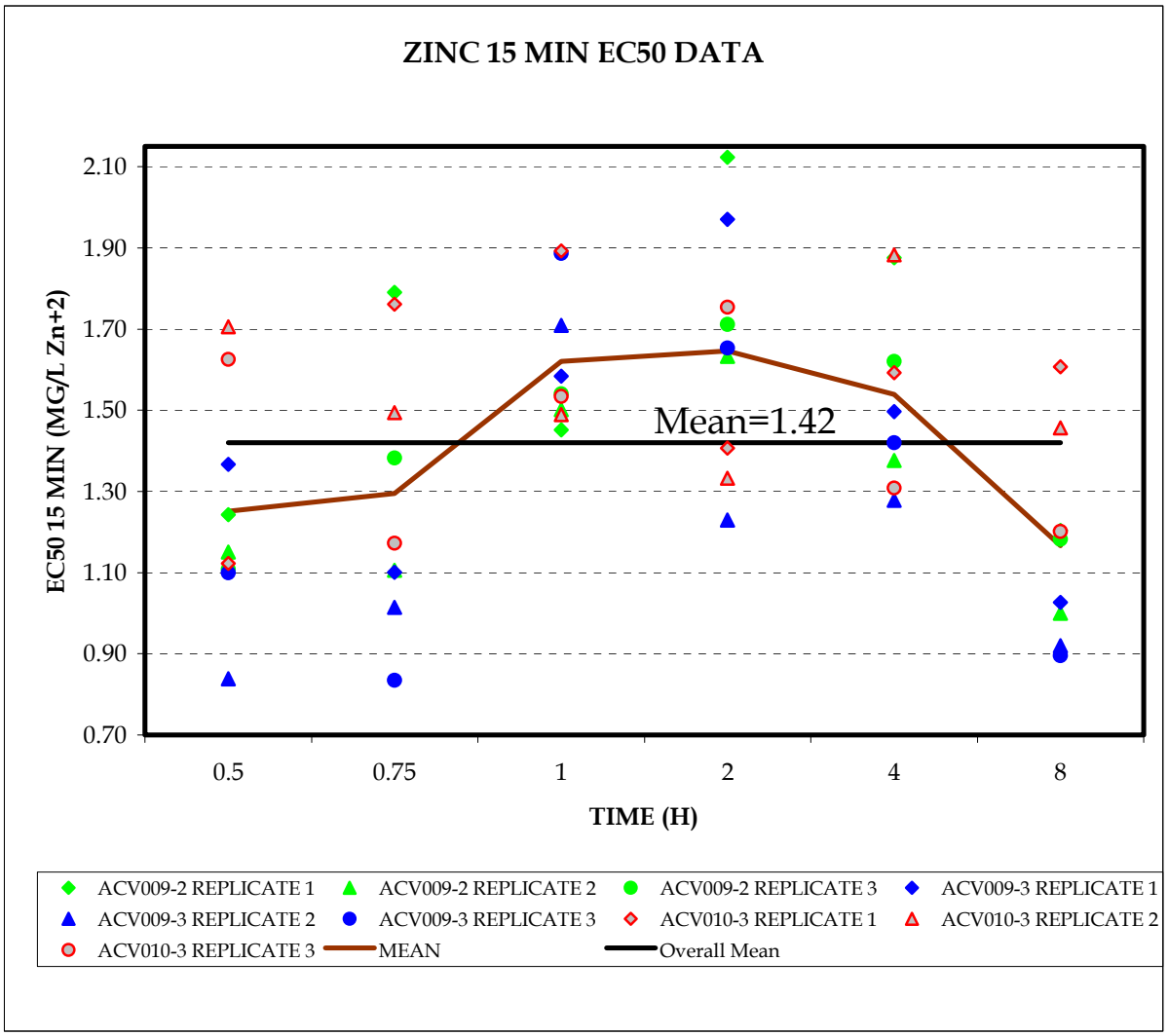


**Table 3. Zinc Sulphate Differences Among Mean $EC_{50}$ Values (mg/L $Zn^{+2}$)**

| | | Time | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Reagent | | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 |
| ACV009-2 | Arithmetic Mean | 1.17 | 1.43 | 1.50 | 1.82 | 1.62 | 1.13 | **0.61*** |
| | Standard Error | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 |
| ACV009-3 | Arithmetic Mean | **1.10*** | **0.98*** | 1.73 | 1.62 | 1.40 | **0.95*** | **0.52*** |
| | Standard Error | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 |
| ACV010-3 | Arithmetic Mean | 1.48 | 1.48 | 1.64 | 1.50 | 1.59 | 1.42 | **0.42*** |
| | Standard Error | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |

Note: Within each reagent, symbol '*' indicates that the mean is significantly different from the mean for 1 hour duration at 95% confidence level

**Table 4. Zinc Sulphate Differences Among Means, Reagent Lots $EC_{50}$ Values (mg/L $Zn^{+2}$)**

| | | Reagent | | | |
|---|---|---|---|---|---|
| Time | | ACV009-2 | ACV009-3 | ACV010-3 | Multiple Comparisons* |
| 0.50 | Adjusted Mean | 1.53 | 1.38 | 1.61 | aaa |
| | Standard Error | 0.08 | 0.08 | 0.08 | |
| 0.75 | Adjusted Mean | 1.52 | 1.37 | 1.60 | aaa |
| | Standard Error | 0.07 | 0.07 | 0.07 | |
| 1.00 | Adjusted Mean | 1.51 | 1.36 | 1.59 | aaa |
| | Standard Error | 0.07 | 0.07 | 0.07 | |
| 2.00 | Adjusted Mean | 1.47 | 1.32 | 1.54 | aaa |
| | Standard Error | 0.07 | 0.07 | 0.07 | |
| 4.00 | Adjusted Mean | 1.39 | 1.25 | 1.45 | aaa |
| | Standard Error | 0.06 | 0.06 | 0.06 | |
| 8.00 | Adjusted Mean | 1.24 | 1.10 | 1.26 | aaa |
| | Standard Error | 0.06 | 0.06 | 0.06 | |
| 24.00 | Adjusted Mean | 0.63 | 0.52 | 0.50 | aaa |
| | Standard Error | 0.16 | 0.16 | 0.16 | |

Note: Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level, i.e. no reagent means at a given time are different when the three letters are the same.

## Table 5: Results of Copper Sulphate Assays

| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L $Cu^{+2}$) | $EC_{50}$ 5 MIN (mg/L $Cu^{+2}$) | $EC_{20}$ 15 MIN (mg/L $Cu^{+2}$) | $EC_{50}$ 15 MIN (mg/L $Cu^{+2}$) |
|---|---|---|---|---|---|---|
| ACV009-2 | 1 | 0.5 | 0.205 | 0.504 | 0.155 | 0.272 |
| ACV009-2 | 1 | 0.75 | 0.197 | 0.407 | 0.103 | 0.247 |
| ACV009-2 | 1 | 1 | 0.215 | 0.353 | 0.105 | 0.208 |
| ACV009-2 | 1 | 2 | 0.251 | 0.545 | 0.133 | 0.268 |
| ACV009-2 | 1 | 4 | 0.281 | 0.478 | 0.171 | 0.340 |
| ACV009-2 | 1 | 8 | 0.328 | 0.576 | 0.214 | 0.396 |
| ACV009-2 | 1 | 24 | 0.185 | 0.433 | 0.120 | 0.387 |
| ACV009-2 | 2 | 0.5 | 0.136 | 0.326 | 0.100 | 0.187 |
| ACV009-2 | 2 | 0.75 | 0.202 | 0.352 | 0.101 | 0.204 |
| ACV009-2 | 2 | 1 | 0.146 | 0.323 | 0.077 | 0.180 |
| ACV009-2 | 2 | 2 | 0.184 | 0.368 | 0.126 | 0.231 |
| ACV009-2 | 2 | 4 | 0.177 | 0.490 | 0.127 | 0.265 |
| ACV009-2 | 2 | 8 | 0.269 | 0.879 | 0.178 | 0.358 |
| ACV009-2 | 2 | 24 | 0.403 | 0.543 | 0.146 | 0.316 |
| ACV009-2 | 3 | 0.5 | 0.133 | 0.253 | 0.093 | 0.156 |
| ACV009-2 | 3 | 0.75 | 0.144 | 0.294 | 0.095 | 0.172 |
| ACV009-2 | 3 | 1 | 0.179 | 0.327 | 0.096 | 0.204 |
| ACV009-2 | 3 | 2 | 0.193 | 0.395 | 0.117 | 0.222 |
| ACV009-2 | 3 | 4 | 0.223 | 0.536 | 0.101 | 0.279 |
| ACV009-2 | 3 | 8 | 0.383 | 0.976 | 0.208 | 0.404 |
| ACV009-2 | 3 | 24 | 0.579 | 1.489 | 0.284 | 0.523 |
| ACV009-3 | 1 | 0.5 | 0.268 | 0.578 | 0.147 | 0.237 |
| ACV009-3 | 1 | 0.75 | 0.150 | 0.744 | 0.080 | 0.214 |
| ACV009-3 | 1 | 1 | 0.212 | 0.603 | 0.110 | 0.231 |
| ACV009-3 | 1 | 2 | 0.160 | 0.593 | 0.129 | 0.222 |
| ACV009-3 | 1 | 4 | 0.493 | 1.034 | 0.213 | 0.406 |
| ACV009-3 | 1 | 8 | 0.395 | 1.317 | 0.226 | 0.411 |
| ACV009-3 | 1 | 24 | 0.538 | 1.955 | 0.210 | 0.463 |
| ACV009-3 | 2 | 0.5 | 0.177 | 0.411 | 0.078 | 0.192 |
| ACV009-3 | 2 | 0.75 | 0.241 | 0.586 | 0.102 | 0.244 |
| ACV009-3 | 2 | 1 | 0.201 | 0.521 | 0.117 | 0.249 |
| ACV009-3 | 2 | 2 | 0.183 | 0.567 | 0.131 | 0.271 |
| ACV009-3 | 2 | 4 | 0.239 | 0.518 | 0.119 | 0.278 |
| ACV009-3 | 2 | 8 | 0.509 | 1.161 | 0.228 | 0.495 |
| ACV009-3 | 2 | 24 | 0.578 | 1.669 | 0.253 | 0.496 |
| ACV009-3 | 3 | 0.5 | 0.218 | 0.505 | 0.137 | 0.240 |
| ACV009-3 | 3 | 0.75 | 0.236 | 0.573 | 0.152 | 0.282 |
| ACV009-3 | 3 | 1 | 0.201 | 0.522 | 0.169 | 0.282 |
| ACV009-3 | 3 | 2 | 0.239 | 0.605 | 0.141 | 0.283 |
| ACV009-3 | 3 | 4 | 0.242 | 0.691 | 0.242 | 0.356 |
| ACV009-3 | 3 | 8 | 0.596 | 0.797 | 0.243 | 0.456 |
| ACV009-3 | 3 | 24 | 0.622 | 1.980 | 0.221 | 0.572 |
| ACV010-3 | 1 | 0.5 | 0.220 | 0.534 | 0.115 | 0.268 |
| ACV010-3 | 1 | 0.75 | 0.251 | 0.640 | 0.126 | 0.290 |

| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L $Cu^{+2}$) | $EC_{50}$ 5 MIN (mg/L $Cu^{+2}$) | $EC_{20}$ 15 MIN (mg/L $Cu^{+2}$) | $EC_{50}$ 15 MIN (mg/L $Cu^{+2}$) |
|---|---|---|---|---|---|---|
| ACV010-3 | 1 | 1 | 0.318 | 0.622 | 0.149 | 0.313 |
| ACV010-3 | 1 | 2 | 0.300 | 0.523 | 0.147 | 0.261 |
| ACV010-3 | 1 | 4 | 0.325 | 0.949 | 0.180 | 0.320 |
| ACV010-3 | 1 | 8 | 0.401 | 0.924 | 0.160 | 0.385 |
| ACV010-3 | 1 | 24 | 0.532 | 1.010 | 0.249 | 0.469 |
| ACV010-3 | 2 | 0.5 | 0.221 | 0.480 | 0.120 | 0.224 |
| ACV010-3 | 2 | 0.75 | 0.385 | 0.654 | 0.192 | 0.331 |
| ACV010-3 | 2 | 1 | 0.290 | 0.558 | 0.120 | 0.245 |
| ACV010-3 | 2 | 2 | 0.231 | 0.447 | 0.112 | 0.246 |
| ACV010-3 | 2 | 4 | 0.258 | 0.463 | 0.153 | 0.290 |
| ACV010-3 | 2 | 8 | 0.332 | 0.557 | 0.214 | 0.378 |
| ACV010-3 | 2 | 24 | 0.444 | 1.077 | 0.231 | 0.481 |
| ACV010-3 | 3 | 0.5 | 0.249 | 0.449 | 0.135 | 0.240 |
| ACV010-3 | 3 | 0.75 | 0.263 | 0.828 | 0.145 | 0.230 |
| ACV010-3 | 3 | 1 | 0.288 | 0.574 | 0.125 | 0.240 |
| ACV010-3 | 3 | 2 | 0.194 | 0.664 | 0.096 | 0.216 |
| ACV010-3 | 3 | 4 | 0.336 | 1.049 | 0.201 | 0.374 |
| ACV010-3 | 3 | 8 | 0.627 | 1.207 | 0.233 | 0.473 |
| ACV010-3 | 3 | 24 | 0.556 | 2.859 | 0.200 | 0.446 |

**Table 6: Copper Sulphate $EC_{50}$ 15 minute Data Summary (mg/L $Cu^{+2}$)**

| | | TIME (H) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| REAGENT LOT | REPLICATE | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 | Mean |
| ACV009-2 | 1 | 0.272 | 0.247 | 0.208 | 0.268 | 0.340 | 0.396 | 0.387 | 0.303 |
| | 2 | 0.187 | 0.204 | 0.180 | 0.231 | 0.265 | 0.358 | 0.316 | 0.249 |
| | 3 | 0.156 | 0.172 | 0.204 | 0.222 | 0.279 | 0.404 | 0.523 | 0.280 |
| ACV009-2 Mean | | 0.205 | 0.208 | 0.197 | 0.240 | 0.295 | 0.386 | 0.409 | 0.277 |
| ACV009-3 | 1 | 0.237 | 0.214 | 0.231 | 0.222 | 0.406 | 0.411 | 0.463 | 0.312 |
| | 2 | 0.192 | 0.244 | 0.249 | 0.271 | 0.278 | 0.495 | 0.496 | 0.318 |
| | 3 | 0.240 | 0.282 | 0.282 | 0.283 | 0.356 | 0.456 | 0.572 | 0.353 |
| ACV009-3 Mean | | 0.223 | 0.247 | 0.254 | 0.259 | 0.347 | 0.454 | 0.510 | 0.328 |
| ACV010-3 | 1 | 0.268 | 0.290 | 0.313 | 0.261 | 0.320 | 0.385 | 0.469 | 0.329 |
| | 2 | 0.224 | 0.331 | 0.245 | 0.246 | 0.290 | 0.378 | 0.481 | 0.314 |
| | 3 | 0.240 | 0.230 | 0.240 | 0.216 | 0.374 | 0.473 | 0.446 | 0.317 |
| ACV010-3 Mean | | 0.244 | 0.284 | 0.266 | 0.241 | 0.328 | 0.412 | 0.465 | 0.320 |
| Overall Mean | | 0.224 | 0.246 | 0.239 | 0.247 | 0.323 | 0.417 | 0.461 | 0.308 |

**Figure 3: Copper Sulphate 15 Minute $EC_{50}$ Data**

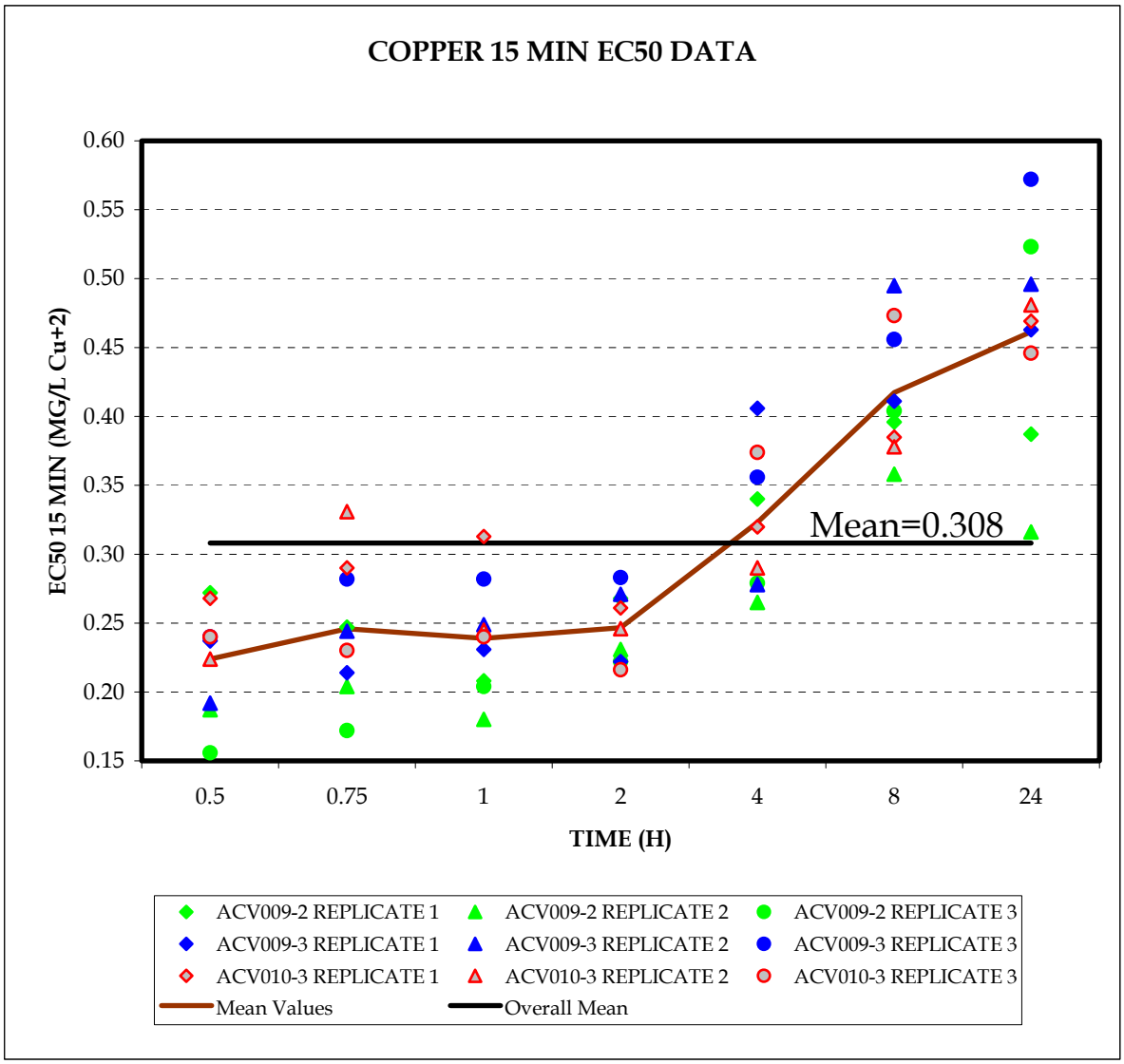


**Figure 4: Copper Sulphate 15 Minute $EC_{50}$ Data (24 Hour Data Removed)**

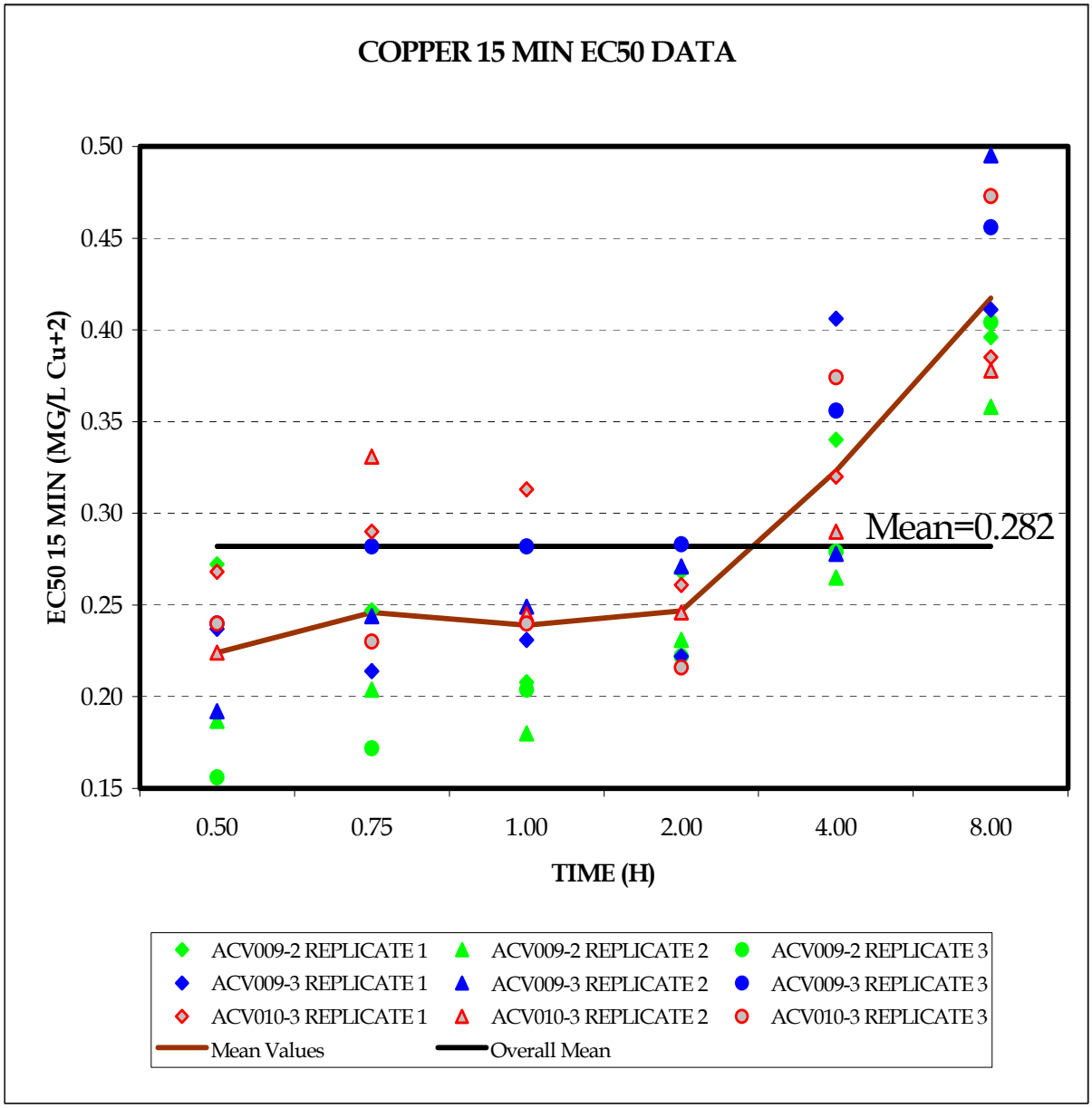


**Figure 5: Copper Sulphate 15 Minute $EC_{50}$ Data (8 and 24 Hour Data Removed)**

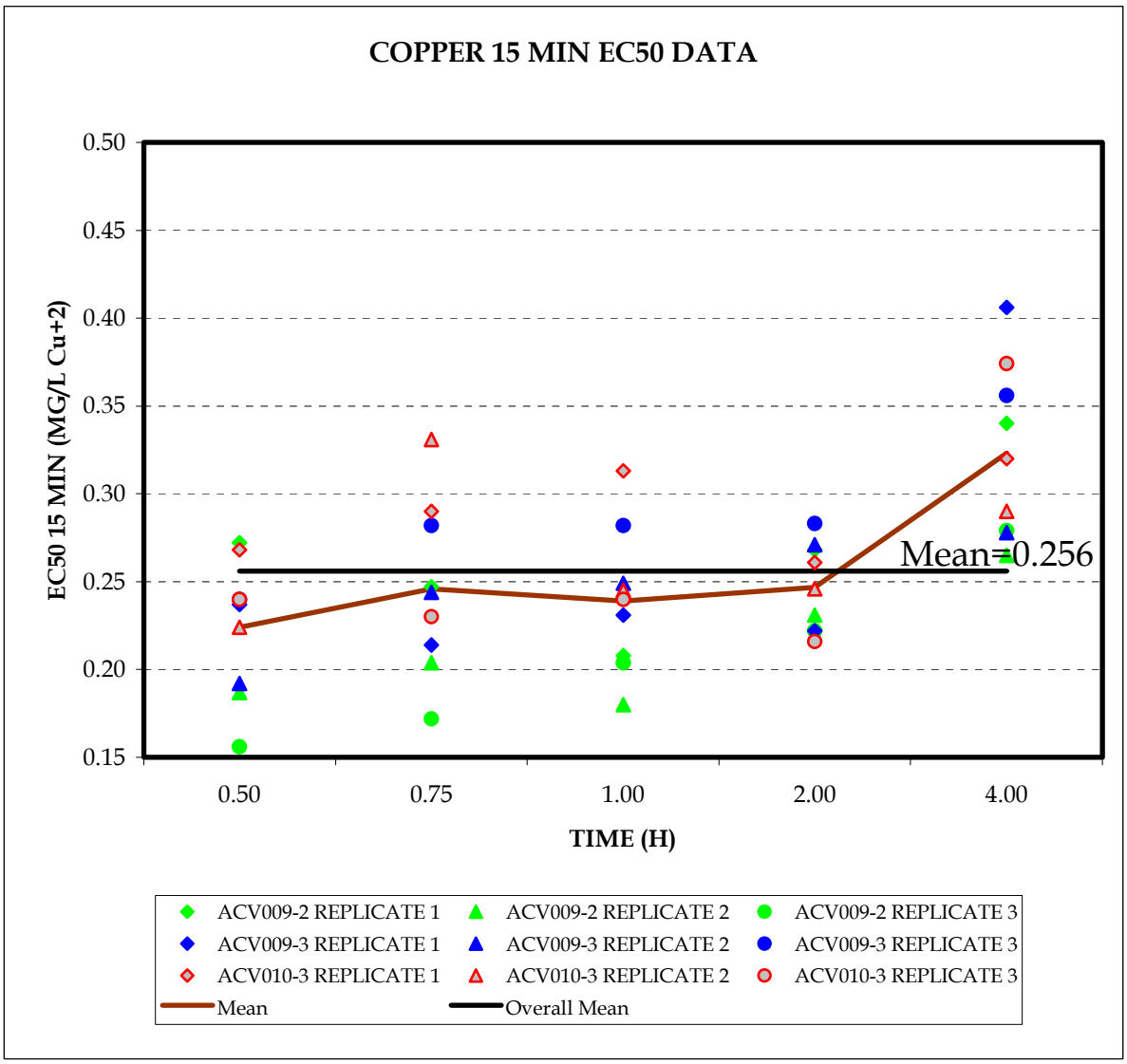


**Table 7. Copper Sulphate Differences Among Mean $EC_{50}$ Values (mg/L $Cu^{+2}$)**

| | | Time | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Reagent | | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 |
| ACV009-2 | Arithmetic Mean | 0.21 | 0.21 | 0.20 | 0.24 | 0.29 | **0.38*** | **0.41*** |
| | Standard Error | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| ACV009-3 | Arithmetic Mean | 0.22 | 0.25 | 0.25 | 0.26 | 0.35 | **0.45*** | **0.51*** |
| | Standard Error | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| ACV010-3 | Arithmetic Mean | 0.24 | 0.28 | 0.27 | 0.24 | 0.33 | **0.41*** | **0.47*** |
| | Standard Error | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |

Note: Within each reagent, symbol '*' indicates that the mean is significantly different from the mean for 1 hour duration at 95% confidence level

**Table 8. Copper Sulphate Differences Among Means, Reagent Lots $EC_{50}$ Values (mg/L $Cu^{+2}$)**

| | | Reagent | | | |
|---|---|---|---|---|---|
| Time | | ACV009-2 | ACV009-3 | ACV010-3 | Multiple Comparisons* |
| 0.50 | Adjusted Mean | 0.23 | 0.26 | 0.27 | aaa |
| | Standard Error | 0.02 | 0.02 | 0.02 | |
| 0.75 | Adjusted Mean | 0.23 | 0.27 | 0.27 | aaa |
| | Standard Error | 0.02 | 0.02 | 0.02 | |
| 1.00 | Adjusted Mean | 0.23 | 0.27 | 0.28 | aaa |
| | Standard Error | 0.01 | 0.01 | 0.01 | |
| 2.00 | Adjusted Mean | 0.24 | 0.28 | 0.29 | aaa |
| | Standard Error | 0.01 | 0.01 | 0.01 | |
| 4.00 | Adjusted Mean | 0.26 | 0.31 | 0.30 | **aba** |
| | Standard Error | 0.01 | 0.01 | 0.01 | |
| 8.00 | Adjusted Mean | 0.30 | 0.35 | 0.34 | **aba** |
| | Standard Error | 0.01 | 0.01 | 0.01 | |
| 24.00 | Adjusted Mean | 0.44 | 0.55 | 0.49 | aaa |
| | Standard Error | 0.03 | 0.03 | 0.03 | |

Note: Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level, i.e. no reagent means at a given time are different when the three letters are the same.

## Table 9: Results of Phenol Assays

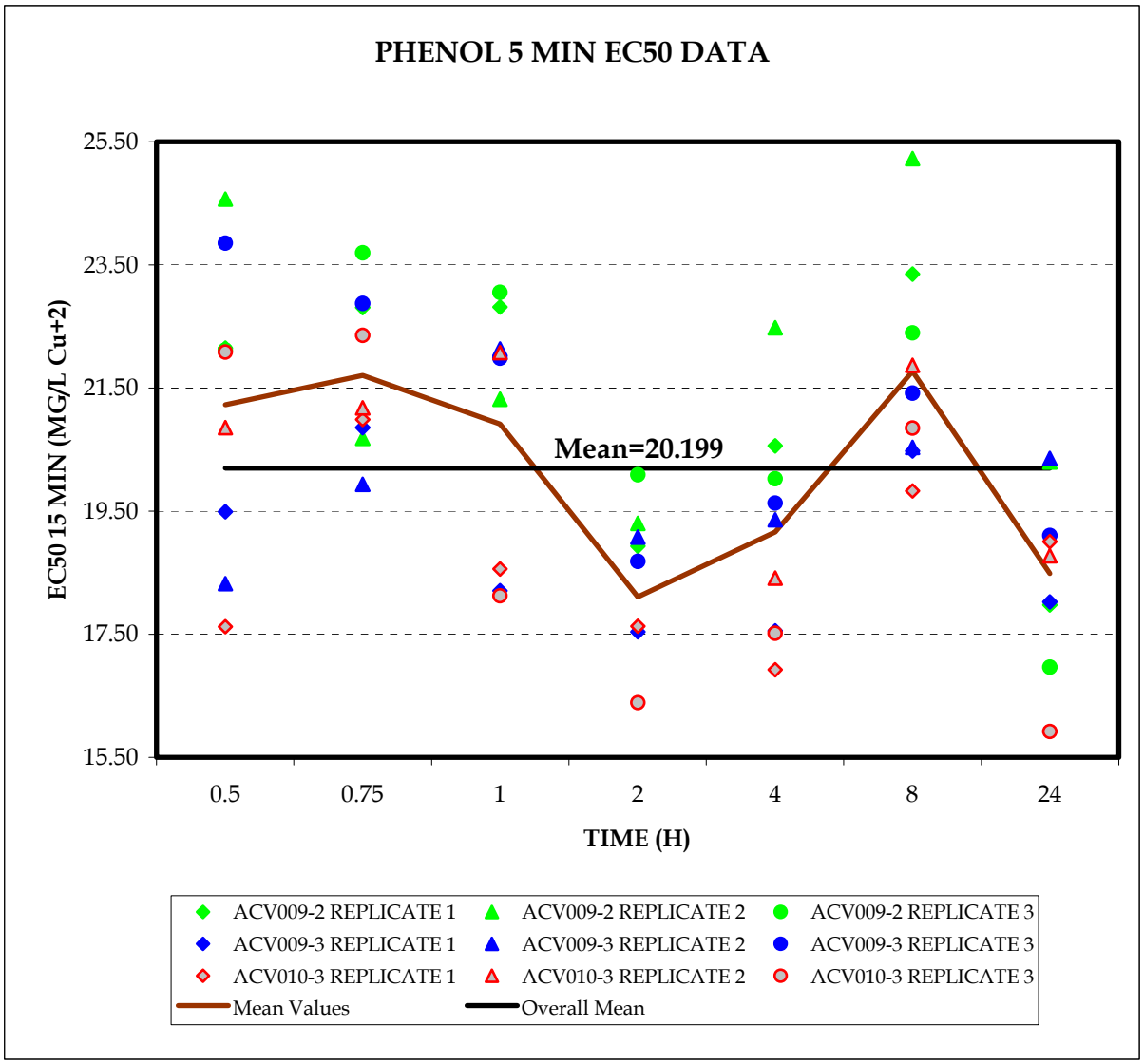
| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L PHENOL) | $EC_{50}$ 5 MIN (mg/L PHENOL) | $EC_{20}$ 15 MIN (mg/L PHENOL) | $EC_{50}$ 15 MIN (mg/L PHENOL) |
|---|---|---|---|---|---|---|
| ACV009-2 | 1 | 0.5 | 4.398 | 22.150 | 5.220 | 24.417 |
| ACV009-2 | 1 | 0.75 | 5.010 | 22.808 | 6.346 | 25.989 |
| ACV009-2 | 1 | 1 | 5.303 | 22.816 | 5.933 | 24.730 |
| ACV009-2 | 1 | 2 | 3.902 | 18.935 | 4.295 | 19.489 |
| ACV009-2 | 1 | 4 | 4.675 | 20.564 | 5.812 | 22.494 |
| ACV009-2 | 1 | 8 | 5.595 | 23.355 | 5.413 | 22.029 |
| ACV009-2 | 1 | 24 | 4.256 | 17.978 | 4.467 | 19.529 |
| ACV009-2 | 2 | 0.5 | 5.793 | 24.571 | 6.584 | 27.061 |
| ACV009-2 | 2 | 0.75 | 4.614 | 20.685 | 6.241 | 24.596 |
| ACV009-2 | 2 | 1 | 4.675 | 21.320 | 5.320 | 22.753 |
| ACV009-2 | 2 | 2 | 3.946 | 19.305 | 4.460 | 20.580 |
| ACV009-2 | 2 | 4 | 5.376 | 22.476 | 5.267 | 23.085 |
| ACV009-2 | 2 | 8 | 5.931 | 25.228 | 6.306 | 24.457 |
| ACV009-2 | 2 | 24 | 5.533 | 20.300 | 5.443 | 20.984 |
| ACV009-2 | 3 | 0.5 | 4.268 | 22.115 | 5.950 | 26.076 |
| ACV009-2 | 3 | 0.75 | 5.942 | 23.697 | 6.523 | 25.556 |
| ACV009-2 | 3 | 1 | 5.448 | 23.056 | 7.561 | 26.862 |
| ACV009-2 | 3 | 2 | 4.561 | 20.091 | 4.986 | 21.124 |
| ACV009-2 | 3 | 4 | 4.122 | 20.027 | 4.670 | 20.900 |
| ACV009-2 | 3 | 8 | 5.430 | 22.395 | 5.509 | 21.977 |
| ACV009-2 | 3 | 24 | 4.024 | 16.966 | 4.977 | 20.895 |
| ACV009-3 | 1 | 0.5 | 3.727 | 19.493 | 4.717 | 22.661 |
| ACV009-3 | 1 | 0.75 | 4.858 | 20.859 | 4.552 | 19.846 |
| ACV009-3 | 1 | 1 | 4.016 | 18.207 | 5.046 | 21.801 |
| ACV009-3 | 1 | 2 | 3.966 | 17.545 | 4.457 | 19.675 |
| ACV009-3 | 1 | 4 | 4.032 | 17.557 | 4.012 | 18.118 |
| ACV009-3 | 1 | 8 | 5.050 | 20.482 | 4.523 | 19.522 |
| ACV009-3 | 1 | 24 | 4.496 | 18.027 | 4.374 | 18.419 |
| ACV009-3 | 2 | 0.5 | 3.609 | 18.326 | 4.363 | 19.968 |
| ACV009-3 | 2 | 0.75 | 4.781 | 19.938 | 5.314 | 22.130 |
| ACV009-3 | 2 | 1 | 5.608 | 22.130 | 6.069 | 22.991 |
| ACV009-3 | 2 | 2 | 4.609 | 19.080 | 5.337 | 20.614 |
| ACV009-3 | 2 | 4 | 4.168 | 19.364 | 4.754 | 19.858 |
| ACV009-3 | 2 | 8 | 4.592 | 20.539 | 4.946 | 21.736 |
| ACV009-3 | 2 | 24 | 4.999 | 20.356 | 6.122 | 22.539 |
| ACV009-3 | 3 | 0.5 | 6.060 | 23.856 | 5.079 | 22.119 |
| ACV009-3 | 3 | 0.75 | 4.874 | 22.871 | 5.173 | 23.098 |
| ACV009-3 | 3 | 1 | 5.359 | 21.983 | 5.206 | 21.766 |
| ACV009-3 | 3 | 2 | 4.284 | 18.682 | 5.116 | 20.487 |
| ACV009-3 | 3 | 4 | 4.389 | 19.629 | 4.538 | 20.119 |
| ACV009-3 | 3 | 8 | 5.706 | 21.415 | 6.202 | 22.906 |
| ACV009-3 | 3 | 24 | 4.662 | 19.102 | 5.109 | 20.703 |
| ACV010-3 | 1 | 0.5 | 3.467 | 17.621 | 3.668 | 18.459 |
| ACV010-3 | 1 | 0.75 | 4.802 | 20.991 | 5.772 | 23.385 |

| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L PHENOL) | $EC_{50}$ 5 MIN (mg/L PHENOL) | $EC_{20}$ 15 MIN (mg/L PHENOL) | $EC_{50}$ 15 MIN (mg/L PHENOL) |
|---|---|---|---|---|---|---|
| ACV010-3 | 1 | 1 | 3.785 | 18.560 | 4.851 | 20.865 |
| ACV010-3 | 1 | 2 | 4.204 | 17.629 | 3.973 | 17.271 |
| ACV010-3 | 1 | 4 | 4.344 | 16.924 | 4.860 | 17.729 |
| ACV010-3 | 1 | 8 | 4.877 | 19.829 | 4.336 | 18.423 |
| ACV010-3 | 1 | 24 | 4.089 | 19.003 | 4.114 | 20.097 |
| ACV010-3 | 2 | 0.5 | 4.743 | 20.862 | 4.959 | 21.464 |
| ACV010-3 | 2 | 0.75 | 5.305 | 21.180 | 6.670 | 24.600 |
| ACV010-3 | 2 | 1 | 5.669 | 22.074 | 5.324 | 21.526 |
| ACV010-3 | 2 | 2 | 3.325 | 15.299 | 3.834 | 16.601 |
| ACV010-3 | 2 | 4 | 4.172 | 18.411 | 4.183 | 17.880 |
| ACV010-3 | 2 | 8 | 5.368 | 21.869 | 5.264 | 21.228 |
| ACV010-3 | 2 | 24 | 4.310 | 18.775 | 4.595 | 19.651 |
| ACV010-3 | 3 | 0.5 | 4.985 | 22.084 | 5.078 | 22.851 |
| ACV010-3 | 3 | 0.75 | 5.582 | 22.353 | 5.926 | 23.313 |
| ACV010-3 | 3 | 1 | 4.173 | 18.125 | 4.666 | 19.353 |
| ACV010-3 | 3 | 2 | 3.626 | 16.390 | 3.519 | 16.544 |
| ACV010-3 | 3 | 4 | 3.890 | 17.516 | 4.334 | 18.666 |
| ACV010-3 | 3 | 8 | 5.298 | 20.849 | 5.415 | 21.594 |
| ACV010-3 | 3 | 24 | 3.730 | 15.916 | 4.213 | 17.878 |

**Table 10: Phenol $EC_{50}$ 5 minute Data Summary (mg/L)**

| | | TIME (H) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| REAGENT LOT | REPLICATE | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 | Mean |
| ACV009-2 | 1 | 22.15 | 22.808 | 22.816 | 18.935 | 20.564 | 23.355 | 17.978 | 21.229 |
| | 2 | 24.571 | 20.685 | 21.32 | 19.305 | 22.476 | 25.228 | 20.2999 | 21.984 |
| | 3 | 22.115 | 23.697 | 23.056 | 20.091 | 20.027 | 22.395 | 16.966 | 21.192 |
| ACV009-2 Mean | | 22.945 | 22.397 | 22.397 | 19.444 | 21.022 | 23.659 | 18.415 | 21.468 |
| ACV009-3 | 1 | 19.493 | 20.859 | 18.207 | 17.545 | 17.557 | 20.482 | 18.027 | 18.881 |
| | 2 | 18.326 | 19.938 | 22.13 | 19.08 | 19.364 | 20.539 | 20.356 | 19.962 |
| | 3 | 23.856 | 22.871 | 21.983 | 18.682 | 19.629 | 21.415 | 19.102 | 21.077 |
| ACV009-3 Mean | | 20.558 | 21.223 | 20.773 | 18.436 | 18.850 | 20.812 | 19.162 | 19.973 |
| ACV010-3 | 1 | 17.621 | 20.991 | 18.56 | 17.629 | 16.924 | 19.829 | 19.003 | 18.651 |
| | 2 | 20.862 | 21.18 | 22.074 | 15.299 | 18.411 | 21.869 | 18.775 | 19.781 |
| | 3 | 22.084 | 22.353 | 18.125 | 16.39 | 17.516 | 20.849 | 15.916 | 19.033 |
| ACV010-3 Mean | | 20.189 | 21.508 | 19.586 | 16.439 | 17.617 | 20.849 | 17.898 | 19.155 |
| Overall Mean | | 21.231 | 21.709 | 20.919 | 18.106 | 19.163 | 21.773 | 18.491 | 20.199 |

**Figure 6: Phenol $EC_{50}$ 5 minute Data**



## Table 11: Phenol $EC_{50}$ 15 minute Data Summary (mg/L)

| | | TIME (H) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| REAGENT LOT | REPLICATE | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 | Mean |
| ACV009-2 | 1 | 24.417 | 25.989 | 24.730 | 19.489 | 22.494 | 22.029 | 19.529 | 22.668 |
| | 2 | 27.061 | 24.596 | 22.753 | 20.580 | 23.085 | 24.457 | 20.984 | 23.359 |
| | 3 | 26.076 | 25.556 | 26.862 | 21.124 | 20.900 | 21.977 | 20.895 | 23.341 |
| ACV009-2 Mean | | 25.851 | 25.380 | 24.782 | 20.398 | 22.160 | 22.821 | 20.469 | 23.123 |
| ACV009-3 | 1 | 22.661 | 19.846 | 21.801 | 19.675 | 18.118 | 19.522 | 18.419 | 20.006 |
| | 2 | 19.968 | 22.130 | 22.991 | 20.614 | 19.858 | 21.736 | 22.539 | 21.405 |
| | 3 | 22.119 | 23.098 | 21.766 | 20.487 | 20.119 | 22.906 | 20.703 | 21.600 |
| ACV009-3 Mean | | 21.583 | 21.691 | 22.186 | 20.259 | 19.365 | 21.388 | 20.554 | 21.004 |
| ACV010-3 | 1 | 18.459 | 23.385 | 20.865 | 17.271 | 17.729 | 18.423 | 20.097 | 19.461 |
| | 2 | 21.464 | 24.600 | 21.526 | 16.601 | 17.880 | 21.228 | 19.651 | 20.421 |
| | 3 | 22.851 | 23.313 | 19.353 | 16.544 | 18.666 | 21.594 | 17.878 | 20.028 |
| ACV010-3 Mean | | 20.925 | 23.766 | 20.581 | 16.805 | 18.092 | 20.415 | 19.209 | 19.970 |
| Overall Mean | | 22.786 | 23.613 | 22.516 | 19.154 | 19.872 | 21.541 | 20.077 | 21.366 |

**Figure 7: Phenol $EC_{50}$ 15 minute Data**

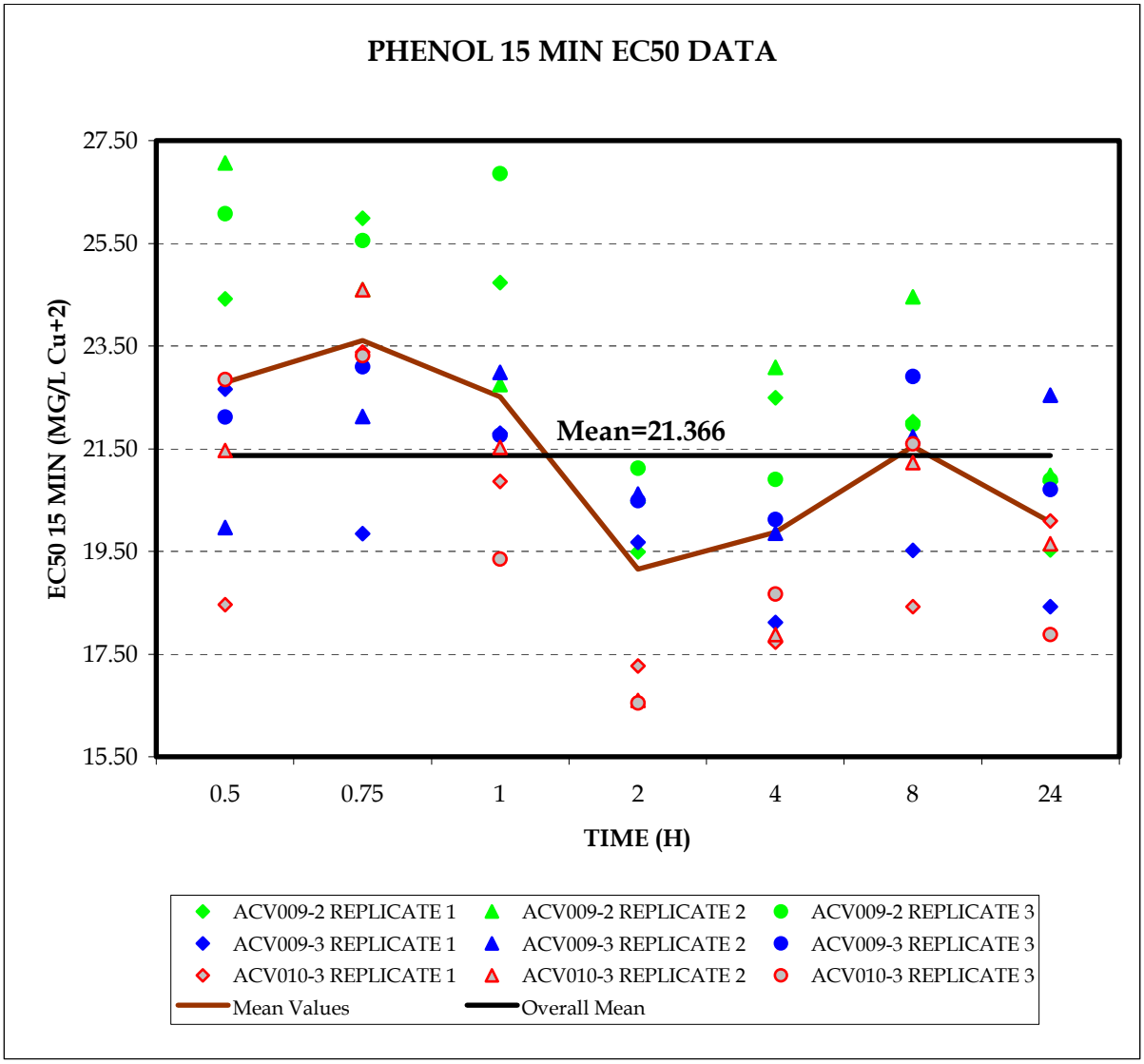


**Table 12. Phenol Differences Among Mean $EC_{50}$ Values (mg/L)**

| | | Time | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Reagent | | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 |
| ACV009-2 | Arithmetic Mean | 25.85 | 25.38 | 24.78 | **20.40*** | 22.16 | 22.82 | **20.47*** |
| | Standard Error | 0.73 | 0.73 | 0.73 | 0.73 | 0.73 | 0.73 | 0.73 |
| ACV009-3 | Arithmetic Mean | 21.58 | 21.69 | 22.19 | 20.26 | 19.37 | 21.39 | 20.55 |
| | Standard Error | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 |
| ACV010-3 | Arithmetic Mean | 20.92 | **23.77*** | 20.58 | **16.81*** | 18.09 | 20.42 | 19.21 |
| | Standard Error | 0.74 | 0.74 | 0.74 | 0.74 | 0.74 | 0.74 | 0.74 |

Note: Within each reagent, symbol '*' indicates that the mean is significantly different from the mean for 1 hour duration at 95% confidence level

**Table 13. Phenol Differences Among Means, Reagent Lots $EC_{50}$ Values (mg/L)**

| | | Reagent | | | |
|---|---|---|---|---|---|
| Time | | ACV009-2 | ACV009-3 | ACV010-3 | Multiple Comparisons* |
| 0.50 | Adjusted mean | 23.99 | 21.17 | 20.27 | **abb** |
| | Standard error | 0.53 | 0.53 | 0.53 | |
| 0.75 | Adjusted mean | 23.94 | 21.16 | 20.26 | **abb** |
| | Standard error | 0.52 | 0.52 | 0.52 | |
| 1.00 | Adjusted mean | 23.90 | 21.16 | 20.24 | **abb** |
| | Standard error | 0.52 | 0.52 | 0.52 | |
| 2.00 | Adjusted mean | 23.74 | 21.12 | 20.19 | **abb** |
| | Standard error | 0.49 | 0.49 | 0.49 | |
| 4.00 | Adjusted mean | 23.41 | 21.06 | 20.07 | **abb** |
| | Standard error | 0.45 | 0.45 | 0.45 | |
| 8.00 | Adjusted mean | 22.75 | 20.93 | 19.84 | **abb** |
| | Standard error | 0.46 | 0.46 | 0.46 | |
| 24.00 | Adjusted mean | 20.13 | 20.42 | 18.93 | aaa |
| | Standard error | 1.12 | 1.12 | 1.12 | |

Note: Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level, i.e. no reagent means at a given time are different when the three letters are the same.

## Table 14: Results of Sodium Dodecyl Sulphate Assays

| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L SDS) | $EC_{50}$ 5 MIN (mg/L SDS) | $EC_{20}$ 15 MIN (mg/L SDS) | $EC_{50}$ 15 MIN (mg/L SDS) |
|---|---|---|---|---|---|---|
| ACV009-2 | 1 | 0.5 | 0.738 | 1.855 | 0.372 | 0.855 |
| ACV009-2 | 1 | 0.75 | 1.180 | 2.038 | 0.480 | 0.953 |
| ACV009-2 | 1 | 1 | 0.597 | 1.575 | 0.290 | 0.736 |
| ACV009-2 | 1 | 2 | 0.598 | 1.466 | 0.269 | 0.654 |
| ACV009-2 | 1 | 4 | 0.859 | 1.757 | 0.287 | 0.721 |
| ACV009-2 | 1 | 8 | 0.608 | 1.762 | 0.226 | 0.632 |
| ACV009-2 | 1 | 24 | 0.897 | 2.544 | 0.332 | 0.973 |
| ACV009-2 | 2 | 0.5 | 0.840 | 2.140 | 0.352 | 0.853 |
| ACV009-2 | 2 | 0.75 | 0.811 | 1.872 | 0.356 | 0.821 |
| ACV009-2 | 2 | 1 | 0.845 | 1.879 | 0.366 | 0.845 |
| ACV009-2 | 2 | 2 | 0.467 | 1.344 | 0.237 | 0.603 |
| ACV009-2 | 2 | 4 | 0.542 | 1.421 | 0.197 | 0.534 |
| ACV009-2 | 2 | 8 | 0.620 | 1.522 | 0.220 | 0.621 |
| ACV009-2 | 2 | 24 | 0.928 | 2.451 | 0.382 | 1.095 |
| ACV009-2 | 3 | 0.5 | 0.962 | 1.917 | 0.418 | 0.920 |
| ACV009-2 | 3 | 0.75 | 0.890 | 1.838 | 0.402 | 0.873 |
| ACV009-2 | 3 | 1 | 0.645 | 1.784 | 0.301 | 0.770 |
| ACV009-2 | 3 | 2 | 0.583 | 1.442 | 0.239 | 0.598 |
| ACV009-2 | 3 | 4 | 0.511 | 1.364 | 0.187 | 0.503 |
| ACV009-2 | 3 | 8 | 0.500 | 1.329 | 0.167 | 0.465 |
| ACV009-2 | 3 | 24 | 1.172 | 2.697 | 0.559 | 1.359 |
| ACV009-3 | 1 | 0.5 | 1.035 | 1.961 | 0.293 | 0.882 |
| ACV009-3 | 1 | 0.75 | 1.026 | 1.996 | 0.391 | 0.979 |
| ACV009-3 | 1 | 1 | 0.945 | 1.861 | 0.395 | 0.862 |
| ACV009-3 | 1 | 2 | 0.872 | 1.783 | 0.365 | 0.805 |
| ACV009-3 | 1 | 4 | 0.578 | 1.597 | 0.220 | 0.593 |
| ACV009-3 | 1 | 8 | 0.423 | 1.528 | 0.164 | 0.523 |
| ACV009-3 | 1 | 24 | 1.057 | 2.523 | 0.365 | 1.174 |
| ACV009-3 | 2 | 0.5 | 1.178 | 1.974 | 0.498 | 1.003 |
| ACV009-3 | 2 | 0.75 | 0.809 | 1.598 | 0.409 | 0.852 |
| ACV009-3 | 2 | 1 | 0.971 | 1.892 | 0.415 | 0.900 |
| ACV009-3 | 2 | 2 | 0.693 | 1.620 | 0.368 | 0.801 |
| ACV009-3 | 2 | 4 | 0.576 | 1.503 | 0.230 | 0.629 |
| ACV009-3 | 2 | 8 | 0.629 | 1.686 | 0.207 | 0.625 |
| ACV009-3 | 2 | 24 | 0.897 | 2.484 | 0.302 | 1.001 |
| ACV009-3 | 3 | 0.5 | 0.827 | 1.673 | 0.346 | 0.818 |
| ACV009-3 | 3 | 0.75 | 0.765 | 1.605 | 0.381 | 0.826 |
| ACV009-3 | 3 | 1 | 0.764 | 1.672 | 0.321 | 0.770 |
| ACV009-3 | 3 | 2 | 0.590 | 1.477 | 0.271 | 0.668 |
| ACV009-3 | 3 | 4 | 0.597 | 1.569 | 0.251 | 0.674 |
| ACV009-3 | 3 | 8 | 0.650 | 1.811 | 0.242 | 0.691 |
| ACV009-3 | 3 | 24 | 1.502 | 5.722 | 0.594 | 1.913 |
| ACV010-3 | 1 | 0.5 | 0.823 | 1.817 | 0.380 | 0.860 |
| ACV010-3 | 1 | 0.75 | 0.832 | 1.924 | 0.361 | 0.895 |

| REAGENT LOT | REPLICATE | TIME (H) | $EC_{20}$ 5 MIN (mg/L SDS) | $EC_{50}$ 5 MIN (mg/L SDS) | $EC_{20}$ 15 MIN (mg/L SDS) | $EC_{50}$ 15 MIN (mg/L SDS) |
|---|---|---|---|---|---|---|
| ACV010-3 | 1 | 1 | 0.798 | 1.743 | 0.378 | 0.854 |
| ACV010-3 | 1 | 2 | 0.775 | 1.615 | 0.417 | 0.848 |
| ACV010-3 | 1 | 4 | 0.601 | 1.419 | 0.333 | 0.727 |
| ACV010-3 | 1 | 8 | 0.704 | 1.345 | 0.487 | 0.819 |
| ACV010-3 | 1 | 24 | 2.737 | 12.471 | 0.942 | 3.024 |
| ACV010-3 | 2 | 0.5 | 0.743 | 1.815 | 0.329 | 0.806 |
| ACV010-3 | 2 | 0.75 | 0.841 | 1.881 | 0.370 | 0.851 |
| ACV010-3 | 2 | 1 | 0.844 | 1.700 | 0.415 | 0.899 |
| ACV010-3 | 2 | 2 | 0.853 | 1.622 | 0.475 | 0.915 |
| ACV010-3 | 2 | 4 | 0.672 | 1.389 | 0.258 | 0.636 |
| ACV010-3 | 2 | 8 | 0.924 | 2.090 | 0.327 | 0.860 |
| ACV010-3 | 2 | 24 | 1.303 | 5.159 | 0.658 | 2.268 |
| ACV010-3 | 3 | 0.5 | 0.693 | 1.606 | 0.360 | 0.782 |
| ACV010-3 | 3 | 0.75 | 0.651 | 1.340 | 0.389 | 0.794 |
| ACV010-3 | 3 | 1 | 0.633 | 1.554 | 0.263 | 0.710 |
| ACV010-3 | 3 | 2 | 0.527 | 1.305 | 0.231 | 0.584 |
| ACV010-3 | 3 | 4 | 0.600 | 1.468 | 0.231 | 0.573 |
| ACV010-3 | 3 | 8 | 0.539 | 1.838 | 0.187 | 0.610 |
| ACV010-3 | 3 | 24 | 1.794 | 8.864 | 0.622 | 2.562 |

**Table 15: Sodium Dodecyl Sulphate $EC_{50}$ 15 minute Data Summary (mg/L)**

| | | TIME (H) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| REAGENT | REPLICATE | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 | Mean |
| ACV009-2 | 1 | 0.855 | 0.953 | 0.736 | 0.654 | 0.721 | 0.632 | 0.973 | 0.789 |
| | 2 | 0.853 | 0.821 | 0.845 | 0.603 | 0.534 | 0.621 | 1.095 | 0.767 |
| | 3 | 0.920 | 0.873 | 0.770 | 0.598 | 0.503 | 0.465 | 1.359 | 0.784 |
| ACV009-2 Mean | | 0.876 | 0.882 | 0.784 | 0.618 | 0.586 | 0.573 | 1.142 | 0.780 |
| ACV009-3 | 1 | 0.882 | 0.979 | 0.862 | 0.805 | 0.593 | 0.523 | 1.174 | 0.831 |
| | 2 | 1.003 | 0.852 | 0.900 | 0.801 | 0.629 | 0.625 | 1.001 | 0.830 |
| | 3 | 0.818 | 0.826 | 0.770 | 0.668 | 0.674 | 0.691 | 1.913 | 0.909 |
| ACV009-3 Mean | | 0.901 | 0.886 | 0.844 | 0.758 | 0.632 | 0.613 | 1.363 | 0.857 |
| ACV010-3 | 1 | 0.860 | 0.895 | 0.854 | 0.848 | 0.727 | 0.819 | 3.024 | 1.147 |
| | 2 | 0.806 | 0.851 | 0.899 | 0.915 | 0.636 | 0.860 | 2.268 | 1.034 |
| | 3 | 0.782 | 0.794 | 0.710 | 0.584 | 0.573 | 0.610 | 2.562 | 0.945 |
| ACV010-3 Mean | | 0.816 | 0.847 | 0.821 | 0.782 | 0.645 | 0.763 | 2.618 | 1.042 |
| Overall Mean | | 0.864 | 0.872 | 0.816 | 0.720 | 0.621 | 0.650 | 1.708 | 0.893 |

**Figure 8: Sodium Dodecyl Sulphate $EC_{50}$ 15 minute Data**

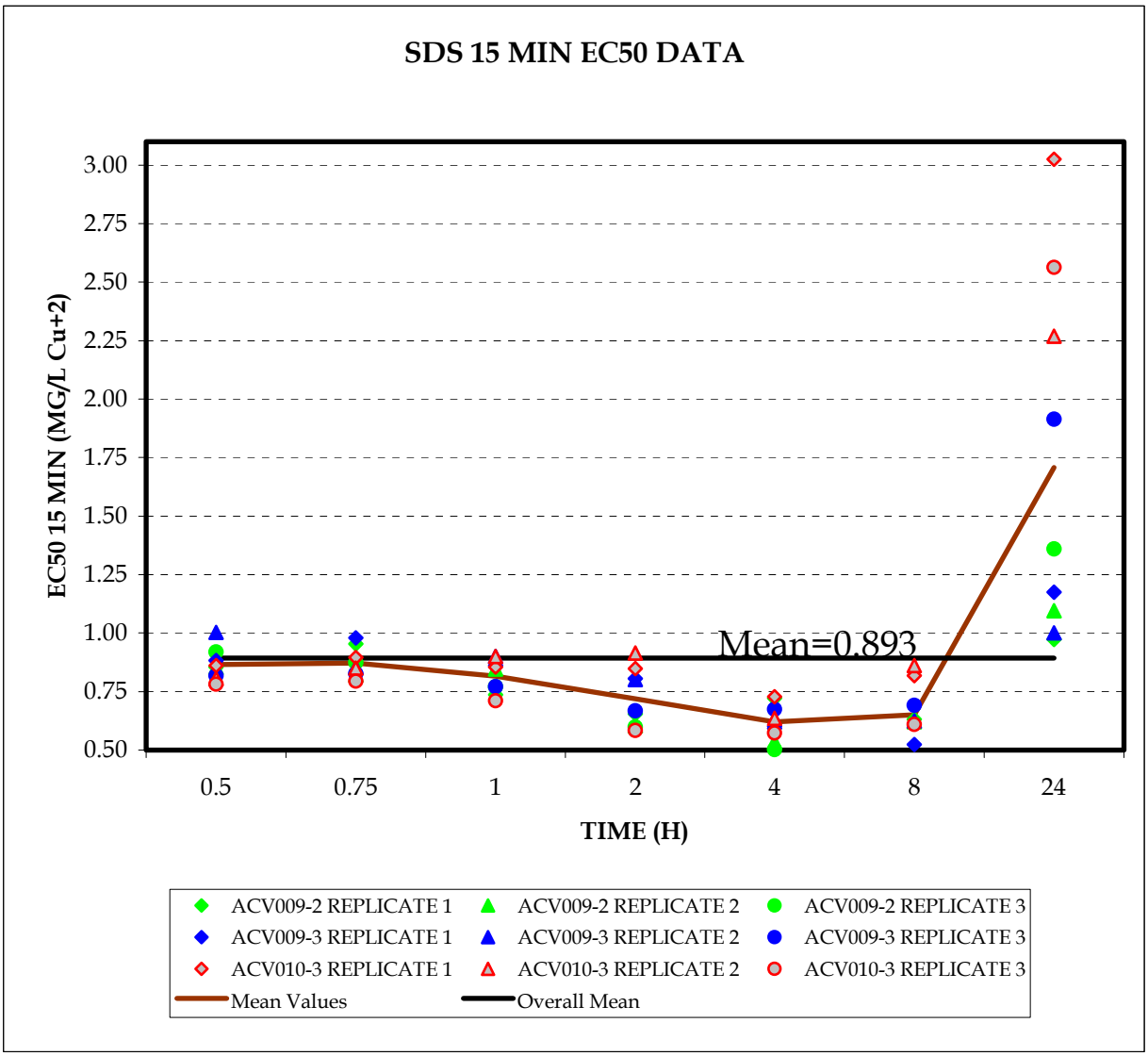


**Figure 9: Sodium Dodecyl Sulphate $EC_{50}$ 15 minute Data (24 hour data removed)**

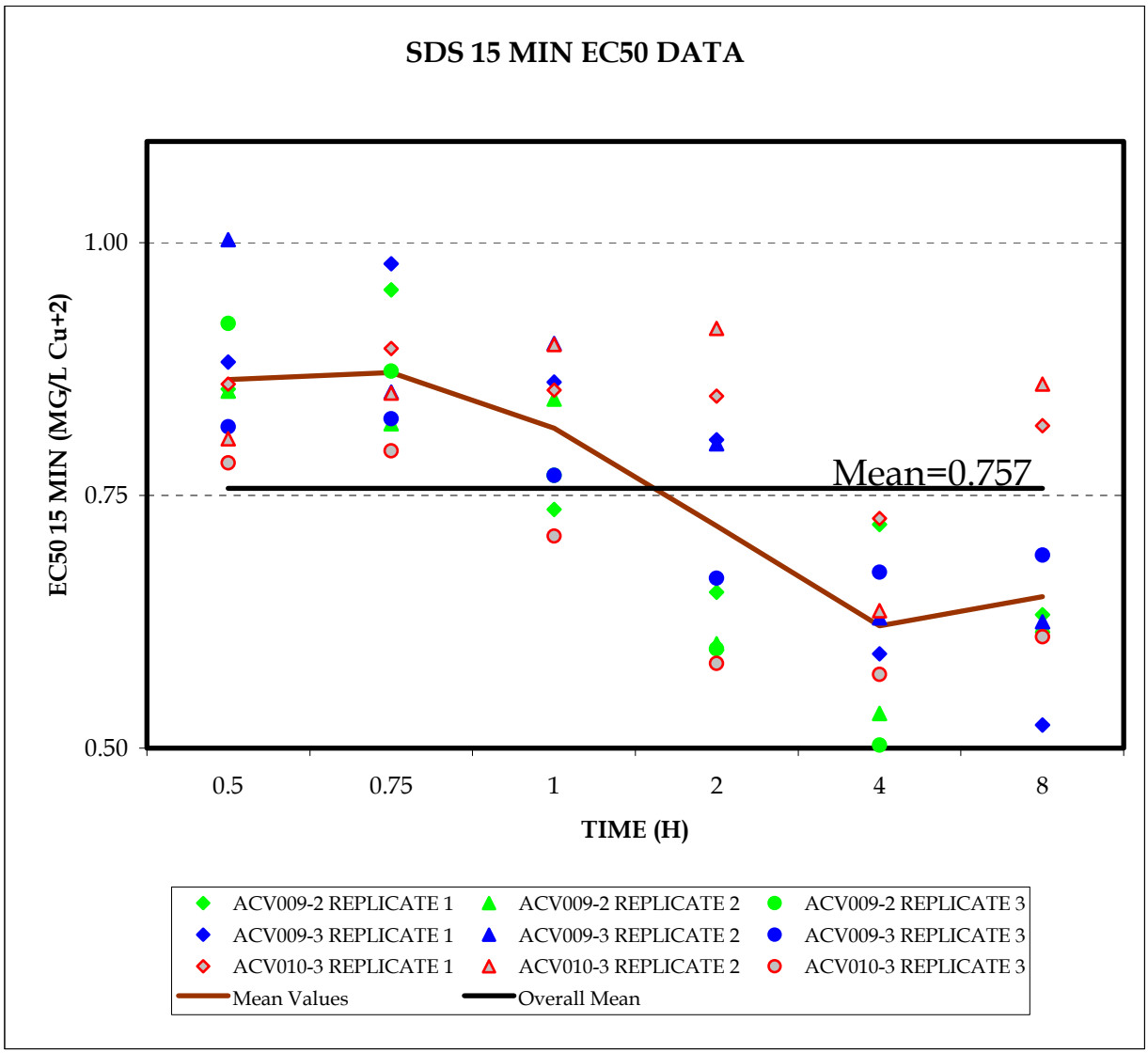


**Table 16. Sodium Dodecyl Sulphate Differences Among Mean $EC_{50}$ Values (mg/L)**

| | | Time | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Reagent | | 0.5 | 0.75 | 1 | 2 | 4 | 8 | 24 |
| ACV009-2 | Arithmetic mean | 0.88 | 0.88 | 0.78 | 0.62 | 0.59 | 0.57 | **1.14*** |
| | Standard error | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| ACV009-3 | Arithmetic mean | 0.90 | 0.89 | 0.84 | 0.76 | 0.63 | 0.61 | **1.36*** |
| | Standard error | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 |
| ACV010-3 | Arithmetic mean | 0.82 | 0.85 | 0.82 | 0.78 | 0.65 | 0.76 | **2.62*** |
| | Standard error | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |

Note: Within each reagent, symbol '*' indicates that the mean is significantly different from the mean for 1 hour duration at 95% confidence level

**Table 16. Sodium Dodecyl Sulphate Differences Among Means, Reagent Lots $EC_{50}$ Values (mg/L)**

| | | Reagent | | | |
|---|---|---|---|---|---|
| Time | | ACV009-2 | ACV009-3 | ACV010-3 | Multiple Comparisons* |
| 0.50 | Adjusted mean | 0.71 | 0.75 | 0.64 | aaa |
| | Standard error | 0.06 | 0.06 | 0.06 | |
| 0.75 | Adjusted mean | 0.71 | 0.75 | 0.66 | aaa |
| | Standard error | 0.06 | 0.06 | 0.06 | |
| 1.00 | Adjusted mean | 0.71 | 0.76 | 0.68 | aaa |
| | Standard error | 0.06 | 0.06 | 0.06 | |
| 2.00 | Adjusted mean | 0.73 | 0.78 | 0.75 | aaa |
| | Standard error | 0.06 | 0.06 | 0.06 | |
| 4.00 | Adjusted mean | 0.76 | 0.82 | 0.91 | aaa |
| | Standard error | 0.05 | 0.05 | 0.05 | |
| 8.00 | Adjusted mean | 0.81 | 0.90 | 1.21 | **aab** |
| | Standard error | 0.06 | 0.06 | 0.06 | |
| 24.00 | Adjusted mean | 1.04 | 1.24 | 2.44 | **aab** |
| | Standard error | 0.13 | 0.13 | 0.13 | |

Note: Within each time point, different letters indicate that the adjusted means are significantly different from each other at the 95% confidence level, i.e. no reagent means at a given time are different when the three letters are the same.