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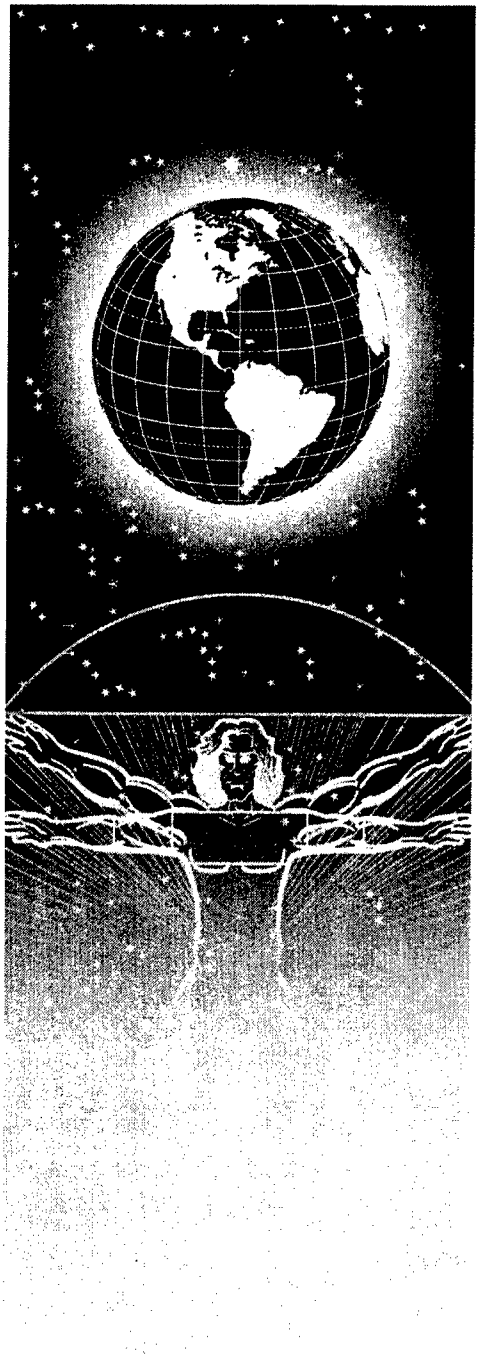

# UNITED STATES AIR FORCE RESEARCH LABORATORY

# PERCHLORATE IN FERTILIZERS

**J.E. Eldridge, 2Lt.**
**D.T. Tsui, Capt.**
**D.R. Mattie, Ph.D.**
OPERATIONAL TOXICOLOGY BRANCH, AFRL/HEST
2856 G STREET
WRIGHT PATTERSON AFB, OH 45433-7400

**J. Crown, Maj.**
AFIT/ENC, BLDG. 640
2950 P STREET
WRIGHT PATTERSON AFB, OH 45433-7765

**R. Scott**
TRC ENVIRONMENTAL SOLUTION, INC.
21 TECHNOLOGY DRIVE
IRVINE, CA 92618

**T. Blackman**
LOCKHEED-MARTIN, INC.
CORPORATE ENVIRONMENT, SAFETY AND HEALTH
2550 NORTH HOLLYWOOD WAY
BURBANK, CA 91505

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**Human Effectiveness Directorate**
**Deployment and Sustainment Division**
**Operational Toxicology Branch**
**2856 G Street**
**Wright-Patterson AFB OH 45433-7400**

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**FOR THE DIRECTOR**

**DAVID R. MATTIE**, PH.D
Acting Branch Chief, Operational Toxicology Branch
Air Force Research Laboratory

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This report documents an inter-laboratory collaborative study on the performance on ion chromatography (IC) methods for perchlorate analysis in lawn and garden fertilizers. Seven government, private, and commercial laboratories participated in the analysis of 34 aqueous suspensions of the test materials, using similar ion chromatography systems. Two method variants (AS-11 and AS-16) were evaluated. Statistical evaluations by paired t-test for the means analysis found good agreements in each of the methods variants and excellent agreement between the methods. The study also found no significant defferences in the performance of the microbore column with respect to the standard bore column. This report also evaluates and compares the performance of IC with respect to capillary eletrophoresis (CE), Raman spectroscopy (RS), and titration for perchlorate analysis. The study found excellent agreement between the ion chromatography and CE results, and little or no agreement between IC and RS or titration. As expected, compared to the IC results, titration is biased high.

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# PREFACE

This report summarizes research that begun in February 1999. Research was completed in August 1999 under Department of the Air Force Contract No. F41624-96-C-9010. This study was a collaborative effort between the Department of the Air Force representing the Inter-Perchlorate Steering Committee, the Perchlorate Study Group, and the United States Environmental Protection Agency National Exposure Research Laboratory, Ecosystems Research Division (EPA/NERL/ERD).

Laboratories who participated in the study were Chemical Testing Service (Diamond Bar, CA), Del Mar Analytical Laboratories (Irvine, CA), American Pacific Corporation (Cedar City, UT), United Technologies Corporation (San Jose, CA), Montgomery Watson Laboratories (Pasadena, CA), Thiokol Corporation (Brigham City, UT), EPA/NERL/ERD (Athens, GA), and AFRL/HEST (Wright-Patterson AFB, OH).

Tommy Blackman (Lockheed Martin, Corporate Environment, Safety and Health, Burbank, CA) and Richard Scott (TRC Environmental Solutions Inc., Irvine, CA) served as the coordinator for the Perchlorate Study Group. MacArthur Long served as the coordinator for EPA/NERL/ERD. Major Steve Channel served as Contract Technical Monitor for the United States Air Force, AFRL/HEST.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| $\alpha$ | probability of Type I error |
| ASRS | Anion Self Regenerating Suppressor |
| CDHS | California Department of Health Services |
| CE | capillary electrophoresis |
| CCD | charge coupled device |
| CV | cycle voltammetry |
| EOF | Electro-osmotic flow |
| HPLC | high performance liquid chromatography |
| i.d. | inner diameter |
| IC | ion chromatography |
| g | gram |
| kV | Kilo-volts |
| ISE | ion selective electrode |
| L | Liter |
| $\mu$g | microgram |
| $\mu$L | micro-liter |
| mL | milliliter |
| mm | millimeter |
| min | minutes |
| m$\Omega$ | mega-Ohm |
| mW | milli-watt |
| mM | milli-molar |
| mmol | milli-mole |
| mV | milli-volt |
| nL | Nanoliter |
| nm | nanometer |
| o.d. | outer diameter |
| ppm | parts per million |

| | |
|---|---|
| ppb | parts per billion |
| %CV | percent coefficient of variation |
| RS | Raman spectroscopy |
| $S_d$ | Stdev, standard deviation |
| $S_{di}$ | standard deviation of the sample measurements between laboratories |
| TDS | total dissolve solids |
| UV | ultra violet |
| V | volts |

# PERCHLORATE IN FERTILIZERS

## 1.0 INTRODUCTION

Perchlorate is a powerful oxidizer used in solid-rocket propellant mixtures, fireworks, and munitions. Inorganic salts of the perchlorate anion are relatively soluble in water. The presence of trace level perchlorate in drinking water poses a potential health risk, due to the interference of perchlorate with the uptake of iodide necessary to produce hormones in the thyroid gland. The EPA's current recommended acceptable level for perchlorate in drinking water is 18 parts per billion (ppb) [1-10]. Heightened awareness of perchlorate as a potential health risk has led to increased activities in the investigation of perchlorate occurrence in the environment [11-12]. Since perchlorate and the ammonium, sodium, and potassium salts of perchlorate are better known in their commercial and industrial applications, studies of occurrence have focused on surface and ground water sources near regions where munitions, aerospace components, and fireworks were manufactured, developed, and tested. However, reports have suggested that naturally occurring perchlorate is present in fertilizers, nitrate deposits from northern Chile (caliche), and minerals from arid environments with chloride deposits [15-17]. These new findings have sparked intense efforts in the study of natural perchlorate occurrence in non-aqueous matrices and the application of existing methodologies to accurately determine perchlorate levels.

The determination of perchlorate at trace (ppb) levels is a difficult analytical task. Early techniques for perchlorate analysis in aqueous and solid matrices, such as gravimetric analysis [18-22], titration [19, 20, 23-25], and liquid-liquid extraction/spectrophotometry [26-31], explored the formation of water insoluble ionic complexes of perchlorate with organic dyes, such as nitron, brilliant green, tetraphenylstibonium, and methylene blue. Because organic dyes are not specific for perchlorate and have preferential selectivity for perchlorate and similar anions, potential exist for those methods to over estimate perchlorate concentrations, leading to biased results.

More specific and selective methods, such as ion pair high performance liquid chromatography (HPLC) [32-33], capillary electrophoresis (CE) [34-40], ion selective electrode (ISE) [40-41], and Raman spectroscopy (RS) [42] have also been used in perchlorate analysis in complex matrices. However, the sensitivity of these methods is limited to the ppm levels.

For the determination of trace level perchlorate in aqueous matrices, ion chromatography coupled with a conductivity detector is the state of the art technology available to most analytical laboratories [43-47]. Several IC methods have been developed for the analysis of trace perchlorate levels. In 1997, the California Department of Health Services (CDHS) using the AS-5 anion separation column developed the so-called CDHS Method for the determination of trace level perchlorate in drinking water [44]. In April 1998, the Dionex Application Laboratory developed an improved IC method, using the AS-11 column, for perchlorate analysis [45-46]. The performances of both AS-5 and AS-11 methods for drinking water and groundwater were validated in an inter-laboratory collaborative study sponsored by the Interagency Perchlorate Steering Committee (IPSC). With respect to aqueous matrices having the same quality as drinking water and groundwater, the collaborative study found no differences between the two methods in terms of bias and accuracy [48]. In March 1999, California Department of Health Services Division of Sanitation Laboratory Services adopted both methods [49], and in July 1999 EPA proposed both methods for national drinking water and groundwater analysis [47].

Although both methods performed similarly, the AS-11 method has gained wider acceptance over the AS-5 because the AS-5 method is incompatible with the newer suppressors and is less rugged for matrices with high total dissolved solids (TDS). The p-cyanophenol organic modifier used in the AS-5 methods is electrochemically active. Incompatible with the newer electrochemical suppressor, the p-cyanophenol may de-grade the suppressor membrane and cause elevated baselines and lower IC performance [48-53]. High TDS effects perchlorate analysis by giving non-specific baseline rise that could mask the perchlorate peak. In a review sponsored by East Valley Water District, Shen and Harrington [54], showed that with respect to mixed water analysis containing TDS at greater 1,000 ppm, the performance of the AS-5 column is not as robust as the AS-11. Furthermore, in separate experiments, Eaton *et al.* and the IPSC analytical subcommittee demonstrated that although samples with high TDS pose a challenge to

both methods, the AS-11 column is more rugged than the AS-5. At greater than 2500 ppm TDS, the AS-5 method could not effectively resolve trace perchlorate at 5 ppb [48, 53-56].

To accommodate samples with high TDS, the AS-16 column was introduced, and an extensive methods development study based on the AS-16 column was completed in May 1999 [52, 53]. The optimized AS-16 method calls for a Dionex IonPac® AS-16 column with a 35-mM mobile phase flowing at 1.25 mL/min. The reported AS-16 method detection limit is 1 ppb, with a 1,000-µL injection loop volume and an ASRS-Ultra suppressor. The AS-16 column, with significantly higher column capacity and more hydrophilic functional groups than the AS-11 column, was developed to accommodate matrices with higher TDS and provide better separation for perchlorate. Whereas the AS-11 is limited in resolving 50-ppb perchlorate at around 6,800 ppm TDS, the AS-16 is capable to resolving 50-ppb perchlorate at TDS levels as high as 20,000 ppm. Shortly after this study began, the EPA proposed the AS-16 column as a replacement for both the AS-5 and AS-11 columns [57].

This report documents the results of a collaborative study conducted by the IPSC Analytical Sub-Committee with the EPA Office of Research and Development, National Exposure Research Laboratory, Ecosystems Research Division in Athens, GA. The objective of this collaborative (co-lab) study is to compare the performance of IC methods for the measurement of perchlorate in the liquid extracts of lawn and garden fertilizers containing high TDS. Additionally, this report evaluates the performance of capillary electrophoresis, titration, and Raman spectroscopy for perchlorate analysis.

# 2.0 METHODS AND MATERIALS

## 2.1 Test Materials

Test materials were purchased from various commercial and retail sources located in Missouri, New York, and California. Appendix A lists the test materials and corresponding sample number, manufacturer, and primary constituents appearing on the manufacture's labels.

All-Purpose Plant Food (Peters Inc.) having different lot numbers was purchased from three different locations: California (ps06), Kansas City, MO (ps05), and Long Island, NY (ps16). Samples ps17 and ps24 are duplicate samples of Fall Fertilizer (Johnathan Green Co.) purchased in Long Island, New York. Samples ps15 and ps21 are duplicate samples of Lawn Restorer (Ringer Inc.) purchased in Long Island, New York. Samples ps02 and ps09 are duplicate samples of Supreme Gardens (Ringer Inc.) purchased from Kansas City, MO. Samples ps08 and ps12 are duplicate samples taken from one lot of Vegetable and Bedding Plant Food bought in Kansas City, MO and sample ps19 was taken from another lot purchased in Long Island.

The caliche sample (ps34, Chilean nitrate) was collected from Region I of the Soquimich Nitrate Works located in Calama, Chile. The langbeinite ore sample (ps30) was obtained from IMC Minerals (Albuquerque, NM). The potassium nitrate used in the Champion (sample number ps31) and Best K-Power (ps32 and ps33) and Chilean Nitrate fertilizers were also collected from the Soquimich Nitrate Works.

Important to note, these raw test materials were heterogeneous. Regardless of mixing time and mesh quality, dividing the raw test materials for laboratory analysis represented sub-sampling of the test materials and the result for each sub-sample is inherently different from another. Although not a factor in the liquid extracts shipped in this study, shipment and storage of bagged fertilizer could bias the results due to changes in moisture content.

Consistent with the preliminary nature of this round robin, a homogenous suspension of subsamples of a nonrepresentative subsample of each fertilizer and raw test material was prepared and shipped to seven laboratories for blind analysis. The suspension was prepared by

mixing the solid sample with deionized water. The mixing ratio was 1 gram of solid sample per 10 mL of de-ionized water. De-ionized water used in this study was Type I reagent grade, with a resistance of 18 mΩ or better. The mixture was shaken over 48 hours, and the liquid phase was removed after the suspension had settled. Approximately 50 mL of the liquid phase from each of the thirty-four test materials was transferred to an amber glass bottle and shipped to the cooperating laboratories for perchlorate analysis. Two 100-µg/L positive controls (LCS1 and LCS2) and one negative control accompanied each set of thirty-four liquid test samples. The 100-µg/L perchlorate controls were prepared gravimetrically from sodium perchlorate (Fisher Scientific, Inc., Lot # 78164) and de-ionized water. The negative control blank was prepared from de-ionized water alone.

## 2.2 Analytical Methods

*Ion Chromatography.*

All seven laboratories performed the ion chromatography analysis on similar ion chromatography systems manufactured by Dionex Corp. of Sunnyvale, CA. A system consists of AS-40 autosampler for sample injection, GP-40 gradient pump for eluent delivery, and CD-20 conductivity detector for detection. Method variations differed in the ion separation columns and eluent conditions; both AS-11 and AS-16 columns were used in this study. Appendix B lists the differences in eluent conditions among laboratories.

Laboratories 3, 4, 6, and 7 employed the AS-11 method with identical method conditions. Perchlorate was chromatographically separated on a Dionex ATC-1 anion trap column, AG-11 guard column (4 mm x 50 mm), and AS-11 anion separation column (4 mm x 250 mm), with 100 mM NaOH mobile phase flowing at 1.0 mL/min. as eluent. All four laboratories used the Anion Self-Regenerating Suppressor-II (ASRS-II) operating in the external water mode for eluent suppression. Laboratory 3 used a 1,000-µL injection volume loop; and labs 4, 6, and 7 used 100-µL injection volume loops.

Laboratory 2 used a micro-bore equivalent of the standard AS-11 method. Instead of the 4-mm guard and separation column, Laboratory 2 used a 2-mm x 50-mm AG-11 guard column and a 2-mm x 250-mm AS-11 column for perchlorate separation. Accordingly, the 100-mM NaOH eluent flow rate was reduced from 1 mL/min to 0.38 mL/min. To avoid overloading the column, the sample injection volume was reduced proportionally from 1,000 µL to 1 µL. Eluent suppression was achieved using an ASRS-Ultra suppressor operating in the external water mode.

Laboratories 1 and 5 used the AS-16 method for perchlorate analysis. For the AS-16 method, perchlorate separation was obtained on a Dionex IonPac AS-16 separation column (4 x 250-mm) with an AG-16 guard column (4 x 50 mm) and an ATC-1 anion trap column. Laboratory 5 used the recommended 1,000-µL sample loop volume and an isocratic program with 35-mM NaOH mobile phase flowing at a rate of 1.25 mL/min. Laboratory 1 used a 50-µL sample loop injection with a gradient program: 20 mM NaOH for 5 minutes and 100-mM NaOH for the remainder of the analysis.

*Capillary Electrophoresis.*

Laboratory 6 performed the CE analysis. A Beckman P/ACE 5000 capillary electrophoresis system was used for indirect UV-detection (214 nM) of perchlorate in fertilizer samples. All data analysis was performed on Beckman System Gold Software. Liquid test materials diluted 1:10 to 1:100 were injected hydrodynamically for 5 seconds on a 47-cm fused silica (40-cm end to detection, 300-µm outside diameter and 75-µm inside diameter). The column temperature and voltage were set at 23°C and 20 kV, respectively. The running buffer consisted of 40-mM phosphate (pH 7.0) containing 2.24-mM pyromellitic acid visualizing agent and 0.5-mM tetradecyl trimethyl ammonium bromide for reduction of the electroosmotic flow. Perchlorate is non-UV active [32]. The displacement of pyromellitic acid by analyte in the running buffer gives negative peaks as the analyte passes the detector. For quantitation, the detector polarity is reversed to give a positive peak. Between sample analyses, the capillary was flushed for 1 minute with deionized water, 4 minutes with 0.1 M NaOH, 4 minutes with water, and 4 minutes with the running buffer. Sample run time was 6 minutes.

*Raman Spectroscopy Analysis.*

Laboratory 6 also performed the RS analysis. For Raman spectroscopy analysis, 5-mL of the undiluted liquid test samples were analyzed on a Kaiser Optical Systems HoloProbe, using 785-nm laser excitation from a diode laser. The laser light was coupled to a remote probe head via a 1.9 meter-long fiber optic cable. The laser light was brought to focus with a series of lenses, at approximately 2.5 inches beyond the end of the probe head assembly. A standard quartz cuvette containing the sample was placed in the path of the beam such that the focus of the beam fell in the center of the cuvette. The power of the laser light at the sample was approximately 135 mW.

Raman scattered light from the sample was collected by the probe head along the same path as the excitation laser beam (i.e., 180° back scattering geometry). The probe head was coupled to a separate 1.9 meter-long fiber optic cable for delivery to the f /1.8 axial transmissive-type spectrograph. A holographic notch filter removed elastically scattered laser light from the probe head and a 50 μm slit focused Raman scattered light through a volume holographic transmissive grating. The dispersed Raman spectrum was collected by a charge coupled device (CCD) detector which allowed simultaneous acquisition of the entire Raman spectrum with useable Stokes Raman shift of about 3280 to 95 $cm^{-1}$ in a single exposure (with spectral resolution of 5 $cm^{-1}$). The CCD detector used a back-illuminated, near-infrared optimized Princeton CCD-1024EHRB chip. The chip was thermoelectrically cooled at –65°C.

*Tetraphenylstibonium Titration.*

Chemical Test Service of Diamond Bar, CA performed tetraphenylstibonium titration of all samples. For titration, the suspension was transferred onto an ion exchange column containing 25 grams of Dowex-1 resin (VWR Inc.) and eluted with 100 mL of 0.01-M potassium bicarbonate solution (VWR Inc.) to remove nitrate, chloride, chlorite, and chlorate ions. The solutions were monitored using a perchlorate selective electrode (Orion Inc., Model 938101), to determine if a portion of the perchlorate eluted in the rinse. If the perchlorate selective electrode indicated over 10-ppm perchlorate, the sample preparation step was repeated with using a larger dilution. When perchlorate was no longer detectable in the effluent by the perchlorate selective electrode, the ion exchange column was rinsed first with 75 mL of 0.05-M sodium fluoroborate

solution followed by 25 mL of the same solution to de-sorb the perchlorate from the ion exchange column. Both rinses were collected. The combined sodium fluoroborate solution was then analyzed using the perchlorate selective electrode. To determine the efficiency of the extraction process, various control samples and duplicate samples were also extracted and analyzed. A 25-mL aliquot was removed from the combined sodium fluoroborate solution, diluted with 10 mL of de-ionized water and titrated with 0.01M (3.6 mg/mL) tetraphenylstibonium sulfate using six drops of bromocresol green as an indicator. The endpoint was clearly visible for each titration, with the bromocresol green color disappearing, producing a clear solution.

## 2.3 Statistical Method

Due to method variations, it would be erroneous to determine the variability in IC measurements by pooling the data from all seven laboratories. There are not enough data points to form a representative population for each method variation. Only three of the laboratories used the same methodology; the others used significant modifications to the method. Therefore, the first goal of this analysis was to investigate the consistency of Laboratories 3, 4, and 7, which used the unaltered AS-11 IC method.

The consistency of data from laboratories 3, 4, and 7 was tested using the paired t-test for a series of null hypotheses:

Ho: data from laboratories 3 and 4 are from the same population

Ho: data from laboratories 3 and 7 are from the same population

Ho: data from laboratories 4 and 7 are from the same population

The paired t-test was appropriate because most of the laboratories involved did not analyze replicates. The paired t-test statistic looks at the normalized differences between analyses of the same sample conducted by two different laboratories. For a given level of significance, the resulting t statistic is compared to zero to see if the difference is significantly different from zero. If the difference is not significantly different from zero, the two sets of

values are assumed to be from the same population, or in practical terms, the same to within a small probability of error.

To avoid undue weighting of the difference between large concentrations and the difference between small concentrations, the data were normalized. Lacking a clear definition of the probability distribution of the population of the measurements of the split samples, the normalization of dividing the measurements by the standard deviation of the sample measurements (values from laboratories 1, 2, 3, 4, 5, and 7) was applied. The normalized difference $d_i$ is

$$d_i = \frac{x_i - M_i}{S_{Di}} - \frac{y_i - M_i}{S_{Di}} = \frac{x_i - y_i}{S_{Di}}$$

where $x_i$ is the analysis of split sample i = ps01, ps02, ps03, ..., ps34 by laboratory x, $y_i$ is the analysis of split sample i = ps01, ps02, ps03, ..., ps34 by laboratory y, $M_i$ is the mean of the sample measurements between laboratories $i = x, y, z, ..., m$ in which m is the total number of laboratories in a pool of results (i.e., $m = 6$ for a pool of results from laboratories 1, 2, 3, 4, 5, and 7), and $S_{Di}$ is the standard deviation of the sample measurements between laboratories for each split sample i = ps01, ps02, ps03, ..., ps34.

Since the only assumption necessary to use the paired t-test is that the $d_i$ values follow a normal distribution, the Shapiro-Wilk test of normality was applied to ensure the set of pair-wise differences in the analysis between any two laboratories was normally distributed. The probability of 0.01 was selected to test normality of these differences. If the population of a set of differences was not normally distributed, the non-parametric Wilcoxon-Signed Rank test was used to test for consistency in measurement between laboratories instead of the paired t-test [57].

The probability $\alpha$ of making a Type I error of rejecting the null hypothesis that mean differences between laboratories is not significantly different from zero, when this is true, was selected as the 0.01 level of significance. Using the Bonferroni [58] approach, the individual pair-wise comparisons of the laboratories were made at $\alpha/k$ significance level, where $k$ is the total number of pair-wise comparisons that need to be made. This limits the chance of making one mistake in the $k$ different paired tests to $\alpha = 0.01$.

Based on these criteria, each test was conducted as follows:

| | |
|---|---|
| Null Hypothesis | Ho: measurements from laboratory x are not significantly different from those of laboratory y |
| Alternative Hypothesis | Ha: measurements from laboratory x are significantly different from those of laboratory y |

Test Statistic $$t = \frac{\sum_{i=1}^{n} d_i}{s_d / \sqrt{n}}$$

Decision Rule If probability of $|t| < \alpha/k$, then reject Ho

When none of the null hypotheses were rejected for the initial comparisons between laboratories 3, 4, and 7, then the measurements for each sample split from labs 3, 4, and 7 were pooled and a "golden mean" was obtained for comparison with other methods to determine consistency. Paired t-tests were conducted between the "golden mean" for the AS-11 method and each IC method variant to see if the hypothesis of measurement consistency can be rejected at the $\alpha/k$ level of significance. Then, the AS-11 mean was compared to CE, RS, and Titration, individually.

In the absence of adequate controls to determine accuracy in this round robin, the term "golden mean" reflects a practical choice of the most likely point of comparison, not necessarily the most accurate point of comparison. For example, other methods like ion chromatography using the new AS-16 column or capillary electrophoresis may prove to be more accurate than ion chromatography using the AS-11 column.

# 3.0 RESULTS AND DISCUSSIONS

## 3.1 Ion Chromatography

Analytical results obtained from ion chromatography analyses are summarized in Appendix C. Laboratory 6 did not report result for sample ps03 because the sample container arrived at Laboratory 6 damaged. Except for Laboratory 6, all laboratories that performed IC analysis submitted the results within a month after sample preparation. Laboratory 6 reported instrument and technical difficulties during the study. The initial set of data from Laboratory 6 showed obvious disagreement with the data from the rest of the laboratories and the data was rejected at the Lab's request. Laboratory 6 re-analyzed the samples three months after sample preparation; these will be evaluated separately.

All seven laboratories using ion chromatography for perchlorate analysis reported non-detects for samples ps10, ps13, and ps17. From individual laboratories, reported method detection limits perchlorate standard prepared in de-ionized water were 5 µg/L or lower. Table 3.1 lists the reported method detection limits in mg perchlorate per kg of test material, after adjusting for injection volume, dilution and extraction ratio. Laboratory 2 reported a higher adjusted detection limit because of the small injection volume required by the microbore column. Method detection limit from Laboratory 6 was unavailable.

**TABLE 3.1 REPORTED IC METHOD DETECTION LIMITS FROM PARTICIPATING LABORATORIES**

| Laboratory | Adjusted Reported Detection Limit (mg/kg) |
|---|---|
| 1 | 6.00 |
| 2 | 40.0 |
| 3 | 0.40 |
| 4 | 3.00 |
| 5 | 1.00 |
| 6 | NA |
| 7 | 4.00 |

Laboratories 3, 4, and 7 used the AS-11 method with the same instrument conditions and parameters; the results were compared by paired two sample tests, as described in Section 2.3. Individual hypothesis tests by paired two sample tests for means analyses are shown in Appendix D. In general, Laboratory 7's results were lower than Laboratory 3's, which were a little lower than Laboratory 4's. The hypothesis of agreement between Laboratory 7 and Laboratory 3 had a p-value of 0.0592, and therefore not rejected. A p-value smaller than $\alpha/3 = 0.0033$ leads to rejection of the null hypothesis. The hypothesis test of agreement between Laboratory 3 and Laboratory 4 had a p-value of 0.1874, and thus failed to reject also. In the last of the three pair-wise combinations, the hypothesis test of agreement between Laboratory 7 and Laboratory 4 had a p-value of 0.0031, which is right at the rejection point of $\alpha/3 = .0033$. Laboratory 4's result for sample ps30 was a statistical outlier, contributing to this difference. Removing the outlier resulted in good agreement between the two laboratories.

All three sets of the normalized differences were approximately normal as discussed in Section II, thus the paired t-test was used. Since the results from laboratories 3, 4, and 7 were not statistically different from each other, it was reasonable to average the three values to obtain a "golden mean" for AS-11 perchlorate concentration in each of the 34 samples. As shown in Appendix E, for each sample, the percent coefficient of variation (% CV) was less than ten, showing good precision and little variability among the laboratories using the standard AS-11 method.

Laboratory 2 used a 2-mm AS-11 microbore column instead of a 4-mm AS-11 standard bore column for perchlorate analysis. The Shapiro-Wilk test of normality was used to compare the sample split differences between the AS-11 "golden mean" results and the results from Laboratory 2. These differences were not normally distributed at a probability of less than 0.01. Therefore non-parametric analysis was used to show that the results from Laboratory 2 were not different from the "golden mean" using the standard AS-11 method. The non-parametric test statistic for the differences, p was 0.295 (Appendix E), indicating good agreement in perchlorate measurements using the AS-11 standard bore method and the microbore.

Laboratories 1 and 5 used an AS-16 column, but the eluent conditions were not the same. A comparison between Laboratory 1 values and AS-11 "golden mean" in a paired t-test is shown in Appendix E, and the results from Laboratory 1 were not different than the "golden mean"

using the AS-11 method. The hypothesis of consistency had a p-value of 0.0101, which when testing at the 0.01 confidence level, failed to reject the null hypothesis. Lab 5 results were also not significantly different from the AS-11 "golden mean" (p-value of 0.0560). Overall, Laboratory 5 reported smaller concentrations than Laboratory 1 in all but 8 of the 33 samples.

Averaging the two AS-16 laboratory measurements and comparing them to the AS-11 "golden mean" values gave the best match of any comparison conducted in this study. The p-value for paired t-test comparison between the mean AS-11 results and the average AS-16 data was 0.9248, indicating that the average of the AS-16 results was very close to the AS-11 results for each sample.

Although Laboratory 6 used the same AS-11 method, comparison of Laboratory 6 results to the AS-11 means did not show agreement. The p-value was 0.0001, indicating less than 0.01% chance that the data from Lab 6 agreed with the mean AS-11 data. Since Laboratory 6 also analyzed the samples by CE and RS, attempts were made to compare Lab 6's IC data to that of RS and CE, and there were no agreement among the data. Null hypothesis tests by paired two sample tests for means analysis for Lab 6' IC and RS data showed absolutely no agreement. Furthermore, there were no agreement between IC and CE, and CE and RS.

Results for the negative control blanks and positive (100-µg/L) perchlorate spikes are shown in Table 3.2. All seven laboratories reported non-detect for the blank negative control. Except for Laboratory 6, all labs reported results within 94 to 104% of the expected value, showing excellent agreement and accuracy. Laboratory 6 did not report results for the spiked controls.

**TABLE 3.2 ION CHROMATOGRAPHY RESULTS OF BLANKS AND 100-µg/L PERCHLORATE SPIKED CONTROLS**

| Laboratory | Blank | Reported LCS1 | Expected LCS1 | Reported LCS2 | Expected LCS2 |
|---|---|---|---|---|---|
| 1 | ND | 96 | 100 | 104 | 100 |
| 2 | ND | 99 | 100 | 94 | 100 |
| 3 | ND | 100 | 100 | 99 | 100 |
| 4 | ND | 94 | 100 | 97 | 100 |
| 5 | ND | 99 | 100 | 98 | 100 |
| 6 | ND | NA | 100 | NA | 100 |
| 7 | ND | 99 | 100 | 99 | 100 |

Although the laboratories reported excellent duplicate recoveries for the spiked controls, duplicate sample analyses were shown to vary widely. The disparity in duplicate sample analyses reflected intra-lot and inter-lot variability. Table 3.3 showed the AS-11 mean values for duplicate sample analyses. Duplicate samples from a same lot of All Natural Lawn Restorer ps15 (5,827 mg/kg) and ps21 (2,767 mg/kg) showed an absolute difference of 58%. The absolute difference for duplicate Supreme Gardens fertilizer sample ps02 (3180 mg/kg) and the duplicate ps09 (4,193 mg/kg) was about 24%. For duplicate Fall Fertilizer samples, 2,360 mg/kg was reported for ps17 and non-detect (ND) for ps24. Three samples taken from three different lots of All-Purpose Plant Food had perchlorate concentrations ranging from 3,093 to 7,303 mg/kg. Samples ps08 (937 mg/kg) and ps12 (550 mg/kg) taken from a same lot of Vegetable Bedding Plant Food showed an absolute difference of 43%. Both ps08 and ps12 were significantly different than the reported result for sample ps19 (2,620 mg/kg) which taken from a different lot of Vegetable/Bedding Plant Food.

**TABLE 3.3 DUPLICATE ANALYSIS OF TEST MATERIALS BY AS-11 METHOD**

| Samples | Brand Name | Purchase Location | Perchlorate Concentration (mg/kg) |
|---|---|---|---|
| ps15 | All Natural Lawn Restorer | Long Island | 5827 |
| ps21 | All Natural Lawn Restorer | Long Island | 2767 |
| ps02 | Supreme Gardens | Kansas City | 3180 |
| ps09 | Supreme Gardens | Kansas City | 4193 |
| ps17 | Fall Fertilizer | Long Island | ND |
| ps24 | Fall Fertilizer | Long Island | 2360 |
| ps08 | Vegetable/Bedding Plant Food | Kansas City | 973 |
| ps12 | Vegetable/Bedding Plant Food | Kansas City | 550 |
| ps19 | Vegetable/Bedding Plant Food | Long Island | 2620 |
| ps06 | All-Purpose Plant Food | California | 3093 |
| ps05 | All-Purpose Plant Food | Kansas City | 6287 |
| ps16 | All-Purpose Plant Food | Long Island | 7303 |

## 3.2 Capillary Electrophoresis

For capillary electrophoresis, a calibration curve was generated by plotting the concentrations of aqueous sodium perchlorate standards (in deionized water) versus absorbance. Solutions of 3, 7, 10, 15, 20, 30, 40, 50 and 60 mg/L sodium perchlorate were used to prepare the standard curve. The correlation coefficient for regression of the calibration curve was better than 0.9973. The limit of detection based on a signal-to-noise ratio of 3:1 for perchlorate was about 3 mg/L (3 ppm). Each solution was analyzed twice and the resulting absorbance values were averaged. Migration times for the perchlorate peak in all 18 analyses used for the standard curve ranged from 3.73 to 3.81 min.

Liquid samples were analyzed as received or diluted 1:10 to 1:100 to avoid interference from a large peak with a migration time matching that of the sulfate anion. Dilution reduced the sulfate signal to the level where the perchlorate peak was easily distinguished and integrated by the instrument if it was present above the detection level. Spike recovery analyses ensured proper peak identifications. Each sample was analyzed in triplicate and an average of triplicate analyses are reported and shown in Appendix C. The average %CV for the 30 samples in which perchlorate was detected by CE was 4.4%, based on triplicate analysis of each sample. No results were reported for ps03 because the sample arrived in a damaged container. No CE data were reported for LCS1 or LCS2. Individual hypothesis testing between the AS-11 mean and capillary electrophoresis by paired two sample tests for means analysis is shown in Appendix F, where agreement is shown with p-value = 0.0225 at the 0.01 level of significance.

## 3.3 Raman Spectroscopy

The Raman spectra for the perchlorate standard showed four bands at 462, 629, 934, and 1113 $cm^{-1}$, consistent with the predicted results using group theory for a tetrahedral molecule [42, 43]. The (3N – 6) rule indicated nine normal vibrational modes. The weak lower frequency band at 462 $cm^{-1}$ was attributed to doubly degenerated deformation modes, *e*. The intense line ($a_1$) at 934 $cm^{-1}$ was assigned to the symmetric stretching and contraction of the Cl-O bonds. This band

was only observed in the isotropic spectrum but not in the anisotropic spectrum. The two remaining week bands were assigned to two triply degenerated modes, $f_2$. The band at 1113 $cm^{-1}$ was attributed to the anti-symmetric Cl-O stretching modes, and the other at 629 $cm^{-1}$ was attributed to the deformation of the anti-symmetric modes. The assignment of the major and minor peaks was consistent to reference values [42, 60].

Interference experiments were conducted to see if the 934 $cm^{-1}$ peak of perchlorate could be obscured by the presence of fertilizer components that are Raman active. Standards of nitrate, sulfate, phosphate, urea, and a mixture of these anions were analyzed with or without perchlorate. Except for phosphate, none of these common components were found to interfere with Raman analysis. Phosphate was found to interfere with perchlorate when pH was above 10.5, as a peak at 937 $cm^{-1}$ was present. However, protonating $PO_4^{3-}$ species to $HPO_4^{2-}$ by lowering the pH to 10.5 or below removed the interference. The pKa of $PO_4^{3-}$ is 12.32. Below the pH of 10.5, less than 1.5% of the phosphate exists as the $PO_4^{3-}$ species. All of the fertilizer samples had pH's below 9.0. Therefore, interference from common fertilizer components is not expected. A few small peaks, not attributed to nitrate, sulfate, phosphate, and urea, were observed also, but they were all far removed from 934 $cm^{-1}$. Confidence level of the 934 $cm^{-1}$ peak of perchlorate at below pH 10.5 is high.

The intense 934 $cm^{-1}$ peak was used for quantitation. Quantification was performed by comparing the ratio of the area of the 934 $cm^{-1}$ peak of perchlorate to the 2329 $cm^{-1}$ peak of atmospheric nitrogen that is observed in all spectra recorded with adequate laser power and exposure time. This approach allowed spectra collected under different conditions to be adequately normalized, and also corrected for minor changes in instrument performance, such as laser power fluctuations. Two sets of 18 perchlorate standards prepared in de-ionized water covering the range from 20 to 3,000 ppm were prepared, and 36 data points were used to generate the calibration line. The calibration line was linear and typically described by the equation $Y = 434 * X - 36.82$; where Y is the perchlorate concentration and X is the ratio of perchlorate and nitrogen peaks. The correlation coefficient value is 0.9980 or better.

Each sample was first run with an exposure time of 20 sec and 5 accumulations co-added, resulting in a total analysis of 7 min per sample. Results obtained by Raman spectroscopy are shown in Appendix C. The reported RS method detection limit is 20 ppm. No result was

reported for ps03 because the sample arrived damaged. The Lab did not report results for LCS1 and LCS2 because they were below their detection level. Additionally, sample ps34, which is supposed to be Chilean Nitrate, does not exhibit a peak for nitrate in the Raman Spectrum.

The t-test comparison shows strong disagreement between the AS-11 means and the RS results with the p-value at 0.0000. Over seventy percent of the RS values were lower than the AS-11 means. The disparity might have been caused by Tyndall scattering from the micro-particles in the solution and fluorescent interference from the organic components of the test materials [42].

Six of the 34 samples exhibited excessive fluorescence; the Raman spectrum could not be observed at the 20-second exposure time. For all but one of the six samples, the problem was overcome by lowering the exposure time until the spectrum background near the 934 $cm^{-1}$ peak fell below 15,000 counts. At or below this level of fluorescence, perchlorate could be observed if present in the sample at above 20 ppm or more. In ps28, the fluorescence was so severe that the baseline could not be lowered below 15,000 counts without decreasing the exposure to such a low level that the atmospheric nitrogen peak used as quantification standard was not observable. In this sample, the sample was shaken with activated charcoal (~50 mg/mL) in order to remove fluorescent interference. After filtering, the extract was clear and was analyzed at a 20-sec exposure with no noticeable fluorescence. Use of activated charcoal is a common technique employed to remove trace-level, high molecular weight organics.

It was suspected that treatment with charcoal would also reduce the level of perchlorate in ps28. This was investigated by analyzing perchlorate standards in distilled water and in other fertilizers with varying levels of perchlorate. In each case, the level of perchlorate was determined before and after charcoal treatment. Charcoal treatment was found to lower the level of perchlorate, but by a constant amount. It was demonstrated that 36 (± 13) percent of the perchlorate was removed, regardless of the nature of the matrix. Thus, the value of the perchlorate reported in Appendix C for ps28 is 1.36 times that actually measured in the charcoal-treated sample.

### 3.4 Tetraphenylstibonium Sulfate Titration

For tetraphenylstibonium sulfate titration, a calibration curve was generated from perchlorate standards at 100, 500, 1,000, and 5,000 ppm. Results obtained from titration are shown in Appendix C. The reported titration method detection limit was 50 ppm (mg/kg). For the positive 100-µg/L LCS1 and LCS2 controls, titration reported 90 and 104 µg/L, respectively. Non-detect was reported for the negative blank. As shown in Appendix E, titration results were significantly different from the AS-11 means with a p-value of 0.0002.

In fitting a model to the AS-11 perchlorate concentration means, the model given below is reasonable. However, the model only explains 51.4% of the variability in the perchlorate numbers, based on phosphorous, nitrogen, and potassium. Sample numbers ps22 and ps31 were having too much influence on the model, so they were removed. A model should be robust, but if small amounts of data are pulling the model around, their effect should be removed. After these samples were removed, the model met all assumptions of the normal error model:

$$\text{Predicted } [ClO_4] = 3057.54 + 205.93*[NO_3^-] - 456.98*[PO_4^{3-}] + 14.69*[PO_4^{3-}]^2$$

What this model illustrates is that $NO_3^-$ is related to the perchlorate level in a linear fashion with a positive slope, so a unit increases in the $NO_3^-$ level results in a 205.93 ppm increase in the sample's $ClO_4^-$ concentration, while holding the $PO_4^{3-}$ level constant. Notice that $PO_4^{3-}$ exhibits a quadratic relationship with $ClO_4^-$, where the predicted $ClO_4^-$ level in a sample decreases as $PO_4^{3-}$ goes from 0 to 15.55, but then the $ClO_4^-$ level increases as $PO_4^{3-}$ increases above 15.55. $K^+$ is not in the model because $K^+$ did not show a direct relationship with $ClO_4^-$. $K^+$ concentration has no predictive capability over $ClO_4^-$ concentration.

This choice of variables in the model appeared robust no matter which Laboratory or method was being modeled. Only the coefficients changed, when, for instance, predicting the perchlorate values for the Titration method, which were significantly different than the AS-11 means. The model had the same variables but the coefficients were:

$$\text{Predicted Perchlorate} = 3454.25 + 219.09*[NO_3^-] - 487.26*[PO_4^{3-}] + 15.76*[PO_4^{3-}]^2$$

The difference in coefficients makes the prediction steeper, so agreement with the AS-11 means is not good. The Y-intercept changed from 3057.54 to 3454.25, indicating that Titration method is generally larger than the AS-11 values even when $NO_3^-$ and $PO_4^{3-}$ levels were at zero.

## 4.0 Conclusions

A successful inter-laboratory collaborative study on the performance of ion chromatography methods for perchlorate analysis in aqueous fertilizer suspensions was conducted. The study included seven government, private and commercial laboratories, and evaluated two method variants (AS-11 and AS-16). Three of the laboratories employed the widely used AS-11 IC method for perchlorate analysis; the paired t-tests for the means analysis showed acceptable agreement with little variability (<10% CV) among the data sets. Two laboratories employed the AS-16 method; the statistical evaluation of the results showed acceptable agreement. This study also showed good agreement between the microbore and the standard bore AS-11 columns, rendering credibility in using the microbore column for perchlorate in limited applications where the analyses are restricted by the sample size.

Furthermore, the study found excellent agreement between two method variants (AS-11 and AS-16). At 99 percent confidence level, the p-value for the means analysis of AS-11 and AS-16 results by paired t-test comparison was 0.9248. The large p-value indicates that there was a very high probability of finding the AS-11 and AS-16 mean values were the same. The good precision between the two methods was also evidenced by the low percent relative deviations in all five labs' results. The average relative standard deviations from the laboratories for all samples were less than seven percent, consistent to that observed in the groundwater and drinking water study [59]. At the conclusion of that study, EPA adopted the AS-16 method as Method 309.0 for perchlorate analysis in groundwater and drinking water; the EPA method 309.0 is currently being recommended for promulgation [57].

This study found poor agreement between ion chromatography and tetraphenylstibonium titration results. Overall, compared to ion chromatography, tetraphenylstibonium titration tends

to over estimate the perchlorate titration values by about fourteen percent. Reasons accounting for the over-estimations have been discussed elsewhere [24]. This study found good agreement between capillary electrophoresis and IC but poor agreement between Raman and IC.

This study found good agreement between capillary electrophoresis (CE) and IC quantitative results. The precision of CE is also good; the average %CV for the 30 fertilizer samples in which perchlorate was detected was 4.4%, based on triplicate analyses. CE, like IC, has no fingerprinting capability for qualitative analysis such as does Raman spectroscopy; however, the precision for peak migration, shown by the %CV of <1, is good enough to provide confidence in peak identify if questionable samples are spiked with perchlorate and the peak area/height increase proportionately with no peak splitting. In summary, this CE method is accurate, precise and moderately fast; a total sample analysis requires less than 20 min. IC is a preferred method because of its greater sensitivity. However, for most of the fertilizer samples CE was sensitive enough for the detection of perchlorate, with a detection limit of about 3-ppm in the fortified matrix. If a laboratory possesses both IC and CE instrumentation, the CE would be very useful for initial screening to determine the dilution factor need for IC analysis. Additionally, the CE is useful for confirmation of IC results, since it is based on an entirely different separation principle and complimentary to IC.

The disparity in the duplicate sample analyses reflected the inherent heterogeneity of the fertilizer samples. As expected for any heterogeneous sample, the lawn and garden fertilizer samples examined in this report exhibited both intra-lot and inter-lot variability. Each sample tested in this study was essentially a sub-sampling of a fertilizer lot, and a single sub-sampling datum taken from a heterogeneous sample can not represent the sample as a whole. Hence, the values as presented in this report were the perchlorate concentrations in the extract of each sub-sample for a given fertilizer, but the values did not necessarily represent the true concentrations in the fertilizer brands examined in this study.

The determination of a true perchlorate concentration for a given type or brand of fertilizer is beyond the scope of this project, since the purpose of this study was to verify methods performance with respect to matrices having the same quality as the test materials. It would be economically unfeasible for the collaborative study group to find the true perchlorate concentration for all 27 brands of fertilizers examined in this study. Because multiple sampling

data are required per sample batch, per lot, and per brand, the number of data points and analyses desired to generate statistically sound values for all of the 27 fertilizer brands would have increased exponentially and overwhelmed the resources available to this collaborative study group. This round robin effort was not to establish or define the occurrence of perchlorate for the final risk assessment. The purpose was to standardize analytical approaches for gaining insight into the occurrence of perchlorate in fertilizers.

Modeling efforts in correlating the perchlorate concentrations to that of potassium, nitrate, and phosphate were unsuccessful. Multiple linear regression model found no correlation between perchlorate and potassium, and the model was only able to predict 51.4% of the variability ($R^2 = 0.5140$) in correlating perchlorate concentration to that of nitrate and phosphate. The poor prediction in the variability was attributed to data quality. Since fertilizers were shown to be heterogeneous, the actual N-P-K values, similar to the perchlorate value, were expected to differ from sub-sample to sub-sample. The N-P-K values used in the predictive modeling effort were provided by the manufacturers and were not experimentally determined for each sub-sample. Manufacturers' N-P-K values were statistical values determined based on multiple sampling. The actual N-P-K values for each sub-sample may have been higher or lower than the values provided the manufacturers.

Although the predictability was low, the model was robust. Regardless of the lab or the method, only the magnitudes or the coefficients changed. The variables (i.e. nitrate and phosphate) having influence on the predictability of variability did not change. This is interesting because the presence of perchlorate has been identified in nitrate and phosphate ores [15, 61-63]. Future perchlorate occurrence study design should carefully incorporate matrix characterization measurements for pH, anions, and cations.

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## APPENDIX A:

## TEST MATERIALS, PURCHASED LOCATIONS, AND MANUFACTURES' INFORMATION

| Samples | Manufacturer | Brand Name | Primary Constituent | Purchase Location | N | P | $K^+$ |
|---|---|---|---|---|---|---|---|
| ps01 | Fertilome | Start-N-Grow Plant Food | Potassium Nitrate | Kansas City, MO | 18 | 6 | 12 |
| ps02 | Ringer | Supreme Gardens | Nitrate of Soda | Kansas City, MO | 7 | 7 | 7 |
| ps03 | Peters | Lawn Food and Iron | Potassium Nitrate | Kansas City, MO | 38 | 4 | 4 |
| ps04 | Hi-Yield | Nitrate of Soda | Nitrate of Soda | Kansas City, MO | 16 | 0 | 0 |
| ps05 | Peters | All Purpose Plant Food | Potassium Nitrate | Kansas City, MO | 20 | 20 | 20 |
| ps06 | Peters | All Purpose Plant Food | Potassium Nitrate | California | 20 | 20 | 20 |
| ps07 | Sudbury | Fertilizer Potash | Potassium Chloride | Kansas City, MO | 0 | 0 | 44 |
| ps08 | Osmocote | Vegetable and Bedding Plant Food | Ammonium Nitrate | Kansas City, MO | 14 | 14 | 14 |
| ps09 | Ringer | Supreme Gardens | Nitrate of Soda | Kansas City, MO | 7 | 7 | 7 |
| ps10 | Acme | Stump Remover | Potassium Nitrate | Kansas City, MO | NA | NA | NA |
| ps11 | Scott's | Miracle-Gro Lawn Food | Potassium Nitrate | Kansas City, MO | 36 | 6 | 6 |
| ps12 | Osmocote | Vegetable and Bedding Plant Food | Ammonium Nitrate | Kansas City, MO | 14 | 14 | 14 |
| ps13 | Shulte | Rose Plus | Potassium Nitrate | Kansas City, MO | 19 | 24 | 24 |
| ps14 | Jobe's | Plant Food Spikes | Potassium Nitrate | Kansas City, MO | 16 | 2 | 6 |
| ps15 | Ringer | All Natural Lawn Restorer | Nitrate of Soda | Long Island, NY | 10 | 2 | 6 |
| ps16 | Peters | All Purpose Plant Food | Potassium Nitrate | Long Island, NY | 20 | 20 | 20 |

| Samples | Manufacturer | Brand Name | Primary Constituent | Purchase Location | $NO_3^+$ | $PO_4^-$ | $K^+$ |
|---|---|---|---|---|---|---|---|
| ps17 | Johnathan Green | Winter Survival Fall Fertilizer | Muriate of Potash | Long Island, NY | 10 | 18 | 20 |
| ps18 | Osmocote | Outdoor and Indoor Plant Food | Ammonium Nitrate | Long Island, NY | 18 | 6 | 12 |
| ps19 | Osmocote | Vegetable and Bedding Plant Food | Ammonium Nitrate | Long Island, NY | 14 | 14 | 14 |
| ps20 | Frank's | All Purpose Concentration Plant Food | Muriate of Potash | Long Island, NY | 15 | 30 | 15 |
| ps21 | Ringer | All Natural Lawn Restorer | Nitrate of Soda | Long Island, NY | 10 | 2 | 6 |
| ps22 | Scott's | Miracle-Grow Lawn Food | Potassium Nitrate | Long Island, NY | 31 | 3 | 9 |
| ps23 | Vigoro | Tomato and Vegetable Plant Food | Muriate of Potash, Sul Po Mag | Long Island, NY | 10 | 8 | 14 |
| ps24 | Johnathan Green | Winter Survival Fall Fertilizer | Muriate of Potash | Long Island, NY | 10 | 18 | 20 |
| ps25 | Best | All Purpose Triple Sixteen | NA | California | 16 | 16 | 16 |
| ps26 | Bandini | Sul Po Mag | NA | California | 0 | 0 | 22 |
| ps27 | Plant Marvel | Matriculture | $KNO_3$ | California | 12 | 31 | 14 |
| ps28 | Orchid | Premium Orchid Food – Bloom Formula | NA | California | 6 | 30 | 30 |
| ps29 | Dexol | Stump Remover | $KNO_3$ | California | NA | NA | NA |
| ps30 | IMC | Langbeinite Ore | Sul Po Mag | New Mexico | NA | NA | NA |
| ps31 | CNC - Champion | Potassium Nitrate | Potassium Nitrate | Albequerque, NM | 14 | 0 | 45 |
| ps32 | Best, K-Power | Prill | Chilean Nitrate | Chile | 14 | 0 | 46 |
| ps33 | Best, K-Power | Prill | Chilean Nitrate | Chile | 14 | 0 | 46 |
| ps34 | NA | Caliche | Sodium Nitrate | Chile | NA | NA | NA |

## APPENDIX B:

## SUMMARY OF ION CHROMATOGRAPHY METHODS

| Laboratory Code | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Separation Column | AS-16 | AS-11 | AS-11 | AS-11 | AS-16 | AS-11 | AS-11 |
| Guard Column | AG-16 | AG-11 (1) | AG-11 | AG-11 | AG-16 | AG-11 | AG-11 |
| Column Size | 4 mm | 2 mm | 4 mm | 4 mm | 4 mm | 4 mm | 4 mm |
| Anion Trap Column | ATC-1 | None | ATC-1 | ATC-1 | ATC-1 | ATC-1 | ATC-1 |
| Suppressor | ASRS-Ultra | ASRS-Ultra | ASRS-II | ASRS-II | ASRS-Ultra | ASRS-II | ASRS-II |
| Suppressor Mode | Ext. Water | Ext. Water | Ext. Water | Ext. Water | Ext. Water | Ext. Water | Ext. Water |
| Regenerant | $H_2O$ | $H_2O$ | $H_2O$ | $H_2O$ | $H_2O$ | $H_2O$ | $H_2O$ |
| Regenerant Flow Rate | 10 mL/min | 12 psi | 10 mL/min | 10 mL/min | 5 mL/min | 25 psi, 3 mL/min | 10 mL/min |
| Autosampler | AS-40 | AS-40 | AS-40 | AS-40 | AS-40 | AS-40 | AS-40 |
| Pump Type | GP-40 | GP-40 | GP-40 | GP-40 | GP-40 | GP-40 | GP-40 |
| Detector Type | CD-20 | CD-20 | CD-20 | CD-20 | CD-20 | CD-20 | CD-20 |
| Sample Size | 50 µL | 1 µL | 1000 µL | 100 µL | 1000 µL | 100 µL | 100 µL |
| Mobile Phase | (2) | 100 mM NaOH | 100 mM NaOH | 100 mM NaOH | 35 mM KOH | 100 mM NaOH | 100 mM NaOH |
| Flow Rate | 1.0 mL/min | 0.38 mL/min | 1.0 mL/min | 1.0 mL/min | 1.25 mL/min | 1.0 mL/min | 1.0 mL/min |

(1) 2-mm x 50-mm AF-11 guard column instead of the 4-mm guard column and separation column

(2) 20 mM NaOH for 5 minutes, 100 mM NaOH for remainder

## APPENDIX C:

## RESULTS OF FERTILIZER ANALYSIS BY TITRATION, ION CHROMATOGRAPHY, CAPILLARY ELECTROPHORESIS (CE), AND RAMAN SPECTROSCOPY (RS)

| Samples | Titration (mg/kg) | Lab1 (mg/kg) | Lab2 (mg/kg) | Lab3 (mg/kg) | Lab4 (mg/kg) | Lab 5 (mg/kg) | Lab 6 (mg/kg) | Lab 7 (mg/kg) | CE (mg/kg) | RS (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| ps01 | 5900 | 4680 | 5150 | 5300 | 4710 | 4872 | 5703 | 4700 | 5557 | 4637 |
| ps02 | 2950 | 3490 | 2360 | 3100 | 3240 | 3077 | 3954 | 3200 | 3752 | 2569 |
| ps03 | 9900 | 8950 | 7730 | 9400 | 8860 | 7678 | NR | 8600 | NR | NR |
| ps04 | 6800 | 7620 | 6300 | 8000 | 7660 | 6908 | 9023 | 7400 | 4255 | 7186 |
| ps05 | 7150 | 6700 | 4760 | 6200 | 6360 | 6898 | 7760 | 6300 | 6535 | 4677 |
| ps06 | 3400 | 3220 | 3680 | 3000 | 3090 | 3128 | 3563 | 3190 | 4399 | 2169 |
| ps07 | 5440 | 4730 | 8830 | 5000 | 4920 | 4369 | 5554 | 4800 | 5821 | 4979 |
| ps08 | 1250 | 1090 | 968 | 970 | 1010 | 903 | 1034 | 940 | 917 | 726 |
| ps09 | 5200 | 4380 | 5120 | 4300 | 4380 | 4049 | 4949 | 3900 | 4039 | 3836 |
| ps10 | < 50.0 | < 6.0 | < 40.0 | < 0.4 | < 3.0 | < 1.0 | < 4.0 | < 0.4 | < 3.0 | < 20.0 |
| ps11 | 8600 | 8160 | 6260 | 8800 | 8290 | 7068 | 8559 | 7400 | 7592 | 7673 |
| ps12 | 750 | 636 | 597 | 530 | 580 | 526 | 593 | 540 | 707 | 286 |
| ps13 | < 50.0 | < 6.0 | < 40.0 | < 0.8 | 0.6 | < 1.0 | ND | 13 | < 3.0 | < 3.00 |
| ps14 | 10350 | 9480 | 8640 | 9000 | 9920 | 6896 | 9688 | 8100 | 9476 | 8514 |
| ps15 | 6140 | 6060 | 5190 | 6100 | 6080 | 4673 | 6278 | 5300 | 6146 | 5867 |
| ps16 | 6980 | 7510 | 5640 | 7400 | 7210 | 6194 | 7439 | 7300 | 7196 | 6079 |
| ps17 | < 50.0 | < 6.0 | < 40.0 | < 2.2 | < 3.0 | < 1.0 | < 4.0 | < 0.4 | < 3.0 | < 3.00 |
| ps18 | 740 | 733 | 832 | 750 | 690 | 904 | 682 | 600 | 495 | 342 |
| ps19 | 3100 | 2650 | 3270 | 2700 | 2660 | 2053 | 2648 | 2500 | 2687 | 2408 |
| ps20 | 6250 | 5380 | 10000 | 4800 | 5560 | 7098 | 5768 | 5400 | 5470 | 4070 |
| ps21 | 3400 | 2960 | 2560 | 2900 | 3000 | 2376 | 2943 | 2400 | 2918 | 2617 |
| ps22 | 1700 | 1549 | 2646 | 1400 | 1530 | 1995 | 1433 | 1300 | 1894 | 1153 |
| ps23 | 450 | 464 | 738 | 340 | 430 | 514 | 391 | 360 | 707 | < 3.00 |
| ps24 | 2850 | 2590 | 2840 | 2200 | 2480 | 2530 | 2600 | 2400 | 3493 | 2618 |
| ps25 | 5250 | 4223 | 3680 | 4000 | 4310 | 3727 | 4586 | 4200 | 4528 | 4343 |
| ps26 | 3550 | 3073 | 4810 | 3200 | 3160 | 2834 | 3474 | 3000 | 3722 | 3391 |
| ps27 | 6300 | 6680 | 7120 | 5500 | 6450 | 6246 | 6816 | 5300 | 7117 | 4650 |
| ps28 | 2800 | 2599 | 3623 | 2200 | 2490 | 3135 | 2840 | 2500 | 3185 | 1690 |
| ps29 | 5800 | 5189 | 5290 | 5400 | 5420 | 4576 | 4819 | 4900 | 7186 | 4699 |
| ps30 | 15600 | 14300 | 20090 | 14000 | 16400 | 13649 | 17397 | 13000 | 15644 | 15734 |
| ps31 | 16800 | 13290 | 14380 | 13000 | 15100 | 11749 | 15603 | 12000 | 13817 | 14120 |
| ps32 | 8200 | 6381 | 6300 | 6100 | 6560 | 5862 | 5477 | 6300 | 7416 | 6214 |
| ps33 | 8250 | 7563 | 2600 | 7400 | 7870 | 7201 | 8018 | 7100 | 8302 | 7278 |
| ps34 | 32800 | 26310 | 32980 | 27000 | 30900 | 26120 | 32454 | 30000 | 26013 | 28183 |

NR = Not Reported

## APPENDIX D:

## PAIRED TWO SAMPLE TESTS FOR MEANS ANALYSIS ON AS-11 METHOD

| **Lab 7 Vs. Laboratory 3** | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.96324 |
| Prob(<W) | 0.3793 |
| Paired t-test | |
| Mean Difference | -0.30738 |
| Std Error | 0.157135 |
| Df | 32 |
| t-Stat | -1.95605 |
| p-value > \|t\| | 0.0592 |

| **Laboratory 7 Vs. Laboratory 4** | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.93970 |
| Prob(<W) | 0.0828 |
| Paired t-test | |
| Mean Difference | -0.53432 |
| Std Error | 0.166783 |
| Df | 32 |
| t-Stat | -3.20367 |
| p-value > \|t\| | 0.0031 |

| **Laboratory 3 Vs. Laboratory 4** | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.933269 |
| Prob(<W) | 0.0532 |
| Paired t-test | |
| Mean Difference | -0.22695 |
| Std Error | 0.168475 |
| Df | 32 |
| t-Stat | -1.34711 |
| p-value > \|t\| | 0.1874 |

## APPENDIX-E:

## PERCHLORATE IN GARDEN AND LAWN FERTILIZERS BY AS-11

| Samples | Lab 7 (mg/kg) | Lab 3 (mg/kg) | Lab 4 (mg/kg) | Average (mg/kg) | Standard Deviation | Percent Coefficient of Variation |
|---|---|---|---|---|---|---|
| ps01 | 4700 | 5300 | 4710 | 4903 | 344 | 7 % |
| ps02 | 3200 | 3100 | 3240 | 3180 | 72 | 2 % |
| ps03 | 8600 | 9400 | 8860 | 8953 | 408 | 5 % |
| ps04 | 7400 | 8000 | 7660 | 7687 | 301 | 4 % |
| ps05 | 6300 | 6200 | 6360 | 6287 | 81 | 1 % |
| ps06 | 3190 | 3000 | 3090 | 3093 | 95 | 3 % |
| ps07 | 4800 | 5000 | 4920 | 4907 | 101 | 2 % |
| ps08 | 940 | 970 | 1010 | 973 | 35 | 4 % |
| ps09 | 3900 | 4300 | 4380 | 4193 | 257 | 6 % |
| ps11 | 7400 | 8800 | 8290 | 8163 | 709 | 9 % |
| ps12 | 540 | 530 | 580 | 550 | 26 | 5 % |
| ps14 | 8100 | 9000 | 9920 | 9007 | 910 | 10 % |
| ps15 | 5300 | 6100 | 6080 | 5827 | 456 | 8 % |
| ps16 | 7300 | 7400 | 7210 | 7303 | 95 | 1 % |
| ps18 | 600 | 750 | 690 | 680 | 67 | 10 % |
| ps19 | 2500 | 2700 | 2660 | 2639 | 106 | 4 % |
| ps20 | 5400 | 4800 | 5560 | 5253 | 401 | 8 % |
| ps21 | 2400 | 2900 | 3000 | 2767 | 71 | 3 % |
| ps22 | 1300 | 1400 | 1530 | 1410 | 115 | 8 % |
| ps23 | 360 | 340 | 430 | 367 | 37 | 10 % |
| ps24 | 2400 | 2200 | 2480 | 2360 | 144 | 6 % |
| ps25 | 4200 | 4000 | 4310 | 4170 | 157 | 4 % |
| ps26 | 3000 | 3200 | 3160 | 3120 | 106 | 3 % |
| ps27 | 5300 | 5500 | 6450 | 5750 | 141 | 2 % |
| ps28 | 2500 | 2200 | 2490 | 2397 | 170 | 7 % |
| ps29 | 4900 | 5400 | 5420 | 5240 | 295 | 6 % |
| ps30 | 13000 | 14000 | 16400 | 14467 | 1747 | 10 % |
| ps31 | 12000 | 13000 | 15100 | 13367 | 1485 | 11 % |
| ps32 | 6300 | 6100 | 6560 | 6320 | 231 | 4 % |
| ps33 | 7100 | 7400 | 7870 | 7457 | 388 | 5 % |
| ps34 | 30000 | 27000 | 30900 | 29300 | 2042 | 7 % |

## APPENDIX - F

## PAIRED TWO SAMPLE TEST FOR MEANS ANALYSIS ON AS-11 VERSUS AS-16, CAPILLARY ELECTROPHORESIS, RAMAN SPECTROSCOPY, AND TITRATION

| AS-11 Average Vs. Laboratory 2 | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.859024 |
| Prob(<W) | 0.0004 |
| Paired t-test (Nonparametric) | |
| Df | 32 |
| t-Stat | -59.500 |
| p-value > \|t\| | 0.295 |

| AS-11 Average Vs. Laboratory 1 (AS-16) | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.948665 |
| Prob(<W) | 0.1516 |
| Paired t-test | |
| Mean Difference | -0.33115 |
| Std Error | 0.121099 |
| Df | 32 |
| t-Stat | -2.73457 |
| p-value > \|t\| | 0.0101 |

| AS-11 Average Vs. Laboratory 5 (AS-16) | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.96364 |
| Prob(<W) | 0.3882 |
| Paired t-test | |
| Mean Difference | 0.35321 |
| Std Error | 0.178093 |
| Df | 32 |
| t-Stat | 1.9833 |
| p-value > \|t\| | 0.0560 |

| AS-11 Average Vs. Lab 6 - IC Analysis | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.882071 |
| Prob(<W) | 0.0029 |
| Paired t-test (Nonparametric) | |
| Df | 29 |
| t-Stat | -182.5 |
| p-value > \|t\| | 0.0001 |

| AS-11 Average Vs. CE Analysis | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.914288 |
| Prob(<W) | 0.0211 |
| Paired t-test | |
| Mean Difference | -1.024154 |
| Std Error | 0.424712 |
| Df | 29 |
| t-Stat | -2.411409 |
| Prob > \|t\| | 0.0225 |

| AS-11 Average Vs. RS Analysis | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.888470 |
| Prob(<W) | 0.0043 |
| Paired t-test (Nonparametric) | |
| Df | 29 |
| t-Stat | 179.5 |
| p-value > \|t\| | 0.0000 |

| AS-11 Vs. Titration | $\alpha = 0.01$ |
|---|---|
| | |
| Shapiro-Wilk Normality test | W=0.927197 |
| Prob(<W) | 0.0350 |
| Paired t-test | |
| Mean Difference | -1.422302 |
| Std Error | 0.341295 |
| Df | 32 |
| t-Stat | -4.167373 |
| Prob > \|t\| | 0.0002 |