# FINAL REPORT

## Validation of a Novel Bioassay for Low-level Perchlorate Determination

## ESTCP Project ER-201030

April 2014

John D. Coates
**University of California, Berkeley**



ESTCP

# Table of Contents i

## List of Appendices

## List of Tables

**Appendix B, Benchtop Bioassay Data**

## List of Figures

**Appendix B, Benchtop Bioassay Data**

**Appendix F, Microplate Reader Bioassay Data**

**Appendix G, Estimate Perchlorate Concentration by SAM Plots of SPE Eluates Analyzed by IC**



**Appendix I, ICPMS Characterization of Groundwater Sources and SPE Eluates**

**Appendix J, Testing to Determine Whether Uranium (VI) Interfers with the Benchtop Bioassay**



**Appendix L, Aspects of Bioassay Testing**

## Abbreviations and Acronyms

| | |
|---|---|
| ANOVA | analysis of variance |
| AU | absorbance units |
| Cld, cld | chlorite dismutase |
| DPRB | dissimilatory perchlorate-reducing bacteria |
| DTAB | decyltrimethyl ammonium bromide |
| EDQW | Environmnetal Data Quality Workgroup |
| EPA | Environmental Protection Agency |
| ESI | electrospray ionization |
| IC | ion chromatography |
| ICPMS | inductively coupled plasma mass spectrometry |
| JPL | Jet Propulsion Laboratory |
| LC/MS | liquid chromatography/mass spectrometry |
| LLNL | Lawrence Livermore National Laboratory |
| LRB | laboratory reagent blank |
| MDS | multidimensional scaling |
| MMR | Massachusetts Military Reservation |
| MOPS | morpholinepropane sulfonic acid |
| MRL | method reporting level |
| MS | mass spectrometry |
| NADH | reduced nicotinamide adenine dinucleotide |
| NAS | National Academy of Sciences |
| Pcr, pcr, PCR | perchlorate reductase |

| | |
|---|---|
| PD | percent difference |
| PI | principal investigator |
| PMS | phenazine methosulfate |
| PMSH | reduced phenazine methosulfate |
| PR | plate reader |
| QA | quality assurance |
| QC | quality control |
| RSD | relative standard of deviation |
| SAM | standard additions method |
| SPE | solid phase extraction |
| SDVB | styrene divinylbenzene |
| UCB | University of California, Berkeley |
| xt | cell-free extract |

## Acknowledgements

The following sites provided invaluable help by providing samples for the conduct of this work: Hill Air Force Base, Naval Weapons Industrial Reserve Plant McGregor, Jet Propulsion Laboratory, Fontana Water Company, Massachusetts Military Reservation, and Lawrence Livermore National Laboratory. Their participation is greatly appreciated.

# EXECUTIVE SUMMARY

The bioassay for low-level perchlorate determination (range approximately 6 to 40 µg/L), which was the focus of the project, is a benchtop enzyme assay combined with a perchlorate concentration and purification step using solid phase extraction (SPE) cartridges (Heinnickel et al., 2011). It is based on perchlorate reductase activity and couples NADH oxidation to perchlorate reduction, with PMS as an electron shuttle. Hence the amount of perchlorate in an aqueous sample can be determined enzymatically by monitoring the amount of NADH oxidized spectrophotometrically (i.e. by measuring the decrease in absorbance at 340 nm). The instrumentation for the bioassay is a UV spectrophotometer. The equipment and materials are relatively inexpensive and the bioassay potentially may be performed by less highly trained personnel than required by IC or LC/MS analytical methods. Thus the benchtop bioassay might supplement more expensive and time consuming analytical methods as a screening test for perchlorate in groundwater to facilitate tasks such as mapping plumes and monitoring perchlorate levels during remediation. The goal of the project was to determine whether results with the benchtop bioassay were in agreement with those of a reference analytical method using diverse groundwater sources. If results with the benchtop bioassay performed in the PI's laboratory were comparable to reference analytical methods, it was planned to develop and test a kit format of the bioassay that could be used in the field.

The enzymatic bioassay for perchlorate was developed under SERDP Project ER 1530[19]. Bioassay results with groundwater collected from three different locations at one site were in good agreement with results obtained by a reference IC method. The levels of perchlorate in these samples were approximately 10, 22, and 71 µg/L. This testing was performed in a 96 well microplate using a microplate reader inside of an anaerobic chamber with an atmosphere of nitrogen and hydrogen. In addition, the benchtop version of the bioassay was developed, using quartz cuvettes, to be performed in ambient laboratory conditions. The benchtop bioassay format performed as well as the microplate reader format with perchlorate standards.

Since different groundwater samples vary in the efficiency of perchlorate recovery at the SPE step, subsamples are spiked with perchlorate according to the standard additions method for quantitating an analyte[14]. After applying perchlorate-spiked groundwater to SPE cartridges, the cartridges are rinsed, and then perchlorate is eluted with an alkaline solution (pH >12) of MOPS and NaCl. The eluates are neutralized to about pH 7.2, added to cuvettes, overlaid with mineral oil in order to provide an aqueous compartment separated from air, and NADH and PMS reactants are added. Anaerobic conditions needed for enzyme activity are generated using the non-enzymatic reduction of PMS by NADH in order to reduce dissolved oxygen to water prior to the addition of enzyme. Addition of enzyme initiates the enzyme reaction. An extract of the perchlorate reducing bacterium *Dechloromonas agitata* strain CKB, which contains the enzyme perchlorate reductase, provides the enzyme source for the assay.

The three performance objectives of the project were as follows (see Table 3-1). 1. Compare benchtop bioassay results from testing in the PI's laboratory to results from a reference analytical method performed by a commercial laboratory. 2. Compare results with a benchtop bioassay kit format tested by site field personnel to the site's routine perchlorate detection method. 3. Evaluate the ease of use of the benchtop bioassay kit format.

Regarding objective 1, although it proved difficult to find partners who could collect the groundwater needed for the study, six groundwater sources were obtained for the project. Success criteria were met for one groundwater source, but were not met for four other groundwater sources. See Table 5-4 for a summary of testing outcomes. Quantitative success criteria could not be applied to the sixth source since the amount of $ClO_4^-$ by the reference method was <MRL. Results for this source were considered to have qualitatively met success criteria. Thus the benchtop bioassay, at least in its current format, is not suitable as a screening test for perchlorate in groundwater. Indepth analysis indicated that oxygen contamination of the bechtop assay, even in the face of several barriers and an active chemical reductant, was the culprit for the unpredictability and poor performance of the bioassay comparison under objective 1. Retesting of the water samples with the bioassay in an anaerobic chamber with a nitrogen/hydrogen atmosphere confirmed this finding and provided a different outcome. In this instance the bioassay accuracy met all performance criteria for groundwater eluates amenable to quantitative testing. Since performance of the bioassay was not high enough to warrant proceeding with development of a kit format for the benchtop bioassay, no testing was done relative to performance objectives 2 and 3. The focus of activities shifted to attempting to identify factors that interfere with the benchtop bioassay in order to determine whether operations and performance could be improved.

It is useful to consider in more detail the performance of the benchtop bioassay with the five groundwater sources that could be quantitatively compared. In addition to the reference method that was performed in a commercial laboratory, analytical testing for perchlorate according to method EPA 314.0 was performed in the PI's laboratory and included in the comparison of methods. The metric used to evaluate accuracy was the percent difference (PD) between results with a method of interest and the results with the reference method tested in a commercial laboratory. A 30 % or less difference was considered to be acceptable accuracy. In addition, for instances where accuracy was acceptable, an ANOVA statistical analysis was used to further evaluate whether bioassay results were significantly different from the reference method.

The benchtop bioassay accuracy was qualatively acceptable for the JPL source. The accuracy was quantitatively acceptable for the Fontana Water Co. source and boarderline for the Hill AFB source. ANOVA testing showed that the methods were not significantly different with the Fontana Water Co. source, whereas they were significantly different for Hill AFB. Additional analysis by the Tukey Method indicated that the benchtop bioassay results were responsible for the difference among the methods for the Hill AFB groundwater. Thus, benchtop bioassay results with Hill AFB groundwater were significantly different from the reference method. Accuracy of the benchtop bioassay was not acceptable (i.e > 30 % PD) for the three other sources: NWIRP McGregor, Massachusetts Military Reservation (MMR), and LLNL. There was a pronounced lack of agreement among replicates tested in the benchtop bioassay for NWIRP McGregor and Massachusetts Military Reservation sources. The reason for this poor performance was not determined but may be due to inefficient reduction of reaction mixtures during the preincubation period before the addition of cell extract. By contrast, with LLNL groundwater, there was good agreement among replicates in the benchtop bioassay testing. Apparently there was interference with the benchtop bioassay seen with the LLNL groundwater. No factor was identified as being responsible for the interference behavior, and interference was

apparently not observed when some LLNL eluate sets were retested using the anaerobic plate reader bioassay format.

It was instructive to retest representative SPE eluate standard additions method sets in the alternative bioassay format, i.e. in a microplate reader in an anaerobic chamber with a nitrogen/hydrogen atmosphere. The accuracy was acceptable for the five groundwater eluates amenable to quantitative testing: Hill AFB, NWIRP McGregor, Fontana Water Co., Massachusetts Military Reservation, and LLNL (Table 5-6). ANOVA and further testing by the Tukey Method (Table 5-7) indicated that the plate reader bioassay and the reference method in a commercial laboratory were not significantly different. Possibly the more reducing conditions used for the plate reader format of the bioassay are responsible for the difference in performance compared to the benchtop format.

In addition to perchlorate, it was shown by ICPMS analysis of groundwater sources and corresponding SPE eluates that other components in groundwater samples are concentrated in SPE eluates. In particular, uranium was concentrated almost to the same degree as perchlorate. Since the level of uranium in SPE eluates of LLNL groundwater was about 9 µM, it seemed possible that uranium might contribute to the interference seen with LLNL groundwater in the benchtop bioassay. However, when uranium (VI) was tested for possible interference with the benchtop bioassay, no effect on perchlorate reductase activity was seen.

# 1.0 INTRODUCTION

## 1.1 BACKGROUND

Perchlorate ($ClO_4^-$) is principally a synthetic compound with a broad assortment of industrial applications ranging from pyrotechnics to lubricating oils[12]. Ammonium perchlorate represents 90% of all perchlorate salts manufactured and is used as an energetic booster or oxidant in solid rocket fuels. Its presence in the environment poses a potential health threat and primarily results from legal historical discharge of unregulated manufacturing waste streams, disposal pond leaching, and the periodic servicing of military inventories. Although a powerful oxidant, under most environmental conditions, perchlorate is highly stable and non-reactive due to the high energy of activation associated with its reduction. Because of the large molecular volume and single anionic charge, perchlorate also has a low affinity for cations and as a result, perchlorate salts are generally highly soluble and completely dissociate in aqueous solution. Perchlorate does not sorb to any significant extent to soils or sediments and, in the absence of any biological interactions, its mobility and fate are largely influenced by the hydrology of the environment[16].

Perchlorate is known to affect mammalian thyroid hormone production and its primary toxicity results from its structural similarity to iodate, which plays an important regulatory role in hormone production by the thyroid gland[3, 18]. Perchlorate readily enters into the food web and can be taken up by forage and edible vegetation[9] as well as plants grown hydroponically[7]. In certain plant species, including tobacco and lettuce, perchlorate accumulates and can persist during processing into final consumer products such as cigarettes and chewing tobacco[8]. Of great concern is a study done in the US on breast and dairy milk showing the presence of perchlorate in almost all samples analyzed[11].

It has been known for more than fifty years that microorganisms can reduce oxyanions of chlorine such as chlorate ($ClO_3^-$) and perchlorate [collectively denoted (per)chlorate] under reducing conditions. The high reduction potential makes them ideal electron acceptors for microbial metabolism and specialized microbes can grow by the anaerobic reductive dissimilation of (per)chlorate to innocuous chloride. Many dissimilatory (per)chlorate-reducing bacteria (DPRB) have been isolated from a broad diversity of environments including both pristine and contaminated soils and sediments[1, 4, 6]. Remediation strategies for perchlorate contamination include making use of DPRB both in-situ and ex-situ, in addition to other technologies[5].

The successful implementation of any remediative strategy is dependent on the accurate identification of the boundaries of the perchlorate plume. Analytical methods currently available include ion chromatography (IC) with conductivity detection, which forms the basis of EPA Method 314.0, and liquid chromatography or IC coupled with electrospray ionization (ESI) mass spectrometry (MS), which forms the basis of EPA Method 6850 or 6860, respectively. These techniques are laborious, expensive, time consuming, and require highly trained personnel, making them unsuitable for the rapid delineation of contaminated environments. An alternative biochemical technique was developed under SERDP Project ER 1530 “An Enzymatic Bioassay for Perchlorate” by Coates, Heinnickel, and Achenbach, 2010[19], which is based on perchlorate reductase activity coupled to NADH oxidation, with PMS as an electron shuttle. The

instrumentation for the bioassay is UV spectrophotometer used to measure the absorbance of NADH at 340 nm.

Bioassay results with groundwater samples from three wells at one collection site were in good agreement with results obtained by a reference IC method. The levels of perchlorate in these samples were approximately 10, 22, and 71 ppb. This testing was performed in a 96 well microplate using a microplate reader inside of an anaerobic chamber with an atmosphere of nitrogen and hydrogen. In addition, a benchtop version of the bioassay was developed using quartz cuvettes to be performed in ambient laboratory conditions. The benchtop bioassay format performed as well as the microplate reader format with perchlorate standards. Thus the benchtop bioassay method appeared to have the potential to supplement more expensive and time consuming analytical methods as a screening test to facilitate tasks such as mapping plumes and monitoring perchlorate levels during remediation.

## 1.2 OBJECTIVE OF THE DEMONSTRATION

The overall project goals of ER-201030 were: 1. Evaluate the sensitivity and reliability of the benchtop perchlorate bioassay as a screening test for perchlorate in the 6 to 40 ppb range, compared to IC or LC/MS analytical techniques as the reference method, using groundwater samples from diverse environments. If bioassay results were consistent with those of the reference method, thereby showing promise as an alternative screening test for perchlorate, proceed to the second goal. 2. Assess the feasibility of using the bioassay in a kit format to monitor perchlorate levels at field sites by developing reagents, an SOP, and providing technical support to field personnel unfamiliar with the assay who would test samples by the bioassay in parallel with the site's usual analytical procedures. Bioassay results would be compared to results from the site's routine perchlorate testing method, and the ease of use would be evaluated by questionnaire and discussions with field testing personnel.

Six sites provided groundwater to meet the first goal. The benchtop bioassay met the criterion for accuracy relative to the reference analytical method for only one groundwater source, and accuracy was qualitatively acceptable with a second source. With a third source, bioassay results did not meet the accuracy criterion, although they were close to the cut-off for acceptance. In addition, bioassay results for this source were significantly different from the reference analytical method by an ANOVA analysis. Finally, the benchtop bioassay accuracy was unacceptable for an additional three groundwater sources. Thus the benchtop bioassay, at least in its current format, is not suitable as a screening test for perchlorate in groundwater. Since performance of the bioassay was not high enough to warrant proceeding with field testing of the bioassay in a kit format, the focus of activities shifted to attempting to identify factors that interfere with the benchtop bioassay in order to determine whether operations and performance could be improved.

## 1.3 REGULATORY DRIVERS

Before 1997, perchlorate was unregulated in the US, however, its discovery in national drinking water resources, prompted the establishment of a federal provisional action level of 18 parts per billion (ppb or μg/L) resulting in a decade of high profile debate over the determination of a final federal action level. In 1998 perchlorate was added to the US EPA Contaminant Candidate List

for drinking water supplies[17], and a final decision regarding the regulatory limit was to be set pending the outcome of ongoing toxicological studies. States such as Massachusetts, New Jersey, and California, adopted their own regulatory levels of 2, 5, and 6 ppb, respectively, in an attempt to limit the health impact of this contaminant while awaiting the outcome of a federal decision[13]. In January 2002, as a result of publication of the first draft of the US EPA review on toxicological and risk assessment data, a revised and lowered health protective standard of 1 μg/L was suggested. Since the findings of this draft assessment were highly controversial to three other federal agencies, the US National Academy of Sciences (NAS) was asked to make an assessment. In January of 2005 the NAS suggested a maximum permissible dose of 0.7 μg/kg/d. This suggestion correlates to a standard of about 23 μg/L for a normal adult person. However, the level would be lower for infants and children based on weight. This is especially poignant due to the recent study done on breast and dairy milk in the US[11]. The highest level detected in breast milk was 92 μg/L, a level 20 times higher than the NAS estimated maximum permissible dose for a baby. Reports of this magnitude are pressuring officials to set a final regulatory limit in the near future, and focus interest on technologies that support perchlorate remediation at contaminated sites. The current EPA Interim Drinking Water Health Advisory for perchlorate exposure is 15 μg/L[24].

# 2.0 TECHNOLOGY

The perchlorate bioassay uses an extract of the perchlorate reducing bacterium *Dechloromonas agitata* strain CKB, which contains the enzyme perchlorate reductase. The bioassay is based on coupling perchlorate reduction to NADH oxidation using phenazine methosulfate (PMS) as an electron shuttle to transfer electrons from NADH to the perchlorate reductase enzyme[2]. Hence the amount of perchlorate in an aqueous sample can be determined enzymatically by the decrease in absorbance at 340 nm. The method also requires prior perchlorate concentration and purification using solid phase extraction (SPE) cartridges by a modification of the procedure described by Thorne[15]. Since different samples vary in the efficiency of perchlorate recovery at the SPE step, subsamples are spiked with perchlorate according to the standard additions method for quantitating an analyte[14]. Cartridges are rinsed and then perchlorate is eluted with an alkaline solution (pH >12) of morpholinepropane sulfonic acid (MOPS) and NaCl. The eluates are neutralized to about pH 7.2, added to cuvettes, and anaerobic conditions needed for enzyme activity are generated using the non-enzymatic reduction of PMS by NADH in order to reduce dissolved oxygen to water.

## 2.1 TECHNOLOGY DESCRIPTION

The benchtop bioassay method for low-level perchlorate determination is a UV spectrophotometric enzyme assay with a prior perchlorate concentration and purification step using solid phase extraction (SPE) cartridges[2]. The equipment and materials are relatively inexpensive and the bioassay potentially may be performed by less highly trained personnel than required by IC or LC/MS analytical methods.

The SPE step uses cartridges and a cartridge conditioning procedure previously described by Thorne (2004) both to concentrate perchlorate[15] and lower the levels of other anions that would interfere with subsequent perchlorate detection, such as nitrate and chlorate ($ClO_3^-$). In addition, a standard additions method[14] is used to compensate for the variable recovery of perchlorate from SPE cartridges. Hence, a 1 L sample of groundwater for bioassay analysis is divided into five 200 mL subsamples. One subsample is not spiked with any perchlorate and the remaining four are spiked with increasing increments of 10 ppb (parts per billion, i.e. µg/L) of perchlorate. Each subsample is applied to a separate conditioned SPE cartridge. To condition cartridges prior to use they are sequentially rinsed with acetone, deionized water, and decyltrimethyl ammonium bromide (DTAB). After a 200 mL subsample is passed through a cartridge, the cartridge is rinsed with 7.5 mL of a solution of 2.5 mM DTAB in 15% acetone in water in order to remove chlorate ($ClO_3^-$), nitrate and other anions. Then perchlorate is eluted using 2 mL of a solution (pH > 12.0) containing 200 mM (MOPS) and 2 M NaCl. Perchlorate potentially could be concentrated 100 fold in eluates compared to the starting levels in subsamples. Figure 2-1 shows a schematic diagram illustrating how groundwater samples were tested by the bioassay method in a benchtop format as well by reference analytical methods.

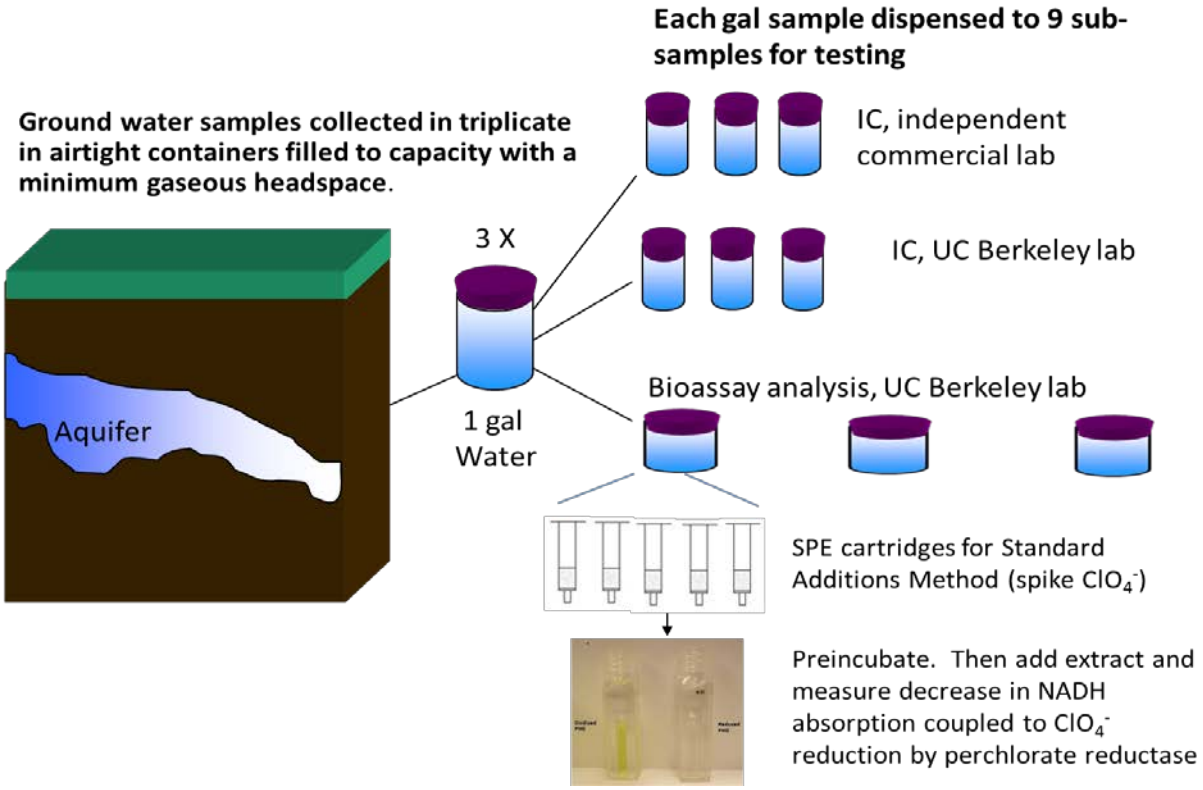



**Figure 2-1. Schematic diagram of groundwater testing by benchtop bioassay and reference analytical methods**

In preparation for the bioassay enzyme step, HCl is added to MOPS-NaCl eluates to adjust the pH to about 7.2, which is the $pK_a$ for a MOPS buffer and ideal for perchlorate reductase activity. 1 mL of this perchlorate sample solution is placed in a quartz cuvette and overlaid with mineral oil to exclude air. Other components of the reaction mixture are then added to provide for both non-enzymatic removal of oxygen during a preincubation interval (see Figure 2-2) and reactants for perchlorate reductase activity (Figure 2-3) when extract is subsequently added to reaction mixtures. NADH quickly reduces PMS non-enzymatically, which yields oxidized $NAD^+$ and reduced PMS. This initial non-enzymatic reduction of PMS is easily observed operationally, since oxidized PMS is yellow, whereas reduced PMS is colorless. In addition to the color change, reaction mixtures also become turbid because the reduced PMS is less soluble than oxidized PMS. The cuvette on the left in Figure 2-1 has PMS in the oxidized state, whereas the cuvette on the right has reduced PMS. Subsequent non-enzymatic reduction of oxygen to water, accompanied by reoxidation of PMS, apparently is a much slower reaction. This proved to be a significant impediment for the benchtop bioassay method, since perchlorate reductase requires

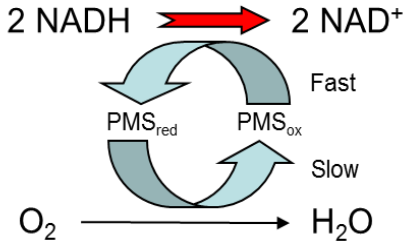



**Figure 2-2. Non-enzymatic reduction of $O_2$ by NADH and PMS**

and anaerobic environment and reaction mixtures must be rendered anoxic before enzyme is added.

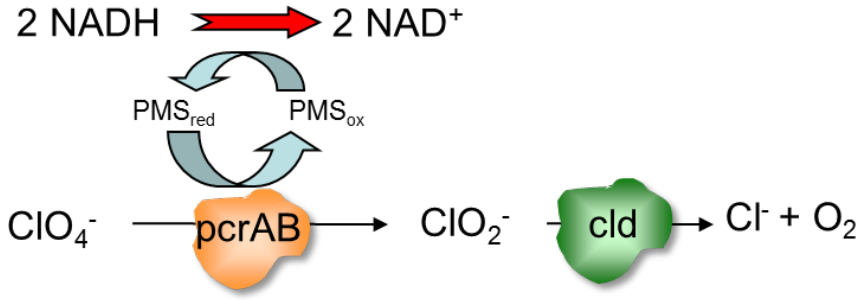



**Figure 2-3. Perchlorate reductase coupled to NADH oxidation**

The enzyme reaction is initiated by adding extract from the CKB strain of *Dechloromonas agitata*, which contains the perchlorate reductase (Pcr) enzyme. PMS and the remaining NADH are then used in the enzymatic reaction to reduce perchlorate. Figure 2-3 shows the components of the NADH-coupled enzyme assay for perchlorate. The reduced PMSH shuttles electrons to PCR so that the perchlorate is reduced to chlorite ($ClO_2^-$) by the enzyme. NADH doesn't interact with the PCR enzyme directly, but is coupled to the PCR reaction through the PMS shuttle component. Because extracts also contain the enzyme chlorite dismutase (Cld), the chlorite PCR product can be dismuted to $Cl^-$ and $O_2$. The $O_2$ generated in the chlorite dismutase reaction can in turn be non-enzymatically reduced by the NADH and PMS (Figure 2-2). Thus the overall stoichiometry when both enzymes are active would be 4 moles NADH oxidized per mole of perchlorate reduced to $Cl^-$.

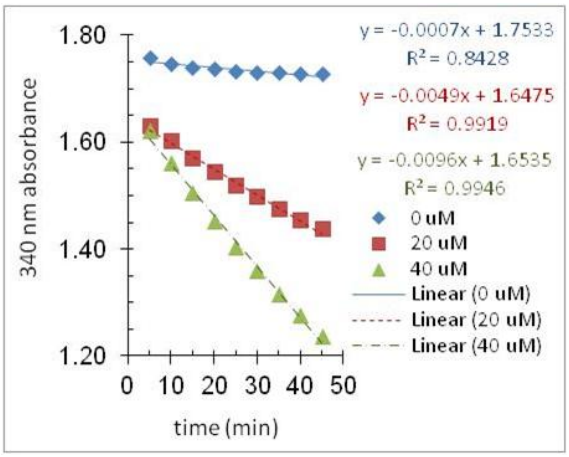



**Figure 2-4. Perchlorate reductase activity in reactions with different starting concentrations of perchlorate**

The oxidation of NADH is measured spectrophotometrically at 340 nm since NADH, but not $NAD^+$, absorbs at this wavelength. As illustrated in Figure 2-3 and discussed above, for each mole of perchlorate reduced, two to four moles of NADH are oxidized. Reactions are incubated at room temperature for about 55 minutes and absorbance measurements are made at 340 nm. The time at which extract is mixed with the other components in a cuvette is taken as the initial time. Absorbance is then measured every 5 minutes from about 10 to 55 minutes to determine the rate of reaction (i.e. the change in 340 nm absorbance/minute). It was envisioned that the kit for field bioassay testing would make use of only two absorption determinations. The first would be after about 10 minutes of reaction and the second after 45 or 55 minutes. For CKB cell extracts with perchlorate reductase activity, the rate of absorption decrease at 340 nm is correlated to the concentration of perchlorate present initially in the reaction mixture (see Figure 2-4). Samples can be analyzed batchwise so that results are obtained for a number of samples simultaneously.

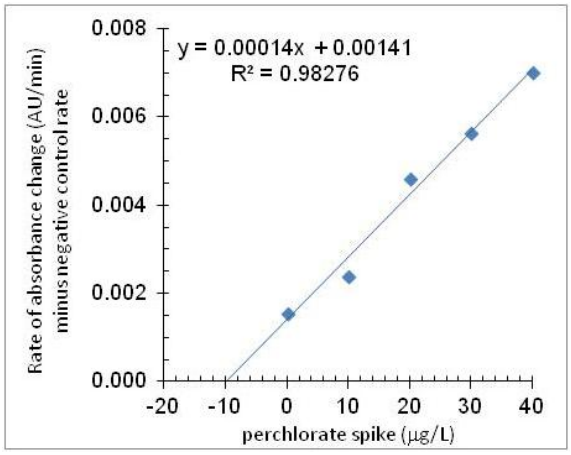



**Figure 2-5. Determination of perchlorate in a groundwater sample by the standard additions method**

Data from the enzyme assay of spiked subsamples are used to determine the amount of perchlorate in a groundwater sample. The rate of reaction (i.e. the change in 340 nm absorbance/minute) is plotted on the y-axis and the amount of perchlorate spike (µg/L) on the x-axis. By the standard additions method, the concentration of perchlorate in the sample equals (-1) times (x-intercept)[14]. Figure 2-5 shows a plot for the determination of perchlorate in SPE eluates of a groundwater sample. In this example, the amount of perchlorate was 9.9 ± 2.0 µg/L.

A summary of results comparing the perchlorate benchtop bioassay with reference methods is listed in Table 5-4.

## 2.2 TECHNOLOGY DEVELOPMENT

The bioassay for perchlorate used for this project was developed during SERDP Project ER 1530[19], also described in Heinnickel et al., 2011[2]. The bioassay makes use of perchlorate reductase, an anaerobic enzyme. Briefly, an earlier assay for perchlorate reductase depended on the use of methyl viologen as the reductant and electron shuttle, and is described in Kengen et al., 1999[10]. However, investigation of this method in ER 1530 indicated that it was unreliable for low perchlorate levels. It was shown in ER 1530 that instead of methyl viologen, NADH could be used as the reductant and that this change was advantageous in several key respects:

1. The empirical ratio of NADH oxidized to perchlorate reduced is similar to theoretical perdiction so that 340 nm absorbance change (i.e. the amount of NADH oxidized) and the amount of perchlorate reduced are quantitatively coupled.
2. NADH is stable to air so that all the necessary reactants for the enzyme reaction (i.e. perchlorate, NADH, and PMS) can be stored in air under ambient conditions.
3. Reaction mixtures must be made anaerobic prior to adding the perchlorate reductase enzyme, which is sensitive to air and requires anaerobic conditions for activity. NADH and PMS react non-enzymatically and could be used to remove oxygen from enzyme reaction mixtures prior to the addition of enzyme.

In addition, several other crucial features were incorporated into the developed bioassay. A cleared cell extract supernatant, rather than purified enzyme protein, is suitable as the enzyme source. This reduces the time and cost of providing enzyme reagent. A pretreatment step using DTAB conditioned SDVB in SPE cartridges, can effectively concentrate perchlorate from groundwater samples. This use of SPE cartridges, previously developed by Thorne[15], was modified by using an alkaline MOPS solution instead of acetone to elute perchlorate from the cartridges. The eluates were suitable for use in perchlorate reductase reaction mixtures after neutralization to pH 7.0 to 7.2 and removal of oxygen. Nitrate and chlorate anions, which are alternative substrates for perchlorate reductase, are removed at the SPE step. Because the

efficiency of perchlorate recovery from SPE cartridges was known to be variable[21] due to groundwater matrix variations, a standard additions method was adopted for the bioassay procedure whereby five 200 mL portions of groundwater were spiked with different amounts of perchlorate (i.e. 0, 10, 20, 30, or 40 μg/L) prior to application to SPE cartridges. Inclusion of 2 M NaCl in the alkaline MOPS eluting solution also was useful in several respects: lowering the background of other NADH oxidizing enzyme reactions, decreasing the solubility of oxygen in eluates, and decreasing diffusion of oxygen into the reaction mixture from the walls of the cuvette. Environmental samples were collected from different locations at the Aerojet Sacramento, CA, site and donated for bioassay analysis. Results with the bioassay (anaerobic plate reader) method were not significantly different from results obtained by a reference IC method[2]. Lastly, a benchtop version of the bioassay was developed in which 1 mL of an enzyme buffer (i.e. MOPS-NaCl solution at about pH 7.2) containing perchlorate could be placed in a quartz cuvette, overlaid with mineral oil to separate the reaction from air, and amended with NADH and PMS to non-enzymatically consume sufficient oxygen during a preincubation period to obtain conditions suitable for perchlorate reductase activity. Reactions were subsequently initiated with the addition of cell extract, and the change in 340 nm absorbance showed a good correlation between the amount of NADH oxidized and the amount of perchlorate reduced[2].

The focus of the current project was on the benchtop bioassay format, first to test environmentally diverse groundwater sources with the bioassay and second, if the performance of the benchtop bioassay was satisfactory, to develop and field test a kit version to be used as a possible perchlorate screening test.

Additional development work was done to improve operations. The benchtop bioassay requires that reactants be added to the aqueous phase under the mineral oil overlay and mixed to obtain a homogeneous solution. Different approaches to mixing were tested. Both repipetting with disposable plastic transfer pipettes and stirring, with disposable inoculating loops or a reusable stirring tool, were satisfactory when tested in reactions with perchlorate standards in enzyme buffer. However, when SPE eluates containing perchlorate were used, stirring seemed to correlate better with less variable non-enzymatic NADH oxidation.

Obtaining sufficiently reduced conditions in reaction mixtures by the non-enzymatic reaction between NADH and PMS proved difficult, particularly when SPE eluates were used. The non-enzymatic oxidation of NADH in the presence of PMS was much greater for the benchtop format than observed when reactions were performed in the anaerobic chamber using the plate reader format. NADH oxidation seen during the preincubation period was variable among replicate SPE eluate sets (see Appendix L, Table L-1) and often of much greater magnitude than occurs during perchlorate reduction by perchlorate reductase. Some consideration was given to the approach of adding reductants, in addition to the NADH and PMS system, to more efficiently reduce SPE eluates prior to bioassay analysis. However, using reductants such as ascorbic acid, dithiothreitol, or dithionite was not attractive, since these reduce PMS[2], and it was thought they would interfere with the stoichiometry of NADH and perchlorate in the bioassay. With regard to the reductant cysteine (an amino acid with a sulfhydral group), PMS is known to chemically react with sulfhydral groups. Mixing oxidized PMS with cysteine resulted in the rapid formation of a red degradation product, showing that cysteine wouldn't be suitable as an additional reductant for the benchtop bioassay. Thus the only approach adopted for benchtop bioassay testing was to extend

the preincubation period during which residual oxygen is removed non-enzymatically and amend reaction mixtures with additional NADH prior to addition of extract to start the enzymatic reaction. Apparently, this procedure was not sufficiently effective or reproducible as a means of making reaction mixtures suitably reduced prior to perchlorate reductase addition.

Development work was also done to improve SPE operations. In the current version of the bioassay, groundwater is applied to SPE cartridges using positive pressure. A condensed nitrogen gas source provides the necessary pressure. Testing was done to determine whether gravity flow would be an effective way of applying samples for perchlorate extraction if large resin beads of SDVB, conditioned with DTAB, were used. 3 g of SDVB (20 % cross-linked) white beads of 20 – 60 mesh were packed into the barrels of 10 mL syringes, conditioned with DTAB, and 200 mL of deionized water containing perchlorate (100 μg/L) was applied by gravity flow. IC analysis of perchlorate in the effluents showed that 96.1 ± 1.6 % of the perchlorate (average of three replicates ± standard deviation) was extracted. (See Appendix K for more detail.) Application of groundwater by gravity flow would be much more convenient and save operator contact time in conducting bioassay testing.

As illustrated in Figure 2-4 of Section 2.1, and studied previously (Coates and Achenbach, unpublished), the rate of 340 nm absorption is correlated to the concentration of perchlorate present initially in the reaction mixture when a CKB extract is used for the bioassay. To evaluate whether this is a general feature of perchlorate reductase enzymes from dissimilatory perchlorate reducing bacteria (DPRB) in the genus *Dechloromonas*, extracts of *D. aromatica* strain RCB were also tested in the bioassay. With the RCB extract, bioassay rates were distinctly less variable at different perchlorate concentrations than is seen with a CKB extract (see Appendix L Section L-5 for more detail). This effect on the rate of reaction was observed in both plate reader and benchtop bioassay formats. Had the development of a kit for the benchtop bioassay gone forward, it might have been advantageous to test RCB extracts for their suitability for that application, since the rate of absorbance change is faster at low perchlorate concentrations when an RCB extract is used.

The stoichiometry of the NADH-coupled perchlorate reductase bioassay was previously studied[19]. In the present study, the stoichiometry was about 4 moles of NADH oxidized per mole of perchlorate reduced using a CKB extract in both the benchtop and plate reader formats (see Appendix L Section L-4 for more detail).

## 2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The major advantages of the perchlorate bioassay technology are that the materials and equipment are relatively inexpensive, operators potentially do not need to be highly trained, and results from the assay can be obtained with a short turnaround time.

A limitation to the bioassay methodology is its complexity. There are three component steps to the overall procedure: use of SPE cartridges to concentrate and purify perchlorate in spiked subsamples, spectrophotometric determination of perchlorate, and data analysis to calculate the perchlorate concentration in the sample by the standard additions method using linear regression. A great difficulty in the current version of the benchtop bioassay is not having an efficient

method of obtaining anaerobic conditions in reaction mixtures prior to the addition of perchlorate reductase. Perchlorate reductase is not active aerobically. Furthermore, lack of anaerobicity favors non-enzymatic NADH oxidation in the presence of PMS and other components in reaction mixtures, thus compromising the specificity of the benchtop bioassay.

# 3.0 PERFORMANCE OBJECTIVES

Performance objectives with the corresponding data requirements, success criteria, and results are listed in Table 3-1.

**Table 3-1. Performance Objectives, Data Requirements, Success Criteria, and Results**

| Performance Objective | Data Requirements | Success Criteria | Results |
|---|---|---|---|
| **Quantitative Performance Objectives** | | | |
| 1. Compare benchtop bioassay results from testing in the PI's laboratory to results from reference analytical method | Results from testing samples for perchlorate in triplicate using:<br>• Benchtop bioassay performed in the PI's laboratory<br>• EPA Method 314.0 or EPA Method 6850 performed in a commercial laboratory<br>• IC EPA Method 314.0 performed in PI's laboratory (data for troubleshooting) | • The percent difference between bioassay and reference method results (from commercial laboratory testing) is 30% or less.<br>• ANOVA statistical analysis shows that results from benchtop bioassay and reference method are not significantly different. | • Success criteria were met for one groundwater source.<br>• Success criteria were not met for four groundwater sources. See Table 5-4 for a summary of testing outcomes.<br>• Quantitative success criteria could not be applied to a sixth source since the amount of $ClO_4^-$ by the reference method was <MRL. Results for this source were considered to have qualitatively met success criteria. |
| 2. Compare results with benchtop bioassay kit format tested by site field personnel to the site's routine method | • Results from benchtop bioassay testing by site field personnel<br>• Results from testing by the site's routine method | • Controls show bioassay is performed correctly<br>• Student t-test shows results from the benchtop bioassay and the site's routine method are not significantly different. | Testing was not done to evaluate this objective.<br><br>Testing for performance objective 1 indicated that the benchtop bioassay in its current format is not suitable for use in the field, thus no testing was performed by field personnel. |
| **Qualitative Performance Objective** | | | |
| Ease of use | Feedback from field personnel on the usability of bioassay kits | Feedback from several field technicians able to perform the bioassay | No feedback was obtained since the benchtop bioassay was not tested by field technicians. |

## 3.1 QUANTITATIVE PERFORMANCE OBJECTIVE 1: COMPARE BENCHTOP BIOASSAY RESULTS TO REFERENCE ANALYTICAL METHOD RESULTS

Quantitative performance objective 1 was to compare results from benchtop bioassay testing performed in the PI's laboratory with results performed by a reference chemical analytical method in a commercial laboratory. The reference analytical methods were either LC/MS tested according to EPA Method 6850, or IC tested according to EPA Method 314.0, which was used for the first three groundwater sources. In addition, IC data were collected in the PI's laboratory according to EPA Method 314.0, which was used to troubleshoot different steps of the bioassay testing procedure.

### 3.1.1 Data Requirements

Three sets of data – one for each of the three methods being compared – were obtained with each groundwater source in order to satisfy the objective. Specifically, the intent was to have project partners at each site collect three 1 gallon containers of groundwater at a designated sampling location and ship them to the UC Berkeley laboratory. Groundwater from each of the gallon containers was subdivided for testing in triplicate by the three methods of interest: 1. the perchlorate reductase benchtop bioassay method, 2. analysis according to EPA Method 6850 or Method 314.0 in a commercial testing laboratory, and 3. IC analysis according to EPA Method 314.0 in the PI's laboratory. Table 5-4 summarizes the results from the three methods, and Table 5-5 has the ANOVA testing. With respect to the testing performed by commercial laboratories, the first three groundwater sources were analyzed by IC according to EPA Method 314.0. Subsequent groundwater sources were analyzed by LC/MS according to EPA Method 6850 because of its greater specificity and sensitivity[20].

The target number of groundwater sampling locations to be used in this component of the study was 10, however, it was only possible to obtain groundwater for the project from 6 locations.

### 3.1.2 Success Criteria

The accuracy of benchtop bioassay estimates of the perchlorate concentration in different groundwater sources was evaluated by comparison to results from the reference analytical method performed in a commercial laboratory. The metric used to determine whether the bioassay level of accuracy was acceptable was the percent difference (i.e. the absolute value of the difference between bioassay and reference method values ÷ the reference method value, expressed as a percent). The criterion for acceptable accuracy was a percent difference (PD) of 30 % or less. In addition, when the accuracy of the bioassay met the acceptance criterion, ANOVA analysis was then used to provide a statistical assessment of possible differences among results obtained by the three methods. The percent confidence interval at which results were deemed significantly different was 95%, i.e. the level of $\alpha = 0.05$ was used.

Furthermore, a statistical comparison was only possible for perchlorate concentrations at or above the MRL for the reference analytical method. The Jet Propulsion Laboratory (JPL) groundwater sample was intentionally collected from a location having historically low perchlorate, in order to determine whether a false positive would be seen with the bioassay. When the JPL sample was tested with the bioassay, the level of perchlorate was $3 \pm 2$ µg/L. Perchlorate was not detected by the reference analytical method in the commercial laboratory or by IC at UCB and was thus considered <MRL, i.e. <2 µg/L or <4 µg/L, respectively. In this case, the bioassay result was considered consistent or qualitatively similar to the result obtained by the more sensitive analytical methods even though no percent difference or statistical comparison could be made.

### 3.1.3 Results

Table 5-4 lists the perchlorate results estimated by the reference method in a commercial laboratory, IC at UCB, and the benchtop bioassay method, as well as the percent difference (PD) values. By the PD comparison, the accuracy of the benchtop bioassay was acceptable for Fontana Water Co. and boarderline for Hill AFB. Results with JPL couldn't be compared quantitatively since the perchlorate from this source was <MRL for the reference method. Since the perchlorate estimated by the benchtop bioassay was low, i.e. 3 µg/L, results were considered qualitatively acceptable. The benchtop bioassay test failed for McGregor NWIRP, Massachusetts Military Reservation (MMR), and Lawrence Livermore National Laboratory (LLNL) groundwater sources, i.e. the PD values were 325, 429, and 92 %, respectively. In most cases, the variation among replicates was greater for the benchtop bioassay than for the other analytical methods. However, the variation was pronounced for the McGregor and MMR testing where relative standard deviations (i.e. the standard deviation ÷ average, expressed as a percent) were 118 and 96 %, respectively. In addition, the average perchlorate estimated with the bioassay was 4 to 5 fold greater than for the reference method, However, for LLNL testing, the average perchlorate estimated with the bioassay was 13 fold lower than for the reference method, and there was good agreement among replicate SPE eluate sets, suggesting there may have been inhibition of the bioassay with this source.

ANOVA was performed using using the Hill AFB and Fontana Water Co. data (see Table 5-5). This analysis showed that the three methods were not significantly different for Fontana Water Co. groundwater. However, the benchtop bioassay was significantly different from the analytical methods for the Hill AFB source.

## 3.2 QUANTITATIVE PERFORMANCE OBJECTIVE 2: COMPARE BENCHTOP BIOASSAY FIELD KIT RESULTS TO SITE'S REFERENCE METHOD RESULTS

Results with the benchtop bioassay did not justify the development of a field kit, hence no testing was done relative to this objective.

## 3.3 QUALITATIVE PERFORMANCE OBJECTIVE: FIELD KIT EASE OF USE ASSESSMENT

No field kit was developed, hence no assessment was done relative to this objective.

# 4.0 SITE DESCRIPTION

## 4.1 SITE SELECTION

The criteria that were used in site selection are listed in Table 4-1. Despite an extended search on the part of both the liaison officer and the PI, it was only possible to identify six sites as groundwater sources for the project.

**Table 4-1. Site Selection Criteria**

| Parameter | Preferred Value(s) | Relative Importance (1-5, with 1 being the highest) |
|---|---|---|
| Known range of perchlorate concentration | 0 to 100 µg/L | 1 |
| Availability of historical hydrological data regarding variability of the zone of sample collection | Yes | 2 |
| Availability of historical data on geochemistry (major anions, cations, and organic constituents), field parameters (DO, ORP, pH, conductance), and the presence of co-contaminants (RDX, etc.) | Yes | 2 |
| Availability of local shipping facilities | Yes | 1 |
| Reasonable site access and personnel available for sample collection | Yes | 1 |

## 4.2 SITE LOCATION AND HISTORY

The sites of groundwater sources are listed in Table 4-2, which briefly summarizes some current or past activities characteristic of missions served by these sites and the range of historical perchlorate levels that have been reported at various locations on the site. Sampling locations chosen for collecting groundwater to use in bioassay testing were sufficiently distant from any ongoing remediation activities that no impact on perchlorate concentration was likely.

**Table 4-2. Sites of Groundwater Sources**

| Project No. | Site | Site Location | Current Site Mission or History | Comments on Historical Perchlorate Testing |
|---|---|---|---|---|
| 102 | Hill Air Force Base | Ogden, UT | F-16 fighter planes and Minuteman ICBs logistics management | Groundwater 3.6–36 µg/L depending on sampling location |
| 103 | Former Naval Weapons Industrial Reserve Plant McGregor | McGregor, TX | Produced solid propellant rocket motors | Navy returned plant to McGregor in 2006. In situ biological treatment system. Groundwater, 2007: 1,300 µg/L in 4 of 39 samples. |
| 104 | Jet Propulsion Laboratory (JPL) | Pasadena, CA | Research and development center | Location selected as the project groundwater source historically had no perchlorate |
| 105 | Fontana Water Company | Fontana, CA | Municipal drinking water supply | Location selected as the project groundwater source is a production well |
| 106 | Massachusetts Military Reservation (MMR) | Cape Cod, MA | Military training facility (Coast Guard, and Army and Air Force National Guard) | Groundwater as high as 200 µg/L, RDX is a co-contaminant at some sampling locations. |

| Project No. | Site | Site Location | Current Site Mission or History | Comments on Historical Perchlorate Testing |
|---|---|---|---|---|
| 107 | Lawrence Livermore National Laboratory (LLNL) | Site 300, Tracy, CA | Explosives test site | Range of concentrations in groundwater, including several wells in the 4 to 40 µg/L range. |

## 4.3 SITE GEOLOGY/HYDROGEOLOGY

Historical information on the geology, hydrogeology, and matrix of sampling locations was considered in order to have groundwater samples as diverse as possible included in the testing.

## 4.4 CONTAMINANT DISTRIBUTION

Historical data on the variability in perchlorate concentration were reviewed to assist in selecting sampling locations that had as low a variability in perchlorate concentration as possible. When possible, locations likely to provide groundwater samples that contained co-contaminants in addition to perchlorate were included in the testing. The ideal perchlorate concentration range was 0 – 40 µg/L. Three locations (JPL, Fontana Water Co., and MMR) had historical perchlorate concentrations less than 10 µg/L.

# 5.0 TEST DESIGN

No new sampling installations were used for this study. Instead, the aim was to coordinate the collection of samples to coincide with the routine groundwater sampling schedule of the different locations included in the study. Groundwater from the different sites was tested to evaluate the performance of the bioassay on various kinds of samples.

## 5.1 CONCEPTUAL EXPERIMENTAL DESIGN

Objective one was to perform bioassay testing on environmentally diverse groundwater samples, defined as groundwaters from a diverse assortment of geographic regions expected to differ with respect to geochemical parameters such as pH, salinity, iron content, temperature, and conductivity. However, the groundwater sources included in the study were collected primarily based on availability. Three labeled gallon containers were collected at each sampling location (see Figure 2-1). These were cooled and shipped overnight along with a chain-of-custody form to the UC Berkeley laboratory where the integrity of the samples was examined and temperature, pH, and conductivity were measured. Each gallon of groundwater was filtered to remove particulate material and subdivided to obtain nine subsamples. The subsamples to be tested by a commercial laboratory were shipped along with chain-of-custody documentation to the testing facility. Subsamples were subsequently tested for perchlorate in triplicate by each method (i.e. benchtop bioassay, a reference analytical method performed by a commercial laboratory, and IC according to EPA method 314.0 at the UC Berkeley laboratory).

Residual sample from each gallon container was stored at 2-8 °C in case additional analytical testing was desirable to identify factors in a sample that might influence bioassay performance.

## 5.2 BASELINE CHARACTERIZATION

Historical data on the perchlorate concentration at sampling locations over several years was used to help select the locations for groundwater sampling. No additional baseline characterization activities were undertaken.

## 5.3 TREATABILITY OR LABORATORY STUDY RESULTS

No treatability or laboratory scale studies were part of the project.

## 5.4 FIELD TESTING

Testing of the perchlorate benchtop bioassay was not carried out since the assay in its current format is not suitable as a screening test for use in the field.

## 5.5 SAMPLING AND ANALYTICAL METHODS

The number of groundwater samples collected for the project and the types of tests performed are summarized in Table 5-1. The analytical methods are listed in Table 5-2. Appendix E

includes information describing the calibration of analytical equipment, quality assurance sampling, decontamination procedures, and sample documentation.

**Table 5-1. Total Number of Groundwater Samples Collected for Benchtop Bioassay, Perchlorate Reference Methods, and Characterization Testing**

| Component | Matrix | No. of Samples | Analyte | QC/QA | Comment or Location |
|---|---|---|---|---|---|
| Bioassay performed in PI's laboratory, and reference analytical methods (EPA 6850 or 314.0 in commercial laboratory, and EPA 314.0 in PI's laboratory) | Ground-water | 17[a] | Perchlorate | Bioassay: include positive and negative controls as well as track enzyme activity in each analysis batch.<br>Analytical methods: confirm instrument performance parameters and include duplicates, spikes and other controls per EPA methods. | 6 sites provided groundwater. Each gallon sample was tested in triplicate for bioassay as well as EPA 314.0 in the PI's laboratory and by reference methods (EPA 314.0 or 6850) in commercial laboratories. |
| | Ground-water | 17[a] | pH and conductive-ity | Confirm instrument performance parameters per instrument manuals. | All samples were tested in PI's lab or at the time of collection. |
| | Ground-water | As needed | Physical and chemical parameters, listed in Table 5-2[b] | Confirm instrument performance parameters per instrument manuals. For IC methods include laboratory duplicates and matrix spikes. | When there was a lack of agreement between bioassay and reference method, more factors were evaluated for possible effects on bioassay performance. |

[a]Each sampling event consisted of collecting three replicate gallons of groundwater. Thus the total number of sample bottles to be tested was 5 x 3 = 15 plus 2 bottles from the NWIRP McGregor location.
[b]Physical and chemical parameters measured included ionic species analyzed by IC (such as nitrate, chlorate, chloride, phosphate, fluoride, nitrite, sulfate, and bromide), and analysis of elements by ICPMS.

### 5.5.1 Analysis Methods

Analysis of perchlorate by the reference analytical methods by commercial laboratories was performed by TestAmerica Laboratories, Inc. and Calscience Environmental Laboratories, Inc. Elements were analyzed by inductively coupled plasma mass spectrometry using a Perkin Elmer SCIEX Elan DRC II Inductively Coupled Plasma Mass Spectrometer (ICPMS). Analyses were conducted at the Lawrence Berkeley National Laboratory by the Analytical Service Center of the Aqueous Geochemistry Laboratory in the Earth Sciences Division.

Detailed operations for performing the bioassay are described in Appendix L.

**Table 5-2. Analytical Methods for Sample Analysis**

| Matrix | Analyte | Method | Container[a] | Preservative[b] | Holding Time |
|---|---|---|---|---|---|
| Ground-water | Perchlorate | Bioassay, EPA 6850, EPA 314.0 | LDPE bottle | 2-8 °C | 28 days |
| | pH | Electrochemical Probe | LDPE bottle | 2-8 °C | Immediately on receipt or at time of collection |
| | Conductivity | Electrochemical Probe | LDPE bottle | 2-8 °C | Immediately on receipt or at time of collection |
| | Common anions[c] | IC | LDPE bottle | 2-8 °C | Not specified |
| | Common elements | ICPMS | LDPE bottle | 2-8 °C | Not specified |
| | Uranium | ICPMS | LDPE bottle | 2-8 °C | Not specified |

[a]One gallon container holds sufficient groundwater to test for perchlorate by bioassay, IC, and LC/MS as well as to determine pH, conductivity, and physical/chemical parameters.
[b]Preservatives are not required for these analyses, however, all samples were stored at 2 – 8°C and shipped chilled on ice or with cold packs.
[c]Ionic species analyzed by IC included nitrate, chlorate, chloride, phosphate, fluoride, nitrite, sulfate, and bromide

### 5.5.2 Statistical Analyses

The bioassay determination of perchlorate is by a standard additions method, and (-1) times the x-intercept of the standard additions method regression line provides the concentration of perchlorate in the sample[14]. Linear regression statistical analysis[22] was performed using Microsoft Excel (Microsoft Corporation, Redmond, Washington). Analysis of variance (ANOVA) statistical testing was performed using Minitab 16.2.3 software (Minitab, Inc., State College, Pennsylvania). Multi-dimensional Scaling (MDS) statistical analysis was performed using Primer 6 software (Primer-E Ltd., Plymouth Marine Laboratory, UK).

## 5.6 SAMPLE TESTING RESULTS

The historical perchlorate levels at the locations from which groundwater was collected, and the conductivity and pH of groundwater samples used for the project are listed in Table 5-3.

Data from the groundwater testing by benchtop bioassay, reference method performed at a commercial laboratory, and IC testing in the PI's laboratory at UCB, are listed in Appendices B, C, and D, respectively. The comparison of results from the three methods, and the accuracy of the benchtop bioassay and UCB IC analysis compared to the results from the reference method performed in a commercial laboratory are listed in Table 5-4. The accuracy of the benchtop bioassay was considered acceptable when the percent difference (PD) from the reference test was 30 % or less. The 30 % value for the acceptability metric was adopted based on a consideration of previous results with the plate reader version of the bioassay, reported by Heinnickel et al.[2]. A t-test analysis of results obtained by the bioassay and by an IC reference method, indicated that the methods were not significantly different ($\alpha = 0.05$) for three different groundwater samples. PD values for the bioassay compared to the IC reference method were 14, 19, and 25 %.

**Table 5-3. Conductivity, pH, and Historical Perchlorate of Groundwater Sources**

| Site | Location | Historical perchlorate (µg/L) | Conductivity (µS/cm) | pH | Volume collected (gallons) |
|---|---|---|---|---|---|
| **102, Hill AFB** | U9-16-007 | 37 | 653 | 8.3 | 3 |
| **103, McGregor NWIRP** | OFFWS-37 | 50 | 616 | 8.0 | 2 |
| **104, JPL** | MW-16 | <1 | 589 | 7.2 | 3 |
| **105, Fontana Water Co.** | F-17C | 8 | 396 | 7.5 | 3 |
| **106, Massachusetts Military Reservation** | J-3 INF | 5.8 | 45 | 6.5 | 3 |
| **107, LLNL** | W-854-1823 | 16 | 968 | 8.0 | 3 |

As noted in Table 5-4, benchtop bioassay results were acceptable for the Fontana Water Co. groundwater (i.e. PD was 18 %) and boarderline with Hill AFB groundwater (i.e. PD was 32 %). The JPL source was collected from a location, which historically has had no detectable perchlorate (Table 5-3), in order to evaluate the potential for false positive results with the benchtop bioassay. The accuracy of the benchtop bioassay with JPL groundwater was considered qualitatively acceptable since the standard deviation for replicate SPE eluate sets was small (i.e. ± 2 µg/L), and the estimated perchlorate was low (i.e. 3 µg/L). It was not possible to make a quantitative PD calculation because the perchlorate by reference methods was <MRL.

The accuracy of the benchtop bioassay was unacceptable for McGregor, MMR and LLNL sources with PD calculated as 325, 429, and 92 %, respectively. The average for the McGregor and MMR benchtop bioassay results were 4 to 5 times higher than the corresponding results with the reference method performed in a commercial laboratory. In addition there was pronounced lack of agreement in perchlorate estimates among the replicate SPE eluate sets for McGregor (range 43 to 720 µg/L) and MMR (range -3 to 83 µg/L). The $r^2$ values for the different SAM regression lines were also variable for these two sources, ranging from 0.002 to 0.627 for McGregor, and from 0.472 to 0.998 for MMR. By contrast, the average for LLNL benchtop bioassay results was 13 times lower than the reference method performed in a commercial laboratory, there was good agreement among replicate SPE eluate sets (i.e. the standard deviation of the mean was ± 3 µg/L), and the $r^2$ values for the different SAM regression lines ranged from 0.953 to 0.999. Thus the benchtop bioassay failed for McGregor and MMR, probably due at least in part to inadequate reduction of the reaction mixtures prior to adding cell extract. However, with the LLNL source, there seemed to be interference with the benchtop bioassay.

Detailed data from benchtop bioassay testing are archived in Appendix B where Table B-1 summarizes for each eluate set the perchlorate concentration determined from standard additions method (SAM) regression lines ± the error of the SAM x-intercept. The averages from all replicates of a source ± standard deviation are also noted in Table B-1 and repeated in Table 5-4. Representative results from SAM regression lines are shown in Figure 5-1. The data shown are a subset of data from Appendix B (i.e. drawn from Figures B-102, 103, 104, 105, 106, and 107). The variability in x-intercepts seen for the McGregor and MMR panels contrast with the much less variable results seen in the panels for the other groundwater sources.

Each batch of benchtop bioassays included a negative control (i.e. no perchlorate added to the reaction mixture) and a 40 µM perchlorate positive control. Usually a 20 µM perchlorate positive control was also included. The negative and positive controls were tested in enzyme buffer,

which contained MOPS and NaCl, pH 7.2, but had not been exposed to SPE cartridges. In all instances, these controls performed in a consistent manner, showing that perchlorate reductase had the expected level of activity. The average x-intercept error was low, ± 1.6 µM perchlorate (n = 15), see Appendix L, Table L-2.

**Table 5-4. Perchlorate Concentration Estimated by Different Methods and Accuracy Evaluated Using Percent Difference Comparisons[a]**

| Source | Method | Perchlorate concentration (µg/L) | | | | | | | | | Average ± SD | PD[b] (%) | Benchtop bioassay accuracy[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A replicates | | | B replicates | | | C replicates | | | | | |
| | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | | | |
| **102, Hill AFB** | **IC Ref. test** | 36 | 40 | 32 | 47 | 44 | 37 | 33 | 35 | 38 | 38±5 | 0 | boarderline |
| | **IC, UCB** | 34 | 35 | 35 | 35 | 35 | 36 | 36 | 36 | 36 | 35±1 | 8 | |
| | **Benchtop** | 23 | 21 | 14 | 20 | 23 | 22 | 31 | 45 | 33 | 26±9 | 32 | |
| **103, McGregor NWIRP** | **IC Ref. test** | 59 | 60 | 61 | 98 | 56 | 55 | NA | NA | NA | 65±16 | 0 | unacceptable |
| | **IC, UCB** | 58 | 58 | 59 | 54 | 55 | 55 | NA | NA | NA | 57±2 | 12 | |
| | **Benchtop** | 43 | 106 | 673 | 58 | 720 | 55 | NA | NA | NA | 276±327 | 325 | |
| **104, JPL** | **IC Ref. test** | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | <2 | NA | qualitatively acceptable |
| | **IC, UCB** | <4 | <4 | <4 | <4 | <4 | <4 | <4 | <4 | <4 | <4 | NA | |
| | **Benchtop** | 3 | 2 | 5 | 0 | 4 | 5 | 4 | 2 | 1 | 3±2 | NA | |
| **105, Fontana Water Co.** | **LC/MS Ref.** | 11 | 11 | 11 | 11 | 11 | 11 | 9.3 | 9.8 | 9.5 | 11±1 | 0 | acceptable |
| | **IC, UCB** | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 10 | 10 | 11±0.4 | 0 | |
| | **Benchtop** | 15 | 8 | 11 | 5 | 8 | 5 | 7 | 9 | 10 | 9±3 | 18 | |
| **106, MMR** | **LC/MS Ref.** | 5.5 | 5.3 | 4.9 | 5.2 | 5.3 | 5.3 | 4.9 | 4.9 | 4.8 | 5.1±0.3 | 0 | unacceptable |
| | **IC, UCB** | 6 | 6 | 6 | 6 | 7 | 6 | 6 | 6 | 6 | 6±0.3 | 18 | |
| | **Benchtop** | -3 | 23 | 51 | 36 | 23 | 9 | 83 | 7 | 18 | 27±26 | 429 | |
| **107, LLNL** | **LC/MS Ref.** | 14 | 14 | 13 | 15 | 14 | 13 | 12 | 13 | 12 | 13±1 | 0 | unacceptable |
| | **IC, UCB** | 16 | 16 | 16 | 15 | 15 | 15 | 16 | 16 | 16 | 16±1 | 23 | |
| | **Benchtop** | 2 | -3 | -4 | 6 | 1 | 0 | 1 | 1 | 2 | 1±3 | 92 | |

[a]NA denotes not applicable, only two gallons of groundwater were obtained for testing source 103, and PD couldn't be calculated for source 104

[b]Percent difference (PD) compared to the results from the reference test performed in a commercial laboratory. PD was calculated as the absolute value (of the average reference test outcome minus the average from testing by another method) ÷ (the average reference test outcome) and expressed as a percent

[c]Bioassay accuracy was considered acceptable when the PD was 30% or less

The data for Hill AFB and Fontana Water Co. (judged to have boarderline and acceptable accuracy, respectively, as shown in Table 5-4), were further analyzed by a one-way ANOVA to examine the data for differences among the methods. The outcomes are listed in Table 5-5. Grouping among the different methods was evaluated by the Tukey Method using 95% simultaneous confidence intervals. This analysis indicated that the methods were significantly different for the Hill AFB source and also that the benchtop bioassay was the cause of this difference. The methods were not significantly different for the Fontana Water Co. groundwater.

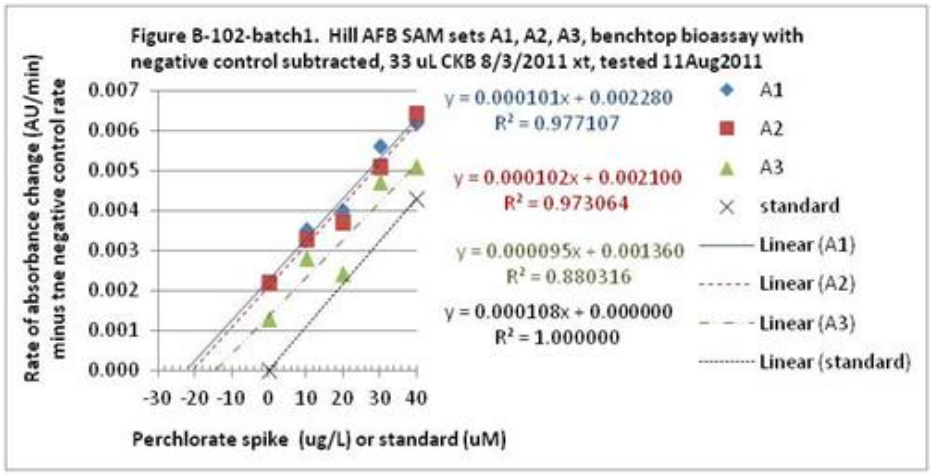
**Table 5-5. ANOVA for Reference Method Performed by a Commercial Laboratory, IC at UCB, and the Benchtop Bioassay, and Grouping Using the Tukey Method**

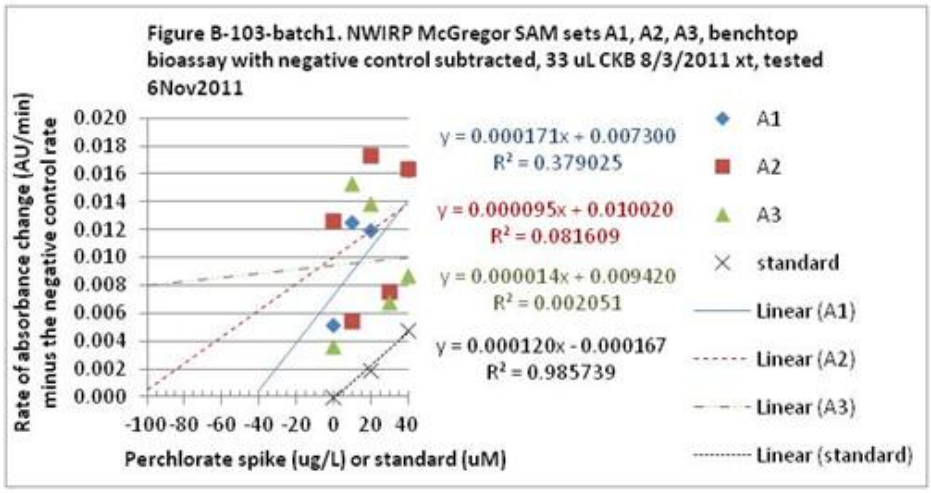
| Source | Average perchlorate results ± SD (µg/L) | | | F | ANOVA P-value | Tests are significantly different (α = 0.05) |
|---|---|---|---|---|---|---|
| | Reference test | IC at UCB | Benchtop bioassay | | | |
| **102, Hill AFB** | 38.000 ± 4.950 | 35.33 ± 0.707 | 25.778 ± 9.176 | 10.21 | 0.001 | yes |
| **105, Fontana Water Co.** | 10.511 ± 0.744 | 10.778 ± 0.441 | 8.778 ± 3.383 | 2.61 | 0.094 | no |
| **Grouping by Tukey Method** | | | | | | |
| **102, Hill AFB** | Group 1 | Group 1 | Group 2 | Benchtop bioassay different from reference analytical methods | | |
| **105, Fontana Water Co.** | NA | NA | NA | Methods are not different from one another, Tukey Method not applied | | |

In summary, the benchtop bioassay in its current form is not suitable as a screening test for perchlorate in groundwater. Six groundwater sources were tested. Results compared to the reference analytical method performed in a commercial laboratory were quantitatively acceptable for the Fontana Water Co. source, and qualitatively acceptable for the JPL source. The benchtop bioassay for the Hill AFB sample was significantly different from the reference method by ANOVA analysis. The benchtop bioassay exhibited characteristics of a failed test for McGregor and MMR sources, in that replicates were highly variable and the average estimate for perchlorate concentration was much higher than for the reference analytical method. Finally, for the LLNL source, there appeared to be interference with the benchtop bioassay since the average estimate for perchlorate concentration was much lower than for the reference analytical method.

## 5.7 TESTING FOR FACTORS THAT MAY IMPACT BIOASSAY PERFORMANCE

Testing was done to investigate factors that could impact bioassay performance. This included: 1. assessing the adequacy of the SPE step in concentrating perchlorate from groundwater and confirming that nitrate would not be extracted along with perchlorate, 2. determining whether SPE eluates of groundwater tested by the plate reader bioassay format – which is performed in an anaerobic chamber – met accuracy criteria, 3. characterizing groundwater samples for other anions by IC, and 4. evaluating the profile of elements in groundwater sources and corresponding SPE eluates by ICPMS.



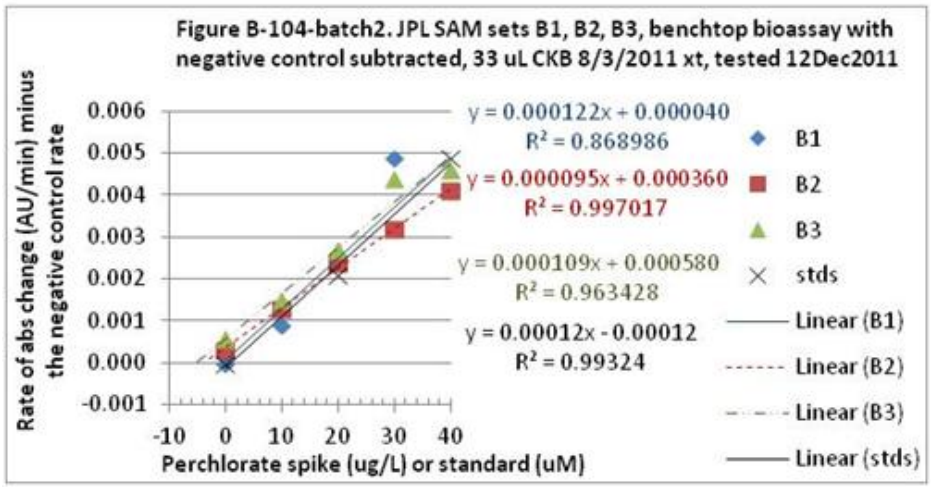
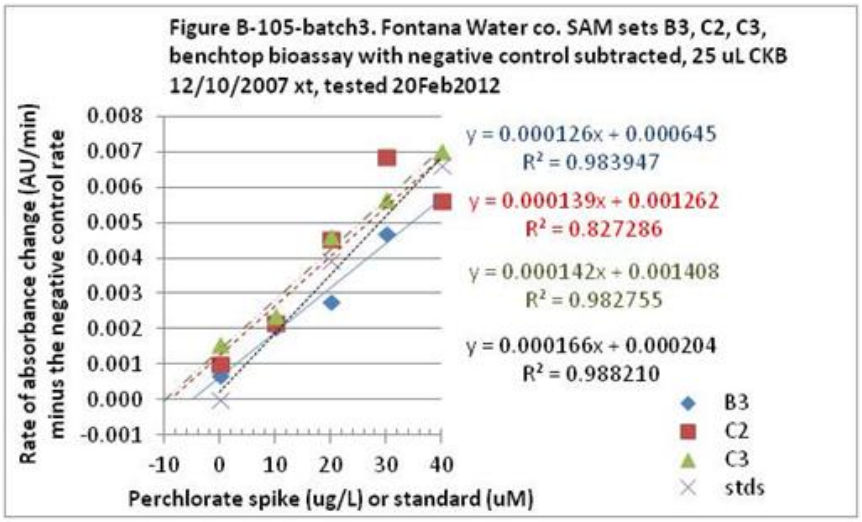
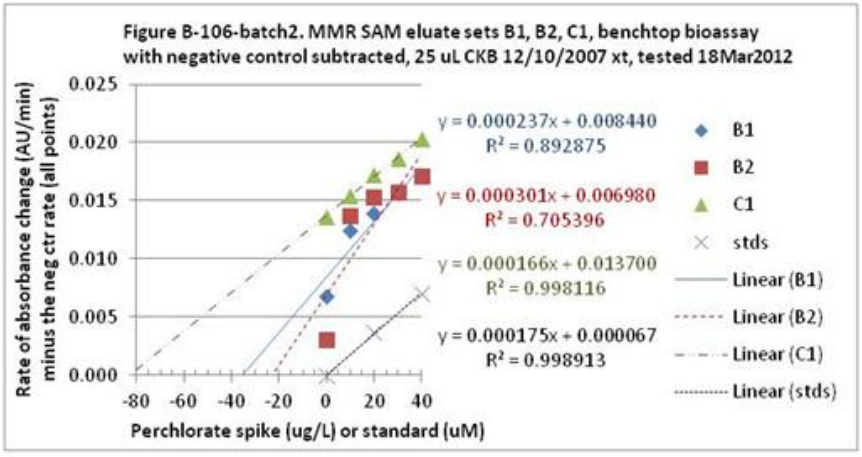




**Figure 5-1. Representative standard additions method (SAM) regression lines from benchtop bioassay testing of the six groundwater sources analyzed in the project**



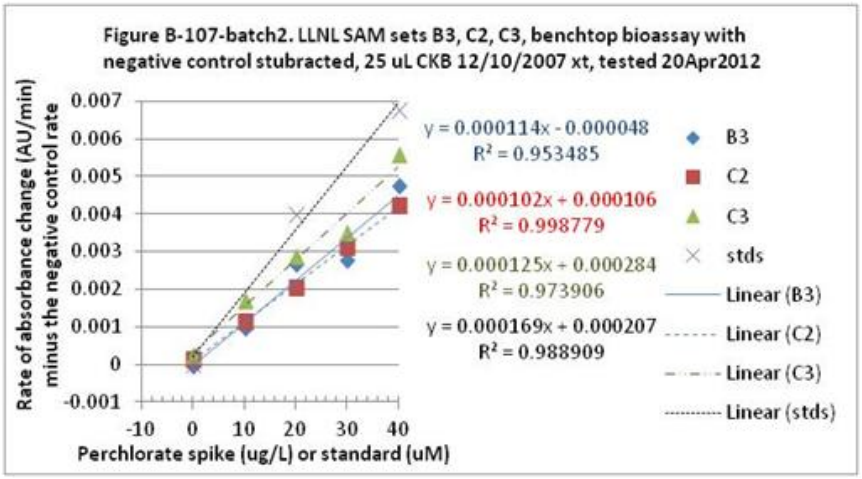




**Figure 5-1, continued. Representative standard additions method (SAM) regression lines from benchtop bioassay testing of the six groundwater sources analyzed in the project**

As discussed in more detail below, these studies indicated the following. 1. The SPE step performed as expected to concentrate perchlorate. 2. The accuracy of the anaerobic plate reader bioassay was acceptable for the five groundwater sources that could be evaluated quantitatively. Apparently the interference phenomenon observed when LLNL SPE eluates were tested by the benchtop bioassay was not seen when testing was repeated in the anaerobic chamber using the plate reader bioassay format. 3. IC analysis for common anions showed that nitrate was present in SPE effluents at about the same concentration as in the corresponding groundwater. In addition, about 9 mg/L of bromide was present in SPE effluents, much more than in the groundwater sources. The probable source of this bromide was the DTAB used to condition cartridges. 4. ICPMS analysis of groundwater and corresponding alkaline MOPS-NaCl SPE eluants showed that uranium is extracted by the SPE step and recovered with good efficiency in SPE eluates. In addition, the amount of bromine present in SPE eluates was much greater than in corresponding groundwater sources or the alkaline MOPS-NaCl solution used to elute cartridges. Again, the probable source of the high bromine in eluates is the DTAB used to condition and rinse cartridges. Eluates prepared from LLNL groundwater had the highest uranium concentration, about 9 µM. Since the presence of uranium might be correlated with the interference of the benchtop bioassay seen with the LLNL source, uranium was tested for possible interference with perchlorate reductase activity by adding it directly to benchtop bioassay reaction mixtures. However, no interference was observed when 1 to 10 µM uranium was present in reaction mixtures in addition to the enzyme substrate, perchlorate (40 µM).

### 5.7.1 Assessment of the Adequacy of the SPE Step

Several approaches were used to confirm that the SPE step met expectations. One was to troubleshoot the unacceptable accuracy performance of the benchtop bioassay with MMR and LLNL groundwater by using IC to reanalyze several SPE eluate sets for perchlorate. Results are archived in Appendix G. Perchlorate concentrations were determined by IC and then plotted by the standard additions method (SAM) to estimate the concentration of perchlorate in groundwater from the x-intercept of the SAM regression line. Three eluate sets were tested in this way for MMR groundwater, and the estimated perchlorate was in good agreement with the reference analytical method for each of the sets tested (see Appendix G, Table G-1). Two eluate sets were tested for LLNL groundwater. The estimated perchlorate was in good agreement with the reference analytical method for the C3 set, but significantly higher for B1 set (see Table G-1). Nevertheless, it is clear that the apparent interference observed with benchtop bioassay testing of LLNL groundwater was not due to failure of the SPE step with respect to the recovery of perchlorate in eluates.

Another approach was to analyze the SPE effluent after groundwater sources were applied to cartridges by the standard protocol (see Appendix H). The amount of nitrate was similar in effluents and groundwater sources. Thus nitrate was not extracted by the SPE step, which thereby effectively separated perchlorate and nitrate. Chlorate is the other anion that could compete with perchlorate as a substrate for perchlorate reductase. However, the groundwater sources included in this study did not contain levels of chlorate that were of concern.

### 5.7.2 Plate Reader Bioassay Data

Three to five SPE eluate sets for different groundwater sources, which had previously been tested by the benchtop bioassay, were retested using the anaerobic plate reader bioassay. The data are archived in Appendix F. Table 5-6 shows the testing results and accuracy assessment for the plate reader format of the bioassay. The accuracy of the plate reader bioassay was considered acceptable when the percent difference (PD) from the reference analytical method, using the same reference values as are listed in Table 5-4, was 30 % or less.

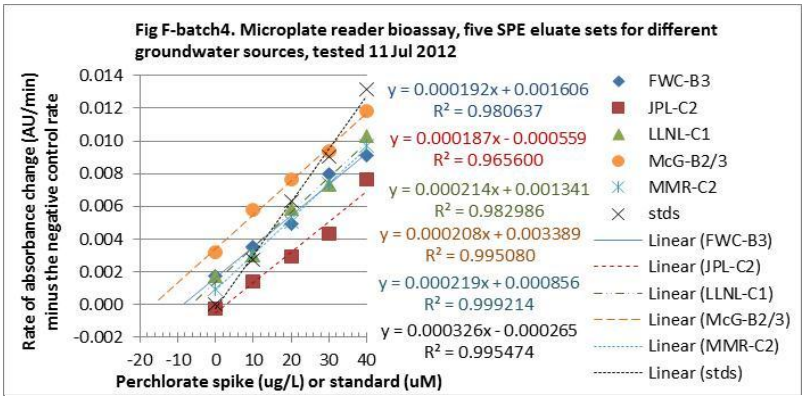



**Figure 5-2. Perchlorate anaerobic plate reader bioassay results for batch 4**

Figure 5-2 shows representa-tive results from the anaerobic plate reader bioassay testing. Pictured is a subset of data archived in Appendix F (i.e. Figure F-4) for plate reader bioassay batch 4. The standard additions method regression lines have high $r^2$ values for all the sources tested. Accuracy was acceptable with the five groundwater sources amenable to quantitative comp-arisons among the methods (Table 5-6). It was considered only qualitatively acceptable for the JPL source, since the estimated perchlorate by the plate reader bioassay was low and the standard deviation of replicate sets was within the range seen for other groundwater sources (i.e. ± 2 to 7 µg/L, see Table 5-6). A quantitative comparison wasn't possible for the JPL source since the reference method results were not quantitative (i.e. <MRL). Table 5-7 shows ANOVA testing for the plate reader bioassay compared to the reference analytical methods tested in a commercial laboratory and IC at UCB (same data as listed in Table 5-4). The three methods were not significantly different for Hill AFB, McGregor NWIRP, and Fontana Water Co. at the 5 % level (i.e. $\alpha$ = 0.05). Although the three tests were significantly different by ANOVA for MMR, grouping by the Tukey Method showed that the three methods grouped together. Thus it seems the assessment of differences among the methods by ANOVA should not overshadow the fact that the accuracy of the plate reader bioassay was acceptable (Table 5-6) as was the IC at UCB accuracy (Table 5-4) relative to the reference method at a commercial laboratory. Finally, ANOVA showed that for LLNL groundwater the three methods were different. Grouping by the Tukey Method showed that the anaerobic plate reader bioassay and the reference method grouped together while the IC at UCB method was the cause of the ANOVA difference.

Each batch of anaerobic plate reader bioassays included at least one negative control well (i.e. no perchlorate added to the reaction mixture). The rate of 340 nm absorbance change/minute for the negative control was subtracted from the rate of NADH oxidation for all the other reaction mixtures (Appendix F, Tables F-2 though F-6). For batches 1, 4, and 5 perchlorate standards were also tested at 10, 20, 30, and 40 µM concentrations. Negative and positive controls were tested in enzyme buffer as previously described for benchtop bioassay controls. Regression lines

**Table 5-6. Perchlorate Concentration Estimated by Plate Reader Bioassay and Accuracy Evaluated Using the Percent Difference Compared to the Reference Analytical Method[a]**

| Source | Method | Perchlorate concentration (µg/L) | | | | | | | | | | | Plate reader bioassay accuracy[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A replicates | | | B replicates | | | C replicates | | | Average ± SD | PD[b] (%) | |
| | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | | | |
| **102, Hill AFB** | **PR assay** | 36 | 43, 27[d] | 23 | 38 | nt | nt | nt | nt | 34 | 34±7 | 11 | acceptable |
| **103, McGregor NWIRP** | **PR assay** | nt | 53 | 45 | nt | 49 | nt | NA | NA | NA | 49±4 | 25 | acceptable |
| **104, JPL** | **PR assay** | nt | nt | nt | -10 | nt | 2 | nt | -3 | nt | -4±6 | NA | qualitatively acceptable |
| **105, Fontana Water Co.** | **PR assay** | 19 | 11 | nt | nt | nt | 8 | nt | nt | 10 | 12±5 | 9 | acceptable |
| **106, MMR** | **PR assay** | nt | 3 | 4 | 8 | nt | nt | nt | 4 | nt | 4.8±2.2 | 6 | acceptable |
| **107, LLNL** | **PR assay** | nt | nt | nt | 13 | 14 | nt | 6 | nt | nt | 11±4 | 15 | acceptable |

[a]The "nt" denotes not tested, NA denotes not applicable

[b]Percent difference (PD) compared to the results from the reference test performed in a commercial laboratory. PD was calculated as the absolute value (of the average reference test outcome minus the average from testing by another method) ÷ (the average reference test outcome) and expressed as a percent

[c]Bioassay accuracy was considered acceptable when the PD was 30% or less

[d]The Hill AFB A2 SPE set was tested by the plate reader bioassay twice. See Appendix F

**Table 5-7. ANOVA for Reference Method Performed by a Commercial Laboratory, IC at UCB, and the Plate Reader Bioassay, and Grouping Using the Tukey Method**

| Source | Average perchlorate results ± SD (µg/L) | | | F | ANOVA P-value | Tests are significantly different ($\alpha = 0.05$) |
|---|---|---|---|---|---|---|
| | Reference test | IC at UCB | Plate reader bioassay | | | |
| **102, Hill AFB** | 38.00 ±4.950 | 35.33 ±0.707 | 33.500±7.342 | 1.72 | 0.203 | no |
| **103, McGregor NWIRP** | 64.83±16.41 | 56.50±2.07 | 49.00±4.00 | 2.29 | 0.144 | no |
| **103, JPL** | <2 | <4 | -3.667 ± 6.028 | NA | NA | qualitatively not different |
| **104, Fontana Water Co.** | 10.511 ±0.744 | 10.778±0.441 | 12.000±4.830 | 0.79 | 0.467 | no |
| **106, MMR** | 5.122±0.249 | 6.111±0.333 | 4.750±2.217 | 4.04 | 0.035 | yes |
| **107, LLNL** | 13.333±1.000 | 15.667±0.500 | 11.000 ± 4.359 | 10.50 | 0.001 | yes |
| **Grouping by Tukey Method[a]** | | | | | | |
| **102, Hill AFB** | NA | NA | NA | NA | | |
| **103, McGregor NWIRP** | NA | NA | NA | NA | | |
| **103, JPL** | NA | NA | NA | NA | | |
| **104, Fontana Water Co.** | NA | NA | NA | NA | | |
| **106, MMR** | Group 1 | Group 1 | Group 1 | Methods not different (group together) | | |
| **107, LLNL** | Group 1 | Group 2 | Group 1 | Plate reader bioassay and reference test are not different. IC at UCB is the cause of the difference by ANOVA analysis | | |

[a]Grouping by the Tukey Method is only performed when the p-value from ANOVA analysis is < 0.05

for plots of the rate of reaction vs. concentration for perchlorate standards intersected the x-axis close to the origin and showed low x-intercept errors (i.e. ± 0.64 µM, n = 3). See Appendix L, Table L-3.

Results with the anaerobic plate reader bioassay support the idea that this format of the bioassay could be an effective laboratory screening test for perchlorate[2]. A possible reservation regarding this suggestion is that generally the plate reader bioassay testing was done after considerable time had passed. Although perchlorate is stable in the environment, ageing of SPE eluates may have made them more amenable to analysis by bioassay methods. It is interesting to note, however, that Hill AFB SPE eluate sets were analyzed on two occasions. The first preceded benchtop bioassay testing, and the second was 11 months later. The average estimate of perchlorate concentration was similar for the two batches (Appendix F, Table F-1), suggesting that for Hill SPE eluate sets, ageing had little impact on results obtained by the anaerobic plate reader bioassay. Additional testing would be needed to resolve this point.

### 5.7.3 OTHER ANIONS IN GROUNDWATER SOURCES

IC analysis was used to determine levels of common anions in the groundwater sources, and the data are listed in Tables H-1 and H-2 in Appendix H. The results showed that the levels of major anions in groundwater sources were not remarkable. Chlorate, which is an alternative substrate for perchlorate reductase, was not present in groundwater sources. Nitrate was not extracted by SPE cartridges. Bromide was much higher in SPE effluents than in groundwater sources and must have come from residual DTAB present in cartridges after conditioning.

### 5.7.4 ELEMENT PROFILES IN GROUNDWATER SOURCES AND SPE ELUATES

Groundwater sources and freshly prepared SPE eluates were analyzed by ICPMS. Results are listed in Appendix I, Table I-1 for groundwater, and Table I-2 for corresponding SPE eluates. Bromine was present at much higher amounts in SPE eluates than in corresponding groundwater sources or the alkaline MOPS-NaCl solution used to elute perchlorate from cartridges. Non-metric multi-dimentional scaling (MDS) analysis of the data on elements from Tables I-1 and I-2 indicated that overall patterns were different for groundwater and SPE eluates. The MDS analysis facilitated an assessment of which elements were more concentrated in SPE eluates than in the corre-sponding groundwater source. As discussed in more depth in Appendix I, uranium was

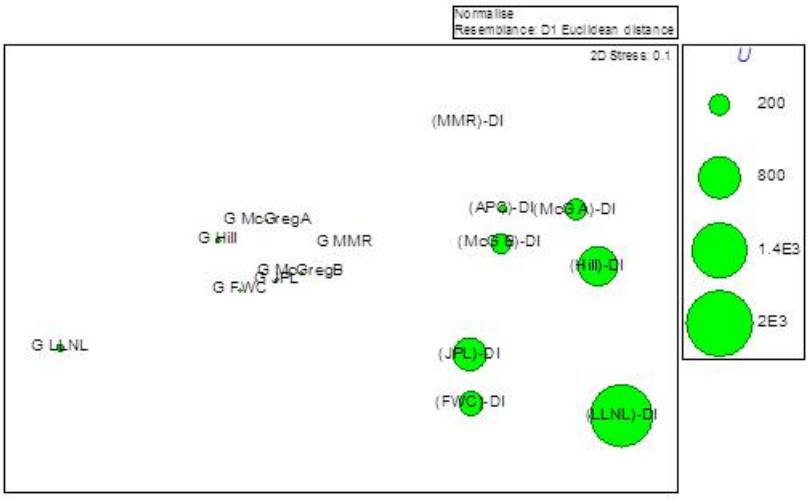



**Figure 5-3. MDS plot based on ICPMS data for groundwater and SPE eluates with superimposed circles representing the concentration of uranium**

concentrated in eluates, and was highest in the LLNL eluate. See Figure 5-3, which shows the panel for uranium from Appendix I, Figure I-4. Relationships among groundwater sources grouped on the left-hand side of the plot, and are identified with the prefix "G". Relationships among SPE eluates grouped on the right-hand side of the plot, and are identified with the suffix "-DI". Superimposed on the MDS plot are circles representing the concentration of uranium. In addition, ICPMS analysis was done on some of the SPE eluate sets from LLNL, Fontana Water Co., and Hill AFB that had previously been tested by the benchtop bioassay (Table I-5). This confirmed that the amount of uranium present in eluates analyzed by bioassay testing was similar to that seen in freshly prepared SPE eluates.

The ICPMS data seemed consistent with the possibility that the presence of fairly high levels of uranium in LLNL SPE eluates might have impacted perchlorate reductase activity in the benchtop bioassay testing.

### 5.7.5 TESTING URANIUM(VI) FOR INTERFERENCE WITH PERCHLORATE REDUCTASE ACTIVITY

Testing was done to determine whether adding uranium (VI) directly to reaction mixtures interfered with the benchtop bioassay for perchlorate (Appendix J). Duplicate reactions containing 40 µM perchlorate and 0, 1, 2, 3, 5, 8, or 10 µM uranium were tested. All reaction mixtures had the same perchlorate reductase activity, showing that the addition of uranium had no effect on enzyme activity.

# 6.0 PERFORMANCE ASSESSMENT

## 6.1 QUANTITATIVE PERFORMANCE OBJECTIVE 1: COMPARE BENCHTOP BIOASSAY RESULTS TO REFERENCE ANALYTICAL METHOD RESULTS

The percent difference between benchtop bioassay results and the reference method performed in a commercial laboratory was the metric used to evaluate the accuracy of the bioassay method. By the PD comparison, the accuracy of the benchtop bioassay was acceptable for Fontana Water Co. and boarderline for Hill AFB (Table 5-4). Results with JPL couldn't be compared quantitatively since the perchlorate from this source was <MRL for the reference method. The benchtop bioassay test failed for McGregor NWIRP, Massachusetts Military Reservation (MMR), and Lawrence Livermore National Laboratory (LLNL) groundwater sources, i.e. the PD values were 325, 429, and 92 %, respectively. In most cases, the variation among replicates was greater for the benchtop bioassay than for the other analytical methods. The lack of agreement was pronounced for the McGregor and MMR testing where relative standard deviations (i.e. the standard deviation ÷ average, expressed as a percent) were 118 and 96 %, respectively. However, for LLNL testing there was good agreement among replicate SPE eluate sets, suggesting there may have been interference with the bioassay for this source.

ANOVA was performed using the Hill AFB and Fontana Water Co. data (see Table 5-5). This analysis showed that the three methods were not significantly different for Fontana Water Co. groundwater. However, the benchtop bioassay was significantly different from the analytical methods for the Hill AFB source.

## 6.2 QUANTITATIVE PERFORMANCE OBJECTIVE 2: COMPARE BENCHTOP BIOASSAY FIELD KIT RESULTS TO SITE'S REFERENCE METHOD RESULTS

Results with the benchtop bioassay did not justify the development of a field kit, hence no testing was done relative to this objective.

## 6.3 QUALITATIVE PERFORMANCE OBJECTIVE: FIELD KIT EASE OF USE ASSESSMENT

No field kit was developed, hence no assessment was done relative to evaluate this objective.

# 7.0 COST ASSESSMENT

A costs assessment for implementing a field screening test for perchlorate based on the benchtop bioassay format is not applicable at this point since the current version of the assay is not suitable for this use.

# 8.0 IMPLEMENTATION ISSUES

Implementation issues are not relevant at this point since the current version of the benchtop bioassay is not suitable for use as a screening test for perchlorate in the field. The accuracy of the benchtop bioassay with different groundwater sources was evaluated by comparison to results from the reference analytical method performed in a commercial laboratory. The metric used to determine whether the bioassay level of accuracy was acceptable was the percent difference. The criterion for acceptable accuracy was a percent difference (PD) of 30 % or less. The reference analytical methods used were either LC/MS tested according to EPA Method 6850, or IC tested according to EPA Method 314.0. In general, the benchtop assay performed poorly. While the accuracy was qualatively acceptable for the JPL source and quantitatively acceptable for the Fontana Water Co. source, it was only boarderline for the Hill AFB source and was not acceptable (i.e > 30 % PD) for the three other sources: NWIRP McGregor, Massachusetts Military Reservation (MMR), and LLNL. There was a pronounced lack of agreement among replicates tested in the benchtop bioassay for NWIRP McGregor and Massachusetts Military Reservation sources.

Retesting of the water samples with the bioassay in an anaerobic chamber with a nitrogen/hydrogen atmosphere provided a different outcome. In this instance the accuracy was acceptable for all five groundwater eluates amenable to quantitative testing: Hill AFB, NWIRP McGregor, Fontana Water Co., Massachusetts Military Reservation, and LLNL. Furthermore, ANOVA and Tukey Method statistical analyses indicated that the anaerobic bioassay and the reference method in a commercial laboratory were not significantly different. This finding pointed to oxygen interference in the benchtop assay as the culprit of inaccuracy. In support of this, creating sufficiently reduced conditions in reaction mixtures by the non-enzymatic reaction between NADH and PMS proved unreliable. The non-enzymatic oxidation of NADH in the presence of PMS was unpredictable and far greater for the benchtop format than observed when reactions were performed in the anaerobic chamber. NADH oxidation seen during the preincubation period was even variable and unpredictable among replicate SPE eluate sets (see Appendix L, Table L-1) and often of much greater magnitude than occurs during perchlorate reduction by perchlorate reductase.

While consideration was given to adding reductants in addition to the NADH and PMS system, to more chemically remove oxygen from the SPE eluates prior to bioassay analysis, this approach was dismissed because of possible interference. As a point in case, mixing of the reductant cysteine (an amino acid with a sulfhydral group) with oxidized PMS resulted in the rapid formation of a red degradation product, showing that cysteine wouldn't be suitable as an additional reductant for the benchtop bioassay. Other reductants such as ascorbic acid, dithiothreitol, or dithionite were not attractive, since these reduce $PMS^2$ directly, and would interfere with the stoichiometry of NADH and perchlorate in the bioassay. Thus the only approach that could be adopted for benchtop bioassay was to extend the preincubation period during which residual oxygen is removed non-enzymatically and amend reaction mixtures with additional NADH prior to addition of extract to start the enzymatic reaction. However, this approach was not sufficiently effective or reproducible as a means of making reaction mixtures suitably reduced prior to perchlorate reductase addition creating unpredictability in the bioassay for perchlorate determination.

## 9.0 REFERENCES


1. Bender KS, Rice MR, Fugate WH, Coates JD, and Achenbach LA. (2004) Metabolic primers for the detection of (per)chlorate-reducing bacteria in the environment and phylogenetic analysis of *cld* gene sequences. Appl Environ Microbiol 70:5651-5658

2. Heinnickel M, Smith SC, Koo J, O'Connor SM, Coates JD. (2011) Design and optimization of a colorimetric bioassay for perchlorate in the ppb range. Environ Sci Tech 45:2958-2964

3. Clark JJ. (2000) In: Perchlorate in the Environment, Urbansky ET (ed.) Kluwer Academic/Plenum, New York

4. Coates JD and Achenbach LA. (2004) Microbial perchlorate reduction: rocket-fuelled metabolism. Nature Rev. Microbiol. 2:569-580

5. Coates JD and Jackson A. (2008) Principles of perchlorate treatment. In: In Situ Bioremediation of Perchlorate in Groundwater. CH Ward and HF Stroo. Norwell, MA, Springer

6. Coates JD, Michaelidou U, O'Connor SM, Bruce RA and Achenbach LA. (2000) The diverse microbiology of (per)chlorate reduction. In: Urbansky ED. Perchlorate in the Environment. Kluwer Academic/Plenum, New York, 257-270

7. Collette TW, Williams TL, Urbansky ET, Magnuson ML, Herbert GN, and Strauss SH. (2003) Analysis of hydroponic fertilizer matrixes for perchlorate: comparison of analytical techniques. Analyst 128:88-97

8. Ericksen GE (1983) The Chilean nitrate deposits. American Scientist 71:366-374

9. Jackson WA et al. (2005) Perchlorate accumulation in forage and edible vegetation. J. Agric. Food Chem. 53:369-373

10. Kengen SW, Rikken GB, Hagen WR, van Ginkel CG, and Stams AJ. (1999) Purification and characterization of (per)chlorate reductase from the chlorate-respiring strain GR-1. J Bacteriol. 181:6706-6711

11. Kirk AB, Smith EE, Tian K, Anderson TA and Dasgupta PK. (2003) Perchlorate in milk. Environmental Science & Technology37:4979-4981

12. Motzer WE. (1998) Perchlorate: problems, detection, and solutions. Environmental Forensics 2:301-311

13. Renner R. (2009) EPA perchlorate decision flawed, say advisers. Environmental Science & Technology 43:553-334

14. Skoog DA, Holler FJ, Nieman TA. (1998) Principles of Instrumental Analysis. Brooks/Cole Thomson Learning, United States of America

15. Thorne PG. (2004) Field screening method for perchlorate in water and soil. ERDC/CRREL TR-04-8

16. Urbansky ET and Brown SK. (2003) Perchlorate retention and mobility in soils. J. Environmental Monitoring 5:455-462

17. USEPA. (1998) Drinking water candidate contaminant list. Washington DC, US Environmental Protection Agency

18. Wolff J. (1998) Perchlorate and the thyroid gland. Pharmacological Reviews 50:89-105

19. Coates JD, Heinnickel M, and Achenbach LA. (2010) An enzymatic bioassay for perchlorate. SERDP-ER-1530-FR. Accession number ADA539494

20. EDQW. (2007) DoD Perchlorate Handbook. Washington DC, US Department of Defense, Environmental Data Quality Workgroup

21. Magnuson ML, Urbansky ET, and Kelty CA. (2000) Determination of perchlorate at trace levels in drinking water by ion-pair extraction with electrospray ionization mass spectrometry. Anal Chem 72:25-29

22. Ott RL, and Longnecker M. (2001) An Introduction to Statistical Methods and Data Analysis, 5th edn. Duxbury, Thomson Learning, United States of America

23. Patterson J, Parette R, Cannon FS, Lutes C, and Henderson T. (2011) Competition of anions with perchlorate for exchange sites on cationic surfactant-tailored GAC. Environ. Eng. Sci. 28:249-256

24. EPA. (2009) Revised Assessment Guidance for Perchlorate. http://www.epa.gov/fedfac/documents/perchlorate_memo_01-08-09.pdf Accessed 14 May 2013

# APPENDICES

## Appendix A: POINTS OF CONTACT

| POINT OF CONTACT<br>Name | ORGANIZATION<br>Name<br>Address | Phone<br>Fax<br>E-mail | Role in Project |
|---|---|---|---|
| Andrea Leeson, Ph.D. | SERDP/ESTCP<br>Environmental Restoration Program Manager<br>901 N. Stuart Street, Suite 303<br>Arlington, VA 22203 | (703) 696-2118<br>(703) 696-2114 (fax)<br>Andrea.Leeson@osd.mil | ESTCP Project Manager |
| John D. Coates, Ph.D. | University of California, Berkeley,<br>271 Koshland Hall, Berkeley, CA 94720-3102 | (510) 643-8455<br>(510) 642-4995 (fax)<br>jdcoates@berkeley.edu | Principal Investigator |
| Nancy E. Ruiz, Ph.D. | Naval Facilities Engineering Service Center,<br>1100 23rd Ave. EV 31<br>Port Hueneme, CA 93043 | (805) 982-1155<br>(805) 982-4304 (fax)<br>Nancy.ruiz@navy.mil | Liaison Officer |
| Kyle Gorder, P.E. | Environmental Restoration Branch<br>Hill Air Force Base, UT 84056 | (801) 775-2559<br>Kyle.gorder@hill.af.mil | Site contact |
| Robert K. Young | Fontana Water Company<br>15966 Arrow Route<br>PO Box 987<br>Fontana, CA 92335 | (909) 822-2201<br>(909) 823-5046 (fax)<br>rkyoung@fontanawater.com | General Manager, Fontana Water Co.<br>Site contact |
| Ben Gregson | Massachusetts Army Natiional Guard<br>Impact Area Groundwater Study Program<br>1803 West Outer Road<br>Camp Edwards, MA 02542-5003 | (508) 968-5821<br>(508) 968-5286 (fax)<br>Benjamin.p.gregson@us.army.mil | MMR Remediation Manager<br>Site contact |
| Victor Madrid | Environmental Restoration Division<br>Lawrence Livermore National Laboratory<br>7000 East Ave.<br>Livermore, CA 94550 | (925) 422-9930<br>Madrid2@llnl.gov | Senior Geologist<br>Site contact |
| Anna Engelbrektson | University of California, Berkeley,<br>271 Koshland Hall, Berkeley, CA 94720-3102 | (510) 642-4972<br>(510) 642-4995 (fax)<br>aengelbrektson@berkeley.edu | Statistical analysis |
| Anne C. Frazer, Ph.D. | University of California, Berkeley,<br>271 Koshland Hall, Berkeley, CA 94720-3102 | (510) 642-4972<br>(510) 642-4995 (fax)<br>acfrazer@berkeley.edu | Project staff |

# Appendix B: BENCHTOP BIOASSAY DATA

Table B-1 summarizes the results from benchtop bioassay testing of SPE eluate sets using the standard additions method to estimate the perchlorate concentration in groundwater sources.

**Table B-1. Perchlorate determined by benchtop bioassay in SPE eluate sets[a]**

| Site | Para-meter[b] | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 | Average ± SD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **102, Hill AFB** | **SPE date** | 27 Jul 2011 | 27 Jul 2011 | 27 Jul 2011 | 6 Aug 2011 | 7 Aug 2011 | 9 Aug 2011 | 7 Aug 2011 | 7 Aug 2011 | 9 Aug 2011 | |
| | **Batch** | *1* | *1* | *1* | *2* | *3* | *2* | *3* | *3* | *2* | |
| | **µg/L** | 23±3 | 21±3 | 14±6 | 20±8 | 23±4 | 22±3 | 31±4 | 45±16 | 33±6 | 26±9 |
| **103, McGre-gor NWIRP[c]** | **SPE date** | 5 Nov 2011 | 5 Nov 2011 | 5Nov 2011 | 7 Nov 2011 | 8 Nov 2011 | 8 Nov 2011 | NA | NA | NA | |
| | **Batch** | *1* | *1* | *1* | *2* | *2* | *2* | | | | |
| | **µg/L** | 43±36 | 106 ±210 | 673 ±8574 | 58±68 | 720 ±3870 | 55±41 | | | | 276±327 |
| **104, JPL** | **SPE date** | 8 Dec 2011 | 8 Dec 2011 | 8 Dec 2011 | 11 Dec 2011 | 11 Dec 2011 | 11 Dec 2011 | 11 Dec 2011 | 11 Dec 2011 | 13 Dec 2011 | |
| | **Batch** | *3* | *1* | *1* | *2* | *2* | *2* | *3* | *3* | *4* | |
| | **µg/L** | 3±2 | 2±9 | 5±3 | 0±5 | 4±1 | 5±3 | 4±3 | 2±4 | 1±1 | 3±2 |
| **105, Fontana Water Co.** | **SPE date** | 13 Feb 2012 | 13 Feb 2012 | 13 Feb 2012 | 14 Feb 2012 | 15 Feb 2012 | 15 Feb 2012 | 17 Feb 2012 | 17 Feb 2012 | 17 Feb 2012 | |
| | **Batch** | *1* | *1* | *1* | *2* | *2* | *3* | *2* | *3* | *3* | |
| | **µg/L** | 15±4 | 8±0 | 11±3 | 5±3 | 8±3 | 5±2 | 7±2 | 9±7 | 10±2 | 9±3 |
| **106, MMR** | **SPE date** | 9 Mar 2012 | 12 Mar 2012 | 12 Mar 2012 | 13 Mar 2012 | 13 Mar 2012 | 13 Mar 2012 | 14 Mar 2012 | 14 Mar 2012 | 14 Mar 2012 | |
| | **Batch** | *1* | *1* | *1* | *2* | *2* | *3* | *2* | *3* | *3* | |
| | **µg/L** | -3±5 | 23±4 | 51±34 | 36±9 | 23±13 | 9±9 | 83±2 | 7±3 | 18±10 | 27±26 |
| **107, LLNL** | **SPE date** | 6 Apr 2012 | 6 Apr 2012 | 9 Apr 2012 | 12 Apr 2012 | 16 Apr 2012 | 16 Apr 2012 | 17 Apr 2012 | 17 Apr 2012 | 17 Apr 2012 | |
| | **Batch** | *3* | *3* | *3* | *1* | *1* | *2* | *1* | *2* | *2* | |
| | **µg/L** | 2±0 | -3±2 | -4±1 | 6±2 | 1±2 | 0±3 | 1±2 | 1±0 | 2±2 | 1±3 |

[a]The x-intercept error from the standard additions method regression line is listed with individual perchlorate estimates. The overall average is also listed for each groundwater source ± the standard deviation. NA denotes not applicable
[b]"SPE date" corresponds to the preparation date for an SPE eluate set. The testing batch that included a specific eluate set is listed in the table, and the date on which batches were tested is listed in the text below.
[c]Only two full gallon samples (bottles A and B) were available from the McGregor groundwater source.

Testing was performed in batches and as many as 18 perchlorate reductase reactions in individual cuvettes were tested at a time. Batches were tested on the following dates:
Hill AFB: batch *1* (11 Aug 2011), batch *2* (11 Aug 2011), batch *3* (12 Aug 2011)
McGregore: batch *1* (6 Nov 2011), batch *2* (9 Nov 2011)
Jet Propulsion Lab: batch *1* (9 Dec 2011), batch *2* (12 Dec 2011), batch *3* (12 Dec 2011), batch *4* (16 Dec 2011)
Fontana Water Company: batch *1* (16 Feb 2012), batch *2* (18 Feb 2012), batch *3* (20 Feb 2012)
Massachusetts Military Reservation: batch *1* (15 Mar 2012), batch *2* (18 Mar 2012), batch *3* (19 Mar 2012)
Lawrence Livermore National Laboratory: batch *1* (20 Apr 2012), batch *2* (23 Apr 2012), batch *3* (24 Apr 2012).

The bioassay rates (i.e. 340 nm absorbance change/minute) used in the standard additions method to determine the perchlorate (µg/L) for SPE eluate sets tested in the different batches of a groundwater source are listed in Tables B-102 (Hill AFB), B-103 (NWIRP McGregor), B-104 (Jet Propulsion Laboratory), B-105 (Fontana Water Co.), B-106 (Massachusetts Military Reservation), and B-107 (Lawence Livermore National Laboratory). The perchlorate spikes added prior to applying samples to SPE cartridges were 0, 10, 20, 30, or 40 µg/L. Each table has an accompanying figure, which shows the standard additions method regression lines for the SPE eluate sets in a particular batch, from which perchlorate concentrations were determined. The Figures are designated as B-102 (Hill AFB), B-103 (NWIRP McGregor), B-104 (Jet Propulsion Laboratory), B-105 (Fontana Water Co.), B-106 (Massachusetts Military Reservation), and B-107 (Lawence Livermore National Laboratory).

Detailed experimental data, i.e. the 340 nm absorbance over time for SPE eluates, are presented last in Appendix B in a series of figures and tables for each batch tested. These show the NADH oxidation during preincubation periods as well as after the addition of extract having perchlorate reductase activity, which constitutes the initial time for bioassay reactions.

## Groundwater sample 102, Hill AFB, location U9-16-007, collected 14Jul2011

**Table B-102. Perchlorate benchtop bioassay analysis of standard additions method sets for Hill AFB groundwater collected 14 Jul 2011 from location U9-16-007[a]**

| Perchlorate Spike (µg/L) | Batch 1 (tested 11 Aug 2011) | | | Batch 2 (tested 11 Aug 2011) | | |
|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | B1 | B3 | C3 |
| 0 | 0.0022 | 0.0022 | 0.0013 | 0.0023 | 0.0026 | 0.0045 |
| 10 | 0.0035 | 0.0033 | 0.0028 | 0.0047 | 0.0042 | 0.0045 |
| 20 | 0.0040 | 0.0037 | 0.0024 | 0.0038 | 0.0046 | 0.0063 |
| 30 | 0.0056 | 0.0051 | 0.0047 | 0.0058 | 0.0066 | 0.0076 |
| 40 | 0.0062 | 0.0064 | 0.0051 | 0.0078 | 0.0074 | 0.0089 |
| **Regression parameters for each set[b]** | | | | | | |
| **y-intercept ± error** | 0.002280 ±0.000219 | 0.002100 ±0.00024 | 0.001360 ±0.000495 | 0.002460 ±0.000719 | 0.002680 ±0.000299 | 0.003980 ±0.000387 |
| **Slope ± error** | 0.000101 ±0.0000089 | 0.000102 ±0.0000098 | 0.000095 ±0.000020 | 0.000121 ±0.000029 | 0.000120 ±0.000012 | 0.000119 ±0.000016 |
| **$r^2$** | 0.9771 | 0.9731 | 0.8803 | 0.8498 | 0.9698 | 0.9496 |
| **x-intercept ± error** | -23±3 | -21±3 | -14±6 | -20±8 | -22±3 | -33±6 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 23±3 | 21±3 | 14±6 | 20±8 | 22±3 | 33±6 |

| Perchlorate Spike (µg/L) | Batch 3 (tested 12 Aug 2011) | | | Perchlorate Standards | | | |
|---|---|---|---|---|---|---|---|
| | B2 | C1 | C2 | µM | Batch 1 | Batch 2 | Batch 3 |
| 0 | 0.0032 | 0.0039 | 0.0033 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 10 | 0.0036 | 0.0045 | 0.0044 | 10 | ND | ND | ND |
| 20 | 0.0048 | 0.0055 | 0.0062 | 20 | ND | ND | ND |
| 30 | 0.0069 | 0.0074 | 0.0069 | 30 | ND | ND | ND |
| 40 | 0.0077 | 0.0083 | 0.0062 | 40 | 0.0043 | 0.0054 | 0.0052 |
| **Regression parameters for each set[b]** | | | | | | | |
| **y-intercept ± error** | 0.002780 ±0.000380 | 0.003580 ±0.000296 | 0.003740 ±0.000640 | | NA | NA | NA |
| **Slope ± error** | 0.000123 ±0.000016 | 0.000117 ±0.000012 | 0.000083 ±0.000026 | | 0.000108 | 0.000135 | 0.000130 |
| **$r^2$** | 0.9544 | 0.9689 | 0.7706 | | NA | NA | NA |
| **x-intercept ± error** | -23±4 | -31±4 | -45±16 | | NA | NA | NA |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 23±4 | 31±4 | 45±16 | | NA | NA | NA |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.0030 (batch 1), 0.0010 (batch 2), 0.0011 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 33 µL of CKB 8/3/2011 extract. ND denotes not determined, NA denotes not applicable

[b]Regression parameters are from the regression line for each set (see Figure B-102). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept

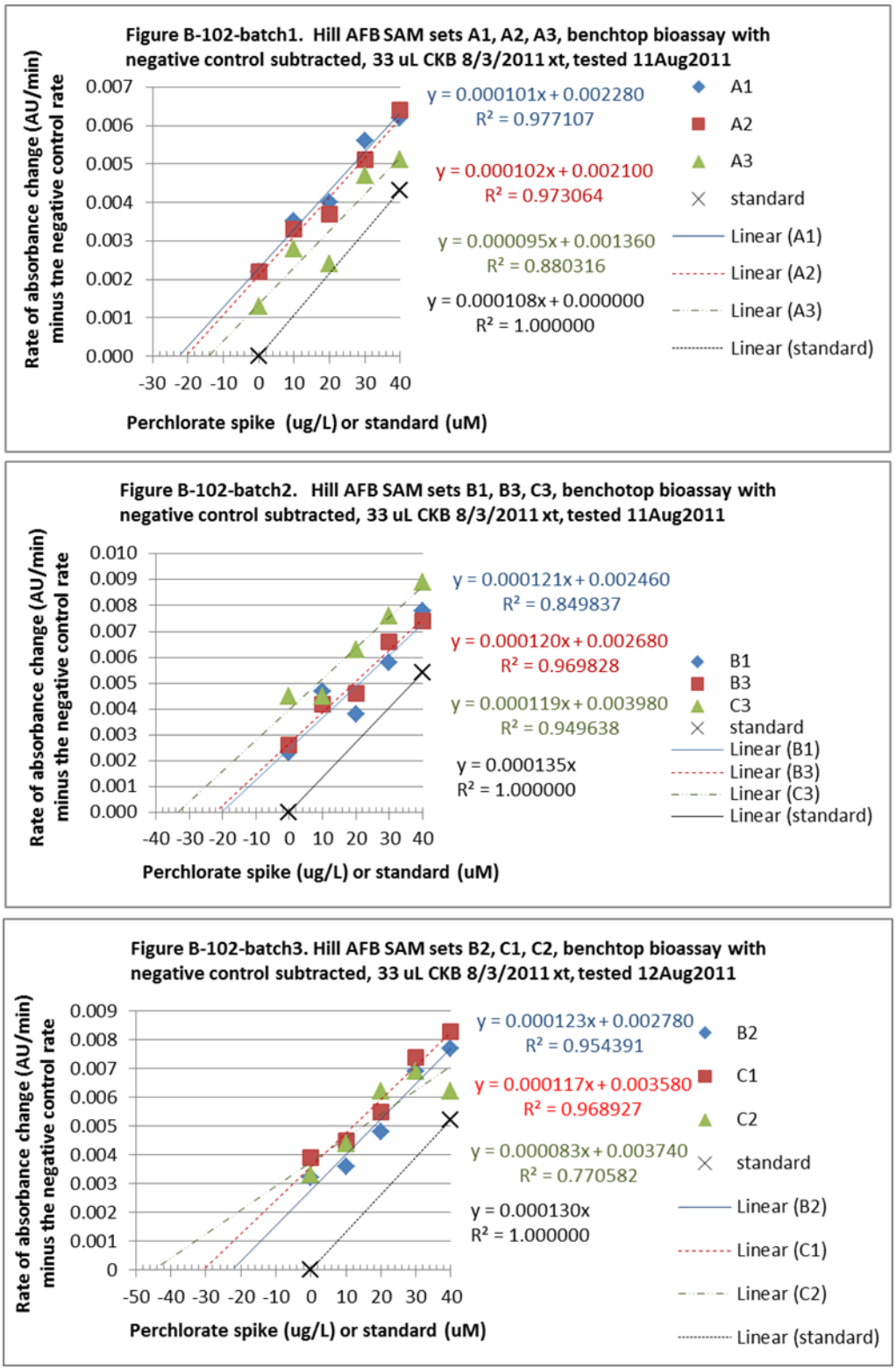



**Figure B-102. Perchlorate benchtop bioassay analysis of standard additions method sets for Hill AFB groundwater collected 14 Jul 2011 from location U9-16-007 (See Table B-102)**

## Groundwater sample 103, NWIRP McGregor, location OFFWS-37, collected 13Oct2011

**Table B-103. Perchlorate benchtop bioassay analysis of standard additions method sets for NWIRP McGregor groundwater collected 13 Oct 2011 from location OFFWS-37[a]**

| Perchlorate Spike (µg/L) | Batch 1 (tested 6 Nov 2011 | | | Batch 2[d] (tested 9 Nov 2011) | | |
|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | B1 | B2 | B3 |
| 0 | 0.0052 | 0.0127 | 0.0037 | 0.0062 | 0.0037 | 0.0011 |
| 10 | 0.0126 | 0.0055 | 0.0154 | 0.0028 | 0.0122 | 0.0122 |
| 20 | 0.0120 | 0.0174 | 0.0139 | 0.0035 | 0.0037 | 0.0096 |
| 30 | 0.0075 | 0.0076 | 0.0068 | 0.0053 | 0.0112 | 0.0136 |
| 40 | 0.0163 | 0.0164 | 0.0087 | 0.0127 | 0.0056 | 0.0135 |
| **Regression parameters for each set[b]** | | | | | | |
| **y-intercept ± error** | 0.007300 ±0.003095 | 0.010020 ±0.004507 | 0.009420 ±0.004367 | 0.003000 ±0.002751 | 0.006720 ±0.003667 | 0.004760 ±0.002856 |
| **Slope ± error** | 0.000171 ±0.000126 | 0.000095 ±0.000184 | 0.000014 ±0.000178 | 0.000155 ±0.000112 | 0.000028 ±0.000150 | 0.000262 ±0.000117 |
| **$r^2$** | 0.3790 | 0.0816 | 0.0021 | 0.3884 | 0.0115 | 0.6273 |
| **x-intercept ± error** | -43±36 | -106±210 | -673±8574 | -19.4±22.6 | -240.0±1290 | -18.2±13.6 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 43±36 | 106±210 | 673±8574 | 58±68 | 720±3870 | 55±41 |

| Perchlorate Standards | | |
|---|---|---|
| **µM** | **Batch 1** | **Batch 2** |
| 0 | 0.0000 | 0.0000 |
| 10 | ND | ND |
| 20 | 0.0019 | 0.0025 |
| 30 | ND | ND |
| 40 | 0.0048 | 0.0042 |
| **Regression parameters for each set[b]** | | |
| **y-intercept ± error** | -0.000167 ±0.000373 | 0.000133 ±0.000298 |
| **Slope ± error** | 0.000120 ±0.000014 | 0.000105 ±0.000012 |
| **$r^2$** | 0.9857 | 0.9881 |
| **x-intercept ± error** | 1±3 | -1±3 |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.0022 (batch 1), 0.0018 (batch 2). Perchlorate reductase was added to cuvettes as the equivalent of 33 µL of CKB 8/3/2011 extract. ND denotes not determined

[b]Regression parameters are from the regression line for each set (see Figure B-103). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept.

[d]Groundwater tested in batch 2 was diluted with two parts deionized water prior to the addition of perchlorate spikes and application to SPE cartridges. Thus the estimate for perchlorate in groundwater for batch 2 was (-1) times the x-intercept times 3 (the dilution factor)

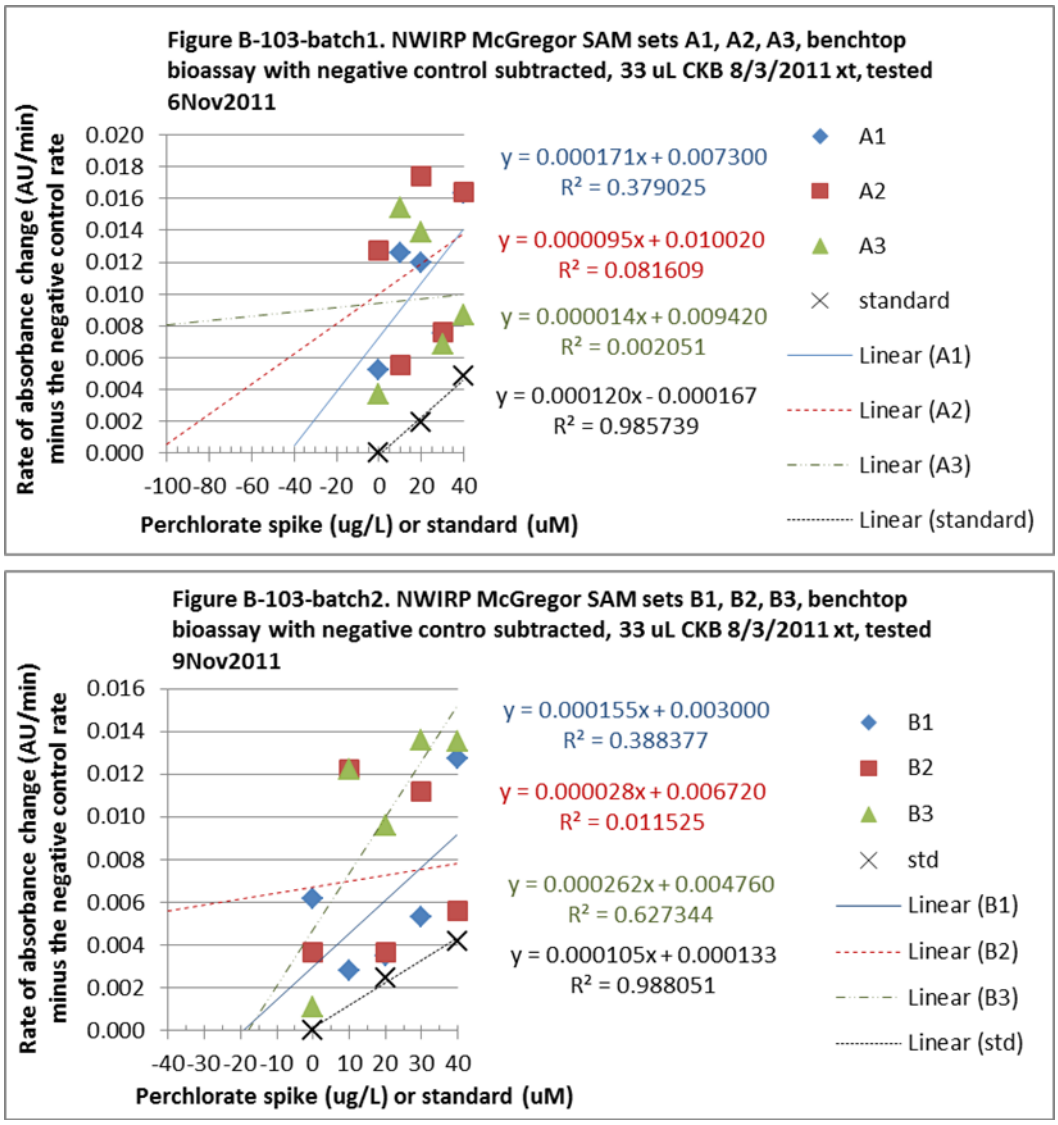



**Figure B-103. Perchlorate benchtop bioassay analysis of standard additions method sets for NWIRP McGregor groundwater collected 13 Oct 2011 from location OFFWS-37 (See Table B-103)**

## Groundwater sample 104, Jet Propulsion Laboratory (JPL), location MW-16, collected 14Nov2011

**Table B-104. Perchlorate benchtop bioassay analysis of standard additions method sets for Jet Propulsion Laboratory groundwater collected 14 Nov 2011 from location MW-16[a]**

| Perchlorate Spike (µg/L) | Batch 1 (tested 9 Dec 2011) | | Batch 2 (tested 12 Dec 2011) | | | Perchlorate Standards | | |
|---|---|---|---|---|---|---|---|---|
| | A2 | A3 | B1 | B2 | B3 | µM | Batch 1 | Batch 2 |
| 0 | 0.0008 | 0.0009 | 0 | 0.0003 | 0.0006 | 0 | 0.0000 | 0.0000 |
| 10 | 0.0019 | 0.0017 | 0.0009 | 0.0013 | 0.0015 | 10 | ND | ND |
| 20 | 0.0025 | 0.0026 | 0.0025 | 0.0024 | 0.0027 | 20 | 0.0023 | 0.0021 |
| 30 | 0.0098 | ND | 0.0049 | 0.0032 | 0.0044 | 30 | ND | ND |
| 40 | 0.0069 | 0.0056 | 0.0041 | 0.0041 | 0.0046 | 40 | 0.0046 | 0.0049 |
| **Regression parameter for each set[b]** | | | | | | | | |
| **y-intercept ± error** | 0.000360 ±0.001888 | 0.000620 ±0.000299 | 0.000040 ±0.000670 | 0.000360 ±0.000073 | 0.000580 ±0.000300 | | 0.000000 ±0.000000 | -0.000117 ±0.00026 |
| **Slope ± error** | 0.000201 ±0.000077 | 0.000119 ±0.000013 | 0.000122 ±0.000027 | 0.000095 ±0.000003 | 0.000109 ±0.000012 | | 0.000115 ±0.000000 | 0.000123 ±0.000010 |
| **$r^2$** | 0.6938 | 0.9764 | 0.8690 | 0.9970 | 0.9634 | | 1.0000 | 0.9932 |
| **x-intercept** | -2±9 | -5±3 | -0±5 | -4±1 | -5±3 | | 0±0 | 1±2 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 2±9 | 5±3 | 0±5 | 4±1 | 5±3 | | NA | NA |

| Perchlorate Spike (µg/L) | Batch 3 (tested 12 Dec 2011) | | | Batch 4 (tested 16 Dec 2011) | Perchlorate Standards | | |
|---|---|---|---|---|---|---|---|
| | A1 | C1 | C2 | C3 | µM | Batch 3 | Batch 4 |
| 0 | 0.0004 | 0.0003 | 0.0004 | nd | 0 | 0.0000 | 0.0000 |
| 10 | 0.0008 | 0.0012 | 0.0013 | 0.0009 | 10 | ND | ND |
| 20 | 0.0023 | 0.003 | 0.002 | 0.0016 | 20 | 0.0028 | 0.0021 |
| 30 | 0.0031 | 0.003 | 0.0028 | 0.0025 | 30 | ND | ND |
| 40 | 0.0039 | 0.0045 | 0.0049 | 0.0033 | 40 | 0.0048 | 0.0047 |
| **Regression parameters for each set[b]** | | | | | | | |
| **y-intercept ± error** | 0.000240 ±0.000205 | 0.000360 ±0.000339 | 0.000180 ±0.000370 | 0.000050 ±0.000072 | | -0.000067 ±0.000149 | -0.000083 ±0.000186 |
| **Slope ± error** | 0.000093 ±0.000008 | 0.000102 ±0.000014 | 0.000105 ±0.000015 | 0.000081 ±0.000003 | | 0.000120 ±0.000006 | 0.000118 ±0.000007 |
| **$r^2$** | 0.9762 | 0.9475 | 0.9417 | 0.9979 | | 0.9977 | 0.9962 |
| **x-intercept** | -3±2 | -4±3 | -2±4 | -1±1 | | 1±1 | 1±2 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 3±2 | 4±3 | 2±4 | 1±1 | | NA | NA |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. ND denotes not determined, NA denotes not applicable. Negative control rates were 0.0005 (batch 1), 0.0007 (batch 2), 0.0006 (batch 3), 0.0006 (batch 4). Perchlorate reductase was added to cuvettes as the equivalent of 33 µL of CKB 8/3/2012 extract. ND denotes not determined, NA denotes not applicable

[b]Regression parameters are from the regression line for each set (see Figure B-104). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept

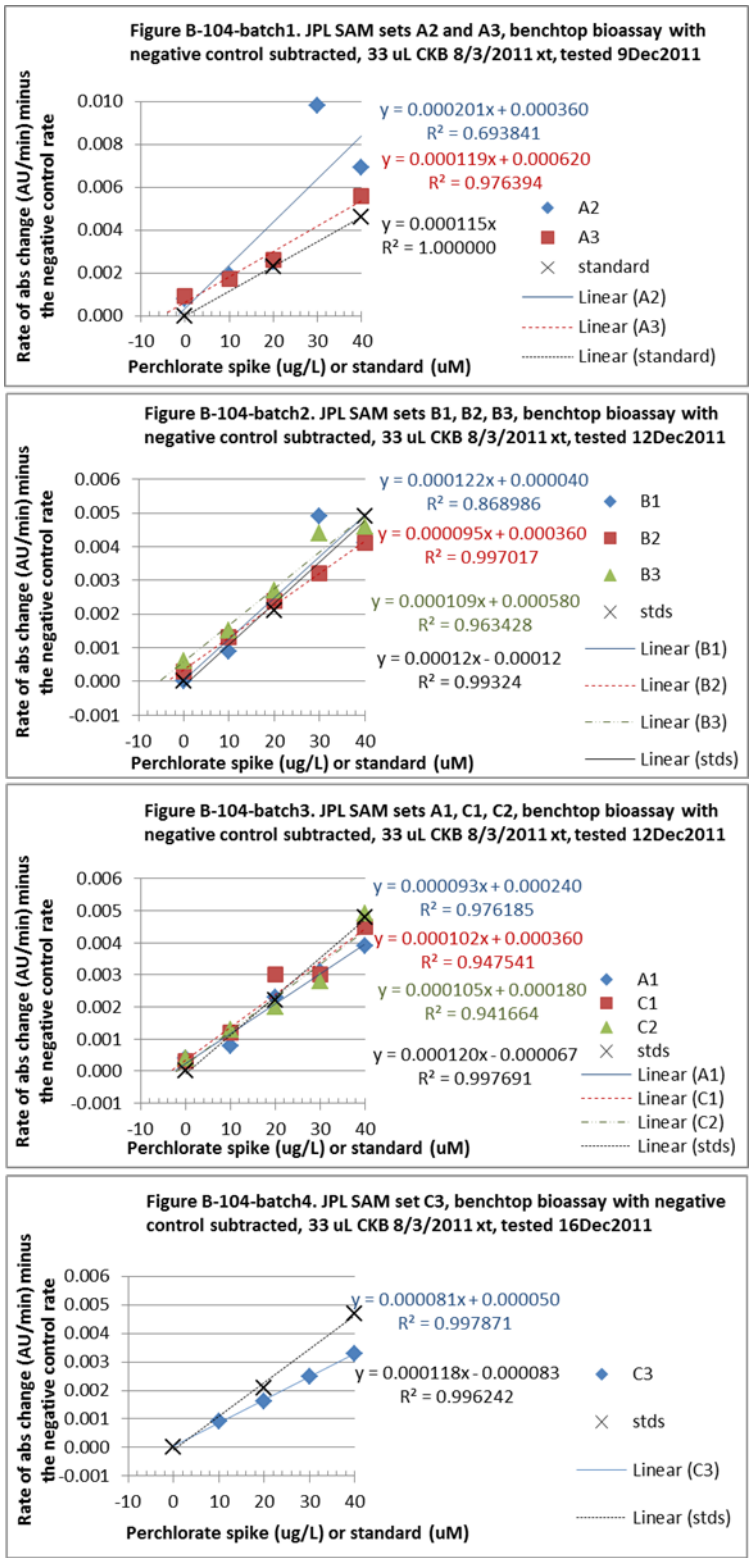



**Figure B-104. Perchlorate benchtop bioassay analysis of standard additions method sets for Jet Propulsion Laboratory groundwater collected 14 Nov 2011 from location MW-16 (See Table B-104)**

## Groundwater sample 105, Fontana Water Company, location F-17C, collected 25Jan2012

**Table B-105. Perchlorate benchtop bioassay analysis of standard additions method sets for Fontana Water Company groundwater collected 25 Jan 2012 from location F-17C[a]**

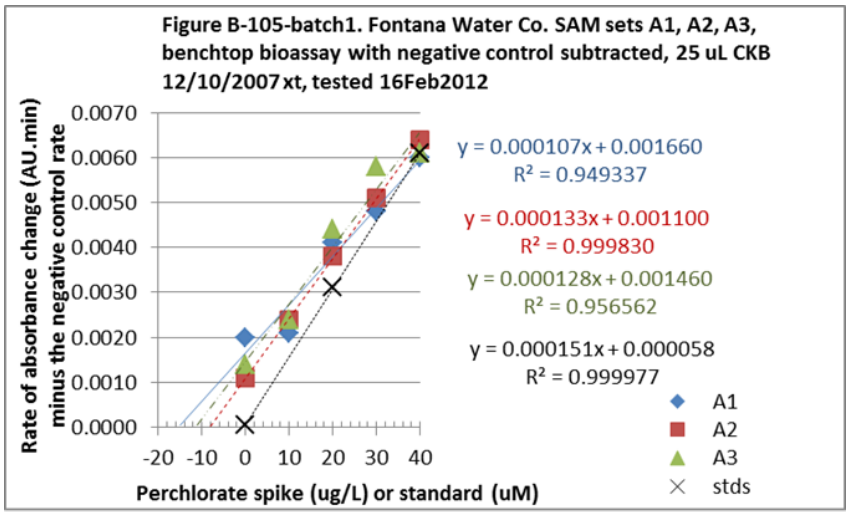
| Perchlorate Spike (µg/L) | Batch 1 (tested 16 Feb 2012) | | | Batch 2 (tested 18 Feb 2012) | | |
|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | B1 | B2 | C1 |
| 0 | 0.0020 | 0.0011 | 0.0014 | 0.0006 | 0.0011 | 0.0011 |
| 10 | 0.0021 | 0.0024 | 0.0024 | 0.0015 | 0.0030 | 0.0033 |
| 20 | 0.0041 | 0.0038 | 0.0044 | 0.0035 | 0.0036 | 0.0046 |
| 30 | 0.0048 | 0.0051 | 0.0058 | 0.0046 | 0.0063 | 0.0060 |
| 40 | 0.0060 | 0.0064 | 0.0061 | 0.0052 | 0.0068 | 0.0086 |
| **Regression parameters for each set[b]** | | | | | | |
| **y-intercept ± error** | 0.001610 ±0.000350 | 0.001050 ±0.000024 | 0.001410 ±0.000386 | 0.000620 ±0.000316 | 0.001220 ±0.000439 | 0.001180 ±0.000316 |
| **Slope ± error** | 0.000107 ±0.000014 | 0.000133 ±0.000001 | 0.000128 ±0.000016 | 0.000123 ±0.000013 | 0.000147 ±0.00018 | 0.000177 ±0.000013 |
| **$r^2$** | 0.9493 | 0.9998 | 0.9566 | 0.9681 | 0.9573 | 0.9843 |
| **x-intercept ± error** | -15±4 | -8±0 | -11±3 | -5±3 | -8±3 | -7±2 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 15±4 | 8±0 | 11±3 | 5±3 | 8±3 | 7±2 |

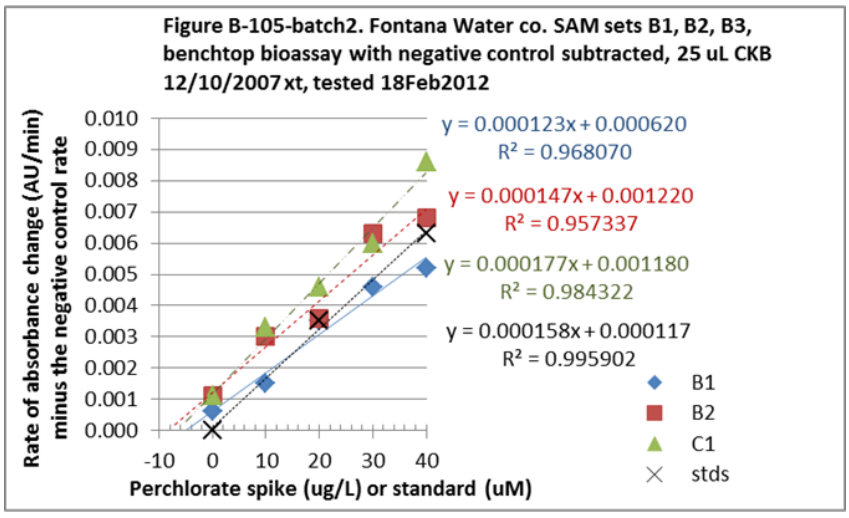
| Perchlorate Spike (µg/L) | Batch 3 (tested 20 Feb 2012) | | | Perchlorate Standards | | | |
|---|---|---|---|---|---|---|---|
| | B3 | C2 | C3 | µM | Batch 1 | Batch 2 | Batch 3 |
| 0 | 0.0007 | 0.0010 | 0.0016 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 10 | 0.0020 | 0.0022 | 0.0024 | 10 | ND | ND | ND |
| 20 | 0.0028 | 0.0045 | 0.0046 | 20 | 0.0031 | 0.0035 | 0.0039 |
| 30 | 0.0047 | 0.0069 | 0.0057 | 30 | ND | ND | ND |
| 40 | 0.0056 | 0.0056 | 0.0070 | 40 | 0.0061 | 0.0063 | 0.0066 |
| **Regression parameters for each set[b]** | | | | | | | |
| **y-intercept ± error** | 0.000645 ±0.000227 | 0.001262 ±0.000897 | 0.001408 ±0.000266 | | 0.000008 ±0.000019 | 0.000117 ±0.000261 | 0.000204 ±0.000467 |
| **Slope ± error** | 0.000126 ±0.000009 | 0.000139 ±0.000037 | 0.000142 ±0.000011 | | 0.000151 ±0.0000008 | 0.000158 ±0.000010 | 0.000166 ±0.000018 |
| **$r^2$** | 0.9839 | 0.8273 | 0.9828 | | 1.0000 | 0.9959 | 0.9882 |
| **x-intercept ± error** | -5±2 | -9±7 | -10±2 | | -0±0 | -1±2 | -1±3 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 5±2 | 9±7 | 10±2 | | NA | NA | NA |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.00054 (batch 1), 0.0004 (batch 2), 0.000455 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 12/10/2007 extract. ND denotes not determined, NA denotes not applicable

[b]Regression parameters are from the regression line for each set (see Figure B-105). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept



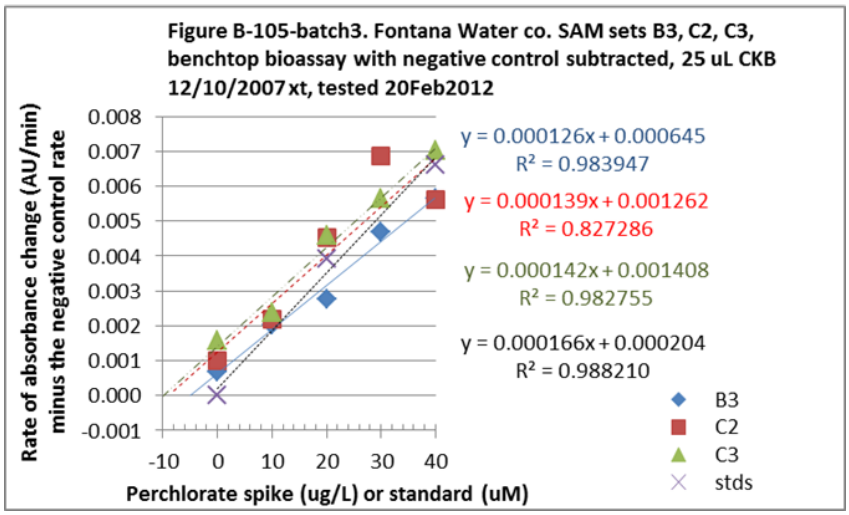




**Figure B-105. Perchlorate benchtop bioassay analysis of standard additions method sets for Fontana Water Company groundwater collected 25 Jan 2012 from location F-17C (See Table B-105)**

## Groundwater sample 106, Massachusetts Military Reservation, location J3-INF, collected 23Feb2012

**Table B-106. Perchlorate benchtop bioassay analysis of standard additions method sets for Massachusetts Military Reservation ground water collected 23 Feb 2012 from location J3-INF[a]**

| Perchlorate Spike (µg/L) | Batch 1 (tested 15 Mar 2012) | | | Batch 2 (tested 18 Mar 2012) | | |
|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | B1 | B2 | C1 |
| 0 | -0.0008 | 0.0032 | 0.0039 | 0.0068 | 0.0031 | 0.0136 |
| 10 | 0.0010 | 0.0061 | 0.0093 | 0.0125 | 0.0137 | 0.0154 |
| 20 | 0.0059 | 0.0074 | 0.0101 | 0.0139 | 0.0153 | 0.0172 |
| 30 | 0.0049 | 0.0082 | 0.0112 | 0.0156 | 0.0158 | 0.0186 |
| 40 | 0.0089 | 0.0105 | 0.0090 | 0.0171 | 0.0171 | 0.0203 |
| **Regression parameters for each set[b]** | | | | | | |
| **y-intercept ± error** | -0.000701 ±0.001127 | 0.003754 ±0.000469 | 0.006235 ±0.001833 | 0.008440 ±0.001161 | 0.006980 ±0.002751 | 0.013700 ±0.000102 |
| **Slope ± error** | 0.000233 ±0.000046 | 0.000166 ±0.000019 | 0.000123 ±0.000075 | 0.000237 ±0.000047 | 0.000301 ±0.000112 | 0.000166 ±0.000004 |
| **$r^2$** | 0.8951 | 0.9617 | 0.4727 | 0.8929 | 0.7054 | 0.9981 |
| **x-intercept ± error** | 3±5 | -23±4 | -51±34 | -36±9 | -23±13 | -83±2 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | -3±5 | 23±4 | 51±34 | 36±9 | 23±13 | 83±2 |

| Perchlorate Spike (µg/L) | Batch 3 (tested 19 Mar 2012) | | | Perchlorate Standards | | | |
|---|---|---|---|---|---|---|---|
| | B3 | C2 | C3 | µM | Batch 1 | Batch 2 | Batch 3 |
| 0 | 0.0005 | 0.0011 | 0.0009 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 10 | 0.0028 | 0.0052 | 0.0053 | 10 | ND | ND | ND |
| 20 | 0.0027 | 0.0090 | 0.0053 | 20 | 0.0023 | 0.0037 | 0.0034 |
| 30 | 0.0063 | 0.0096 | 0.0063 | 30 | ND | ND | ND |
| 40 | 0.0046 | 0.0130 | 0.0069 | 40 | 0.0053 | 0.0070 | 0.0069 |
| **Regression parameters for each set[b]** | | | | | | | |
| **y-intercept ± error** | 0.001040 ±0.001041 | 0.001940 ±0.000849 | 0.002340 ±0.001037 | | -0.000118 ±0.000264 | 0.000067 ±0.000149 | 0.000017 ±0.000037 |
| **Slope ± error** | 0.000117 ±0.000043 | 0.000282 ±0.000035 | 0.000130 ±0.000042 | | 0.000132 ±0.000010 | 0.000175 ±0.0000058 | 0.000173 ±0.0000014 |
| **$r^2$** | 0.7164 | 0.9566 | 0.7588 | | 0.9940 | 0.9989 | 0.9999 |
| **x-intercept ± error** | -9±9 | -7±3 | -18±10 | | 1±2 | -0±1 | 0±0 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 9±9 | 7±3 | 18±10 | | NA | NA | NA |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control. Negative control rates were 0.000969 (batch 1), 0.0003 (batch 2), 0.0007 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 12/10/2007 extract. ND denotes not determined, NA denotes not applicable

[b]Regression parameters are from the regression line for each set (see Figure B-106). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept

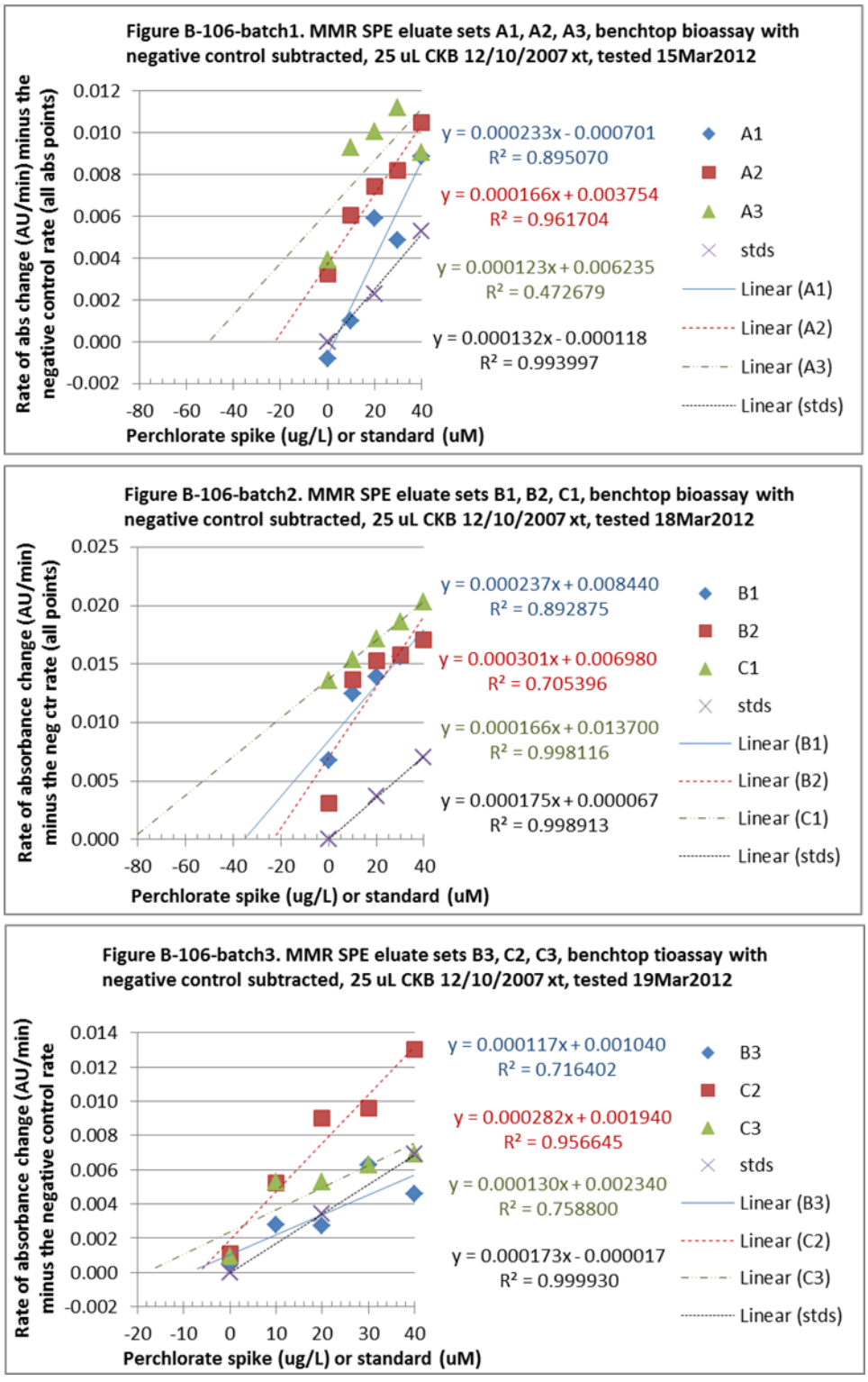



**Figure B-106. Perchlorate benchtop bioassay analysis of standard additions method sets for Massachusetts Military Reservation groundwater collected 23 Feb 2012 from location J3-INF (See Table B-106)**

## Groundwater sample 107, Lawrence Livermore National Laboratory-site 300, location W-854-1823, collected 29 March 2012

**Table B-107. Perchlorate benchtop bioassay analysis of standard additions method sets for Lawrence Livermore National Laboratory groundwater collected 29 March 2012 from location W-854-1823[a]**

| Perchlorate Spike (µg/L) | Batch 1 (tested 20 Apr 2012) | | | Batch 2 (tested 23 Apr 2012) | | |
|---|---|---|---|---|---|---|
| | B1 | B2 | C1 | B3 | C2 | C3 |
| 0 | 0.0005 | 0.0003 | 0.0002 | -0.00004 | 0.0001 | 0.0003 |
| 10 | 0.0012 | 0.0011 | 0.0013 | 0.0010 | 0.0012 | 0.0017 |
| 20 | 0.0025 | 0.0016 | 0.0026 | 0.0027 | 0.0021 | 0.0029 |
| 30 | 0.0033 | 0.0030 | 0.0033 | 0.0028 | 0.0031 | 0.0035 |
| 40 | 0.0038 | 0.0041 | 0.0050 | 0.0047 | 0.0042 | 0.0056 |
| **Regression parameters for each set[b]** | | | | | | |
| **y-intercept ± error** | 0.000520 ±0.000181 | 0.000120 ±0.000220 | 0.000154 ±0.000185 | 0.000048 ±0.000354 | 0.000106 ±0.000050 | 0.000284 ±0.000288 |
| **Slope ± error** | 0.000087 ±0.000007 | 0.000095 ±0.000009 | 0.000116 ±0.000008 | 0.000114 ±0.000014 | 0.000102 ±0.000002 | 0.000125 ±0.000012 |
| **$r^2$** | 0.9789 | 0.9738 | 0.9875 | 0.9535 | 0.9988 | 0.9739 |
| **x-intercept ± error** | -6±2 | -1±2 | -1±2 | 0±3 | -1±0 | -2±2 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 6±2 | 1±2 | 1±2 | 0±3 | 1±0 | 2±2 |

| Perchlorate Spike (µg/L) | Batch 3 (tested 24 Apr 2012) | | | Perchlorate Standards | | | |
|---|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | µM | Batch 1 | Batch 2 | Batch 3 |
| 0 | 0.0002 | -0.0004 | -0.0003 | 0 | 0.0000 | 0.0000 | 0.0000 |
| 10 | 0.0014 | 0.0008 | 0.0006 | 10 | ND | ND | ND |
| 20 | 0.0024 | 0.0019 | 0.0016 | 20 | 0.0039 | 0.0040 | 0.0041 |
| 30 | 0.0035 | 0.0035 | 0.0029 | 30 | ND | ND | ND |
| 40 | 0.0048 | 0.0038 | 0.0036 | 40 | 0.0070 | 0.0068 | 0.0076 |
| **Regression parameters for each set[b]** | | | | | | | |
| **y-intercept ± error** | 0.000230 ±0.000054 | -0.000304 ±0.000270 | -0.000360 ±0.000144 | | 0.000133 ±0.000298 | 0.000207 ±0.000462 | 0.000107 ±0.000239 |
| **Slope ± error** | 0.000112 ±0.000002 | 0.000111 ±0.000011 | 0.000102 ±0.000006 | | 0.000175 ±0.000012 | 0.000169 ±0.000018 | 0.000189 ±0.0000092 |
| **$r^2$** | 0.9988 | 0.9714 | 0.9902 | | 0.9957 | 0.9889 | 0.9976 |
| **x-intercept ± error** | -2±0 | 3±2 | 4±1 | | -1±2 | -1±3 | -1±1 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 2±0 | -3±2 | -4±1 | | NA | NA | NA |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. Negative control rates were 0.0007 (batch 1), 0.00089 (batch 2), 0.00061 (batch 3). Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 12/10/2007 extract

[b]Regression parameters are from the regression line for each set (see Figure B-107). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The estimated concentration of analyte (perchlorate) in a groundwater set by the standard additions method is equal to (-1) times the x-intercept

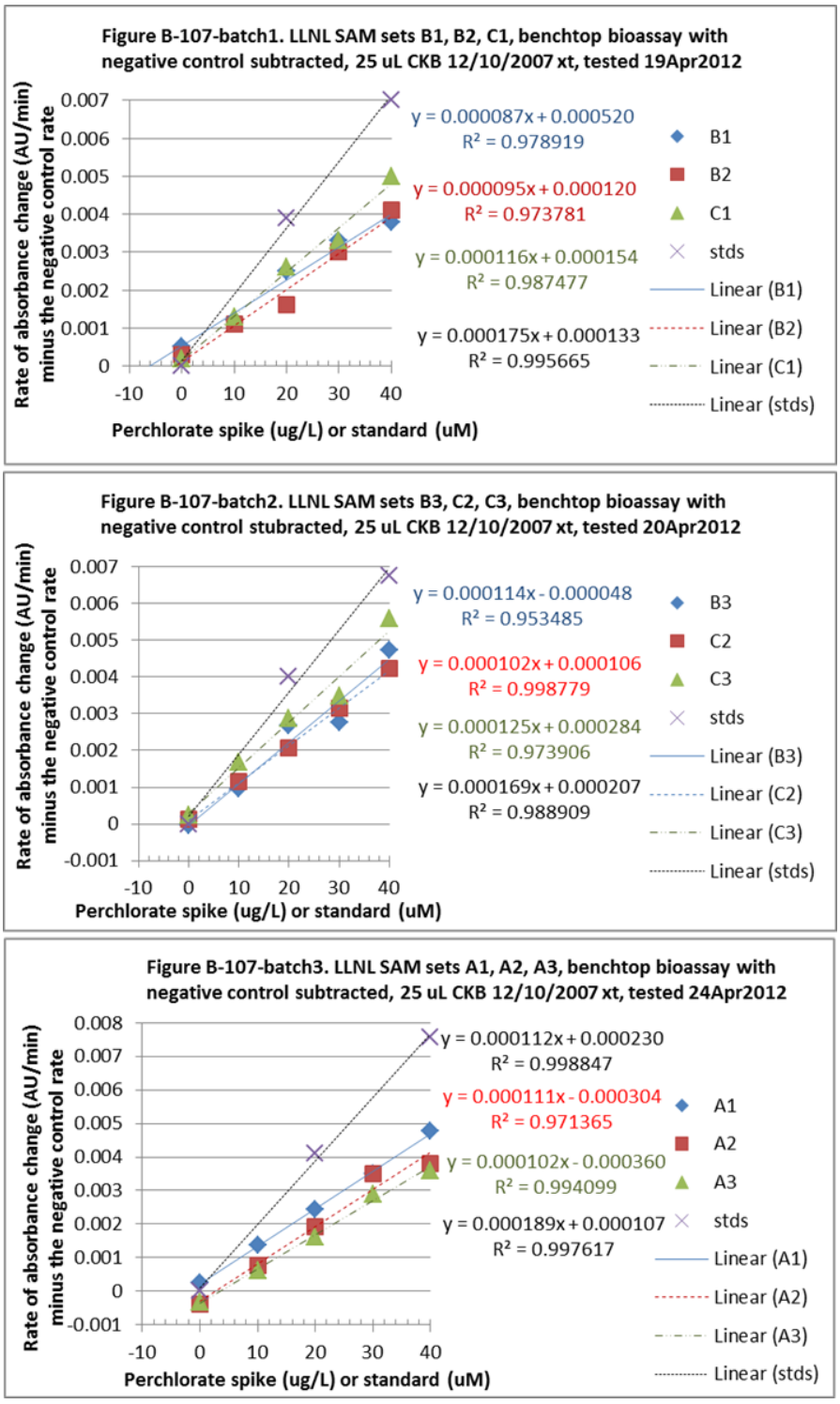



**Figure B-107. Perchlorate benchtop bioassay analysis of standard additions method sets for Lawrence Livermore National Laboratory groundwater collected 29 March 2012 from location W-854-1823 (See Table 107)**

## Groundwater sample 102, Hill AFB, location U9-16-007, collected 14Jul2011

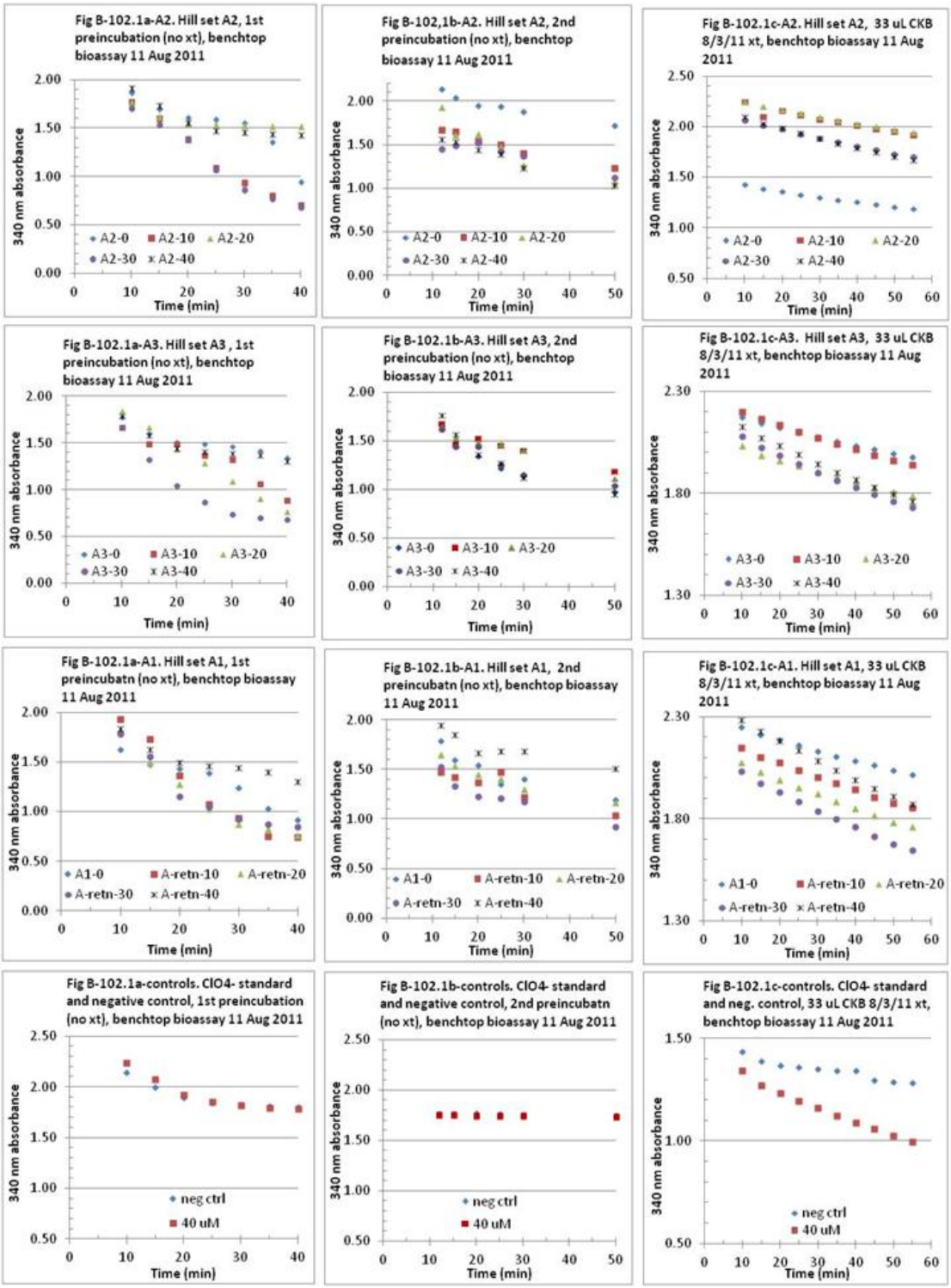



**Figure B-102.1. Hill AFB, benchtop bioassay batch 1, sets A2, A3, A1, and controls. Test date 11 Aug 2011.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-102.1a, b, and c

Data for Figure B-102.1.
Groundwater sample 102, Hill AFB. Standards addition method benchtop bioassay, batch1, 11Aug2011. SDVB/DTAB eluate sets A2, A3, A1, perchlorate 40 µM standard, and negative control (no $ClO_4^-$ present)

**Table B-102.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 11Aug2011**

| | Set A2 | | | | | Set A3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 |
| 10 | 1.8625 | 1.7631 | 1.7668 | 1.7046 | 1.9052 | 1.7844 | 1.6659 | 1.8462 | 1.6736 | 1.7943 |
| 15 | 1.6924 | 1.5940 | 1.6128 | 1.5294 | 1.7266 | 1.6042 | 1.4900 | 1.6654 | 1.3228 | 1.5872 |
| 20 | 1.6023 | 1.3787 | 1.5492 | 1.3811 | 1.5488 | 1.5142 | 1.4907 | 1.4399 | 1.0436 | 1.4434 |
| 25 | 1.5835 | 1.0809 | 1.5322 | 1.0687 | 1.4733 | 1.4916 | 1.3748 | 1.2860 | 0.8689 | 1.4087 |
| 30 | 1.5503 | 0.9337 | 1.5235 | 0.8570 | 1.4472 | 1.4671 | 1.3218 | 1.0985 | 0.7374 | 1.3869 |
| 35 | 1.3525 | 0.7977 | 1.5176 | 0.7695 | 1.4339 | 1.4156 | 1.0650 | 0.9046 | 0.7063 | 1.3713 |
| 40 | 0.9420 | 0.7026 | 1.5160 | 0.6763 | 1.4254 | 1.3412 | 0.8944 | 0.7726 | 0.6811 | 1.3033 |

| | Set A1 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | neg ctrl | 40 µM |
| 10 | 1.6221 | 1.9338 | 1.8169 | 1.7814 | 1.8385 | 2.1392 | 2.2380 |
| 15 | 1.4760 | 1.7303 | 1.4942 | 1.5526 | 1.6277 | 1.9991 | 2.0706 |
| 20 | 1.4332 | 1.3595 | 1.2769 | 1.1541 | 1.4912 | 1.8898 | 1.9221 |
| 25 | 1.3905 | 1.0699 | 1.0284 | 1.0468 | 1.4584 | 1.8416 | 1.8542 |
| 30 | 1.2398 | 0.9305 | 0.8696 | 0.9211 | 1.4427 | 1.8244 | 1.8175 |
| 35 | 1.0292 | 0.7479 | 0.8198 | 0.8717 | 1.3994 | 1.8108 | 1.7920 |
| 40 | 0.9167 | 0.7397 | 0.7544 | 0.8478 | 1.3032 | 1.7986 | 1.7790 |

**Table B-102.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 11Aug2011**

| | Set A2 | | | | | Set A3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 |
| 12 | 2.1339 | 1.6730 | 1.9280 | 1.4466 | 1.5559 | 1.6627 | 1.6734 | 1.6528 | 1.6206 | 1.7645 |
| 15 | 2.0353 | 1.6518 | 1.6136 | 1.4894 | 1.5295 | 1.5046 | 1.4617 | 1.5283 | 1.4465 | 1.5663 |
| 20 | 1.9470 | 1.5365 | 1.6233 | 1.5203 | 1.4364 | 1.3453 | 1.5261 | 1.4835 | 1.4428 | 1.3585 |
| 25 | 1.9402 | 1.5030 | 1.4706 | 1.4190 | 1.3935 | 1.2226 | 1.4519 | 1.4702 | 1.2573 | 1.2720 |
| 30 | 1.8736 | 1.3979 | 1.2651 | 1.3690 | 1.2297 | 1.1559 | 1.3946 | 1.4057 | 1.1463 | 1.1207 |
| 50 | 1.7186 | 1.2347 | 1.0620 | 1.1164 | 1.0318 | 0.9744 | 1.1861 | 1.1139 | 1.0423 | 0.9525 |

| | Set A1 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | neg ctrl | 40 µM |
| 12 | 1.7892 | 1.4723 | 1.6463 | 1.5260 | 1.9479 | 1.7666 | 1.7510 |
| 15 | 1.5937 | 1.4220 | 1.5311 | 1.3355 | 1.8479 | 1.7651 | 1.7502 |
| 20 | 1.5400 | 1.3726 | 1.4464 | 1.2286 | 1.6648 | 1.7700 | 1.7480 |
| 25 | 1.3519 | 1.4742 | 1.4046 | 1.2125 | 1.6877 | 1.7593 | 1.7479 |
| 30 | 1.4028 | 1.2173 | 1.2963 | 1.1732 | 1.6840 | 1.7553 | 1.7466 |
| 50 | 1.1924 | 1.0354 | 1.1676 | 0.9251 | 1.5090 | 1.7470 | 1.7352 |

**Table B-102.1c. Batch 1, 340 nm absorbance, 33 uL CKB 8/3/2011 extract, 11Aug2011**

| | Set A2 | | | | | Set A3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** |
| 10 | 1.4337 | 2.2431 | 2.2505 | 2.0699 | 2.0968 | 2.1733 | 2.1996 | 2.0337 | 2.0792 | 2.1247 |
| 15 | 1.3862 | 2.0995 | 2.2079 | 2.0180 | 2.0342 | 2.1429 | 2.1638 | 1.9868 | 2.0254 | 2.0725 |
| 20 | 1.3607 | 2.1617 | 2.1728 | 1.9769 | 1.9886 | 2.1203 | 2.1363 | 1.9616 | 1.9844 | 2.0343 |
| 25 | 1.3317 | 2.1202 | 2.1389 | 1.9321 | 1.9350 | 2.0961 | 2.1016 | 1.9349 | 1.9430 | 1.9895 |
| 30 | 1.3002 | 2.0779 | 2.1022 | 1.8898 | 1.8855 | 2.0741 | 2.0714 | 1.9060 | 1.9009 | 1.9438 |
| 35 | 1.2771 | 2.0482 | 2.0660 | 1.8480 | 1.8358 | 2.0529 | 2.0413 | 1.8771 | 1.8607 | 1.9020 |
| 40 | 1.2565 | 2.0111 | 2.0365 | 1.8094 | 1.7931 | 2.0331 | 2.0138 | 1.8564 | 1.8281 | 1.8652 |
| 45 | 1.2338 | 1.9839 | 2.0059 | 1.7742 | 1.7512 | 2.0132 | 1.9874 | 1.8295 | 1.7947 | 1.8265 |
| 50 | 1.2115 | 1.9517 | 1.9747 | 1.7356 | 1.7094 | 1.9924 | 1.9618 | 1.8064 | 1.7589 | 1.7917 |
| 55 | 1.1928 | 1.9208 | 1.9489 | 1.7062 | 1.6732 | 1.9765 | 1.9374 | 1.7842 | 1.7284 | 1.7587 |
| **Rate[a]** | 0.0052 | 0.0063 | 0.0067 | 0.0081 | 0.0094 | 0.0043 | 0.0058 | 0.0054 | 0.0077 | 0.0081 |
| **$r^2$** | 0.9869 | 0.9165 | 0.9970 | 0.9967 | 0.9961 | 0.9958 | 0.9973 | 0.9931 | 0.9950 | 0.9970 |
| **Rate – neg ctrl rate** | 0.0022 | 0.0033 | 0.0037 | 0.0051 | 0.0064 | 0.0013 | 0.0028 | 0.0024 | 0.0047 | 0.0051 |

| | Set A1 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **neg ctrl** | **40 µM** |
| 10 | 2.2504 | 2.1482 | 2.0753 | 2.0344 | 2.2852 | 1.4374 | 1.3419 |
| 15 | 2.2109 | 2.1024 | 2.0270 | 1.9744 | 2.2265 | 1.3892 | 1.2730 |
| 20 | 2.1877 | 2.0731 | 1.9877 | 1.9290 | 2.1795 | 1.3699 | 1.2343 |
| 25 | 2.1608 | 2.0354 | 1.9516 | 1.8835 | 2.1325 | 1.3617 | 1.1959 |
| 30 | 2.1324 | 2.0014 | 1.9199 | 1.8381 | 2.0829 | 1.3514 | 1.1589 |
| 35 | 2.1058 | 1.9720 | 1.8825 | 1.7964 | 2.0383 | 1.3445 | 1.1241 |
| 40 | 2.0816 | 1.9412 | 1.8498 | 1.7583 | 1.9909 | 1.3411 | 1.0911 |
| 45 | 2.0613 | 1.9051 | 1.8168 | 1.7140 | 1.9473 | 1.2951 | 1.0601 |
| 50 | 2.0361 | 1.8761 | 1.7826 | 1.6762 | 1.9078 | 1.2886 | 1.0252 |
| 55 | 2.0135 | 1.8519 | 1.7585 | 1.6461 | 1.8723 | 1.2854 | 0.9972 |
| **Rate[a]** | 0.0052 | 0.0065 | 0.0070 | 0.0086 | 0.0092 | 0.0030 | 0.0073 |
| **$r^2$** | 0.9959 | 0.9971 | 0.9962 | 0.9957 | 0.9974 | 0.9282 | 0.9892 |
| **Rate – neg ctrl rate** | 0.0022 | 0.0035 | 0.0040 | 0.0056 | 0.0062 | 0.0000 | 0.0043 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-102.1c-A1, A2, A3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

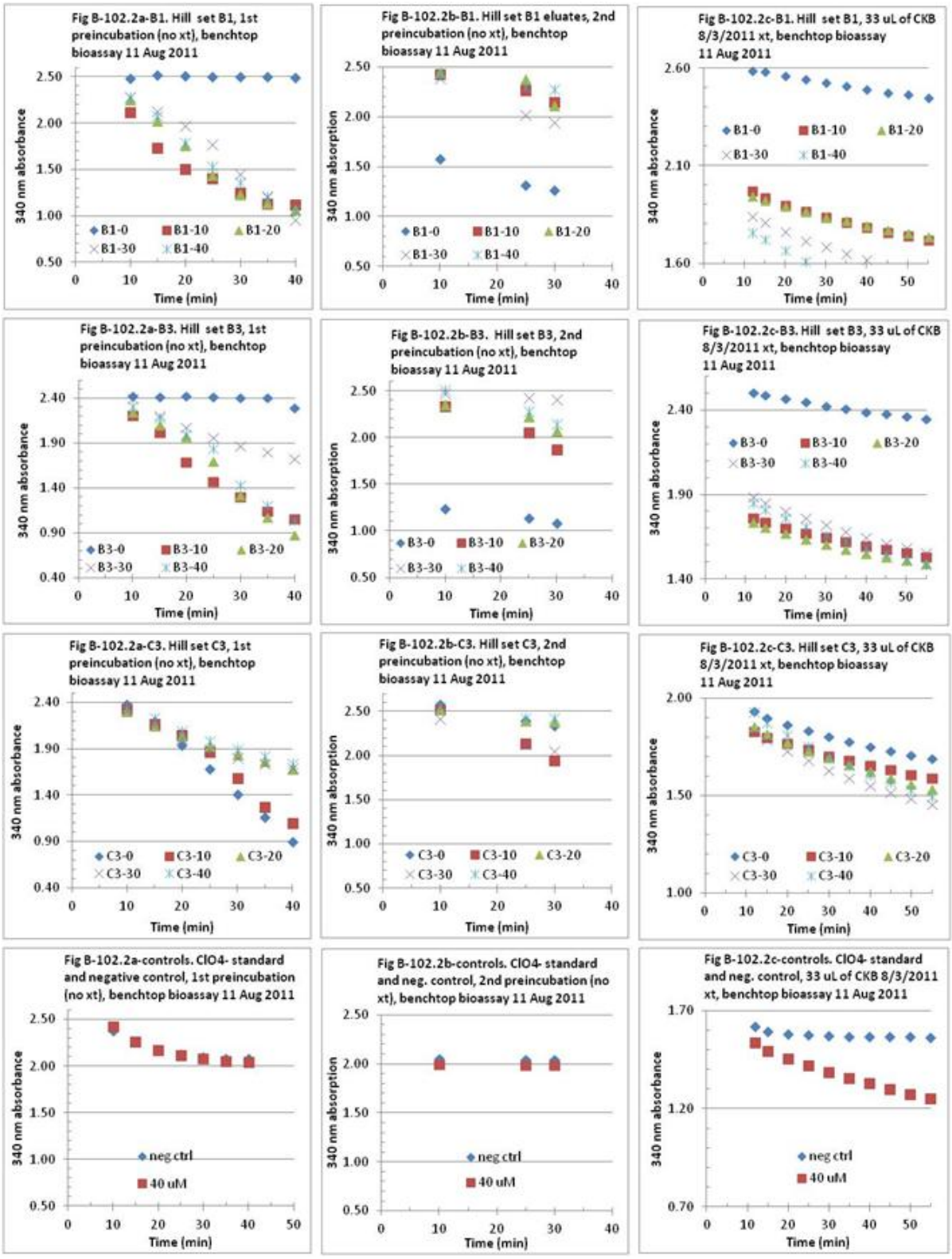



**Figure B-102.2. Hill AFB, benchtop bioassay batch2, sets B1, B3, C3, and controls. Test date 11 Aug 2011.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-102.2a, b, and c

Data for Figure B-102.2.
Groundwater sample 102, Hill AFB. Standards addition method benchtop bioassay batch2, 11Aug2011. SDVB/DTAB eluate sets B1, B3, C3, perchlorate 40 µM standard, and negative control (no $ClO_4^-$ present)

**Table B-102.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 11Aug2011**

| | Set B1 | | | | | Set B3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 |
| 10 | 2.4859 | 2.1126 | 2.2573 | 2.2794 | 2.2803 | 2.4300 | 2.2079 | 2.2464 | 2.3950 | 2.3164 |
| 15 | 2.5237 | 1.7340 | 2.0269 | 2.1272 | 2.0842 | 2.4134 | 2.0227 | 2.0998 | 2.2091 | 2.1806 |
| 20 | 2.5059 | 1.5079 | 1.7602 | 1.9676 | 1.7891 | 2.4234 | 1.6930 | 1.9700 | 2.0773 | 1.9976 |
| 25 | 2.5025 | 1.4078 | 1.4392 | 1.7696 | 1.5353 | 2.4125 | 1.4702 | 1.7026 | 1.9597 | 1.8365 |
| 30 | 2.4997 | 1.2524 | 1.2341 | 1.4476 | 1.3558 | 2.4045 | 1.3026 | 1.3177 | 1.8693 | 1.4354 |
| 35 | 2.4968 | 1.1314 | 1.1404 | 1.2163 | 1.2009 | 2.4029 | 1.1442 | 1.0730 | 1.8060 | 1.2031 |
| 40 | 2.4931 | 1.1214 | 1.0700 | 0.9608 | 1.0667 | 2.2946 | 1.0587 | 0.8755 | 1.7247 | 1.0336 |

| | Set C3 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 |
| (min) | C3-0 | C3-10 | C3-20 | C3-30 | C3-40 | neg ctrl | 40 µM |
| 10 | 2.3736 | 2.3256 | 2.3038 | 2.3124 | 2.3493 | 2.3734 | 2.4259 |
| 15 | 2.1921 | 2.1675 | 2.1464 | 2.1547 | 2.2285 | 2.2560 | 2.2608 |
| 20 | 1.9307 | 2.0401 | 2.0373 | 2.0096 | 2.0934 | 2.1675 | 2.1671 |
| 25 | 1.6772 | 1.8660 | 1.9252 | 1.8915 | 1.9800 | 2.1212 | 2.1102 |
| 30 | 1.4061 | 1.5782 | 1.8350 | 1.7932 | 1.8855 | 2.0934 | 2.0757 |
| 35 | 1.1623 | 1.2644 | 1.7590 | 1.7341 | 1.8132 | 2.0797 | 2.0531 |
| 40 | 0.8922 | 1.0931 | 1.6799 | 1.6861 | 1.7372 | 2.0750 | 2.0434 |

**TableB- 102.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 11Aug2011**

| | Set B1 | | | | | Set B3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 |
| 10 | 1.5777 | 2.4230 | 2.4398 | 2.3806 | 2.4226 | 1.2302 | 2.3364 | 2.3508 | 2.4721 | 2.5059 |
| 25 | 1.3095 | 2.2667 | 2.3720 | 2.0184 | 2.2992 | 1.1371 | 2.0478 | 2.2218 | 2.4245 | 2.2803 |
| 30 | 1.2563 | 2.1430 | 2.1141 | 1.9409 | 2.2686 | 1.0833 | 1.8696 | 2.0733 | 2.4046 | 2.1444 |

| | Set C3 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 |
| (min) | C3-0 | C3-10 | C3-20 | C3-30 | C3-40 | neg ctrl | 40 µM |
| 10 | 2.5723 | 2.5140 | 2.5154 | 2.4157 | 2.5582 | 2.0529 | 1.9909 |
| 25 | 2.3888 | 2.1379 | 2.3917 | 2.1235 | 2.4241 | 2.0487 | 1.9886 |
| 30 | 2.3352 | 1.9404 | 2.3794 | 2.0498 | 2.4192 | 2.0476 | 1.9865 |

**Table B-102.2c. Batch 2, 340 nm absorbance, 33 uL CKB 8/3/2011 extract, 11Aug2011**

| | Set B1 | | | | | Set B3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B1-0** | **B1-10** | **B1-20** | **B1-30** | **B1-40** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** |
| 12 | 2.5867 | 1.9682 | 1.9443 | 1.8385 | 1.7582 | 2.5025 | 1.7615 | 1.7358 | 1.8853 | 1.8502 |
| 15 | 2.5825 | 1.9339 | 1.9204 | 1.8074 | 1.7212 | 2.4873 | 1.7366 | 1.7065 | 1.8489 | 1.8105 |
| 20 | 2.5619 | 1.8967 | 1.8928 | 1.7601 | 1.6642 | 2.4689 | 1.7023 | 1.6686 | 1.8033 | 1.7586 |
| 25 | 2.5423 | 1.8659 | 1.8636 | 1.7154 | 1.6103 | 2.4470 | 1.6723 | 1.6349 | 1.7616 | 1.7094 |
| 30 | 2.5252 | 1.8337 | 1.8373 | 1.6816 | 1.5612 | 2.4244 | 1.6432 | 1.6036 | 1.7186 | 1.6608 |
| 35 | 2.5080 | 1.8083 | 1.8167 | 1.6508 | 1.5223 | 2.4101 | 1.6193 | 1.5773 | 1.6818 | 1.6254 |
| 40 | 2.4899 | 1.7829 | 1.7900 | 1.6180 | 1.4799 | 2.3863 | 1.5966 | 1.5520 | 1.6433 | 1.5840 |
| 45 | 2.4753 | 1.7578 | 1.7716 | 1.5905 | 1.4432 | 2.3797 | 1.5749 | 1.5304 | 1.6123 | 1.5480 |
| 50 | 2.4644 | 1.7415 | 1.7547 | 1.5671 | 1.4114 | 2.3642 | 1.5532 | 1.5108 | 1.5841 | 1.5146 |
| 55 | 2.4489 | 1.7164 | 1.7346 | 1.5409 | 1.3771 | 2.3472 | 1.5319 | 1.4893 | 1.5544 | 1.4837 |
| **Rate[a]** | 0.0033 | 0.0057 | 0.0048 | 0.0068 | 0.0088 | 0.0036 | 0.0052 | 0.0056 | 0.0076 | 0.0084 |
| **$r^2$** | 0.9954 | 0.9839 | 0.9901 | 0.9847 | 0.9889 | 0.9901 | 0.9895 | 0.9834 | 0.9912 | 0.9903 |
| **Rate – neg ctrl rate** | 0.0023 | 0.0047 | 0.0038 | 0.0058 | 0.0078 | 0.0026 | 0.0042 | 0.0046 | 0.0066 | 0.0074 |

| | Set C3 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** |
| **(min)** | **C3-0** | **C3-10** | **C3-20** | **C3-30** | **C3-40** | **neg ctrl** | **40 µM** |
| 12 | 1.9315 | 1.8312 | 1.8554 | 1.8294 | 1.9283 | 1.6174 | 1.5345 |
| 15 | 1.9002 | 1.8005 | 1.8177 | 1.7851 | 1.8740 | 1.5911 | 1.4909 |
| 20 | 1.8635 | 1.7686 | 1.7718 | 1.7285 | 1.8124 | 1.5786 | 1.4522 |
| 25 | 1.8316 | 1.7365 | 1.7312 | 1.6802 | 1.7548 | 1.5734 | 1.4180 |
| 30 | 1.8010 | 1.7039 | 1.6926 | 1.6311 | 1.7000 | 1.5694 | 1.3842 |
| 35 | 1.7776 | 1.6812 | 1.6612 | 1.5910 | 1.6550 | 1.5660 | 1.3561 |
| 40 | 1.7516 | 1.6549 | 1.6263 | 1.5510 | 1.6082 | 1.5648 | 1.3270 |
| 45 | 1.7299 | 1.6324 | 1.5910 | 1.5182 | 1.5666 | 1.5649 | 1.2991 |
| 50 | 1.7077 | 1.6093 | 1.5619 | 1.4858 | 1.5306 | 1.5647 | 1.2719 |
| 55 | 1.6913 | 1.5885 | 1.5336 | 1.4554 | 1.4934 | 1.5607 | 1.2515 |
| **Rate[a]** | 0.0055 | 0.0055 | 0.0073 | 0.0086 | 0.0099 | 0.0010 | 0.0064 |
| **$r^2$** | 0.9831 | 0.9891 | 0.9917 | 0.9857 | 0.9872 | 0.6744 | 0.9854 |
| **Rate – neg ctrl rate** | 0.0045 | 0.0045 | 0.0063 | 0.0076 | 0.0089 | 0.0000 | 0.0054 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-102.2c-B1, B3, C3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

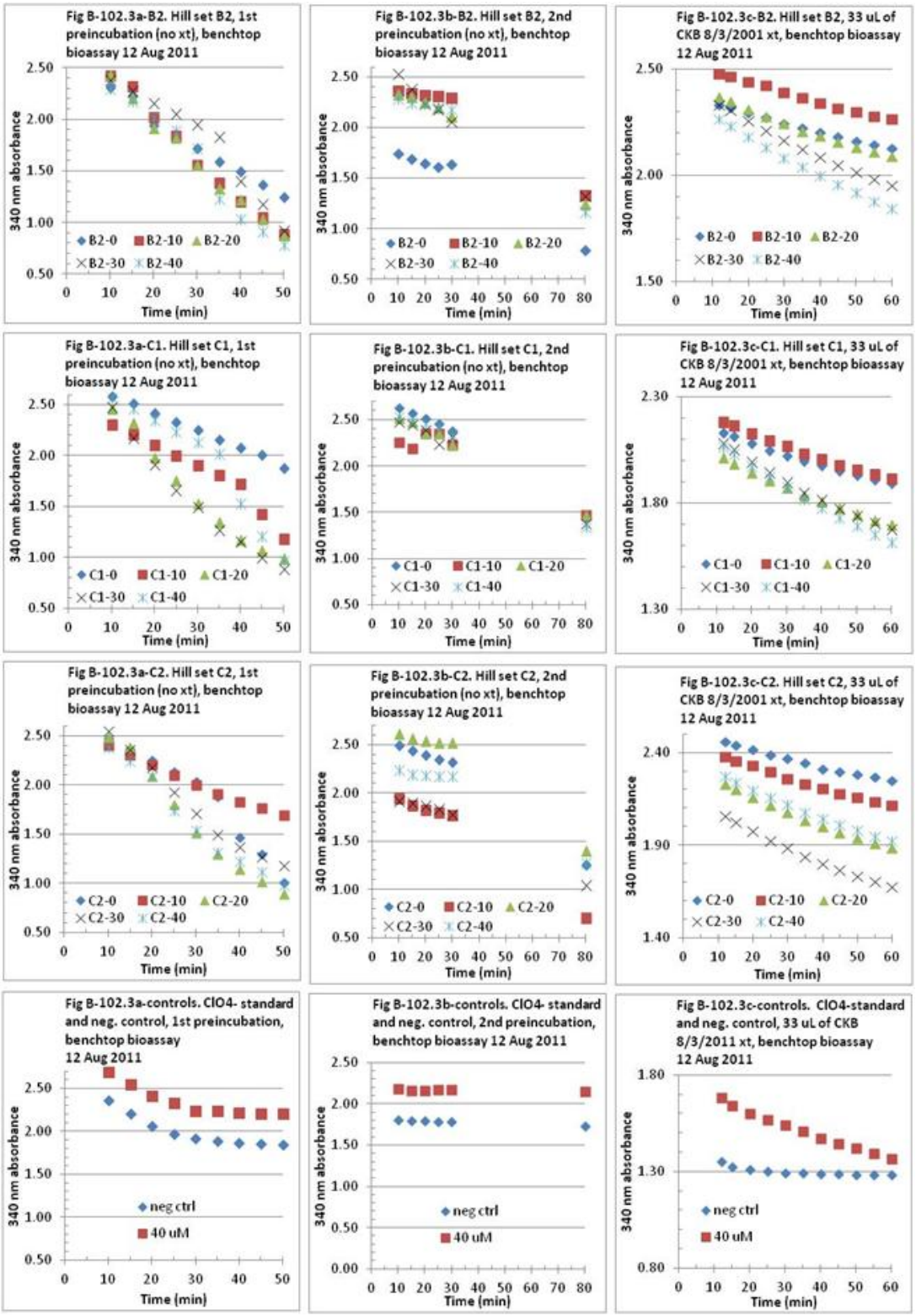



**Figure B-102.3. Hill AFB, benchtop bioassay batch 3, sets B2, C1, C2, and controls. Test date 12 Aug 2011.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-102.3a, b, and c

Data for Figure B-102.3.
Groundwater sample 102, Hill AFB. Standards addition method benchtop bioassay, batch3, 12Aug2011. SDVB/DTAB eluate sets B2, C1, C2, perchlorate 40 µM standard, and negative control (no $ClO_4^-$ present)

**Table B-102.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 12Aug2011**

| | Set B2 | | | | | Set C1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 |
| 10 | 2.3134 | 2.4195 | 2.4315 | 2.3745 | 2.2926 | 2.5830 | 2.3077 | 2.4654 | 2.4781 | 2.5657 |
| 15 | 2.2200 | 2.3203 | 2.2031 | 2.2719 | 2.1715 | 2.5116 | 2.2104 | 2.3192 | 2.1787 | 2.4654 |
| 20 | 1.9419 | 2.0148 | 1.9122 | 2.1578 | 2.0208 | 2.4209 | 2.1030 | 1.9852 | 1.9181 | 2.3513 |
| 25 | 1.8475 | 1.8413 | 1.8205 | 2.0484 | 1.8863 | 2.3318 | 2.0005 | 1.7538 | 1.6662 | 2.2354 |
| 30 | 1.7188 | 1.5511 | 1.5567 | 1.9470 | 1.7177 | 2.2513 | 1.9058 | 1.5292 | 1.4963 | 2.1296 |
| 35 | 1.5870 | 1.3814 | 1.3315 | 1.8326 | 1.2278 | 2.1585 | 1.8125 | 1.3472 | 1.2719 | 2.0158 |
| 40 | 1.4931 | 1.2006 | 1.2190 | 1.3968 | 1.0311 | 2.0818 | 1.7200 | 1.1761 | 1.1568 | 1.5353 |
| 45 | 1.3689 | 1.0474 | 1.0301 | 1.1730 | 0.9118 | 2.0086 | 1.4307 | 1.0662 | 1.0032 | 1.2058 |
| 50 | 1.2490 | 0.8870 | 0.8727 | 0.9208 | 0.7853 | 1.8799 | 1.1834 | 0.9928 | 0.8889 | 0.9633 |

| | Set C2 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 |
| (min) | C2-0 | C2-10 | C2-20 | C2-30 | C2-40 | neg ctrl | 40 µM |
| 10 | 2.4780 | 2.4038 | 2.4852 | 2.5482 | 2.3803 | 2.3652 | 2.6926 |
| 15 | 2.3744 | 2.3048 | 2.3890 | 2.3610 | 2.2458 | 2.2023 | 2.5497 |
| 20 | 2.2519 | 2.2037 | 2.0922 | 2.1801 | 2.0945 | 2.0636 | 2.4157 |
| 25 | 2.1390 | 2.0966 | 1.8028 | 1.9269 | 1.7369 | 1.9709 | 2.3288 |
| 30 | 2.0360 | 2.0006 | 1.5143 | 1.7142 | 1.5442 | 1.9168 | 2.2407 |
| 35 | 1.8882 | 1.9068 | 1.2964 | 1.5002 | 1.3187 | 1.8824 | 2.2400 |
| 40 | 1.4716 | 1.8275 | 1.1413 | 1.3689 | 1.2287 | 1.8634 | 2.2153 |
| 45 | 1.3011 | 1.7682 | 1.0228 | 1.2755 | 1.1139 | 1.8536 | 2.2112 |
| 50 | 1.0065 | 1.6930 | 0.8927 | 1.1771 | 0.9807 | 1.8450 | 2.2047 |

**Table B-102.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 12Aug2011**

| | Set B2 | | | | | Set C1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 |
| 10 | 1.7453 | 2.3666 | 2.3337 | 2.5291 | 2.2828 | 2.6303 | 2.2625 | 2.5150 | 2.4757 | 2.5335 |
| 15 | 1.6905 | 2.3409 | 2.3012 | 2.3846 | 2.2458 | 2.5692 | 2.1945 | 2.4789 | 2.4534 | 2.4869 |
| 20 | 1.6430 | 2.3236 | 2.2485 | 2.2334 | 2.2306 | 2.5158 | 2.3529 | 2.3529 | 2.3913 | 2.4633 |
| 25 | 1.6143 | 2.3144 | 2.1954 | 2.1816 | 2.1990 | 2.4545 | 2.3578 | 2.3578 | 2.2385 | 2.4209 |
| 30 | 1.6363 | 2.2954 | 2.1122 | 2.0586 | 2.1813 | 2.3842 | 2.2313 | 2.2313 | 2.3175 | 2.3405 |
| 80 | 0.7864 | 1.3310 | 1.2459 | 1.3230 | 1.1678 | 1.4480 | 1.4756 | 1.4756 | 1.3737 | 1.3415 |

| | Set A1 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 |
| (min) | C2-0 | C2-10 | C2-20 | C2-30 | C2-40 | neg ctrl | 40 µM |
| 10 | 2.4959 | 1.9391 | 2.6133 | 1.9124 | 2.2372 | 1.8060 | 2.1811 |
| 15 | 2.4343 | 1.8689 | 2.5638 | 1.8958 | 2.1935 | 1.8020 | 2.1664 |
| 20 | 2.3951 | 1.8237 | 2.5363 | 1.8664 | 2.1843 | 1.7956 | 2.1628 |
| 25 | 2.3486 | 1.7944 | 2.5245 | 1.8286 | 2.1703 | 1.7902 | 2.1747 |
| 30 | 2.3227 | 1.7661 | 2.5167 | 1.7751 | 2.1729 | 1.7847 | 2.1692 |
| 80 | 1.2512 | 0.7018 | 1.4017 | 1.0449 | 1.2719 | 1.7357 | 2.1469 |

**TableB- 102.3c. Batch 3, 340 nm absorbance, 33 uL CKB 8/3/2011 extract, 12Aug2011**

| | Set B2 | | | | | Set C1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B2-0** | **B2-10** | **B2-20** | **B2-30** | **B2-40** | **C1-0** | **C1-10** | **C1-20** | **C1-30** | **C1-40** |
| 10 | 2.3334 | 2.4789 | 2.3716 | 2.3344 | 2.2674 | 2.1316 | 2.1794 | 2.0125 | 2.0811 | 2.0648 |
| 15 | 2.3194 | 2.4662 | 2.3500 | 2.3055 | 2.2326 | 2.1126 | 2.1633 | 1.9854 | 2.0506 | 2.0294 |
| 20 | 2.2932 | 2.4409 | 2.3099 | 2.2557 | 2.1827 | 2.0776 | 2.1264 | 1.9428 | 1.9926 | 1.9726 |
| 25 | 2.2741 | 2.4222 | 2.2768 | 2.2098 | 2.1334 | 2.0482 | 2.0917 | 1.9047 | 1.9424 | 1.9201 |
| 30 | 2.2442 | 2.3913 | 2.2468 | 2.1675 | 2.0841 | 2.0225 | 2.0651 | 1.8696 | 1.8960 | 1.8697 |
| 35 | 2.2232 | 2.3665 | 2.2069 | 2.1242 | 2.0390 | 1.9959 | 2.0306 | 1.8336 | 1.8479 | 1.8188 |
| 40 | 2.2047 | 2.3405 | 2.1845 | 2.0861 | 1.9986 | 1.9752 | 2.0047 | 1.8044 | 1.8119 | 1.7749 |
| 45 | 2.1818 | 2.3161 | 2.1583 | 2.0490 | 1.9554 | 1.9501 | 1.9769 | 1.7739 | 1.7724 | 1.7296 |
| 50 | 2.1630 | 2.2979 | 2.1325 | 2.0165 | 1.9175 | 1.9311 | 1.9552 | 1.7455 | 1.7388 | 1.6899 |
| 55 | 2.1435 | 2.2782 | 2.1120 | 1.9831 | 1.8779 | 1.9094 | 1.9326 | 1.7175 | 1.7035 | 1.6494 |
| 60 | 2.1296 | 2.2640 | 2.0898 | 1.9548 | 1.8440 | 1.8933 | 1.9141 | 1.6946 | 1.6732 | 1.6131 |
| **Rate[a]** | 0.0043 | 0.0047 | 0.0059 | 0.0080 | 0.0088 | 0.0050 | 0.0056 | 0.0066 | 0.0085 | 0.0094 |
| **$r^2$** | 0.9956 | 0.9954 | 0.9899 | 0.9936 | 0.9961 | 0.9908 | 0.9922 | 0.9925 | 0.9907 | 0.9953 |
| **Rate – neg ctrl rate** | 0.0032 | 0.0036 | 0.0048 | 0.0069 | 0.0077 | 0.0039 | 0.0045 | 0.0055 | 0.0074 | 0.0083 |

| | Set C2 | | | | | Controls | |
|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** |
| **(min)** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** | **neg ctrl** | **40 µM** |
| 10 | 2.4598 | 2.3776 | 2.2327 | 2.0568 | 2.2742 | 1.3522 | 1.6854 |
| 15 | 2.4432 | 2.3568 | 2.2031 | 2.0240 | 2.2393 | 1.3252 | 1.6436 |
| 20 | 2.4166 | 2.3305 | 2.1601 | 1.9763 | 2.1962 | 1.3112 | 1.6012 |
| 25 | 2.3869 | 2.2998 | 2.1169 | 1.9264 | 2.1577 | 1.3031 | 1.5701 |
| 30 | 2.3711 | 2.2585 | 2.0768 | 1.8847 | 2.1211 | 1.2970 | 1.5418 |
| 35 | 2.3440 | 2.2308 | 2.0343 | 1.8394 | 2.0777 | 1.2931 | 1.5089 |
| 40 | 2.3130 | 2.2064 | 2.0002 | 1.8021 | 2.0462 | 1.2908 | 1.4739 |
| 45 | 2.2988 | 2.1768 | 1.9685 | 1.7649 | 2.0101 | 1.2881 | 1.4450 |
| 50 | 2.2852 | 2.1595 | 1.9400 | 1.7347 | 1.9810 | 1.2869 | 1.4229 |
| 55 | 2.2675 | 2.1364 | 1.9106 | 1.7033 | 1.9479 | 1.2867 | 1.3937 |
| 60 | 2.2511 | 2.1182 | 1.8845 | 1.6742 | 1.9225 | 1.2864 | 1.3701 |
| **Rate[a]** | 0.0044 | 0.0055 | 0.0073 | 0.0080 | 0.0073 | 0.0011 | 0.0063 |
| **$r^2$** | 0.9881 | 0.9906 | 0.9914 | 0.9911 | 0.9941 | 0.7214 | 0.9891 |
| **Rate – neg ctrl rate** | 0.0033 | 0.0044 | 0.0062 | 0.0069 | 0.0062 | 0.0000 | 0.0052 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-102.3c-B2, C1, C2, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

## Groundwater sample 103, NWIRP McGregor, location OFFWS-37, collected 13Oct2011

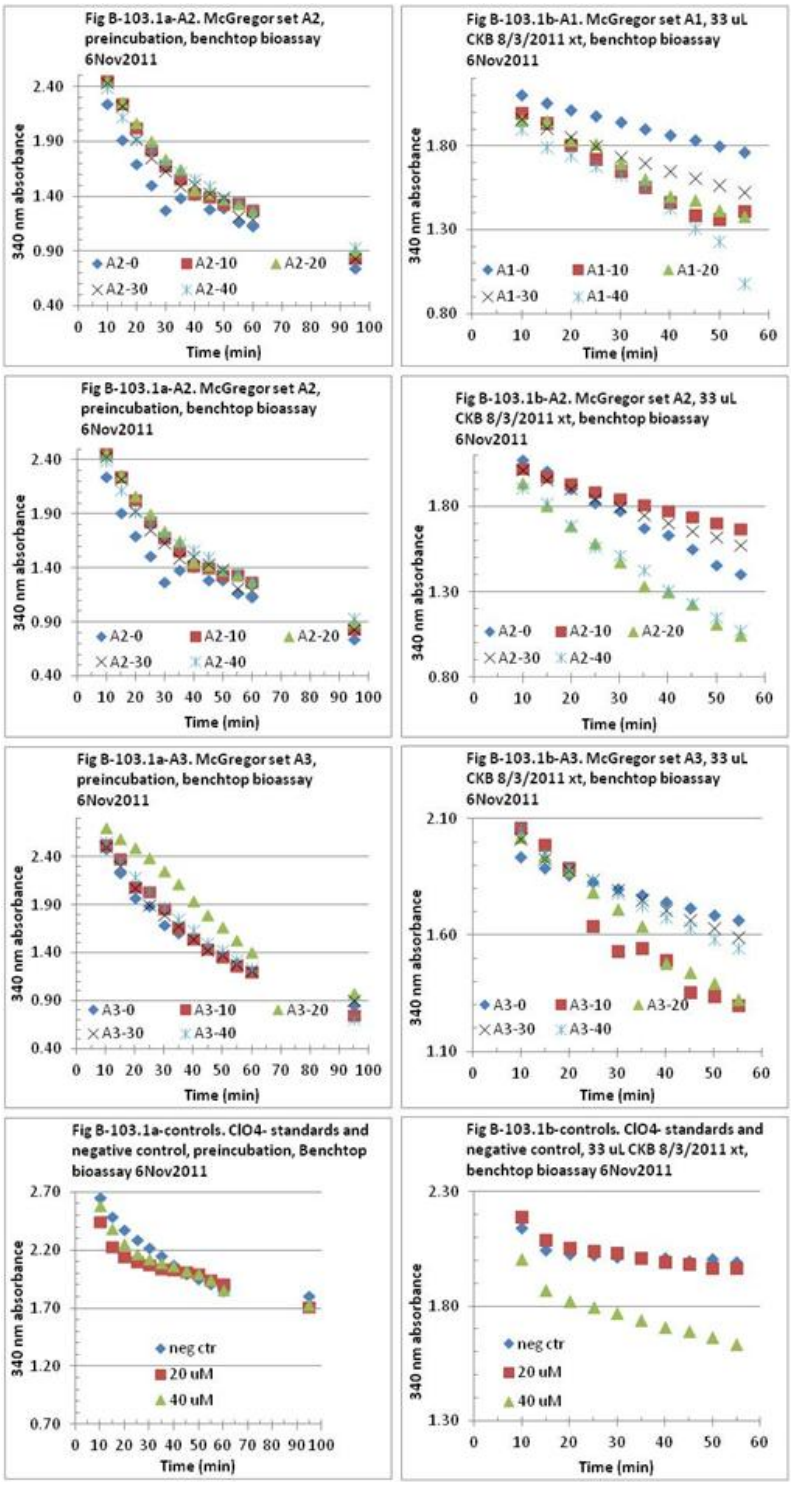



**Figure B-103.1. NWIRP McGregor, benchtop bioassay batch 1, sets A1, A2, A3, and controls. Test date 6 Nov 2011.** Preincubation without extract (**a**), 33 µL CKB 8/3/2011 extract (**b**). Data values displayed are listed in Tables B-103.1a and b

Data for Figure B-103.1.
Groundwater sample 103, NWIRP McGregor. Standards addition method benchtop bioassay, batch1, 6Nov2011. SDVB/DTAB eluate sets A1, A2, A3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-103.1a. Batch 1, 340 nm absorbance, preincubation without extract, 6Nov2011**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 2.2645 | 2.3078 | 2.2392 | 2.3064 | 2.4394 | 2.2466 | 2.4591 | 2.4589 | 2.4332 | 2.396 |
| 15 | 2.0144 | 2.0811 | 2.0734 | 2.0437 | 2.2297 | 1.9145 | 2.2483 | 2.2671 | 2.2269 | 2.1274 |
| 20 | 1.8236 | 1.8082 | 1.9306 | 1.7740 | 2.0732 | 1.6968 | 2.0284 | 2.0677 | 1.9298 | 1.9210 |
| 25 | 1.6299 | 1.6840 | 1.7853 | 1.7711 | 1.8700 | 1.5096 | 1.8247 | 1.9060 | 1.7508 | 1.8312 |
| 30 | 1.6129 | 1.6055 | 1.7528 | 1.6203 | 1.7678 | 1.2757 | 1.6760 | 1.7423 | 1.6366 | 1.7011 |
| 35 | 1.5801 | 1.4855 | 1.6115 | 1.5698 | 1.6993 | 1.3855 | 1.5629 | 1.6497 | 1.4967 | 1.6224 |
| 40 | 1.4800 | 1.4026 | 1.6044 | 1.5218 | 1.5407 | 1.4135 | 1.4186 | 1.4609 | 1.5147 | 1.5634 |
| 45 | 1.3185 | 1.3807 | 1.4984 | 1.4296 | 1.6040 | 1.2854 | 1.3967 | 1.4295 | 1.4338 | 1.5001 |
| 50 | 1.3149 | 1.3839 | 1.4726 | 1.3002 | 1.5211 | 1.2923 | 1.3342 | 1.3788 | 1.3929 | 1.3758 |
| 55 | 1.2469 | 1.1947 | 1.3731 | 1.2686 | 1.4269 | 1.1712 | 1.3362 | 1.3328 | 1.2175 | 1.3635 |
| 60 | 1.2434 | 1.2435 | 1.2916 | 1.2373 | 1.4122 | 1.1280 | 1.2723 | 1.2699 | 1.1957 | 1.2673 |
| 95 | 0.9023 | 0.9566 | 0.9756 | 0.9665 | 0.9882 | 0.7441 | 0.8351 | 0.9015 | 0.8380 | 0.9386 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.4897 | 2.5215 | 2.7024 | 2.5045 | 2.5565 | 2.6513 | 2.4469 | 2.5813 |
| 15 | 2.2345 | 2.3818 | 2.5927 | 2.3117 | 2.3649 | 2.4855 | 2.2320 | 2.3887 |
| 20 | 1.9780 | 2.0834 | 2.4973 | 2.0762 | 2.1973 | 2.3790 | 2.1466 | 2.2565 |
| 25 | 1.8933 | 2.0390 | 2.3864 | 1.8995 | 2.0480 | 2.2949 | 2.0974 | 2.1665 |
| 30 | 1.6888 | 1.8546 | 2.2571 | 1.7959 | 1.8839 | 2.2239 | 2.0755 | 2.1277 |
| 35 | 1.6056 | 1.6545 | 2.1202 | 1.6804 | 1.7537 | 2.1488 | 2.0438 | 2.0900 |
| 40 | 1.5489 | 1.5398 | 1.9397 | 1.5401 | 1.6373 | 2.0722 | 2.0348 | 2.0666 |
| 45 | 1.4458 | 1.4361 | 1.7987 | 1.4289 | 1.4979 | 1.9948 | 2.0148 | 2.0242 |
| 50 | 1.3661 | 1.3541 | 1.6734 | 1.3729 | 1.4258 | 1.9564 | 2.0003 | 1.9993 |
| 55 | 1.2991 | 1.2584 | 1.5380 | 1.2864 | 1.3277 | 1.9116 | 1.9441 | 1.9419 |
| 60 | 1.2122 | 1.2018 | 1.4138 | 1.2065 | 1.2414 | 1.8502 | 1.9117 | 1.8616 |
| 95 | 0.8522 | 0.7494 | 0.9774 | 0.9062 | 0.7069 | 1.8055 | 1.7164 | 1.7307 |

**Table B-103.1b. Batch 1, 340 nm absorbance, 33 μL CKB 8/3/2011 extract, 6Nov2011**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 2.1022 | 1.9965 | 1.9541 | 1.9588 | 1.9009 | 2.0717 | 2.0194 | 1.9409 | 2.0157 | 1.9109 |
| 15 | 2.0540 | 1.9320 | 1.9437 | 1.9018 | 1.7903 | 2.0072 | 1.9717 | 1.8045 | 1.9573 | 1.8235 |
| 20 | 2.0134 | 1.8028 | 1.8333 | 1.8486 | 1.7426 | 1.9064 | 1.9334 | 1.6853 | 1.9035 | 1.6928 |
| 25 | 1.9760 | 1.7164 | 1.8069 | 1.7995 | 1.6791 | 1.8201 | 1.8864 | 1.5869 | 1.8474 | 1.5616 |
| 30 | 1.9415 | 1.6499 | 1.6924 | 1.7298 | 1.6237 | 1.7761 | 1.8479 | 1.4742 | 1.7975 | 1.5141 |
| 35 | 1.9005 | 1.5544 | 1.6002 | 1.6969 | 1.5607 | 1.6778 | 1.8086 | 1.3342 | 1.7505 | 1.4263 |
| 40 | 1.8642 | 1.4604 | 1.4997 | 1.6498 | 1.4245 | 1.6311 | 1.7725 | 1.2997 | 1.7042 | 1.3118 |
| 45 | 1.8306 | 1.3858 | 1.4748 | 1.6052 | 1.3044 | 1.5523 | 1.7374 | 1.2323 | 1.6555 | 1.2336 |
| 50 | 1.7975 | 1.3593 | 1.4170 | 1.5611 | 1.2296 | 1.4571 | 1.7035 | 1.1147 | 1.6202 | 1.1508 |
| 55 | 1.7624 | 1.4097 | 1.3775 | 1.5211 | 0.9788 | 1.4065 | 1.6709 | 1.0460 | 1.5742 | 1.0752 |
| **Rate[a]** | 0.0074 | 0.0148 | 0.0142 | 0.0097 | 0.0185 | 0.0149 | 0.0077 | 0.0196 | 0.0098 | 0.0186 |
| **$r^2$** | 0.9980 | 0.9475 | 0.9799 | 0.9955 | 0.9537 | 0.9963 | 0.9969 | 0.9875 | 0.9966 | 0.9935 |
| **Rate – neg ctrl rate** | 0.0052 | 0.0126 | 0.0120 | 0.0075 | 0.0163 | 0.0127 | 0.0055 | 0.0174 | 0.0076 | 0.0164 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 1.9371 | 2.0630 | 2.0212 | 2.0138 | 2.0541 | 2.1422 | 2.1893 | 2.0068 |
| 15 | 1.8877 | 1.9871 | 1.9427 | 1.9272 | 1.9476 | 2.0482 | 2.0894 | 1.8723 |
| 20 | 1.8573 | 1.8910 | 1.8820 | 1.8806 | 1.8909 | 2.0271 | 2.0567 | 1.8221 |
| 25 | 1.8277 | 1.6396 | 1.7873 | 1.8363 | 1.8369 | 2.0221 | 2.0428 | 1.7969 |
| 30 | 1.7983 | 1.5316 | 1.7116 | 1.7937 | 1.7831 | 2.0166 | 2.0314 | 1.7692 |
| 35 | 1.7708 | 1.5462 | 1.6402 | 1.7522 | 1.7316 | 2.0123 | 2.0127 | 1.7405 |
| 40 | 1.7409 | 1.4951 | 1.4803 | 1.7070 | 1.6799 | 2.0109 | 1.9924 | 1.7099 |
| 45 | 1.7169 | 1.3540 | 1.4435 | 1.6662 | 1.6316 | 1.9999 | 1.9827 | 1.6895 |
| 50 | 1.6856 | 1.3371 | 1.3930 | 1.6291 | 1.5837 | 2.0080 | 1.9681 | 1.6633 |
| 55 | 1.6655 | 1.2994 | 1.3246 | 1.5907 | 1.5434 | 1.9948 | 1.9673 | 1.6359 |
| **Rate[a]** | 0.0059 | 0.0176 | 0.0161 | 0.0090 | 0.0109 | 0.0022 | 0.0041 | 0.0070 |
| **$r^2$** | 0.9936 | 0.9308 | 0.9897 | 0.9898 | 0.9883 | 0.5851 | 0.8399 | 0.9095 |
| **Rate – neg ctrl rate** | 0.0037 | 0.0154 | 0.0139 | 0.0068 | 0.0087 | 0.0000 | 0.0019 | 0.0048 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-103.1b-A1, A2, A3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

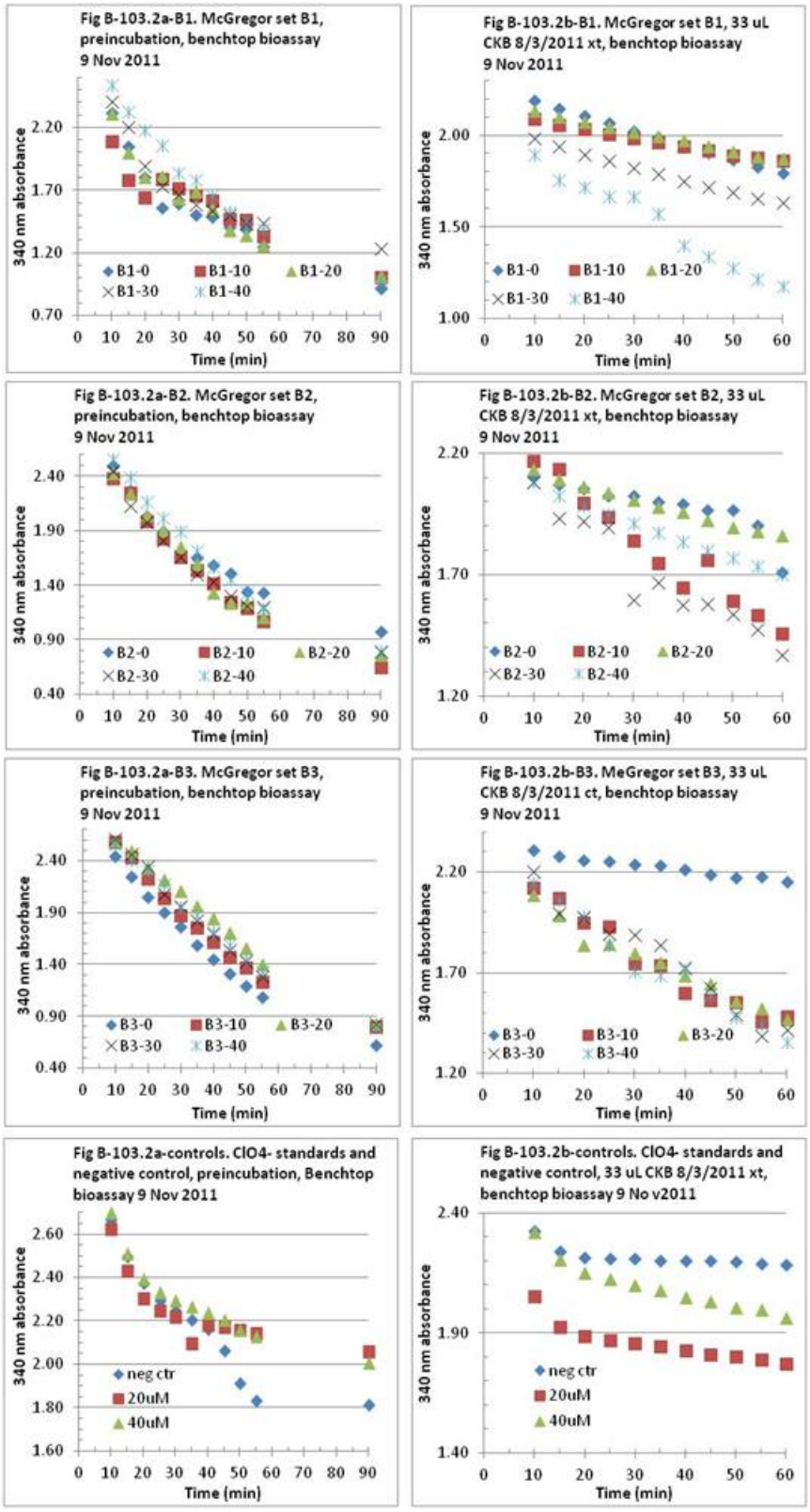



**Figure B-103.2. NWIRP McGregor, benchtop bioassay batch 2, sets B1, B2, B3, and perchlorate controls. Test date 9 Nov 2011.** Preincubation without extract (**a**), 33 µL CKB 8/3/2011 extract (**b**). Data values displayed are listed in Tables B-103.2 a and b.

Data for Figure B-103.2.
Groundwater sample 103, NWIRP McGregor. Standards addition method benchtop bioassay, batch 2, 9Nov2011. SDVB/DTAB eluate sets B1, B2, B3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-103.2a. Batch 2, 340 nm absorbance, preincubation without extract, 9Nov2011**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B1-0** | **B1-10** | **B1-20** | **B1-30** | **B1-40** | **B2-0** | **B2-10** | **B2-20** | **B2-30** | **B2-40** |
| 10 | 2.3139 | 2.0903 | 2.3112 | 2.4031 | 2.5373 | 2.4912 | 2.3778 | 2.4279 | 2.4463 | 2.5585 |
| 15 | 2.0500 | 1.7817 | 2.0029 | 2.2015 | 2.3227 | 2.2670 | 2.2445 | 2.2385 | 2.1265 | 2.3891 |
| 20 | 1.8034 | 1.6416 | 1.8049 | 1.8930 | 2.1787 | 2.0275 | 1.9859 | 2.0375 | 1.9760 | 2.1636 |
| 25 | 1.5596 | 1.7873 | 1.8119 | 1.7323 | 2.0544 | 1.8897 | 1.8278 | 1.9041 | 1.8095 | 2.0094 |
| 30 | 1.5959 | 1.7117 | 1.6299 | 1.6684 | 1.8352 | 1.7159 | 1.6580 | 1.7511 | 1.6601 | 1.8897 |
| 35 | 1.5028 | 1.6607 | 1.6879 | 1.5840 | 1.7807 | 1.6436 | 1.5371 | 1.5917 | 1.5013 | 1.7147 |
| 40 | 1.4826 | 1.6197 | 1.5515 | 1.5357 | 1.6566 | 1.5808 | 1.4089 | 1.3282 | 1.4283 | 1.5618 |
| 45 | 1.4072 | 1.4727 | 1.3841 | 1.5052 | 1.5288 | 1.5043 | 1.2464 | 1.2382 | 1.3056 | 1.4507 |
| 50 | 1.3862 | 1.4588 | 1.3395 | 1.4370 | 1.4487 | 1.3416 | 1.1912 | 1.2315 | 1.2070 | 1.3304 |
| 55 | 1.2513 | 1.3308 | 1.2561 | 1.4353 | 1.3906 | 1.3309 | 1.0668 | 1.1048 | 1.1937 | 1.1796 |
| 90 | 0.9158 | 1.0072 | 1.0116 | 1.2340 | 0.9761 | 0.9721 | 0.6452 | 0.7547 | 0.7878 | 0.7928 |

| | Set B3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.4353 | 2.5778 | 2.5881 | 2.6034 | 2.5456 | 2.6659 | 2.6265 | 2.6973 |
| 15 | 2.2468 | 2.4314 | 2.4854 | 2.4584 | 2.4011 | 2.4957 | 2.4331 | 2.5110 |
| 20 | 2.0428 | 2.2203 | 2.3327 | 2.3448 | 2.3046 | 2.3742 | 2.3040 | 2.3916 |
| 25 | 1.9015 | 2.0390 | 2.2108 | 2.0781 | 2.1601 | 2.2954 | 2.2474 | 2.3315 |
| 30 | 1.7594 | 1.8703 | 2.1096 | 1.9592 | 1.9520 | 2.2471 | 2.2164 | 2.2926 |
| 35 | 1.5850 | 1.7531 | 1.9588 | 1.8175 | 1.8661 | 2.2050 | 2.0964 | 2.2668 |
| 40 | 1.4503 | 1.6139 | 1.8385 | 1.6872 | 1.7109 | 2.1621 | 2.1790 | 2.2397 |
| 45 | 1.3086 | 1.4699 | 1.6969 | 1.5382 | 1.5829 | 2.0639 | 2.1727 | 2.2030 |
| 50 | 1.1894 | 1.3703 | 1.5554 | 1.4228 | 1.4450 | 1.9119 | 2.1561 | 2.1579 |
| 55 | 1.0808 | 1.2275 | 1.3969 | 1.2834 | 1.3264 | 1.8309 | 2.1429 | 2.1293 |
| 90 | 0.6181 | 0.7947 | 0.8297 | 0.8273 | 0.7822 | 1.8134 | 2.0603 | 2.0080 |

**Table B-103.2b. Batch 2, 340 nm absorbance, 33uL CKB 8/3/2011 extract, 9Nov2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B1-0** | **B1-10** | **B1-20** | **B1-30** | **B1-40** | **B2-0** | **B2-10** | **B2-20** | **B2-30** | **B2-40** |
| 10 | 2.1911 | 2.0876 | 2.1359 | 1.9860 | 1.8945 | 2.1021 | 2.1659 | 2.1318 | 2.0767 | 2.0811 |
| 15 | 2.1486 | 2.0582 | 2.1027 | 1.9402 | 1.7535 | 2.0711 | 2.1325 | 2.0931 | 1.9328 | 2.0233 |
| 20 | 2.1075 | 2.0359 | 2.0755 | 1.8973 | 1.7143 | 2.0518 | 1.9944 | 2.0633 | 1.9170 | 1.9825 |
| 25 | 2.0670 | 2.0076 | 2.0479 | 1.8603 | 1.6679 | 2.0225 | 1.9341 | 2.0351 | 1.8957 | 1.9477 |
| 30 | 2.0258 | 1.9849 | 2.0186 | 1.8214 | 1.6681 | 2.0218 | 1.8407 | 2.0065 | 1.5943 | 1.9085 |
| 35 | 1.9897 | 1.9631 | 1.9965 | 1.7866 | 1.5709 | 2.0003 | 1.7462 | 1.9793 | 1.6669 | 1.8712 |
| 40 | 1.9513 | 1.9407 | 1.9706 | 1.7511 | 1.4014 | 1.9914 | 1.6460 | 1.9560 | 1.5761 | 1.8351 |
| 45 | 1.9078 | 1.9190 | 1.9385 | 1.7182 | 1.3350 | 1.9666 | 1.7582 | 1.9211 | 1.5799 | 1.7977 |
| 50 | 1.8663 | 1.8910 | 1.9100 | 1.6910 | 1.2780 | 1.9655 | 1.5935 | 1.8919 | 1.5381 | 1.7658 |
| 55 | 1.8282 | 1.8780 | 1.8851 | 1.6545 | 1.2141 | 1.9014 | 1.5334 | 1.8788 | 1.4752 | 1.7332 |
| 60 | 1.7945 | 1.8592 | 1.8754 | 1.6328 | 1.1768 | 1.7089 | 1.4558 | 1.8599 | 1.3706 | 1.7026 |
| **Rate[a]** | 0.0080 | 0.0046 | 0.0053 | 0.0071 | 0.0145 | 0.0055 | 0.0140 | 0.0055 | 0.0130 | 0.0074 |
| **$r^2$** | 0.9996 | 0.9963 | 0.9962 | 0.9947 | 0.9716 | 0.7499 | 0.9574 | 0.9936 | 0.9138 | 0.9957 |
| **Rate – neg ctrl rate** | 0.0062 | 0.0028 | 0.0035 | 0.0053 | 0.0127 | 0.0037 | 0.0122 | 0.0037 | 0.0112 | 0.0056 |

| | Set B3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.3063 | 2.1212 | 2.0860 | 2.2011 | 2.1433 | 2.3231 | 2.0520 | 2.3226 |
| 15 | 2.2765 | 2.0692 | 1.9818 | 1.9903 | 2.0568 | 2.2377 | 1.9253 | 2.2060 |
| 20 | 2.2577 | 1.9457 | 1.8358 | 1.9707 | 1.9751 | 2.2164 | 1.8849 | 2.1511 |
| 25 | 2.2504 | 1.9258 | 1.8417 | 1.8920 | 1.8348 | 2.2086 | 1.8712 | 2.1258 |
| 30 | 2.2382 | 1.7450 | 1.7936 | 1.8845 | 1.7021 | 2.2087 | 1.8573 | 2.1009 |
| 35 | 2.2286 | 1.7346 | 1.7497 | 1.8349 | 1.6838 | 2.2010 | 1.8430 | 2.0765 |
| 40 | 2.2121 | 1.5952 | 1.6849 | 1.7220 | 1.7116 | 2.2008 | 1.8286 | 2.0496 |
| 45 | 2.1846 | 1.5631 | 1.6408 | 1.6242 | 1.5847 | 2.1995 | 1.8113 | 2.0297 |
| 50 | 2.1716 | 1.5512 | 1.5544 | 1.4922 | 1.4755 | 2.1975 | 1.8022 | 2.0064 |
| 55 | 2.1750 | 1.4569 | 1.5211 | 1.3859 | 1.4384 | 2.1869 | 1.7885 | 1.9968 |
| 60 | 2.1507 | 1.4824 | 1.4663 | 1.4140 | 1.3534 | 2.1829 | 1.7710 | 1.9638 |
| **Rate[a]** | 0.0029 | 0.0140 | 0.0114 | 0.0154 | 0.0153 | 0.0018 | 0.0043 | 0.0060 |
| **$r^2$** | 0.9785 | 0.9549 | 0.9718 | 0.9607 | 0.9686 | 0.5885 | 0.8109 | 0.9078 |
| **Rate – neg ctrl rate** | 0.0011 | 0.0122 | 0.0096 | 0.0136 | 0.0135 | 0.0000 | 0.0025 | 0.0042 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-103.2b-B1, B2, B3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

## Groundwater sample 104, Jet Propulsion Laboratory (JPL), location MW-16, collected 14Nov2011

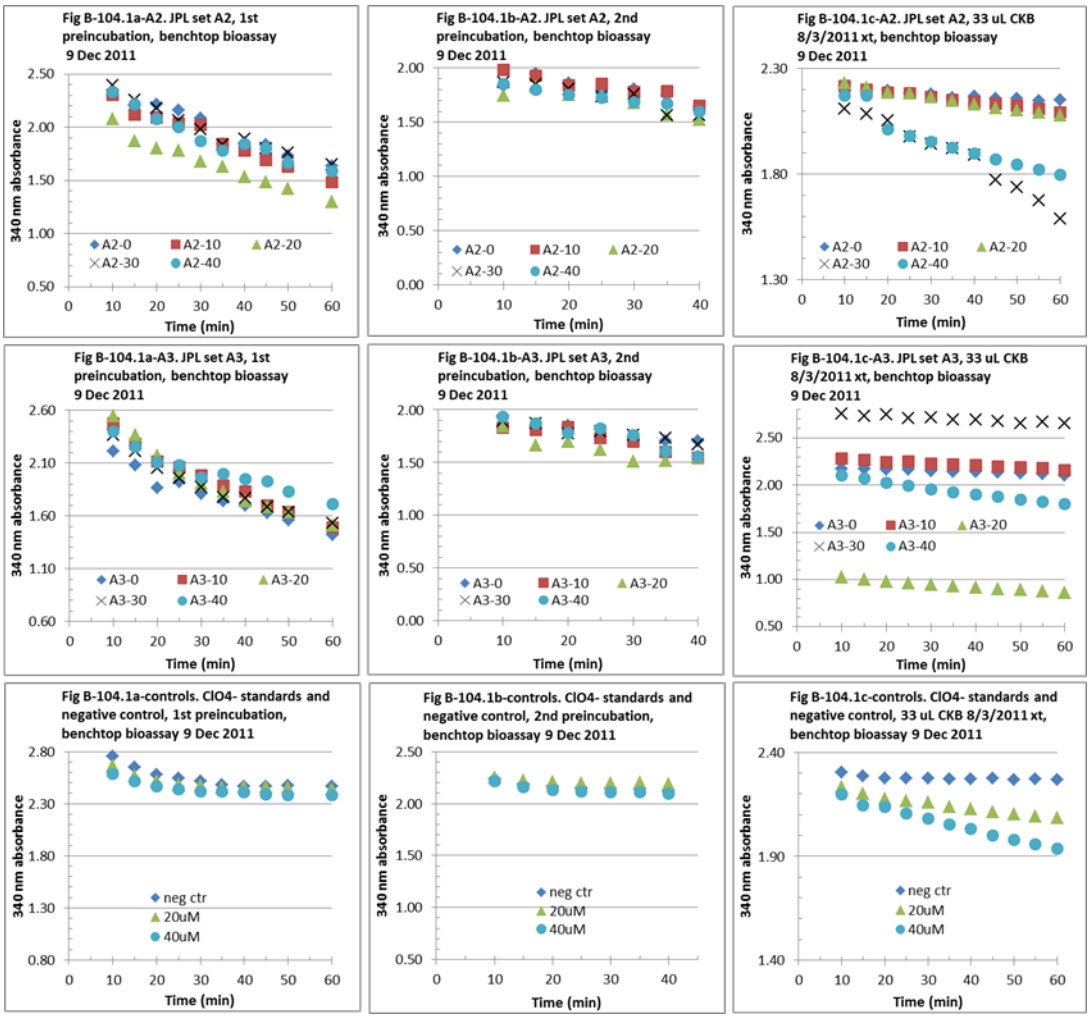



**Figure B-104.1. Jet Propulsion Laboratory, benchtop bioassay batch 1, sets A1, A2, A3, and perchlorate controls. Test date 9 Dec 2011.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.1a, b, and c

Data for Figure B-104.1.
Groundwater sample 104, Jet Propulsion Laboratory. Standards addition method benchtop bioassay, batch 1, 9Dec2011. SDVB/DTAB eluate sets A2, A3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present). With respect to set A1, the 340 nm absorption time course was aberrant for A1-0, A1-10 and A1-20. Testing was performed again for set A1 in batch 3 on 12Dec2011.

**Table B-104.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 9Dec2011**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 |
| 10 | 2.3943 | 2.3416 | 2.1412 | 2.1757 | 2.1370 | 2.3049 | 2.3059 | 2.0737 | 2.3926 | 2.3293 |
| 15 | 2.1682 | 2.2454 | 2.0449 | 2.0763 | 1.9785 | 2.1986 | 2.1160 | 1.8683 | 2.2594 | 2.2128 |
| 20 | 2.1829 | 2.2619 | 2.0939 | 2.0772 | 1.9287 | 2.2180 | 2.0920 | 1.7979 | 2.1801 | 2.0740 |
| 25 | 2.1048 | 2.0375 | 1.9508 | 2.0233 | 1.9130 | 2.1586 | 2.0326 | 1.7805 | 2.0617 | 2.0029 |
| 30 | 2.0610 | 2.0058 | 1.9037 | 1.8546 | 1.7703 | 2.0820 | 2.0236 | 1.6740 | 1.9800 | 1.8692 |
| 35 | 1.8877 | 1.9809 | 1.8353 | 1.7701 | 1.6535 | 1.8399 | 1.8421 | 1.6252 | 1.8382 | 1.7831 |
| 40 | 1.7825 | 1.8259 | 1.7920 | 1.7253 | 1.5459 | 1.8483 | 1.7822 | 1.5317 | 1.8882 | 1.8426 |
| 45 | 1.8005 | 1.7662 | 1.6840 | 1.6857 | 1.4965 | 1.8353 | 1.6874 | 1.4845 | 1.7983 | 1.8039 |
| 50 | 1.6480 | 1.6895 | 1.4631 | 1.6353 | 1.4392 | 1.7337 | 1.6257 | 1.4211 | 1.7623 | 1.6645 |
| 60 | 1.6000 | 1.6436 | 1.5053 | 1.4924 | 1.3313 | 1.6363 | 1.4815 | 1.2987 | 1.6525 | 1.5884 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 | neg ctr | 20 μM | 40 μM |
| 10 | 2.2121 | 2.4792 | 2.5494 | 2.3661 | 2.4031 | 2.7556 | 2.6647 | 2.5831 |
| 15 | 2.0789 | 2.2814 | 2.3642 | 2.2173 | 2.2570 | 2.6463 | 2.5620 | 2.5126 |
| 20 | 1.8641 | 2.1190 | 2.1694 | 2.0560 | 2.1129 | 2.5797 | 2.5135 | 2.4683 |
| 25 | 1.9229 | 2.0587 | 2.0028 | 1.9574 | 2.0833 | 2.5460 | 2.4812 | 2.4410 |
| 30 | 1.8126 | 1.9844 | 1.9077 | 1.8760 | 1.9605 | 2.5175 | 2.4710 | 2.4199 |
| 35 | 1.7406 | 1.8863 | 1.8291 | 1.7763 | 2.0010 | 2.4792 | 2.4523 | 2.4177 |
| 40 | 1.6988 | 1.8346 | 1.7411 | 1.7670 | 1.9494 | 2.4674 | 2.4597 | 2.4107 |
| 45 | 1.6291 | 1.6979 | 1.6836 | 1.6834 | 1.9266 | 2.4686 | 2.4575 | 2.3921 |
| 50 | 1.5618 | 1.6334 | 1.6370 | 1.6358 | 1.8282 | 2.4706 | 2.4505 | 2.3851 |
| 60 | 1.4214 | 1.4922 | 1.5139 | 1.5307 | 1.7113 | 2.4638 | 2.4407 | 2.3809 |

**Table B-104.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 9Dec2011**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 |
| 10 | 1.8524 | 1.8397 | 1.7184 | 1.7051 | 1.8537 | 1.8323 | 1.9801 | 1.7471 | 1.8704 | 1.8546 |
| 15 | 1.8350 | 1.8002 | 1.7106 | 1.7685 | 1.7968 | 1.9487 | 1.9253 | 1.8276 | 1.8442 | 1.7973 |
| 20 | 1.8017 | 1.8737 | 1.6901 | 1.7166 | 1.7244 | 1.8609 | 1.8418 | 1.7552 | 1.8065 | 1.7545 |
| 25 | 1.7685 | 1.7564 | 1.5684 | 1.6259 | 1.7396 | 1.7673 | 1.8510 | 1.7361 | 1.7396 | 1.7284 |
| 30 | 1.7118 | 1.7094 | 1.5348 | 1.6629 | 1.7239 | 1.8076 | 1.7774 | 1.6747 | 1.7604 | 1.6839 |
| 35 | 1.6092 | 1.6266 | 1.5262 | 1.5125 | 1.6908 | 1.7482 | 1.7848 | 1.5705 | 1.5627 | 1.6687 |
| 40 | 1.6076 | 1.5120 | 1.4880 | 1.4199 | 1.6563 | 1.6452 | 1.6490 | 1.5239 | 1.5633 | 1.5978 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.9299 | 1.8286 | 1.8401 | 1.8889 | 1.9329 | 2.2387 | 2.2522 | 2.2118 |
| 15 | 1.8487 | 1.8121 | 1.6649 | 1.8715 | 1.8718 | 2.1872 | 2.2231 | 2.1560 |
| 20 | 1.8536 | 1.8352 | 1.6956 | 1.7756 | 1.7787 | 2.1675 | 2.2084 | 2.1297 |
| 25 | 1.8140 | 1.7356 | 1.6185 | 1.7928 | 1.8213 | 2.1511 | 2.1950 | 2.1188 |
| 30 | 1.7411 | 1.6966 | 1.5063 | 1.7587 | 1.7592 | 2.1429 | 2.1976 | 2.1124 |
| 35 | 1.7017 | 1.6032 | 1.5166 | 1.7299 | 1.6070 | 2.1400 | 2.1980 | 2.1110 |
| 40 | 1.7061 | 1.5441 | 1.5526 | 1.6720 | 1.5595 | 2.1188 | 2.1901 | 2.0987 |

**Table B-104.1c. Batch 1, 340 nm absorbance, 33 μL CKB 8/3/2011 extract, 9Dec2011**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 2.2190 | 2.3045 | 2.0855 | 2.2512 | 2.0401 | 2.2168 | 2.2180 | 2.2299 | 2.1103 | 2.1725 |
| 15 | 2.1680 | 2.2950 | 2.0635 | 2.2217 | 2.0087 | 2.1980 | 2.2016 | 2.2095 | 2.0861 | 2.1729 |
| 20 | 2.1243 | 2.2656 | 2.0538 | 2.2057 | 1.9812 | 2.1969 | 2.1862 | 2.1851 | 2.0556 | 2.0125 |
| 25 | 2.0914 | 2.2102 | 2.0274 | 2.1779 | 1.9431 | 2.1838 | 2.1816 | 2.1830 | 1.9778 | 1.9801 |
| 30 | 2.0145 | 2.1592 | 2.0099 | 2.1563 | 1.9188 | 2.1810 | 2.1646 | 2.1649 | 1.9423 | 1.9543 |
| 35 | 1.9456 | 2.0426 | 1.9950 | 2.1345 | 1.8922 | 2.1617 | 2.1516 | 2.1476 | 1.9222 | 1.9239 |
| 40 | 1.9136 | 2.0280 | 1.9754 | 2.1163 | 1.8644 | 2.1675 | 2.1413 | 2.1296 | 1.8896 | 1.8966 |
| 45 | 1.9269 | 1.8435 | 1.9519 | 2.0995 | 1.8400 | 2.1595 | 2.1325 | 2.1123 | 1.7733 | 1.8693 |
| 50 | 1.9381 | 1.8067 | 1.8939 | 2.0750 | 1.8196 | 2.1602 | 2.1223 | 2.1032 | 1.7376 | 1.8456 |
| 55 | 1.8506 | 1.7941 | 1.8146 | 2.0618 | 1.7920 | 2.1476 | 2.1089 | 2.0917 | 1.6738 | 1.8205 |
| 60 | 1.8460 | 1.7159 | 1.7405 | 2.0462 | 1.7749 | 2.1509 | 2.0932 | 2.0784 | 1.5893 | 1.7978 |
| **Rate[a]** | 0.0075 | 0.0131 | 0.0062 | 0.0041 | 0.0053 | 0.0013 | 0.0024 | 0.0030 | 0.0103 | 0.0074 |
| **$r^2$** | 0.9327 | 0.9631 | 0.8887 | 0.9944 | 0.9947 | 0.9190 | 0.9950 | 0.9911 | 0.9748 | 0.9101 |
| **Rate – neg ctrl rate** | 0.0070 | 0.0126 | 0.0057 | 0.0036 | 0.0048 | 0.0008 | 0.0019 | 0.0025 | 0.0098 | 0.0069 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.1725 | 2.2827 | 1.0230 | 2.7528 | 2.1074 | 2.3044 | 2.2329 | 2.1989 |
| 15 | 2.1729 | 2.2640 | 0.9989 | 2.7371 | 2.0758 | 2.2871 | 2.2007 | 2.1458 |
| 20 | 2.1641 | 2.2414 | 0.9771 | 2.7452 | 2.0226 | 2.2749 | 2.1759 | 2.1363 |
| 25 | 2.1661 | 2.2494 | 0.9619 | 2.7092 | 1.9918 | 2.2772 | 2.1651 | 2.1047 |
| 30 | 2.1490 | 2.2301 | 0.9473 | 2.7164 | 1.9588 | 2.2762 | 2.1579 | 2.0807 |
| 35 | 2.1407 | 2.2230 | 0.9317 | 2.6938 | 1.9265 | 2.2734 | 2.1372 | 2.0530 |
| 40 | 2.1397 | 2.2104 | 0.9146 | 2.6948 | 1.8997 | 2.2704 | 2.1265 | 2.0319 |
| 45 | 2.1310 | 2.1959 | 0.9017 | 2.6810 | 1.8747 | 2.2747 | 2.1143 | 2.0005 |
| 50 | 2.1271 | 2.1881 | 0.8879 | 2.6587 | 1.8466 | 2.2684 | 2.1014 | 1.9791 |
| 55 | 2.1223 | 2.1827 | 0.8766 | 2.6697 | 1.8233 | 2.2712 | 2.0914 | 1.9592 |
| 60 | 2.1010 | 2.1596 | 0.8635 | 2.6570 | 1.8013 | 2.2677 | 2.0843 | 1.9378 |
| **Rate[a]** | 0.0014 | 0.0022 | 0.0031 | absorbance | 0.0061 | 0.0005 | 0.0028 | 0.0051 |
| **$r^2$** | 0.9536 | 0.9765 | 0.9907 | out of | 0.9883 | 0.6282 | 0.9691 | 0.9925 |
| **Rate – neg ctrl rate** | 0.0009 | 0.0017 | 0.0026 | range for assay | 0.0056 | 0.0000 | 0.0023 | 0.0046 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.1c-A1, A2, A3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

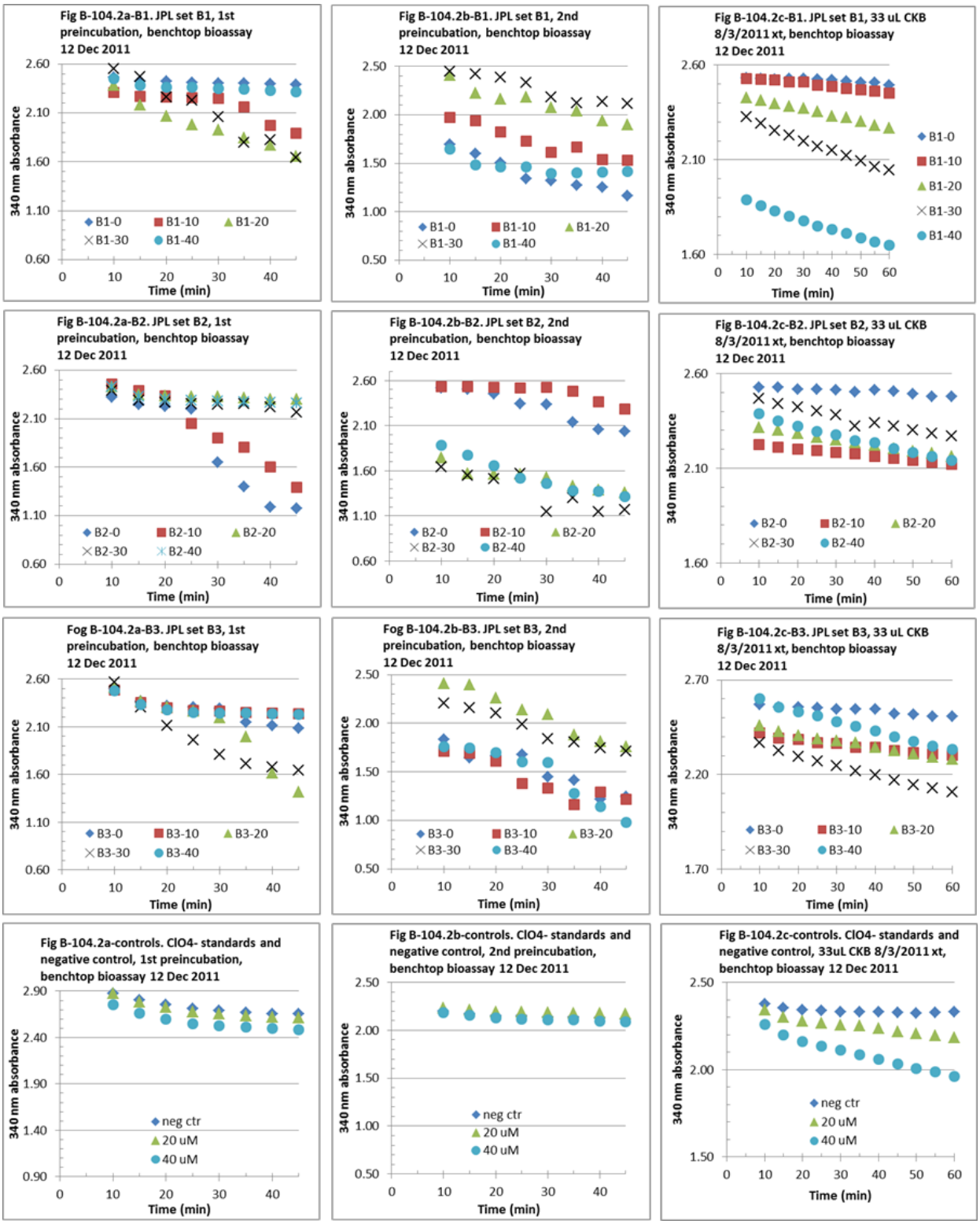



**Figure B-104.2. Jet Propulsion Laboratory, benchtop bioassay batch 2, sets B1, B2, B3, and perchlorate controls. Test date 12 Dec 2011.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.2a, b, and c

Data for Figure B-104.2.
Groundwater sample 104, JPL. Standards addition method benchtop bioassay, batch 2, 12Dec2011. SDVB/DTAB eluate sets B1, B2, B3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present).

**Table B-104.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 12Dec2011**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 2.4634 | 2.3118 | 2.3850 | 2.5522 | 2.4483 | 2.3237 | 2.4587 | 2.4320 | 2.3882 | 2.4304 |
| 15 | 2.4249 | 2.2708 | 2.1852 | 2.4715 | 2.3841 | 2.2487 | 2.3873 | 2.3517 | 2.2881 | 2.3248 |
| 20 | 2.4226 | 2.2615 | 2.0699 | 2.2627 | 2.3645 | 2.2295 | 2.3359 | 2.3360 | 2.2679 | 2.2978 |
| 25 | 2.4122 | 2.2563 | 1.9821 | 2.2301 | 2.3631 | 2.1991 | 2.0521 | 2.3290 | 2.2534 | 2.2874 |
| 30 | 2.4073 | 2.2471 | 1.9270 | 2.0610 | 2.3502 | 1.6504 | 1.9024 | 2.3250 | 2.2477 | 2.2824 |
| 35 | 2.4022 | 2.1628 | 1.8424 | 1.7954 | 2.3442 | 1.3987 | 1.8040 | 2.3160 | 2.2569 | 2.2745 |
| 40 | 2.3951 | 1.9716 | 1.7716 | 1.8280 | 2.3307 | 1.1905 | 1.6051 | 2.3032 | 2.2219 | 2.2698 |
| 45 | 2.3908 | 1.8932 | 1.6596 | 1.6419 | 2.3170 | 1.1732 | 1.3913 | 2.3024 | 2.1676 | 2.2627 |

| | Set B3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 | neg ctr | 20 μM | 40 μM |
| 10 | 2.4969 | 2.4832 | 2.5119 | 2.5638 | 2.4740 | 2.8765 | 2.8713 | 2.7535 |
| 15 | 2.3660 | 2.3539 | 2.3637 | 2.3034 | 2.3281 | 2.8016 | 2.7854 | 2.6625 |
| 20 | 2.3191 | 2.2961 | 2.3020 | 2.1131 | 2.2743 | 2.7520 | 2.7285 | 2.5952 |
| 25 | 2.3006 | 2.2683 | 2.2656 | 1.9573 | 2.2509 | 2.7137 | 2.6752 | 2.5513 |
| 30 | 2.2890 | 2.2601 | 2.1916 | 1.8067 | 2.2443 | 2.6876 | 2.6582 | 2.5273 |
| 35 | 2.1479 | 2.2483 | 1.9954 | 1.7125 | 2.2395 | 2.6704 | 2.6340 | 2.5116 |
| 40 | 2.1105 | 2.2435 | 1.6178 | 1.6797 | 2.2315 | 2.6545 | 2.6178 | 2.4959 |
| 45 | 2.0799 | 2.2363 | 1.4153 | 1.6395 | 2.2265 | 2.6515 | 2.6097 | 2.4848 |

**Table B-104.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 12Dec2011**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 1.6961 | 1.9759 | 2.4111 | 2.4506 | 1.6441 | 2.5178 | 2.5299 | 1.7467 | 1.6392 | 1.8851 |
| 15 | 1.6003 | 1.9399 | 2.2250 | 2.4196 | 1.4857 | 2.5032 | 2.5285 | 1.5702 | 1.5450 | 1.7732 |
| 20 | 1.5027 | 1.8210 | 2.1626 | 2.3893 | 1.4666 | 2.4543 | 2.5243 | 1.5641 | 1.5140 | 1.6551 |
| 25 | 1.3449 | 1.7259 | 2.1867 | 2.3325 | 1.4657 | 2.3438 | 2.5205 | 1.5645 | 1.5697 | 1.5190 |
| 30 | 1.3243 | 1.6165 | 2.0783 | 2.1805 | 1.3978 | 2.3317 | 2.5211 | 1.5236 | 1.1427 | 1.4572 |
| 35 | 1.2736 | 1.6661 | 2.0396 | 2.1239 | 1.4013 | 2.1363 | 2.4837 | 1.4307 | 1.3006 | 1.3791 |
| 40 | 1.2504 | 1.5397 | 1.9380 | 2.1348 | 1.4097 | 2.0553 | 2.3646 | 1.3881 | 1.1490 | 1.3718 |
| 45 | 1.1630 | 1.5311 | 1.8968 | 2.1146 | 1.4186 | 2.0393 | 2.2812 | 1.3592 | 1.1713 | 1.3140 |

| | Set B3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.8305 | 1.7089 | 2.4114 | 2.2029 | 1.7606 | 2.1945 | 2.2302 | 2.1858 |
| 15 | 1.6461 | 1.6899 | 2.3948 | 2.1572 | 1.7426 | 2.1709 | 2.2147 | 2.1550 |
| 20 | 1.6635 | 1.6074 | 2.2593 | 2.1021 | 1.7008 | 2.1524 | 2.1940 | 2.1258 |
| 25 | 1.6793 | 1.3757 | 2.1353 | 1.9920 | 1.6035 | 2.1464 | 2.1876 | 2.1125 |
| 30 | 1.4478 | 1.3287 | 2.0895 | 1.8396 | 1.5949 | 2.1335 | 2.1873 | 2.1073 |
| 35 | 1.4154 | 1.1610 | 1.8822 | 1.8092 | 1.2775 | 2.1342 | 2.1768 | 2.1052 |
| 40 | 1.2143 | 1.2909 | 1.8105 | 1.7433 | 1.1434 | 2.1305 | 2.1743 | 2.0947 |
| 45 | 1.2427 | 1.2128 | 1.7561 | 1.7094 | 0.9808 | 2.1181 | 2.1724 | 2.0887 |

**Table B-104.2c. Batch 2, 340 nm absorbance, 33 μL CKB 8/3/2011 extract, 12Dec2011**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B1-0** | **B1-10** | **B1-20** | **B1-30** | **B1-40** | **B2-0** | **B2-10** | **B2-20** | **B2-30** | **B2-40** |
| 10 | 2.5335 | 2.5297 | 2.4267 | 2.3264 | 1.8881 | 2.5269 | 2.2250 | 2.3171 | 2.4682 | 2.3893 |
| 15 | 2.5298 | 2.5254 | 2.4133 | 2.2933 | 1.8565 | 2.5257 | 2.2101 | 2.3018 | 2.4390 | 2.3488 |
| 20 | 2.5255 | 2.5230 | 2.3959 | 2.2542 | 1.8294 | 2.5166 | 2.2019 | 2.2852 | 2.4225 | 2.3235 |
| 25 | 2.5287 | 2.5129 | 2.3822 | 2.2297 | 1.8022 | 2.5159 | 2.1925 | 2.2644 | 2.4019 | 2.2959 |
| 30 | 2.5270 | 2.5098 | 2.3706 | 2.1970 | 1.7758 | 2.5148 | 2.1819 | 2.2505 | 2.3815 | 2.2757 |
| 35 | 2.5240 | 2.4949 | 2.3554 | 2.1715 | 1.7512 | 2.5021 | 2.1761 | 2.2364 | 2.3240 | 2.2468 |
| 40 | 2.5214 | 2.4876 | 2.3321 | 2.1487 | 1.7308 | 2.5133 | 2.1627 | 2.2218 | 2.3410 | 2.2367 |
| 45 | 2.5136 | 2.4782 | 2.3230 | 2.1232 | 1.7098 | 2.5049 | 2.1507 | 2.2050 | 2.3219 | 2.2057 |
| 50 | 2.5087 | 2.4695 | 2.3029 | 2.0940 | 1.6883 | 2.4930 | 2.1415 | 2.1889 | 2.3024 | 2.1830 |
| 55 | 2.5078 | 2.4632 | 2.2823 | 2.0643 | 1.6667 | 2.4780 | 2.1314 | 2.1793 | 2.2826 | 2.1641 |
| 60 | 2.4945 | 2.4513 | 2.2683 | 2.0440 | 1.6476 | 2.4794 | 2.1211 | 2.1642 | 2.2720 | 2.1405 |
| **Rate[a]** | 0.0007 | 0.0016 | 0.0032 | 0.0056 | 0.0048 | 0.0010 | 0.0020 | 0.0031 | 0.0039 | 0.0048 |
| **$r^2$** | 0.8750 | 0.9848 | 0.9960 | 0.9964 | 0.9948 | 0.8739 | 0.9974 | 0.9972 | 0.9655 | 0.9931 |
| **Rate – neg ctrl rate** | 0.0000 | 0.0009 | 0.0025 | 0.0049 | 0.0041 | 0.0003 | 0.0013 | 0.0024 | 0.0032 | 0.0041 |

| | Set B3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.5710 | 2.4200 | 2.4588 | 2.3692 | 2.6019 | 2.3773 | 2.3433 | 2.2582 |
| 15 | 2.5578 | 2.3918 | 2.4290 | 2.3256 | 2.5551 | 2.3529 | 2.3001 | 2.1986 |
| 20 | 2.5575 | 2.3843 | 2.4083 | 2.2966 | 2.5326 | 2.3423 | 2.2785 | 2.1636 |
| 25 | 2.5517 | 2.3692 | 2.3898 | 2.2710 | 2.5112 | 2.3405 | 2.2684 | 2.1366 |
| 30 | 2.5454 | 2.3647 | 2.3784 | 2.2452 | 2.4813 | 2.3323 | 2.2577 | 2.1109 |
| 35 | 2.5462 | 2.3444 | 2.3694 | 2.2193 | 2.4539 | 2.3317 | 2.2534 | 2.0856 |
| 40 | 2.5447 | 2.3453 | 2.3444 | 2.1994 | 2.4299 | 2.3316 | 2.2364 | 2.0595 |
| 45 | 2.5232 | 2.3284 | 2.3269 | 2.1714 | 2.3982 | 2.3290 | 2.2202 | 2.0326 |
| 50 | 2.5197 | 2.3178 | 2.3096 | 2.1465 | 2.3764 | 2.3239 | 2.2080 | 2.0065 |
| 55 | 2.5071 | 2.3107 | 2.2913 | 2.1279 | 2.3523 | 2.3280 | 2.1970 | 1.9866 |
| 60 | 2.5069 | 2.3028 | 2.2819 | 2.1074 | 2.3345 | 2.3335 | 2.1848 | 1.9599 |
| **Rate[a]** | 0.0013 | 0.0022 | 0.0034 | 0.0051 | 0.0053 | 0.0007 | 0.0028 | 0.0056 |
| **$r^2$** | 0.9575 | 0.9759 | 0.9914 | 0.9927 | 0.9950 | 0.6239 | 0.9580 | 0.9857 |
| **Rate – neg ctrl rate** | 0.0006 | 0.0015 | 0.0027 | 0.0044 | 0.0046 | 0.0000 | 0.0021 | 0.0049 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.2c-B1, B2, B3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

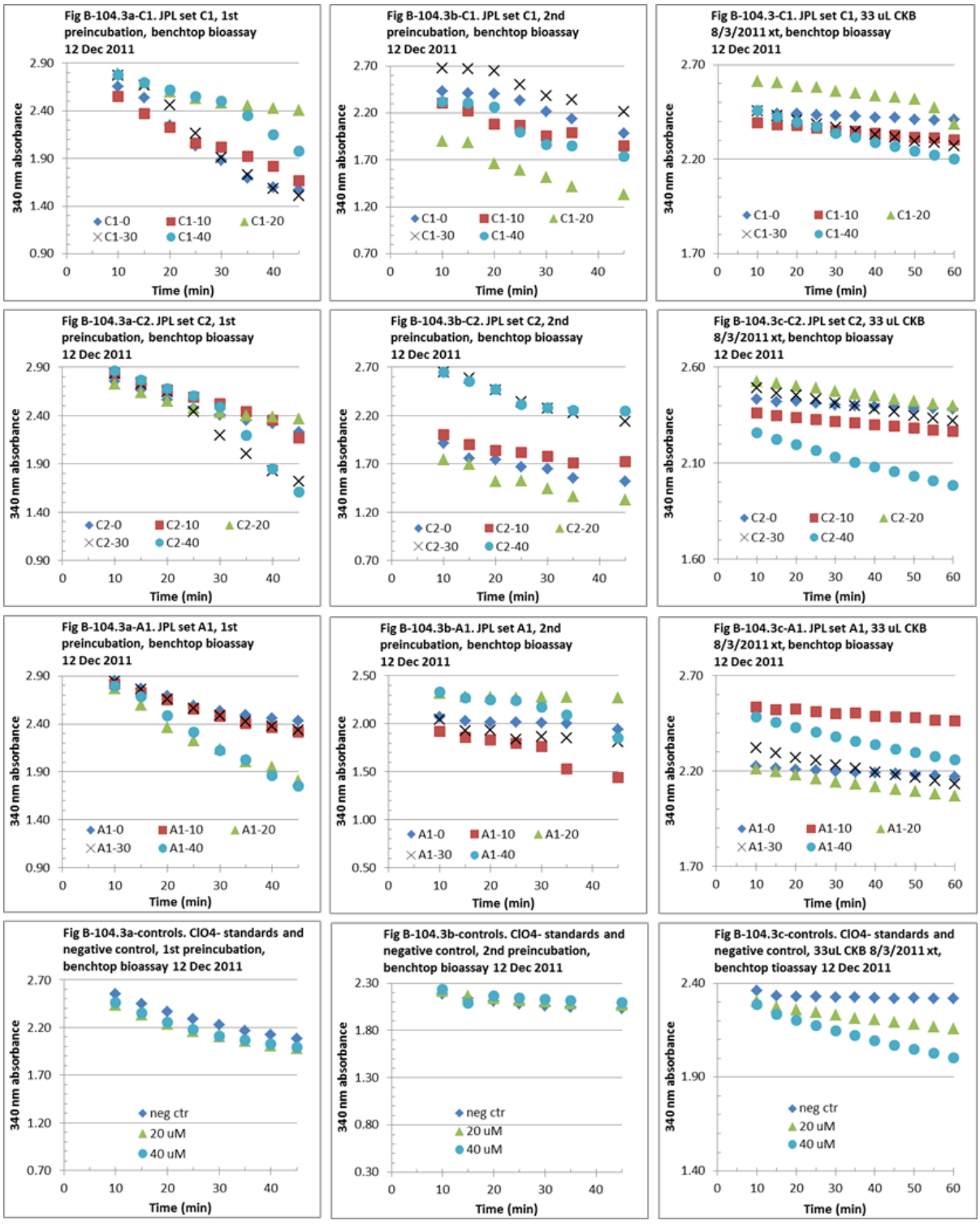



**Figure B-104.3. Jet Propulsion Laboratory, benchtop bioassay batch 3, sets C1, C2, A1, and perchlorate controls. Test date 12 Dec 2011.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 µL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.3a, b, and c

Data for Figure B-104.3.
Groundwater sample 104, JPL. Standards addition method benchtop bioassay, batch 3, 12Dec2011. SDVB/DTAB eluate sets C1, C2, A1 (repeated 9Dec2011 testing), perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present).

**Table B-104.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 12Dec2011**

| | Set C1 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **C1-0** | **C1-10** | **C1-20** | **C1-30** | **C1-40** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** |
| 10 | 2.6527 | 2.5525 | 2.7903 | 2.7720 | 2.7782 | 2.7564 | 2.8326 | 2.7287 | 2.8348 | 2.8590 |
| 15 | 2.5382 | 2.3727 | 2.6994 | 2.6710 | 2.6969 | 2.6624 | 2.7464 | 2.6341 | 2.7247 | 2.7646 |
| 20 | 2.2440 | 2.2267 | 2.6019 | 2.4588 | 2.6195 | 2.5630 | 2.6653 | 2.5465 | 2.6475 | 2.6798 |
| 25 | 2.0360 | 2.0584 | 2.5324 | 2.1676 | 2.5489 | 2.4762 | 2.5915 | 2.4748 | 2.4410 | 2.6047 |
| 30 | 1.8783 | 2.0216 | 2.4791 | 1.9089 | 2.5017 | 2.4051 | 2.5240 | 2.4299 | 2.1976 | 2.4907 |
| 35 | 1.6954 | 1.9249 | 2.4541 | 1.7301 | 2.3526 | 2.3532 | 2.4372 | 2.3980 | 2.0030 | 2.1973 |
| 40 | 1.6006 | 1.8215 | 2.4236 | 1.5834 | 2.1519 | 2.3148 | 2.3527 | 2.3852 | 1.8285 | 1.8502 |
| 45 | 1.5716 | 1.6677 | 2.4093 | 1.5090 | 1.9781 | 2.2308 | 2.1710 | 2.3662 | 1.7168 | 1.6123 |

| | Set A1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.8525 | 2.8139 | 2.7650 | 2.8432 | 2.7920 | 2.5546 | 2.4364 | 2.4642 |
| 15 | 2.7700 | 2.7270 | 2.5963 | 2.7615 | 2.6862 | 2.4468 | 2.3277 | 2.3485 |
| 20 | 2.6894 | 2.6471 | 2.3595 | 2.6588 | 2.4871 | 2.3644 | 2.2347 | 2.2544 |
| 25 | 2.5911 | 2.5560 | 2.2256 | 2.5606 | 2.3163 | 2.2857 | 2.1596 | 2.1762 |
| 30 | 2.5328 | 2.4766 | 2.1374 | 2.4883 | 2.1206 | 2.2238 | 2.1020 | 2.1120 |
| 35 | 2.4942 | 2.4069 | 2.0078 | 2.4212 | 2.0250 | 2.1652 | 2.0531 | 2.0654 |
| 40 | 2.4586 | 2.3628 | 1.9496 | 2.3737 | 1.8596 | 2.1240 | 2.0081 | 2.0246 |
| 45 | 2.4298 | 2.3118 | 1.8073 | 2.3358 | 1.7551 | 2.0841 | 1.9775 | 1.9914 |

**Table B-104.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 12Dec2011**

| | Set C1 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 | C2-0 | C2-10 | C2-20 | C2-30 | C2-40 |
| 10 | 2.4330 | 2.3058 | 1.8969 | 2.6797 | 2.3218 | 1.9104 | 2.0018 | 1.7432 | 2.6498 | 2.6475 |
| 15 | 2.4127 | 2.2243 | 1.8815 | 2.6707 | 2.3026 | 1.7583 | 1.9012 | 1.6942 | 2.5901 | 2.5507 |
| 20 | 2.4011 | 2.0835 | 1.6601 | 2.6503 | 2.2649 | 1.7410 | 1.8352 | 1.5201 | 2.4644 | 2.4706 |
| 25 | 2.3299 | 2.0688 | 1.5929 | 2.4998 | 1.9968 | 1.6651 | 1.8177 | 1.5278 | 2.3446 | 2.3169 |
| 30 | 2.2129 | 1.9547 | 1.5144 | 2.3839 | 1.8660 | 1.6475 | 1.7782 | 1.4417 | 2.2772 | 2.2782 |
| 35 | 2.1365 | 1.9873 | 1.4131 | 2.3437 | 1.8511 | 1.5552 | 1.7119 | 1.3609 | 2.2291 | 2.2512 |
| 45 | 1.9836 | 1.8523 | 1.3317 | 2.2152 | 1.7399 | 1.5215 | 1.7196 | 1.3253 | 2.1391 | 2.2494 |

| | Set A1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | neg ctr | 20 µM | 40 µM |
| 10 | 2.0691 | 1.9179 | 2.3212 | 2.0470 | 2.3351 | 2.1836 | 2.2151 | 2.2379 |
| 15 | 2.0305 | 1.8561 | 2.2867 | 1.9299 | 2.2707 | 2.1362 | 2.1669 | 2.0909 |
| 20 | 2.0200 | 1.8328 | 2.2785 | 1.9314 | 2.2525 | 2.1079 | 2.1389 | 2.1661 |
| 25 | 2.0169 | 1.7945 | 2.2688 | 1.8394 | 2.2411 | 2.0801 | 2.1146 | 2.1455 |
| 30 | 2.0096 | 1.7623 | 2.2753 | 1.8644 | 2.1719 | 2.0639 | 2.1014 | 2.1292 |
| 35 | 2.0036 | 1.5322 | 2.2787 | 1.8546 | 2.0906 | 2.0495 | 2.0843 | 2.1189 |
| 45 | 1.9432 | 1.4415 | 2.2715 | 1.8142 | 1.8487 | 2.0308 | 2.0642 | 2.0987 |

**Table B-104.3c. Batch 3, 340 nm absorbance, 33 μL CKB 8/3/2011 extract, 12Dec2011**

| | Set C1 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **C1-0** | **C1-10** | **C1-20** | **C1-30** | **C1-40** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** |
| 10 | 2.4517 | 2.3921 | 2.6115 | 2.4544 | 2.4573 | 2.4350 | 2.3619 | 2.5267 | 2.4929 | 2.2603 |
| 15 | 2.4419 | 2.3820 | 2.6057 | 2.4312 | 2.4247 | 2.4191 | 2.3462 | 2.5151 | 2.4649 | 2.2231 |
| 20 | 2.4417 | 2.3776 | 2.5848 | 2.4066 | 2.3973 | 2.4219 | 2.3390 | 2.5019 | 2.4537 | 2.1970 |
| 25 | 2.4337 | 2.3632 | 2.5803 | 2.3860 | 2.3720 | 2.4130 | 2.3280 | 2.4909 | 2.4341 | 2.1649 |
| 30 | 2.4301 | 2.3499 | 2.5604 | 2.3665 | 2.3380 | 2.4010 | 2.3160 | 2.4730 | 2.4135 | 2.1316 |
| 35 | 2.4272 | 2.3465 | 2.5474 | 2.3469 | 2.3158 | 2.3983 | 2.3106 | 2.4617 | 2.3980 | 2.1057 |
| 40 | 2.4210 | 2.3359 | 2.5359 | 2.3313 | 2.2879 | 2.3968 | 2.3007 | 2.4499 | 2.3827 | 2.0809 |
| 45 | 2.4193 | 2.3271 | 2.5295 | 2.3145 | 2.2660 | 2.3937 | 2.2920 | 2.4336 | 2.3683 | 2.0562 |
| 50 | 2.4103 | 2.3168 | 2.5182 | 2.2967 | 2.2443 | 2.3862 | 2.2814 | 2.4227 | 2.3482 | 2.0307 |
| 55 | 2.4049 | 2.3119 | 2.4740 | 2.2897 | 2.2204 | 2.3868 | 2.2725 | 2.4098 | 2.3348 | 2.0084 |
| 60 | 2.4091 | 2.3019 | 2.3868 | 2.2706 | 2.2021 | 2.3810 | 2.2670 | 2.3993 | 2.3198 | 1.9849 |
| **Rate[a]** | 0.0009 | 0.0018 | 0.0036 | 0.0036 | 0.0051 | 0.0010 | 0.0019 | 0.0026 | 0.0034 | 0.0055 |
| **$r^2$** | 0.9685 | 0.9935 | 0.8462 | 0.9896 | 0.9955 | 0.9387 | 0.9945 | 0.9986 | 0.9962 | 0.9954 |
| **Rate – neg ctrl rate** | 0.0003 | 0.0012 | 0.0030 | 0.0030 | 0.0045 | 0.0004 | 0.0013 | 0.0020 | 0.0028 | 0.0049 |

| | Set A1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.2258 | 2.5352 | 2.2128 | 2.3208 | 2.4849 | 2.3592 | 2.3042 | 2.2861 |
| 15 | 2.2151 | 2.5210 | 2.1965 | 2.2938 | 2.4564 | 2.3330 | 2.2730 | 2.2339 |
| 20 | 2.2090 | 2.5246 | 2.1792 | 2.2698 | 2.4298 | 2.3295 | 2.2574 | 2.2039 |
| 25 | 2.2054 | 2.5135 | 2.1595 | 2.2551 | 2.4047 | 2.3286 | 2.2442 | 2.1744 |
| 30 | 2.1987 | 2.5003 | 2.1436 | 2.2321 | 2.3818 | 2.3258 | 2.2296 | 2.1466 |
| 35 | 2.1954 | 2.5032 | 2.1336 | 2.2143 | 2.3581 | 2.3242 | 2.2146 | 2.1209 |
| 40 | 2.1890 | 2.4869 | 2.1174 | 2.1952 | 2.3384 | 2.3223 | 2.2045 | 2.0945 |
| 45 | 2.1863 | 2.4827 | 2.1043 | 2.1787 | 2.3135 | 2.3186 | 2.1918 | 2.0708 |
| 50 | 2.1815 | 2.4797 | 2.0942 | 2.1663 | 2.2979 | 2.3211 | 2.1811 | 2.0483 |
| 55 | 2.1761 | 2.4663 | 2.0806 | 2.1493 | 2.2781 | 2.3190 | 2.1671 | 2.0259 |
| 60 | 2.1689 | 2.4646 | 2.0690 | 2.1310 | 2.2611 | 2.3183 | 2.1565 | 2.0037 |
| **Rate[a]** | 0.0010 | 0.0014 | 0.0029 | 0.0037 | 0.0045 | 0.0006 | 0.0028 | 0.0054 |
| **$r^2$** | 0.9878 | 0.9731 | 0.9927 | 0.9935 | 0.9945 | 0.6403 | 0.9835 | 0.9883 |
| **Rate – neg ctrl rate** | 0.0004 | 0.0008 | 0.0023 | 0.0031 | 0.0039 | 0.0000 | 0.0022 | 0.0048 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.3c-C1, C2, C3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

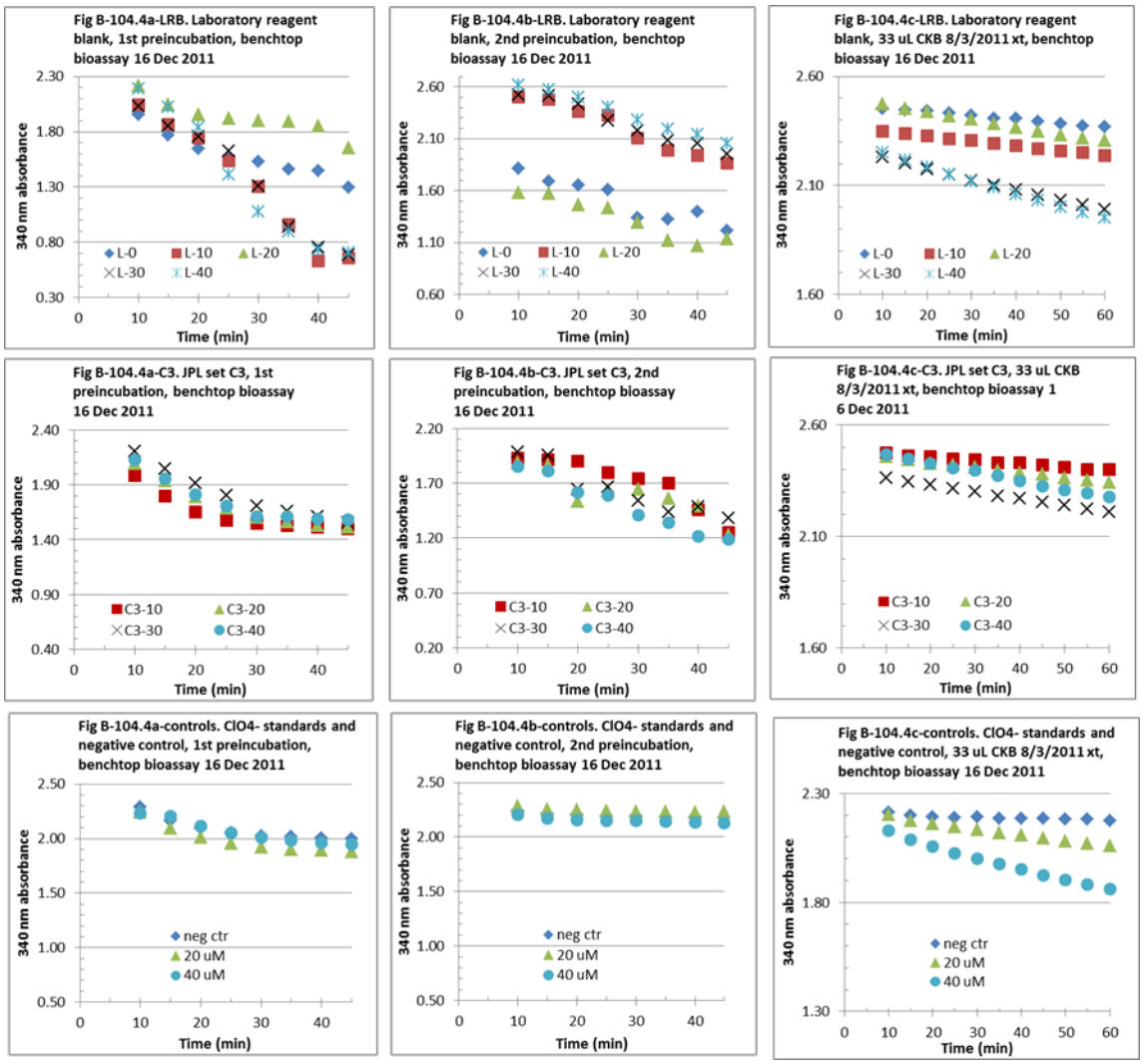



**Figure B-104.4. Jet Propulsion Laboratory, benchtop bioassay batch 4, set C3, and Laboratory Reagent Blank set, and perchlorate controls. Test date 16 Dec 2011.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 33 uL CKB 8/3/2011 extract (**c**). Data values displayed are listed in Tables B-104.4a, b, and c

Data for Figure B-104.4.
Groundwater sample 104, Jet Propulsion Laboratory. Standards addition method benchtop bioassay, batch 4, 16Dec2011. SDVB/DTAB eluate set C3, Laboratory Reagent Blank (L), perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present).

**Table B-104.4a. Batch 4, 340 nm absorbance, 1st preincubation without extract, 16Dec2011**

| | Set L | | | | | Set C3 | | | |
|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 |
| (min) | L-0 | L-10 | L-20 | L-30 | L-40 | C3-10 | C3-20 | C3-30 | C3-40 |
| 10 | 1.9544 | 2.0421 | 2.2120 | 2.0305 | 2.1901 | 1.9854 | 2.0940 | 2.2075 | 2.1290 |
| 15 | 1.7679 | 1.8618 | 2.0423 | 1.8519 | 2.0197 | 1.7956 | 1.9320 | 2.0464 | 1.9578 |
| 20 | 1.6481 | 1.7451 | 1.9537 | 1.7522 | 1.8375 | 1.6534 | 1.7908 | 1.9126 | 1.8097 |
| 25 | 1.5697 | 1.5384 | 1.9205 | 1.6237 | 1.4160 | 1.5789 | 1.6849 | 1.8033 | 1.7053 |
| 30 | 1.5260 | 1.3002 | 1.9003 | 1.3091 | 1.0771 | 1.5464 | 1.6068 | 1.7042 | 1.6120 |
| 35 | 1.4631 | 0.9601 | 1.8915 | 0.9397 | 0.8985 | 1.5269 | 1.5595 | 1.6567 | 1.6081 |
| 40 | 1.4499 | 0.6294 | 1.8521 | 0.7524 | 0.7351 | 1.5125 | 1.5308 | 1.6084 | 1.5921 |
| 45 | 1.2943 | 0.6603 | 1.6557 | 0.6851 | 0.7035 | 1.5024 | 1.5165 | 1.5550 | 1.5800 |

| | Controls | | |
|---|---|---|---|
| Time | rxn 10 | rxn 11 | rxn 12 |
| (min) | neg ctr | 20 μM | 40 μM |
| 10 | 2.2915 | 2.2376 | 2.2311 |
| 15 | 2.1636 | 2.0967 | 2.2066 |
| 20 | 2.0932 | 2.0105 | 2.1121 |
| 25 | 2.0532 | 1.9560 | 2.0551 |
| 30 | 2.0276 | 1.9216 | 2.0128 |
| 35 | 2.0152 | 1.9016 | 1.9859 |
| 40 | 2.0073 | 1.8895 | 1.9633 |
| 45 | 1.9986 | 1.8808 | 1.9460 |

**Table B-104.4b. Batch 4, 340 nm absorbance, 2nd preincubation without extract, 16Dec2011**

| Time | Set L | | | | | Set C3 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 |
| (min) | L-0 | L-10 | L-20 | L-30 | L-40 | C3-10 | C3-20 | C3-30 | C3-40 |
| 10 | 1.8113 | 2.5006 | 1.5760 | 2.5217 | 2.6174 | 1.9279 | 1.8872 | 1.9809 | 1.8547 |
| 15 | 1.6865 | 2.4813 | 1.5738 | 2.5144 | 2.5728 | 1.9142 | 1.8632 | 1.9541 | 1.8115 |
| 20 | 1.6548 | 2.3600 | 1.4608 | 2.4305 | 2.4989 | 1.9001 | 1.5368 | 1.6468 | 1.6182 |
| 25 | 1.6069 | 2.3214 | 1.4317 | 2.2762 | 2.4062 | 1.7970 | 1.6285 | 1.6621 | 1.5863 |
| 30 | 1.3385 | 2.1062 | 1.2914 | 2.1781 | 2.2795 | 1.7441 | 1.6424 | 1.5397 | 1.4130 |
| 35 | 1.3229 | 1.9909 | 1.1178 | 2.0806 | 2.1924 | 1.6986 | 1.5588 | 1.4358 | 1.3418 |
| 40 | 1.3995 | 1.9386 | 1.0649 | 2.0574 | 2.1409 | 1.4582 | 1.4941 | 1.4889 | 1.2199 |
| 45 | 1.2155 | 1.8620 | 1.1344 | 1.9548 | 2.0550 | 1.2534 | 1.2297 | 1.3789 | 1.1901 |

| Time | Controls | | |
|---|---|---|---|
| | rxn 10 | rxn 11 | rxn 12 |
| (min) | neg ctr | 20 μM | 40 μM |
| 10 | 2.2386 | 2.2794 | 2.2055 |
| 15 | 2.2122 | 2.2566 | 2.1729 |
| 20 | 2.2039 | 2.2475 | 2.1574 |
| 25 | 2.1917 | 2.2363 | 2.1514 |
| 30 | 2.1940 | 2.2360 | 2.1465 |
| 35 | 2.1867 | 2.2301 | 2.1393 |
| 40 | 2.1826 | 2.2282 | 2.1356 |
| 45 | 2.1794 | 2.2301 | 2.1276 |

**Table B-104.4c. Batch 4, 340 nm absorbance, 33 μmL CKB 8/3/2011 extract, 16Dec2011**

| | Set L | | | | | Set C3 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** |
| **(min)** | **L-0** | **L-10** | **L-20** | **L-30** | **L-40** | **C3-10** | **C3-20** | **C3-30** | **C3-40** |
| 10 | 2.4526 | 2.35 | 2.4741 | 2.2302 | 2.2517 | 2.4747 | 2.4575 | 2.3645 | 2.4695 |
| 15 | 2.4451 | 2.3385 | 2.4534 | 2.2029 | 2.2176 | 2.4622 | 2.4453 | 2.347 | 2.4488 |
| 20 | 2.4423 | 2.3282 | 2.4373 | 2.1745 | 2.1841 | 2.4583 | 2.4281 | 2.3336 | 2.4289 |
| 25 | 2.431 | 2.3134 | 2.4187 | 2.1487 | 2.1498 | 2.4488 | 2.4203 | 2.3142 | 2.4079 |
| 30 | 2.4202 | 2.3059 | 2.4035 | 2.1236 | 2.1206 | 2.4452 | 2.4103 | 2.3032 | 2.3977 |
| 35 | 2.4062 | 2.2929 | 2.3841 | 2.1005 | 2.0894 | 2.4306 | 2.3968 | 2.2825 | 2.3727 |
| 40 | 2.4074 | 2.2808 | 2.3651 | 2.0792 | 2.0592 | 2.4308 | 2.3887 | 2.2715 | 2.3496 |
| 45 | 2.3935 | 2.2698 | 2.3468 | 2.0564 | 2.0311 | 2.4204 | 2.3803 | 2.2553 | 2.3271 |
| 50 | 2.383 | 2.2592 | 2.3318 | 2.0317 | 2.0019 | 2.4104 | 2.3648 | 2.241 | 2.3093 |
| 55 | 2.3745 | 2.2496 | 2.3159 | 2.0117 | 1.9761 | 2.4016 | 2.3535 | 2.2246 | 2.2961 |
| 60 | 2.3678 | 2.2376 | 2.3054 | 1.991 | 1.9506 | 2.3994 | 2.3426 | 2.2115 | 2.2768 |
| **Rate[a]** | 0.0018 | 0.0022 | 0.0034 | 0.0048 | 0.0060 | 0.0015 | 0.0022 | 0.0031 | 0.0039 |
| **$r^2$** | 0.9895 | 0.9990 | 0.9976 | 0.9976 | 0.9980 | 0.9889 | 0.9964 | 0.0025 | 0.9972 |
| **Rate – neg ctrl rate** | 0.0012 | 0.0016 | 0.0028 | 0.0042 | 0.0054 | 0.0009 | 0.0016 | 0.9987 | 0.0033 |

| | Controls | | |
|---|---|---|---|
| **Time** | **rxn 10** | **rxn 11** | **rxn 12** |
| **(min)** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.2112 | 2.2017 | 2.1295 |
| 15 | 2.1977 | 2.1747 | 2.0881 |
| 20 | 2.1931 | 2.1597 | 2.0575 |
| 25 | 2.1880 | 2.1459 | 2.0264 |
| 30 | 2.1914 | 2.1339 | 2.0006 |
| 35 | 2.1852 | 2.1200 | 1.9752 |
| 40 | 2.1853 | 2.1068 | 1.9523 |
| 45 | 2.1833 | 2.0948 | 1.9257 |
| 50 | 2.1799 | 2.0810 | 1.9020 |
| 55 | 2.1804 | 2.0709 | 1.8816 |
| 60 | 2.1754 | 2.0581 | 1.8598 |
| **Rate[a]** | 0.0006 | 0.0027 | 0.0053 |
| **$r^2$** | 0.8460 | 0.9906 | 0.0047 |
| **Rate – neg ctrl rate** | 0.0000 | 0.0021 | 0.9925 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-104.4c-L, C3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

## Groundwater sample 105, Fontana Water Company, location F-17C, collected 25Jan2012

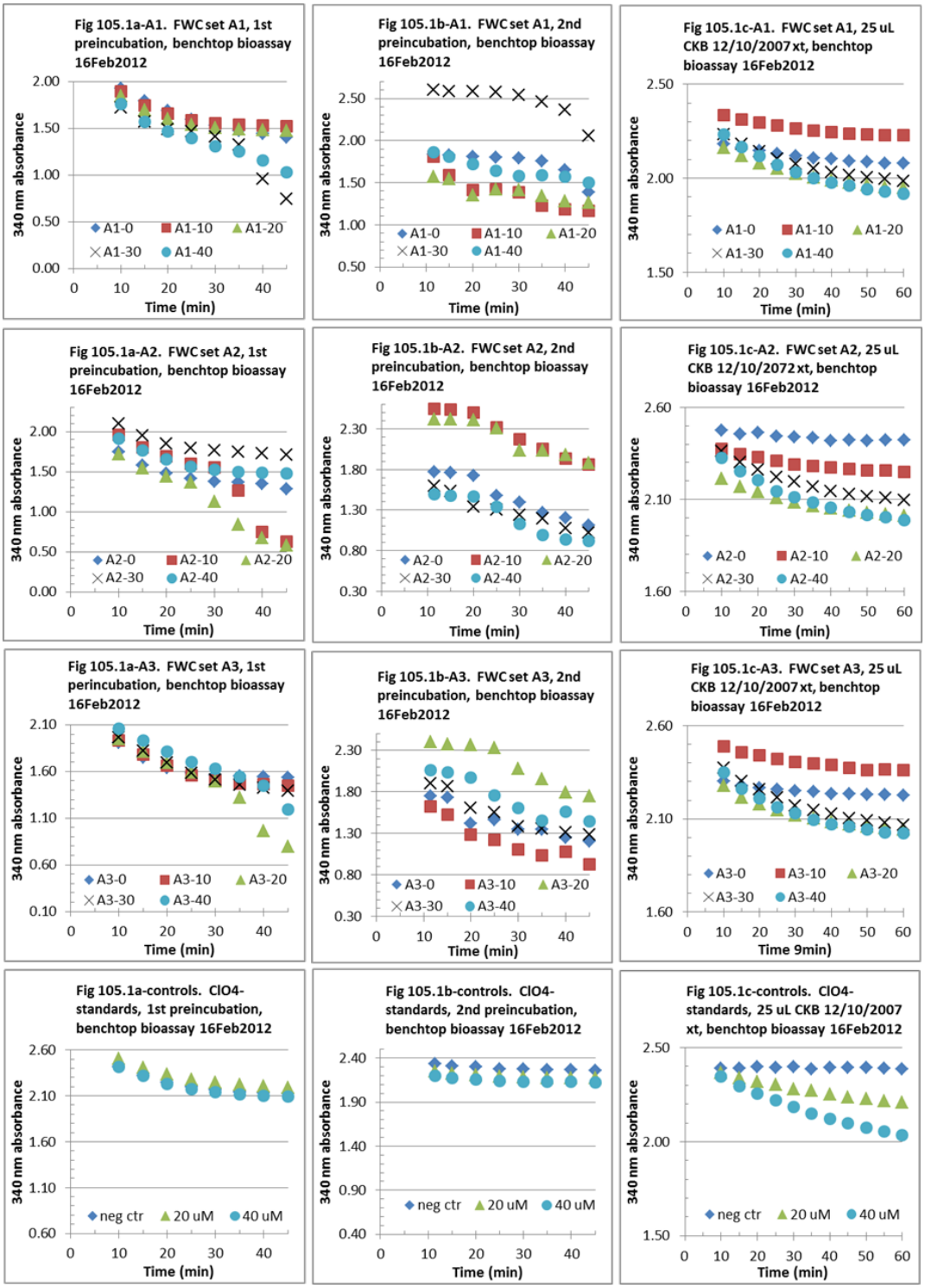



**Figure B-105.1. Fontana Water Company, benchtop bioassay batch 1, sets A3, A2, A1, and perchlorate controls. Test date 16 Feb 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-105.1a, b, and c

Data for Figure B-105.1.
Groundwater sample 105, FWC. Standards addition method benchtop bioassay, batch 1, 16Feb2012. SDVB/DTAB eluate sets A1, A2, A3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-105.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 16Feb2012**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 1.9230 | 1.8909 | 1.8462 | 1.7218 | 1.7545 | 1.7499 | 1.9642 | 1.7218 | 2.1005 | 1.9130 |
| 15 | 1.7900 | 1.7431 | 1.6975 | 1.5639 | 1.5709 | 1.5792 | 1.8058 | 1.5460 | 1.9493 | 1.7612 |
| 20 | 1.6843 | 1.6517 | 1.6005 | 1.4872 | 1.4638 | 1.4760 | 1.6918 | 1.4412 | 1.8510 | 1.6547 |
| 25 | 1.5931 | 1.5821 | 1.5336 | 1.4611 | 1.3904 | 1.4124 | 1.6033 | 1.3650 | 1.7899 | 1.5650 |
| 30 | 1.5244 | 1.5497 | 1.5036 | 1.4126 | 1.3028 | 1.3777 | 1.5515 | 1.1278 | 1.7622 | 1.5235 |
| 35 | 1.4733 | 1.5324 | 1.4884 | 1.3200 | 1.2529 | 1.3634 | 1.2686 | 0.8452 | 1.7454 | 1.4998 |
| 40 | 1.4394 | 1.5262 | 1.4802 | 0.9589 | 1.1520 | 1.3524 | 0.7465 | 0.6715 | 1.7294 | 1.4882 |
| 45 | 1.3979 | 1.5239 | 1.4744 | 0.7450 | 1.0257 | 1.2878 | 0.6274 | 0.5826 | 1.7100 | 1.4780 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 1.9088 | 1.9317 | 1.9496 | 1.9634 | 2.0600 | 2.4179 | 2.5017 | 2.4181 |
| 15 | 1.7485 | 1.7847 | 1.8172 | 1.8232 | 1.9304 | 2.3207 | 2.4086 | 2.3174 |
| 20 | 1.6388 | 1.6601 | 1.6969 | 1.6975 | 1.8148 | 2.2424 | 2.3317 | 2.2309 |
| 25 | 1.5841 | 1.5630 | 1.5914 | 1.5868 | 1.7024 | 2.1993 | 2.2790 | 2.1699 |
| 30 | 1.5626 | 1.5074 | 1.4920 | 1.5115 | 1.6299 | 2.1659 | 2.2448 | 2.1393 |
| 35 | 1.5537 | 1.4739 | 1.3190 | 1.4569 | 1.5412 | 2.1450 | 2.2193 | 2.1129 |
| 40 | 1.5439 | 1.4615 | 0.9605 | 1.4218 | 1.4470 | 2.1321 | 2.2052 | 2.1003 |
| 45 | 1.5375 | 1.4514 | 0.7966 | 1.3909 | 1.1970 | 2.1246 | 2.1909 | 2.0917 |

**Table B-105.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 16Feb2012**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 |
| 11.5 | 1.8327 | 1.8113 | 1.5685 | 2.6006 | 1.8633 | 1.7701 | 2.5433 | 2.4171 | 1.5911 | 1.4909 |
| 15 | 1.8248 | 1.5879 | 1.5494 | 2.5894 | 1.8084 | 1.7602 | 2.5375 | 2.4190 | 1.5324 | 1.4779 |
| 20 | 1.8072 | 1.4141 | 1.3483 | 2.5820 | 1.7219 | 1.7188 | 2.5042 | 2.4074 | 1.3400 | 1.4632 |
| 25 | 1.8009 | 1.4236 | 1.4246 | 2.5731 | 1.6466 | 1.4763 | 2.3184 | 2.3091 | 1.2982 | 1.3344 |
| 30 | 1.7956 | 1.3859 | 1.4134 | 2.5408 | 1.5808 | 1.3929 | 2.1739 | 2.0362 | 1.2425 | 1.1298 |
| 35 | 1.7594 | 1.2255 | 1.3404 | 2.4636 | 1.5850 | 1.2667 | 2.0558 | 2.0358 | 1.1926 | 0.9892 |
| 40 | 1.6507 | 1.1850 | 1.2815 | 2.3672 | 1.5686 | 1.2010 | 1.9351 | 1.9803 | 1.0760 | 0.9375 |
| 45 | 1.3841 | 1.1645 | 1.2600 | 2.0584 | 1.5048 | 1.1095 | 1.8580 | 1.8765 | 1.0166 | 0.9226 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 | neg ctr | 20 µM | 40 µM |
| 11.5 | 1.7512 | 1.6273 | 2.4048 | 1.8968 | 2.0589 | 2.3324 | 2.2478 | 2.1963 |
| 15 | 1.7339 | 1.5227 | 2.3768 | 1.8682 | 2.0313 | 2.3126 | 2.2211 | 2.1743 |
| 20 | 1.4205 | 1.2859 | 2.3627 | 1.6041 | 1.9720 | 2.2965 | 2.2062 | 2.1533 |
| 25 | 1.4655 | 1.2245 | 2.3257 | 1.5552 | 1.7566 | 2.2745 | 2.1924 | 2.1401 |
| 30 | 1.3444 | 1.1023 | 2.0791 | 1.3838 | 1.6034 | 2.2771 | 2.1838 | 2.1328 |
| 35 | 1.3444 | 1.0387 | 1.9545 | 1.3573 | 1.4565 | 2.2701 | 2.1766 | 2.1297 |
| 40 | 1.2487 | 1.0775 | 1.7965 | 1.3098 | 1.5584 | 2.2688 | 2.1741 | 2.1285 |
| 45 | 1.2037 | 0.9252 | 1.7518 | 1.2867 | 1.4424 | 2.2617 | 2.1710 | 2.1228 |

**Table B-105.1c. Batch 1, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 16Feb2012**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 2.1830 | 2.3346 | 2.1616 | 2.2333 | 2.2320 | 2.4763 | 2.3741 | 2.2134 | 2.3629 | 2.3261 |
| 15 | 2.1601 | 2.3125 | 2.1175 | 2.1809 | 2.1651 | 2.4571 | 2.3455 | 2.1675 | 2.3022 | 2.2541 |
| 20 | 2.1441 | 2.2965 | 2.0799 | 2.1418 | 2.1183 | 2.4618 | 2.3305 | 2.1384 | 2.2627 | 2.2047 |
| 25 | 2.1304 | 2.2793 | 2.0499 | 2.1037 | 2.0710 | 2.4446 | 2.3083 | 2.1089 | 2.2210 | 2.1429 |
| 30 | 2.1185 | 2.2640 | 2.0222 | 2.0739 | 2.0311 | 2.4410 | 2.2910 | 2.0844 | 2.1964 | 2.1124 |
| 35 | 2.1044 | 2.2537 | 2.0043 | 2.0519 | 1.9988 | 2.4345 | 2.2826 | 2.0642 | 2.1696 | 2.0826 |
| 40 | 2.1006 | 2.2455 | 1.9861 | 2.0329 | 1.9773 | 2.4181 | 2.2725 | 2.0513 | 2.1442 | 2.0545 |
| 45 | 2.0919 | 2.2360 | 1.9755 | 2.0140 | 1.9606 | 2.4243 | 2.2656 | 2.0376 | 2.1286 | 2.0296 |
| 50 | 2.0860 | 2.2332 | 1.9625 | 2.0034 | 1.9411 | 2.4201 | 2.2591 | 2.0267 | 2.1157 | 2.0166 |
| 55 | 2.0800 | 2.2271 | 1.9544 | 1.9942 | 1.9287 | 2.4219 | 2.2555 | 2.0184 | 2.1089 | 2.0035 |
| 60 | 2.0781 | 2.2268 | 1.9472 | 1.9838 | 1.9188 | 2.4238 | 2.2485 | 2.0118 | 2.0967 | 1.9879 |
| **Rate[a]** | 0.0020 | 0.0021 | 0.0041 | 0.0048 | 0.0060 | 0.0011 | 0.0024 | 0.0038 | 0.0051 | 0.0064 |
| **$r^2$** | 0.9386 | 0.9272 | 0.9266 | 0.9308 | 0.9299 | 0.8326 | 0.9240 | 0.9305 | 0.9281 | 0.9290 |
| **Rate – neg ctrl rate** | 0.0020 | 0.0021 | 0.0041 | 0.0048 | 0.0060 | 0.0011 | 0.0024 | 0.0038 | 0.0051 | 0.0064 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 2.3035 | 2.4874 | 2.2791 | 2.3717 | 2.3500 | 2.3901 | 2.3670 | 2.3449 |
| 15 | 2.2795 | 2.4560 | 2.2152 | 2.3002 | 2.2634 | 2.3888 | 2.3384 | 2.2938 |
| 20 | 2.2666 | 2.4419 | 2.1804 | 2.2589 | 2.2108 | 2.3971 | 2.3196 | 2.2561 |
| 25 | 2.2585 | 2.4200 | 2.1447 | 2.2137 | 2.1627 | 2.3949 | 2.3016 | 2.2211 |
| 30 | 2.2486 | 2.4055 | 2.1201 | 2.1724 | 2.1288 | 2.3978 | 2.2781 | 2.1832 |
| 35 | 2.2454 | 2.3966 | 2.1011 | 2.1472 | 2.0961 | 2.3873 | 2.2697 | 2.1509 |
| 40 | 2.2328 | 2.3878 | 2.0828 | 2.1266 | 2.0725 | 2.3949 | 2.2519 | 2.1218 |
| 45 | 2.2330 | 2.3748 | 2.0705 | 2.1044 | 2.0571 | 2.3920 | 2.2358 | 2.0963 |
| 50 | 2.2288 | 2.3610 | 2.0595 | 2.0898 | 2.0415 | 2.3942 | 2.2288 | 2.0750 |
| 55 | 2.2283 | 2.3671 | 2.0494 | 2.0773 | 2.0258 | 2.3887 | 2.2161 | 2.0546 |
| 60 | 2.2260 | 2.3632 | 2.0426 | 2.0674 | 2.0216 | 2.3877 | 2.2096 | 2.0333 |
| **Rate[a]** | 0.0014 | 0.0024 | 0.0044 | 0.0058 | 0.0061 | 0.00005 | 0.0031 | 0.0061 |
| **$r^2$** | 0.8772 | 0.9221 | 0.9077 | 0.9278 | 0.9044 | 0.0551 | 0.9745 | 0.9819 |
| **Rate – neg ctrl rate** | 0.0014 | 0.0024 | 0.0044 | 0.0058 | 0.0061 | 0.0000 | 0.0031 | 0.0061 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-105.1c-A1, A2, A3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

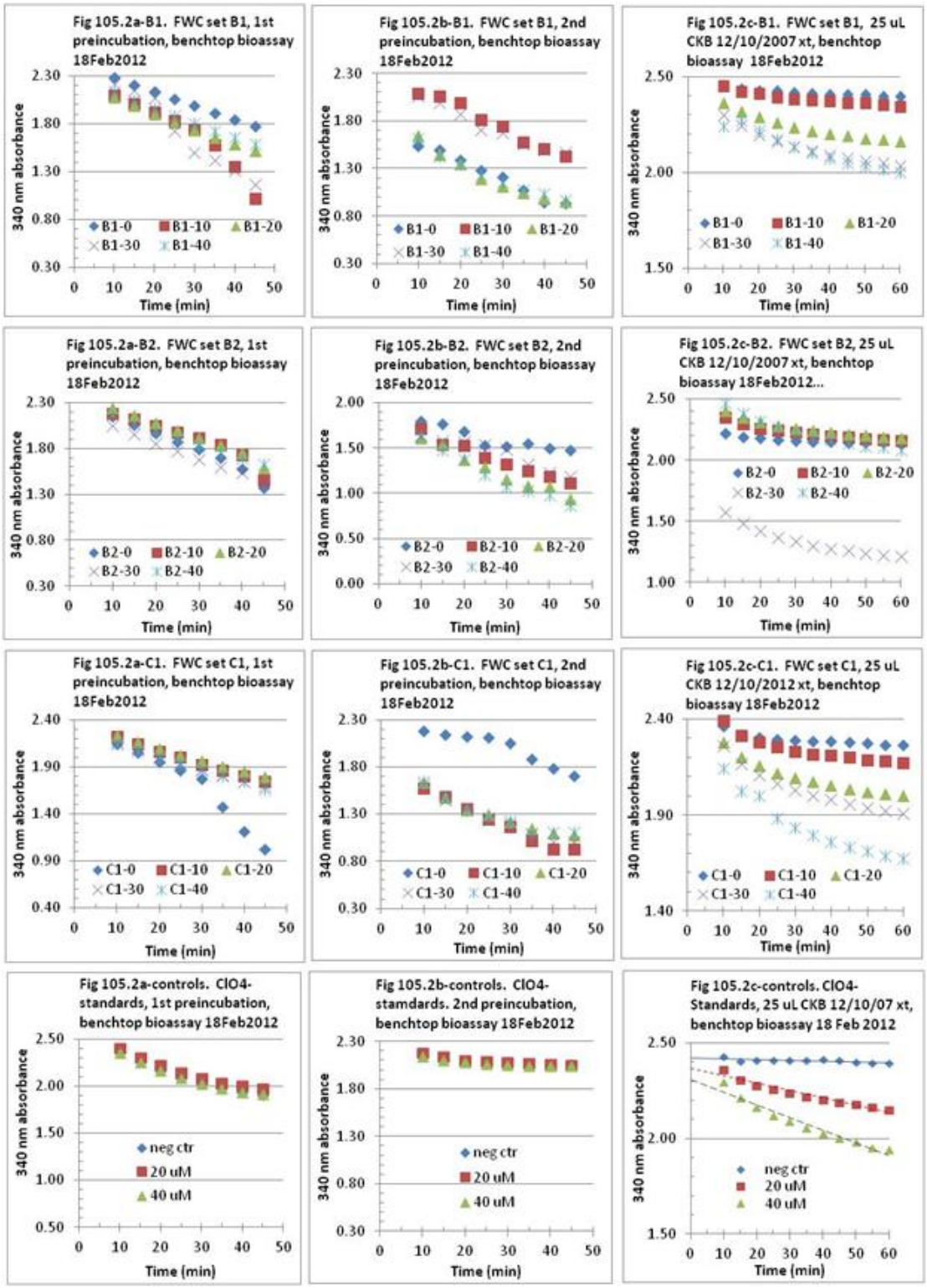



**Figure B-105.2. Fontana Water Company, benchtop bioassay batch 1, sets B1, B2, C1, and perchlorate controls. Test date 18 Feb 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-105.2a, b, and c

Data for Figure B-105.2.
Groundwater sample 105, FWC. Standards addition method benchtop bioassay, batch 2, 18Feb2012. SDVB/DTAB eluate sets B1, B2, C1, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-105.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 18Feb2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 2.2801 | 2.0992 | 2.0786 | 2.2084 | 2.1386 | 2.1486 | 2.1762 | 2.2446 | 2.0511 | 2.1678 |
| 15 | 2.2074 | 2.0096 | 1.9841 | 2.1425 | 2.0498 | 2.0582 | 2.1147 | 2.1624 | 1.9558 | 2.0807 |
| 20 | 2.1308 | 1.9220 | 1.8945 | 2.0640 | 1.9606 | 1.9641 | 2.0494 | 2.0750 | 1.8516 | 1.9888 |
| 25 | 2.0524 | 1.8338 | 1.8123 | 1.7263 | 1.8807 | 1.8730 | 1.9766 | 1.9936 | 1.7667 | 1.9099 |
| 30 | 1.9856 | 1.7351 | 1.7286 | 1.4922 | 1.8031 | 1.7912 | 1.9117 | 1.9120 | 1.6766 | 1.8296 |
| 35 | 1.9079 | 1.5749 | 1.6529 | 1.4210 | 1.7233 | 1.6950 | 1.8464 | 1.8356 | 1.6005 | 1.7631 |
| 40 | 1.8404 | 1.3461 | 1.5826 | 1.3057 | 1.6502 | 1.5767 | 1.7329 | 1.7539 | 1.5289 | 1.6903 |
| 45 | 1.7735 | 1.0163 | 1.5201 | 1.1632 | 1.5843 | 1.3672 | 1.4533 | 1.5895 | 1.4286 | 1.6273 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 | neg ctr | 20 μM | 40 μM |
| 10 | 2.1373 | 2.2153 | 2.2250 | 2.1598 | 2.1376 | 2.3677 | 2.4107 | 2.3533 |
| 15 | 2.0459 | 2.1400 | 2.1601 | 2.0848 | 2.0640 | 2.2720 | 2.3080 | 2.2597 |
| 20 | 1.9434 | 2.0595 | 2.0852 | 2.0059 | 1.9904 | 2.1691 | 2.2264 | 2.1667 |
| 25 | 1.8544 | 1.9936 | 2.0188 | 1.9369 | 1.9240 | 2.0894 | 2.1483 | 2.0912 |
| 30 | 1.7625 | 1.9211 | 1.9547 | 1.8673 | 1.8599 | 2.0226 | 2.0827 | 2.0267 |
| 35 | 1.4699 | 1.8611 | 1.9012 | 1.8050 | 1.7975 | 1.9754 | 2.0381 | 1.9788 |
| 40 | 1.2101 | 1.7971 | 1.8423 | 1.7416 | 1.7342 | 1.9411 | 2.0035 | 1.9420 |
| 45 | 1.0200 | 1.7407 | 1.7898 | 1.6904 | 1.6614 | 1.9104 | 1.9726 | 1.9183 |

**Table B-105.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 18Feb2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 1.5322 | 2.0822 | 1.6420 | 2.0604 | 1.6076 | 1.7988 | 1.7085 | 1.6089 | 1.6275 | 1.6400 |
| 15 | 1.4891 | 2.0547 | 1.4480 | 1.9917 | 1.4245 | 1.7661 | 1.5365 | 1.5446 | 1.5808 | 1.4712 |
| 20 | 1.3840 | 1.9918 | 1.3412 | 1.8712 | 1.3469 | 1.6795 | 1.5254 | 1.3686 | 1.5625 | 1.3703 |
| 25 | 1.2807 | 1.8125 | 1.1914 | 1.7005 | 1.2294 | 1.5236 | 1.3866 | 1.2903 | 1.5354 | 1.2073 |
| 30 | 1.2087 | 1.7413 | 1.1053 | 1.6688 | 1.1440 | 1.5126 | 1.3179 | 1.1506 | 1.4861 | 1.0715 |
| 35 | 1.0721 | 1.5746 | 1.0367 | 1.5487 | 1.0679 | 1.5439 | 1.2434 | 1.0794 | 1.3226 | 1.0267 |
| 40 | 0.9378 | 1.5071 | 0.9781 | 1.4947 | 1.0254 | 1.4949 | 1.1802 | 1.0697 | 1.2197 | 0.9709 |
| 45 | 0.9259 | 1.4206 | 0.9543 | 1.4681 | 0.9637 | 1.4724 | 1.1090 | 0.9336 | 1.1883 | 0.8632 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 | neg ctr | 20 µM | 40 µM |
| 10 | 2.1783 | 1.5741 | 1.6293 | 1.6066 | 1.6373 | 2.1720 | 2.1827 | 2.1407 |
| 15 | 2.1359 | 1.4820 | 1.4731 | 1.4411 | 1.4789 | 2.1307 | 2.1395 | 2.1026 |
| 20 | 2.1175 | 1.3514 | 1.3372 | 1.3658 | 1.3335 | 2.1018 | 2.1061 | 2.0803 |
| 25 | 2.1035 | 1.2412 | 1.2930 | 1.2274 | 1.2648 | 2.0897 | 2.0898 | 2.0646 |
| 30 | 2.0481 | 1.1585 | 1.2112 | 1.1941 | 1.2235 | 2.0802 | 2.0777 | 2.0544 |
| 35 | 1.8826 | 1.0105 | 1.1415 | 1.0795 | 1.0945 | 2.0677 | 2.0739 | 2.0465 |
| 40 | 1.7777 | 0.9267 | 1.0890 | 1.0019 | 1.1064 | 2.0660 | 2.0620 | 2.0418 |
| 45 | 1.7034 | 0.9210 | 1.0756 | 1.0008 | 1.1006 | 2.0583 | 2.0547 | 2.0374 |

**Table B-105.2c. Batch 2, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 18Feb2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B1-0** | **B1-10** | **B1-20** | **B1-30** | **B1-40** | **B2-0** | **B2-10** | **B2-20** | **B2-30** | **B2-40** |
| 10 | 2.4537 | 2.4517 | 2.3646 | 2.2965 | 2.2396 | 2.2207 | 2.3513 | 2.4011 | 1.5784 | 2.4633 |
| 15 | 2.4380 | 2.4188 | 2.3170 | 2.2441 | 2.2700 | 2.1923 | 2.2962 | 2.3479 | 1.4854 | 2.3819 |
| 20 | 2.4235 | 2.4094 | 2.2899 | 2.1953 | 2.2166 | 2.1792 | 2.2602 | 2.3141 | 1.4201 | 2.3186 |
| 25 | 2.4238 | 2.3921 | 2.2608 | 2.1626 | 2.1717 | 2.1665 | 2.2427 | 2.2852 | 1.3703 | 2.2692 |
| 30 | 2.4156 | 2.3795 | 2.2365 | 2.1340 | 2.1326 | 2.1587 | 2.2303 | 2.2620 | 1.3347 | 2.2285 |
| 35 | 2.4107 | 2.3748 | 2.2154 | 2.1119 | 2.1013 | 2.1539 | 2.2155 | 2.2445 | 1.3026 | 2.1875 |
| 40 | 2.4040 | 2.3729 | 2.2026 | 2.0888 | 2.0719 | 2.1495 | 2.2002 | 2.2299 | 1.2769 | 2.1648 |
| 45 | 2.4081 | 2.3631 | 2.1898 | 2.0743 | 2.0488 | 2.1418 | 2.1917 | 2.2155 | 1.2572 | 2.1349 |
| 50 | 2.4056 | 2.3601 | 2.1755 | 2.0606 | 2.0318 | 2.1429 | 2.1761 | 2.2052 | 1.2400 | 2.1165 |
| 55 | 2.3942 | 2.3542 | 2.1691 | 2.0476 | 2.0124 | 2.1401 | 2.1686 | 2.1907 | 1.2243 | 2.1055 |
| 60 | 2.3981 | 2.3414 | 2.1605 | 2.0336 | 2.0019 | 2.1327 | 2.1554 | 2.1841 | 1.2136 | 2.0839 |
| **Rate[a]** | 0.0010 | 0.0019 | 0.0039 | 0.005 | 0.0056 | 0.0015 | 0.0034 | 0.0040 | 0.0067 | 0.0072 |
| **$r^2$** | 0.8697 | 0.9102 | 0.9361 | 0.9375 | 0.9585 | 0.8626 | 0.9173 | 0.9295 | 0.9061 | 0.9339 |
| **Rate – neg ctrl rate** | 0.0006 | 0.0015 | 0.0035 | 0.0046 | 0.0052 | 0.0011 | 0.0030 | 0.0036 | 0.0063 | 0.0068 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C1-0** | **C1-10** | **C1-20** | **C1-30** | **C1-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 2.3629 | 2.3905 | 2.2776 | 2.2568 | 2.1398 | 2.4286 | 2.3626 | 2.2939 |
| 15 | 2.3147 | 2.3112 | 2.1996 | 2.1631 | 2.0221 | 2.4023 | 2.3067 | 2.2141 |
| 20 | 2.3024 | 2.2793 | 2.1549 | 2.1075 | 1.9982 | 2.4115 | 2.2785 | 2.1640 |
| 25 | 2.2925 | 2.2540 | 2.1148 | 2.0624 | 1.8837 | 2.4094 | 2.2554 | 2.1204 |
| 30 | 2.2870 | 2.2306 | 2.0897 | 2.0284 | 1.8343 | 2.4091 | 2.2348 | 2.0900 |
| 35 | 2.2828 | 2.2151 | 2.0702 | 1.9984 | 1.7927 | 2.4085 | 2.2187 | 2.0558 |
| 40 | 2.2836 | 2.2070 | 2.0520 | 1.9771 | 1.7586 | 2.4120 | 2.2035 | 2.0267 |
| 45 | 2.2771 | 2.1973 | 2.0326 | 1.9530 | 1.7309 | 2.4091 | 2.1894 | 2.0012 |
| 50 | 2.2722 | 2.1859 | 2.0196 | 1.9366 | 1.7081 | 2.3985 | 2.1764 | 1.9799 |
| 55 | 2.2634 | 2.1780 | 2.0066 | 1.9194 | 1.6849 | 2.3963 | 2.1614 | 1.9541 |
| 60 | 2.2606 | 2.1685 | 2.0005 | 1.9065 | 1.6721 | 2.3938 | 2.1486 | 1.9399 |
| **Rate[a]** | 0.0015 | 0.0037 | 0.0050 | 0.0064 | 0.0090 | 0.0004 | 0.0039 | 0.0067 |
| **$r^2$** | 0.7818 | 0.8589 | 0.9028 | 0.9168 | 0.9224 | 0.5495 | 0.9473 | 0.9571 |
| **Rate – neg ctrl rate** | 0.0011 | 0.0033 | 0.0046 | 0.0060 | 0.0086 | 0.0000 | 0.0035 | 0.0063 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-105.2c-B1, B2, C1, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

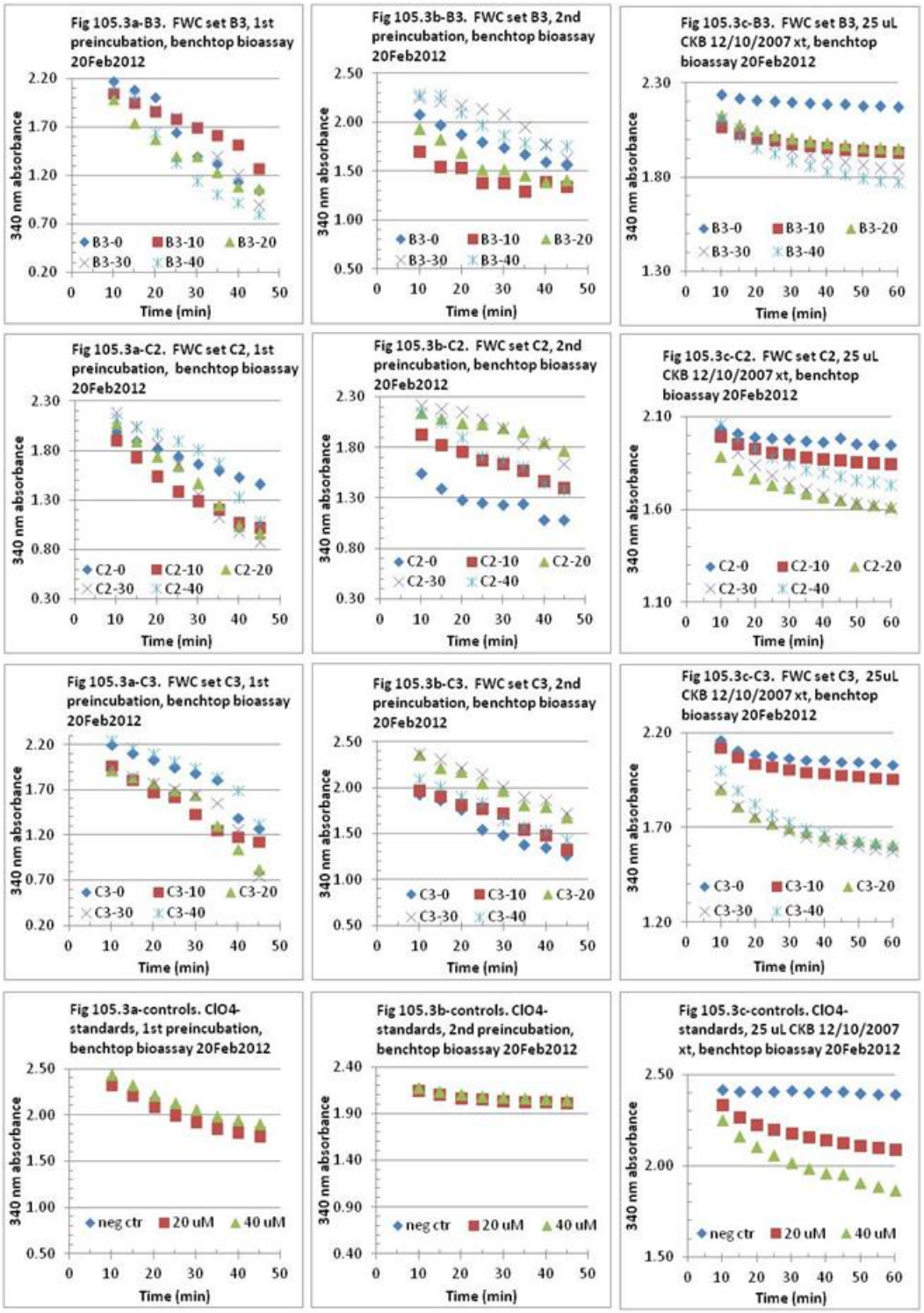



**Figure B-105.3. Fontana Water Company, benchtop bioassay batch 3, sets B3, C2, C3, and perchlorate controls. Test date 20 Feb 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-105.3a, b, and c

Data for Figure B-105.3.
Groundwater sample 105, FWC. Standards addition method benchtop bioassay, batch 3, 20Feb2012. SDVB/DTAB eluate sets B3, C2, C3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-105.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 20Feb2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** |
| 10 | 2.1828 | 2.0495 | 1.9912 | 2.0485 | 2.1322 | 1.9906 | 1.9027 | 2.0722 | 2.1856 | 2.1212 |
| 15 | 2.0955 | 1.9519 | 1.7463 | 1.9700 | 2.0120 | 1.8968 | 1.7329 | 1.8989 | 2.0383 | 2.0357 |
| 20 | 2.0098 | 1.8675 | 1.5811 | 1.8885 | 1.6519 | 1.8211 | 1.5427 | 1.7435 | 1.8665 | 1.9705 |
| 25 | 1.6513 | 1.7848 | 1.4013 | 1.8117 | 1.3347 | 1.7452 | 1.3885 | 1.6459 | 1.6627 | 1.8895 |
| 30 | 1.4025 | 1.6947 | 1.4044 | 1.7040 | 1.1466 | 1.6668 | 1.2888 | 1.4712 | 1.3558 | 1.8070 |
| 35 | 1.3295 | 1.6165 | 1.2328 | 1.4041 | 1.0083 | 1.5990 | 1.2034 | 1.2527 | 1.1305 | 1.6738 |
| 40 | 1.1371 | 1.5232 | 1.0893 | 1.2211 | 0.9259 | 1.5319 | 1.0656 | 1.0407 | 0.9831 | 1.3281 |
| 45 | 1.0533 | 1.2807 | 1.0675 | 0.9060 | 0.8092 | 1.4586 | 1.0168 | 0.9610 | 0.8783 | 1.0753 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C3-0** | **C3-10** | **C3-20** | **C3-30** | **C3-40** | **neg ctr** | **20 μM** | **40 μM** |
| 10 | 2.1981 | 1.9695 | 1.9187 | 1.9349 | 2.2580 | 2.3908 | 2.3186 | 2.4298 |
| 15 | 2.1104 | 1.8113 | 1.8434 | 1.8516 | 2.1727 | 2.2732 | 2.2059 | 2.3171 |
| 20 | 2.0325 | 1.6793 | 1.7770 | 1.7807 | 2.1037 | 2.1738 | 2.0874 | 2.2071 |
| 25 | 1.9540 | 1.6200 | 1.6953 | 1.7168 | 2.0215 | 2.0833 | 1.9967 | 2.1263 |
| 30 | 1.8816 | 1.4332 | 1.6486 | 1.6537 | 1.9517 | 2.0070 | 1.9188 | 2.0504 |
| 35 | 1.8091 | 1.2542 | 1.3098 | 1.5655 | 1.8448 | 1.9464 | 1.8519 | 1.9876 |
| 40 | 1.3919 | 1.1871 | 1.0515 | 1.2716 | 1.6984 | 1.8981 | 1.8036 | 1.9433 |
| 45 | 1.2746 | 1.1285 | 0.8235 | 0.7484 | 1.3327 | 1.8627 | 1.7641 | 1.9047 |

**Table B-105.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 20Feb2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 | C2-0 | C2-10 | C2-20 | C2-30 | C2-40 |
| 10 | 2.0805 | 1.7026 | 1.9349 | 2.2533 | 2.2802 | 1.5414 | 1.9325 | 2.1465 | 2.2173 | 2.1428 |
| 15 | 1.9741 | 1.5461 | 1.8200 | 2.2094 | 2.2705 | 1.3962 | 1.8202 | 2.0904 | 2.1822 | 2.0535 |
| 20 | 1.8706 | 1.5362 | 1.6916 | 2.1734 | 2.0913 | 1.2793 | 1.7613 | 2.0425 | 2.1523 | 1.8999 |
| 25 | 1.7965 | 1.3742 | 1.5162 | 2.1309 | 1.9743 | 1.2523 | 1.6775 | 2.0269 | 2.0780 | 1.7163 |
| 30 | 1.7411 | 1.3812 | 1.5152 | 2.0787 | 1.8642 | 1.2334 | 1.6363 | 1.9949 | 1.9949 | 1.6649 |
| 35 | 1.6729 | 1.2912 | 1.4528 | 1.9537 | 1.7825 | 1.2379 | 1.5702 | 1.9572 | 1.8339 | 1.6120 |
| 40 | 1.5958 | 1.3895 | 1.3876 | 1.7648 | 1.7770 | 1.0858 | 1.4660 | 1.8495 | 1.8334 | 1.4528 |
| 45 | 1.5630 | 1.3402 | 1.4085 | 1.6316 | 1.7537 | 1.0829 | 1.4037 | 1.7702 | 1.6398 | 1.3820 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C3-0 | C3-10 | C3-20 | C3-30 | C3-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.9256 | 1.9695 | 2.3624 | 2.3697 | 2.0848 | 2.1651 | 2.1475 | 2.1783 |
| 15 | 1.8627 | 1.9041 | 2.2165 | 2.3109 | 2.0052 | 2.1152 | 2.1033 | 2.1395 |
| 20 | 1.7602 | 1.8105 | 2.1690 | 2.2116 | 1.8986 | 2.0817 | 2.0662 | 2.1091 |
| 25 | 1.5406 | 1.7721 | 2.0434 | 2.1413 | 1.8319 | 2.0622 | 2.0501 | 2.0865 |
| 30 | 1.4841 | 1.7185 | 1.9666 | 2.0033 | 1.6447 | 2.0480 | 2.0354 | 2.0746 |
| 35 | 1.3803 | 1.5423 | 1.8072 | 1.8977 | 1.5689 | 2.0416 | 2.0272 | 2.0650 |
| 40 | 1.3476 | 1.4779 | 1.7882 | 1.8604 | 1.5283 | 2.0333 | 2.0210 | 2.0569 |
| 45 | 1.2655 | 1.3211 | 1.6788 | 1.7158 | 1.4294 | 2.0285 | 2.0168 | 2.0481 |

**Table B-105.3c. Batch 3, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 20Feb2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** |
| 10 | 2.2369 | 2.0671 | 2.1285 | 2.1129 | 2.1002 | 2.0365 | 1.9916 | 1.8854 | 1.9990 | 2.0603 |
| 15 | 2.2182 | 2.0289 | 2.0775 | 2.0569 | 2.0182 | 2.0075 | 1.9507 | 1.8144 | 1.9053 | 1.9830 |
| 20 | 2.2066 | 2.0059 | 2.0482 | 2.0113 | 1.9581 | 1.9908 | 1.9285 | 1.7687 | 1.8391 | 1.9260 |
| 25 | 2.2012 | 1.9938 | 2.0234 | 1.9746 | 1.9238 | 1.9836 | 1.9087 | 1.7317 | 1.7858 | 1.8830 |
| 30 | 2.1975 | 1.9781 | 2.0047 | 1.9422 | 1.8868 | 1.9760 | 1.8958 | 1.7157 | 1.7427 | 1.8503 |
| 35 | 2.1917 | 1.9661 | 1.9930 | 1.9164 | 1.8603 | 1.9682 | 1.8826 | 1.6862 | 1.7071 | 1.8169 |
| 40 | 2.1863 | 1.9537 | 1.9801 | 1.8998 | 1.8308 | 1.9640 | 1.8729 | 1.6667 | 1.6807 | 1.7982 |
| 45 | 2.1851 | 1.9477 | 1.9720 | 1.8805 | 1.8135 | 1.9826 | 1.8645 | 1.6486 | 1.6577 | 1.7768 |
| 50 | 2.1775 | 1.9421 | 1.9617 | 1.8668 | 1.7955 | 1.9542 | 1.8562 | 1.6347 | 1.6375 | 1.7590 |
| 55 | 2.1779 | 1.9355 | 1.9553 | 1.8513 | 1.7797 | 1.9499 | 1.8508 | 1.6233 | 1.6210 | 1.7477 |
| 60 | 2.1703 | 1.9286 | 1.9501 | 1.8442 | 1.7737 | 1.9461 | 1.8458 | 1.6135 | 1.6059 | 1.7341 |
| **Rate[a]** | 0.0011 | 0.0025 | 0.0031 | 0.0051 | 0.0061 | 0.0015 | 0.0026 | 0.0050 | 0.0073 | 0.0061 |
| **$r^2$** | 0.9185 | 0.9163 | 0.8938 | 0.9342 | 0.9192 | 0.8231 | 0.9102 | 0.9195 | 0.9197 | 0.9200 |
| **Rate – neg ctrl rate** | 0.0007 | 0.0020 | 0.0028 | 0.0047 | 0.0056 | 0.0010 | 0.0022 | 0.0045 | 0.0069 | 0.0056 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C3-0** | **C3-10** | **C3-20** | **C3-30** | **C3-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 2.1630 | 2.1220 | 1.9014 | 1.9169 | 2.0033 | 2.4183 | 2.3326 | 2.2504 |
| 15 | 2.0177 | 2.0707 | 1.8132 | 1.8185 | 1.8975 | 2.4075 | 2.2688 | 2.1623 |
| 20 | 2.0863 | 2.0382 | 1.7579 | 1.7587 | 1.8246 | 2.4080 | 2.2245 | 2.1048 |
| 25 | 2.0760 | 2.0204 | 1.7229 | 1.7223 | 1.7714 | 2.4064 | 2.2004 | 2.0575 |
| 30 | 2.0682 | 2.0053 | 1.6959 | 1.6813 | 1.7309 | 2.4118 | 2.1788 | 2.0165 |
| 35 | 2.0581 | 1.9923 | 1.6739 | 1.6535 | 1.6956 | 2.4007 | 2.1584 | 1.9869 |
| 40 | 2.0548 | 1.9849+ | 1.6577 | 1.6304 | 1.6694 | 2.4061 | 2.1430 | 1.9577 |
| 45 | 2.0479 | 1.9773 | 1.6431 | 1.6138 | 1.6450 | 2.4050 | 2.1259 | 1.9523 |
| 50 | 2.0449 | 1.9700 | 1.6313 | 1.5990 | 1.6252 | 2.3956 | 2.1097 | 1.9068 |
| 55 | 2.0398 | 1.9627 | 1.6225 | 1.5875 | 1.6089 | 2.3915 | 2.1000 | 1.8870 |
| 60 | 2.0336 | 1.9580 | 1.6129 | 1.5781 | 1.5948 | 2.3902 | 2.0903 | 1.8647 |
| **Rate[a]** | 0.0020 | 0.0028 | 0.0051 | 0.0061 | 0.0075 | 0.0005 | 0.0044 | 0.0071 |
| **$r^2$** | 0.7982 | 0.8643 | 0.8671 | 0.8893 | 0.9068 | 0.7773 | 0.9225 | 0.9460 |
| **Rate – neg ctrl rate** | 0.0016 | 0.0024 | 0.0046 | 0.0057 | 0.0070 | 0.0000 | 0.0039 | 0.0066 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-105.3c-B3, C2, C3, and controls. $r^2$ is the correlation c
oefficient for the corresponding regression line for each reaction mixture

## Groundwater sample 106, Massachusetts Military Reservation, location J3-INF, collected 23Feb2012

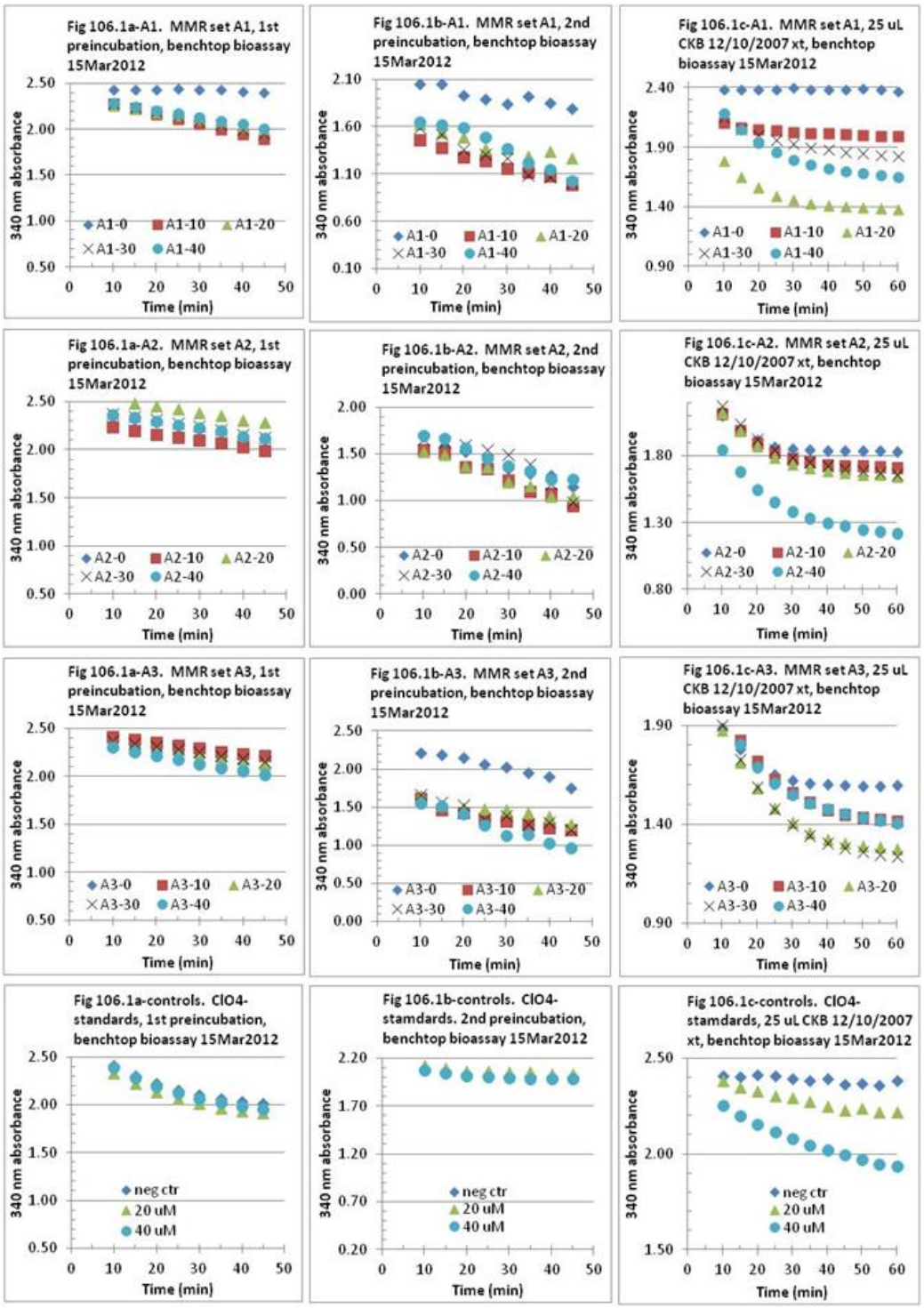



**Figure B-106.1. Massachusetts Military Reservation, benchtop bioassay batch 1, sets A1, A2, A3, and perchlorate controls. Test date 15 Mar 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-106.1a, b, and c

Data for Figure B-106.1.
Groundwater sample 106, Massachusetts Military Reservation. Standards addition method benchtop bioassay, batch 1, 15Mar2012. SDVB/DTAB eluate sets A1, A2, A3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-106.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 15Mar2012**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 |
| 10 | 2.4409 | 2.2802 | 2.2652 | 2.2844 | 2.2912 | 2.3424 | 2.2320 | 2.5058 | 2.3757 | 2.3686 |
| 15 | 2.4348 | 2.2253 | 2.2250 | 2.2365 | 2.2499 | 2.3112 | 2.1912 | 2.4794 | 2.3453 | 2.3325 |
| 20 | 2.4413 | 2.1714 | 2.1918 | 2.1938 | 2.2114 | 2.2804 | 2.1600 | 2.4501 | 2.3097 | 2.2967 |
| 25 | 2.4457 | 2.1186 | 2.1555 | 2.1472 | 2.1728 | 2.2486 | 2.1274 | 2.4184 | 2.2779 | 2.2598 |
| 30 | 2.4359 | 2.0608 | 2.1192 | 2.0970 | 2.1354 | 2.2104 | 2.0967 | 2.3877 | 2.2410 | 2.2211 |
| 35 | 2.4396 | 2.0028 | 2.0796 | 2.0510 | 2.0970 | 2.1777 | 2.0633 | 2.3493 | 2.2008 | 2.1923 |
| 40 | 2.4194 | 1.9450 | 2.0320 | 2.0061 | 2.0597 | 2.1368 | 2.0324 | 2.3044 | 2.1547 | 2.1404 |
| 45 | 2.4091 | 1.8938 | 2.0003 | 1.9589 | 2.0096 | 2.1052 | 1.9933 | 2.2813 | 2.1310 | 2.1186 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 | neg ctr | 20 μM | 40 μM |
| 10 | 2.3553 | 2.4137 | 2.3370 | 2.3784 | 2.3051 | 2.4136 | 2.3371 | 2.3886 |
| 15 | 2.3111 | 2.3819 | 2.2943 | 2.3418 | 2.2536 | 2.3021 | 2.2249 | 2.2791 |
| 20 | 2.2833 | 2.3555 | 2.2659 | 2.3126 | 2.2144 | 2.2260 | 2.1342 | 2.1908 |
| 25 | 2.2473 | 2.3235 | 2.2349 | 2.2835 | 2.1744 | 2.1581 | 2.0642 | 2.1234 |
| 30 | 2.2109 | 2.2936 | 2.2089 | 2.2565 | 2.1318 | 2.1044 | 2.0135 | 2.0676 |
| 35 | 2.1725 | 2.2621 | 2.1789 | 2.2179 | 2.0921 | 2.0661 | 1.9659 | 2.0205 |
| 40 | 2.1369 | 2.2387 | 2.1402 | 2.1846 | 2.0616 | 2.0306 | 1.9328 | 1.9841 |
| 45 | 2.0911 | 2.2130 | 2.1262 | 2.1586 | 2.0220 | 2.0111 | 1.9123 | 1.9589 |

**Table B-106.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 15Mar2012**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 |
| 10 | 2.0500 | 1.4545 | 1.6303 | 1.5825 | 1.6484 | 1.6864 | 1.5474 | 1.5291 | 1.6588 | 1.7001 |
| 15 | 2.0528 | 1.3738 | 1.5401 | 1.5123 | 1.6152 | 1.5897 | 1.5146 | 1.4974 | 1.6247 | 1.6661 |
| 20 | 1.9255 | 1.2801 | 1.4813 | 1.3561 | 1.5893 | 1.5258 | 1.3665 | 1.3741 | 1.5943 | 1.5628 |
| 25 | 1.8878 | 1.2334 | 1.3615 | 1.3009 | 1.4836 | 1.3756 | 1.3458 | 1.3629 | 1.5475 | 1.4565 |
| 30 | 1.8327 | 1.1534 | 1.3678 | 1.2669 | 1.3636 | 1.3421 | 1.2142 | 1.1938 | 1.4897 | 1.3685 |
| 35 | 1.9150 | 1.1123 | 1.2865 | 1.0862 | 1.2183 | 1.2888 | 1.0962 | 1.1629 | 1.3873 | 1.3195 |
| 40 | 1.8487 | 1.0734 | 1.3391 | 1.0625 | 1.1415 | 1.2672 | 1.0724 | 1.0442 | 1.1744 | 1.2330 |
| 45 | 1.7884 | 0.9804 | 1.2682 | 1.0007 | 1.0288 | 1.1442 | 0.9455 | 1.0489 | 0.9845 | 1.2260 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 | neg ctr | 20 µM | 40 µM |
| 10 | 2.2102 | 1.6194 | 1.6533 | 1.6677 | 1.5550 | 2.0961 | 2.1251 | 2.0771 |
| 15 | 2.1836 | 1.4683 | 1.5293 | 1.5649 | 1.5082 | 2.0625 | 2.0923 | 2.0472 |
| 20 | 2.1513 | 1.4218 | 1.4880 | 1.5288 | 1.4197 | 2.0378 | 2.0679 | 2.0185 |
| 25 | 2.0683 | 1.3487 | 1.4712 | 1.3435 | 1.2589 | 2.0241 | 2.0593 | 1.9994 |
| 30 | 2.0214 | 1.3151 | 1.4590 | 1.3842 | 1.1331 | 2.0123 | 2.0496 | 1.9906 |
| 35 | 1.9566 | 1.2655 | 1.4208 | 1.2769 | 1.1339 | 2.0120 | 2.0486 | 1.9869 |
| 40 | 1.9006 | 1.2313 | 1.3795 | 1.2983 | 1.0275 | 1.9993 | 2.0366 | 1.9823 |
| 45 | 1.7525 | 1.1989 | 1.2813 | 1.2061 | 0.9601 | 2.0070 | 2.0329 | 1.9841 |

**Table B-106.1c. Batch 1, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 15Mar2012**

| | Set A1 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 2.3866 | 2.1112 | 1.7855 | 2.1574 | 2.1916 | 2.1053 | 2.1148 | 2.1229 | 2.1751 | 1.8475 |
| 15 | 2.3894 | 2.0698 | 1.6545 | 2.0516 | 2.0505 | 1.9950 | 1.9899 | 1.9864 | 2.0375 | 1.6824 |
| 20 | 2.3848 | 2.0490 | 1.5626 | 2.0040 | 1.9426 | 1.9184 | 1.8957 | 1.8732 | 1.9212 | 1.5495 |
| 25 | 2.3915 | 2.0475 | 1.4974 | 1.9616 | 1.8597 | 1.8703 | 1.8237 | 1.7905 | 1.8441 | 1.4527 |
| 30 | 2.4003 | 2.0288 | 1.4592 | 1.9292 | 1.7986 | 1.8496 | 1.7773 | 1.7387 | 1.7906 | 1.3846 |
| 35 | 2.3905 | 2.0235 | 1.4329 | 1.9030 | 1.7540 | 1.8426 | 1.7508 | 1.7083 | 1.7545 | 1.3347 |
| 40 | 2.3862 | 2.0213 | 1.4165 | 1.8853 | 1.7226 | 1.8375 | 1.7309 | 1.6877 | 1.7278 | 1.2982 |
| 45 | 2.3853 | 2.0132 | 1.4075 | 1.8636 | 1.6982 | 1.8399 | 1.7249 | 1.6759 | 1.7048 | 1.2734 |
| 50 | 2.3930 | 2.0016 | 1.3972 | 1.8500 | 1.6832 | 1.8380 | 1.7232 | 1.6620 | 1.6862 | 1.2499 |
| 55 | 2.3898 | 1.9988 | 1.3906 | 1.8378 | 1.6695 | 1.8394 | 1.7200 | 1.6577 | 1.6764 | 1.2360 |
| 60 | 2.3714 | 1.9977 | 1.3847 | 1.8287 | 1.6500 | 1.8309 | 1.7095 | 1.6456 | 1.6637 | 1.2160 |
| **Rate[a]** | 0.00014 | 0.00195 | 0.00687 | 0.00582 | 0.00984 | 0.00420 | 0.00703 | 0.00839 | 0.00918 | 0.01143 |
| **$r^2$** | 0.1062 | 0.8689 | 0.7832 | 0.8860 | 0.8658 | 0.6339 | 0.7710 | 0.8000 | 0.8455 | 0.8598 |
| **Rate – neg ctrl rate** | -0.00083 | 0.00098 | 0.00590 | 0.00485 | 0.00886 | 0.00323 | 0.00606 | 0.00742 | 0.00821 | 0.01046 |

| | Set A3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 1.8868 | 1.9595 | 1.8746 | 1.8974 | 1.9685 | 2.4090 | 2.3824 | 2.2540 |
| 15 | 1.7777 | 1.8238 | 1.7123 | 1.7245 | 1.8053 | 2.4006 | 2.3451 | 2.1963 |
| 20 | 1.7005 | 1.7151 | 1.5819 | 1.5856 | 1.6887 | 2.4099 | 2.3268 | 2.1540 |
| 25 | 1.6500 | 1.6265 | 1.4828 | 1.4777 | 1.6057 | 2.4091 | 2.3021 | 2.1131 |
| 30 | 1.6188 | 1.5587 | 1.4073 | 1.3966 | 1.5488 | 2.3926 | 2.2916 | 2.0792 |
| 35 | 1.6059 | 1.5084 | 1.3579 | 1.3410 | 1.5064 | 2.3831 | 2.2743 | 2.0466 |
| 40 | 1.6008 | 1.4729 | 1.3231 | 1.3046 | 1.4784 | 2.3923 | 2.2478 | 2.0195 |
| 45 | 1.5943 | 1.4455 | 1.3010 | 1.2785 | 1.4535 | 2.3629 | 2.2282 | 1.9956 |
| 50 | 1.5930 | 1.4313 | 1.2882 | 1.2582 | 1.4345 | 2.3680 | 2.2370 | 1.9721 |
| 55 | 1.5904 | 1.4229 | 1.2816 | 1.2458 | 1.4186 | 2.3572 | 2.2178 | 1.9449 |
| 60 | 1.5943 | 1.4138 | 1.2725 | 1.2355 | 1.4044 | 2.3808 | 2.2195 | 1.9380 |
| **Rate[a]** | 0.00484 | 0.01024 | 0.01102 | 0.01218 | 0.01001 | 0.00097 | 0.00325 | 0.00623 |
| **$r^2$** | 0.69411 | 0.8646 | 0.8315 | 0.84826 | 0.8488 | 0.7138 | 0.9443 | 0.9718 |
| **Rate – neg ctrl rate** | 0.00387 | 0.00927 | 0.01005 | 0.01121 | 0.00904 | 0.00000 | 0.00228 | 0.00526 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-106.1c-A1, A2, A3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

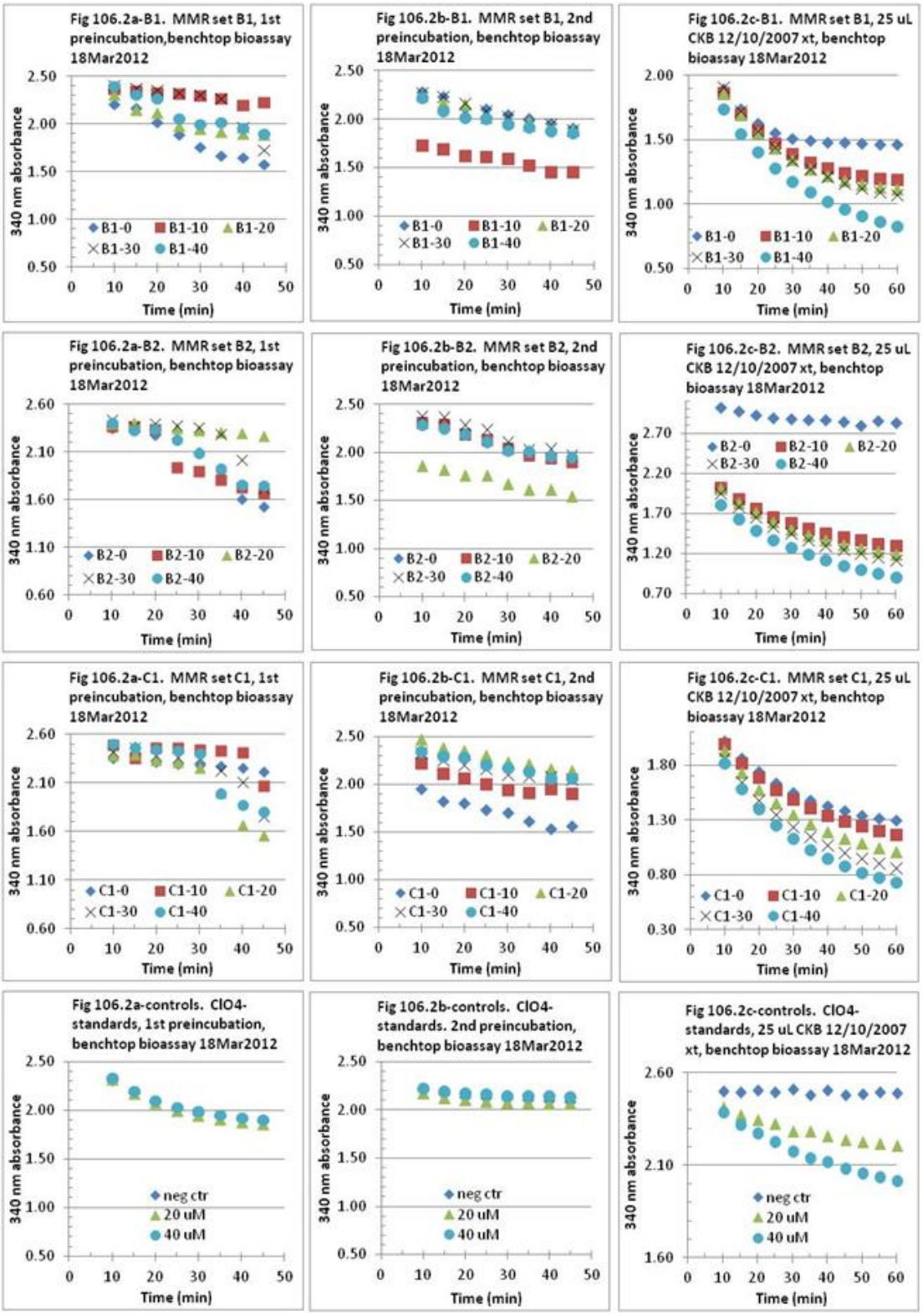



**Figure B-106.2. Massachusetts Military Reservation, benchtop bioassay batch 2, sets B1, B2, C1, and perchlorate controls. Test date 18 Mar 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-106.2a, b, and c

Data for Figure B-106.2.
Groundwater sample 106, Massachusetts Military Reservation. Standards addition method benchtop bioassay, batch 2, 18Mar2012. SDVB/DTAB eluate sets B1, B2, C1, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-106.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 18Mar2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 2.2103 | 2.3737 | 2.3161 | 2.4108 | 2.4061 | 2.3494 | 2.3720 | 2.3991 | 2.4405 | 2.4131 |
| 15 | 2.1718 | 2.3530 | 2.1530 | 2.3783 | 2.3149 | 2.3603 | 2.3760 | 2.4153 | 2.3720 | 2.3325 |
| 20 | 2.0213 | 2.3367 | 2.1268 | 2.3577 | 2.2679 | 2.2815 | 2.3402 | 2.3608 | 2.4015 | 2.3404 |
| 25 | 1.8963 | 2.3222 | 1.9861 | 2.3348 | 2.0620 | 2.2419 | 1.9416 | 2.3493 | 2.3867 | 2.2357 |
| 30 | 1.7669 | 2.3023 | 1.9534 | 2.3127 | 2.0036 | 2.1042 | 1.9063 | 2.3271 | 2.3613 | 2.0887 |
| 35 | 1.6768 | 2.2759 | 1.9178 | 2.2715 | 2.0256 | 1.9021 | 1.8170 | 2.3143 | 2.2938 | 1.9290 |
| 40 | 1.6519 | 2.2056 | 1.9002 | 1.9705 | 1.9637 | 1.6088 | 1.7299 | 2.3042 | 2.0178 | 1.7667 |
| 45 | 1.5837 | 2.2300 | 1.8933 | 1.7330 | 1.9038 | 1.5274 | 1.6758 | 2.2764 | 1.7263 | 1.7568 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 | neg ctr | 20 μM | 40 μM |
| 10 | 2.3576 | 2.4963 | 2.3816 | 2.4218 | 2.5006 | 2.3096 | 2.3176 | 2.3385 |
| 15 | 2.4735 | 2.3558 | 2.3990 | 2.4606 | 2.4602 | 2.1773 | 2.1740 | 2.1984 |
| 20 | 2.3181 | 2.4614 | 2.3348 | 2.3833 | 2.4478 | 2.0835 | 2.0739 | 2.1047 |
| 25 | 2.3005 | 2.4603 | 2.3163 | 2.3685 | 2.4338 | 2.0192 | 1.9978 | 2.0346 |
| 30 | 2.2900 | 2.4445 | 2.2582 | 2.3540 | 2.4084 | 1.9714 | 1.9464 | 1.9918 |
| 35 | 2.2759 | 2.4386 | 2.0024 | 2.2283 | 1.9904 | 1.9341 | 1.9092 | 1.9572 |
| 40 | 2.2587 | 2.4148 | 1.6685 | 2.1155 | 1.8788 | 1.9124 | 1.8803 | 1.9316 |
| 45 | 2.2215 | 2.0694 | 1.5593 | 1.7562 | 1.8092 | 1.8901 | 1.8589 | 1.9077 |

**Table B-106.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 18Mar2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 2.2789 | 1.7322 | 2.2361 | 2.2664 | 2.2185 | 3.1301 | 2.3090 | 1.8634 | 2.3804 | 2.2875 |
| 15 | 2.2347 | 1.6866 | 2.1710 | 2.2308 | 2.0802 | 3.1447 | 2.2851 | 1.8206 | 2.3648 | 2.2439 |
| 20 | 2.1072 | 1.6175 | 2.1500 | 2.1596 | 2.0086 | 3.1670 | 2.1929 | 1.7592 | 2.2884 | 2.1927 |
| 25 | 2.1027 | 1.6077 | 2.0107 | 2.0800 | 1.9995 | 3.1756 | 2.1250 | 1.7586 | 2.2355 | 2.1045 |
| 30 | 2.0393 | 1.5927 | 1.9785 | 2.0227 | 1.9404 | 3.1572 | 2.0380 | 1.6757 | 2.1060 | 2.0154 |
| 35 | 2.0074 | 1.5202 | 1.9616 | 1.9385 | 1.9148 | 3.0999 | 1.9717 | 1.6126 | 2.0328 | 2.0101 |
| 40 | 1.9569 | 1.4526 | 1.9268 | 1.9466 | 1.8773 | 3.1722 | 1.9365 | 1.6136 | 2.0390 | 1.9638 |
| 45 | 1.9045 | 1.4553 | 1.8843 | 1.8859 | 1.8589 | 3.1078 | 1.9025 | 1.5458 | 1.9749 | 1.9540 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.9513 | 2.2224 | 2.4707 | 2.3237 | 2.3434 | 2.2069 | 2.1763 | 2.2239 |
| 15 | 1.8229 | 2.1063 | 2.3756 | 2.2491 | 2.2840 | 2.1607 | 2.1275 | 2.1925 |
| 20 | 1.7945 | 2.0561 | 2.3459 | 2.1962 | 2.2760 | 2.1336 | 2.1035 | 2.1755 |
| 25 | 1.7269 | 1.9974 | 2.3028 | 2.1585 | 2.2036 | 2.1212 | 2.0861 | 2.1627 |
| 30 | 1.6986 | 1.9383 | 2.2336 | 2.1010 | 2.1692 | 2.1085 | 2.0722 | 2.1476 |
| 35 | 1.6053 | 1.9105 | 2.2127 | 2.0791 | 2.1332 | 2.1032 | 2.0720 | 2.1442 |
| 40 | 1.5320 | 1.9470 | 2.1548 | 2.0775 | 2.0600 | 2.0903 | 2.0669 | 2.1407 |
| 45 | 1.5553 | 1.8963 | 2.1362 | 2.0438 | 2.0564 | 2.0876 | 2.0600 | 2.1348 |

**Table B-106.2c. Batch 2, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 18Mar2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B1-0** | **B1-10** | **B1-20** | **B1-30** | **B1-40** | **B2-0** | **B2-10** | **B2-20** | **B2-30** | **B2-40** |
| 10 | 1.8896 | 1.8630 | 1.8578 | 1.9093 | 1.7359 | 3.0142 | 2.0237 | 2.0036 | 1.9492 | 1.8108 |
| 15 | 1.7375 | 1.7036 | 1.6894 | 1.7220 | 1.5476 | 2.9676 | 1.8754 | 1.8314 | 1.7825 | 1.6311 |
| 20 | 1.6237 | 1.5748 | 1.5510 | 1.5670 | 1.4006 | 2.9207 | 1.7548 | 1.7141 | 1.6482 | 1.4879 |
| 25 | 1.5506 | 1.4725 | 1.4365 | 1.4403 | 1.2756 | 2.8933 | 1.6542 | 1.5904 | 1.5348 | 1.3664 |
| 30 | 1.5077 | 1.3883 | 1.3452 | 1.3376 | 1.1747 | 2.8720 | 1.5761 | 1.5011 | 1.4437 | 1.2656 |
| 35 | 1.4895 | 1.3225 | 1.2742 | 1.2703 | 1.0904 | 2.8670 | 1.5034 | 1.4252 | 1.3620 | 1.1803 |
| 40 | 1.4763 | 1.2791 | 1.2169 | 1.2098 | 1.0195 | 2.8646 | 1.4512 | 1.3624 | 1.2979 | 1.1101 |
| 45 | 1.4745 | 1.2395 | 1.1785 | 1.1588 | 0.9584 | 2.8409 | 1.4000 | 1.3066 | 1.2398 | 1.0446 |
| 50 | 1.4692 | 1.2185 | 1.1515 | 1.1221 | 0.9076 | 2.7903 | 1.3589 | 1.2608 | 1.1926 | 0.9920 |
| 55 | 1.4638 | 1.1981 | 1.1288 | 1.0917 | 0.8644 | 2.8523 | 1.3205 | 1.2241 | 1.1467 | 0.9471 |
| 60 | 1.4623 | 1.1889 | 1.1180 | 1.0687 | 0.8247 | 2.8341 | 1.2889 | 1.1914 | 1.1099 | 0.9025 |
| **Rate[a]** | 0.0071 | 0.0128 | 0.0142 | 0.0159 | 0.0174 | 0.0034 | 0.0140 | 0.0156 | 0.0161 | 0.0174 |
| **$r^2$** | 0.7073 | 0.8879 | 0.8955 | 0.9077 | 0.9415 | 0.7845 | 0.9437 | 0.9411 | 0.9478 | 0.9495 |
| **Rate – neg ctrl rate** | 0.0068 | 0.0125 | 0.0139 | 0.0156 | 0.0171 | 0.0031 | 0.0137 | 0.0153 | 0.0158 | 0.0171 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C1-0** | **C1-10** | **C1-20** | **C1-30** | **C1-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 2.0208 | 1.9931 | 1.9304 | 1.8600 | 1.8218 | 2.5001 | 2.4122 | 2.3888 |
| 15 | 1.8620 | 1.8213 | 1.7345 | 1.6475 | 1.5832 | 2.4954 | 2.3699 | 2.3182 |
| 20 | 1.7396 | 1.6906 | 1.5790 | 1.4819 | 1.4027 | 2.5024 | 2.3460 | 2.2718 |
| 25 | 1.6344 | 1.5762 | 1.4520 | 1.3463 | 1.2512 | 2.4941 | 2.3237 | 2.2291 |
| 30 | 1.5495 | 1.4874 | 1.3486 | 1.2382 | 1.1316 | 2.5103 | 2.2840 | 2.1735 |
| 35 | 1.4809 | 1.4108 | 1.2654 | 1.1464 | 1.0297 | 2.4815 | 2.2838 | 2.1387 |
| 40 | 1.4278 | 1.3449 | 1.1944 | 1.0695 | 0.9482 | 2.5055 | 2.2552 | 2.1198 |
| 45 | 1.3829 | 1.2896 | 1.1353 | 1.0028 | 0.8779 | 2.4783 | 2.2367 | 2.0833 |
| 50 | 1.3456 | 1.2448 | 1.0866 | 0.9511 | 0.8224 | 2.4836 | 2.2279 | 2.0549 |
| 55 | 1.3165 | 1.2023 | 1.0466 | 0.9038 | 0.7729 | 2.4939 | 2.2180 | 2.0370 |
| 60 | 1.2943 | 1.1669 | 1.0115 | 0.8646 | 0.7338 | 2.4896 | 2.2081 | 2.0136 |
| **Rate[a]** | 0.0139 | 0.0157 | 0.0175 | 0.0189 | 0.0206 | 0.0003 | 0.0040 | 0.0073 |
| **$r^2$** | 0.9241 | 0.9431 | 0.9324 | 0.9362 | 0.9309 | 0.2005 | 0.9560 | 0.9684 |
| **Rate – neg ctrl rate** | 0.0136 | 0.0154 | 0.0172 | 0.0186 | 0.0203 | 0.0000 | 0.0037 | 0.0070 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-106.2c-B1, B2, C1, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

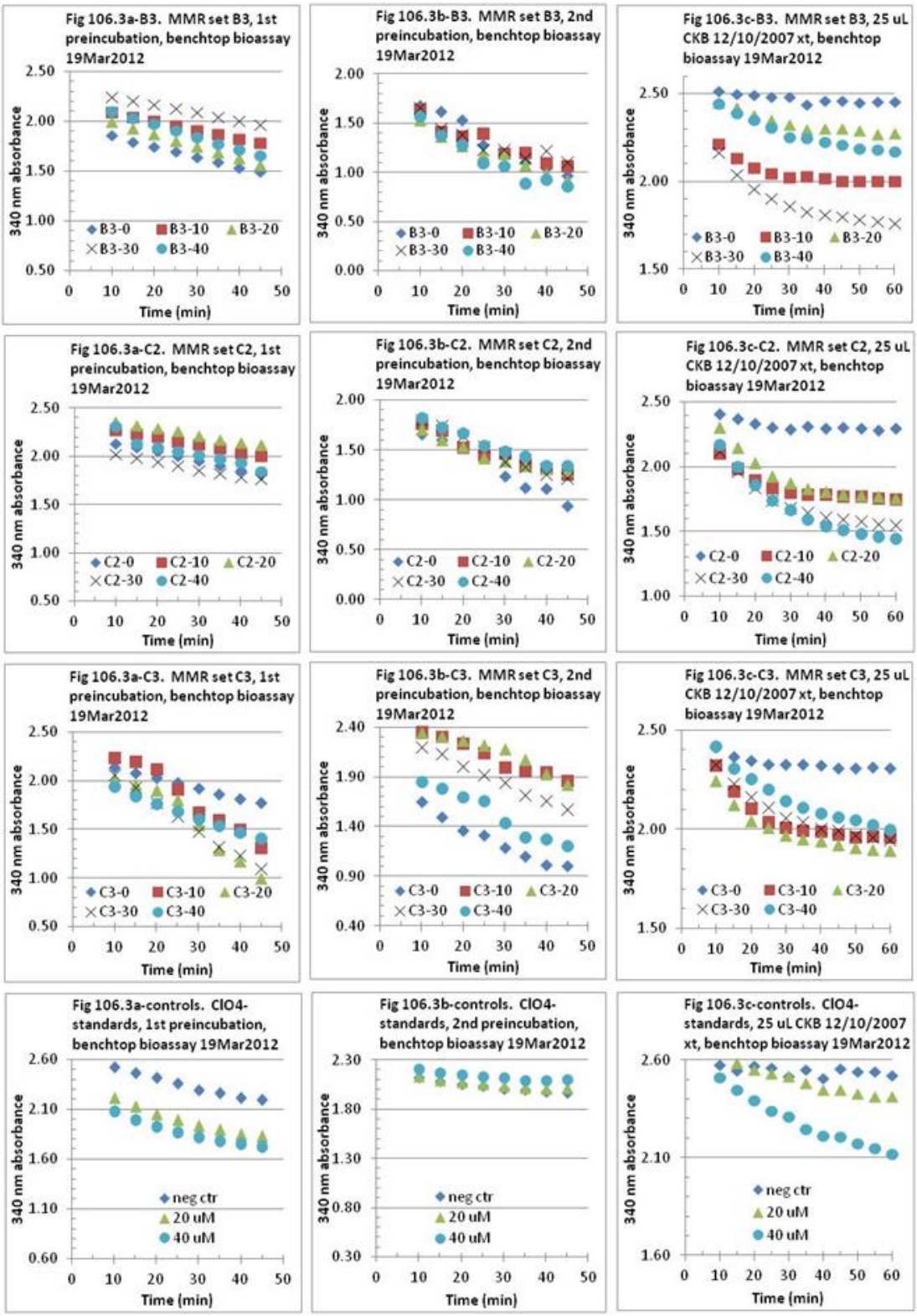



**Figure B-106.3. Massachusetts Military Reservation, benchtop bioassay batch 3, sets B3, C2, C3, and perchlorate controls. Test date 19 Mar 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-106.3a, b, and c

Data for Figure B-106.3.
Groundwater sample 106, Massachusetts Military Reservation. Standards addition method benchtop bioassay, batch 3, 19Mar2012. SDVB/DTAB eluate sets B3, C2, C3, perchlorate 20 and 40 μM standards, and negative control (no $ClO_4^-$ present)

**Table B-106.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 19Mar2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 | C2-0 | C2-10 | C2-20 | C2-30 | C2-40 |
| 10 | 1.8629 | 2.0898 | 1.9954 | 2.2470 | 2.1055 | 2.1376 | 2.2721 | 2.3612 | 2.0295 | 2.3176 |
| 15 | 1.7995 | 2.0445 | 1.9267 | 2.2092 | 2.0330 | 2.0906 | 2.2326 | 2.3268 | 1.9847 | 2.1341 |
| 20 | 1.7471 | 2.0018 | 1.8704 | 2.1721 | 1.9729 | 2.0521 | 2.1996 | 2.2915 | 1.9453 | 2.0972 |
| 25 | 1.6948 | 1.9611 | 1.8071 | 2.1283 | 1.9100 | 2.0026 | 2.1589 | 2.2621 | 1.9050 | 2.0601 |
| 30 | 1.6391 | 1.9126 | 1.7506 | 2.0890 | 1.8386 | 1.9541 | 2.1237 | 2.2124 | 1.8600 | 2.0113 |
| 35 | 1.5887 | 1.8717 | 1.6904 | 2.0451 | 1.7740 | 1.9041 | 2.0858 | 2.1776 | 1.8315 | 1.9754 |
| 40 | 1.5377 | 1.8256 | 1.6346 | 2.0066 | 1.7205 | 1.8581 | 2.0383 | 2.1405 | 1.7880 | 1.9404 |
| 45 | 1.4930 | 1.7863 | 1.5685 | 1.9671 | 1.6629 | 1.8184 | 2.0043 | 2.1294 | 1.7635 | 1.8482 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C3-0 | C3-10 | C3-20 | C3-30 | C3-40 | neg ctr | 20 μM | 40 μM |
| 10 | 2.1390 | 2.2406 | 2.0143 | 2.0577 | 1.9434 | 2.5247 | 2.2171 | 2.0814 |
| 15 | 2.0836 | 2.1938 | 1.9568 | 1.9305 | 1.8461 | 2.4663 | 2.1320 | 2.0024 |
| 20 | 2.0293 | 2.1151 | 1.8995 | 1.7718 | 1.7658 | 2.4213 | 2.0604 | 1.9359 |
| 25 | 1.9792 | 1.9108 | 1.8053 | 1.6328 | 1.6832 | 2.3626 | 1.9971 | 1.8752 |
| 30 | 1.9251 | 1.6773 | 1.5471 | 1.4830 | 1.6024 | 2.2948 | 1.9365 | 1.8239 |
| 35 | 1.8628 | 1.5996 | 1.2884 | 1.3178 | 1.5337 | 2.2730 | 1.9015 | 1.7874 |
| 40 | 1.8184 | 1.4992 | 1.1714 | 1.2374 | 1.4677 | 2.2173 | 1.8575 | 1.7612 |
| 45 | 1.7724 | 1.3147 | 1.0020 | 1.0969 | 1.4118 | 2.2011 | 1.8363 | 1.7267 |

**Table B-106.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 19Mar2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 | C2-0 | C2-10 | C2-20 | C2-30 | C2-40 |
| 10 | 1.6760 | 1.6379 | 1.5307 | 1.6630 | 1.5667 | 1.6609 | 1.7577 | 1.7024 | 1.7959 | 1.8150 |
| 15 | 1.6241 | 1.4186 | 1.3711 | 1.4451 | 1.3805 | 1.5983 | 1.6961 | 1.5951 | 1.7422 | 1.7209 |
| 20 | 1.5299 | 1.3663 | 1.2683 | 1.3854 | 1.2845 | 1.5323 | 1.5237 | 1.5325 | 1.6338 | 1.6699 |
| 25 | 1.2773 | 1.3984 | 1.1865 | 1.2276 | 1.0966 | 1.4292 | 1.4459 | 1.4181 | 1.5293 | 1.5436 |
| 30 | 1.1792 | 1.1972 | 1.1899 | 1.2264 | 1.0663 | 1.2352 | 1.4615 | 1.3891 | 1.3741 | 1.4830 |
| 35 | 1.1040 | 1.1973 | 1.0764 | 1.1413 | 0.8955 | 1.1226 | 1.3448 | 1.3389 | 1.3297 | 1.4326 |
| 40 | 1.0442 | 1.0984 | 0.9848 | 1.2253 | 0.9340 | 1.1063 | 1.3142 | 1.3128 | 1.2551 | 1.3437 |
| 45 | 0.9707 | 1.0657 | 0.9226 | 1.1042 | 0.8633 | 0.9404 | 1.2576 | 1.2896 | 1.2156 | 1.3403 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C3-0 | C3-10 | C3-20 | C3-30 | C3-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.6446 | 2.3520 | 2.3380 | 2.1925 | 1.8495 | 2.1270 | 2.1383 | 2.2108 |
| 15 | 1.4947 | 2.2982 | 2.3025 | 2.1244 | 1.7775 | 2.0896 | 2.0940 | 2.1802 |
| 20 | 1.3535 | 2.2350 | 2.2636 | 2.0018 | 1.6926 | 2.0572 | 2.0752 | 2.1555 |
| 25 | 1.3092 | 2.1418 | 2.2173 | 1.9187 | 1.6549 | 2.0361 | 2.0583 | 2.1397 |
| 30 | 1.1892 | 1.9915 | 2.1761 | 1.8396 | 1.4311 | 2.0108 | 2.0364 | 2.1282 |
| 35 | 1.0992 | 1.9510 | 2.0714 | 1.7168 | 1.2894 | 2.0024 | 2.0230 | 2.1026 |
| 40 | 1.0092 | 1.9478 | 1.9302 | 1.6589 | 1.2731 | 1.9835 | 2.0145 | 2.1009 |
| 45 | 1.0019 | 1.8600 | 1.8202 | 1.5715 | 1.2005 | 1.9669 | 2.0155 | 2.1103 |

**Table B-106.3c. Batch 3, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 19Mar2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** |
| 10 | 2.5139 | 2.2167 | 2.4512 | 2.1673 | 2.4452 | 2.4058 | 2.1040 | 2.3013 | 2.1170 | 2.1716 |
| 15 | 2.4978 | 2.1364 | 2.4216 | 2.0434 | 2.3896 | 2.3660 | 1.9832 | 2.1478 | 1.9617 | 2.0020 |
| 20 | 2.4934 | 2.0782 | 2.3782 | 1.9594 | 2.3518 | 2.3282 | 1.8972 | 2.0265 | 1.8379 | 1.8610 |
| 25 | 2.4811 | 2.0468 | 2.3502 | 1.9030 | 2.3071 | 2.2989 | 1.8376 | 1.9218 | 1.7405 | 1.7384 |
| 30 | 2.4836 | 2.0260 | 2.3222 | 1.8617 | 2.2522 | 2.2845 | 1.8008 | 1.8714 | 1.6777 | 1.6623 |
| 35 | 2.4367 | 2.0287 | 2.2987 | 1.8271 | 2.2476 | 2.3068 | 1.7865 | 1.8280 | 1.6399 | 1.5949 |
| 40 | 2.4613 | 2.0167 | 2.3036 | 1.8146 | 2.2282 | 2.2969 | 1.7805 | 1.8040 | 1.6079 | 1.5419 |
| 45 | 2.4612 | 2.0052 | 2.3030 | 1.8000 | 2.2111 | 2.3025 | 1.7721 | 1.7852 | 1.5903 | 1.5080 |
| 50 | 2.4494 | 2.0019 | 2.2942 | 1.7871 | 2.1893 | 2.2946 | 1.7659 | 1.7750 | 1.5742 | 1.4839 |
| 55 | 2.4568 | 2.0031 | 2.2721 | 1.7757 | 2.1810 | 2.2795 | 1.7557 | 1.7697 | 1.5532 | 1.4593 |
| 60 | 2.4568 | 2.0006 | 2.2732 | 1.7619 | 2.1718 | 2.2979 | 1.7475 | 1.7542 | 1.5435 | 1.4449 |
| **Rate[a]** | 0.0012 | 0.0035 | 0.0034 | 0.0070 | 0.0053 | 0.0018 | 0.0059 | 0.0097 | 0.0103 | 0.0137 |
| **$r^2$** | 0.6789 | 0.7220 | 0.8669 | 0.8328 | 0.9223 | 0.5768 | 0.7493 | 0.8097 | 0.8386 | 0.8854 |
| **Rate – neg ctrl rate** | 0.0005 | 0.0028 | 0.0027 | 0.0063 | 0.0046 | 0.0011 | 0.0052 | 0.0090 | 0.0096 | 0.0130 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C3-0** | **C3-10** | **C3-20** | **C3-30** | **C3-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 2.4128 | 2.3200 | 2.2415 | 2.3251 | 2.4162 | 2.5742 | 2.6104 | 2.5141 |
| 15 | 2.3620 | 2.1891 | 2.1243 | 2.2269 | 2.3080 | 2.5447 | 2.5850 | 2.4477 |
| 20 | 2.3442 | 2.1015 | 2.0399 | 2.1617 | 2.2531 | 2.5709 | 2.5532 | 2.3974 |
| 25 | 2.3274 | 2.0351 | 2.0058 | 2.1073 | 2.2018 | 2.5613 | 2.5319 | 2.3430 |
| 30 | 2.3280 | 2.0093 | 1.9687 | 2.0531 | 2.1418 | 2.5176 | 2.5162 | 2.3096 |
| 35 | 2.3247 | 1.9928 | 1.9517 | 2.0351 | 2.1065 | 2.5522 | 2.4817 | 2.2486 |
| 40 | 2.3216 | 1.9865 | 1.9418 | 2.0034 | 2.0808 | 2.5062 | 2.4495 | 2.2151 |
| 45 | 2.3060 | 1.9740 | 1.9199 | 1.9925 | 2.0592 | 2.5560 | 2.4474 | 2.2112 |
| 50 | 2.3065 | 1.9613 | 1.9070 | 1.9679 | 2.0447 | 2.5439 | 2.4305 | 2.1767 |
| 55 | 2.3108 | 1.9646 | 1.8952 | 1.9573 | 2.0239 | 2.5418 | 2.4140 | 2.1521 |
| 60 | 2.3080 | 1.9572 | 1.8896 | 1.9440 | 1.9969 | 2.5206 | 2.4128 | 2.1213 |
| **Rate[a]** | 0.0016 | 0.0060 | 0.0060 | 0.0070 | 0.0076 | 0.0007 | 0.0041 | 0.0076 |
| **$r^2$** | 0.7128 | 0.7325 | 0.8176 | 0.8932 | 0.9207 | 0.2760 | 0.9690 | 0.9658 |
| **Rate – neg ctrl rate** | 0.0009 | 0.0053 | 0.0053 | 0.0063 | 0.0069 | 0.0000 | 0.0034 | 0.0069 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-106.3c-B3, C2, C3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

## Groundwater sample 107, Lawrence Livermore National Laboratory-site 300, location W-854-1823, collected 29 March 2012

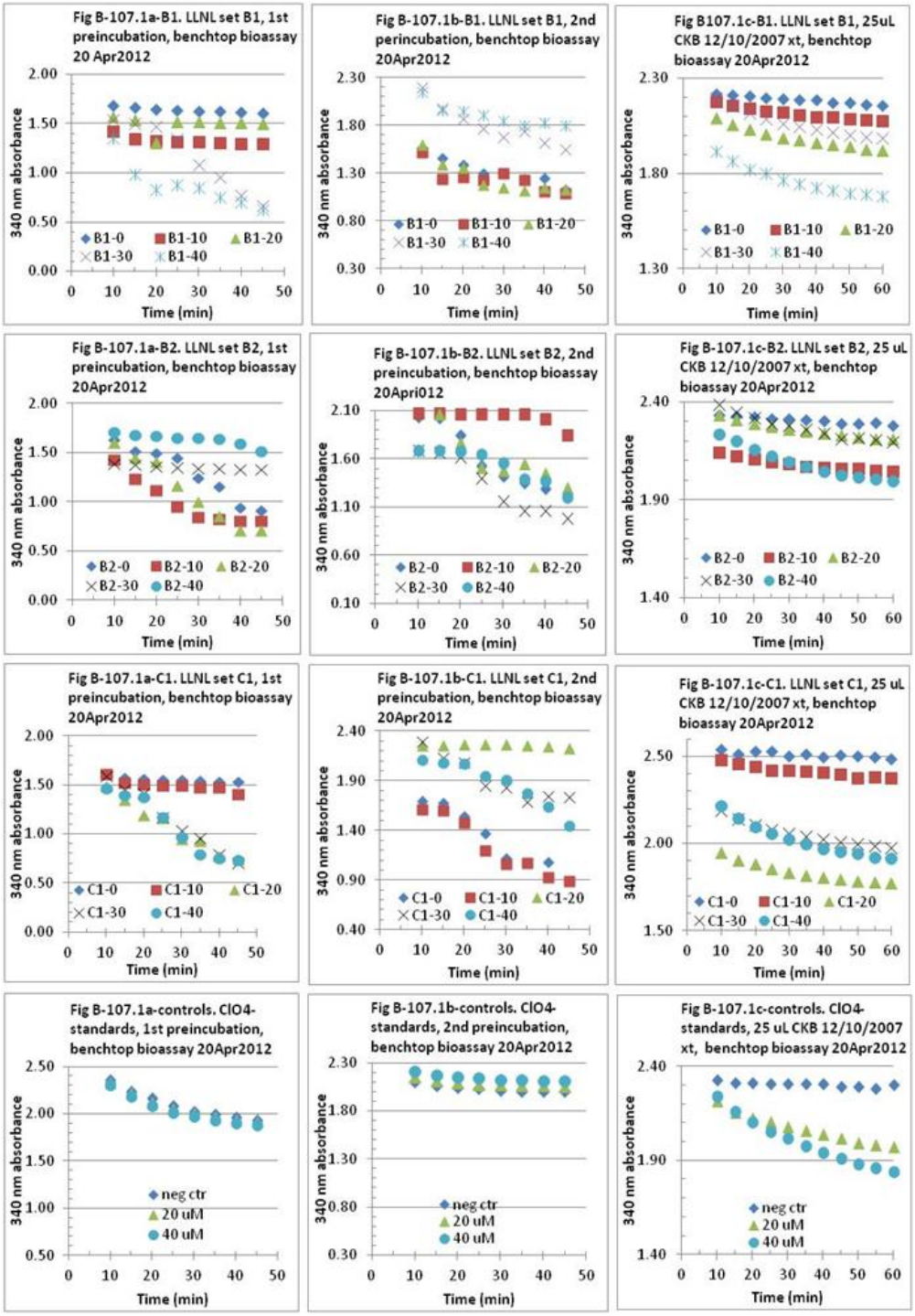



**Figure B-107.1. Lawrence Livermore National Laboratory-site 300, batch 1, sets B1, B2, C1, and perchlorate controls. Test date 20 Apr 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 (**c**). Data values displayed are listed in Tables B-107.1a, b, and c

Data for Figure B-107.1.
Groundwater sample 107, LLNL-site 300. Standards addition method benchtop bioassay, batch 1, 20April2012: SDVB/DTAB eluate sets B1, B2, C1, perchlorate 20 and 40 µM standards, and negative control (no $ClO_4^-$ present)

**Table B-107.1a. Batch 1, 340 nm absorbance, 1st preincubation without extract, 20April2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 1.6840 | 1.4191 | 1.5650 | 1.5357 | 1.3548 | 1.6318 | 1.4263 | 1.6015 | 1.3934 | 1.7087 |
| 15 | 1.6609 | 1.3451 | 1.5426 | 1.5003 | 0.9903 | 1.5195 | 1.2314 | 1.4463 | 1.3769 | 1.6782 |
| 20 | 1.6446 | 1.3291 | 1.3050 | 1.4750 | 0.8296 | 1.4943 | 1.1146 | 1.4132 | 1.3621 | 1.6690 |
| 25 | 1.6346 | 1.3201 | 1.5250 | 1.3479 | 0.8789 | 1.4455 | 0.9506 | 1.1711 | 1.3541 | 1.6544 |
| 30 | 1.6277 | 1.3135 | 1.5202 | 1.0839 | 0.8529 | 1.2461 | 0.8509 | 1.0072 | 1.3455 | 1.6546 |
| 35 | 1.6222 | 1.3046 | 1.5114 | 0.9625 | 0.7545 | 1.1538 | 0.8261 | 0.8586 | 1.3390 | 1.6397 |
| 40 | 1.6179 | 1.2999 | 1.5073 | 0.7694 | 0.7026 | 0.9419 | 0.8056 | 0.7105 | 1.3370 | 1.5929 |
| 45 | 1.6108 | 1.2952 | 1.5025 | 0.6672 | 0.6260 | 0.9139 | 0.8084 | 0.7099 | 1.3355 | 1.5198 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.6087 | 1.6130 | 1.4837 | 1.5928 | 1.4623 | 2.3656 | 2.3285 | 2.3073 |
| 15 | 1.5718 | 1.5223 | 1.3492 | 1.4603 | 1.3978 | 2.2380 | 2.2145 | 2.1863 |
| 20 | 1.5610 | 1.5007 | 1.1966 | 1.4159 | 1.3804 | 2.1663 | 2.1204 | 2.0901 |
| 25 | 1.5513 | 1.4939 | 1.1613 | 1.1770 | 1.1698 | 2.0834 | 2.0500 | 2.0211 |
| 30 | 1.5495 | 1.4904 | 0.9508 | 1.0366 | 0.9716 | 2.0319 | 2.0026 | 1.9753 |
| 35 | 1.5429 | 1.4793 | 0.9335 | 0.9563 | 0.7966 | 1.9937 | 1.9648 | 1.9368 |
| 40 | 1.5368 | 1.4755 | 0.7697 | 0.7974 | 0.7555 | 1.9662 | 1.9372 | 1.9061 |
| 45 | 1.5360 | 1.4066 | 0.7410 | 0.7101 | 0.7393 | 1.9387 | 1.9132 | 1.8878 |

**Table B-107.1b. Batch 1, 340 nm absorbance, 2nd preincubation without extract, 20Apr2012**

| Time | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B1-0 | B1-10 | B1-20 | B1-30 | B1-40 | B2-0 | B2-10 | B2-20 | B2-30 | B2-40 |
| 10 | 1.5585 | 1.5218 | 1.5997 | 2.1958 | 2.1520 | 2.0326 | 2.0790 | 2.1122 | 1.6893 | 1.6865 |
| 15 | 1.4630 | 1.2384 | 1.3899 | 1.9784 | 1.9556 | 2.0249 | 2.0759 | 2.0625 | 1.6719 | 1.6851 |
| 20 | 1.3959 | 1.2660 | 1.3658 | 1.8706 | 1.9537 | 1.8523 | 2.0666 | 1.7723 | 1.6245 | 1.6847 |
| 25 | 1.2987 | 1.2332 | 1.1859 | 1.7730 | 1.9057 | 1.5323 | 2.0631 | 1.5123 | 1.4026 | 1.6454 |
| 30 | 1.2991 | 1.3020 | 1.1484 | 1.6779 | 1.8541 | 1.4181 | 2.0683 | 1.4805 | 1.1663 | 1.5630 |
| 35 | 1.2428 | 1.2330 | 1.1225 | 1.7433 | 1.8013 | 1.3480 | 2.0618 | 1.5511 | 1.0607 | 1.3937 |
| 40 | 1.2489 | 1.1089 | 1.1508 | 1.6196 | 1.8264 | 1.2949 | 2.0176 | 1.4516 | 1.0658 | 1.3756 |
| 45 | 1.1287 | 1.0925 | 1.1322 | 1.5558 | 1.8036 | 1.2480 | 1.8508 | 1.3070 | 0.9834 | 1.2009 |

| Time | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C1-0 | C1-10 | C1-20 | C1-30 | C1-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.6966 | 1.6121 | 2.2530 | 2.2906 | 2.1095 | 2.0942 | 2.1490 | 2.2106 |
| 15 | 1.6815 | 1.5991 | 2.2553 | 2.1352 | 2.0823 | 2.0635 | 2.1092 | 2.1710 |
| 20 | 1.5468 | 1.4774 | 2.2664 | 2.0805 | 2.0748 | 2.0392 | 2.0907 | 2.1463 |
| 25 | 1.3719 | 1.1995 | 2.2674 | 1.8479 | 1.9508 | 2.0279 | 2.0718 | 2.1367 |
| 30 | 1.1215 | 1.0647 | 2.2653 | 1.8373 | 1.9080 | 2.0141 | 2.0724 | 2.1166 |
| 35 | 1.0698 | 1.0750 | 2.2586 | 1.6903 | 1.7725 | 2.0034 | 2.0583 | 2.1149 |
| 40 | 1.0831 | 0.9262 | 2.2506 | 1.7452 | 1.6385 | 1.9992 | 2.0550 | 2.1102 |
| 45 | 0.8942 | 0.8904 | 2.2303 | 1.7419 | 1.4518 | 1.9973 | 2.0524 | 2.1077 |

**Table B-107.1c. Batch 1, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 20April2012**

| | Set B1 | | | | | Set B2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B1-0** | **B1-10** | **B1-20** | **B1-30** | **B1-40** | **B2-0** | **B2-10** | **B2-20** | **B2-30** | **B2-40** |
| 10 | 2.2146 | 2.1745 | 2.0921 | 2.1955 | 1.9147 | 2.3328 | 2.1395 | 2.3311 | 2.3832 | 2.2336 |
| 15 | 2.2128 | 2.1541 | 2.0552 | 2.1483 | 1.8625 | 2.3276 | 2.1193 | 2.3047 | 2.3464 | 2.1969 |
| 20 | 2.2069 | 2.1391 | 2.0285 | 2.1148 | 1.8209 | 2.3219 | 2.1056 | 2.2857 | 2.3159 | 2.1566 |
| 25 | 2.1962 | 2.1257 | 2.0048 | 2.0875 | 1.7974 | 2.3111 | 2.0921 | 2.2706 | 2.2867 | 2.1186 |
| 30 | 2.1919 | 2.1187 | 1.9839 | 2.0618 | 1.7616 | 2.3092 | 2.0814 | 2.2580 | 2.2764 | 2.0905 |
| 35 | 2.1856 | 2.1068 | 1.9727 | 2.0459 | 1.7416 | 2.3083 | 2.0672 | 2.2490 | 2.2559 | 2.0687 |
| 40 | 2.1834 | 2.0942 | 1.9579 | 2.0294 | 1.7245 | 2.3018 | 2.0642 | 2.2397 | 2.2397 | 2.0450 |
| 45 | 2.1724 | 2.0935 | 1.9486 | 2.0132 | 1.7108 | 2.2846 | 2.0581 | 2.2283 | 2.2107 | 2.0236 |
| 50 | 2.1708 | 2.0856 | 1.9382 | 2.0023 | 1.6956 | 2.2859 | 2.0559 | 2.2198 | 2.2118 | 2.0134 |
| 55 | 2.1587 | 2.0798 | 1.9248 | 1.9917 | 1.6890 | 2.2933 | 2.0480 | 2.2103 | 2.2049 | 2.0055 |
| 60 | 2.1575 | 2.0749 | 1.9216 | 1.9829 | 1.6795 | 2.2786 | 2.0428 | 2.2090 | 2.1928 | 1.9948 |
| **Rate[a]** | 0.0012 | 0.0019 | 0.0032 | 0.0040 | 0.0045 | 0.0010 | 0.0018 | 0.0023 | 0.0037 | 0.0048 |
| **$r^2$** | 0.9867 | 0.9518 | 0.9411 | 0.9396 | 0.9279 | 0.9271 | 0.9364 | 0.9560 | 0.9476 | 0.9476 |
| **Rate – neg ctrl rate** | 0.0005 | 0.0012 | 0.0025 | 0.0033 | 0.0038 | 0.0003 | 0.0011 | 0.0016 | 0.0030 | 0.0042 |

| | Set C1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C1-0** | **C1-10** | **C1-20** | **C1-30** | **C1-40** | **neg ctr** | **20 uM** | **40 uM** |
| 10 | 2.5426 | 2.4790 | 1.9490 | 2.1912 | 2.2147 | 2.3273 | 2.2147 | 2.2411 |
| 15 | 2.5119 | 2.4583 | 1.9052 | 2.1393 | 2.1480 | 2.3091 | 2.1567 | 2.1581 |
| 20 | 2.5307 | 2.4440 | 1.8799 | 2.1055 | 2.0967 | 2.3091 | 2.1228 | 2.1042 |
| 25 | 2.5284 | 2.4221 | 1.8540 | 2.0806 | 2.0579 | 2.3080 | 2.1010 | 2.0532 |
| 30 | 2.5043 | 2.4197 | 1.8309 | 2.0572 | 2.0255 | 2.3072 | 2.0798 | 2.0171 |
| 35 | 2.5160 | 2.4147 | 1.8170 | 2.0393 | 1.9995 | 2.3085 | 2.0601 | 1.9765 |
| 40 | 2.4963 | 2.4109 | 1.8051 | 2.0225 | 1.9730 | 2.3060 | 2.0381 | 1.9434 |
| 45 | 2.5060 | 2.3990 | 1.7945 | 2.0086 | 1.9551 | 2.2888 | 2.0197 | 1.9098 |
| 50 | 2.5002 | 2.3753 | 1.7852 | 1.9970 | 1.9418 | 2.2898 | 1.9920 | 1.8828 |
| 55 | 2.4963 | 2.3830 | 1.7780 | 1.9864 | 1.9223 | 2.2787 | 1.9824 | 1.8588 |
| 60 | 2.4855 | 2.3747 | 1.7710 | 1.9779 | 1.9135 | 2.2987 | 1.9729 | 1.8382 |
| **Rate[a]** | 0.00089 | 0.0020 | 0.0033 | 0.0040 | 0.0057 | 0.0007 | 0.0046 | 0.0077 |
| **$r^2$** | 0.7382 | 0.9401 | 0.9208 | 0.9343 | 0.9344 | 0.6885 | 0.9644 | 0.9664 |
| **Rate – neg ctrl rate** | 0.00019 | 0.0013 | 0.0026 | 0.0033 | 0.0050 | 0.0000 | 0.0039 | 0.0070 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-107.1c-B1, B2, C1, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

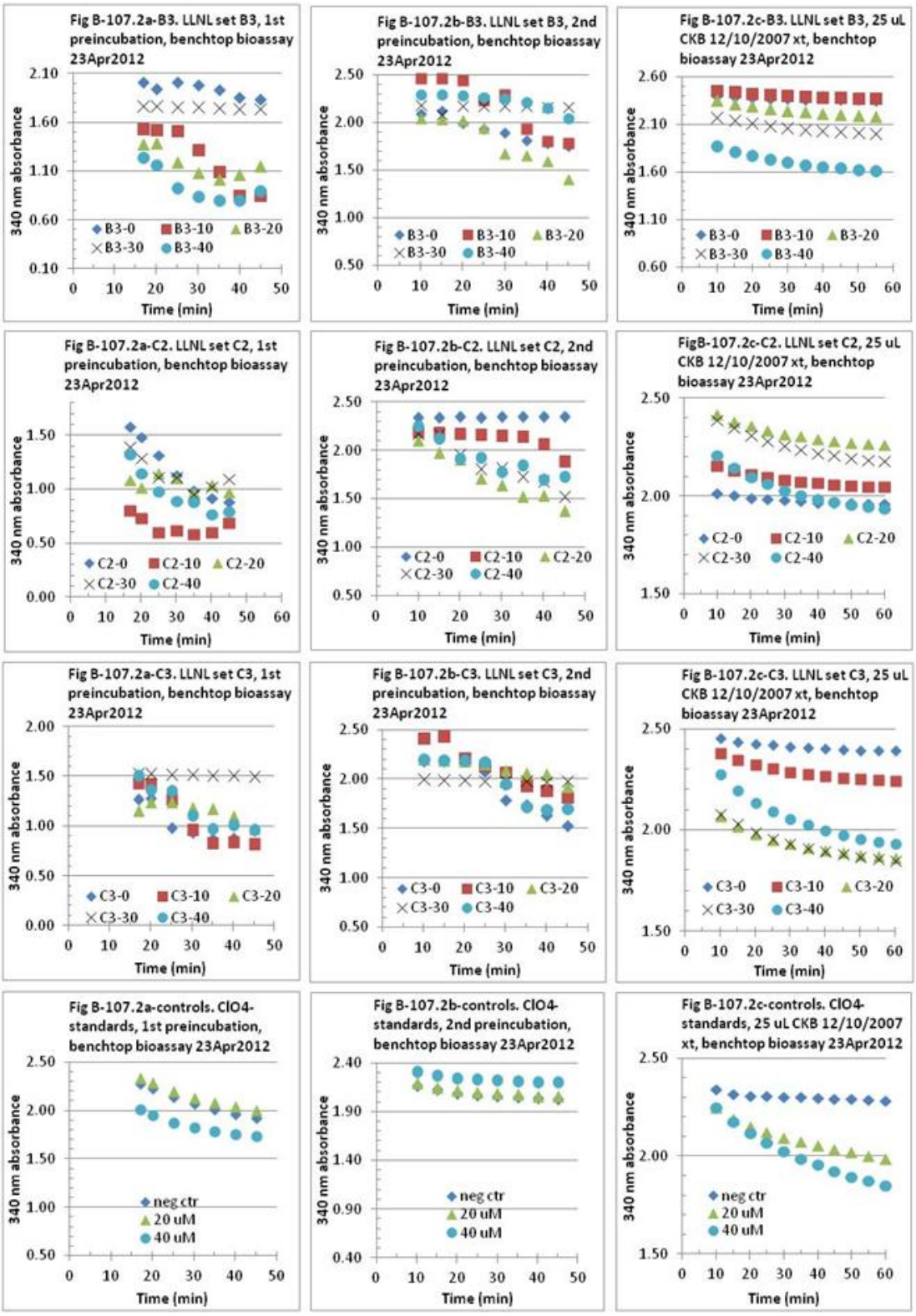



**Figure B-107.2. Lawrence Livermore National Laboratory-site 300, batch 2, sets B3, C2, C3, and perchlorate controls. Test date 23 Apr 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-107.2a, b, and c

Data for Figure B-107.2.
Groundwater sample 107, Lawrence Livermore National Laboratory-site 300. Standards addition method benchtop bioassay, batch 2, 23April2012. SDVB/DTAB eluate sets B3, C2, C3, perchlorate 20 and 40 µM standards, and negative control (no $ClO_4^-$ present)

**Table B-107.2a. Batch 2, 340 nm absorbance, 1st preincubation without extract, 23April2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** |
| 17 | 2.0098 | 1.5291 | 1.3783 | 1.7714 | 1.2431 | 1.5810 | 0.8077 | 1.0874 | 1.3944 | 1.3240 |
| 20 | 1.9438 | 1.5202 | 1.3843 | 1.7678 | 1.1597 | 1.4841 | 0.7385 | 1.0148 | 1.2945 | 1.1497 |
| 25 | 2.0065 | 1.5150 | 1.1924 | 1.7603 | 0.9255 | 1.3204 | 0.6064 | 1.1487 | 1.1162 | 0.9852 |
| 30 | 1.9780 | 1.3225 | 1.0821 | 1.7531 | 0.8380 | 1.1338 | 0.6226 | 1.1082 | 1.1175 | 0.8941 |
| 35 | 1.9359 | 1.0943 | 1.0142 | 1.7462 | 0.8023 | 0.9936 | 0.5829 | 0.9731 | 0.9560 | 0.8879 |
| 40 | 1.8531 | 0.8491 | 1.0646 | 1.7394 | 0.8030 | 0.9169 | 0.6044 | 1.0474 | 1.0271 | 0.7742 |
| 45 | 1.8364 | 0.8512 | 1.1491 | 1.7381 | 0.9037 | 0.8861 | 0.6881 | 0.9695 | 1.0925 | 0.7998 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C3-0** | **C3-10** | **C3-20** | **C3-30** | **C3-40** | **neg ctr** | **20 µM** | **40 µM** |
| 17 | 1.2759 | 1.4336 | 1.1584 | 1.5349 | 1.5141 | 2.2807 | 2.3384 | 2.0127 |
| 20 | 1.2794 | 1.4255 | 1.2399 | 1.5313 | 1.3663 | 2.2305 | 2.2874 | 1.9568 |
| 25 | 0.9863 | 1.2740 | 1.2415 | 1.5251 | 1.3566 | 2.1414 | 2.1955 | 1.8792 |
| 30 | 0.9369 | 0.9638 | 1.1866 | 1.5189 | 1.1081 | 2.0744 | 2.1351 | 1.8268 |
| 35 | 0.8693 | 0.8351 | 1.1794 | 1.5135 | 0.9737 | 2.0159 | 2.0819 | 1.7890 |
| 40 | 0.8763 | 0.8448 | 1.0959 | 1.5099 | 1.0134 | 1.9692 | 2.0396 | 1.7554 |
| 45 | 0.9601 | 0.8270 | 0.9715 | 1.5058 | 0.9621 | 1.9290 | 2.0044 | 1.7337 |

**Table B-107.2b. Batch 2, 340 nm absorbance, 2nd preincubation without extract, 23April2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | B3-0 | B3-10 | B3-20 | B3-30 | B3-40 | C2-0 | C2-10 | C2-20 | C2-30 | C2-40 |
| 10 | 2.0996 | 2.4718 | 2.0445 | 2.1906 | 2.3000 | 2.3413 | 2.1909 | 2.1005 | 2.1753 | 2.2464 |
| 15 | 2.1257 | 2.4712 | 2.0404 | 2.0845 | 2.2971 | 2.3371 | 2.1819 | 1.9790 | 2.1609 | 2.1171 |
| 20 | 1.9941 | 2.4478 | 2.0284 | 2.1802 | 2.2917 | 2.3472 | 2.1702 | 1.9029 | 1.9552 | 1.9186 |
| 25 | 1.9412 | 2.2426 | 1.9446 | 2.1744 | 2.2681 | 2.3405 | 2.1643 | 1.7123 | 1.8058 | 1.9232 |
| 30 | 1.8998 | 2.2984 | 1.6743 | 2.1749 | 2.2539 | 2.3467 | 2.1548 | 1.6410 | 1.8138 | 1.7807 |
| 35 | 1.8189 | 1.9436 | 1.6619 | 2.1747 | 2.2143 | 2.3489 | 2.1456 | 1.5175 | 1.7277 | 1.8502 |
| 40 | 1.7932 | 1.8100 | 1.5995 | 2.1724 | 2.1565 | 2.3521 | 2.0665 | 1.5340 | 1.6824 | 1.7020 |
| 45 | 1.7554 | 1.7863 | 1.4055 | 2.1695 | 2.0484 | 2.3500 | 1.8847 | 1.3696 | 1.5249 | 1.7267 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | C3-0 | C3-10 | C3-20 | C3-30 | C3-40 | neg ctr | 20 µM | 40 µM |
| 10 | 2.1811 | 2.4118 | 2.2049 | 2.0052 | 2.2023 | 2.1649 | 2.1943 | 2.3108 |
| 15 | 2.1822 | 2.4366 | 2.1922 | 1.9958 | 2.1947 | 2.1251 | 2.1479 | 2.2735 |
| 20 | 2.1808 | 2.2138 | 2.1827 | 1.9866 | 2.1915 | 2.0890 | 2.1168 | 2.2432 |
| 25 | 2.0798 | 2.1397 | 2.1560 | 1.9846 | 2.1758 | 2.0704 | 2.0946 | 2.2305 |
| 30 | 1.7909 | 2.0685 | 2.0908 | 1.9802 | 1.9486 | 2.0531 | 2.0805 | 2.2193 |
| 35 | 1.7442 | 1.9298 | 2.0631 | 1.9786 | 1.7207 | 2.0434 | 2.0731 | 2.2103 |
| 40 | 1.6334 | 1.8832 | 2.0452 | 1.9757 | 1.6887 | 2.0359 | 2.0600 | 2.2051 |
| 45 | 1.5243 | 1.8166 | 1.9244 | 1.9781 | 1.7024 | 2.0311 | 2.0554 | 2.2065 |

**Table B-107.2c. Batch 2, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 23April2012**

| | Set B3 | | | | | Set C2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **Rxn 3** | **rxn 4** | **rxn 5** | **rxn6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **B3-0** | **B3-10** | **B3-20** | **B3-30** | **B3-40** | **C2-0** | **C2-10** | **C2-20** | **C2-30** | **C2-40** |
| 10 | 2.3994 | 2.4505 | 2.3508 | 2.1683 | 1.8736 | 2.0146 | 2.1563 | 2.4162 | 2.3911 | 2.2096 |
| 15 | 2.3896 | 2.4414 | 2.3081 | 2.1354 | 1.8122 | 2.0035 | 2.1318 | 2.3818 | 2.3483 | 2.1473 |
| 20 | 2.3797 | 2.4195 | 2.2843 | 2.1058 | 1.7651 | 1.9931 | 2.1123 | 2.3604 | 2.3098 | 2.0985 |
| 25 | 2.3731 | 2.4095 | 2.2601 | 2.0796 | 1.7261 | 1.9868 | 2.0970 | 2.3378 | 2.2812 | 2.0620 |
| 30 | 2.3648 | 2.3973 | 2.2387 | 2.0582 | 1.6947 | 1.9799 | 2.0825 | 2.3184 | 2.2562 | 2.0308 |
| 35 | 2.3626 | 2.3853 | 2.2244 | 2.0436 | 1.6684 | 1.9752 | 2.0751 | 2.3045 | 2.2378 | 2.0068 |
| 40 | 2.3910 | 2.3826 | 2.2090 | 2.0278 | 1.6487 | 1.9687 | 2.0672 | 2.2912 | 2.2201 | 1.9863 |
| 45 | 2.3596 | 2.3771 | 2.2000 | 2.0150 | 1.6341 | 1.9685 | 2.0595 | 2.2841 | 2.2096 | 1.9730 |
| 50 | 2.3554 | 2.3710 | 2.1879 | 2.0088 | 1.6194 | 1.9647 | 2.0551 | 2.2728 | 2.1957 | 1.9557 |
| 55 | 2.3533 | 2.3671 | 2.1825 | 2.0006 | 1.6088 | 1.9617 | 2.0511 | 2.2680 | 2.1868 | 1.9450 |
| 60 | 2.3481 | 2.3704 | 2.1762 | 1.9925 | 1.5969 | 1.9624 | 2.0481 | 2.2620 | 2.1809 | 1.9370 |
| **Rate[a]** | 0.00085 | 0.00186 | 0.00357 | 0.00366 | 0.00563 | 0.00102 | 0.00205 | 0.00295 | 0.00403 | 0.00513 |
| **$r^2$** | 0.6261 | 0.9421 | 0.9422 | 0.9464 | 0.9319 | 0.9151 | 0.9114 | 0.9349 | 0.9296 | 0.9197 |
| **Rate – neg ctrl rate** | -0.00004 | 0.00097 | 0.00268 | 0.00277 | 0.00474 | 0.00013 | 0.00116 | 0.00206 | 0.00314 | 0.00424 |

| | Set C3 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **C3-0** | **C3-10** | **C3-20** | **C3-30** | **C3-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 2.4554 | 2.3814 | 2.0674 | 2.0801 | 2.2765 | 2.3435 | 2.2524 | 2.2501 |
| 15 | 2.4389 | 2.3481 | 2.0157 | 2.0312 | 2.1982 | 2.3199 | 2.1931 | 2.1760 |
| 20 | 2.4266 | 2.3227 | 1.9798 | 1.9913 | 2.1366 | 2.3065 | 2.1511 | 2.1176 |
| 25 | 2.4205 | 2.3052 | 1.9529 | 1.9584 | 2.0946 | 2.3069 | 2.1223 | 2.0704 |
| 30 | 2.4139 | 2.2884 | 1.9315 | 1.9309 | 2.0553 | 2.3037 | 2.0964 | 2.0270 |
| 35 | 2.4078 | 2.2785 | 1.9145 | 1.9118 | 2.0259 | 2.3050 | 2.0736 | 1.9901 |
| 40 | 2.4048 | 2.2667 | 1.9006 | 1.8959 | 2.0004 | 2.2963 | 2.0535 | 1.9570 |
| 45 | 2.4001 | 2.2591 | 1.8888 | 1.8792 | 1.9745 | 2.2935 | 2.0344 | 1.9262 |
| 50 | 2.3929 | 2.2533 | 1.8784 | 1.8669 | 1.9567 | 2.2913 | 2.0203 | 1.8978 |
| 55 | 2.3966 | 2.2485 | 1.8711 | 1.8593 | 1.9448 | 2.2895 | 2.0030 | 1.8753 |
| 60 | 2.3944 | 2.2417 | 1.8617 | 1.8495 | 1.9341 | 2.2860 | 1.9890 | 1.8524 |
| **Rate[a]** | 0.00114 | 0.00258 | 0.00376 | 0.00438 | 0.00647 | 0.00089 | 0.00489 | 0.00765 |
| **$r^2$** | 0.8921 | 0.9124 | 0.9117 | .09272 | 0.9255 | 0.9067 | 0.9504 | 0.9677 |
| **Rate – neg ctrl rate** | 0.00025 | 0.00169 | 0.00287 | 0.00349 | 0.00558 | 0.00000 | 0.00400 | 0.00676 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-107.2c-B3, C2, C3, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

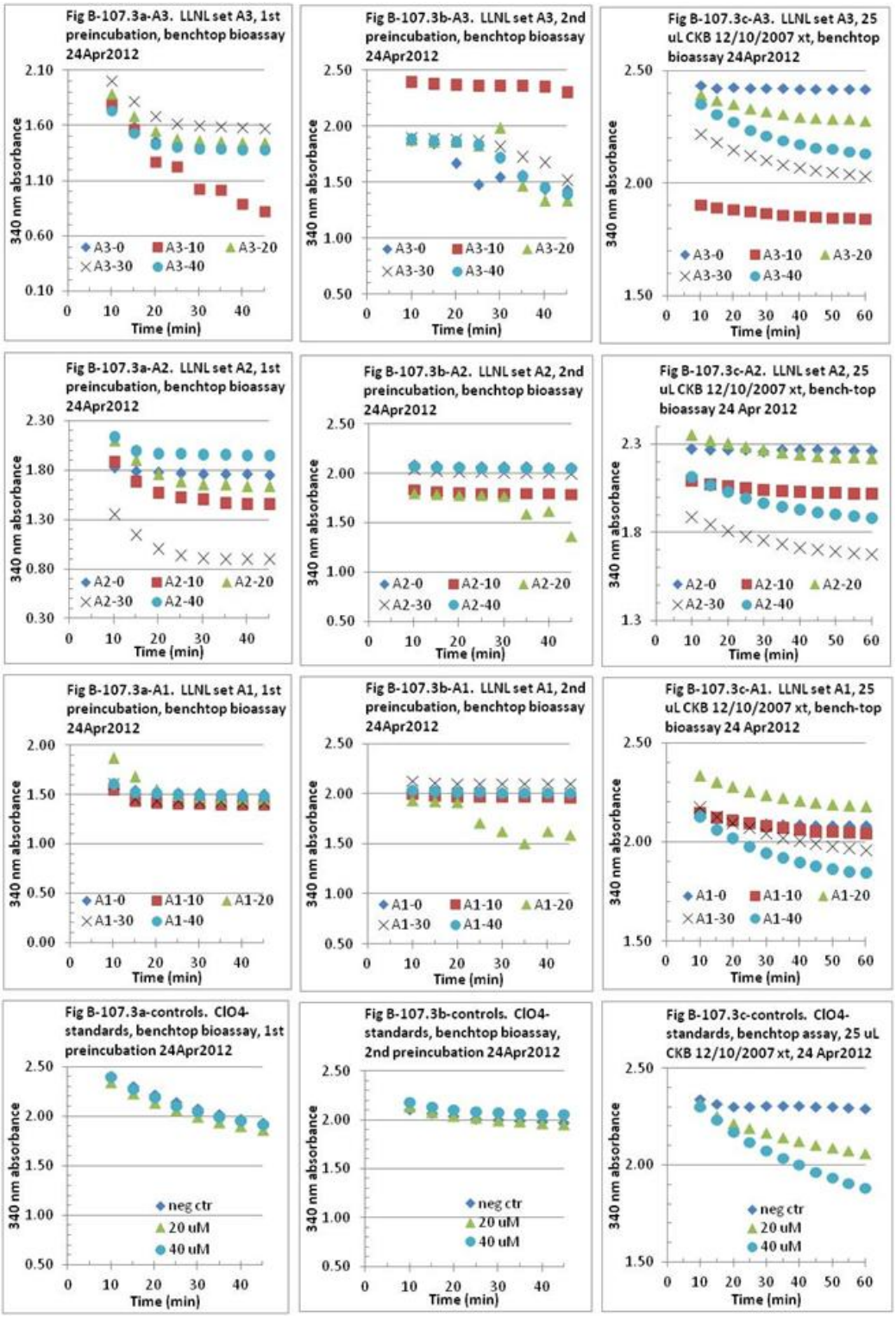



**Figure B-107.3. Lawrence Livermore National Laboratory-site 300, batch 3, sets A3, A2, A1, and perchlorate controls. Test date 24 Apr 2012.** 1st preincubation without extract (**a**), 2nd preincubation without extract (**b**), 25 µL CKB 12/10/2007 extract (**c**). Data values displayed are listed in Tables B-107.3a, b, and c

Data for Figure B-107.3.
Groundwater sample 107, Lawrence Livermore National Laboratory-site 300. Standards addition method benchtop bioassay, batch 3, 24April2012. SDVB/DTAB eluate sets A3, A2, A1, perchlorate 20 and 40 µM standards, and negative control (no $ClO_4^-$ present)

**Table B-107.3a. Batch 3, 340 nm absorbance, 1st preincubation without extract, 24April2012**

| | Set A3 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 1.6840 | 1.4191 | 1.5650 | 1.5357 | 1.3548 | 1.6318 | 1.4263 | 1.6015 | 1.3934 | 1.7087 |
| 15 | 1.6609 | 1.3451 | 1.5426 | 1.5003 | 0.9903 | 1.5195 | 1.2314 | 1.4463 | 1.3769 | 1.6782 |
| 20 | 1.6446 | 1.3291 | 1.3050 | 1.4750 | 0.8296 | 1.4943 | 1.1146 | 1.4132 | 1.3621 | 1.6690 |
| 25 | 1.6346 | 1.3201 | 1.5250 | 1.3479 | 0.8789 | 1.4455 | 0.9506 | 1.1711 | 1.3541 | 1.6544 |
| 30 | 1.6277 | 1.3135 | 1.5202 | 1.0839 | 0.8529 | 1.2461 | 0.8509 | 1.0072 | 1.3455 | 1.6546 |
| 35 | 1.6222 | 1.3046 | 1.5114 | 0.9625 | 0.7545 | 1.1538 | 0.8261 | 0.8586 | 1.3390 | 1.6397 |
| 40 | 1.6179 | 1.2999 | 1.5073 | 0.7694 | 0.7026 | 0.9419 | 0.8056 | 0.7105 | 1.3370 | 1.5929 |
| 45 | 1.6108 | 1.2952 | 1.5025 | 0.6672 | 0.6260 | 0.9139 | 0.8084 | 0.7099 | 1.3355 | 1.5198 |

| | Set A1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 1.6087 | 1.6130 | 1.4837 | 1.5928 | 1.4623 | 2.3656 | 2.3285 | 2.3073 |
| 15 | 1.5718 | 1.5223 | 1.3492 | 1.4603 | 1.3978 | 2.2380 | 2.2145 | 2.1863 |
| 20 | 1.5610 | 1.5007 | 1.1966 | 1.4159 | 1.3804 | 2.1663 | 2.1204 | 2.0901 |
| 25 | 1.5513 | 1.4939 | 1.1613 | 1.1770 | 1.1698 | 2.0834 | 2.0500 | 2.0211 |
| 30 | 1.5495 | 1.4904 | 0.9508 | 1.0366 | 0.9716 | 2.0319 | 2.0026 | 1.9753 |
| 35 | 1.5429 | 1.4793 | 0.9335 | 0.9563 | 0.7966 | 1.9937 | 1.9648 | 1.9368 |
| 40 | 1.5368 | 1.4755 | 0.7697 | 0.7974 | 0.7555 | 1.9662 | 1.9372 | 1.9061 |
| 45 | 1.5360 | 1.4066 | 0.7410 | 0.7101 | 0.7393 | 1.9387 | 1.9132 | 1.8878 |

**Table B-107.3b. Batch 3, 340 nm absorbance, 2nd preincubation without extract, 24Apr2012**

| | Set A3 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | rxn 1 | rxn 2 | rxn 3 | rxn 4 | rxn 5 | rxn 6 | rxn 7 | rxn 8 | rxn 9 | rxn 10 |
| (min) | A3-0 | A3-10 | A3-20 | A3-30 | A3-40 | A2-0 | A2-10 | A2-20 | A2-30 | A2-40 |
| 10 | 1.5585 | 1.5218 | 1.5997 | 2.1958 | 2.1520 | 2.0326 | 2.0790 | 2.1122 | 1.6893 | 1.6865 |
| 15 | 1.4630 | 1.2384 | 1.3899 | 1.9784 | 1.9556 | 2.0249 | 2.0759 | 2.0625 | 1.6719 | 1.6851 |
| 20 | 1.3959 | 1.2660 | 1.3658 | 1.8706 | 1.9537 | 1.8523 | 2.0666 | 1.7723 | 1.6245 | 1.6847 |
| 25 | 1.2987 | 1.2332 | 1.1859 | 1.7730 | 1.9057 | 1.5323 | 2.0631 | 1.5123 | 1.4026 | 1.6454 |
| 30 | 1.2991 | 1.3020 | 1.1484 | 1.6779 | 1.8541 | 1.4181 | 2.0683 | 1.4805 | 1.1663 | 1.5630 |
| 35 | 1.2428 | 1.2330 | 1.1225 | 1.7433 | 1.8013 | 1.3480 | 2.0618 | 1.5511 | 1.0607 | 1.3937 |
| 40 | 1.2489 | 1.1089 | 1.1508 | 1.6196 | 1.8264 | 1.2949 | 2.0176 | 1.4516 | 1.0658 | 1.3756 |
| 45 | 1.1287 | 1.0925 | 1.1322 | 1.5558 | 1.8036 | 1.2480 | 1.8508 | 1.3070 | 0.9834 | 1.2009 |

| | Set A1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| Time | rxn 11 | rxn 12 | rxn 13 | rxn 14 | rxn 15 | rxn 16 | rxn 17 | rxn 18 |
| (min) | A1-0 | A1-10 | A1-20 | A1-30 | A1-40 | neg ctr | 20 µM | 40 µM |
| 10 | 1.6966 | 1.6121 | 2.2530 | 2.2906 | 2.1095 | 2.0942 | 2.1490 | 2.2106 |
| 15 | 1.6815 | 1.5991 | 2.2553 | 2.1352 | 2.0823 | 2.0635 | 2.1092 | 2.1710 |
| 20 | 1.5468 | 1.4774 | 2.2664 | 2.0805 | 2.0748 | 2.0392 | 2.0907 | 2.1463 |
| 25 | 1.3719 | 1.1995 | 2.2674 | 1.8479 | 1.9508 | 2.0279 | 2.0718 | 2.1367 |
| 30 | 1.1215 | 1.0647 | 2.2653 | 1.8373 | 1.9080 | 2.0141 | 2.0724 | 2.1166 |
| 35 | 1.0698 | 1.0750 | 2.2586 | 1.6903 | 1.7725 | 2.0034 | 2.0583 | 2.1149 |
| 40 | 1.0831 | 0.9262 | 2.2506 | 1.7452 | 1.6385 | 1.9992 | 2.0550 | 2.1102 |
| 45 | 0.8942 | 0.8904 | 2.2303 | 1.7419 | 1.4518 | 1.9973 | 2.0524 | 2.1077 |

**Table B-107.3c. Batch 3, 340 nm absorbance, 25 µL CKB 12/10/2007 extract, 24April2012**

| | Set A3 | | | | | Set A2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 1** | **rxn 2** | **rxn 3** | **rxn 4** | **rxn 5** | **rxn 6** | **rxn 7** | **rxn 8** | **rxn 9** | **rxn 10** |
| **(min)** | **A3-0** | **A3-10** | **A3-20** | **A3-30** | **A3-40** | **A2-0** | **A2-10** | **A2-20** | **A2-30** | **A2-40** |
| 10 | 2.3994 | 2.4505 | 2.3508 | 2.1683 | 1.8736 | 2.0146 | 2.1563 | 2.4162 | 2.3911 | 2.2096 |
| 15 | 2.3896 | 2.4414 | 2.3081 | 2.1354 | 1.8122 | 2.0035 | 2.1318 | 2.3818 | 2.3483 | 2.1473 |
| 20 | 2.3797 | 2.4195 | 2.2843 | 2.1058 | 1.7651 | 1.9931 | 2.1123 | 2.3604 | 2.3098 | 2.0985 |
| 25 | 2.3731 | 2.4095 | 2.2601 | 2.0796 | 1.7261 | 1.9868 | 2.0970 | 2.3378 | 2.2812 | 2.0620 |
| 30 | 2.3648 | 2.3973 | 2.2387 | 2.0582 | 1.6947 | 1.9799 | 2.0825 | 2.3184 | 2.2562 | 2.0308 |
| 35 | 2.3626 | 2.3853 | 2.2244 | 2.0436 | 1.6684 | 1.9752 | 2.0751 | 2.3045 | 2.2378 | 2.0068 |
| 40 | 2.3910 | 2.3826 | 2.2090 | 2.0278 | 1.6487 | 1.9687 | 2.0672 | 2.2912 | 2.2201 | 1.9863 |
| 45 | 2.3596 | 2.3771 | 2.2000 | 2.0150 | 1.6341 | 1.9685 | 2.0595 | 2.2841 | 2.2096 | 1.9730 |
| 50 | 2.3554 | 2.3710 | 2.1879 | 2.0088 | 1.6194 | 1.9647 | 2.0551 | 2.2728 | 2.1957 | 1.9557 |
| 55 | 2.3533 | 2.3671 | 2.1825 | 2.0006 | 1.6088 | 1.9617 | 2.0511 | 2.2680 | 2.1868 | 1.9450 |
| 60 | 2.3481 | 2.3704 | 2.1762 | 1.9925 | 1.5969 | 1.9624 | 2.0481 | 2.2620 | 2.1809 | 1.9370 |
| **Rate[a]** | 0.00027 | 0.00121 | 0.00222 | 0.00359 | 0.00420 | 0.00021 | 0.00139 | 0.00252 | 0.00412 | 0.00440 |
| **$r^2$** | 0.7706 | 0.9441 | 0.9124 | 0.9357 | 0.9259 | 0.4909 | 0.9007 | 0.9187 | 0.9370 | 0.9288 |
| **Rate – neg ctrl rate** | -0.00034 | 0.00060 | 0.00161 | 0.00298 | 0.00359 | -0.00040 | 0.00078 | 0.00191 | 0.00351 | 0.00379 |

| | Set A1 | | | | | Controls | | |
|---|---|---|---|---|---|---|---|---|
| **Time** | **rxn 11** | **rxn 12** | **rxn 13** | **rxn 14** | **rxn 15** | **rxn 16** | **rxn 17** | **rxn 18** |
| **(min)** | **A1-0** | **A1-10** | **A1-20** | **A1-30** | **A1-40** | **neg ctr** | **20 µM** | **40 µM** |
| 10 | 2.4554 | 2.3814 | 2.0674 | 2.0801 | 2.2765 | 2.3435 | 2.2524 | 2.2501 |
| 15 | 2.4389 | 2.3481 | 2.0157 | 2.0312 | 2.1982 | 2.3199 | 2.1931 | 2.1760 |
| 20 | 2.4266 | 2.3227 | 1.9798 | 1.9913 | 2.1366 | 2.3065 | 2.1511 | 2.1176 |
| 25 | 2.4205 | 2.3052 | 1.9529 | 1.9584 | 2.0946 | 2.3069 | 2.1223 | 2.0704 |
| 30 | 2.4139 | 2.2884 | 1.9315 | 1.9309 | 2.0553 | 2.3037 | 2.0964 | 2.0270 |
| 35 | 2.4078 | 2.2785 | 1.9145 | 1.9118 | 2.0259 | 2.3050 | 2.0736 | 1.9901 |
| 40 | 2.4048 | 2.2667 | 1.9006 | 1.8959 | 2.0004 | 2.2963 | 2.0535 | 1.9570 |
| 45 | 2.4001 | 2.2591 | 1.8888 | 1.8792 | 1.9745 | 2.2935 | 2.0344 | 1.9262 |
| 50 | 2.3929 | 2.2533 | 1.8784 | 1.8669 | 1.9567 | 2.2913 | 2.0203 | 1.8978 |
| 55 | 2.3966 | 2.2485 | 1.8711 | 1.8593 | 1.9448 | 2.2895 | 2.0030 | 1.8753 |
| 60 | 2.3944 | 2.2417 | 1.8617 | 1.8495 | 1.9341 | 2.2860 | 1.9890 | 1.8524 |
| **Rate[a]** | 0.00085 | 0.00199 | 0.00304 | 0.00410 | 0.00537 | 0.00061 | 0.00471 | 0.00817 |
| **$r^2$** | 0.8301 | 0.9211 | 0.9330 | 0.9408 | 0.9264 | 0.6284 | 0.9495 | 0.9750 |
| **Rate – neg ctrl rate** | 0.00024 | 0.00138 | 0.00243 | 0.00349 | 0.00476 | 0.00000 | 0.00410 | 0.00756 |

[a]Rate is absorbance change per minute, determined from the slopes in Figures B-107.3c-A3, A2, A1, and controls. $r^2$ is the correlation coefficient for the corresponding regression line for each reaction mixture

# Appendix C: PERCHLORATE IN GROUNDWATER SAMPLES TESTED BY REFERENCE METHODS IN COMMERCIAL LABORATORIES

**Table C-1. Perchlorate (μg/L) determined by reference analytical methods in commercial laboratories[a]**

| Site | 102, Hill AFB | 103, NWIRP McGregor | 104, Jet Propulsion Laboratory | 105, Fontana Water Co. | 106, Massachusetts Military Reservation | 107, Lawrence Livermore National Laboratory |
|---|---|---|---|---|---|---|
| **Location** | **U9-16-007** | **OFFWS-37** | **MW-16** | **F-17C** | **J3-INF** | **W-854-1823** |
| **Collection date** | **14Jul2011** | **13Oct2011** | **14Nov2011** | **25Jan2012** | **23Feb2012** | **29Mar2012** |
| **Analysis date** | **22Jul2011** | **19Oct2011** | **21Nov2011** | **09Feb2012** | **06Mar2012** | **12Apr2012** |
| **Instrumentation** | **IC** | **IC** | **IC** | **LC/MS** | **LC/MS** | **LC/MS** |
| **EPA method** | **314.0** | **314.0** | **314.0** | **6850** | **6850** | **6850** |
| **Bottle A** | | | | | | |
| **Replicate 1** | 36 | 59 | ND | 11 | 5.5 | 14 |
| **Replicate 2** | 40 | 60 | ND | 11 | 5.3 | 14 |
| **Replicate 3** | 32 | 61 | ND | 11 | 4.9 | 13 |
| **Bottle B** | | | | | | |
| **Replicate 1** | 47 | 98 | ND | 11 | 5.2 | 15 |
| **Replicate 2** | 44 | 56 | ND | 11 | 5.3 | 14 |
| **Replicate 3** | 37 | 55 | ND | 11 | 5.3 | 13 |
| **Bottle C** | | | | | | |
| **Replicate 1** | 33 | NA | ND | 9.3 | 4.9 | 12 |
| **Replicate 2** | 35 | NA | ND | 9.8 | 4.9 | 13 |
| **Replicate 3** | 38 | NA | ND | 9.5 | 4.8 | 12 |
| **Average ± SD** | 38±5 | 65±16 | <2 | 11±1 | 5.1±0.3 | 13±1 |

[a]NA designates not applicable and ND designates none detected, *i.e.* less than the 2 μg/L reporting limit. The average is listed ± standard deviation

# Appendix D: PERCHLORATE IN GROUNDWATER SAMPLES TESTED BY EPA METHOD 314.0 AT UNIVERSITY OF CALIFORNIA, BERKELEY

**Table D-1. Perchlorate (μg/L) determined by EPA Method 314.0 at University of California, Berkeley[a]**

| Site | 102, Hill AFB | 103, NWIRP McGregor | 104, Jet Propulsion Laboratory | 105, Fontana Water Co. | 106, Massachusetts Military Reservation | 107, Lawrence Livermore National Laboratory |
|---|---|---|---|---|---|---|
| **Location** | **U9-16-007** | **OFFWS-37** | **MW-16** | **F-17C** | **J3-INF** | **W-854-1823** |
| **Collection date** | **14Jul2011** | **13Oct2011** | **14Nov2011** | **25Jan2012** | **23Feb2012** | **29Mar2012** |
| **Analysis date** | **11Aug2011** | **09Nov2011** | **14Dec2011** | **24Feb2012** | **22Mar2012** | **25Apr2012** |
| **Instrumentation** | **IC** | **IC** | **IC** | **IC** | **IC** | **IC** |
| **EPA method** | **314.0** | **314.0** | **314.0** | **314.0** | **314.0** | **314.0** |
| **Bottle A** | | | | | | |
| **Replicate 1** | 34 | 58 | ND | 11 | 6 | 16 |
| **Replicate 2** | 35 | 58 | ND | 11 | 6 | 16 |
| **Replicate 3** | 35 | 59 | ND | 11 | 6 | 16 |
| **Bottle B** | | | | | | |
| **Replicate 1** | 35 | 54 | ND | 11 | 6 | 15 |
| **Replicate 2** | 35 | 55 | ND | 11 | 7 | 15 |
| **Replicate 3** | 36 | 55 | ND | 11 | 6 | 15 |
| **Bottle C** | | | | | | |
| **Replicate 1** | 36 | NA | ND | 11 | 6 | 16 |
| **Replicate 2** | 36 | NA | ND | 10 | 6 | 16 |
| **Replicate 3** | 36 | NA | ND | 10 | 6 | 16 |
| **Average ± SD** | 35±1 | 57±2 | <4 | 11±0 | 6±0 | 16±1 |

[a]NA designates not applicable and ND designates none detected, *i.e.* less than the 4 μg/L reporting limit. The average is listed ± standard deviation

# Appendix E: CALIBRATION PROCEDURES, QUALITY ASSURANCE SAMPLING, DECONTAMINATION PROCEDURES, AND SAMPLE DOCUMENTATION

## E-1.0 CALIBRATION OF ANALYTICAL EQUIPMENT

Ion chromatographs and analytical balances receive anual preventive maintenance inspection and calibration by the manufacturer or the manufacturer's designate. In addition, calibration standards are analyzed with each batch of samples analyzed by ion chromatography.

Conductivity standards are analyzed before use as specified in the operator's manual.

Standards for the pH meter are used daily to calibrate the pH meter, as specified in the operator's manual.

The alignment of the UV/Vis spectrophotometer is checked before use.

## E-2.0 QUALITY ASSURANCE SAMPLING

Three one gallon amounts of groundwater were collected successively in different bottles for each sampling event. This provided field replicates for testing.

The contents of each bottle were subsequently tested in triplicate by each of the three perchlorate analysis methods (benchtop bioassay, IC or LC/MS performed by a commercial laboratory, and IC performed in the laboratory of the PI)

## E-3.0 DECONTAMINATION PROCDURES

Decontamination procedures are not applicable to the project.

## E-4.0 SAMPLE DOCUMENTATION

### E-4.1 SHIPPING AND COLLECTION OF GROUNDWATER SAMPLES

Bottles were prelabeled and shipped in a sealed cooler with a chain-of-custody form to the site. The samples were collected by site personnel who shipped them to the PI's laboratory for next day delivery in the sealed cooler with the chain-of-custody form.

In addition to documenting receipt of the shipment on the chain-of-custody form, receipt was also documented in the bound laboratory notebook dedicated to the project.

### E-4.2 DOCUMENTATION FOR SUBSAMPLES ANALYZED BY COMMERCIAL LABORATORIES

Triplicate subsamples were prepared from each gallon of groundwater in labeled bottles. These were shipped for next day delivery in a sealed cooler with a chain-of-custody form to the commercial laboratory.
The operations undertaken to prepare these subsamples were documented in the bound laboratory notebook dedicated to the project.

### E-4.3 DOCUMENTATION FOR SUBSAMPLES ANALYZED IN THE PI'S LABORATORY

Triplicate subsamples for bioassay analysis and IC analysis in the PI's laboratory were prepared in tubes or bottles and project-specific labels identified the contents with regard to groundwater source and the dates of source collection and subsample preparation.

### E-4.4 DOCUMENTATION FOR SPE ELUATE PREPARATION AND BENCHTOP BIOASSAY TESTING

Steps in the preparation of SPE eluates and the dates operations were performed were documented on project specific test records.

Absorbance readings at 340 nm for perchlorate reductase reactions in cuvettes were measured at timed intervals. These data were recorded on test records and constitute the primary data for benchtop bioassay testing.

# Appendix F: MICROPLATE READER BIOASSAY DATA

In addition to benchtop bioassay testing (see Appendix B), 3 to 5 SPE eluate sets from the different groundwater sources were tested by the plate reader format of the bioassay, which is performed in an anerobic chamber using a 96-well microplate reader. Results are presented in Table F-1. There were five testing batches, identified with Roman numerals in Table F-1.

**Table F-1. Perchlorate determined by plate reader bioassay for some SPE eluate sets with storage for various periods at 2 – 8 °C prior to analysis[a]**

| Site | Para-meter[b] | A1 | A2 | | A3 | B1 | B2 | B3 | C1 | C2 | C3 | Average ± SD[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 102, Hill AFB | SPE date | 27 Jul 2011 | 27 Jul 2011 | | 27 Jul 2011 | 6 Aug 2011 | - | - | - | - | 9 Aug 2011 | 34±10 (batch I) |
| | PR batch | I | I | V | I | V | - | - | - | - | V | |
| | µg/L | 36±4 | 43 ±14 | 27 ±5 | 23±4 | 38±17 | - | - | - | - | 34±4 | 33±6 (batch V) |
| 103, NWIRP McGre-gor | SPE date | - | 5 Nov 2011 | | 5 Nov 2011 | - | 8 Nov 2011 | - | NA | NA | NA | |
| | PR batch | - | III | | II | - | IV | - | | | | |
| | µg/L | - | 53±6 | | 45±12 | - | 49±4 | - | | | | 49±4 |
| 104, JPL | SPE date | - | 8 Dec 2011 | | - | - | - | 11 Dec 2011 | - | 11 Dec 2011 | - | |
| | PR batch | - | II | | - | - | - | III | - | IV | - | |
| | µg/L | - | -10±7 | | - | - | - | 2±2 | - | -3±3 | - | -4±6 |
| 105, Fontana Water Co. | SPE date | 13 Feb 2012 | 13 Feb 2012 | | - | - | - | 15 Feb 2012 | - | - | 17 Feb 2012 | |
| | PR batch | II | III | | - | - | - | IV | - | - | V | |
| | µg/L | 19±5 | 11±1 | | - | - | - | 8±2 | - | - | 10±1 | 12±5 |
| 106, MMR | SPE date | - | 12 Mar 2012 | | 12 Mar 2012 | 13 Mar 2012 | - | - | - | 14 Mar 2012 | - | |
| | PR batch | - | V | | II | III | - | - | - | IV | - | |
| | µg/L | - | 3±1 | | 4±3 | 8±2 | - | - | - | 4±0.4 | - | 5±2 |
| 107, LLNL | SPE date | - | - | | - | 12 Apr 2012 | 16 Apr 2012 | - | 17 Apr 2012 | - | - | |
| | PR batch | - | - | | - | II | III | - | IV | - | - | |
| | µg/L | - | - | | - | 13±3 | 14±3 | - | 6±2 | - | - | 11±4 |

[a]NA denotes not applicable, "-" denotes that there is no data for this cell of the table

[b]SPE date corresponds to the preparation date for an SPE eluate set. The plate reader bioassay batches are identified as I (tested on 5 Aug 2011), II (tested on 29 Jun 2012), III (tested on 6 Jul 2012), and IV and V (tested successively on 11 Jul 2012)

[c]The spread around the average is given as the standard deviation

The bioassay rates (i.e. 340 nm absorbance change/minute) used in the standard additions method to determine the perchlorate (µg/L) for an SPE eluate set are listed for the different

batches in Tables F-2 (batch I), F-3 (batch II), F-4 (batch III), F-5 (batch IV), and F-6 (batch V). Each is accompanied by a figure showing the standard additions method regression lines for the SPE eluate sets in the batch, from which the perchlorate concentration is determined. Additionally, several subsequent figures for each batch present the experimental data, i.e. the 340 nm absorbance over time for eluates. The perchlorate spikes added were 0, 10, 20, 30, or 40 µg/L.

**Table F-2. Perchlorate estimated in plate reader bioassay batch 1 by the standard additions method for Hill SPE eluate sets A1, A2, A3, tested 5 Aug 2011[a]**

| Perchlorate Spike (µg/L) | 102, Set A1 | 102, Set A2 | 102, Set A3 | Perchlorate Standards | |
|---|---|---|---|---|---|
| | | | | µM | Rate |
| **0** | 0.001153 | 0.001118 | 0.000884 | 0 | 0.000000 |
| **10** | 0.001532 | 0.002108 | 0.001328 | 10 | 0.000640 |
| **20** | 0.001877 | 0.002169 | 0.001389 | 20 | 0.001160 |
| **30** | 0.002006 | 0.002247 | 0.001917 | 30 | 0.001655 |
| **40** | 0.002535 | 0.002689 | 0.002445 | 40 | 0.002283 |
| **Regression parameters for each set[b]** | | | | | |
| **y-intercept ± error** | 0.0011728 ±0.000075 | 0.0014100 ±0.00022 | 0.0008503 ±0.00012 | | 0.0000314 ±0.000036 |
| **Slope ± error** | 0.0000324 ±0.0000031 | 0.0000328 ±0.0000092 | 0.0000371 ±0.0000048 | | 0.0000558 ±0.000001 |
| **$r^2$** | 0.9736 | 0.8080 | 0.9529 | | 0.9979 |
| **x-intercept ± error** | -36±4.2 | -43±14 | -23±4.3 | | -0.56±0.65 |
| **$ClO_4^-$ in groundwater ± error, (µg/L)[c]** | 36±4 | 43±14 | 23±4 | | NA |

[a]The eluate sets were tested in duplicate and the values listed are the average rate (340 nm AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the background NADH oxidation rate in the negative control for the batch. The negative control rate was 0.000501 AU/minute. Perchlorate reductase was added to microplate wells in 10 µL of CKB 8/3/2011 extract. NA denotes not applicable

[b]Regression parameters are from the regression line for each set (see Figure F-1). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * $\sqrt{((\text{slope error/slope})^2 + (\text{y-intercept error/y-intercept})^2)}$

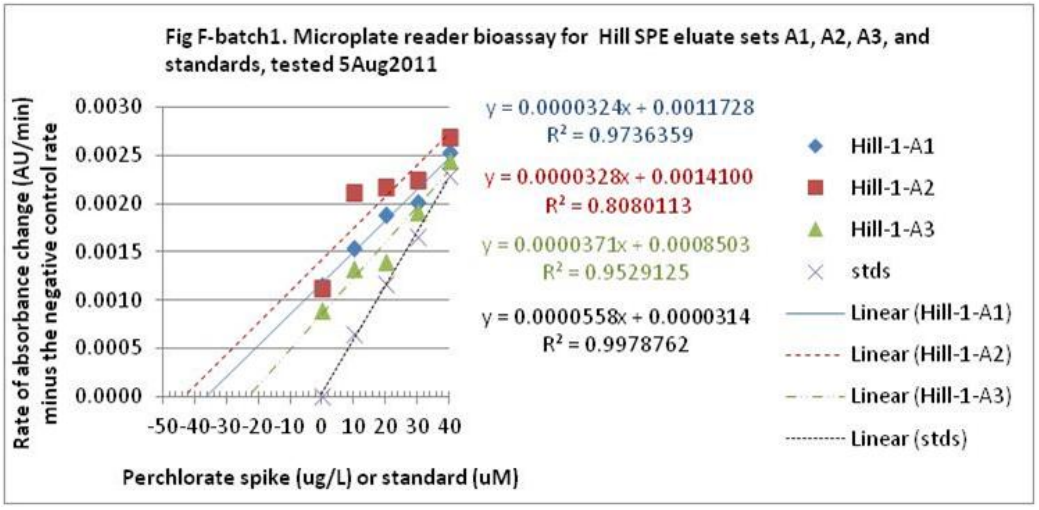



**Figure F-1. Perchlorate by the standard additions method in plate reader bioassay batch 1 for Hill SPE eluate sets A1, A2, A3, and control standards, tested 5 Aug 2011**

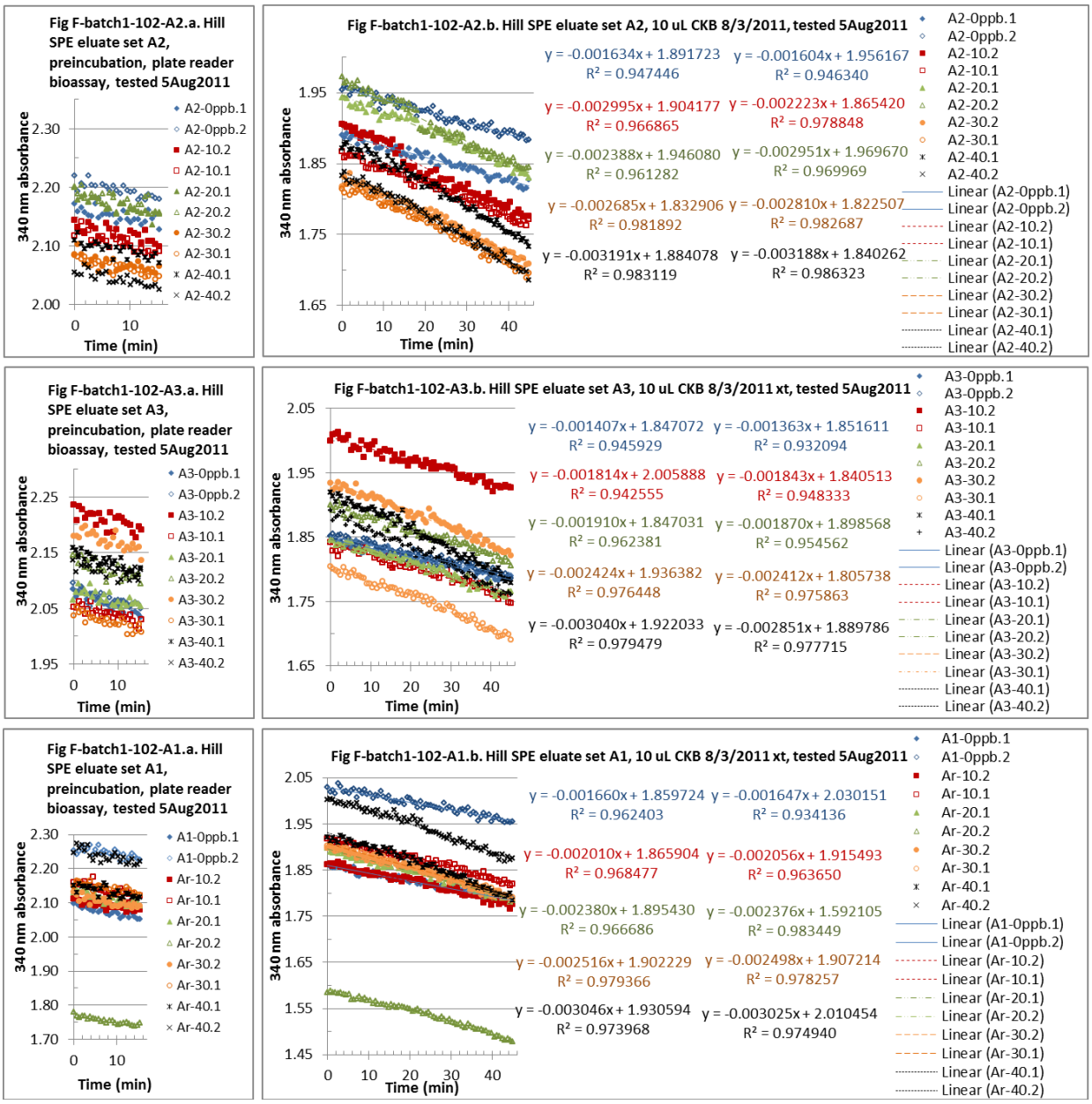



**Figure F-1i. Plate reader bioassay batch 1 absorbance change for SPE eluate sets Hill A1, A2, and A3, 10 µL CKB 8/3/2011 extract, tested 5 Aug 2011**

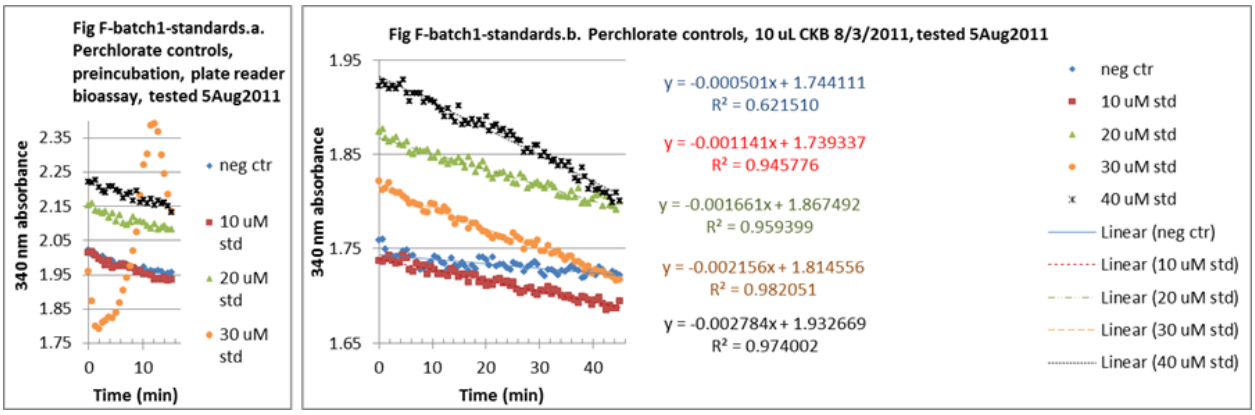



**Figure F-1ii. Plate reader bioassay batch 1 absorbance change for control standards, 10 µL CKB 8/3/2011 extract, tested 5 Aug 2011**

**Table F-3. Perchlorate estimated in plate reader bioassay batch 2 by the standard additions method for SPE eluate sets FWC-A1, JPL-B1, LLNL-B1, McGregor-A3, and MMR-A3, tested 29 Jun 2012[a]**

| Perchlorate Spike (µg/L) | 105, Fontana Water Co-A1 | 104, JPL-B1 | 107, LLNL-B1 | 103, NWIRP McGregor–A3 | 106, MMR-A3 |
|---|---|---|---|---|---|
| 0 | 0.001286 | -0.001376 | 0.000527 | 0.003735 | 0.000526 |
| 10 | 0.001739 | 0.000011 | 0.001468 | 0.003979 | 0.000827 |
| 20 | 0.002006 | 0.001501 | 0.001854 | 0.004189 | 0.001904 |
| 30 | 0.002631 | 0.002788 | 0.002515 | 0.006197 | 0.003207 |
| 40 | 0.003762 | 0.002039 | 0.002765 | 0.006419 | 0.003563 |
| **Regression parameters for each set[b]** | | | | | |
| **y-intercept ± error** | 0.001116 ±0.00023 | -0.000929 ±0.00062 | 0.000721 ±0.00016 | 0.003387 ±0.00043 | 0.000315 ±0.00025 |
| **Slope ± error** | 0.000058 ±0.0000093 | 0.000096 ±0.000025 | 0.000055 ±0.0000064 | 0.000076 ±0.000018 | 0.000085 ±0.000010 |
| **$r^2$** | 0.9293 | 0.8277 | 0.9614 | 0.8589 | 0.9584 |
| **x-intercept ± error** | -19±4.9 | 9.7±6.9 | -13±3.2 | -45±12 | -3.7±3.0 |
| **$ClO_4^-$ in groundwater ± error, (µg/L)[c]** | 19±5 | -10±7 | 13±3 | 45±12 | 4±3 |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate (n = 5) was 0.000466 AU/minute. Perchlorate reductase was added to microplate wells in 10 µL of CKB 5/8/2012 extract

[b]Regression parameters are from the regression line for each set (see Figure F-2). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike)

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * $\sqrt{((\text{slope error/slope})^2 + (\text{y-int error/y-int})^2)}$

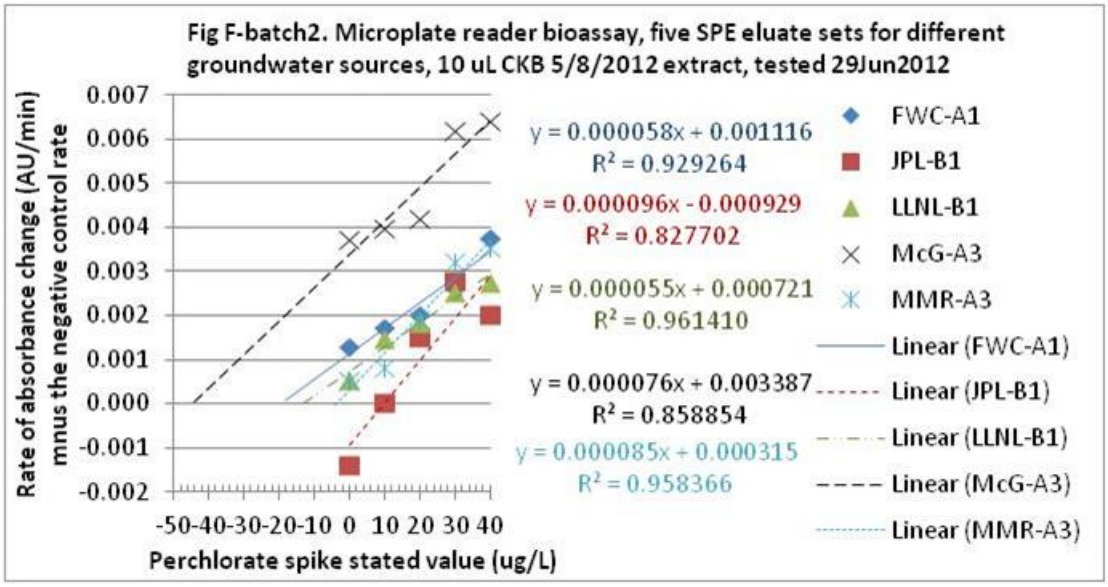



**Figure F-2. Perchlorate estimated in plate reader bioassay batch 2 by the standard additions method for SPE eluate sets FWC A1, JPL B1, LLNL B1, NWIRP McGregor A3, and MMR A3, tested 29 Jun 2012**

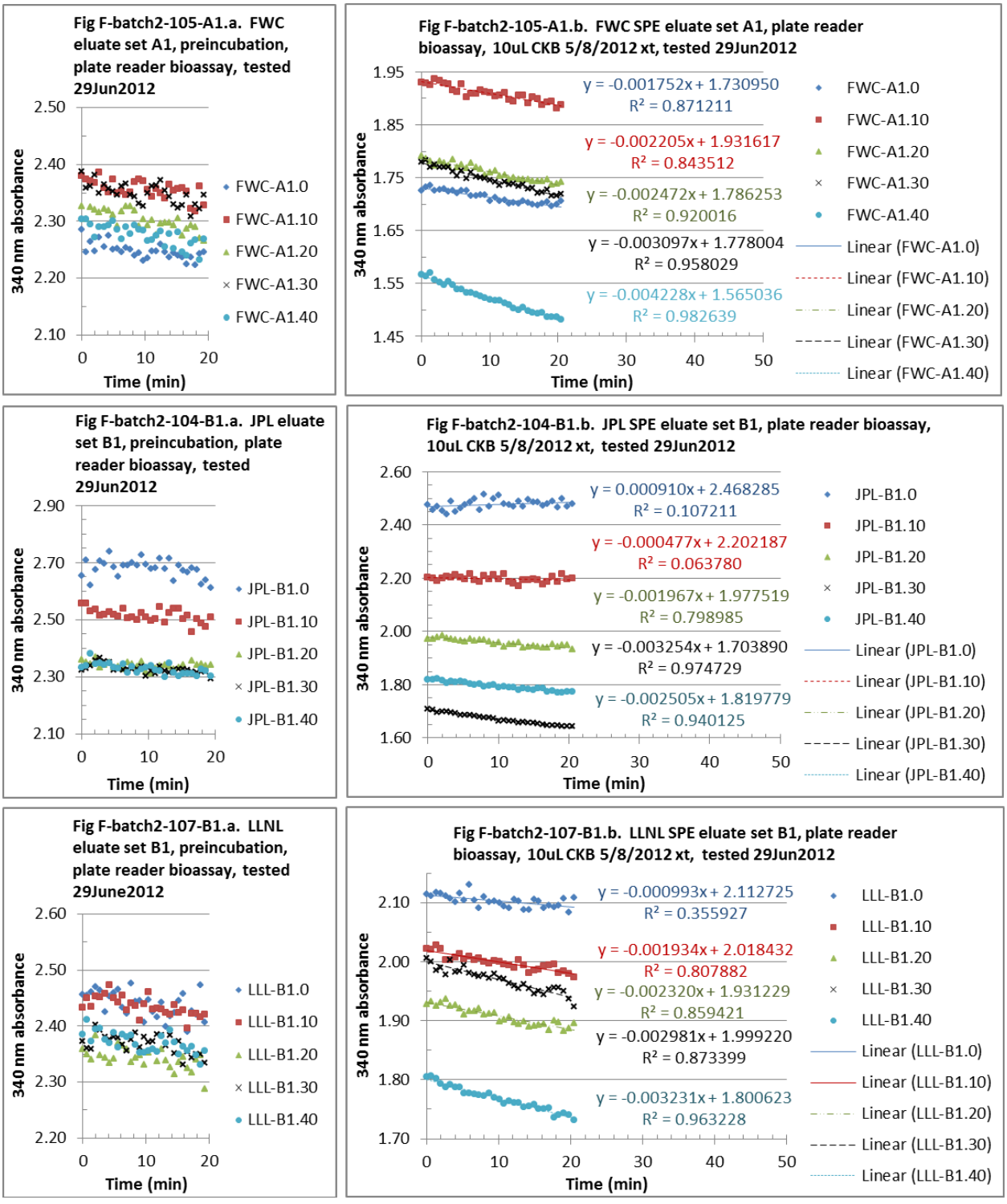



**Figure F-2i. Plate reader bioassay batch 2 absorbance change for SPE eluate sets FWC A1, JPL B1, and LLNL B1, 10 µL CKB 5/8/2012 extract, tested 29 Jun 2012**

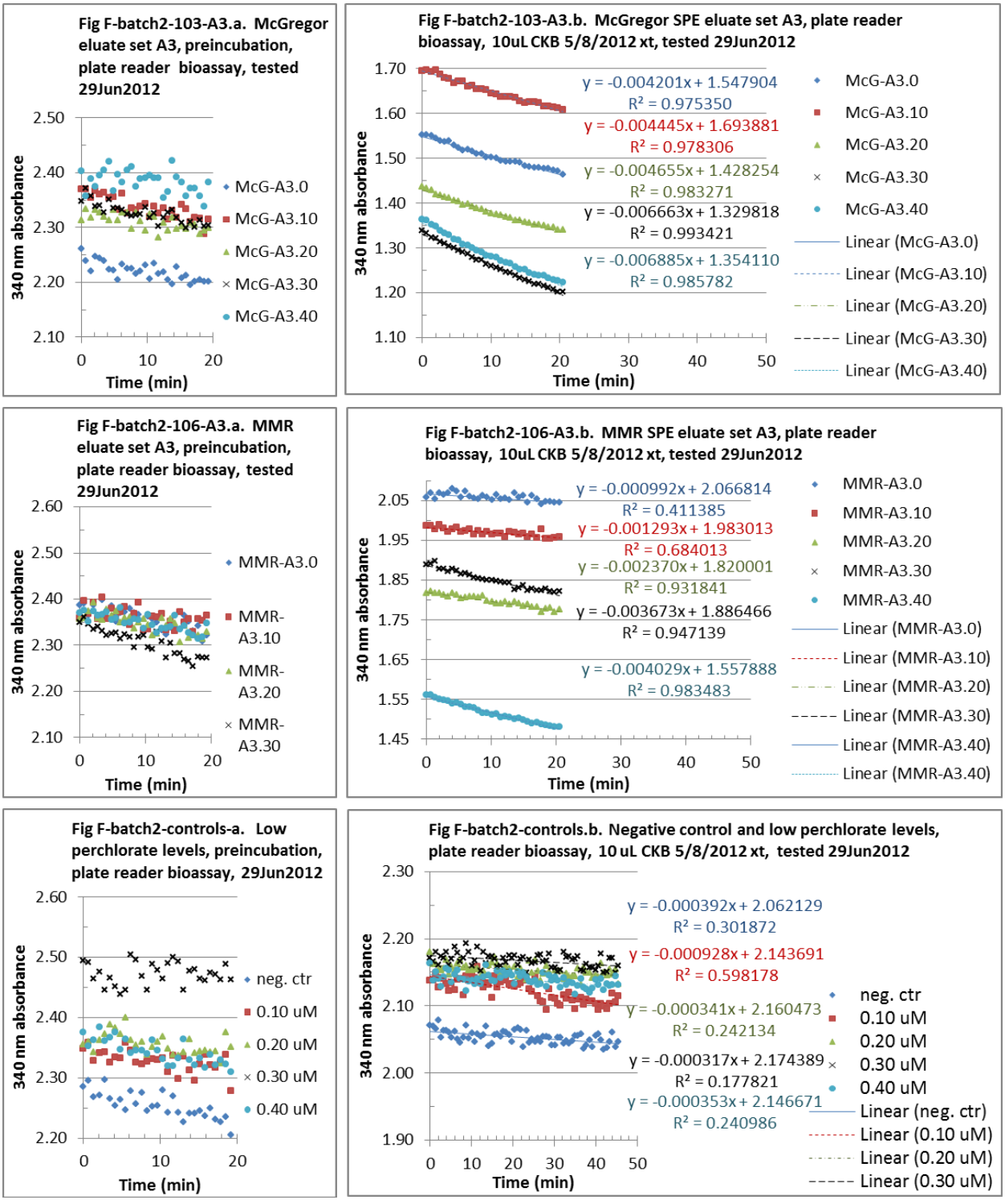



**Figure F-2ii. Plate reader bioassay batch 2 absorbance change for SPE eluate sets McGregor A3, MMR A3, and negative control and low perchlorate levels of standards, 10 µL CKB 5/8/2012 extract, tested 29 Jun 2012**

**Table F-4. Perchlorate estimated in plate reader bioassay batch 3 by the standard additions method for SPE eluate sets FWC-A2, JPL-B3, LLNL-B2, NWIRP McGregor-A2, and MMR-B1, tested 6 Jul 2012[a]**

| Perchlorate Spike (µg/L) | 105, FWC-A2 | 104, JPL-B3 | 107, LLNL-B2 | 103, NWIRP McGregor-A2 | 106, MMR-B1 |
|---|---|---|---|---|---|
| 0 | 0.0025738 | 0.0004478 | 0.0024498 | 0.0101648 | 0.0021588 |
| 10 | 0.0047358 | 0.0026238 | 0.0039738 | 0.0117088 | 0.0033608 |
| 20 | 0.0071228 | 0.0037818 | 0.0047168 | 0.0136018 | 0.0064428 |
| 30 | 0.0090988 | 0.0072858 | 0.0069978 | 0.0147608 | 0.0081748 |
| 40 | 0.0118418 | 0.0084258 | 0.0087638 | 0.0179888 | 0.0107048 |
| **Regression parameters for each set[b]** | | | | | |
| **y-intercept ± error** | 0.002495 ±0.00015 | 0.000389 ±0.00047 | 0.00225 ±0.00034 | 0.009905 ±0.00045 | 0.001787 ±0.00036 |
| **Slope ± error** | 0.000229 ±0.0000062 | 0.000206 ±0.000019 | 0.000157 ±0.000014 | 0.000187 ±0.000018 | 0.000219 ±0.000015 |
| **$r^2$** | 0.9978 | 0.9744 | 0.9772 | 0.9720 | 0.9866 |
| **x-intercept ± error** | -11±0.73 | -1.9±2.3 | -14±2.5 | -53±5.7 | -8.2±1.7 |
| **$ClO_4^-$ in groundwater ± error, (µg/L)[c]** | 11±1 | 2±2 | 14±3 | 53±6 | 8±2 |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate was 0.000463 (n = 5). Perchlorate reductase was added to wells in 10 µL of CKB 5/8/2012 extract

[b]Regression parameters are from the regression line for each set (see Figure F-3). The slope is the rate of absorbance change per µg/L of spike (AU/minute/ppb of perchlorate spike)

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * √ ((slope error/slope)$^2$ + (y-intercept error/y-intercept)$^2$)

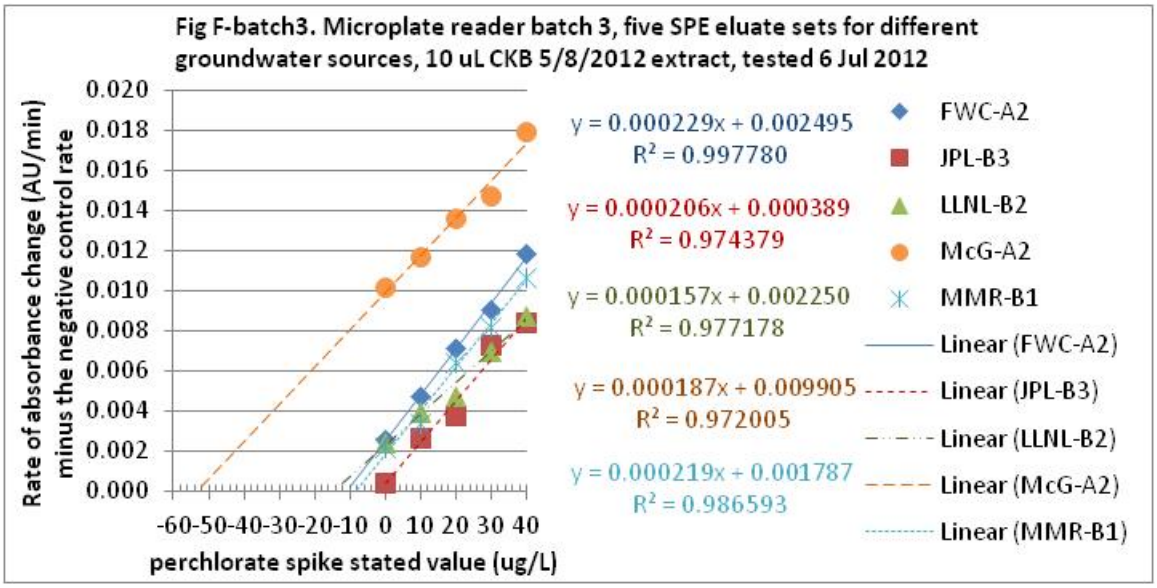



**Figure F-3. Perchlorate estimated in plate reader bioassay batch 3 by the standard additions method for SPE eluate sets FWC A2, JPL B3, LLNL B2, NWIRP McGregor A2, and MMR B1, tested 6 Jul 2012**

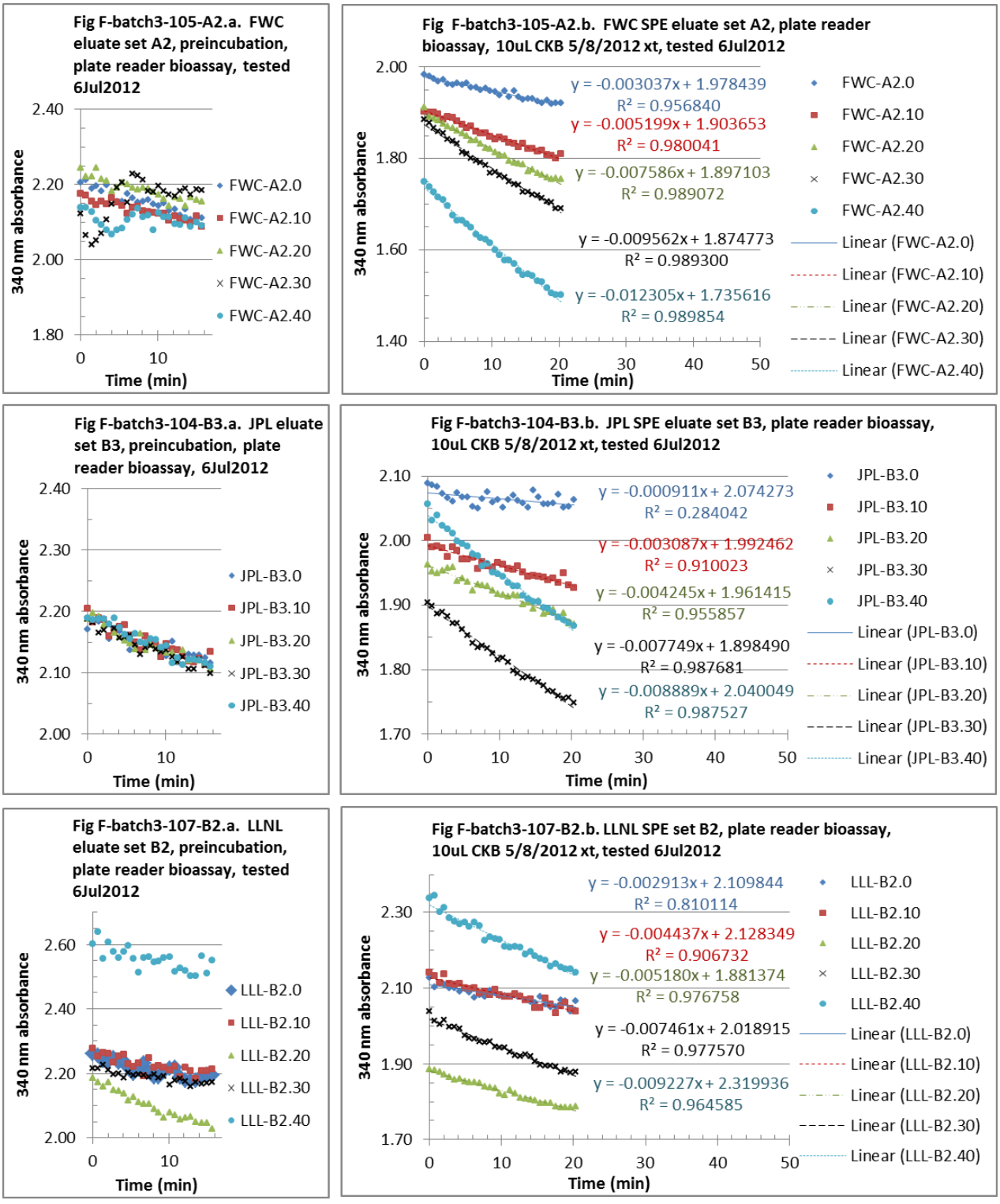



**Figure F-3i. Plate reader bioassay batch 3 absorbance change for SPE eluate sets FWC A2, JPL B3, and LLNL B2, 10 µL CKB 5/8/2012 extract, tested 6 Jul 2012**

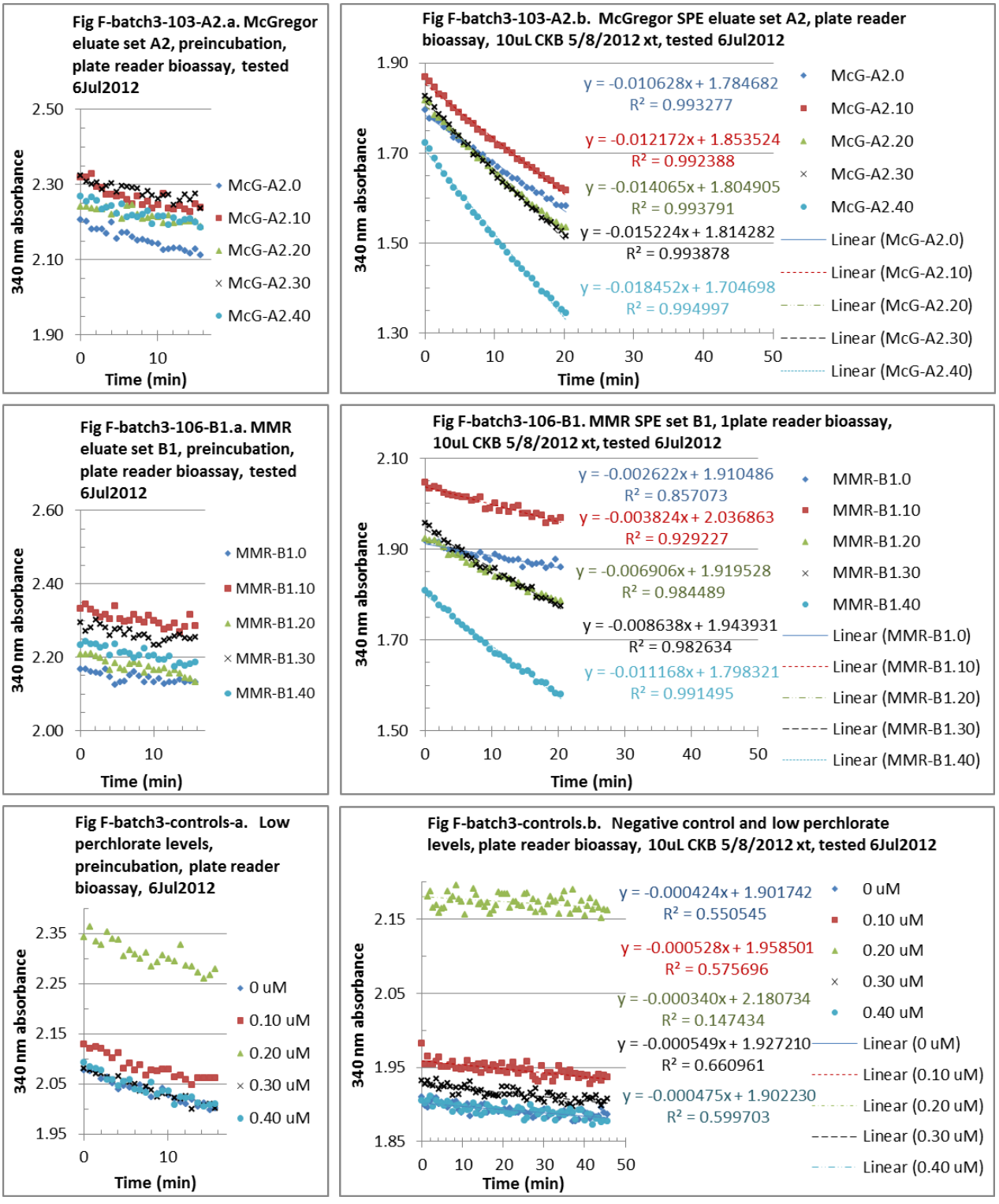



**Figure F-3ii. Plate reader bioassay batch 3 absorbance change for SPE eluate sets McGregor A2, MMR B1, and negative control and low perchlorate standards, 10 µL CKB 5/8/2012 extract, tested 6 Jul 2012**

**Table F-5. Perchlorate estimated in plate reader bioassay batch 4 by the standard additions method for SPE eluate sets FWC-B3, JPL-C2, LLNL-C1, NWIRP McGregor-B2/3, and MMR-C2, tested 11 Jul 2012[a]**

| Perchlorate Spike (µg/L) | 105, FWC-B3 | 104, JPL-C2 | 107, LLNL-C1 | 103, NWIRP McGregor-B2/3[b] | 106, MMR-C2 | Perchlorate Standards | |
|---|---|---|---|---|---|---|---|
| | | | | | | µM | Rate |
| 0 | 0.001735 | -0.000299 | 0.001726 | 0.003209 | 0.000921 | 0 | 0 |
| 10 | 0.003507 | 0.001417 | 0.002994 | 0.005764 | 0.003065 | 10 | 0.002746 |
| 20 | 0.004892 | 0.002903 | 0.00583 | 0.007623 | 0.005078 | 20 | 0.006317 |
| 30 | 0.007966 | 0.004286 | 0.007298 | 0.009355 | 0.007373 | 30 | 0.009047 |
| 40 | 0.009083 | 0.007635 | 0.010291 | 0.011832 | 0.009693 | 40 | 0.013132 |
| **Regression parameters for each set[c]** | | | | | | | |
| **y-intercept ± error** | 0.001606 ±0.00038 | -0.000559 ±0.00050 | 0.001341 ±0.00040 | 0.003389 ±0.00021 | 0.000856 ±0.000087 | -0.0002646 ±0.00031 | |
| **Slope ± error** | 0.000192 ±0.000016 | 0.000187 ±0.000020 | 0.000214 ±0.000016 | 0.000208 ±0.0000085 | 0.000219 ±0.0000035 | 0.000326 ±0.000013 | |
| **$r^2$** | 0.9806 | 0.9656 | 0.9830 | 0.9951 | 0.9992 | 0.9954 | |
| **x-intercept ± error** | -8.4±2.1 | 3.0±2.7 | -6.3±1.9 | -16±1.2 | -3.9±0.40 | 0.81±0.95 | |
| **$ClO_4^-$ in groundwater ± error (µg/L)[d]** | 8±2 | -3±3 | 6±2 | 49±4 | 4±0.4 | NA | |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the different bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate was 0.000773 (n = 3). Perchlorate reductase was added to wells in 10 µL CKB 5/8/2012 extract

[b]The samples from McGregor groundwater bottle B were diluted 1/3 with deionized water prior to spiking with perchlorate and application to SPE cartridges. Thus the estimated level of perchlorate in groundwater is equal to (3)(-1)(x-intercept) for the B2 eluate set

[c]Regression parameters are from the regression line for each set (see Figure F-4). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standard

[d]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * √ ((slope error/slope)$^2$ + (y-intercept error/y-intercept)$^2$)

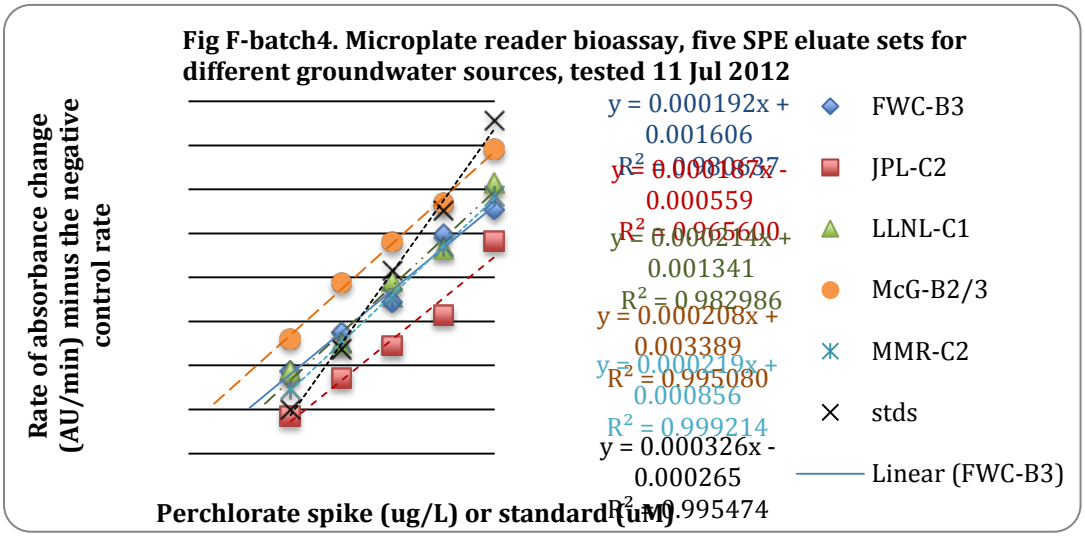



**Figure F-4. Perchlorate estimated in plate reader bioassay batch 4 by the standard additions method for SPE eluate sets FWC B3, JPL C2, LLNL C1, McGregor B2/3, MMR C2, and control standards, tested 11 Jul 2012**

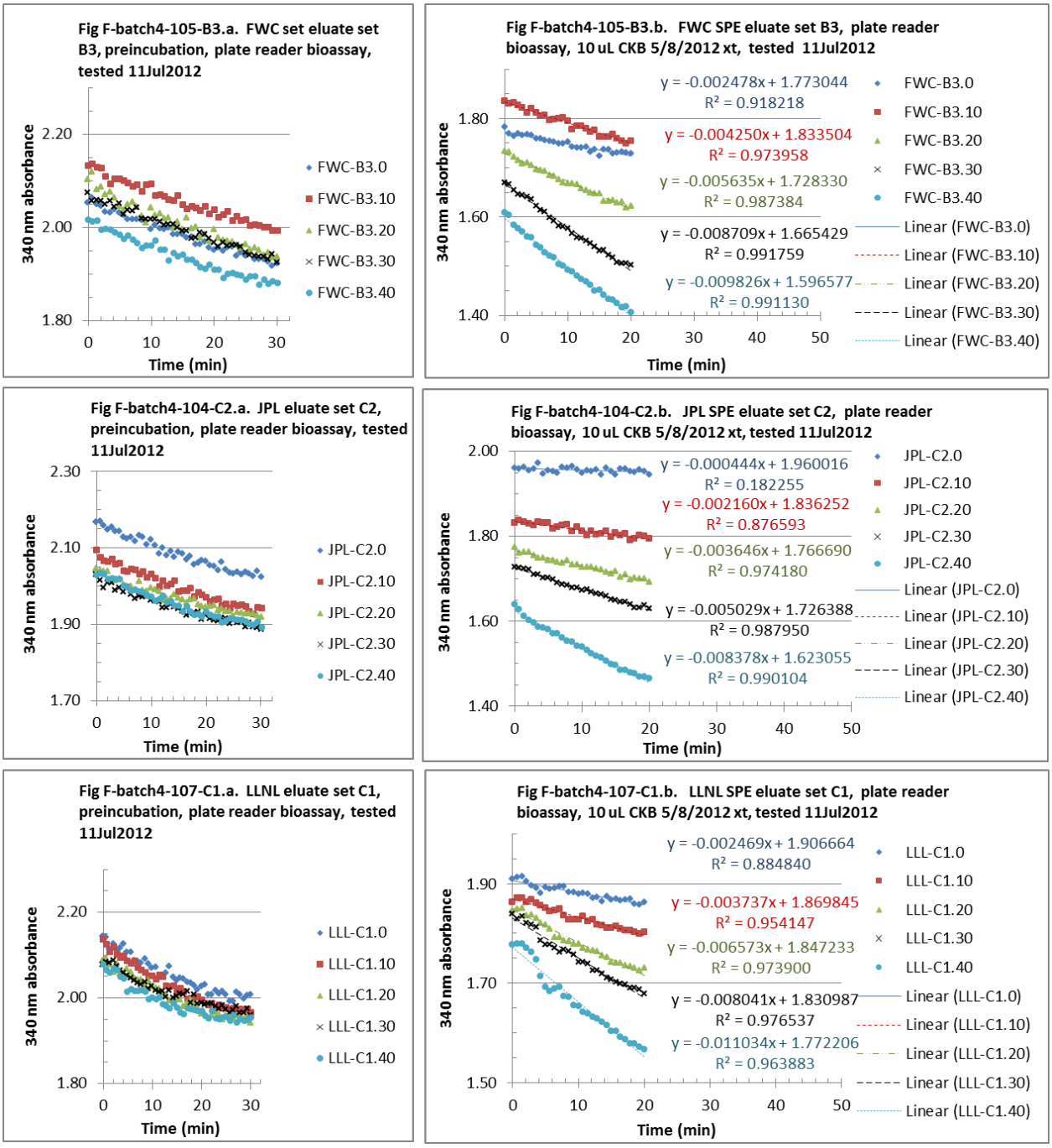



**Figure F-4i. Plate reader bioassay batch 4 absorbance change for SPE eluate sets FWC B3, JPL C2, and LLNL C1, 10 µL CKB 5/8/2012 extract, tested 11 Jul 2012**

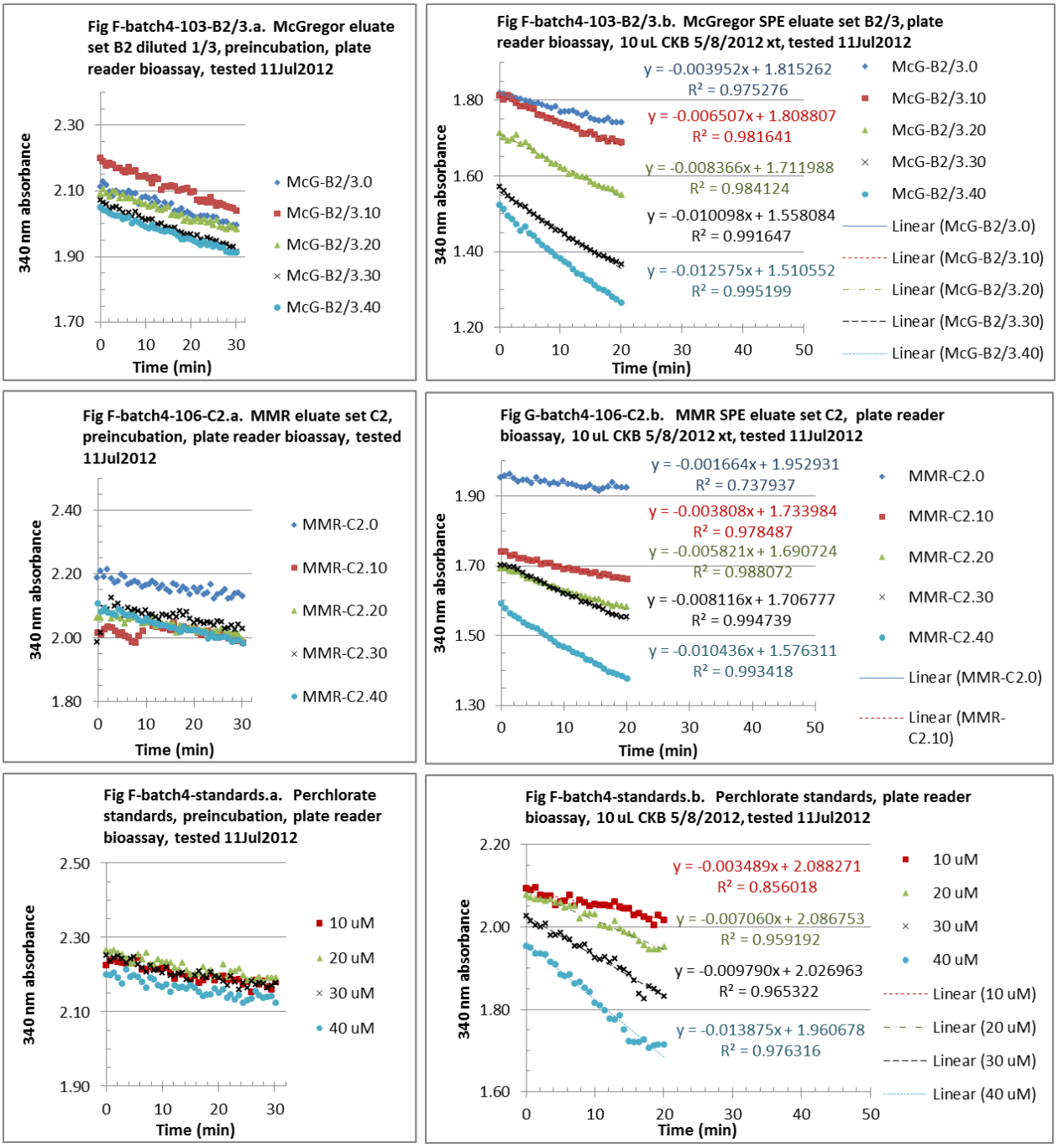



**Figure F-4ii. Plate reader bioassay batch 4 absorbance change for SPE eluate sets McGregor B2/3, MMR C2, and perchlorate standards, 10 µL CKB 5/8/2012 extract, tested 11 Jul 2012**

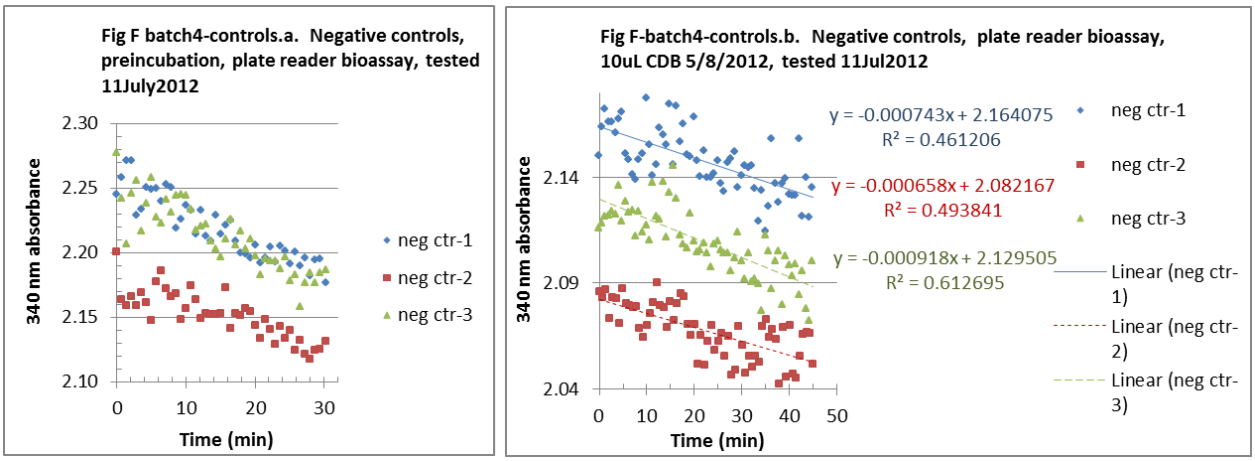



**Figure F-4iii. Plate reader bioassay batch 4 absorbance change for negative control wells, 10 µL CKB 5/8/2012 extract, tested 11 Jul 2012**

**Table F-6. Perchlorate estimated in plate reader bioassay batch 5 by the standard additions method for SPE eluate sets Hill A2, B1, C3, FWC-C3, and MMR-A2 , tested 11 Jul 2012[a]**

| Perchlorate Spike (µg/L) | 102, Hill-A2 | 102, Hill-B1 | 102, Hill-C3 | 105, FWC-C3 | 106, MMR-A2 | Perchlorate Standards | |
|---|---|---|---|---|---|---|---|
| | | | | | | µM | Rate |
| 0 | 0.004067 | 0.005696 | 0.005723 | 0.002066 | 0.001068 | 0 | 0.000000 |
| 10 | 0.007270 | 0.005752 | 0.006529 | 0.003389 | 0.003109 | 10 | 0.002802 |
| 20 | 0.008763 | 0.005370 | 0.007901 | 0.005620 | 0.005728 | 20 | 0.005926 |
| 30 | 0.009496 | 0.007739 | 0.010139 | 0.007918 | 0.008759 | 30 | 0.008990 |
| 40 | 0.011846 | 0.010738 | 0.011760 | 0.009353 | 0.011030 | 40 | 0.012205 |
| **Regression parameters for each set[b]** | | | | | | | |
| **y-intercept ± error** | 0.004732 ±0.00056 | 0.004645 ±0.0011 | 0.005274 ±0.00036 | 0.001849 ±0.00025 | 0.000824 ±0.00022 | | -0.000135 ±0.00010 |
| **Slope ± error** | 0.000178 ±0.000023 | 0.000121 ±0.000044 | 0.000157 ±0.000014 | 0.000191 ±0.000010 | 0.000256 ±0.0000091 | | 0.000306 ±0.0000041 |
| **$r^2$** | 0.9527 | 0.7137 | 0.9750 | 0.9913 | 0.9962 | | 0.9995 |
| **x-intercept ± error** | -27±4.7 | -38±17 | -34±3.8 | -9.7±1.4 | -3.2±0.88 | | 0.44±0.33 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[c]** | 27±5 | 38±17 | 34±4 | 10±1 | 3±1 | | NA |

[a]Values are the rate of 340 nm absorbance change (AU/minute) due to NADH oxidation for the differnt bioassay reaction mixtures minus the average background NADH oxidation rate in negative controls for the batch. There were no duplicates tested for the eluate sets. The negative control rate was 0.000941 (n = 2). Perchlorate reductase was added to wells in 10 µL CKB 5/8/2012 extract

[b]Regression parameters are from the regression line for each set (see Figure F-5). The slope is the rate of absorbance change per ppb of spike (AU/minute/ppb of perchlorate spike), or AU/minute/µM perchlorate standardl

[c]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * $\sqrt{}$ ((slope error/slope)$^2$ + (y-intercept error/y-intercept)$^2$)

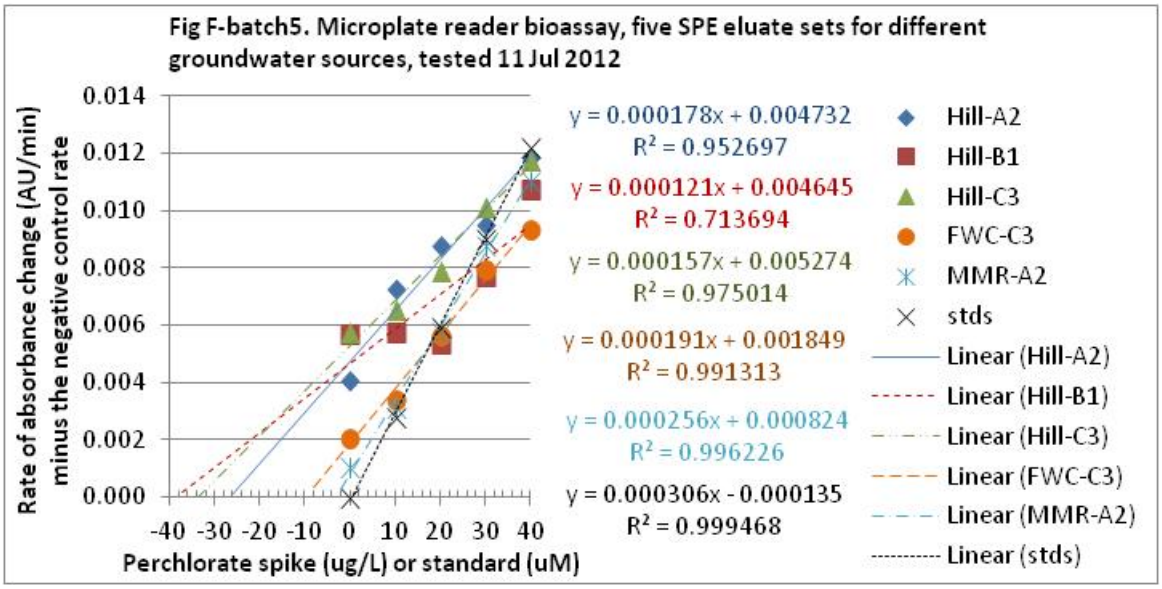



**Figure F-5. Perchlorate estimated in plate reader bioassay batch 5 by the standard additions method for SPE eluate sets Hill A2, B1, C3, FWC C3, MMR A2, and control standards, tested 11 Jul 2012**

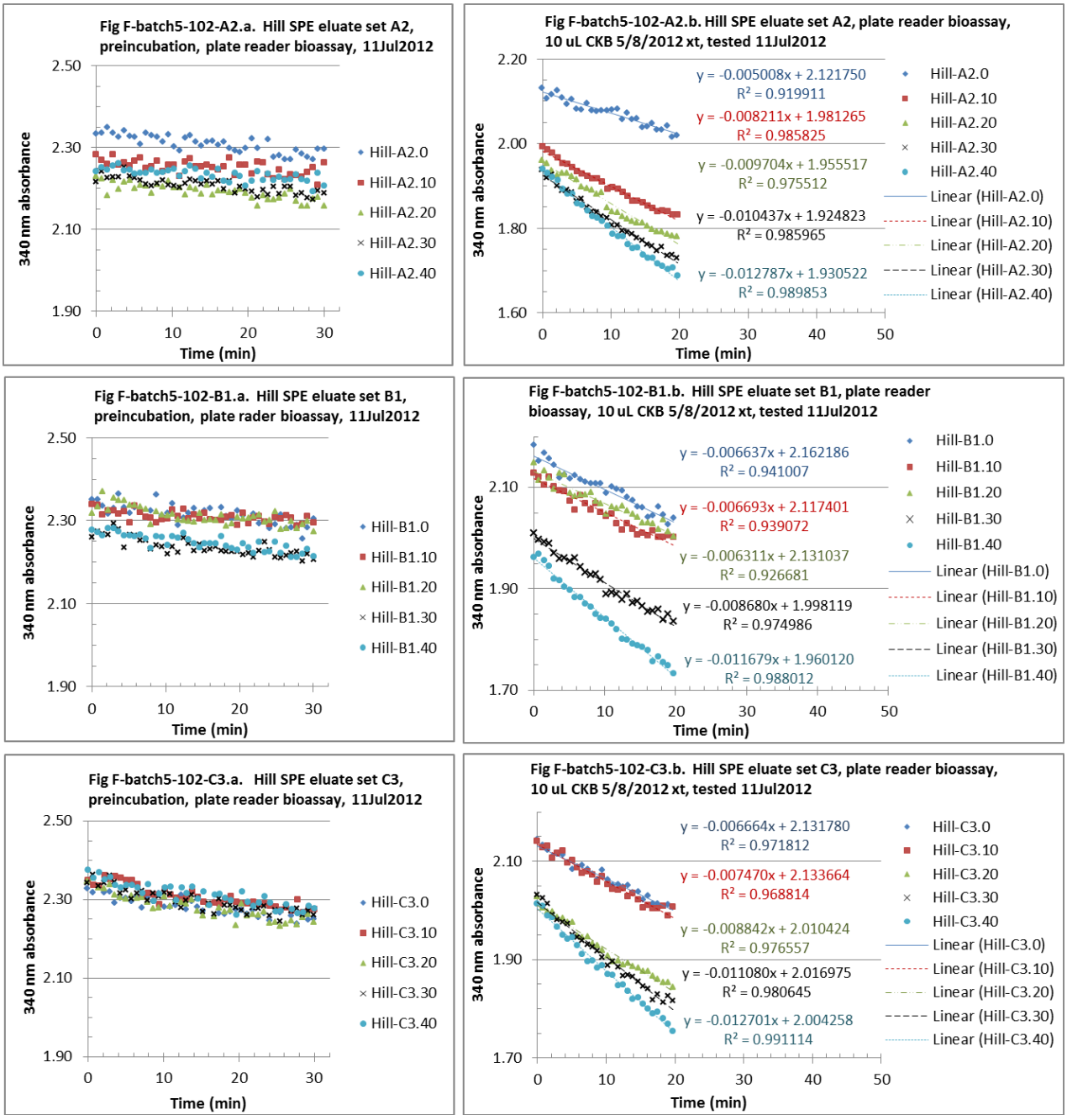



**Figure F-5i. Plate reader bioassay batch 5 absorbance change for SPE eluate sets Hill A2, B1, and C3, 10 µL CKB 5/8/2012 extract, tested 11 Jul 2012**

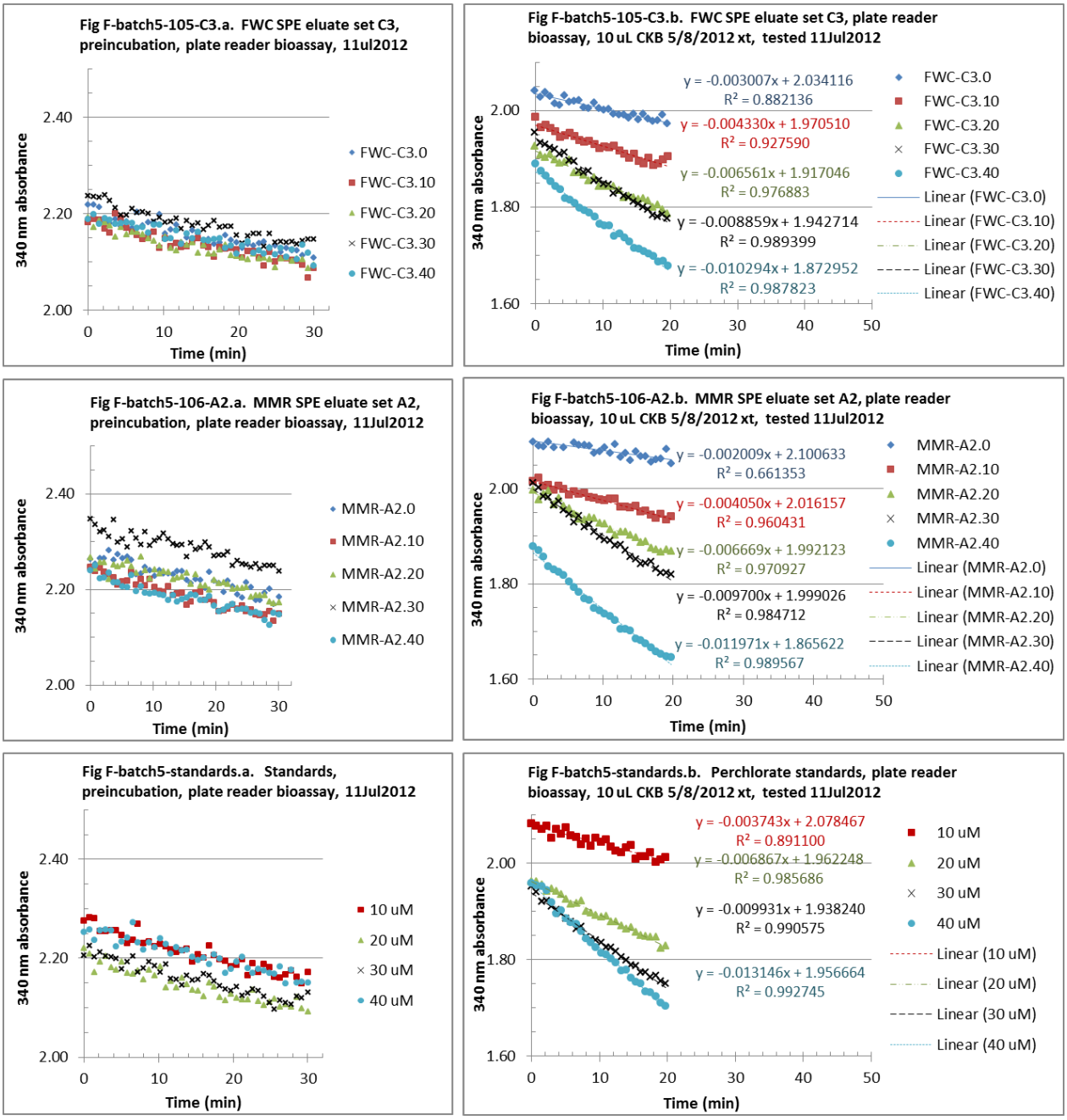



**Figure F-5ii. Plate reader bioassay batch 5 absorbance change for SPE eluate sets FWC C3, MMR A2, and perchlorate standards, 10 µL CKB 5/8/2012 extract, tested 11 Jul 2012**

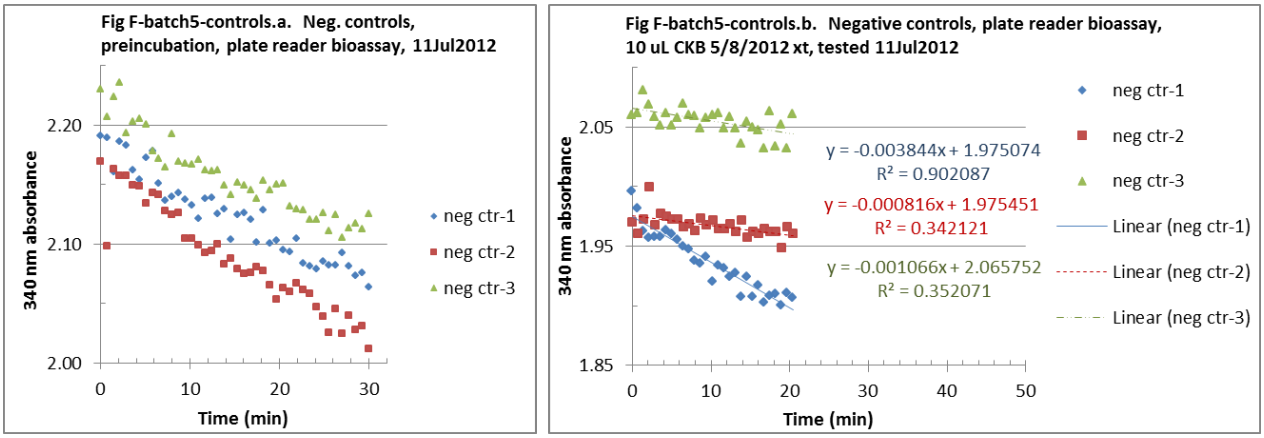



**Figure F-5iii. Plate reader bioassay batch 5 absorbance change for negative control wells, 10 µL CKB 5/8/2012 extract, tested 11 Jul 2012**

# Appendix G: ESTIMATE PERCHLORATE CONCENTRATION BY STANDARD ADDITIONS METHOD PLOTS OF SPE ELUATES ANALYZED BY IC

Benchtop bioassay results were not consistent with historical perchlorate levels for several groundwater sources, including MMR and LLNL (Table 5-4). To help evaluate whether the SPE concentration and purification step had performed according to expectation, six SPE eluate sets were analyzed for perchlorate by IC and the results used for standard additions method estimates of perchlorate concentration in groundwater. Results are listed in Table G-1.

**Table G-1. Perchlorate in groundwater estimated by the standard additions method for some SPE eluate sets using data from ion chromatographic determination of perchlorate in eluates[a]**

| Site | Para-meter[b] | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 | Average ± SD[c] | PD (%) | Histor-ical $ClO_4^-$ (µg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **104, JPL** | **SPE date** | 8 Dec 2011 | nt | nt | nt | nt | nt | nt | nt | nt | | | <1 |
| | **Analysis date** | 10 Dec 2011 | nt | nt | nt | nt | nt | nt | nt | nt | | | |
| | **µg/L** | -1±1 | nt | nt | nt | nt | nt | nt | nt | nt | -1 | NA | |
| **106, MMR** | **SPE date** | 9 Mar 2012 | nt | nt | nt | 13 Mar 2012 | nt | nt | nt | 14 Mar 2012 | | | 5.8 |
| | **Analysis date** | 28Mar 2012 | nt | nt | nt | 28Mar 2012 | nt | nt | nt | 28Mar 2012 | | | |
| | **µg/L** | 5±1 | nt | nt | nt | 5±1 | nt | nt | nt | 5±1 | 5±0.3 | 2 | |
| **107, LLNL** | **SPE date** | nt | nt | nt | 12 Apr 2012 | nt | nt | nt | nt | 17 Apr 2012 | | | 16 |
| | **Analysis date** | nt | nt | nt | 27Apr 2012 | nt | nt | nt | nt | 27Apr 2012 | | | |
| | **µg/L** | nt | nt | nt | 20±2 | nt | nt | nt | nt | 16±4 | 18±2 | 38 | |

[a]nt denotes that there is no data for this cell of the table, PD is the percent difference compared to the reference analytical method performed in a commercial laboratory as listed in Table 5-4

[b]SPE date corresponds to the preparation date for an SPE eluate set. The date of IC analysis is also noted

[c]For MMR eluate sets the spread around the average is given as the standard deviation, for LLNL eluate sets it is the range/2

The IC data for the SPE eluate sets tested from each groundwater source are listed in Tables G-104 (JPL), G-106 (MMR), and G-107 (LLNL). Each of these tables is accompanied by a figure showing the standard additions method regression lines for the SPE eluate sets, from which the perchlorate concentration is determined. The perchlorate spikes added to groundwater prior to the SPE step were 0, 10, 20, 30, or 40 µg/L).

**Table G-104. Perchlorate IC analysis of SPE eluate set A1 for Jet Propulsion Laboratory groundwater collected 14 Nov 2011 from location MW-16**

| Perchlorate Spike (µg/L) | Perchlorate (µM) (IC analysis on 10 Dec 2011) |
|---|---|
| | Set A1 |
| 0 | 0 |
| 10 | 6.7 |
| 20 | 16.7 |
| 30 | 25.1 |
| 40 | 35.8 |
| **Regression parameters for SPE set[a]** | |
| **y-intercept ± error** | -1.1273 ± 0.9065 |
| **Slope ± error** | 0.9000 ± 0.0370 |
| **$r^2$** | 0.9950 |
| **x-intercept ± error** | 1.3±1.0 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[b]** | 1 ± 1 |

[a]Regression parameters are from the standard additions method regression line for the set (see Figure G-104).
[b]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * √ ((slope error/slope)$^2$ + (y-intercept error/y-intercept)$^2$)

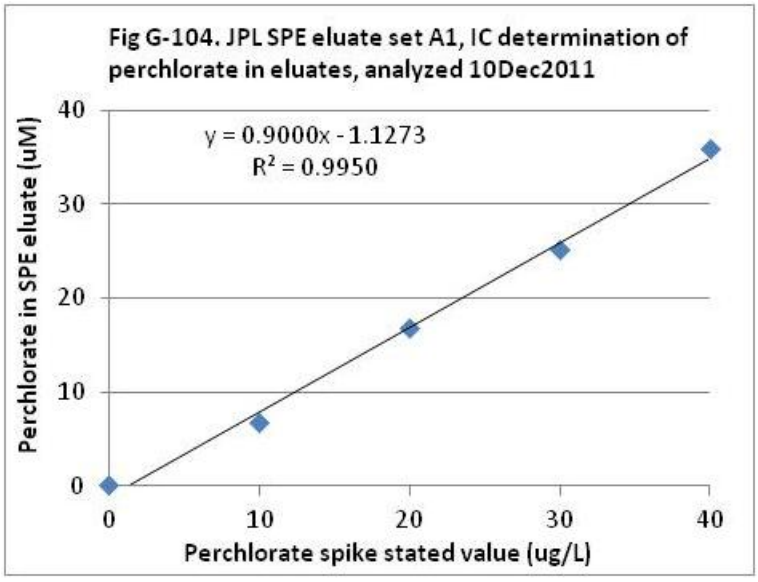



**Figure G-104. Perchlorate estimated by the standard additions method for JPL SPE eluate set A1 using data from IC determination of perchlorate in eluates**

**Table G-106. Perchlorate IC analysis of SPE eluate sets A1, B2, and C3 for Massachusetts Militry Reservation groundwater collected 23 Feb 2012 from location J3-INF**

| Perchlorate Spike (μg/L) | Perchlorate (μM) (IC analysis on 28 Mar 2012) | | |
|---|---|---|---|
| | Set A1 | Set B2 | Set C3 |
| 0( | 4.4 | 3.6 | 4.7 |
| 10 | 12.7 | 12.8 | 13.1 |
| 20 | 20.2 | 19.8 | 19.7 |
| 30 | 30.7 | 27.8 | 29.3 |
| 40 | 38.3 | 36.0 | 37.6 |
| **Regression parameters for SPE sets[a]** | | | |
| **y-intercept ± error** | 4.1012 ± 0.6513 | 4.0507 ± 0.3999 | 4.4460 ± 0.5833 |
| **Slope ± error** | 0.8575 ± 0.0266 | 0.7971 ± 0.0163 | 0.8214 ± 0.0238 |
| **$r^2$** | 0.9971 | 0.9987 | 0.9975 |
| **x-intercept** | -4.8±0.77 | -5.1±0.51 | -5.4±0.73 |
| **$ClO_4^-$ in groundwater ± error (μg/L)[b]** | 5 ± 1 | 5 ± 1 | 5 ± 1 |

[a]Regression parameters are from the standard additions method regression line for a set (see Figure G-106).
[b]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * √ ((slope error/slope)$^2$ + (y-intercept error/y-intercept)$^2$)

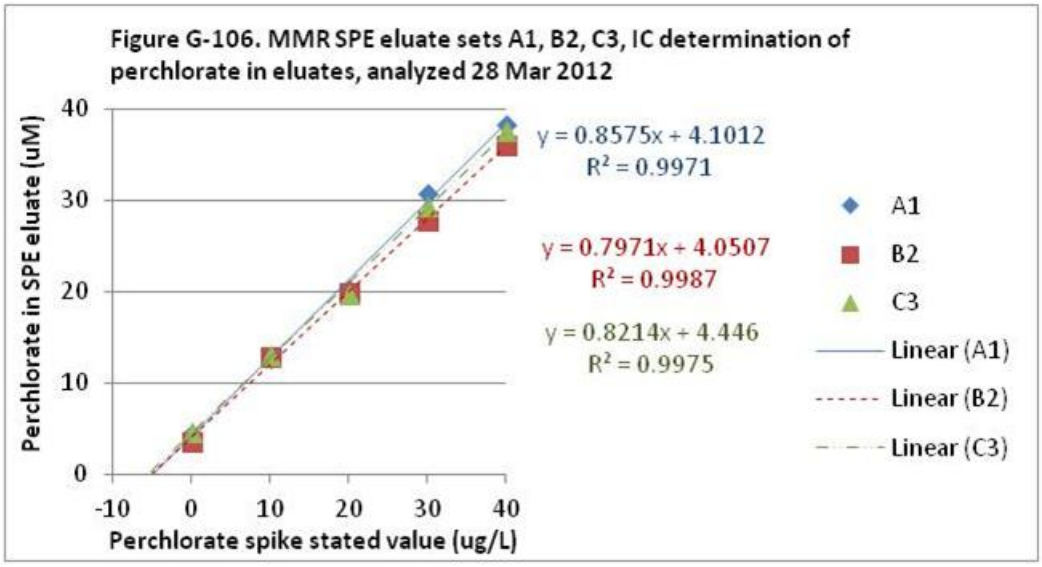



**Figure G-106. Perchlorate estimated by the standard additions method for MMR SPE eluate sets A1, B2, and C3 using data from IC determination of perchlorate in eluates**

**Table G-107. Perchlorate IC analysis of SPE eluate sets B1 and C3 for Lawrence Livermore National Laboratory groundwater collected 29 Mar 2012 from location W-854-1823**

| Perchlorate Spike (µg/L) | Perchlorate (µM) (IC analysis on 27 Apr 20120 | |
|---|---|---|
| | Set B1 | Set C3 |
| 0 | 10.9 | 9.0 |
| 10 | 15.8 | 19.4 |
| 20 | 24.2 | 23.3 |
| 30 | 28.3 | 26.4 |
| 40 | 32.8 | 37.1 |
| **Regression parameters for SPE sets[a]** | | |
| **y-intercept ± error** | 11.1333 ± 1.0236 | 10.4222 ± 2.0007 |
| **Slope ± error** | 0.5633 ± 0.0418 | 0.6322± 0.0817 |
| **$r^2$** | 0.9838 | 0.9523 |
| **x-intercept** | -20±2.3 | -16±3.8 |
| **$ClO_4^-$ in groundwater ± error (µg/L)[b]** | 20 ± 2 | 16 ± 4 |

[a]Regression parameters are from the standard additions method regression line for a set (see Figure G-107).
[b]The concentration of analyte (perchlorate) by the standard additions method is equal to (-1) times the x-intercept ± the x-intercept error = (estimated $ClO_4^-$ concentration) * $\sqrt{}$ ((slope error/slope)$^2$ + (y-intercept error/y-intercept)$^2$)

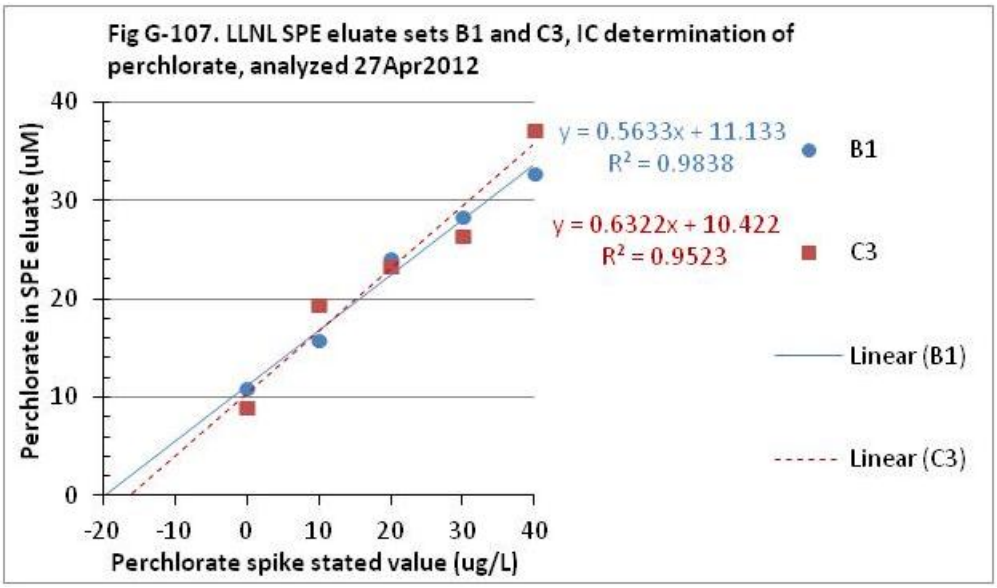



**Figure G-107. Perchlorate estimated by the standard additions method for LLNL SPE eluate sets B1 and C3 using data from IC determination of perchlorate in eluates**

# Appendix H: CHARACTERIZATION OF GROUNDWATER SAMPLES BY ION CHROMATOGRAPHY FOR CHLORIDE, NITRATE, SULFATE, FLUORIDE, NITRITE, BROMIDE, CHLORATE, AND PHOSPHATE

Groundwater was tested for perchlorate by the benchtop bioassay and reference analytical methods, subsequently it was stored 2 – 8 °C. Groundwater samples and a deionized water (i.e. DI) control were tested for other anions by IC on 21 and 23 May 2012. See Table H-1.

**Table H-1. Anions in groundwater sources or deionized water by ion chromatography**

| Anion | 102, Hill (mg/L) | 103A, McGregor (mg/L) | 103B, McGregor (mg/L) | 104, JPL (mg/L) | 105, FWC (mg/L) | 106, MMR (mg/L) | 107, LLNL (mg/L) | DI control (mg/L) |
|---|---|---|---|---|---|---|---|---|
| **Chloride** | 51 | 27 | 28 | 47 | 11 | 11 | 157 | <1 |
| **Nitrate** | 11 | 21 | 18 | 6 | 32 | 1 | 24 | <1 |
| **Sulfate** | 31 | 81 | 90 | 42 | 15 | 6 | 19 | <1 |
| **Fluoride** | <0.4 | 0.6 | 0.8 | 0.8 | <0.2 | <0.2 | <0.6 | <0.2 |
| **Nitrite** | <2 | <2 | <2 | <2 | <1 | <1 | <3 | <1 |
| **Bromide** | <2 | <2 | <2 | <2 | <1 | <1 | <3 | <1 |
| **Chlorate** | <0.8 | <0.8 | <0.8 | <0.8 | <0.4 | <0.4 | <1.2 | <0.4 |
| **Phosphate** | <4 | <4 | <4 | <4 | <2 | <2 | <6 | <2 |

Groundwater samples and a DI control (i.e. LRB) were applied to SPE cartridges according to the standard protocol on 4 Jun 2012, and the effluents were analyzed by IC for anions on 8 Jun 2012. Results are listed in Table H-2. The anions listed were not extracted by SPE cartridges.

**Table H-2. Anions in SPE effluents by ion chromatography**

| Anion | 102, Hill (mg/L) | 103A, McGregor (mg/L) | 103B, McGregor (mg/L) | 104, JPL (mg/L) | 105, FWC (mg/L) | 106, MMR (mg/L) | 107, LLNL (mg/L) | LRB control (mg/L) | Extracted by SPE cartridge |
|---|---|---|---|---|---|---|---|---|---|
| **Chloride** | 52 | 25 | 26 | 48 | 11 | 11 | 155 | <1 | No |
| **Nitrate** | 11 | 20 | 18 | 7 | 29 | <1 | 28 | <1 | No |
| **Sulfate** | 28 | 79 | 88 | 39 | 13 | 4 | 17 | <1 | No |
| **Fluoride** | 0.3 | 0.4 | 0.5 | 0.6 | 0.3 | <0.2 | 0.6 | <0.2 | No |
| **Nitrite** | <1 | <1 | <1 | <1 | <1 | <1 | <3 | <1 | No |
| **Bromide**[a] | 9 | 9 | 9 | 9 | 9 | 9 | 10 | 6 | NA |
| **Chlorate** | <0.4 | <0.4 | <0.4 | <0.4 | <0.4 | <0.4 | <1.2 | <0.4 | No |
| **Phosphate** | <2 | <2 | <2 | <2 | <2 | <2 | <6 | <2 | No |

[a]SPE cartridges were conditioned with decyltrimethyl ammonium bromide (DTAB). It is likely that residual DTAB in the cartridges was responsible for the bromide seen in the SPE effluents

# Appendix I: ICPMS CHARACTERIZATION OF GROUNDWATER SOURCES AND SPE ELUATES

Groundwater sources and freshly prepared SPE eluates from groundwaster sources were analyzed by ICPMS to characterize the pattern of elements present. It was anticipated that this information might provide insight into factors that could impact the performance of the benchtop bioassay. Results of the groundwater testing are listed in Table I-1, and of the SPE eluates in Table I-2.

**Table I-1. Elements detected in groundwater samples by ICPMS (5 Jun 2012)**

| Row No. | Element | 102, Hill (µg/L) | 103A, McGregor (µg/L) | 103B, McGregor (µg/L) | 104, JPL (µg/L) | 105, FWC (µg/L) | 106, MMR (µg/L) | 107, LLNL (µg/L) | Deion-ized water (µg/L) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Li | 21.989 | 16.974 | 21.701 | 3.329 | 2.836 | 0.699 | 96.808 | 0.001 |
| 2 | B | 37.424 | 112.275 | 136.568 | 43.066 | 0 | 0 | 1518.960 | 0.217 |
| 3 | Na | 28606.923 | 31726.54 | 34541.261 | 31284.948 | 17149.138 | 7009.164 | 124190.113 | 63.53 |
| 4 | Mg | 23497.595 | 4196.883 | 5411.412 | 19987.998 | 9069.109 | 1516.18 | 14867.993 | 0.16 |
| 5 | Al | 0 | 0 | 0 | 3.744 | 0 | 2.198 | 0 | 0.069 |
| 6 | Si | 8935.548 | 6510.043 | 6930.117 | 13351.408 | 8834.639 | 3185.705 | 17060.435 | 0.505 |
| 7 | K | 4885.533 | 1373.443 | 1427.469 | 3154.717 | 1598.434 | 541.295 | 7418.409 | 3.197 |
| 8 | Ca | 21105.449 | 34760.428 | 37101.644 | 40566.559 | 34990.280 | 1641.695 | 24419.616 | 2.893 |
| 9 | Sc | 1.710 | 0.710 | 0.729 | 2.953 | 1.626 | 0 | 4.280 | 0 |
| 10 | Ti | 0 | 0.555 | 1.294 | 1.479 | 0.370 | 0.555 | 0.370 | 0.023 |
| 11 | V | 0 | 0 | 0 | 11.244 | 6.052 | 0 | 5.693 | 0.011 |
| 12 | Cr | 16.961 | 0 | 0 | 0 | 0 | 0 | 0 | 0.071 |
| 13 | Mn | 0 | 0 | 0 | 0 | 0 | 0.287 | 0.262 | 0.001 |
| 14 | Fe | 513.072 | 585.614 | 465.305 | 526.120 | 388.798 | 388.909 | 523.356 | 0 |
| 15 | Co | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.001 |
| 16 | Ni | 0 | 0 | 0 | 0 | 0 | 0.777 | 0 | 0.014 |
| 17 | Cu | 0 | 0 | 0 | 0 | 0 | 2.032 | 1.058 | 0.003 |
| 18 | Zn | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4.76 |
| 19 | Ga | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 |
| 20 | As | 0.621 | 0.078 | 0.466 | 4.504 | 0.544 | 0.155 | 3.960 | 0.005 |
| 21 | Br | 0 | 77.617 | 198.063 | 0 | 49.068 | 53.083 | 670.535 | 7.628 |
| 22 | Rb | 0.783 | 1.539 | 0 | 0.047 | 0.116 | 0.951 | 12.154 | 0 |
| 23 | Sr | 219.569 | 400.705 | 516.038 | 470.656 | 295.742 | 18.403 | 762.416 | 0 |
| 24 | Y | 0 | 0 | 0 | 0 | 0 | 0.005 | 0.021 | 0 |
| 25 | Zr | 0.052 | 0.080 | 0 | 0.068 | 0.104 | 0.057 | 0.052 | 0 |
| 26 | Nb | 0 | 0 | 0 | 0 | 0 | 0 | 0.006 | 0 |
| 27 | Mo | 6.141 | 1.175 | 1.668 | 3.944 | 4.146 | 0.251 | 9.412 | 0 |
| 28 | Ru | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | Rh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | Pd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | Cd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | In | 0 | 0 | 0 | 0 | 0.031 | 0.013 | 0.018 | 0 |
| 33 | Sn | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | Sb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | Cs | 0.013 | 0.027 | 0.017 | 0.01 | 0.003 | 0.003 | 0.094 | 0 |
| 36 | Ba | 141.769 | 40.520 | 38.967 | 82.818 | 44.902 | 3.289 | 53.018 | 0.143 |

**Table I-1, continued**

| Row No. | Element | 102, Hill (µg/L) | 103A, McGregor (µg/L) | 103B, McGregor (µg/L) | 104, JPL (µg/L) | 105, FWC (µg/L) | 106, MMR (µg/L) | 107, LLNL (µg/L) | Deion -ized water (µg/L) |
|---|---|---|---|---|---|---|---|---|---|
| 37 | La | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 38 | Ce | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 39 | Pr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | Nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 | Sm | 0 | 0 | 0 | 0 | 0 | 0 | 0.034 | 0 |
| 42 | Eu | 0.010 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | Gd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | Tb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | Dy | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 46 | Ho | 0.020 | 0.004 | 0.004 | 0.008 | 0.012 | 0.020 | 0.016 | 0 |
| 47 | Er | 0.000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | Tm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | Yb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | Lu | 0 | 0.003 | 0 | 0 | 0 | 0 | 0 | 0 |
| 51 | Ir | 0.619 | 0.319 | 0.480 | 0.491 | 0.788 | 0.263 | 0.726 | 0.001 |
| 52 | Pt | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 53 | Tl | 0.042 | 0.084 | 0.092 | 0 | 0.127 | 0 | 0.148 | 0 |
| 54 | Pb | 0 | 0 | 0 | 0 | 0 | 0.086 | 0.057 | 0 |
| 55 | Bi | 0 | 0 | 0.005 | 0.005 | 0.010 | 0 | 0.010 | 0 |
| 56 | Th | 0.185 | 0.168 | 0.129 | 0.101 | 0.236 | 0.056 | 0.247 | 0 |
| 57 | U | 11.468 | 3.121 | 3.904 | 7.566 | 3.53 | 0 | 26.115 | 0.003 |

**Table I-2. Elements detected in SPE eluate samples by ICPMS (5 Jun 2012)[a]**

| Row No. | Element | 102, Hill (µg/L) | 103A, Mcgregor (µg/L) | 103B, Mcgregr (µg/L) | 104, JPL (µg/L) | 105, FWC (µg/L) | 106, MMR (µg/L) | 107, LLNL (µg/L) | LRB Eluate (µg/L)[b] | MOPS – NaCl solution (µg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Li | 13 | 12 | 13 | 12 | 9 | 12 | 11 | 8 | 13 |
| 2 | B | 5021 | 4605 | 4761 | 4718 | 4673 | 4849 | 5315 | 4854 | 4856 |
| 3 | Na | S | S | S | S | S | S | S | S | S |
| 4 | Mg | 39 | 22 | 83 | 25 | 26 | 20 | 30 | 18 | 12 |
| 5 | Al | 1895 | 1834 | 1843 | 1856 | 1849 | 1767 | 1869 | 1897 | 1812 |
| 6 | Si | 53263 | 51783 | 52544 | 69028 | 71741 | 68053 | 72420 | 71895 | 51231 |
| 7 | K | 2439 | 2317 | 2325 | 2036 | 2069 | 1962 | 2247 | 2068 | 2388 |
| 8 | Ca | 1412 | 1111 | 1209 | 2497 | 2752 | 2511 | 2935 | 2467 | 1080 |
| 9 | Sc | 19 | 16 | 18 | 22 | 20 | 21 | 22 | 19 | 18 |
| 10 | Ti | 136 | 130 | 140 | 150 | 146 | 143 | 148 | 136 | 206 |
| 11 | V | 176 | 169 | 170 | 166 | 161 | 159 | 173 | 155 | 173 |
| 12 | Cr | 184 | 59 | 65 | 838 | 918 | 794 | 894 | 824 | 81 |
| 13 | Mn | 0.65 | 0.25 | 0.12 | 0.22 | 0.34 | 0.11 | 0.115 | 0 | 0.43 |
| 14 | Fe | 94 | 56 | 55 | 31 | 0 | 2 | 0 | 0 | 0 |
| 15 | Co | 0.44 | 0.86 | 0.51 | 0.90 | 0 | 0.25 | 1.85 | 0 | 0.14 |
| 16 | Ni | 0 | 0 | 0.042 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | Cu | 21 | 18 | 15 | 16 | 16 | 19 | 34 | 11 | 17 |

**Table I-2, continued**

| Row No. | Element | 102, Hill (µg/L) | 103A, Mcgregor (µg/L) | 103B, Mcgregor (µg/L) | 104, JPL (µg/L) | 105, FWC (µg/L) | 106, MMR (µg/L) | 107, LLNL (µg/L) | LRB Eluate (µg/L)[b] | MOPS – NaCl solution (µg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| 18 | Zn | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 |
| 19 | Ga | 0.57 | 0.56 | 0.48 | 0.32 | 0.48 | 0.41 | 0.38 | 0.29 | 0.37 |
| 20 | As | 116 | 117 | 114 | 116 | 107 | 110 | 111 | 108 | 130 |
| 21 | Br | 724983 | 720631 | 736332 | 197877 | 183996 | 198868 | 190967 | 210507 | 7735 |
| 22 | Rb | 1.95 | 1.87 | 1.86 | 2.16 | 1.87 | 1.92 | 1.88 | 2.00 | 1.63 |
| 23 | Sr | 12 | 10 | 11 | 9 | 8 | 11 | 11 | 9 | 12 |
| 24 | Y | 0.005 | 0 | 0 | 0.021 | 0 | 0.121 | 0 | 0.016 | 0.011 |
| 25 | Zr | 6 | 5 | 5 | 4 | 4 | 3 | 4 | 2 | 4 |
| 26 | Nb | 0.064 | 0.11 | 0.045 | 0.077 | 0.155 | 0.064 | 0.116 | 0.084 | 0.148 |
| 27 | Mo | 45 | 6 | 7 | 16 | 22 | 4 | 39 | 2 | 0 |
| 28 | Ru | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | Rh | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | Pd | 0 | 0.021 | 0 | 0 | 0.021 | 0 | 0 | 0 | 0 |
| 31 | Cd | 0.035 | 0 | 0.039 | 0.079 | 0.035 | 0 | 0 | 0.040 | 0 |
| 32 | In | 0.004 | 0 | 0 | 0.004 | 0.004 | 0 | 0 | 0 | 0 |
| 33 | Sn | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | Sb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 35 | Cs | 0.074 | 0.074 | 0.043 | 0.05 | 0.05 | 0.04 | 0.063 | 0.053 | 0.053 |
| 36 | Ba | 34 | 23 | 23 | 22 | 24 | 21 | 25 | 23 | 23 |
| 37 | La | 0.004 | 0 | 0 | 0.03 | 0.03 | 0.013 | 0.017 | 0 | 0 |
| 38 | Ce | 0 | 0 | 0 | 0 | 0 | 0.041 | 0.157 | 0 | 0 |
| 39 | Pr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | Nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 41 | Sm | 0 | 0 | 0 | 0 | 0.025 | 0 | 0 | 0 | 0 |
| 42 | Eu | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.009 | 0 |
| 43 | Gd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44 | Tb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 |
| 45 | Dy | 0 | 0 | 0 | 0 | 0 | 0.008 | 0 | 0 | 0 |
| 46 | Ho | 0.008 | 0.008 | 0 | 0 | 0.02 | 0.012 | 0.004 | 0.028 | 0.024 |
| 47 | Er | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 48 | Tm | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 49 | Yb | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | Lu | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 51 | Ir | 0.078 | 0.056 | 0.075 | 0.300 | 0.338 | 0.206 | 0.257 | 0.131 | 0.075 |
| 52 | Pt | 0 | 0 | 0 | 0 | 0.026 | 0.027 | 0 | 0.104 | 0 |
| 53 | Tl | 0.034 | 0.035 | 0.028 | 0.067 | 0.035 | 0.028 | 0.084 | 0.141 | 0.202 |
| 54 | Pb | 1.30 | 1.07 | 0.94 | 0.97 | 1.48 | 0.95 | 1.10 | 0.91 | 2.36 |
| 55 | Bi | 0 | 0 | 0 | 0 | 0.01 | 0 | 0 | 0.005 | 0.005 |
| 56 | Th | 0 | 0 | 0 | 0 | 0.073 | 0 | 0 | 0.073 | 0 |
| 57 | U | 707 | 190 | 178 | 501 | 262 | 0 | 1779 | 0 | 0 |

[a]SPE eluates were prepared 4 Jun 2012 and stored at 2-8 °C until shortly before ICPMS analysis the next day. 200 mL of groundwater were applied to an SPE cartridge, rinsed, and eluted with alkaline MOPS-NaCl solution. Groundwater applied to SPE cartridges was from retains that had been stored at 2-8 °C since the preparation of sample aliquots for perchlorate testing by bioassay and reference analytical methods: Hill (B-retain), McGregor (A and B-retains), JPL (A-retain), FWC (B-retain), MMR (A-retain), and LLNL (A-retain). "S" indicates element was above the range of concentration for analysis.

[b]The LRB eluate was obtained by applying 200 mL of deionized water to an SPE cartridge and eluting with the alkaline MOPS-NaCl solution according to the standard procedure.

The elements that had the highest variation in concentration among the different SPE eluates were considered possible candidates for impacting bioassay performance. The amounts of these in groundwater and SPE eluates were analyzed by non-metric multidimensional scaling (MDS). The MDS analysis indicated that five elements were concentrated in SPE eluates relative to their levels in groundwater (see Figure I-4) while 3 were higher in groundwater compared to their levels in SPE eluates (see Figure I-5). Table I-3 shows the µM concentration of these elements in SPE eluates. Also shown are the concentrations in the LRB eluate as well as the alkaline MOPS-NaCl solution used to elute the SPE cartridges.

**Table I-3. Elements concentrated in SPE eluates**

| Row No.[a] | Element | 102, Hill (µM) | 103A, Mcgregor (µM) | 103B, Mcgregor (µM) | 104, JPL (µM) | 105, FWC (µM) | 106, MMR (µM) | 107, LLNL (µM) | LRB Eluant (µM) | MOPS - NaCl solution (µM) |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | Cr | 3.5 | 1.1 | 1.3 | 16 | 18 | 15 | 17 | 15.8 | 1.6 |
| 15 | Co | 0.0075 | 0.015 | 0.0087 | 0.015 | 0 | 0.0042 | 0.031 | 0 | 0.002 |
| 17 | Cu | 0.33 | 0.23 | 0.24 | 0.25 | 0.25 | 0.30 | 0.54 | 0.17 | 0.27 |
| 27 | Mo | 0.47 | 0.063 | 0.073 | 0.17 | 0.23 | 0.042 | 0.41 | 0.02 | 0 |
| 57 | U | 3.0 | 0.80 | 0.75 | 2.1 | 1.1 | 0 | 7.5 | 0 | 0 |

[a]The row number refers to the row numbers used in Tables I-1 and I-2

The data shown in Table I-3 suggested that the relatively high uranium in the LLNL eluate might correlate with the interference effect noted in the benchtop bioassay with SPE eluates from this source. With respect to the other elements shown in Table I-3, cobalt levels were low (i.e. 0.031 µM or less) from all groundwater sources and thus less likely to impact bioassay performance. Copper levels were the same order of magnitude in the alkaline MOPS-NaCl solution as in SPE eluates and thus not likely to be a factor correlated with the difference in benchtop bioassay performance observed between LLNL SPE eluates, for which interference occurred, and the enzyme control reactions tested in the same batches where no interference was seen (see Figure B-107). Molybdenum concentrations showed about an 11-fold difference in concentration among the SPE eluates, however, molybdenum concentrations weren't very different for the Fontana Water Co. (FWC) and LLNL eluates. In benchtop bioassay testing, the results with FWC eluates were in better agreement with reference analytical methods than for LLNL eluates (Table 5-4). Chromium levels were high in many eluates (i.e. 16 to 18 µM), but since levels were high with both FWC and LLNL, chromium seems unlikely to correlate with the difference in benchtop bioassay performance between them.

The capacity of SPE cartridges employed in this project to concentrate uranium is shown in Table I-4 and Figure I-1. Removal of uranium from groundwater by ion exchange processes is a well described phenomenon[23], and so finding that uranium is concentrated in SPE eluates is not an unexpected observation for the SPE processing step. The average percent recovery of uranium in SPE eluates ± standard deviation was 68 ± 10. In addition, several SPE eluate sets for LLNL, FWC, and Hill, which had previously been tested for perchlorate by the benchtop bioassay, were analyzed for uranium by ICPMS (Table I-5 and Figure I-2). The uranium content of these older SPE eluates was similar to that presented in Table I-4. For comparison, the SPE eluates and

groundwater sources examined by ICPMS were also analyzed by IC for perchlorate (Table I-6, and Figure I-3). The recovery of perchlorate in SPE eluates ranged from 39 to 96 percent.

**Table I-4. Comparison of uranium in groundwater sources and SPE eluates**

| | Groundwater | | SPE eluates | | | | |
|---|---|---|---|---|---|---|---|
| **Sample Name** | **Concen-tration[a] (μg/L)** | **Concen-tration normalized to LLNL** | **Concen-tration[b] (μg/L)** | **Concen-tration normalized to LLNL** | **Percent uranium recovered in eluate** | **Concen-tration factor** | **Concen-tration[c] (μM)** |
| **107, LLNL** | 26 | 1.00 | 1779 | 1.00 | 68 | 68 | 7.5 |
| **102, Hill AFB** | 11 | 0.42 | 707 | 0.40 | 64 | 64 | 3.0 |
| **104, JPL** | 7.6 | 0.29 | 501 | 0.28 | 66 | 66 | 2.1 |
| **105, FWC** | 3.5 | 0.13 | 262 | 0.15 | 75 | 75 | 1.1 |
| **103, Mcgregor-A** | 3.1 | 0.12 | 190 | 0.11 | 61 | 61 | 0.8 |
| **103, McGregor-B** | 3.9 | 0.15 | 178 | 0.10 | 46 | 46 | 0.8 |
| **106, MMR** | 0 | 0.00 | 0 | 0.0001 | NA | NA | 0.0008 |

[a]Data are from row 57 of Table I-1
[b]Data are from row 57 of Table I-2
[c]Data are from Table I-3

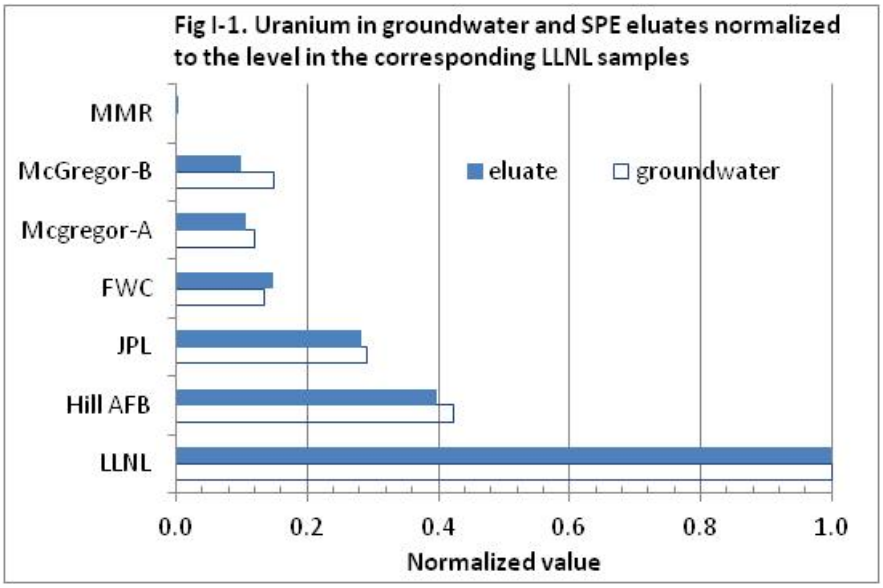



**Figure I-1. Uranium detected in groundwater and SPE eluates by ICPMS (5 Jun 2012)**

**Table I-5. Uranium detected by ICPMS (24 Jul 2012) in some SPE eluate sets previously tested by benchtop bioassay for perchlorate**

| SPE eluate source | $ClO_4^-$ spike (µg/L) | Uranium in SPE eluates (µg/L) | | | | | Pooled average for uranium in SPE eluates[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| **107, LLNL** | | B2 | C1 | B3 | C3 | Replicate C3 | **(µg/L)** | **Normalized to LLNL** | **(µM)** |
| | 0 | 1,892 | 2,121 | 2,046 | 2,099 | 2,094 | 2076 ±42 | 1.00±0.02 | 8.7 ±0.16 |
| | 10 | 2,542 | 2,022 | 2,158 | 2,207 | 2,191 | | | |
| | 20 | 1,599 | 2,133 | 2,062 | 2,087 | 2,064 | | | |
| | 30 | 2,034 | 1,935 | 2,207 | 2,060 | 2,036 | | | |
| | 40 | 1,980 | 2,097 | 2,053 | 2,113 | 2,080 | | | |
| | Average ±SD | 2,009 ±342 | 2,062 ±83 | 2,105 ±73 | 2,113 ±56 | 2,093 ±59 | | | |
| **105, FWC** | | A2 | B1 | | | | | | |
| | 0 | 297 | 292 | | | | 292 ±3 | 0.14±0.002 | 1.2 ±0.01 |
| | 10 | 292 | 277 | | | | | | |
| | 20 | 296 | 298 | | | | | | |
| | 30 | 284 | 284 | | | | | | |
| | 40 | 305 | 291 | | | | | | |
| | Average ±SD | 295 ±8 | 288 ±8 | | | | | | |
| **102, Hill AFB** | | C3 | B3 | | | | | | |
| | 0 | 761 | 737 | | | | 735 ±23 | 0.35±0.01 | 3.1 ±0.10 |
| | 10 | 760 | 651 | | | | | | |
| | 20 | 760 | 682 | | | | | | |
| | 30 | 776 | 792 | | | | | | |
| | 40 | 732 | 696 | | | | | | |
| | Average ±SD | 758 ±16 | 712 ±55 | | | | | | |

[a]The spread about the average is ± standard deviation for LLNL, or ± range/2 for FWC and Hill

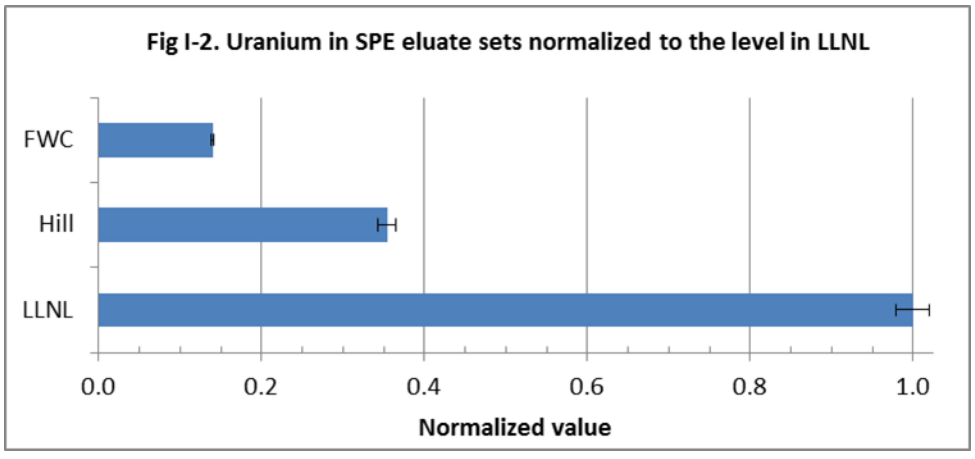



**Figure I-2. Uranium detected by ICPMS (24 Jul 2012) in some SPE eluate sets previously tested by benchtop bioassay for perchlorate.** Pooled averages for the SPE eluate sets from the three groundwater sources were normalized to the LLNL pooled average. The error bars correspond to the standard deviation (LLNL) or the range/2 (FWC and Hill)

**Table I-6. Perchlorate detected by IC in groundwater and SPE eluates[a]**

| Sample Name | Groundwater | | SPE eluates | | | |
|---|---|---|---|---|---|---|
| | Concen-tration (µg/L) | Concentrations normalized to McGregor-A | Concen-tration (µg/L) | Concentrations normalized to McGregor-A | Percent perchlorate recovered in eluate | Concen-tration factor |
| **107, LLNL** | 14 | 0.23 | 1060 | 0.40 | 76 | 76 |
| **102, Hill AFB** | 36 | 0.59 | 1720 | 0.65 | 48 | 48 |
| **104, JPL** | NA | NA | NA | NA | NA. | NA |
| **105, FWC** | 12 | 0.20 | 910 | 0.35 | 76 | 76 |
| **103, McGregor-A** | 61 | 1.00 | 2630 | 1.00 | 43 | 43 |
| **103, McGrebor-B** | 53 | 0.87 | 2060 | 0.78 | 39 | 39 |
| **106, MMR** | 7 | 0.11 | 670 | 0.25 | 96 | 96 |

[a]SPE eluates were prepared 4 Jun 2012. Groundwater and these SPE eluates had also been analyzed by ICPMS to characterize the elements present (see Tables I-1, I-2, I-3, I-4, and Fig. I-1). Storage was at 2-8 °C prior to IC testing

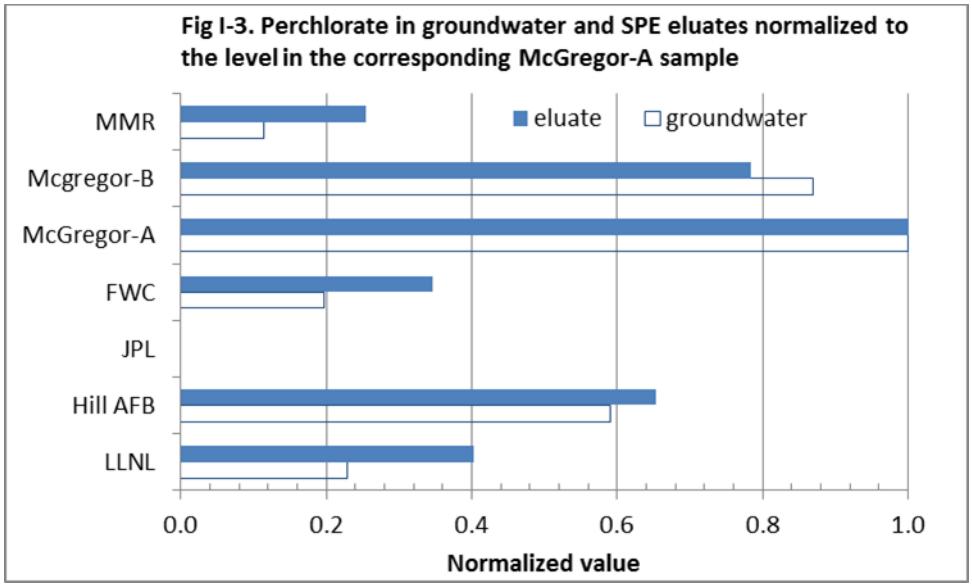



**Figure I-3. Perchlorate in groundwater and SPE eluates detected by IC.** These samples had previously been analyzed by ICPMS for uranium (see Table I-4 and Fig. I-1) and other elements (Tables I-1, I-2 and I-3).

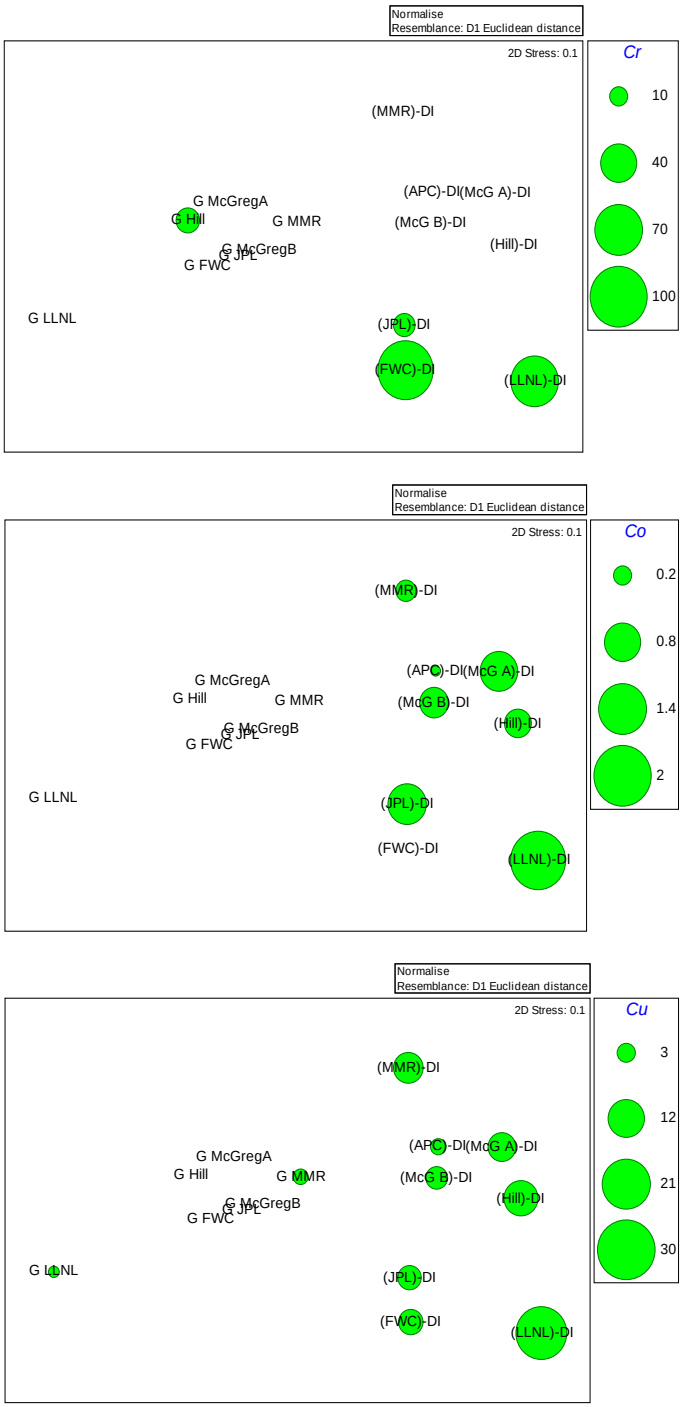



**Figure I-4. Elements that are concentrated by the SPE step.** An MDS plot was obtained based on normalized ICPMS data for elements in groundwater sources (denoted with a "G" prefix) and their corresponding SPE eluates. Bubble plots were obtained by superimposing circles of increasing size that represent increasing element concentrations. A different element is featured in each panel. The scale in the legends show the actual concentration of elements in groundwater (µg/L) or the difference between the element concentration in an eluent minus its concentration in the deionized water eluate blank. Elements shown in different panels are Cr, Co, Cu, Mo and U

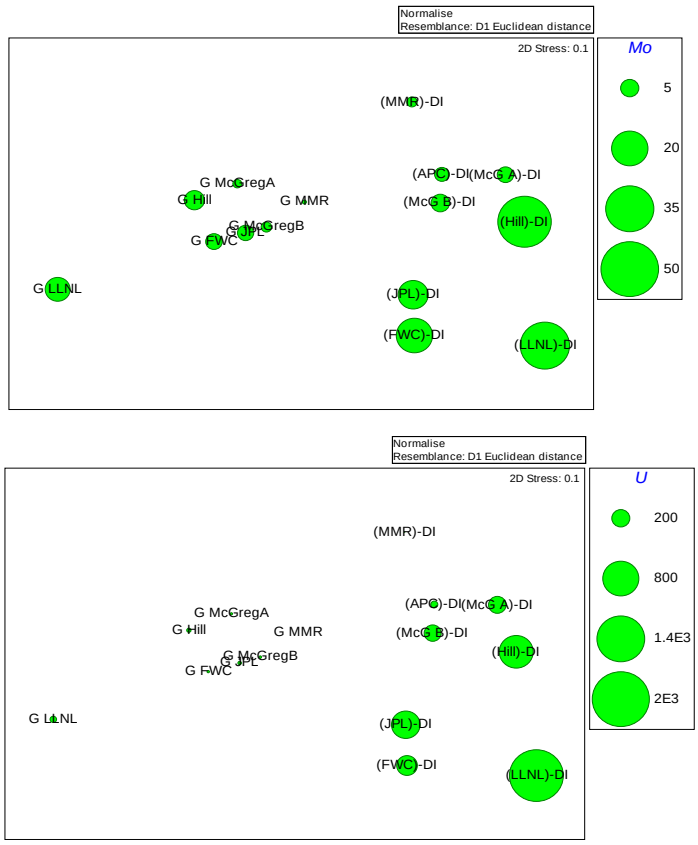



**Figure I-4, continued. Elements that are concentrated by the SPE step**

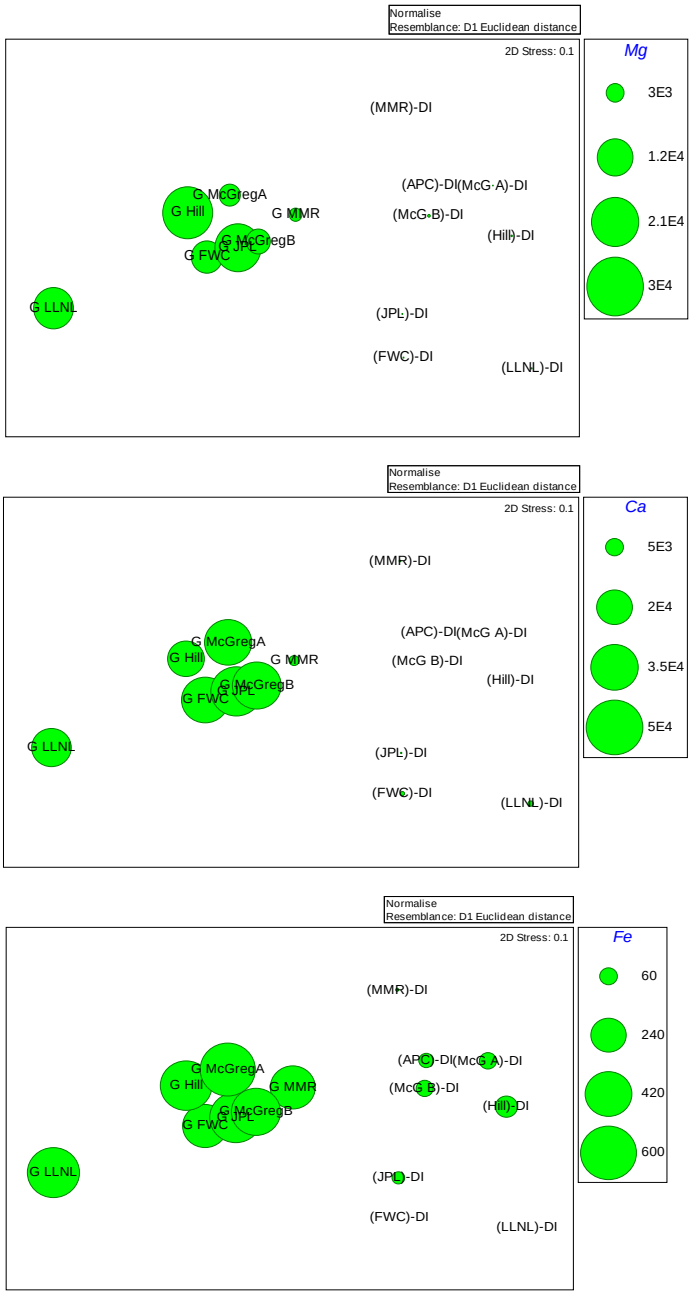



**Figure I-5. Elements that are at lower levels in SPE eluates than in the corresponding groundwater source.** Bubble plots were obtained by superimposing, on the MDS plot described in the Fig. I-4 legend, circles of increasing size that represent increasing element concentrations. A different element is featured in each panel. The scale in the legends show the actual concentration of elements in groundwater (µg/L) or the difference between the element concentration in an eluent minus its concentration in the deionized water eluate blank. Elements shown in different panels are Mg, Ca, and Fe

# Appendix J: TESTING TO DETERMINE WHETHER URANIUM (VI) INTERFERES WITH THE BENCHTOP BIOASSAY

To test whether uranium (VI) would have an effect on perchlorate reductase activity in the benchtop bioassay, reaction mixtures containing 40 µM perchlorate and 0, 1, 2, 3, 5, 8, or 10 µM uranium (VI) were analyzed by the benchtop bioassay. These uranium concentrations cover the range observed in SPE eluates of groundwater (Tables I-4 and I-5). Results for the reaction mixtures are shown in Figure J-1 and listed in Table J-1 . There was no apparent difference in perchlorate reductase activity absence or presence of uranium (VI).

**Table J-1. Benchtop bioassay perchlorate reductase activity in the presence of various amounts of uranium (VI)**

| Reaction category | Reaction mixture description | Concentration in reaction mixtures | | Perchlorate reductase activity[a] | | |
|---|---|---|---|---|---|---|
| | | $ClO_4^-$ (µM) | U(VI) (µM) | Rate (AU/min) | Rate – negative control rate (AU/min) | $r^2$ for rate regression lines |
| **Controls** | negative control | 0 | 0 | 0.0015 | 0.0000 | 0.9896 |
| | 10 µM U, replicate 1 | 0 | 10 | 0.0015 | 0.0000 | 0.9829 |
| | 10 µM U, replicate 2 | 0 | 10 | 0.0013 | -0.0002 | 0.9958 |
| **Test mixtures** | 40 µM per, 0 µM U | 40 | 0 | 0.0110 | 0.0095 | 0.9850 |
| | 40 µM per, 1 µM U, replicate 1 | 40 | 1 | 0.0110 | 0.0095 | 0.9851 |
| | 40 µM per, 1 µM U, replicate 2 | 40 | 1 | 0.0113 | 0.0098 | 0.9873 |
| | 40 µM per, 2 µM U, replicate 1 | 40 | 2 | 0.0110 | 0.0095 | 0.9863 |
| | 40 µM per, 2 µM U, replicate 2 | 40 | 2 | 0.0108 | 0.0093 | 0.9867 |
| | 40 µM per, 3 µM U, replicate 1 | 40 | 3 | 0.0113 | 0.0098 | 0.9862 |
| | 40 µM per, 3 µM U, replicate 2 | 40 | 3 | 0.0115 | 0.0100 | 0.9805 |
| | 40 µM per, 5 µM U, replicate 1 | 40 | 5 | 0.0112 | 0.0097 | 0.9840 |
| | 40 µM per, 5 µM U, replicate 2 | 40 | 5 | 0.0116 | 0.0101 | 0.9853 |
| | 40 µM per, 8 µM U, replicate 1 | 40 | 8 | 0.0117 | 0.0102 | 0.9837 |
| | 40 µM per, 8 µM U, replicate 2 | 40 | 8 | 0.0113 | 0.0098 | 0.9863 |
| | 40 µM per, 10 µM U, replicate 1 | 40 | 10 | 0.0120 | 0.0105 | 0.9857 |
| | 40 µM per, 10 µM U, replicate 2 | 40 | 10 | 0.0144 | 0.0129 | 0.9665 |
| | Test mixtures average ± SD | | | | 0.0100 ± 0.0009 | |

[a]Perchlorate reductase activity was measured as the 340 nm absorbance (AU) change per minute due to NADH oxidation. Perchlorate reductase was added to cuvettes as the equivalent of 25 µL of CKB 5/8/2012 extract.

## DESCRIPTION OF URANIUM SOLUTION AND MODIFIED ENZYME BUFFER USED FOR THE TESTING

The uranium (VI) solution added to reaction mixtures was prepared from a laboratory stock solution of uranium dichloride oxide trihydrate ($UO_2Cl_2 – 3\ H_2O$) in deionized water. This was

further diluted in a pH 7.3 buffer containing 45 mM MOPS and 30 mM $NaHCO_3$ to obtain a 100 µM solution, which was added to the reaction mixtures.

The buffer used for the benchtop bioassay was the same as a LRB (i.e. laboratory reagent blank). It was prepared by applying 200 mL of deionized water to each of 25 SPE cartridges that had been conditioned with DTAB (decyltrimethyl ammonium bromide). After rinsing cartridges with a mixture of acetone and DTAB, and eluting with alkaline MOPS-NaCl by the standard procedure, eluates were pooled, neutralized to pH 7.1 by addition of 1 M HCl, and stored at 2-8 °C until use.

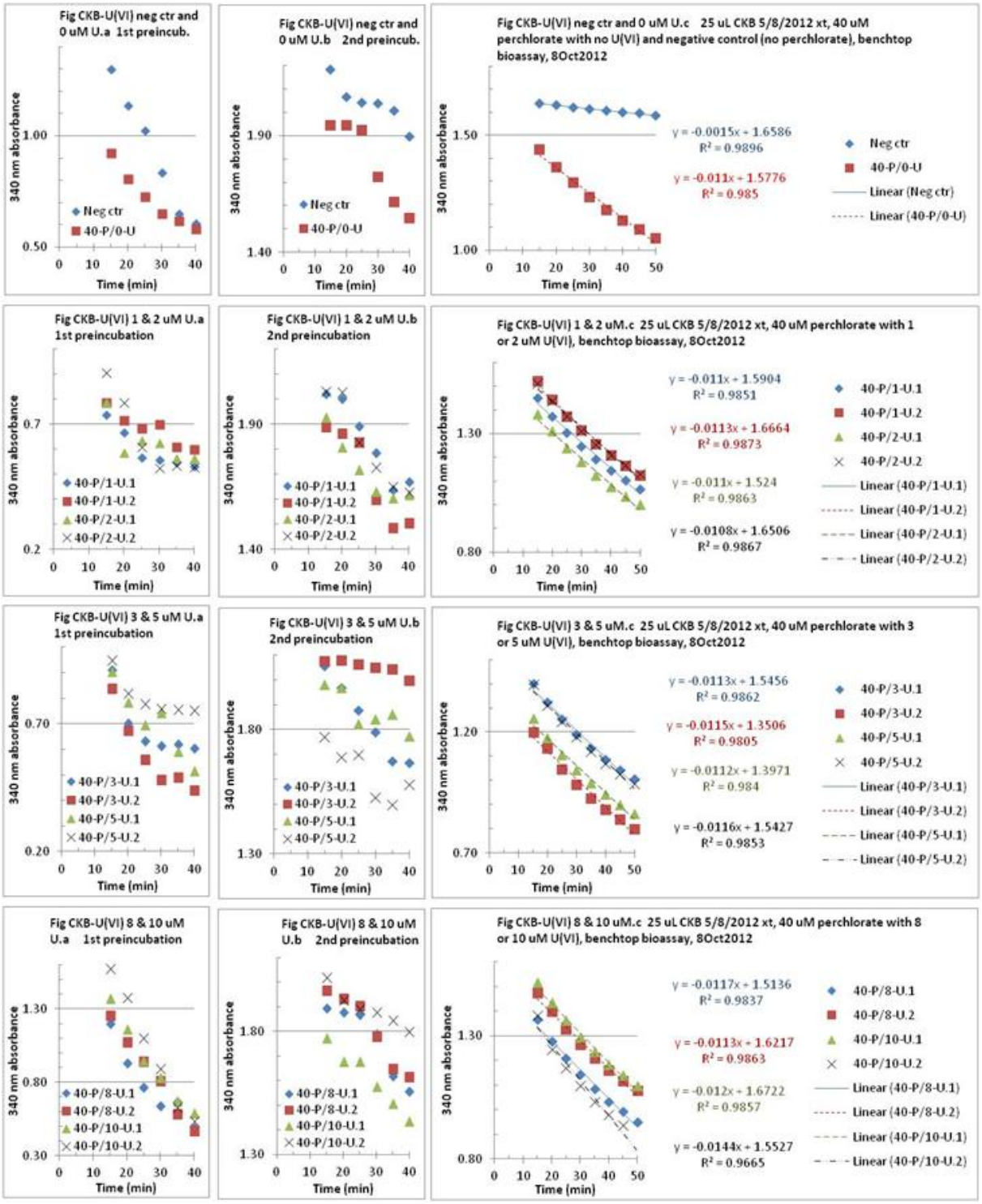



**Figure J-1. Benchtop bioassay testing to determine the effect of uranium (VI) on perchlorate reductase activity.** Reaction mixtures contained 40 μM perchlorate and 0, 1, 2, 3, 5, 8, or 10 μM of uranium (VI). There were two preincubation intervals before the reactions were initiated by the addition of cell extract. NADH was added after each preincubation period.

# Appendix K. SOLID PHASE EXTRACTION OF PERCHLORATE USING LARGE RESIN BEADS OF STYRENE DIVINYLBENZENE

Preliminary testing was done to evaluate using gravity flow over large beads of styrene divinylbenzene (SDVB) to extract perchlorate from a solution. Syrene divinylbenzene (20 % cross-linked) white beads, 20 – 60 mesh (Strem Chemical, Inc.) were used. The efficiency of perchlorate extraction was compared to that seen with standard SPE cartridges.

A 3 g amount of large SDVB beads was placed in the barrel of a 10 mL syringe and held in place with glass wool placed at the bottom and top of the bead bed. The bead bed was rinsed sequentially with 150 mL acetone, then 300 mL deionized water, and then conditioned with 15 mL of 25 mM DTAB. After each liquid had passed through the column, air was forced through the bed several times to remove residual fluid trapped in the bed.

For comparison, solid phase extraction cartridges (Phenomenex, Inc., Torrance, CA, USA) containing 200 mg of SDVB resin were prepared for use by the standard procedure of rinsing each cartridge sequentially with 10 mL acetone, then 20 mL deionized water, and conditioning with 1 mL of 25 mM DTAB. After each liquid had passed through, air was forced through the cartridge to remove residual fluid trapped in the resin bed.

200 mL of perchlorate solution (100 µg/L) was applied with gravity flow to triplicate large bead columns and the effluent flow-through solutions were collected for subsequent perchlorate analysis by IC. In parallel, 200 mL of the perchlorate solution was applied to triplicate SPE cartridges by the standard method of applying pressure from a nitrogen compressed gas cylinder to force the solution through the resin at a moderate flow rate. The effluent solutions were collected for subsequent perchlorate analysis.

Table K-1 shows the efficiency of perchlorate removal from the effluent by the gravity flow procedure and removal by the standard SPE cartridge method. The percent of perchlorate extracted by the gravity flow procedure was 96.1 ± 1.6 (average of 3 syringes ± standard deviation), compared to 100 percent with the standard SPE cartridge procedure whereby samples are applied under pressure.

**Table K-1. Perchlorate extraction by gravity flow through columns of large SDVB beads compared to SPE SDVB cartridges with sample applied under pressure**

| Sample description | Perchlorate (µg/L) | Estimated percent of perchlorate extracted[a] |
|---|---|---|
| Perchlorate solution applied to large bead columns and SPE cartridges | | |
| Replicate 1 | 87.7 | NA |
| Replicate 2 | 88.1 | NA |
| Replicate 3 | 88.5 | NA |
| Average ± SD | 88.1 ± 0.4 | |
| Effluent from large bead SDVB columns | | |
| Column 1 | 1.9 | 97.9 |
| Column 2 | 4.6 | 94.7 |
| Column 3 | 3.8 | 95.7 |
| Average ± SD | 3.4 ± 1.4 | 96.1 ± 1.6 |
| Effluent from SPE cartridges | | |
| Cartridge 1 | None detected | 100 |
| Cartridge 2 | None detected | 100 |
| Cartridge 3 | None detected | 100 |

[a]NA denotes not applicable

-

# Appendix L: ASPECTS OF BIOASSAY TESTING

## L-1.0 PREPARATION OF CELL EXTRACTS

Extracts of strain CKB are identified by the date of preparation. Those used in benchtop and plate reader bioassay testing and are listed in tables L-2 and L-3, respectively.

The 12/10/2007 extract was prepared as previously described[19]. A similar procedure was used to prepare the 8/3/2011 and 5/8/2012 extracts.

Briefly, cell cultures were grown anaerobically on phosphate medium containing 10 mM acetate and 10 mM chlorate. Cells in late exponential growth phase were harvested by centrifugation. The cell pellet was resuspended in anaerobic MOPS buffer (50 mM, pH 7.2) by vortexing inside an anaerobic chamber. The cell suspension was repelleted and the cell pellet was stored in a – 80 °C freezer.

To prepare extracts, a thawed cell pellet was suspended in 50 mM MOPS (pH 7.2) anaerobic buffer inside an anaerobic chamber. Prior to use, the buffer was amended with sufficient DNase (Sigma-Aldrich Corporation, DNase I from bovine pancreas) and lysozyme (Thermo Scientific Pierce, product no. 89833) to obtain final concentrations of 0.01 mg DNase/mL and 0.1 mg lysozyme/mL in the cell suspension. The amount of this buffer used was 5 mL per gram of cell pellet wet weight. The mixture of cells and degradative enzymes was incubated at room temperature for about 30 to 60 minutes before the sonication step. The tube with the cell suspension was transferred from the anaerobic chamber to a laboratory bench, placed in ice bucket, and flushed with $N_2$ prior to disruption by sonication. Cells were disrupted by 5 to 6 sonication bursts (30 seconds each) with a 30 second pause between bursts.

An anaerobic chamber was used to transfer the sonicated material to polycarbonate centrifuge tubes (capped with a three-part screw cap assembly). Tubes were centrifuged in a preparative ultracentrifuge using a Ti 70.1 rotor for 1 hour at 35000 rpm (i.e. 112,000 x *g*). After centrifugation, tubes were opened inside an anaerobic chamber. The clear upper portion of the supernatant was removed with a Pasteur pipette, mixed with anaerobic glycerol to a final concentration of about 8 %, and aliquots were distributed to microfuge tubes. The tubes with freshly prepared extract were removed from the anaerobic chamber and flash frozen in liquid $N_2$. The tubes with frozen extract were stored in a – 80 °C freezer until use.

## L-2.0 BENCHTOP BIOASSAY PROCEDURE

### L-2.1 SET-UP CUVETTES FOR BENCHTOP BIOASSAY

Prepare a dilution of CKB extract to be added to reaction mixtures. In an anaerobic chamber, dilute the extract 1/3 or 1/4-fold in anaerobic 50 mM MOPS (pH 7.2) buffer contained in a Hungate tube. Add NADH and PMS to the buffer prior to adding extract to help further reduce the buffer. The amount of NADH and PMS added should bring their final concentrations in the

diluted extract to the same concentrations as are used in cuvette reaction mixtures (see below). Store diluted extract in an ice bucket until use.

Deliver 1 mL of SPE eluate (neutralized to about pH 7.2 with 1N HCl) to a cuvette, add 16 µL NADH (45 mM in 0.01 M NaOH), and overlay with 0.9 mL of mineral oil. For a perchlorate standards reaction, deliver 0.95 mL of enzyme buffer (200 mM MOPS, 2M NaCl, adjusted to pH 7.2 with HCl) to a cuvette, add 50 µL of deionized water (for the negative control) or 20-fold concentrated solution of perchlorate standard, 16 µL NADH, and overlay with 0.9 mL of mineral oil.

Deliver 32 µL of PMS (4 – 5 mM in deionized water) to the aqueous phase below the mineral oil overlay. Mix. Follow absorbance change at 340 nm at 5 minute intervals to monitor the non-enzymatic oxidation of NADH. After 45 – 50 minutes, calculate how much NADH should be added to the cuvette to restore the 340 nm absorbance to about 2.0. Add the NADH below the mineral oil overly, mix, and again monitor the non-enzymatic oxidation of NADH for 45 – 50 minutes. Again calculate how much NADH to add to restore the 340 nm absorbance to about 2.0.

Add the NADH. Then add 0.1 mL of diluted CKB cell extract below the mineral oil overlay using a 1 mL syringe to transfer diluted extract from the Hungate tube to the reaction mixture. Mix. Measure the 340 absorbance every 5 minutes for about 50 minutes to monitor the progress of the perchlorate reductase reaction.

The millimolar extinction coefficient for NADH at a wavelength of 340 nm is 6.22. The extinction coefficient was used to convert 340 nm absorbance units to µmoles of NADH oxidized per mL of reaction mixture. Semi-micro quartz cuvettes with a 1 cm light path that accommodate a nominal volume of 1.4 mL, but which have a standard threaded top so that the actual total capacity is about 2.5 mL, were used (Starna Cells, Inc).

## L-2.2 RECORD OF NADH SUPPLEMENTATION MADE TO BENCHTOP BIOASSAY REACTIONS

As discussed in the text of this report, preincubation of reaction mixtures containing NADH and PMS was used to reduce SPE eluate (as well as negative and perchlorate standard control) reactions prior to the addition of extract. Reaction mixtures need to be mixed by stirring or repipetting after additions are made in order to obtain homogeneity. The mixing process unavoidably also introduces some oxygen into the mixture.

Table L-1 lists the average amount of NADH supplement added to reactions for each benchtop bioassay batch. Also listed is the number of times the reaction mixtures were mixed in order to achieve homogeneity. For instance, reactions were mixed for the first time immediately after adding PMS, and 340 nm absorbance was then monitored for the first preincubation interval.. For batches tested with all sources except Hill AFB and McGregor NWIRP, NADH supplementation was performed after the first preincubation, after which reactions were mixed for the second time, and absorbance monitored again for the second preincubation interval. An additional NADH supplement was added after the second preincubation, followed immediately by addition of diluted extract, and reaction mixtures were then mixed for the third time.

Absorbance readings after extract addition provide the data for determining the rate of enzyme activity (AU change/minute). For Hill AFB and McGregor NWIRP testing, the number of NADH supplements, the number of times reaction mixtures were mixed, and the number of preincubation intervals varied as indicated in Table L-1.

**Table L-1. Supplementation of benchtop bioassay reaction mixtures with NADH at the end of preincubation intervals and the total number of times reaction mixtures were mixed**

| Batch | SPE eluates | | | | Negative and standard controls | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of supple-ments | No. of times mixed | Average[a] ± SD (µL) | No. of reac-tions | No. of supple-ments | No. of times mixed | Average[a] ± SD (µL) | No. of reac-tions |
| **102, Hill AFB[b]** | | | | | | | | |
| 1 (11Aug2011) | 2 | 3 | 10.0 ± 0.76 | 15 | 0 | 1 | 4.0 ± 0.0 | 2 |
| 2 (11Aug2011 | 1 | 3[c] | 7.7 ± 0.70 | 15 | 0 | 1 | 0 | 2 |
| 3 (12Aug2011) | 2 | 3 | 13.1 ± 1.03 | 15 | 0 | 1 | 0 | 2 |
| **103, McGregor NWIRP[d]** | | | | | | | | |
| 1 (6Nov2011) | 1 | 2 | 8.9 ± 0.40 | 15 | 1 | 2 | 4.9 ± 0.17 | 3 |
| 2 (9Nov2011) | 1 | 2 | 8.7 ± 0.72 | 15 | 1 | 2 | 4.1 ± 0.75 | 3 |
| **104, JPL** | | | | | | | | |
| 1 (9Dec2011) | 2 | 3 | 10.3 ± 0.60 | 15 | 2 | 3 | 4.3 ± 0.30 | 3 |
| 2 (12Dec2011) | 2 | 3 | 9.3 ± 0.87 | 15 | 2 | 3 | 3.8 ± 0.49 | 3 |
| 3 (12Dec2011) | 2 | 3 | 7.8 ± 0.91 | 15 | 2 | 3 | 6.5 ± 0 | 3 |
| 4 (16Dec2011) | 2 | 3 | 11.4 ± 0.61 | 9 | 2 | 3 | 5.5 ± 0.45 | 3 |
| **105, FWC** | | | | | | | | |
| 1 (16Feb2012) | 2 | 3 | 12.0 ± 0.60 | 15 | 2 | 3 | 4.6 ± 0.52 | 3 |
| 2 (18Feb2012) | 2 | 3 | 11.8 ± 0.98 | 15 | 2 | 3 | 6.1 ± 0.15 | 3 |
| 3 (20Feb2012) | 2 | 3 | 12.2 ± 0.42 | 15 | 2 | 3 | 6.5 ± 0.35 | 3 |
| **106, MMR** | | | | | | | | |
| 1 (15Mar2012) | 2 | 3 | 9.6 ± 1.55 | 15 | 2 | 3 | 6.2 ± 0.20 | 3 |
| 2(18Mar2012) | 2 | 3 | 7.7 ± 2.20 | 15 | 2 | 3 | 6.0 ± 0 | 3 |
| 3 (19Mar2012) | 2 | 3 | 10.9 ± 1.24 | 15 | 2 | 3 | 6.3 ± 0.92 | 3 |
| **107, LLNL** | | | | | | | | |
| 1 (20Apr2012) | 2 | 3 | 12.7 ± 1.13 | 15 | 2 | 3 | 5.7 ± 0.10 | 3 |
| 2 (23Apr2012) | 2 | 3 | 10.8 ± 2.06 | 15 | 2 | 3 | 5.6 ± 0.23 | 3 |
| 3 (24Apr2012) | 2 | 3 | 9.2 ± 1.63 | 15 | 2 | 3 | 6.0 ± 0.35 | 3 |

[a]Values are the average µL of NADH stock solution added to reaction mixtures at the end of both preincubation intervals

[b]Only reaction mixtures containing SPE eluates were supplemented with additional NADH. That is, NADH was not added to negative and positive control reaction mixtures after the preincubation intervals

[c]For the Hill AFB SPE eluates B1-(0 spike) and B3-(0 spike), reactions were only mixed 2 times instead of 3. The other SPE elutate reactions were mixed 3 times

[d]There was only one preincubation interval used for McGregor testing. NADH was added at the end of the preincubation period prior to the addition of cell extract

## L-2.3 PARAMETERS FROM REGRESSION LINES OBTAINED WITH PERCHLORATE STANDARDS (0, 20, 40 µM) IN BENCHTOP BIOASSAY TESTING

Negative and positive control reactions were used to check that perchlorate reductase activity was present for each batch of reactions. The rate (change in absorbance units/minute) for the negative control was subtracted from the rate of reaction for SPE eluates or perchlorate standards. Regression lines for the rate of absorbance change for standards (minus the rate for the negative control) were calculated and the slope and $r^2$ values are listed in Table L-2. Figures showing the regression lines for perchlorate controls are in Figures B-102, 103, 104, 105, 106, and 107. Standards regression lines were used to calculate the x-intercepts listed in the table. Clearly, $r^2$ correlation coefficients were high when standards were tested in the benchtop bioassay, and the average error for x-intercepts was low, i.e. ± 1.6 µM perchlorate (n = 15).

**Table L-2. Results from benchtop bioassay testing of perchlorate standards[a,b]**

| Row | Batch ID | Test date | x-intercept | | Slope parameters | | $r^2$ | Extract/reaction (µL), and CKB extract date |
|---|---|---|---|---|---|---|---|---|
| | | | (µM) | ± error (µM) | slope | ± error | | |
| 1 | Hill #1 | 11Aug2011 | ND | NA | 0.000108 | NA | 1.0 | 33, 8/3/2011 |
| 2 | Hill #2 | 11Aug2011 | ND | NA | 0.000135 | NA | 1.0 | 33, 8/3/2011 |
| 3 | Hill #3 | 12Aug2011 | ND | NA | 0.000130 | NA | 1.0 | 33, 8/3/2011 |
| 4 | McG #1 | 6Nov2011 | 1.4 | 3.1 | 0.000120 | 0.000014 | 0.9857 | 33, 8/3/2011 |
| 5 | McG #2 | 9Nov2011 | -1.3 | 2.8 | 0.000105 | 0.000012 | 0.9881 | 33, 8/3/2011 |
| 6 | JPL #1 | 9Dec2011 | 0.0 | 0.0 | 0.000115 | 0.000000 | 1.0000 | 33, 8/3/2011 |
| 7 | JPL #2 | 12Dec2011 | 0.95 | 2.1 | 0.000123 | 0.000010 | 0.9932 | 33, 8/3/2011 |
| 8 | JPL #3 | 12Dec2011 | 0.56 | 1.2 | 0.000120 | 0.000006 | 0.9977 | 33, 8/3/2011 |
| 9 | JPL #4 | 16Dec2011 | 0.71 | 1.6 | 0.000118 | 0.000007 | 0.9962 | 33, 8/3/2011 |
| 10 | FWC #1 | 16Feb2012 | -0.055 | 0.12 | 0.000151 | 0.0000007 | 1.0000 | 25, 12/10/2007 |
| 11 | FWC #2 | 18Feb2012 | -0.74 | 1.7 | 0.000158 | 0.000010 | 0.9959 | 25, 12/10/2007 |
| 12 | FWC #3 | 20Feb2012 | -1.2 | 2.8 | 0.000166 | 0.000018 | 0.9882 | 25, 12/10/2007 |
| 13 | MMR #1 | 15Mar2012 | 0.90 | 2.0 | 0.000132 | 0.000010 | 0.9940 | 25, 12/10/2007 |
| 14 | MMR #2 | 18Mar2012 | -0.38 | 0.85 | 0.000175 | 0.000006 | 0.9989 | 25, 12/10/2007 |
| 15 | MMR #3 | 19Mar2012 | 0.097 | 0.22 | 0.000173 | 0.000001 | 0.9999 | 25, 12/10/2007 |
| 16 | LLNL #1 | 20Apr2012 | -0.76 | 1.7 | 0.000175 | 0.000012 | 0.9957 | 25, 12/10/2007 |
| 17 | LLNL #2 | 23Apr2012 | -1.2 | 2.7 | 0.000169 | 0.000018 | 0.9889 | 25, 12/10/2007 |
| 18 | LLNL #3 | 24Apr2012 | -0.56 | 1.3 | 0.000189 | 0.000009 | 0.9976 | 25, 12/10/2007 |
| | | Average | -0.11 | 1.61 | | | | |

[a]Slope is the 340 nm absorbance/minute/µM perchlorate. The negative control rate (AU/min) was subtracted from rate values before the regression line was plotted. Negative control rates for each batch are listed in the footnotes to Appendix B Tables B-102, 103, 104, 105, 106, and 107. Parameters of slope and $r^2$ are from Excel regression analysis (LINEST function).

[b]For the Hill batches, only negative control and 40 µM perchlorate standard reactions were tested. Hence, no regression line could be determined and the "slope" is only estimated from the two data points: 0 and 40 µM. For all other testing, three data points were used to generate regression lines: 0 (i.e. negative control), 20 and 40 µM perchlorate standards. ND denotes not determined, NA denotes not applicable

# L-3.0 ANAEROBIC PLATE READER BIOASSAY PROCEDURE

## L-3.1 SET-UP MICROPLATE WELLS FOR PLATE READER BIOASSAY

Deliver 276 µL of SPE eluate (neutralized to about pH 7.2 with 1 N HCl) to a microplate well. Add 4 µL NADH (45 mM in 0.01 M NaOH), and 10 µL PMS (4 – 5 mM in deionized water and mix with the tip of the pipettor. Preincubate for 45 to 60 minutes to be sure the SPE eluate is non-enzymatically reduced by the NADH/PMS system. Add 10 µL CKB cell extract and mix again. By contrast with the benchtop bioassay format, the CKB extract is not diluted before adding to reaction mixtures. Furthermore, reaction mixtures are not supplemented with additional NADH after the preincubation interval. The 340 nm absorbance change is measured for 20 to 45 minutes to determine the rate of the perchlorate reductase reaction, using the kinetics program of the plate reader.

A negative control (i.e. no perchlorate present) should be included in each batch and the rate of NADH oxidation for the negative control subtracted from the rate of reaction for all other reaction mixtures. Positive control reaction mixtures containing perchlorate standards should also be included with each microplate batch of reactions. For the negative and positive control reactions, deliver 261 µL of enzyme buffer (200 mM MOPS, 2 M NaCl, adjusted to pH 7.2 with HCl) to a well. Add 15 µL of deionized water (negative control) or 15 µL of a 20-fold concentrated solution of perchlorate standard, 4 µL NADH, and 10 µL PMS. Mix the contents of the well, and preincubate. Monitor the 340 nm absorbance change during preincubation. Add 10 µL CKB cell extract and mix again. Measure the 340 nm absorbance change for 20 to 45 minutes to determine the rate of the perchlorate reductase reaction.

Costar 96-well non-sterile acrylic microplates with a UV transparent flat bottom were used (Corning, Inc.). The final volume of reaction mixtures was 0.3 mL per well. The relationship between 340 nm absorbance and NADH concentration when the well volume is 0.3 mL was empirically determined to be 4.959 absorbance units per mM. This factor was used to convert 340 nm absorbance units to µmoles of NADH oxidized per mL of reaction mixture.

## L-3.2 PARAMETERS FROM REGRESSION LINES OBTAINED WITH PERCHLORATE STANDARDS (0, 10, 20, 30, 40 µM) IN PLATE READER BIOASSAY TESTING

The rate (change in absorbance units/minute) for the negative control was subtracted from the rate of reaction for wells containing SPE eluates or perchlorate standards. Regression lines for the rate of absorbance change for standards (minus the rate for the negative control) were calculated and parameters for these are listed in Table L-3. Figures showing the regression lines for the perchlorate controls are in Figures F-1, 4, and 5. Standards regression lines were used to calculate the x-intercepts listed in the table. The $r^2$ correlation coefficients were high when standards were tested in the plate reader bioassay, and the average error for x-intercepts was low, i.e. ± 0.64 µM perchlorate (n = 3).

**Table L-3. Results from plate reader bioassay testing of perchlorate standards[a,b]**

| Row | Batch ID | Test date | x-intercept | | Slope parameters | | $r^2$ | Extract/reaction (µL), and CKB extract date |
|---|---|---|---|---|---|---|---|---|
| | | | (µM) | ± error (µM) | Slope | ± error | | |
| 1 | Batch 1 | 5Aug2011 | -0.56 | 0.65 | 0.000056 | 0.000001 | 0.9979 | 10, 8/3/2011 |
| 2 | Batch 2 | 29Jun2012 | ND | NA | ND | NA | NA | 10, 5/8/2012 |
| 3 | Batch 3 | 6Jul2012 | ND | NA | ND | NA | NA | 10, 5/8/2012 |
| 4 | Batch 4 | 11Jul2012 | 0.81 | 0.95 | 0.000326 | 0.000013 | 0.9955 | 10, 5/8/2012 |
| 5 | Batch 5 | 11Jul2012 | 0.44 | 0.33 | 0.000306 | 0.000004 | 0.9995 | 10, 5/8/2012 |
| | | Average | 0.23 | 0.64 | | | | |

[a]Slope is the 340 nm absorbance/minute/µM perchlorate. The negative control rate (AU/min) was subtracted from rate values before the regression line was plotted. Negative control rates for each batch are listed in the footnotes to Appendix F Tables F-2, 3, 4, 5, and 6. Parameters of slope and $r^2$ are from Excel regression analysis (LINEST function).

[b]For batch1 duplicate wells were tested with 0, 10, 20, 30, and 40 µM of perchlorate, and the regression line was plotted with the average of the duplicate wells. No regression line was plotted for batches 2 and 3 – because, by mistake, the perchlorate standards added were below the limit of detection; these wells were averaged and used as the negative control rate. For batches 4 and 5, single wells with 0, 10, 20, 30, or 40 µM perchlorate were plotted to obtain the standard curves.

## L-4.0 CONFIRMATION OF STOICHIOMETRY OF NADH OXIDIZED TO PERCHLORATE REDUCED IN BENCHTOP AND PLATE READER BIOASSAYS

The stoichiometry of the NADH-coupled perchlorate reductase assay was previously studied[19] and has been described by Heinnickel et al.[2]. Confirmatory testing was performed with the CKB 5/8/2012 extract using both the benchtop and plate reader formats of the bioassay. The initial concentration of perchlorate in reaction mixtures was 40 µM.

For the benchtop bioassay, the equivalent of 25 µL of undiluted extract was used for each reaction mixture. Figure L-1.i. shows the 340 nm absorbance for the negative control (no perchlorate added to the cuvette) and the 40 µM perchlorate reaction mixture for a 45 minute

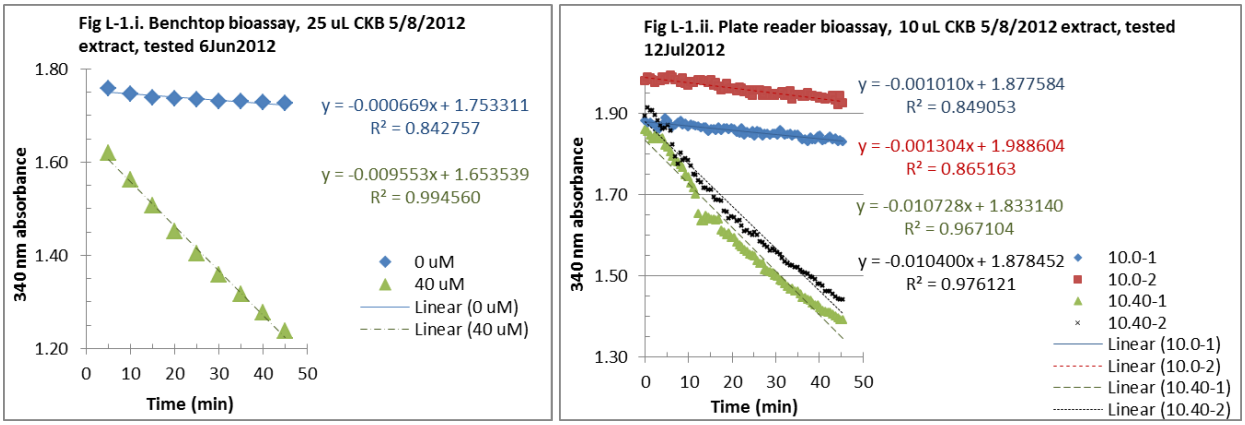



**Figure L-1. 340 nm absobance change for benchtop or plate reader bioassays using the CKB 5/8/2012 extract. (i)** Data from the benchtop bioassay is in the left hand panel, **(ii)** the right hand panel has the data from the plate reader bioassay

incubation period. To measure perchlorate reduction directly, a second reaction mixture with 40 μM perchlorate was tested in parallel. At 10 minute intervals, 60 μL samples were removed and diluted 100-fold to stop the enzyme reaction. Perchlorate concentration was subsequently determined by IC in the diluted samples. Table L-4 lists the perchlorate reduced and NADH oxidized in the parallel benchtop bioassay reaction mixtures over a 40 minute incubation period. Figure L-2.i shows the plot of these data. The slope corresponds to the stoichiometry for the μmoles of NADH oxidized per μmole of perchlorate reduced. The parameters for the regression line were calculated, and are also listed in Table L-4.

**Table L-4. Perchlorate reduced and NADH oxidized in parallel reaction mixtures for benchtop and plate reader bioassays**

| **Benchtop bioassay tested 6Jun2012** | | | **Plate reader bioassay tested 12Jul2012** | | |
|---|---|---|---|---|---|
| **Time (min)** | **Perchlorate reduced (μmoles)** | **NADH oxidized (μmoles)** | **Time (min)** | **Average Perchlorate reduced (μmoles)** | **Average NADH oxidized (μmoles)** |
| 0 | 0.0000 | 0.0000 | 0 | 0.00000 | 0.00000 |
| 10 | 0.0069 | 0.0167 | 10 | 0.00176 | 0.00604 |
| 20 | 0.0134 | 0.0335 | 20 | 0.00285 | 0.01449 |
| 30 | 0.0162 | 0.0502 | 30 | 0.00471 | 0.01881 |
| 40 | 0.0196 | 0.0670 | 40 | 0.00575 | 0.02340 |
| **Regression parameters for benchtop or plate reader bioassay results** | | | | | |
| **Slope ± error** | 3.3256 ± 0.3781 | | 4.0868 ± 0.3834 | | |
| **y-intercept ± error** | -0.0038 ± 0.0050 | | 0.0002 ± 0.0014 | | |
| **$r^2$** | 0.9627 | | 0.9743 | | |

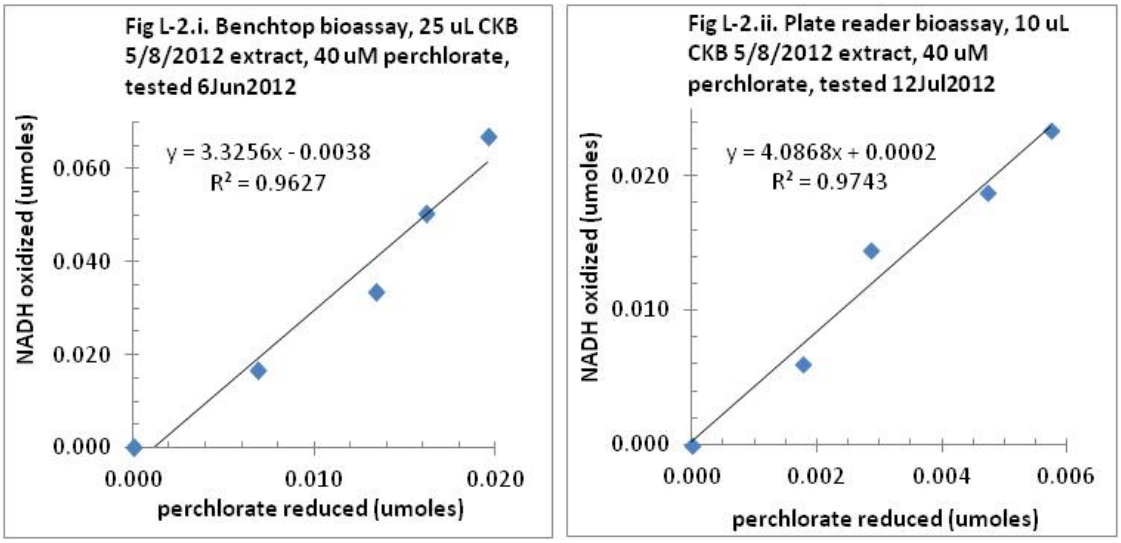



**Figure L-2. Stoichiometry of perchlorate reduction and NADH oxidation in benchtop and plate reader bioassays. (i)** Data from the benchtop bioassay is in the left hand panel, **(ii)** the right hand panel has the data from the plate reader bioassay

For the plate reader bioassay, 10 µL of extract was used for each reaction well. Figure L-1.ii shows the 340 nm absorbance for duplicate negative control and 40 µM perchlorate reaction mixtures over an incubation period of about 45 minutes. In parallel, a second microplate was used to measure perchlorate reduction directly. This plate had 12 replicate wells containing reaction mixtures with 40 µM perchlorate. Each well was sampled only once. At 10 minute intervals, duplicate 60 µL samples were diluted 100-fold to stop the enzyme reaction. Perchlorate concentration was subsequently determined by IC in the diluted samples. Table L-4 lists the perchlorate reduced and NADH oxidized in the parallel bioassay reaction mixtures. Figure L-2.ii shows the plot of these data, with the slope corresponding to the stoichiometry for the µmoles of NADH oxidized per µmole of perchlorate reduced. The parameters for the regression line were calculated, and are also listed in Table L-4.

The observed stoichiometry for µmoles of NADH oxidized per µmole of perchlorate reduced was 3.3 ± 0.38 with the benchtop bioassay and 4.1 ± 0.38 with the plate reader bioassay. As discussed in Section 2.1 above, a stoichiometry of 2 would be expected if only perchlorate reductase were active in reaction mixtures, and a stoichiometry of 4 is possible if chlorite dismutase is also active.

## L-5.0 CONTRAST BETWEEN CKB AND RCB EXTRACTS REGARDING PERCHLORATE REDUCTASE RATES AT DIFFERENT PERCHORATE CONCENTRATIONS

The activity (340 nm absorbance change/minute) of extracts of *Dechloromonas agitata* strain CKB shows a clear dependance on the initial concentration of perchlorate (Coates and Achenbach, unpublished). Bioassay testing was also done with extracts of another strain in the *Dechloromonas* genus, *Dechloromonas aromatica* strain RCB, to determine whether a similar degree of dependence on initial perchlorate concentration would be observed.

Both strains were cultivated on the same medium, and the procedure described in Section L-1.0 was used to prepare extracts with both strains on 8/3/2011. The plate reader bioassay format was used as described in Section L-3.0, and the reactions with both strains were performed simultaneously in the same microplate. Perchlorate concentrations of 0, 10, 20, 40, and 500 µM were tested in triplicate for each extract. The reactions without perchlorate are the negative controls. Figure L-3 shows the 340 nm absorbance change for all the reactions over the course of about 46 minutes. The rates of absorbance change with the RCB extract were clearly biphasic for the lower perchlorate concentrations. The faster rate phase was from 0 to about 4 to 5 minutes of reaction for 10 µM perchlorate, from 0 to 11 minutes for 20 µM perchlorate, and from 0 to 31 minutes for 40 µM perchlorate. Rates for 500 µM perchlorate were not biphasic during the interval over which absorbance changes were monitored.

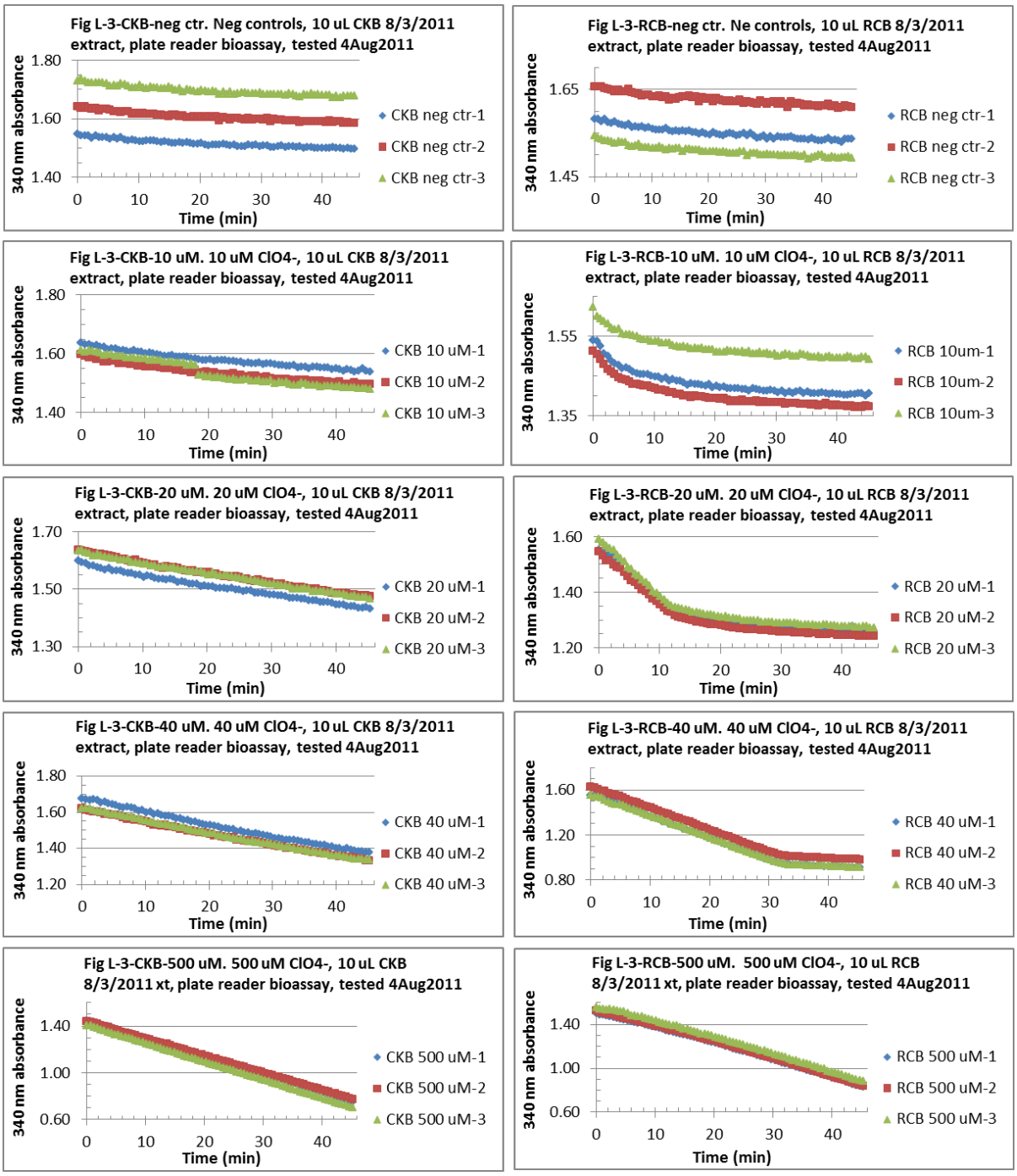



**Figure L-3. 340 nm absorbance change with CKB and RCB extracts at different initial perchlorate concentrations**

Table L-5 lists the average rates of 340 nm absorbance change for each test condition as well as the standard deviation for the replicates. Also listed are the average rates after subtracting the average negative control rate. With the RCB reactions, different time intervals were used to determine the absorbance change during the faster rate phase, and these intervals are also listed in the table. Since there was a small difference in the negative control rates depending on the time interval, negative control rates were calculated for the different intervals. These negative control rates these are also listed in the table.

**Table L-5. Rates of perchlorate reductase activity with CKB and RCB extracts for various initial perchlorate concentrations**

| Initial $ClO_4^-$ (µM) | CKB 8/3/2011 extract | | | | RCB 8/3/2011 extract | | | |
|---|---|---|---|---|---|---|---|---|
| | Time interval (min) | Average rate | | Average rate – neg. ctr | Time interval (min) | Average rate | | Average rate – neg. ctr |
| | | (AU/min) | SD | (AU/min) | | (AU/min) | SD | (AU/min) |
| 10 | 0 to 16.05 | 0.00290 | 0.00018 | 0.00106 | 0 to 4.2 | 0.01541 | 0.00253 | 0.01251 |
| 20 | 0 to 16.05 | 0.00423 | 0.00006 | 0.00239 | 0 to 10.1 | 0.01959 | 0.00119 | 0.01735 |
| 40 | 0 to 16.05 | 0.00722 | 0.00028 | 0.00538 | 0 to 29.7 | 0.01935 | 0.00016 | 0.01817 |
| 500 | 0 to 16.05 | 0.01516 | 0.00050 | 0.01332 | 0 to 45.3 | 0.01516 | 0.00012 | 0.01424 |
| **Negative controls** | | | | | | | | |
| 0 | 0 to 16.05 | 0.00184 | 0.00009 | 0.00000 | 0 to 4.2 | 0.00290 | 0.00046 | 0.00000 |
| 0 | | | | | 0 to 10.1 | 0.00225 | 0.00014 | 0.00000 |
| 0 | | | | | 0 to 29.7 | 0.00118 | 0.00005 | 0.00000 |
| 0 | | | | | 0 to 45.3 | 0.00092 | 0.00005 | 0.00000 |

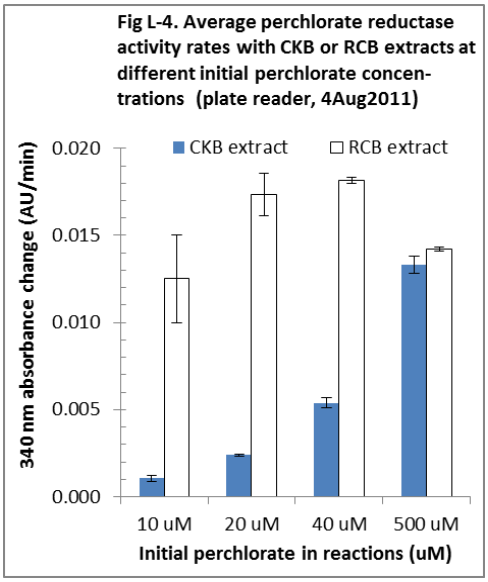



**Figure L-4. Perchlorate reductase rates with CKB or RCB extracts.** Error bars show the standard deviation of triplicate determinations

Figure L-4 shows a comparison of the average rates of absorbance change, after subtracting the average negative control rate, for each concentration of perchlorate tested with the two extracts. Had the development of a kit for the benchtop bioassay gone forward, it might have been advantageous to test RCB extracts for their suitability for such an application, since the rate of absorbance change is faster at low perchlorate concentrations when an RCB extract is used. The basis of the difference between the extracts might be due to an operationally lower $K_m$ for perchlorate with the RCB strain.