

EVALUATION OF X-RAY FLUORESCENCE (XRF)

LEAD DETECTION METHOD FOR CANDY

by

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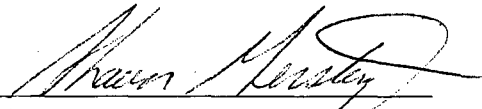
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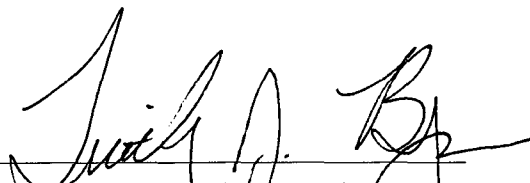
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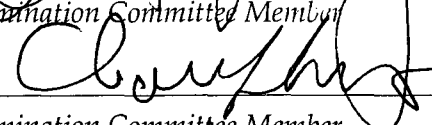
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ABSTRACT

Evaluation of X-Ray Fluorescence (XRF) Lead Detection Method for Candy

by

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The harmful effects of childhood exposure to environmental lead continue to be a major health concern. Due to the significance of this hazard, a Healthy People 2010 objective was set to reduce all young children's blood lead levels to less than 10 micrograms per deciliter. Identification and removal of lead-contaminated candies is an integral part of the primary prevention of lead poisoning in children.

This research examined the efficacy of a protocol to use a portable XRF to screen candies for lead contamination. Method Detection Limits (MDLs) of 5.45 ppm and 7.05 ppm were determined in the Bulk Sample and Plastics Modes, respectively, using 45 fortified analytical samples with a candy matrix. Results also indicated that the XRF-determined concentrations were significantly different from the actual concentrations, as determined via Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Regression analysis established predictive relationships between XRF data and the actual concentration of lead in candy.

The XRF's current ability to screen candies may be limited to wrappers and highly contaminated samples, as candies are typically seen at concentrations below the MDL. Future research should be done to improve the sensitivity of the XRF, in conjunction with collaborative efforts to gather and disseminate information on the dangers of lead-contaminated candies.

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CHAPTER 1

INTRODUCTION

Although the effects of acute lead poisoning from food and industrial exposure have been described for over four thousand years, the first reports of chronic lead poisoning weren't observed until 1892, in Brisbane, Australia. These original reports were met with pervasive disbelief that lead toxicity could cause chronic damage when ingested by children (Gibson, 1892). As evidence continued to mount, this misconception was finally discarded in 1943 with the first long-term study of children who had recovered from acute lead poisonings. Survivors of childhood exposure to lead were found to exhibit ongoing behavioral disorders, learning difficulties, and difficulty in school (Byers & Lord, 1943).

As protective policies regarding industrial lead exposure in the United States improve, the occupational exposure to lesser, chronic exposures, especially those in children (Needleman, 2004). In 2002, the CDC reported that 890,000 American children had elevated blood lead levels, most likely resulting from low-level chronic exposure to lead-contaminated products (CDC, 2006). Although lead-based paint is the major contributor, research also indicates several other possible contributing culprits for lead poisonings. One of these factors is lead-contaminated candies, imported into the United States from countries all over the world.

In April of 2004, the *Orange County Register* addressed increasing concerns about lead-poisoning by running an investigative story that detailed fourteen imported candies with dangerous levels of lead contamination. The *Register* provided over 180 tests on candy and wrappers to substantiate and supplement the sparse existing federal and state data. The article outlined the migration of these candies into American markets and provided data collected by the public health focus regarding lead poisoning is shifting from acute, high-magnitude state of California that indicated dangerous levels of lead in about one quarter of Mexican candies tested (McKim, Sharon & Heisel, 2004).

The California Department of Health and Safety surveyed candies from across the state in April of 2004, and found 112 distinct brands of candies with dangerous levels of lead. Of those 112 candies, 84 were made in Mexico, 8 were made in other countries and 20 were of unknown origin. Although these candies tested above the maximum safety concentrations for lead, the state of California took action in only 11 cases, and health officials across the country have only rarely pulled candy from the shelves (McKim, Sharon, & Heisel, 2004). Several barriers, including lack of screening, restrict health departments and the FDA from effectively controlling the inflow of these candies into the United States. In 2003, legislation that would have increased testing on candies, established clear procedures for issuing health advisories and spread information to parents and health care workers, was defeated by budgetary concerns, lobbying by major candy manufacturers and a lack of cohesive support from state and federal health officials (Quintero-Somaini et.al., 2004).

One major barrier to systematic action against candy manufacturers is the lack of cost-effective methods for identification of contaminated candies. Currently, the FDA-

approved methods for analyzing lead concentrations in candies include analysis by graphite furnace atomic absorption spectrometry (GRAAS) or inductively coupled plasma mass spectrometry (ICP-MS). The machine and procedure is costly and time consuming. Additionally, these methods require large amounts of the candies and include a time-intensive process.

Ongoing research has indicated that X-Ray fluorescence (XRF) could potentially serve as an effective primary screening tool for identifying lead-contaminated candies. The technique, which has been shown as a valuable tool for identifying lead in paint, soil, dust wipes and bone, has yet to be approved as a screening method for testing candy or candy packaging materials for lead contamination. Although use of the XRF, as well as results from this technique, are used in one study testing candies for lead, the article does not discuss the methodology or limits of the XRF (Lynch et al., 2000).

Research at the University of Nevada, Las Vegas (UNLV), examined the XRF as a tool for screening candies. They examined procedures for an XRF protocol for testing candies and their packaging items, and found the XRF to be an effective tool at identifying the presence of lead in candy as these data were compared to graphite furnace analysis. Furthermore, the research at UNLV found the XRF to be especially valuable at quickly screening large numbers of samples at a low cost. Researchers cautioned that the XRF is not as effective at low levels of lead contamination, but recommended the XRF for use in initial screening of candies. Although the limit of detection was unknown, it appeared to be above the FDA's food action limit of 0.1 ppm (Donnelly, 2007).

Understanding the XRF's limits of detection (LOD) for use in screening lead-contaminated candies is important for defining its practicality and reliability. This study

will evaluate the method detection limit (MDL) for use of the XRF in screening lead-contaminated candies and their packaging materials. Candy samples with known concentrations of lead and fortified analytical samples that have been spiked with lead will be used to find the lower limit of detection and the degree of accuracy at all concentrations. Different modes for detection, along with effective procedures for analysis, will be examined in relation to screening for lead contaminated candy.

CHAPTER 2

LITERATURE REVIEW

Toxicology of Lead in Humans

Lead is a versatile metal that targets many organs of the body. In the central nervous system, where lead can distort enzymes and structural proteins, its effects are the most serious and irreversible (Bailey & Kitchen, 1985). Additionally, many of lead's damaging effects can be attributed to its ability to compete with or mimic calcium. Even at very low concentrations, lead can compete with calcium for binding sites throughout the body. In the central nervous system, lead can affect neuronal signaling by competing with cerebellar phosphokinase C (Markovac & Goldstein, 1988). Lead can also inhibit calcium's passage through the cell membrane (Simons, 1993). When lead is absorbed by the mitochondria, where it distorts the cristae, cellular respiration is inhibited and other calcium reactions, including energy coupling, are affected (Holtzman et.al., 1984).

Research has continued to indicate that there is no safe threshold BLL for lead in young children (CDC, 2005). Permanent deleterious effects of chronic lead exposure have been observed in children with BLLs well below 10 µg/dL (Needleman, 2004 & CDC, 2005). Often, the first visible symptoms of lead toxicity are exhibited as mild behavioral alterations or flu-like symptoms, which can easily go undiagnosed. At increasing doses, clinical symptoms become more obvious, with abdominal pain,

arthralgia, clumsiness and headache presenting as the most common early signs of encephalopathy. Untreated, the condition may progress to include loss of consciousness, stupor and convulsions. Many children who recover from clinical encephalopathy retain serious, life-long cognitive, attention, and behavioral impairments (Needleman, et.al, 1990). Lead can also cause other serious long-term effects, ranging from hypertension and renal failure to adverse effects on reproduction (Rice, 1992, Vupputuri, et.al, 2003).

High Risk Populations

The majority of lead poisonings, from any lead exposure, occur in minority, urban, low-income families (CDC, 2005, Aldnsdown & Yule, 1986, Gorospe & Gerstenberger, 2008). Research at the New York City Lead Poisoning Prevention Program found that in 2000, about one third (33%) of children with BLLs at or above 20 µg/dL were Hispanic (New York City Department of Health and Mental Hygiene, 2002). In a predominantly Hispanic area of Miami, Florida, 55 percent of homes exceeded the EPA's action standards for lead (Gasana & Chamorro, 2002). In Santa Clara, California, twenty percent of US-born Latino children had elevated blood levels at their routine childhood screenings (Snyder, Mohle-Boetani, Palla & Fenstersheib, 1995). Almost 90 percent of the lead-poisoning victims in Orange County, California in 2004 were Latino children, and at least half of those children were believed to have been exposed to lead-contaminated candies (Bailey & Kitchen, 1985). Despite this evidence, Mexican and Latin Americans continue to eat imported lead-contaminated candies at an alarming rate. Recent research in California found that 37 percent of Latino households reported eating candy imported from Mexico (Mack, 2006).

Children of any background are especially susceptible to lead poisoning. The CDC estimates that about one million US children under the age of five have elevated blood lead levels (BLLs) (CDC, 2002). Children have a disparately high risk for lead poisoning, due to several factors: Pica behavior, or hand-to-mouth activity, is commonly observed in infants and young children and can significantly increase their risk. The intestines of children absorb lead more readily than the intestines of adults and their developing central nervous system is more vulnerable than an adult's (Needleman, 2004). Additionally, children are more frequent consumers of the types of lead-contaminated candies discussed in this article.

Lead in the Environment

Lead is a naturally occurring non-nutrient metal that is malleable, resistant to weathering and heat, and a good conductor, making it useful in a wide variety of products and, therefore, present throughout the environment in air, water and food products. Additionally, lead is acid resistant and shows chemical stability in air, water, and soil (EPA National Trends in Lead, 2006). Although these attributes contribute to its commercial usefulness, they are the same traits that lead to the availability to and accumulation of lead within an organism.

In 1970, lead in gasoline, which had long been used as an anti-knocking agent, was banned by the US Legislature. Seven years later, the maximum concentrations of lead in paints were set at 0.06% (Lynch et.al., 2000). The removal of lead from gasoline and paints led to considerable decreases in environmental lead exposure, but chronic exposures to lower concentrations of environmental lead still exist today in the United States and throughout the world. Although the uses of lead are gradually being phased

out, lead is currently used in many commercial products, including storage batteries, cable covering, noise control materials, chemical-resistant linings, ammunition, ceramic glazes, pigments, glass paints, plastic stabilizers, caulk, sheets and pipe for X-Ray and nuclear shielding, and lead alloys used in bearings, brass, bronze, and some solders (ATSDR, 2005). It is also present in household items such as zippers, furniture paint, mini-blinds, and some herbal remedies and mineral supplements.

Once present in the environment, lead is persistent in the air, water, and soil. Lead in the atmosphere comes from a variety of natural and anthropogenic sources, including volcanic eruptions, natural lead dusts, and the burning of leaded gasoline. Particulate lead in the air may deposit close to the source or travel up to thousands of miles from its original location (EPA National Trends in Lead, 2006). Lead will remain in the atmosphere for an average of ten days, depending on particle size, shape, emission source, metrological patterns, and local geography, before it is removed via precipitation or gravitational settling (Millstone, 1997, & Ratcliffe, 1981).

Lead generally contaminates a water sources via leachate from aerial fallout, soils or rocks, or contamination within a distribution system (Ratcliffe, 1981). Solubility of lead in water, which is a function of pH, hardness, salinity, and presence of organic materials, is highest in soft, acidic water (ASDTR Toxicological Profile, 2005). Decreases in the pH of rain water and water runoff may, therefore, increase the rate of lead leaching into water delivery systems.

Lead released into the environment from various sources settles on soil, sediment, foliage, and other surfaces (EPA National Trends in Lead, 2006). Accumulation of lead in soil is a reflection of the rate of lead decomposition from the atmosphere. The highest

contamination is, therefore, observed near highways, power plants, and smelters (WHO, 1077). In soil, variations in pH, organic content, temperature and exposure to an air source can affect its movement through the environment. Variations of the environment can make the pathway of lead extremely difficult to predict or manage (Landsdown & Yule, 1986).

Lead in Food and Candy

The primary route of exposure to lead is through ingestion. There are two processes whereby foods can become contaminated with lead: environmental contamination of the product or ingredients prior to manufacturing and cross-contamination of the product during or after the manufacturing process (Harrison and Laxen, 1981). As discussed, environmental contamination of lead is persistent in air, soil, and water. Animals may inhale contaminated air, or ingest contaminated water or plants (Ratcliffe, 1981). Plants may be contaminated via atmospheric deposition of environmental lead or via uptake from lead present in the soil (ATSDR, 2005). Studies that examined pH, organic content, temperature and exposure to an air in relation to plant uptake of soil-based lead concluded that the lead content of an edible plant is not a reliably measurable source of lead contamination (Landsdown & Yule, 1986). More current research, however, is starting to connect the lead found on chili peppers or tamarind fruit with lead from gasoline emissions (Gerstenberger, Cross, Donnelly & Fels, 2005).

Foods grown, stored or processed in the presence of lead can contain high concentrations of contamination (FDA, 2005). Additional risk can occur when foods are cooked in water that contains lead. It is estimated that cans soldered with lead contribute

to between ten and forty percent of lead poisonings (Oregon DHS, 2005). In the 1980s, the United States eliminated the use of lead in welding, in an effort to reduce measured lead levels in the US diet. Although cans in the United States are no longer soldered using lead, foreign manufacturers still use this technique. The health risks increase when lead leaches into the food from the can.

One major area of concern in the United States comes from foods bought at international markets, swap meets, ice cream trucks and other popular, less-regulated sources. Historically, lead has been used as an additive in foods or food supplements around the world to impart a sweet taste, a yellow or orange color or to increase the weight of the product (Kakosy, Hudak & Naray, 1996). The FDA has found high lead concentrations in curry powder, food coloring from Iraq, prune concentrate from France, duck eggs from Taiwan, and raisins from Turkey, as well as candies from Mexico, Brazil, the Philippines and other South American countries (Lynch, Boatright & Moss, 2000 & Wagner et al., 2005).

The US FDA defines *Mexican style* candy as “candy which contains ingredients popular in Mexico, such as chili and tamarind, which are not typically found in domestic candy in the US.” The level of concern for imported “Mexican style” candies is 0.1 mg/kg (FDA, 2007). This category includes powdered mixes, composed of salt, chili powder, sugar and flavoring. The powders are often sprinkled on fruits or vegetables, mixed into drinks or eaten as candy out of the container. Tamarind products, made from the pulp of the fruit from the tamarind tree, are imported from a number of Asian and Latin American countries. Tamarind candies and jams are a leading source of candy-related lead poisoning. “Mexican style” candies, which are consumed by both adults and

children, are sold in retail outlets, ice cream trucks, or simply brought into the United States from Mexico for personal consumption (FDA, 2007).

In response to a case of suspected candy-related lead poisoning, the Oklahoma City-County Health Department tested two distinct types of tamarind lollipops, as well as their packaging. Although the majority of these candies, 83.1 percent, were purchased in Mexico, 11.6 percent were purchased in the United States. Over half of the lollipops tested exceeded the FDA's level of concern for tamarind candies and their wrappers (CDC, 2000). Researchers in Oklahoma also used models and prediction methods to analyze the impact that those candies could have on the average BLLs of children in the United States. They concluded that lead concentrations were high enough in the two types of tamarind suckers analyzed that the maximum FDA tolerable food intake for children, 6.0 ug per day, would be exceeded if a child were to consume just one quarter to one-half of one of these candies. They also predicted that an average consumption of one of those lead-contaminated candies per day could result in a 43 to 84 percent increase in the mean BLL for children ages six to 84 months (Lynch, Boatright & Moss, 2000).

Perhaps the most extensive available database for lead-contaminated candies is presented online by the California Department of Public Health's Food and Drug Branch. This branch has committed to preventing the sale of adulterated candy to infants, young children, and pregnant women, in accordance with Assembly Bill 121, and has collected and tested candy samples since 1993 (California Department of Health, Food and Drug Branch, 2008). Those samples that tested above the FDA action limit of 0.1 parts per million (ppm) can be seen in Table 1.

Table 1: Candy with lead concentrations above regulatory limits, 2007-08 ⁽¹⁾.*

Candy Name	Number of Candies Sampled	Number of Candies That Tested Above Regulatory Limit ⁽²⁾	Range of Lead Concentration (ppm) ⁽³⁾	Mean Lead Concentration (ppm) ⁽⁴⁾
BarrieChicle	2	2	0.25 - 0.26	0.255
Barrlito, Liquid Chili Snack	4	4	0.14 - 0.15	0.145
Bibi Rainbow chewing gum, assorted flavors	4	4	0.63 - 0.78	0.760
Chaca Chaca Chacatrozo with salt and chili	6	2	ND - 0.30	0.122
De La Rosa Pulparindo (Extra Hot)	4	4	0.12 - 0.18	0.135
Dulces Yosi Mega Pack Toys with Bubble Gum	2	2	0.57 - 0.58	0.575
Ego Hao Jin Bang	2	2	0.70 - 0.73	0.715
Huevines Confitados Sabor Chocolate	2	2	0.19 - 0.20	0.195
Indy Cerillos, Spicy & sour candy lollipop, watermelon flavor	6	2	ND - 0.14	0.040
Indy Dedos, Spicy and sour candy	26	3	ND - 0.17	0.033
Indy Mini Dedos, Spicy and Sour	8	8	0.11 - 0.13	0.118
Lucas Limon	4	4	0.20 - 0.43	0.345
Lucas Limon con Chili (Baby Lucas)	4	4	0.38 - 0.48	0.418
Lucky Country Aussie Style Soft Gourmet Licorice Black All Natural	4	4	0.13 - 0.15	0.14
Miguello, Salt/Sugar Mix	4	4	0.12 - 0.13	0.125
Qi Cai Bang	2	2	0.60 - 0.61	0.605
Shaiky Pop, Tamarind candy lollipop with chili powder	2	2	0.11	0.11
Tama Roca Banderilla	20	8	ND - 0.14	0.076
Tamanlorin	2	2	0.21 - 0.23	0.22
Tamanzela, tamarind lollipop coated with chili powder	2	2	0.71	0.71
Tarritos, liquid chili snack	4	4	0.12 - 0.17	0.14

* Table adapted from CA Department of Public Health, Food and Drug Branch's. "AB 121 Lead in Candy Analysis Data - 2007 & 2008"

- (1) Candies tested by the California Food and Drug Laboratory
- (2) The state and federal regulatory limit for lead in candy at the time of these analyses was 0.10 parts per million (ppm).
- (3) ND denotes that the test result was below the quantitative limit for the method used and that this ND level was less than 0.10 ppm and in some instances less than 0.01 ppm.
- (4) Mean concentrations of lead were calculated by adding all sample concentrations together. In cases where some samples tested below the quantitative limit (ND), these samples were considered to have no lead for the purpose of finding the mean.

Lead in Ink and Wrappers

Foodstuffs can also become contaminated with lead when their wrappers or containers are contaminated and lead leaches from wrappers into food. This could be a particular problem in cases where the food is acidic. Some acidic candies, such as those made from tamarinds, chili peppers and tejocote fruits, have been shown to leach materials from their wrappers (Oregon DHS, 2005). The risk continues to increase when the wrappers are used as chewing paper or when children put the wrappers into their mouths. Lead ink is also found in pottery or glazes, and can leach out of the earthenware through the process of cooking.

- The lead-based inks used on candy wrappers in Mexico and Latin America have been a difficult regulatory issue for almost fifteen years. The FDA acceptable concentration for food contact surfaces is 7 mg/kg. For ceramic ware, a common culprit in lead-poisoning cases, the FDA acceptable concentration is ≤ 3 mg/kg (FDA, 1997). The US Consumer Product Safety Commission, in conjunction with the FDA and other concerned entities, has tried to halt the import of these products into the country, with little success. A survey of Latin American candies found in Southern Nevada showed some imported candies to contain 1.46 ± 0.27 mg/kg lead in their wrappers and straws (Gerstenberger et al, 2007).

Published Case Reports

In 2004, California reported that of approximately 1,000 cases of elevated BLLs between May 2001 and January 2002, imported candies were identified as major contributing sources in 173 of those cases. The average BLL for the candy-related cases was 24.3 μ g/dL. The candy-related lead poisonings were found in 17 counties across the

state (New York City Department of Health and Mental Hygiene, 2002 & CDC, 2002).

Although case reports on lead-poisonings have not historically acknowledged lead-contaminated candies as a contributing source of exposure, these candies are increasingly becoming a new source of lead exposure, especially for Latino children.

Table 2: Cases of Elevated BLLs from Imported Candies and/ or Packaging

Reference	Age/ Sex	Location	Candy Description	Lead Content (mg/kg)	BLL (μ g/ dL)	Inter- vention	Reported Outcome
CDC, 2002	4/M ⁱ	Stanislaus County, CA	Imported Mexican candies, Dulmex- Bolorindo lollipops	None reported	88.0	Chelation therapy	None reported
	6/F ¹				69.0		
CDC, 2002	4/ M	Fresno County, CA	Imported candy wrapper	16,000	26.0	None reported	BLL decreased to 13.2 μ g/dL after 21 months
CDC, 2002	2/ M	Orange County, CA	Various imported tamarind fruit candies, including a Dulmex- Bolorindo lollipop	Stick: 404	26.0	None reported	None reported
				Wrapper: 21,000			
				Candy: 0.2			
				Seed: 0.3			
CDC, 2002	4/M	Los Angeles County, CA	Imported candies from Mexico	None reported	22.0	Candy consump-tion dis-continued	BLL decreased to 11 μ g/ dL after 17 months
CDC, 1998	6/M ii, 2, a	CA	Tamarind candy jam products in ceramic jars, from Mexico	None reported	59.0	Chelation therapy	None reported
	>18 /F ⁱ				26.0		
	3 ^{3, a}				50.0		
	7 ^{3, a}				57.0		
Lynch et. al., 2000	<6/?	OK	Tamarind suckers		48.0	Home investigation and candy discont.	Initial increase in BLL

ⁱ Offspring (Same number denotes a parent-child relationship)

^{1, 2, 3} Siblings (Same number denotes siblings from one family)

^a Cousins (Same number denotes cousins)

XRF Analysis of Lead

One of the vital factors in effective identification and removal of threatening lead-based products is the timely and cost-effective analysis of that substance. Historically, the high costs and time requirements of laboratory-based analyses have been barriers to the process. Use of field portable X-Ray fluorescence (XRF) spectrometry has become a common and useful analytical tool for on-site screening and rapid analysis of contaminant elements.

1. Theory

The XRF is able to identify elements because each atom fluoresces at a specific energy when excited by an X-Ray. X-Ray photons from the XRF create inner shell vacancies within the atom. An outer shell electron fills that space as the atom relaxes to ground states. This process releases photons with energy in the X-Ray region of the electromagnetic spectrum equal to the energy difference. Detectors on the XRF machine are able to identify the element due to its characteristic emitted X-Ray. By comparing the observed intensities of the X-Ray to a known standard, the machine can quickly quantify the amount of lead (Kalnicky & Singhvi, 2001).

2. Detection Limits

Detection capabilities of the XRF improve as time increases, as background noise decreases, and as sensitivity increases. Detection limits are dependent on both the element and the matrix, and most elements are still detectable below typical site action levels. Qualitative results depend largely on the application and intended use of the data, as well as the calibration of the XRF using a sample matrix. A sample matrix is designed

through laboratory procedures to minimize interfering elements that are commonly found in a specific environmental application. (Kalnicky & Singhvi, 2001).

The limit of detection for the XRF can be found in multiple ways, but the most commonly applied method states that the detection limit (DL) is, “that amount that gives a net intensity equal to three times the standard counting error of the background intensity.” The DL may also be defined in terms of the precision of repeat measurements on a standard sample. In order to determine the DL, the US EPA recommends the measurement of a sample that has a concentration of analyte close to the expected DL. The EPA defines the method detection limit as, “the minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero.” To estimate the method detection limit, the standard deviation of non-consecutive replicate measurements must be multiplied by the rounded Student’s t-factor, as seen in the following equation:

$$MDL = 3\sigma$$

where σ is the standard deviation for the replicates and the Student’s t-factor is approximately equal to 3 (Federal Register, 1984).

CHAPTER 3

QUESTIONS, OBJECTIVES, AND HYPOTHESES

Research Questions

- What is the method detection limit (MDL) at which the Niton® XRF, in each mode, becomes an effective screening tool for lead in candy and candy packaging materials?
- Which XRF modes are most effective at determining the concentration of lead in candies and their packaging materials?
- Can the XRF be used as an effective and reliable screening tool for identifying lead in contaminated candies?

Objectives

- This study will attempt to determine the Niton® XRF's method detection limit (MDL) for each mode when analyzing lead found in candies and their packaging materials.
- The study will evaluate the various modes of the XRF in relation to identification of lead in contaminated candies and modify the protocol for using the XRF to identify lead in candies accordingly.
- The study will examine the confidence limits for detection and the accuracy of the XRF at various increasing concentrations of lead.

- This study will attempt to create a scale that compares the units for each mode (ppm, mg/cm², and ug/cm²) for candy samples.

Hypotheses

Hypothesis 1: Method Detection Limit

The method detection limit for use of the Niton® XRF in Plastics and Bulk Sample Modes for use in identifying lead in contaminated candies will be found between 10 and 15 parts per million.

Use the EPA procedure for method detection limit to find the Method Detection Limit (MDL) for each mode of the Niton® XRF. The standard deviation for ten non-consecutive replicate measurements will be multiplied by the student t-factor to determine the MDL.

Hypothesis 2: Analytical Method Comparison

The XRF can be used quantitatively to screen lead concentrations above the MDL in candies and candy packaging materials. The mean XRF values for each concentration above the determined MDL will be statistically equivalent to the mean GFAAS concentration values.

Examine the mean XRF analyses for a given set of fortified candy samples vs. the mean graphite furnace analysis of lead concentration for the same candy samples.

Hypothesis 3: Mode Comparison

The Niton® XRF Plastics mode will have the most sensitive limit of detection.

Niton® XRF data for each mode will be examined and the GFAAS-determined concentration at the MDL for each mode will be compared. An ANOVA will test for

significance between MDLs for each mode and post hoc analysis will examine the relationship between modes.

Hypothesis 4: Predictive Relationship

There is a significant predictive relationship between the XRF modes and the actual concentration of lead in candy, as determined by the GFAAS.

Regression analysis will be used to find the predictive relationship between the mean XRF-determined concentration and the mean GFAAS-determined concentration, as well as confidence limits at each concentration, for each mode. If necessary, data without homoscedasticity will be transformed to allow for linear regression. Additionally, data points below the XRF limit of detection (coded as ND) will be replaced with one half of the MDL, as determined in this study. The 100(1- α) percent prediction interval for each MDL will be determined based on the linear regression equation.

Hypothesis 5: Practical Mode

The Niton® XRF Plastics and Bulk Sample Modes will be the most useful analyses of the concentration of lead in contaminated candies, because they report data in parts per million, ppm, which is most comparable to graphite furnace analysis and FDA action limits.

CHAPTER 4

METHODOLOGY AND DATA COLLECTION

Sample Collection

Based on the Orange County Register report, previous consumption studies and opportunistic sampling at local markets and swap meets, approximately thirty brands of candies were selected for an initial survey. Candy samples were obtained from merchants, vendors and volunteers in Clark County, Nevada. Approximately five- to eight-hundred candies, as well as their packaging materials, were analyzed in all modes using the Niton® XRF. An XRF method developed by researchers at the University of Nevada, Las Vegas, was used to screen the samples (Donnelly & Gerstenberger, 2007).

Samples were cataloged, recorded and sorted into concentration levels. Thirty-three candies and wrappers were collected as range-finding samples, with lead concentrations categorized as high (>40 ppm or >1.0 ug/cm²), medium (0.6-0.99 ug/cm²), low (0 – 0.59 ug/cm²) or no lead, as indicated by the XRF.

Pelon Pelo Rico ® was chosen for use in a candy matrix, because of its ingredients and because it typically contains low lead contamination. The candy matrix was developed by homogenizing candies in heated water at 300 degrees for two hours. Analytical portions of the candy matrix were spiked with lead, vortexed for one minute, and the fortified solution was placed in a soil sample cup with a mylar X-Ray film

covering the top. A method blank of candy matrix was created in the same manner, without the lead. Fortified analytical portions (FAPs) were spiked with known amounts of lead to create a range of samples within five times the predicted limit of detection (2.0 to 75.0 ppm). Fifteen samples were created to establish a detection limit range and 30 samples were created within the range, for a total of 45 samples.

Analysis

The initial candy samples, with concentrations between zero and 1.32 $\mu\text{g}/\text{cm}^2$ of lead, were taken to the FDA for ICP-MS analysis in March of 2008. Results from the FDA and subsequent XRF and graphite furnace analysis were used to compare the XRF values with the ICP-MS values for lead concentration. This information was used to develop an estimated detection limit range between 10.0 ppm and 15.0 ppm. Based on this expected range, fortified analytical portions (N=30) were created with lead concentrations ranging between 2.0 and 75.0 ppm. Once a detection limit range was established, fortified analytical portions (N=15) were created with lead concentrations within that more precise range.

Fortified analytical portions were created using the sample candy matrix. Approximately ten milliliters of candy matrix were poured into 50 mL centrifuge tubes. A known amount of lead was added to the sample to establish the desired lead concentration. The sample was then vortexed for sixty seconds and poured into a soil cup and covered with 6 μ Mylar® X-Ray film.

The fortified analytical portions were then treated as “bulk samples,” according to the protocol initially proposed by Donnelly and Gerstenberger, in 2007, and outlined in Appendix A. The soil cup was placed, film down, in the XRF stand. Ten replicate tests

with the XRF were done in thin layer, bulk soil sample, paint, and plastics modes. Two XRF devices were used for this analysis. The XLt 7972W model was used for analysis in Plastics Mode. This model uses a low power (1.0 W) X-Ray tube excitation source to analyze bulk samples for multiple elements. Another device, the XLp 303A model, was used for the Paint, Bulk Soil, and Thin Layer Modes. The XLp models utilize a sealed cadmium (^{109}Cd) radioisotope source for excitation.

Using the EPA's procedure for the determination of the method detection limit, the standard deviation of the sample with the lowest detectable concentration was used to find the method detection limit for each mode. The spiked samples were then analyzed using a Perkin Elmer Graphite Furnace Atomic Absorption Spectrometer (GFAAS) 600 (Perkin Elmer, Shelton, CT), in order to compare results to the XRF analysis. Figure 1 outlines the process.

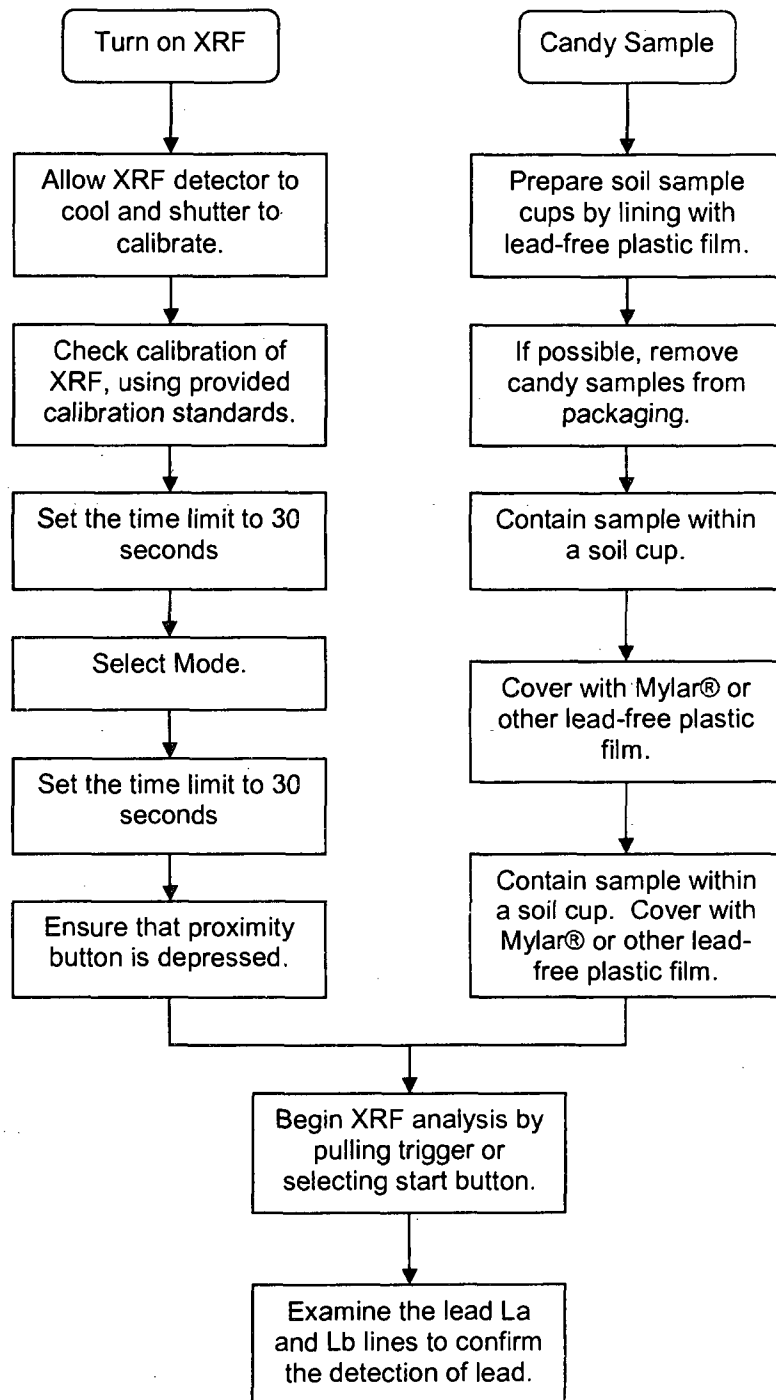


Figure 1: Flow diagram of method to analyze samples for lead

Candy sample analysis at the UNLV laboratory was performed using graphite furnace atomic absorption spectrometry (GFAAS). The GFAAS method, adapted from the FDA's method for determination of lead in food, is summarized as follows (FDA, 2002):

Preparation of Analytical Samples for GFAAS

1. Quality Control Samples

Quality control samples are used to determine if analytes, preparation procedure or sample matrix contribute bias to the results. The following quality control samples were prepared and included with each batch of samples:

- 3 Method Blanks (MBK)– 1 g of reagent water, processed using all the same reagents and exposed to all laboratory ware;
- 1 Standard Reference Material (SRM)– A known sample of milk powder, with uncertainties that do not exceed 20 percent (95% confidence). The SRM is treated as an analytical sample in all aspects of the analysis; &
- 1 Check Solution– An analytical portion, typically with a concentration of 40 ppm, that was spiked with a known amount of lead before decomposition; used to analyze percent recovery.

2. Representative Samples

Representative homogenate samples are prepared by acid digestion, using heated concentrated nitric acid. Samples are heated in the nitric acid until they are completely dissolved and the solution is clear of solids. The solution is adjusted to 50 mL with nitric acid. A pipette is used to transfer 0.5 mL of the sample to a clean decomposition vessel

liner. Any material on the walls of the liner was washed down with no more than 3 mL of deionized water.

Sample Preparation by Microwave Digestion

Ten mL trace metals grade HNO₃ were added to each vessel liner. Two method blanks were included with each batch to assess contamination. A milk powder Pb standardized reference material was assessed with each series. Vessels were hand-sealed and samples were digested in an Anton-Parr Multiwave 3000 microwave digester (PerkinElmer, Multiwave 3000; Shelton, CT). Power was ramped for over 25 minutes until 200°C was reached and this temperature was maintained for 10 minutes. After vessels cooled, solutions were diluted to 200 mL with DI water. Dilution was performed into a clean, disposable 250 mL polyethylene bottle for storage.

GFAAS Analysis

1. Instrument Setup and Standardization

The graphite furnace (PerkinElmer, AAnalyst600; Shelton, CT) and gas were turned on and the lead hollow cathode lamp was allowed to warm up for twenty minutes. Optical alignment of the furnace and alignment of the autosampler tip were checked each day. The graphite tube was inspected and replaced if needed. The wavelength for lead was set to 283.3 nm. The instrument was programmed to perform three replicate injections for each analytical solution. An instrument stability check was performed and problems were corrected if the short term precision was >5% RSD. The instrument was standardized using the standard blank and approximately six standard solutions.

2. Determination

All solutions were analyzed three times and the mean concentration was used in calculations. The standards, blanks, and analytical solutions were analyzed and the instrument diluted any solutions with concentrations above the highest standard solution. The instrument factors in dilutions to find the concentration of lead in the analytical portion.

Statistical Approach

XRF data was input into a Microsoft Windows Excel datasheet SPSS for Windows, Student Version 16.0 (SPSS Inc., Chicago, IL). The mean XRF analyses were taken from ten consecutive readings for a given set of fortified candy samples. The limit of detection was determined for each FAP sample concentration, in each XRF mode. The standard deviation for ten non-consecutive replicate measurements was multiplied by the student t-factor to determine the MDL, using the following equation:

$$\text{MDL} = 3\sigma.$$

This MDL has above a 99 percent confidence limit. An ANOVA will test for significance between MDLs for each mode and post hoc analysis will examine the relationship between modes.

Analysis of variance, with a within-subject or repeated measures design, was used to compare the means of GFAAS-determined concentrations and each XRF mode. The overall test used was a Wilk's λ . Bonferroni pos hoc analysis was used to determine the comparative relationships.

Finally, a linear regression model was performed analyzing the predictive relationship between the XRF modes and the actual concentration, as determined by GFAAS. The 100(1- α) percent prediction interval for each MDL was determined based

on the linear regression equation. A scatter dot plot was graphed for each XRF mode against the GFAAS mean data.

Quality Assurance and Quality Control

All reusable labware was cleaned sufficiently for trace element analysis. Cleaning procedures included washing in lead-rinsing laboratory detergent, 5 reagent water rinses, soaking for at least 4 hours in 10 percent nitric acid and a final reagent water rinse. Reusable labware was covered with aluminum after being washed. All gloves were powder free vinyl gloves to avoid the possible contamination that can occur when latex gloves are used. High purity reagents were used for all analyses, as well as high purity argon. Reagent water meets specifications for ASTM Type I water.

The GFAAS method requires the monitoring of several quality control parameters. Assessment of standardization, analyte recovery, interferences, accuracy, and contamination must be monitored during routine analysis, in order to ensure data quality and reliability. Analytical performance was recorded throughout the process and trends were analyzed periodically for potential problems.

Laboratory reference values were estimated using the XRF. The upper limits for each analyte were established by measuring the absorbance of at least 6 standards, with the concentration of the two highest standards near the estimated upper limit. Instrument sensitivity checks were performed prior to each analysis by running standardized solutions. The characteristic mass, m_0 , for each standard must have been within 20 percent of the expected value for the analysis to continue. Recovery results from blanks and standards must have been $100 \pm 5\%$ of expected value for the analysis to continue. On the standard curve, the value for the correlation coefficient, R^2 , must be ≥ 0.998 , with all

samples lying in the range of control concentration. If displays of the curve indicated which standard was outside the acceptable standard concentration parameters, re-standardization for that standard was performed. Otherwise, a new set of standards was analyzed until all QA/QC guidelines were met.

CHAPTER 5

RESULTS

Each fortified analytical portion (FAP) was analyzed with the Niton® XRF ten times in each mode, and then digested, diluted, and analyzed via GFAAS. GFAAS-determined concentration was considered the actual concentration of lead in the fortified analytical portions (FAPs) for the purposes of this study. As discussed in the Methods section, the Plastics Mode is an application of the Niton® XLt XRF, which contains a low power X-Ray tube excitation source. All other modes are applications of the XLp XRF, which utilizes a sealed cadmium source. Data was recorded for all modes, using both XRFs, and can be seen in Table C in the Appendix. Statistical analysis was performed with SPSS for Windows, Student Version 16.0 (SPSS Inc., Chicago, IL). The EPA procedure for method detection limit (MDL), whereby the standard deviation of ten replicate measurements was multiplied by the rounded Student's t-factor, was utilized to determine the MDL for this protocol. The Niton ® XRF Paint Mode was unable to detect lead at any concentration within the expected range. It was, therefore, omitted from initial statistical analysis.

Table 3 shows the average minimum concentration at which ten consecutive measurements could be performed, the standard deviation, and the resulting MDL for each remaining mode. Each MDL has a confidence limit above 99 percent. The lowest

concentration detected by the XRF was found in the Plastics Mode, however, the lowest MDL was observed in Bulk Mode. The hypothesis that the method detection limit for use of the Niton ® XRF in both Plastics and Bulk Sample Modes for use in identifying lead in contaminated candies would be found between 10 and 15 parts per million was supported by this analysis. Additionally, the Thin Layer Mode also had an MDL within this range, as determined by GFAAS analysis.

Table 3: Method Detection Limit for All Modes (n=10 per MDL, per mode)

Mode	Average Actual Concentration, determined by GFAAS (ppm)	Average Concentration, determined by XRF	Standard Deviation	MDL = 3σ (units vary by mode)
Plastics	12.35	10.59 ppm	2.35	7.05 ppm
Bulk	8.56	9.83 ppm	1.82	5.45 ppm
Thin Layer	19.77	2.32 μg/cm ²	0.40	1.19 μg/cm ²
Paint *	N/A	N/A	N/A	N/A

*Paint Mode MDL was too high to be analyzed

The GFAAS-determined concentrations of the fortified analytical portions at the MDL were compared using an ANOVA within-subject design, or repeated measures ANOVA. Due to the differences in units, Thin Layer and Paint Mode data were not analyzed. Data was examined for normality using a histogram with a normal curve. Although normality was not perfect for each mode, ANOVA is robust enough to handle some non-normality (Lindman, 1974). A Wilk's λ overall test was conducted, with Bonferroni post hoc comparison of results. The mean measurements differed significantly among the three methods ($F_{2,24} = 15.128$, $p < 0.001$). The XRF modes did not differ ($t = 1.39$, $df = 24$, $p = 0.531$); however, GFAAS measurements differed from both Bulk Mode ($t = 5.61$, $df = 24$, $p < 0.001$) and Plastics Mode ($t = 4.83$, $df = 24$, $p < 0.001$). Results

of the within-subject ANOVA and Bonferroni post hoc comparison can be seen in Tables 4 and 5.

Table 4: Estimates of XRF and GFAAS Measurements

Factor			95% Confidence Interval	
	Mean (ppm)	Standard Error	Lower Bound	Upper Bound
GFAAS	45.08	3.97	36.90	53.26
Bulk XRF Mode	37.41	3.88	29.42	45.40
Plastics XRF Mode	38.33	4.07	29.95	46.71

Table 5: Bonferroni Post Hoc Comparison of XRF vs. GFAAS Measurements

Factor 1 (I)	Factor 2 (J)				95 % Confidence Intervals for Difference	
		(I - J) Mean Difference	Standard Error	Sig.	Lower Bound	Upper Bound
GFAAS	Bulk	7.67	1.37	<0.001	4.16	11.18
	Plastics	6.75	1.40	<0.001	3.16	10.33
Bulk	GFAAS	-7.67	1.37	<0.001	-11.18	-4.16
	Plastics	-0.92	0.67	0.53	-2.63	0.78
Plastics	GFAAS	-7.67	1.40	<0.001	-10.33	-3.16
	Bulk	0.92	0.67	0.53	-0.78	2.63

Results of the within-subject ANOVA indicate that the XRF modes are equal to one another, but not significantly equivalent to the GFAAS measurements. These results necessitate the rejection of both Hypothesis 2 and Hypothesis 3, because the mean values of the XRF measurements were not equal to the mean GFAAS measurements, and because the Plastics Mode was no more sensitive than the Bulk Sample Mode. The significant difference between the measurements acquired by both XRF modes and the measurements acquired via GFAAS indicate that the XRF cannot be utilized to precisely

quantify the concentration of lead in a sample of candy, using this protocol. Linear regression analysis was, therefore, performed to determine if a predictive relationship exists between each XRF mode and the actual concentration, as determined by GFAAS.

The assumptions of linear regression were all met by the mean data values.

Homoscedasticity of variance was examined visually with a normal curve. No clustering appeared when residuals were plotted against the x values. All other assumptions of linear regression were analyzed and determined to be acceptable. The R^2 values for Bulk and Plastics Modes were 0.89 and 0.885, indicating a high level of correlation. Data for the linear regression analysis of the mean data for GFAAS and XRF Bulk and Plastics modes can be seen in Table 6 and the regression plots can be seen in Figures 2 through 4.

Table 6: Coefficients for Linear Regression Analysis

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95 % Confidence Interval for B	
	B	Standard Error				Lower Bound	Upper Bound
(Constant)	6.940	2.778	0.945	2.498	0.019	1.229	12.651
Bulk	1.004	0.068		14.708	<0.001	0.864	1.144
(Constant)	6.935	2.338	0.949	2.966	0.006	2.160	11.709
Plastics	0.971	0.059		16.456	<0.001	0.851	1.092
(Constant)	9.919	3.248	0.939	3.054	0.006	3.183	16.655
TLM	5.559	0.436		12.751	<0.001	4.655	6.464

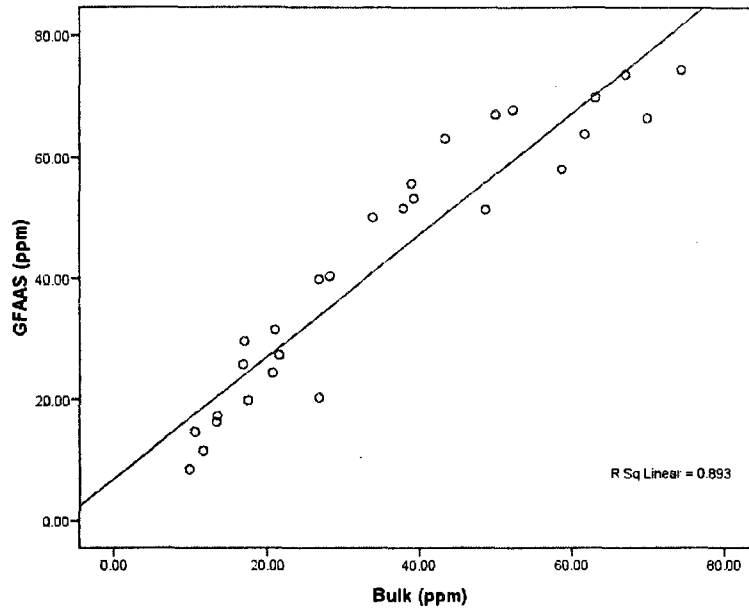


Figure 2: Linear Regression Plot, Mean Bulk XRF Mode vs. Actual (GFAAS)

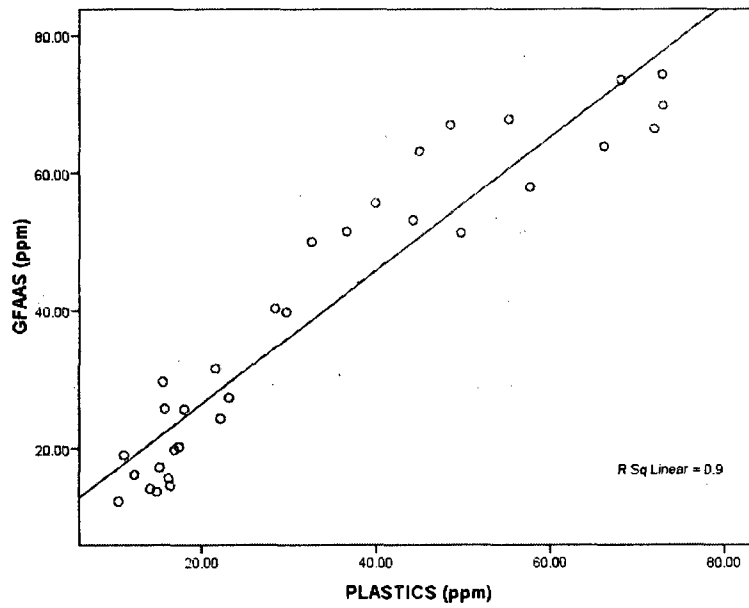


Figure 3: Linear Regression Plot, Mean Plastics XRF Mode Vs. Actual (GFAAS)

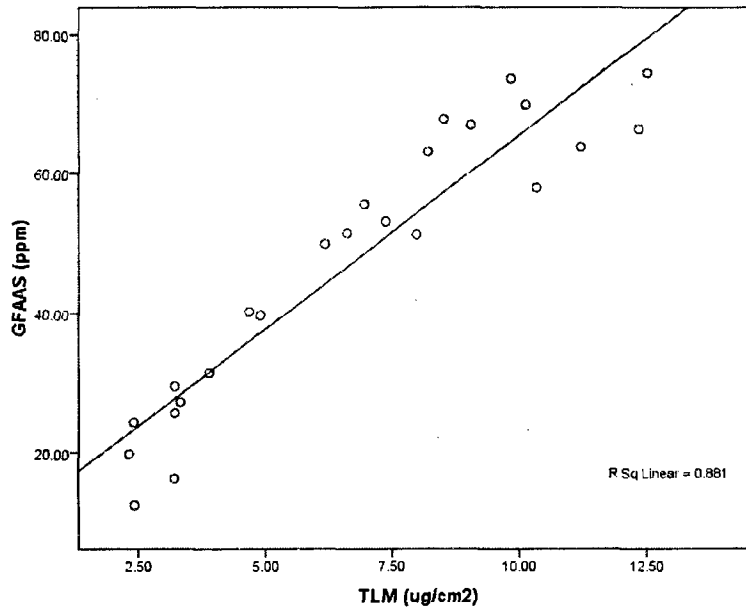


Figure 4: Linear Regression Plot, Mean Thin Layer XRF Mode Vs. Actual (GFAAS)

The subsequent linear regression equation for the Bulk Soil Sample mode is:

$$\text{Mean GFAAS (Actual) Concentration} = \text{Mean Bulk Soil Sample Concentration} (0.1.004) + (6.940).$$

The linear regression equation for the Plastics mode is:

$$\text{Mean GFAAS (Actual) Concentration} = \text{Mean Plastics Concentration} (0.971) + (6.935).$$

The linear regression equation for the Plastics mode is:

$$\text{Mean GFAAS (Actual) Concentration} = \text{Mean TLM Concentration} (5.559) + (9.919).$$

Results of the linear regression analysis indicate a significant predictive relationship between each XRF mode and the actual concentration of lead in candy, as determined by XRF. Therefore, Hypothesis 4, which stated that such a relationship would be established, could not be rejected.

CHAPTER 6

DISCUSSION

The Orange County Register report on lead-contaminated candies ignited a nation-wide interest in imported candies that encouraged federal, state, and local health districts, practitioners, researchers, and parents to question the inconsistently high concentrations of lead found in some of these candies. Regardless of this interest, the candies are still sold in stores, flea markets, and in street vendors and carts, and the United State's Food and Drug Administration (FDA) is unable to adequately protect American children who consume these candies. The US Center for Disease Control (CDC) found that in 1999 through 2002, 1.6% of American children aged 1 to 5 years had blood lead levels greater than or equal to 10 micrograms per deciliter (US CDC, 2005). Recognizing the significance of this finding, a Healthy People 2010 objective was set to reduce all young children's blood lead levels to less than 10 micrograms per deciliter (USDHHS, 2000).

In order to reach this goal, evidence supports the importance of a shift in primary prevention of lead exposure in children. Removal of contaminated candies is an important part of this goal. The two major components of this effort are education and identification and removal of candies. X-Ray fluorescence spectrometry provides a rapid, non-destructive, mobile screening of multiple elements. Its use for identifying lead

has been established in lead-based paint, soil, and bone. If this technology could be applied to quickly, effectively, and non-destructively identifying lead-contaminated candies, it would provide the most cost-efficient method for preventing childhood exposure to lead through contaminated candies.

This research examined the efficacy of a protocol to screen candies for lead contamination. The XRF has proven to be useful for estimating concentrations of lead in candies with lead contamination above the method detection limit (MDL), for the method outlined in Appendix A. An MDL of 5.45 ppm was determined for this method in Bulk Sample Mode and an MDL of 7.05 ppm was found in Plastics Mode. Results of an ANOVA test indicate that the mean concentrations above the MDL for both Plastics and Bulk Sample Modes are significantly equal to one another. Therefore, public health workers may use either the XLt or XLp XRFs, in Bulk or Plastics Modes, to screen candies for lead contamination.

Results also indicated that the XRF-determined means in either mode were significantly different from the actual mean concentrations, as determined via a Graphite Furnace Atomic Absorption Spectrometer (GFAAS). Linear regression analysis established a predictive relationship between the XRF-acquired data, in each mode, and the actual concentration of lead, as determined by the GFAAS. The equations provided by the linear regression analysis can be used to estimate the actual concentration of lead in candies using XRF data, with 95 percent confidence.

The modes and the models of XRFs affected the detection and applicability of the method. The Plastics Mode is an application of the Niton® XLt XRF, which contains a low power X-Ray tube excitation source. All other modes are applications of the XLp

XRF, which utilizes a sealed cadmium source. The XLp is the most common XRF, because it is utilized for home investigations of lead in paint. The XLt, however, requires less training because it operates with a low power X-Ray tube instead of a radioisotope excitation source.

The Thin Layer Mode alone was not considered as practical as the Bulk Soil Sample and Plastics Modes because of its units ($\mu\text{g}/\text{cm}^2$). This study proposes a linear regression equation that can be used to predict the actual concentration of lead based on the Thin Layer XRF Modes. This simple calculation can also be used for converting units, and can be utilized by public health workers who are operating with Thin Layer Mode only, or who want to convert their results. Use of the conversion equation,

$$\text{Mean GFAAS (Actual) Concentration(ppm)} = \text{Mean TLM Concentration } (\mu\text{g}/\text{cm}^2) (5.559) + (9.919).$$

will make it relatively simple for public health workers with an XRF in Thin Layer Mode to estimate what their data would equate to in parts per million. It should be noted that this conversion equation only applies to candies measured according to this protocol, using a 10 mL soil sample cup. The conversion equation will not necessarily apply to any other protocol, medium, or sample depth.

Although the information gathered in this research is extremely valuable for protocol development, it does highlight some limitations of use of the XRF for detecting contaminated candies in the field. The Niton[®] XRF Paint Mode was unable to detect lead at any concentration within the expected range. It was, therefore, considered an impractical mode for analyzing lead contamination in candies, according to this protocol. The candy chosen as a matrix for this research was chosen because it contains many ingredients common to “Mexican candy,” as defined by the FDA. It was also chosen

because it is low in salt concentration, which interferes with the GFAAS analytical method. Other candies, however, may have different proportions and ingredients, thus representing a different matrix. The protocol and performance measurements outlined in this paper should apply to most candies with similar ingredients, but results may vary in candies that are significantly different from this candy matrix.

The most important limitation of the XRF pertains to the concentration of candies typically seen in the United States. As seen in Table 1, the mean concentration of candies identified by the California FDA in 2007-2008 as having lead concentrations above the state and federal regulatory limit was 0.2849 ppm lead. Although this concentration is almost three times higher than the regulatory limit for lead in candy, it is well below the MDL of 5.45 ppm for the XRF determined in this study.

Candy wrappers and other packaging items have been shown to contain lead contamination in concentrations above 1000 ppm. The lead in these wrappers has the potential to leach into candies, especially when the candies are acidic. Additionally, wrappers, sticks, spoons, straws, and other non-candy packaging items are exposed to the acidic conditions of saliva as children eat the candy. Although the risk of leaching and the dangers posed by wrappers has not been well researched, the potential threat that these wrappers poses could be easily identified by the XRF. Additionally, the Paint and Thin Layer Modes may prove to be more effective and practical at identifying lead contamination in the thin surface of wrappers. This is an important area of research that should be addressed in future studies.

Additionally, improvement of the sensitivity of the XRF for identifying lead in candies should be investigated. If possible, manufacturers of XRF handheld devices may

develop algorithms that specifically identify candy as a matrix, thus improving its detection abilities. Future research can also examine the effects of salt and other ingredient composition on the sensitivity of the XRF. Additionally, public health officials working with X-Ray fluorescence to identify contaminated candies can work with the manufacturer to calibrate their instrument to improve sensitivity.

Perhaps most importantly, future efforts must include plans to collect and disseminate information on the dangers of lead-contaminated candies and foods. These efforts must begin by updating lead exposure risk questionnaires used during screening to include questions on all known exposures, including contaminated candies. Clinicians must understand the risk of exposure to lead-contaminated imported candies and advise their patients on the potential dangers. Clinicians, researchers, and public health workers at local, state, and federal levels must collaborate to improve education, pool data, and develop intervention guidelines. Manufacturers of candies identified as contaminated should be monitored and efforts should be made to improve their practices or ban their products. By improving the rapid detection of lead-contaminated candies, increasing education on the dangers of exposure, and continuing research and collaboration to identify and remove highly contaminated candies, the goal of reducing this threat can be achieved.

APPENDIX

TEST METHOD FOR DETERMINATION OF LEAD CONCENTRATION IN CANDY BY X-RAY FLUORESCENCE SPECTROMETRY (XRF) IN BULK SOIL SAMPLE MODE

I. SCOPE AND APPLICATION

- a. This test method covers the determination of lead concentration in candies. The concentration of lead in any candy is restricted by the FDA to an action limit of 0.1 mg/kg (ppm).
- b. The applicable range of this method is from 5.45 mg/kg to percent levels.
- c. This method and interpretation of the results should be restricted to use by, or under the supervision of, analysts experienced in the operation of an X-Ray fluorescence spectrometer.

II. SUMMARY OF METHOD

A candy sample, contained in a disposable plastic soil sample cup, is loaded into an X-Ray fluorescence (XRF) spectrometer stand. The intensities of the emitted photons are measured and concentration is thereby determined by the XRF.

III. APPARATUS AND MATERIALS

- a. XRF spectrometer, either energy dispersive or wavelength dispersive. The instrument must be able to accurately resolve and measure the intensity of lead with acceptable precision.
- b. Disposable sample cups with suitable plastic film, such as Mylar®, or lead-free plastic wrap.
- c. Testing stand, designed for soil cups.
- d. Candy samples.

IV. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

a. Sampling

There are two methods of sample preparation that should be considered when analyzing candy samples by XRF: in situ and discrete sampling.

- i. For direct analysis of candies (in situ), the XRF instrument may be taken to the sample location and the candy should be directly analyzed. This sampling method provides flexibility by allowing

efficient collection of data for a large number of samples that can be used for rapid decision-making in the field.

- ii. Discrete sampling (removal of physical sample) requires more time and effort, but allows for greater analytical accuracy and precision. Additionally, it allows for an unlimited number of samples and time for analysis.

b. Preservation

If discrete sampling is performed, special attention should be paid to storage of candy samples. Sample should be stored in a dry area, away from UV radiation, moisture, and other hazards. If necessary, candies may be preserved in a freezer to maintain quality.

c. Handling

Candies should be handled with powder free vinyl gloves to avoid contamination. All candies should be placed carefully into soil cups. If candy is spilled onto the surface of the XRF or clean preparation table, it should be immediately cleaned. The XRF can be used to test for residual contamination on the workstation.

V. PHYSICAL MATRIX EFFECTS

Variations in the characteristics of the candy sample may contribute to physical matrix effects and must be monitored. These variations include parameters such as ingredients, concentration, moisture, size, uniformity, heterogeneity, and texture. These parameters are also influenced by the condition of the sample. When prepared candy samples are stored in XRF cups, settling effects may also bias results. Vortexing soil sample cups prior to XRF analysis helps to homogenize the particles within the soil sample cup.

VI. PROCEDURE

a. Preparation

- i. Dosimeters should be worn by all persons while operating the XRF.
- ii. XRF should be stored in locked case, with battery separate from the device.
- iii. Initialization of the XRF requires cooling down of the detector and shutter calibration, which takes several minutes.
- iv. Prepare soil sample cups by lining bottom end with lead-free plastic film.
- v. Candy samples can be poured or placed into soil cup. Samples that do not fit into soil cup should be cut to fit within the soil cup. Consideration should be paid to the fact that analyses of samples in wrappers will include the concentration of lead in the wrapper, which may not be indicative of the concentration of lead in the edible portion of candy. It is recommended that, if possible, all candy be removed from its wrapper and other packaging materials prior to analysis.
- vi. Place candy samples film-down in the stand.

- b. Calibration and standardization
 - i. Calibration checks should be performed prior to analysis, following analysis, and every four hours during analysis.
 - ii. Three calibration checks should be performed for each calibration standard, using a high, medium, and low calibration standard. The average of the three readings for each level should be within the acceptable range for that standard.
 - iii. If calibration checks do not fall within the acceptable range, the manufacturer must be contacted, in order to re-calibrate the XRF.

- c. Analysis (See Figure A)
 - i. Manipulate candy to ensure that the sample can be contained within the soil cup. Place or pour candy into soil cup.
 - ii. Place sample within soil cup into upright stand, with sample film-down so that candy is pressed to film and film is flush with XRF detection window.
 - iii. Set the time limit to 30 seconds. This setting is typically found under "Hardware Setup".
 - iv. Under "Mode," select the "Bulk Sample" Mode on the XRF.
 - v. Select the "Soil Sample" Mode on the XRF.
 - vi. Ensure that proximity button is depressed.
 - vii. Begin XRF analysis by pressing trigger or selecting start button when using a computer.
 - viii. Examine the lead La and Lb lines to confirm that the detection of lead is correct. Spectra should exhibit lines at 10.55 and 12.61, respectively.

VII. QUALITY ASSURANCE/ QUALITY CONTROL AND DATA INTERPRETATION

For each data collection, regardless of sampling method, a quality assurance (QA) objective must be specified that corresponds to the ultimate data use objective. The US EPA has defined three objectives for assessing and substantiating data collection: QA1, QA2, and QA3. For XRF analysis of candy samples, both QA1 and QA2 can apply:

- A. QA1 is a screening objective used to afford a quick, preliminary assessment of site contamination, and is suitable for data collection activities that involve rapid, non-rigorous methods of analysis and QA.
- B. QA2 is a verification objective used to verify screened data (field or laboratory). A minimum of 10% verification of results is required. For candy, this requires verification using one of the by a US EPA-approved laboratory method (such as graphite furnace atomic absorption spectrometry (GFAAS) or inductively coupled plasma (ICP) analysis). This objective is suitable for data collection activities that require qualitative and/or quantitative verification of all or a select portion (10% or more) of the data. QA2 is intended to give a level of confidence for a select portion of the preliminary data.

VIII. METHOD PERFORMANCE

- a. These data are based on 45 data points, each representing ten replicate analyses of a fortified analytical portion of candy.
- b. Precision: The precision of the method, as determined by the statistical examination of laboratory test results is as follows:
 - i. Method Detection Limit - The EPA defines the method detection limit as, “the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.” To estimate the method detection limit, the standard deviation of non-consecutive replicate measurements must be multiplied by the rounded Student’s t-factor, as seen in the following equation:

$$MDL = 3\sigma$$

where σ is the standard deviation for the replicates and the Student’s t-factor is approximately equal to 3 (Federal Register, 1984). The MDL for this protocol was determined to be 5.45, based on this equation:

$$MDL = 3\sigma = 3(1.82) = 5.45 \text{ mg/kg}$$

where the standard deviation was found for ten non-consecutive replicate measurements at the minimum detectable concentration of 9.83 mg/kg.

- ii. Repeatability – The difference between successive results obtained by the same operator with the same apparatus under constant operating conditions on identical test material would exceed the following values only in one case in 20. The repeatability coefficient is a precision measure which represents the value below which the absolute difference between two repeated test results may be expected to lie with a probability of 95% (see Table 1):

$$\text{Repeatability} = 5.72 \sqrt{x}$$

where x is the average value of ten results, in mg/kg.

- iii. Reproducibility – The difference between two single and independent results obtained by different operators working on identical test material would exceed the following values only in 1 case in 20 (see Table 1):

$$\text{Reproducibility} = 9.83 \sqrt{x}$$

where x is the average value of ten results, in mg/kg.

- iv. Bias – The bias of this test method varies with concentration, as shown in Table 2:

$$\text{Bias} = \text{Concentration found} - \text{Concentration expected}$$

VIII. Reference

Federal Register (1984), Vol. 49, No. 209.

Table A: Repeatability and Reproducibility for Method to Determine Concentration of Lead in Candy by X-Ray Fluorescence, Bulk Mode

Approximate Average Value (mg/kg)	Repeatability (mg/kg)	Reproducibility (mg/kg)
9	17.3156	29.7575
12	20.2197	34.7481
16	23.4429	40.2874
20	26.2023	45.0296
22	26.5269	45.5873
28	30.3316	52.1259
33	33.2887	57.2077
37	35.2261	60.5372
42	35.853	61.6146
44	37.6722	64.7408
49	40.4336	69.4864
53	41.3545	71.0889
58	43.8131	75.2942
63	44.9237	77.2027
67	46.8338	80.4854
70	47.8279	82.1937
74	49.3124	84.7481
80	50.9186	87.5052
86	53.2808	91.5647

Table B: Recovery and Bias data for Method to Determine Concentration of Lead in Candy by X-Ray Fluorescence, Bulk Soil Sample Mode

Concentration expected (mg/kg)	Concentration found (mg/kg)	Bias (mg/kg)	Percent Bias (%)
9.528	8.692	-0.836	-8.774
10.27	28.119	17.849	173.7975
14.56	9.164	-5.396	-37.06
16.19	13.419	-2.771	-17.116
18.72	12.49556	-6.22444	-33.25
25.69	16.797	-8.893	-34.617
27.28	21.507	-5.773	-21.162
29.55	16.985	-12.565	-42.521
31.48	20.984	-10.496	-33.342
39.78	26.725	-13.055	-32.818
49.98	33.869	-16.111	-32.235
51.29	48.723	-2.567	-5.005
51.51	37.926	-13.584	-26.372
53.14	39.288	-13.852	-26.067
55.59	38.905	-16.685	-30.014
57.94	58.67	0.73	1.2599
63.13	43.376	-19.754	-31.291
63.83	61.682	-2.148	-3.365
66.41	69.915	3.505	5.2778
67.01	49.968	-17.042	-25.432
67.84	52.27	-15.57	-22.951
69.87	63.128	-6.742	-9.649
73.63	67.039	-6.591	-8.952
74.42	74.328	-0.092	-0.124

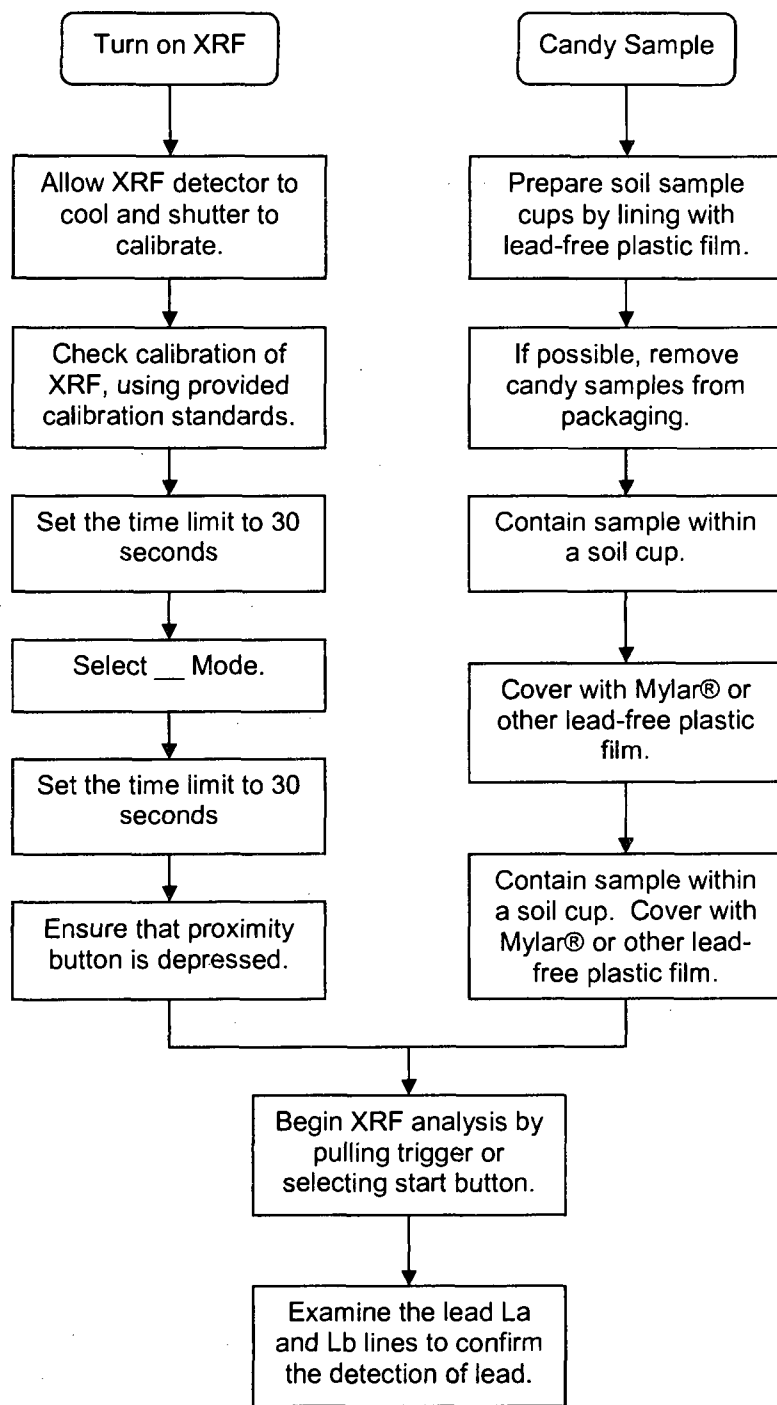


Figure A: Test Method for Determination of Lead Concentration in Candy by X-Ray Fluorescence Spectrometry (XRF)

DATA VALUES FOR ALL SAMPLES

Table C: Data Values for Samples

Sample (Read)	GFAAS-Determined Concentration	XRF-Determined Concentration		
		Bulk Sample Mode (ppm)	Thin Layer Mode ($\mu\text{g}/\text{cm}^2$)	Plastics Mode (ppm)
FAP 01 (01)		nd	nd	nd
FAP 01 (02)		nd	nd	nd
FAP 01 (03)		nd	nd	nd
FAP 01 (04)		nd	nd	nd
FAP 01 (05)		nd	nd	nd
FAP 01 (06)		nd	nd	nd
FAP 01 (07)		nd	nd	nd
FAP 01 (08)	11.04	nd	nd	nd
FAP 01 (09)	10.22	nd	nd	nd
FAP 01 (10)	9.632	nd	nd	nd
FAP 01 (Mean)	10.3 ppm	nd	nd	nd
FAP 02 (01)		9.48	nd	nd
FAP 02 (02)		nd	1.61	nd
FAP 02 (03)		8.92	1.43	nd
FAP 02 (04)		8.43	1.36	8.03
FAP 02 (05)		nd	1.64	nd
FAP 02 (06)		8.75	2.23	nd
FAP 02 (07)		nd	1.79	8.99
FAP 02 (08)	9.484	nd	1.79	nd
FAP 02 (09)	9.513	nd	1.49	nd
FAP 02 (10)	9.588	7.88	nd	nd
FAP 02 (Mean)	9.528 ppm	nd	nd	nd
FAP 03 (01)		8.05	nd	nd
FAP 03 (02)		8.6	1.61	nd
FAP 03 (03)		nd	1.43	nd
FAP 03 (04)		nd	1.36	8.03
FAP 03 (05)		8.87	1.64	nd
FAP 03 (06)		10.62	2.23	nd
FAP 03 (07)		9.68	1.79	8.99
FAP 03 (08)	14.09	nd	1.79	nd
FAP 03 (09)	14.87	nd	1.49	nd
FAP 03 (10)	14.72	nd	nd	nd

FAP 03 (Mean)	14.56 ppm	nd	nd	nd
FAP 04 (01)		14.04	2.41	8.23
FAP 04 (02)		nd	2.62	9.03
FAP 04 (03)		10.75	2.02	13.56
FAP 04 (04)		14.66	1.75	11.32
FAP 04 (05)		18	1.87	12.88
FAP 04 (06)		9.42	1.85	11.32
FAP 04 (07)	19.01	11.28	1.6	12.5
FAP 04 (08)	18.61	10.09	nd	9.05
FAP 04 (09)	18.54	11.59	2.52	8.75
FAP 04 (10)	18.72	12.63	1.48	14.92
FAP 04 (Mean)	19.01 ppm	nd	nd	11.156
FAP 05 (01)		13.56	2.15	8.18
FAP 05 (02)		10.78	1.4	16.61
FAP 05 (03)		15.9	2.93	12.36
FAP 05 (04)		14.41	3.3	13.49
FAP 05 (05)		13.48	3.26	8.25
FAP 05 (06)		14.16	2.52	10.77
FAP 05 (07)		17.83	2.98	13.06
FAP 05 (08)	16.51	14.42	1.92	12.48
FAP 05 (09)	15.82	9.55	2.1	14.47
FAP 05 (10)	16.25	10.1	2.86	14.25
FAP 05 (Mean)	16.19 ppm	13.419ppm	3.208 ug/cm2	12.392 ppm
FAP 06 (01)		14.94	2.52	13.41
FAP 06 (02)		19.5	2.43	19.36
FAP 06 (03)		16.56	3.18	11.84
FAP 06 (04)		16.91	2.87	13.55
FAP 06 (05)		14.1	3.12	13.26
FAP 06 (06)		18.85	2.52	19.11
FAP 06 (07)		13.77	4.34	13.37
FAP 06 (08)	25.45	19.97	4.29	16.56
FAP 06 (09)	25.89	15.02	2.9	21.67
FAP 06 (10)	25.74	18.35	3.91	16.21
FAP 06 (Mean)	25.69 ppm	16.797 ppm	3.208 ug/cm2	15.834 ppm
FAP 07 (01)		16.17	2.94	18.13
FAP 07 (02)		15.32	2.74	16.02
FAP 07 (03)		19.91	3.99	15.97
FAP 07 (04)		22.43	2.75	16.59
FAP 07 (05)		12.26	3.48	17.41
FAP 07 (06)		17.56	3.51	9.36
FAP 07 (07)		26.19	3.06	16.27

FAP 07 (08)	29.92	10.13	2.7	19.1
FAP 07 (09)	29.22	14.32	3.21	13.01
FAP 07 (10)	29.52	15.56	3.7	14.53
FAP 07 (Mean)	29.55 ppm	16.985 ppm	3.208 ug/cm2	15.639 ppm
FAP 08 (01)		20.98	3.89	19.94
FAP 08 (02)		20.23	4.06	25.5
FAP 08 (03)		22.55	3.89	24.32
FAP 08 (04)		19.23	3.39	20.79
FAP 08 (05)		14.39	4.19	22.16
FAP 08 (06)		19.26	3.36	21.52
FAP 08 (07)		22.83	3.81	19.21
FAP 08 (08)	31.96	23.75	4.31	20.3
FAP 08 (09)	31.27	24.49	3.95	21.44
FAP 08 (10)	31.2	22.13	4.05	21.34
FAP 08 (Mean)	31.48 ppm	20.984 ppm	3.890 ug/cm2	21.652 ppm
FAP 09 (01)		22.16	3.44	18.99
FAP 09 (02)		22.26	3.52	22.37
FAP 09 (03)		20.06	3.26	25.16
FAP 09 (04)		19.41	3.5	26.67
FAP 09 (05)		20.43	2.68	25.84
FAP 09 (06)		22.08	3.68	21.49
FAP 09 (07)		18.53	3.01	24.49
FAP 09 (08)	26.96	18.67	3.46	25.16
FAP 09 (09)	27.37	24.6	3.26	20.39
FAP 09 (10)	27.5	26.87	3.38	21.08
FAP 09 (Mean)	27.28 ppm	21.507 ppm	3.319 ug/cm2	23.164 ppm
FAP 10		<i>Sample lost</i>		
FAP 11 (01)		29.25	5.1	30.79
FAP 11 (02)		31.01	4.88	30.09
FAP 11 (03)		26.81	5.79	27.48
FAP 11 (04)		30.5	3.84	22.41
FAP 11 (05)		26.35	3.57	32.7
FAP 11 (06)		26.84	5.54	27.42
FAP 11 (07)		29.4	4.32	27.19
FAP 11 (08)	39.51	27.5	4.08	23.79
FAP 11 (09)	40.25	26.84	4.47	31.47
FAP 11 (10)	41.05	26.69	5.19	30.45
FAP 11 (Mean)	40.27 ppm	28.119 ppm	4.678 ug/cm2	28.379 ppm
FAP 12 (01)		32.18	4.74	27.76
FAP 12 (02)		26.05	5.64	35.4
FAP 12 (03)		24.8	4.26	31.74

FAP 12 (04)		22.48	3.52	31.24
FAP 12 (05)		24.68	4.93	31.04
FAP 12 (06)		26.8	5.66	25.21
FAP 12 (07)		28.63	5.27	25.87
FAP 12 (08)	39.16	30.31	4.08	28.95
FAP 12 (09)	39.34	24.79	5.46	30.11
FAP 12 (10)	40.83	26.53	5.48	29.51
FAP 12 (Mean)	39.777 ppm	26.725 ppm	4.904 ug/cm2	29.683 ppm
FAP 13 (01)		37.42	7.38	32.64
FAP 13 (02)		38.67	6.89	32.27
FAP 13 (03)		37.62	6.33	40.56
FAP 13 (04)		36	6.4	41.79
FAP 13 (05)		37.88	7.05	39.16
FAP 13 (06)		33.08	6.63	33.32
FAP 13 (07)		39.51	5.69	39.25
FAP 13 (08)	55.22	39.93	6.77	29.15
FAP 13 (09)	49.87	41.02	6.04	40.57
FAP 13 (10)	49.46	38.13	6.94	36.99
FAP 13 (Mean)	51.517 ppm	37.926 ppm	6.612 ug/cm2	36.57 ppm
FAP 14 (01)		32.24	6.66	27.02
FAP 14 (02)		39.29	5.26	38.571
FAP 14 (03)		37.06	7.26	33.064
FAP 14 (04)		34.85	6.61	30.153
FAP 14 (05)		28.4	6.4	37.649
FAP 14 (06)		32.27	5.02	36.653
FAP 14 (07)		32.45	5.9	32.241
FAP 14 (08)	49.46	33.39	6.04	28.65
FAP 14 (09)	50.41	38.14	6.46	29.505
FAP 14 (10)	50.07	30.6	6.12	31.711
FAP 14 (Mean)	49.98 ppm	33.869 ppm	6.173 ug/cm2	32.522 ppm
FAP 15 (01)		38.16	8.21	50.23
FAP 15 (02)		40.43	5.5	40.66
FAP 15 (03)		45.46	5.22	51.33
FAP 15 (04)		32.03	7.62	37.89
FAP 15 (05)		36.52	8.24	46.53
FAP 15 (06)		38.64	7.72	39.32
FAP 15 (07)		39.01	7.77	40.9
FAP 15 (08)	55.4	44.55	7.5	45.35
FAP 15 (09)	52.08	37.91	7.67	45.75
FAP 15 (10)	51.95	40.17	8.33	44.47
FAP 15 (Mean)	53.14 ppm	39.288 ppm	7.378 ug/cm2	44.243 ppm

FAP 16 (01)		48.9	7.86	43.24
FAP 16 (02)		47.45	7.97	40.75
FAP 16 (03)		44.76	8.84	45.79
FAP 16 (04)		47.75	9.39	53.46
FAP 16 (05)		46.64	8.84	49.95
FAP 16 (06)		56.76	11.6	51.52
FAP 16 (07)		54.24	8.93	47.96
FAP 16 (08)	69.06	49.2	9.87	53.8
FAP 16 (09)	65.53	54.51	8.67	48.1
FAP 16 (10)	66.44	49.47	8.53	50.88
FAP 16 (Mean)	67.01 ppm	49.968 ppm	9.05 ug/cm2	48.545 ppm
FAP 17 (01)		37.49	7.36	39.5
FAP 17 (02)		38.37	5.84	35.29
FAP 17 (03)		38.88	7.84	42.79
FAP 17 (04)		43.43	6.89	37.33
FAP 17 (05)		39.95	6.76	42.62
FAP 17 (06)		34.35	6.22	41.36
FAP 17 (07)		38.03	6.71	39.15
FAP 17 (08)	57.43	42.29	6.66	35.88
FAP 17 (09)	56.07	40.74	7.78	45.03
FAP 17 (10)	53.27	35.52	7.53	40.37
FAP 17 (Mean)	55.59 ppm	38.905 ppm	6.959 ug/cm2	39.932 ppm
FAP 18 (01)		37.05	9.56	44.26
FAP 18 (02)		42.11	7.7	46.35
FAP 18 (03)		42.08	8.51	39.33
FAP 18 (04)		45.29	8.11	47.13
FAP 18 (05)		48.16	8.53	42.33
FAP 18 (06)		45.5	8.43	44.88
FAP 18 (07)		44.2	8.64	46.9
FAP 18 (08)	62.36	45.5	7.48	48.84
FAP 18 (09)	63.16	42.03	7.65	44.94
FAP 18 (10)	63.88	41.84	7.46	44.98
FAP 18 (Mean)	63.13 ppm	43.376 ppm	8.207 ug/cm2	44.994 ppm
FAP 19 (01)		49.67	8.56	55.01
FAP 19 (02)		54.46	8.13	58.2
FAP 19 (03)		49.73	8.78	58.88
FAP 19 (04)		50.51	7.77	61.26
FAP 19 (05)		56.26	8.62	54.84
FAP 19 (06)		46.73	8.48	45.78
FAP 19 (07)		63.23	8.62	46.48
FAP 19 (08)	68.88	50.27	8.69	61.39
FAP 19 (09)	68.54	53.78	8.44	59.56

FAP 19 (10)	66.09	48.06	9.15	51.36
FAP 19 (Mean)	67.84 ppm	52.27 ppm	8.524 ug/cm2	55.276 ppm
FAP 20 (01)		64.71	11.58	59.57
FAP 20 (02)		61.73	9.51	54.88
FAP 20 (03)		56.96	9.47	61.66
FAP 20 (04)		57.32	10.36	66.78
FAP 20 (05)		55.87	10.51	51.59
FAP 20 (06)		53.47	10.57	56.31
FAP 20 (07)		64.34	10.77	52.56
FAP 20 (08)	58.47	60.65	10.7	57.07
FAP 20 (09)	60.18	54.31	10.26	60.52
FAP 20 (10)	55.16	57.34	9.67	56.05
FAP 20 (Mean)	57.94 ppm	58.67 ppm	10.34 ug/cm2	57.699 ppm
FAP 21 (01)		51.21	8.54	51.07
FAP 21 (02)		50.32	7.62	52.24
FAP 21 (03)		48.31	7.25	52.95
FAP 21 (04)		49.25	7.4	50.44
FAP 21 (05)		45.79	7.94	51.54
FAP 21 (06)		45.7	7.31	45.32
FAP 21 (07)		55.2	9.38	47.39
FAP 21 (08)	51.66	45.19	8.52	46.83
FAP 21 (09)	50.53	47.13	7.99	51.61
FAP 21 (10)	51.66	49.13	7.87	48
FAP 21 (Mean)	51.29 ppm	48.723 ppm	7.982 ug/cm2	49.739 ppm
FAP 22 (01)		76.52	11.59	80.48
FAP 22 (02)		78.18	12.84	72.62
FAP 22 (03)		71.11	11.73	73.44
FAP 22 (04)		75.83	12.92	68.98
FAP 22 (05)		64.92	13.19	74.81
FAP 22 (06)		79.62	12.39	69.33
FAP 22 (07)		72.44	13.3	67.22
FAP 22 (08)	77.71	84.48	12.17	75.6
FAP 22 (09)	71.66	68.17	13.14	71.23
FAP 22 (10)	73.9	72.01	12	75.31
FAP 22 (Mean)	74.42 ppm	74.328 ppm	12.527 ug/cm2	72.902 ppm
FAP 23 (01)		65.82	11.78	66.7
FAP 23 (02)		59	11.18	70.26
FAP 23 (03)		61.44	11.06	65.14
FAP 23 (04)		64.81	11.77	71.43
FAP 23 (05)		61.7	10.32	59.42
FAP 23 (06)		57.42	11.05	55.17

FAP 23 (07)		54.08	10.91	65.93
FAP 23 (08)	67.55	65.81	11.52	64.15
FAP 23 (09)	62.4	65.07	11.17	72.72
FAP 23 (10)	61.53	61.67	11.31	71.01
FAP 23 (Mean)	63.83 ppm	61.682 ppm	11.207 ug/cm2	66.193 ppm
FAP 24 (01)		70.49	10.98	81.64
FAP 24 (02)		72.97	12.92	72.4
FAP 24 (03)		66.75	12.77	73.27
FAP 24 (04)		69.4	12.03	68.7
FAP 24 (05)		83.15	12.21	68.82
FAP 24 (06)		61.17	12.65	71.64
FAP 24 (07)		73.9	12.97	81.59
FAP 24 (08)	69.42	66.12	12.3	68.11
FAP 24 (09)	64.43	74.41	12.42	63.49
FAP 24 (10)	65.39	60.79	12.26	69.99
FAP 24 (Mean)	66.41 ppm	69.915 ppm	12.351 ug/cm2	71.965 ppm
FAP 25 (01)		59.46	10.08	66.93
FAP 25 (02)		64.26	10.4	65.65
FAP 25 (03)		60.06	9.92	78.12
FAP 25 (04)		62.7	10.45	81.31
FAP 25 (05)		58.33	10.1	81.1
FAP 25 (06)		60.59	10.01	69.69
FAP 25 (07)		64.67	10.02	69.9
FAP 25 (08)	74.74	72.1	8.75	72.51
FAP 25 (09)	67.53	65.87	10.45	70.48
FAP 25 (10)	67.33	63.24	11.16	73.74
FAP 25 (Mean)	69.87 ppm	63.128 ppm	10.134 ug/cm2	72.943 ppm
FAP 26 (01)		67.76	10.06	70.16
FAP 26 (02)		68.26	8.82	63.9
FAP 26 (03)		62.9	9.3	66.19
FAP 26 (04)		68.21	9.11	64.19
FAP 26 (05)		66.9	10.77	70.98
FAP 26 (06)		64.87	9.86	69.55
FAP 26 (07)		68.48	9.85	66.69
FAP 26 (08)	73.62	68.37	10.26	65.47
FAP 26 (09)	73.9	62.81	10.38	64.57
FAP 26 (10)	73.38	71.83	10.04	80.27
FAP 26 (Mean)	73.63 ppm	67.039 ppm	9.845 ug/cm2	68.197 ppm
FAP 27 (01)		70.69	11.37	77.00
FAP 27 (02)		71.68	11.88	79.62
FAP 27 (03)		80.42	12.34	85.36

FAP 27 (04)		72.49	11.67	78.83
FAP 27 (05)		68.8	11.99	75.08
FAP 27 (06)		76.83	10.68	85.53
FAP 27 (07)		82.2	10.7	72.7
FAP 27 (08)		64.13	12.18	72.29
FAP 27 (09)		62.7	11.12	80.32
FAP 27 (10)		72.78	10.98	74.51
FAP 27 (Mean)		72.272 ppm	11.491 ug/cm2	78.124 ppm
FAP 28 (01)		94.62	11.43	74.15
FAP 28 (02)		94.45	12.92	77.32
FAP 28 (03)		87.8	12.57	89.72
FAP 28 (04)		87.11	12.18	75.7
FAP 28 (05)		83.94	12.1	75.59
FAP 28 (06)		80.84	12.57	81.25
FAP 28 (07)		98.45	12.37	79.24
FAP 28 (08)		93.39	12.58	86.21
FAP 28 (09)		86.25	12.1	85.68
FAP 28 (10)		86.12	12.43	79.26
FAP 28 (Mean)		89.297 ppm	12.325 ug/cm2	80.412 ppm
FAP 29 (01)		81.24	12.38	87.25
FAP 29 (02)		74.28	12.84	81.16
FAP 29 (03)		75.8	10.48	93.73
FAP 29 (04)		70.05	13.41	90.51
FAP 29 (05)		84.77	13.79	85.07
FAP 29 (06)		77.74	12.84	91.84
FAP 29 (07)		79.96	12.77	87.42
FAP 29 (08)		83.44	13.02	91.19
FAP 29 (09)		78.45	12.89	83.48
FAP 29 (10)		86.7	11.86	81.27
FAP 29 (Mean)		79.243 ppm	12.628 ug/cm2	87.292 ppm
FAP 30 (01)		90.39	14.53	85.7
FAP 30 (02)		78.62	15.41	77.09
FAP 30 (03)		81.25	14.01	88.29
FAP 30 (04)		98.22	13.56	84.12
FAP 30 (05)		94.9	15.22	86.74
FAP 30 (06)		84.95	15.81	93.93
FAP 30 (07)		81.16	15.21	81.04
FAP 30 (08)		75.17	13.28	87.02
FAP 30 (09)		86.06	14.54	85.81
FAP 30 (10)		96.94	13.64	85.75
FAP 30 (Mean)		86.766 ppm	14.521 ug/cm2	85.549 ppm

MDL 03 (01)		11.77	1.37	16.08
MDL 03 (02)		15.04	nd	16.16
MDL 03 (03)		10.42	1.92	16.34
MDL 03 (04)		16.08	1.65	16.95
MDL 03 (05)		13.4	1.31	10.58
MDL 03 (06)		14.29	1.5	17.21
MDL 03 (07)		17.35	1.79	14.59
MDL 03 (08)	17.60	13.72	1.74	17.06
MDL 03 (09)	16.79	14.63	1.72	9.56
MDL 03 (10)	17.32	8.69	nd	17.48
MDL 03 (Mean)	17.24 ppm	13.539 ppm	nd	15.201 ppm
MDL 04 (01)		12.25	1.034	10.33
MDL 04 (02)		13.05	nd	13.53
MDL 04 (03)		9.18	1.648	nd
MDL 04 (04)		9.99	1.557	12.77
MDL 04 (05)		10.6	1.12	12.61
MDL 04 (06)		12.2	1.08	13.31
MDL 04 (07)		10.33	nd	14.55
MDL 04 (08)	11.94	10.75	1.32	12.55
MDL 04 (09)	11.5	13.47	1.22	10.2
MDL 04 (10)	11.51	14.75	1.46	9.04
MDL 04 (Mean)	11.65 ppm	11.657 ppm	nd	nd
MDL 05 (01)		8.72	2.2	9.01
MDL 05 (02)		11.56	2.97	9.98
MDL 05 (03)		8.89	2.89	13.74
MDL 05 (04)		14.6	2.89	8.11
MDL 05 (05)		8.77	2.58	12.47
MDL 05 (06)		11.75	1.66	8.5
MDL 05 (07)		8.82	2.88	13.65
MDL 05 (08)	11.92	8.24	2.14	8.86
MDL 05 (09)	12.22	nd	1.94	8.48
MDL 05 (10)	12.92	8.86	2.09	13.05
MDL 05 (Mean)	12.35 ppm	nd	2.424 ug/cm2	10.585 ppm
MDL 06 (01)		8.82	1.69	8.86
MDL 06 (02)		13.02	2.02	8.3
MDL 06 (03)		9.33	2.02	8.93
MDL 06 (04)		8.37	1.93	nd
MDL 06 (05)		10.6	3.65	10.04
MDL 06 (06)		7.61	nd	nd
MDL 06 (07)		11.1	1.72	10.26
MDL 06 (08)	8.803	7.85	2.18	nd
MDL 06 (09)	8.474	9.57	2.58	nd

MDL 06 (10)	8.417	12.07	2.26	10.32
MDL 06 (Mean)	8.565 ppm	9.834 ppm	nd	nd
MDL 07 (01)		8.32	1.73	17
MDL 07 (02)		7.73	nd	9.17
MDL 07 (03)		nd	nd	8.17
MDL 07 (04)		nd	1.61	14.39
MDL 07 (05)		nd	nd	22.92
MDL 07 (06)		nd	1.87	17.79
MDL 07 (07)		nd	nd	16.3
MDL 07 (08)	14.03	nd	nd	12.47
MDL 07 (09)	13.77	nd	nd	17.69
MDL 07 (10)	13.46	8.01	nd	13.47
MDL 07 (Mean)	13.76 ppm	nd	nd	14.937 ppm
MDL 08 (01)		9.62	1.63	17.16
MDL 08 (02)		10.49	nd	16.91
MDL 08 (03)		10.87	1.63	17.98
MDL 08 (04)		11.66	2.33	15.58
MDL 08 (05)		11.54	1.58	12.11
MDL 08 (06)		9.75	2.09	12.15
MDL 08 (07)		10.16	1.45	16.36
MDL 08 (08)	14.49	14.4	2.15	17.78
MDL 08 (09)	14.82	8.88	1.57	19.68
MDL 08 (10)	14.55	8.59	1.53	18.92
MDL 08 (Mean)	14.62 ppm	10.596 ppm	nd	16.463 ppm
MDL 09 (01)		15.13	1.14	15.83
MDL 09 (02)		nd	nd	13.73
MDL 09 (03)		11.14	1.19	13.93
MDL 09 (04)		15.12	nd	9.16
MDL 09 (05)		15.12	nd	18.49
MDL 09 (06)		nd	1.07	10.76
MDL 09 (07)		nd	1.36	12.31
MDL 09 (08)	14.29	nd	nd	13.43
MDL 09 (09)	13.61	nd	nd	18.7
MDL 09 (10)	14.65	nd	nd	15.56
MDL 09 (Mean)	14.18 ppm	nd	nd	14.19 ppm
MDL 10 (01)		9.53	1.87	14.84
MDL 10 (02)		nd	1.53	17.42
MDL 10 (03)		nd	1.34	15.61
MDL 10 (04)		nd	1.25	14.81
MDL 10 (05)		nd	1.75	12.69
MDL 10 (06)		nd	nd	14.52

MDL 10 (07)		nd	1.32	18.94
MDL 10 (08)	15.74	13.49	nd	17.6
MDL 10 (09)	15.57	nd	nd	20.37
MDL 10 (10)	15.79	nd	1.27	15.81
MDL 10 (Mean)	15.7 ppm	nd	nd	16.261 ppm
MDL 11 (01)			1.4	16.16
MDL 11 (02)			2.25	16.68
MDL 11 (03)			1.23	26.57
MDL 11 (04)			nd	16.27
MDL 11 (05)			nd	16.65
MDL 11 (06)			2.12	16.49
MDL 11 (07)			1.99	25.18
MDL 11 (08)	25.37		1.48	15.6
MDL 11 (09)	25.93		1.46	14.24
MDL 11 (10)	25.58		1.67	16.76
MDL 11 (Mean)	25.63 ppm		nd	18.06 ppm
MDL 12 (01)		19.79	2.38	14.77
MDL 12 (02)		18.09	1.62	15.17
MDL 12 (03)		12.1	2.78	14.31
MDL 12 (04)		11.29	1.4	8.5
MDL 12 (05)		12.83	2.05	18.59
MDL 12 (06)		9.56	1.54	11.04
MDL 12 (07)		8.73	1.99	9.59
MDL 12 (08)		11.85	2.15	13.89
MDL 12 (09)		13.87	2.32	10.23
MDL 12 (10)		15.21	1.65	14.38
MDL 12 (Mean)		13.332 ppm	1.988 ug/cm2	13.047 ppm
MDL 13 (01)		14.7	2.09	22.76
MDL 13 (02)		20.82	2.37	26.62
MDL 13 (03)		19.63	2.42	18.38
MDL 13 (04)		18.21	1.59	15.35
MDL 13 (05)		16.57	2.34	16.81
MDL 13 (06)		12.65	nd	24.85
MDL 13 (07)		16.38	2.66	17.74
MDL 13 (08)		15.2	2.47	17.22
MDL 13 (09)		20.09	1.81	16.98
MDL 13 (10)		19.3	3.13	14.99
MDL 13 (Mean)		17.355 ppm	nd	19.17 ppm
MDL 14 (01)		10.53	2.28	16.12
MDL 14 (02)		21.44	1.93	15.07
MDL 14 (03)		17.26	2.08	16.79

MDL 14 (04)		22.54	2.72	13.57
MDL 14 (05)		20.97	2.48	21.57
MDL 14 (06)		16.71	1.56	20.73
MDL 14 (07)		11.43	2.74	12.78
MDL 14 (08)	19.98	18.08	2.12	15.65
MDL 14 (09)	20.01	23.05	2.77	20.48
MDL 14 (10)	19.33	12.28	2.45	16.01
MDL 14 (Mean)	19.77 ppm	17.429 ppm	2.313 ug/cm2	16.877 ppm
MDL 15 (01)		21.53	2.52	24.79
MDL 15 (02)		17.38	2.84	21.24
MDL 15 (03)		23.65	1.15	15.29
MDL 15 (04)		20.66	2.22	24.49
MDL 15 (05)		19.58	2.84	21.44
MDL 15 (06)		21.71	2.55	23.18
MDL 15 (07)		21.84	2.42	29.14
MDL 15 (08)	24.58	21.08	2.44	21.66
MDL 15 (09)	24.44	24.35	2.29	22.73
MDL 15 (10)	23.9	14.79	2.79	17.79
MDL 15 (Mean)	24.31 ppm	20.657 ppm	2.406 ug/cm2	22.175 ppm
MDL 16 (01)		38.11	1.02	15.13
MDL 16 (02)		19.72	1.06	20.34
MDL 16 (03)		39.99	nd	24.71
MDL 16 (04)		23.78	1.59	15.32
MDL 16 (05)		35.67	1.47	15.07
MDL 16 (06)		23.76	1.5	19.52
MDL 16 (07)		26.45	0.96	17.85
MDL 16 (08)	20.14	16.14	1.04	19.39
MDL 16 (09)	20.27	23.45	1.83	13.25
MDL 16 (10)	20.3	19.83	0.99	13.82
MDL 16 (Mean)	20.24 ppm	26.69 ppm	nd	17.44 ppm
MDL 17 (01)		nd	nd	10.74
MDL 17 (02)		nd	nd	nd
MDL 17 (03)		nd	nd	nd
MDL 17 (04)		nd	nd	11.82
MDL 17 (05)		nd	nd	16.4
MDL 17 (06)		nd	nd	11.21
MDL 17 (07)		nd	nd	nd
MDL 17 (08)	11.01	nd	nd	11.38
MDL 17 (09)	12.01	nd	nd	14.44
MDL 17 (10)	12.61	nd	nd	10.11
MDL 17 (Mean)	11.88 ppm	nd	nd	nd

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