ChemRisk®

Chromium in Blood Evaluation

Submitted to: **Dr. Paul Van Buynder, MBBS MPH FAFPHM**
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Submitted by: **ChemRisk Canada, a division of ChemRisk LLP**
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ChemRisk®

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Dr. Paul Van Buynder, MBBS MPH FAFPHM
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Dear Dr. Van Buynder,

ChemRisk Canada, a division of ChemRisk LLP ('ChemRisk') is pleased to submit the enclosed report prepared for your Agency regarding interpretation of plasma and erythrocyte chromium levels in blood. Our interpretation is based on summaries of data obtained following collection of blood samples from residents that live in proximity to a residential community that was potentially impacted by a chromic acid spill.

Based on our statistical assessment and evaluation, and based on the information currently available and provided to us, we found no statistical difference in chromium levels in either the plasma or erythrocytes for residents that live in proximity to the spill Site when compared to a control population that lives outside of the spill area. This indicates that residential exposure to chromium is no different than that found in the normal control population.

If you have any questions regarding the content of this report, that you contact me directly at 707-527-2615, or via email at: bfinley@chemrisk.com at your convenience.

Sincerely,
Chem*Risk* LLP

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CONTENTS

TABLES

APPENDICES

1 Introduction and Objectives

ChemRisk® Canada, a division of ChemRisk® LLP ('ChemRisk') was retained by the Deputy Chief Medical Officer of Health, Dr. Paul Van Buynder, to conduct a review and statistical evaluation of data regarding chromium blood levels. The chromium in blood data was obtained following sampling and analysis of a sample population who purport that they were exposed to chromium as a result of a spill of chromic acid in groundwater in proximity to their residential neighbourhood (i.e., the Evergreen Park Subdivision, located outside of Fredericton, New Brunswick, Canada; herein referred to as the 'community'). The blood sampling program was conducted in partnership with the local Medical Officer of Health, the provincial Deputy Chief Medical Officer of Health, provincial Department of Health staff, and local hospital laboratory personnel. The monitoring program included the determination of chromium in plasma, in addition to chromium levels in red blood cells (RBCs). The blood monitoring program was established after the spill event occurred, and was completed between September-October, 2009.

The assessment of blood chromium was conducted using recognized sampling and analytical techniques, and included approximately 21 members of the sample population (i.e., 'cases') that lived in close proximity to where the spill occurred (the 'spill site'). Multiple samples were collected for each individual, and the results were tabulated using Excel. This information was submitted electronically to ChemRisk for review and independent statistical evaluation. The results of the blood sampling for chromium in plasma and red blood cells were assessed using a number of statistical tests to determine whether there was a significant difference between the sample population to that of the control group (i.e., the group that did not live in proximity to the Site). This comparison was used to determine whether any individual resident or the residents as a group had elevated chromium levels which might be indicative of potential exposure through contact with groundwater supplies.

2 Background and Study Design

2.1 Spill Event

In October 2008, a 'chromium trioxide' (i.e., 'chromic acid') spill event occurred at the 'Custom Machine and Hardchrome' company adjacent (and possibly down-gradient) to the Evergreen Park Subdivision ('the community') (Appendix A, Location Key). This particular community is located on the outskirts of Fredericton, New Brunswick. Shortly after the spill event, on or around October 28, 2008, the New Brunswick Department of Health (through the Medical Officer of Health's office) issued what is referred to as a "health advisory" to several dozen homeowners and approximately twenty businesses located in the Evergreen Park area. The advisory indicated that the community should not consume their well water or use it for bathing, and in general to reduce contact with groundwater until further notice.

Remedial work began shortly after the spill event; however, given the limited information available regarding groundwater flow direction (reported to be towards the north, and possibly away (down-gradient) from the residential community), and moreover as a result of the initial health advisory issued by the Department of Health, community residents became concerned about the potential for exposure

to chromium in groundwater through various direct and indirect exposure pathways including consumption of groundwater (through oral ingestion). Soon afterwards, several local residents had requested blood tests for chromium from their family physicians, and apparently one such result that was outside of the laboratory reference range was then reported to Public Health.

This prompted the local medical officer of health to initiate recruitment of individuals living in the community for the purpose of testing their blood for chromium. The blood testing (i.e., biomonitoring) included assessment of chromium in plasma and red blood cells (RBCs, erythocytes). Although the spill appears to have occurred down-gradient of the residential community, it was agreed that blood sampling would be the most definitive means to determine whether chromium exposure had occurred for residents living in the community.

2.2 Contaminants of Potential Concern

It was reported that an overflowing tank containing 'chromic acid' likely caused the spill, and the spill occurred at the industrial site (MOH, personal communication, 2009). In general, chromic acid connotes a collection of compounds generated by the acidification of solutions containing chromate and dichromate anions or the dissolving of chromium trioxide in sulfuric acid. Often the species are assigned the formulas H_2CrO_4 and $H_2Cr_2O_7$. The anhydride of these "chromic acids" is chromium trioxide, also called chromium (VI) oxide (i.e., CrO_3); industrially, this compound is sometimes sold and known as 'chromic acid'. Chromic acid displays chromium in an oxidation state of +6 (or VI), which is referred to as 'hexavalent chromium'. Additional characteristics regarding chromium and chromium related forms are presented in the following sections.

2.2.1 Chromium (Cr)

Briefly, chromium (Cr) is a naturally occurring element, which is frequently found in rocks within the earth's crust. By virtue of its ubiquity, chromium can be found and detected in different animal tissues and plant species. It is also found as a natural constituent in soil and in volcanic dust and gasses (Goyer, 2001). Chromium is present in the environment in several different forms. The most common forms are CrIII (trivalent) and CrVI (also referred to as hexavalent chromium). Additional details regarding these forms are provided below.

2.2.2 Trivalent Chromium (Cr III)

Trivalent chromium occurs naturally in the environment and can be found in many different foods such as seafood, in addition to a variety of vegetables. Chromium (III) is considered to be an essential trace nutrient; its essentiality is related to its function as a component of the 'glucose tolerance factor' (i.e., chromodulin; Jacquamet et al., 2003), therefore it is required for normal physiological and homeostatic functioning of the human body (Stoecker, 1996 as referenced in Goyer, 2001). Every person has detectable trivalent chromium levels in their blood. On the basis of its essentiality, the U.S. Food and Nutrition Board of the United States Academy of Sciences has determined that an estimated safe and adequate daily intake for chromium in adults ranges from 50 to 200 µg (NRC, 1989). The Institute of

Medicine (IOM, 2001) of the U.S. National Academy of Sciences estimates that an adequate intake of chromium is between 20-45 µg chromium III/day for adolescents and adults and is normally required for healthy homeostatic functioning. Chromium is typically excreted from the human body at approximately 10 µg/day for humans in the absence of exposure (e.g., occupational exposure, etc.). Critical reviews regarding the adsorption, distribution, and metabolism of chromium in humans following ingestion of drinking water are provided by Finley et al. (1997) and Kerger et al. (1996).

Cr(III) does not easily cross the membrane of the red blood cell (RBC); Cr(III) that is ingested and crosses into the bloodstream largely remains in the plasma compartment of the blood. Hence, ingestion of Cr(III) may cause an increase in plasma chromium levels, but does not result in an increase in RBC chromium levels (except at very high doses). The increased plasma levels of Cr(III) typically return to "background" within hours of ingestion due to the rapid clearance of Cr(III) from the bloodstream.

2.2.3 Hexavalent Chromium

Hexavalent (i.e., Cr VI) forms of chromium do not occur naturally, rather they are man-made. Chromium (VI) compounds are widely used as corrosion inhibitors, in the manufacture of pigments, in metal finishing and chrome plating, in stainless-steel production, in leather tanning, and in wood preservatives (Costa 1997, ATSDR 2008). When hexavalent chromium is ingested, (i.e., consumption of drinking water containing hexavalent chromium; chromic acid), most or all of the hexavalent chromium is converted to trivalent chromium in the stomach (due to the acidic conditions of the stomach) before the remainder is absorbed into the bloodstream.

Unlike Cr(III), any Cr(VI) that is absorbed into the bloodstream can cross the RBC membrane, where it rapidly binds to intracellular proteins, including hemoglobin (Kerger et al., 1997). The life of a RBC is approximately 120 days (Shemin and Rittenberg, 1946). Hence, if the amount of ingested Cr(VI) is sufficient to overwhelm the reductive capacity of the gastrointestinal tract, both plasma and RBC chromium levels may be elevated for a few hours after ingestion, while RBC chromium levels may remain elevated for several weeks.

2.3 Biological Monitoring for Chromium

In general, biological monitoring (e.g., sampling of blood or urine) can be an efficient and effective tool for monitoring individual exposure for many compounds, including chromium. The most reliable measure of hexavalent or trivalent chromium exposure, and the most direct way to determine if one's exposure is potentially higher than normal, is to sample the blood.

As mentioned previously, there are two compartments in the whole blood: the RBCs and the plasma; while chromium plasma levels can be useful for evaluating recent exposure to Cr(III) and Cr(VI) compounds, chromium RBC levels provide information regarding exposure to Cr(VI).

It is important to note that blood chromium levels (both plasma and RBC) for any individual may vary widely throughout the day. This is primarily due to fluctuations in dietary chromium intake and

excretion. Therefore, in order to properly assess typical chromium levels in an individual or group of individuals, it is usually necessary to collect more than one sample from each individual. In this analysis, up to 3 samples were collected from each study participant. Also, as noted earlier, all individuals have measurable levels of chromium in their plasma and RBCs due to chromium in the diet, and therefore the mere presence of chromium in either of these blood compartments is not indicative of an unusual or elevated chromium exposure. In order to determine whether unusual or elevated exposure to chromium has occurred, it is necessary to compare the blood chromium levels in the "potentially exposed" group to a normal or "background" value.

2.4 Study Design

2.4.1 Study Area and Subjects

Residents (initially n = 23) were recruited from the Evergreen Park Subdivision area (the community), which is the residential community that is in close proximity to the Melissa Street chromic acid spill site (i.e., spill from the 'Custom Machine and Hardchrome' facility). It was reported that in the course of the blood sample collection efforts, two residents opted to drop out of the study.

For comparison purposes, a "control" or "background" group was established. Individuals in the control group did not have any potential for contact with groundwater from the spill site. In general, individuals in the control population lived and worked in locations fairly distant from the spill site than the case group. The 'control' group (n=25) included workers from the laboratory that performed the analytical test, in addition to the Medical Officer of Health. The study population consisted of 10 males and 11 females (n = 21), while the control group consisted of twenty-one (21) females and four (4) males (n = 25). There were no significant differences between the average age of cases and controls. The study group was equally represented by males and females, while the control group had a relatively much higher proportion of females.

Because the blood chromium levels for any particular individual can vary throughout the day (e.g., as a result of diet, fasting, intake of dietary supplements such as vitamin and mineral supplements, etc.), three (3) samples were obtained from each subject over the course of a three-week sampling period. In order to establish 'background', or baseline levels of chromium in blood, the group of control subjects was also sampled up to three times over a three-week period.

During the recruitment and sampling process, attempts were made to interview (i.e., survey) all subjects. The summaries of the surveys were made available for review to ChemRisk. Four control group subjects did not respond to the questionnaires, which included a summary of gender, age, tobacco use and exposure to environmental tobacco use, potential exposure to chromium through dental care (i.e., mercury amalgam fillings), vitamin consumption, in addition to potential for exposure to chromium through the use of household products. None of the residents or control subjects reported being currently employed in any industries where chromium exposure might occur, such as chrome plating, dry-cleaning, welding, or printing. Two case subjects reported previously working in the welding industry, while 1 control subject reported working in the welding industry.

2.4.2 Collection Procedure for Plasma

Blood samples (up to three samples collected over a three week period) were obtained in such a way as to minimize contamination. Specifically, the conventional use of a needle and syringe was avoided for the purpose of minimizing its potential as a contributor of chromium (Versieck et al., 1982; Minoia et al., 1992; Christiansen et al., 1993). No directions were provided to subjects regarding consumption of seafood (which often contain high levels of chromium) or dietary supplements (including chromium supplements) and no subjects were requested to fast prior to sampling.

The procedure used for collecting plasma samples in the study is described as follows:

1. For each individual, one (1) tube of blood was collected using either a 10 ml glass Sodium Heperanized green top 'vacutainer' tube or an alternative Royal Blue Royal Blue top trace metal tube or Green top tube. The vacutainer tube was obtained from Becton Dickinson (Ref# 366480).

2. Following collection, the vacutainer tube was gently mixed, then centrifuged with the stopper on for 15 minutes. The samples were required to be spun and separated within 30 minutes (i.e., separation of plasma from the red blood cells (i.e., erythrocytes).

3. Transfer the plasma, using a polypropylene transfer pipette into a 7 ml Sarstedt polypropylene tube.

4. Store at 4ºC, or -20ºC until mailing to the laboratory for analysis.

The RBC samples were also kept on ice until analysis was carried out. Trace metal analysis was conducted within a very short period (i.e., < than 1 month) of blood collection.

2.4.3 Chemical Analysis

The samples were analyzed at the 'London Trace Metal laboratory' located in London, Ontario. The analysis was conducted using an ICP-MS (Model Finnigan Element High Resolution ICP-MS). The Finnigan MAT Element High Resolution ICP-MS combines an ion source (or ICP), which operates at temperatures in excess of 8000 K and a double focusing magnetic sector mass spectrometer used as a detector to separate the elements and their isotopes for subsequent detection and measurement. Resolutions of 380,4800,and 10,500 amu are attainable. This method reports only the total amount of chromium in the sample, i.e., it does not distinguish between Cr(III) and Cr(VI).

The analytical procedure included the requisite number of blanks, standards traceable to NIST and commercially available plasma and blood controls (personal communication, ChemRisk and London Trace Metal Laboratory, 2009).

2.5 Statistical Analysis

The analytical data and summary results following testing for chromium in plasma and RBCs for residents and control population were provided to ChemRisk in Excel spreadsheet format through the custody of the Medical Officer of Health, Dr. Cristin Muecke, MD.

Data were calculated and analyzed using SAS (SAS/STAT® Software, 100 SAS Campus Drive, Cary, NC). Descriptive statistics including arithmetic mean (AM), median, mode, in addition to variability measures (SD, variance, range) were calculated using SAS (refer to Appendix B).

Because of potential variability within samples, all data for the case- and control groups were pooled together prior to statistical evaluation. A statistical analysis (i.e., comparison using statistical approaches) between the two groups was used to determine whether any individual resident or the residents as a group have elevated chromium levels when compared to the control population. In this study, the statistical test was a 't-test'[1]. The t-test was performed using SAS/Stat software. The results of the statistical analysis are appended in Appendix C. All statistical calculations were performed at the 95% confidence level.

A summary of results obtained from the chromium analysis are summarized in Table 2-1 for the resident population, while the results for the control population (i.e., background subjects) are summarized and provided in Table 2-2.

Table 2-1: Chromium levels in plasma and RBCs for residents living in the Evergreen Residential Subdivision (Pooled results). Data result presented as nmol/L

Subject # and Date of Test #1	Chromium (Plasma) Result	Chromium Erythrocyte Result	Date of Test #2	Chromium (Plasma) Result	Chromium Erythrocyte Result	Date of Test #3	Chromium (Plasma) Result	Chromium Erythrocyte Result
Subject 1 - 23-Sep-09	4.81	6.70	30-Sep-09	4.81	22.70	07-Oct-09	4.04	6.2
Subject 2 - 23-Sep-09	5.96	8.70	30-Sep-09	5.38	12.50	07-Oct-09	2.12	3.1
Subject 3 - 23-Sep-09	5.00	3.80	30-Sep-09	4.23	8.80	07-Oct-09	3.85	6
Subject 4 - 23-Sep-09	4.42	2.50	30-Sep-09	9.23	3.10	07-Oct-09	4.04	2.9
Subject 5 -23-Sep-09	3.27	5.00	30-Sep-09	3.46	4.20	07-Oct-09	5	3.1
Subject 6 -23-Sep-09	3.08	5.60	30-Sep-09	7.12	5.20	07-Oct-09	3.56	3.5
Subject 7 -23-Sep-09	4.42	4.20	30-Sep-09	4.81	9.60	07-Oct-09	4.62	3.3
Subject 8 -23-Sep-09	4.23	2.10	30-Sep-09	1.76	13.10	07-Oct-09	7.12	4.2
Subject 9 -23-Sep-09	4.04	1.90	30-Sep-09	5.19	9.40	07-Oct-09	26.54	8.3
Subject 10 -23-Sep-09	5.19	6.30	30-Sep-09	2.88	25.20	07-Oct-09	4.23	6.2
Subject 11 -23-Sep-09	5.58	3.50	30-Sep-09	4.04	2.30	07-Oct-09	4.23	4.4
Subject 12 -23-Sep-09	5.38	6.20	30-Sep-09	5.38	3.80	07-Oct-09	6.35	9.8
Subject 13 -23-Sep-09	4.62	9.40	30-Sep-09	5.77	6.70	07-Oct-09	5.19	3.3

[1] The t-test is the most commonly used method to evaluate the differences in means between two groups. For example, the t-test can be used to test for a difference in test scores between a group of patients who were given a drug and a control group who received a placebo.

Subject # and Date of Test #1	Chromium (Plasma) Result	Chromium Erythrocyte Result	Date of Test #2	Chromium (Plasma) Result	Chromium Erythrocyte Result	Date of Test #3	Chromium (Plasma) Result	Chromium Erythrocyte Result
Subject 14 -23-Sep-09	3.08	15.60	30-Sep-09	5.96	8.70	07-Oct-09	3.85	3.1
Subject 15 -23-Sep-09	6.15	5.40	30-Sep-09	5.38	5.60	07-Oct-09	7.5	4
Subject 16 -30-Sep-09	4.23	5.40	07-Oct-09	4.42	11.20	14-Oct-09	6.15	8.1
Subject 17 -30-Sep-09	4.81	5.60	07-Oct-09	7.12	3.50	14-Oct-09	5.77	14.2
Subject 18 -30-Sep-09	3.46	8.30	07-Oct-09	5.77	10.20	14-Oct-09	4.23	4.6
Subject 19 -30-Sep-09	1.92	4.20	07-Oct-09	3.65	13.70	20-Oct-09	4.42	2.3
Subject 20 -30-Sep-09	5.77	7.70	07-Oct-09	3.85	7.90	14-Oct-09	5.00	4.8
Subject 21 -30-Sep-09	3.08	8.80	07-Oct-09	4.04	11.00	14-Oct-09	4.42	20
Subject 22 -30-Sep-09	5.93	15.20	07-Oct-09	7.88	3.50	14-Oct-09	7.88	3.7
Subject 23 -07-Oct-09	5.19	5.00	14-Oct-09	5.58	2.30	21-Oct-09	5	5

Table 2-2: Chromium levels in plasma and erythrocyte for control (i.e., background) population (pooled results) living outside of the influence of the spill site. The results are presented in nmol/L.

Subject Number and Date of Test #1	Chromium Result	Chromium Erythrocyte Result	Date of Test #2	Chromium Result	Chromium Erythrocyte Result	Date of Test #3	Chromium Result	Chromium Erythrocyte Result
Subject 1 -23-Sep-09	5.38	3.80	30-Sep-09	5.19	7.50	26-Oct-09	4.04	2.5
Subject 2 -08-Oct-09	6.73	4.40	14-Oct-09	7.50	7.90	23-Oct-09	5.58	20.4
Subject 3 -07-Oct-09	4.62	5.80	14-Oct-09	5.00	4.80	21-Oct-09	2.88	2.3
Subject 4 -07-Oct-09	4.23	8.10	14-Oct-09	5.38	7.90	21-Oct-09	5.38	15.6
Subject 5 -08-Oct-09	4.42	15.60	14-Oct-09	6.54	12.30	21-Oct-09	3.08	5.6
Subject 6 -07-Oct-09	4.04	5.80	14-Oct-09	6.73	5.20	21-Oct-09	4.81	7.3
Subject 7 -07-Oct-09	4.81	9.60	14-Oct-09	5.19	14.00	21-Oct-09	3.08	10.8
Subject 8 -07-Oct-09	7.31	3.70	14-Oct-09	4.04	18.80	22-Oct-09	4.62	7.5
Subject 9 -07-Oct-09	4.81	11.50	13-Oct-09	7.12	18.10	21-Oct-09	6.35	7.5
Subject 10 -07-Oct-09	5.38	6.70	14-Oct-09	7.31	6.70	21-Oct-09	5	2.9
Subject 11 -14-Oct-09	5.38	17.30	21-Oct-09	3.85	5.00	28-Oct-09	5.58	4
Subject 12 -08-Oct-09	6.54	23.30	14-Oct-09	7.12	3.70	21-Oct-09	5.77	9.8
Subject 13 -08-Oct-09	6.35	9.40	14-Oct-09	3.65	7.30	22-Oct-09	9.62	8.8
Subject 14 -08-Oct-09	4.42	5.20	14-Oct-09	4.23	8.50	21-Oct-09	7.31	5.6
Subject 15 -07-Oct-09	4.81	13.70	14-Oct-09	5.96	4.40	21-Oct-09	8.08	10.2
Subject 16 -14-Oct-09	4.81	4.20	21-Oct-09	3.08	4.80	28-Oct-09	5.96	6.5
Subject 17 -07-Oct-09	4.23	14.00	14-Oct-09	6.54	10.40	21-Oct-09	9.42	11.7
Subject 18 -07-Oct-09	5.19	3.80	14-Oct-09	5.58	4.80	22-Oct-09	4.81	3.8
Subject 19 -08-Oct-09	5.96	11.20	14-Oct-09	4.23	3.70	21-Oct-09	5	6.2
Subject 20 -07-Oct-09	3.65	9.60	14-Oct-09	4.42	8.30	21-Oct-09	2.12	5.0
Subject 21 -14-Oct-09	6.15	7.30	21-Oct-09	4.62	2.70	28-Oct-09	7.5	3.1
Subject 22 -14-Oct-09	4.62	6.00	21-Oct-09	3.65	4.80	28-Oct-09	5.58	3.7
Subject 23 -14-Oct-09	4.04	14.40	22-Oct-09	4.81	5.80	28-Oct-09	3.08	4.8
Subject 24 -14-Oct-09	5.58	6.50	21-Oct-09	5.38	2.70	28-Oct-09	2.5	6.3
Subject 25 -14-Oct-09	5.38	21.00	21-Oct-09	1.92	7.90	28-Oct-09	2.69	3.7

3 Results

In general, results for any given individual were fairly consistent across the three sampling periods. The only 'outlier' appears to be the plasma chromium value of 26.54 nmol/L measured in Subject #9 on 7th of October, 2009. This value is clearly elevated beyond any other plasma values measured in the resident or control group. Interestingly, it is elevated far beyond the plasma values measured earlier in that same individual on 23rd September, 2009 and 30th September, 2009 (4.04 and 5.19 nmol/L, respectively). It is also curious to note that the RBC chromium levels for this individual were not increased on the same date. This data point is most likely a result of 1) contamination during sample collection or analysis, 2) laboratory error, or 3) prior consumption of a Cr(III) dietary supplement or some food group that has a high chromium content. A follow up test that was recommended returned a normal plasma (5.00 nmol/L) and RBC chromium concentration (4.8 nmol/L).

The general statistical measures (i.e., mean and standard deviation) for the two pooled data sets are presented below in Table (3-1). The average chromium concentration in plasma was identical for the case and control groups: 5.17 nmol/L, and there was no significant difference (p = 0.99) between the case- and control group plasma chromium concentration.

Table 3-1: Basic statistical measures for pooled datasets- plasma and erythrocyte chromium levels (nmol/L)

	Cases – 'Residents' (n=23)	Controls (n=25)	Statistical difference
Mean Plasma	5.17 – SD (2.94)	5.17 – SD (1.53)	No statistical difference (p = 0.99)
Mean RBC	7.13 – SD (4.80)	8.07 – SD (4.81)	No statistical difference (p = 0.24)

SD – Standard deviation; p = p-value

Similarly, there was no statistical difference between the average values measured in the RBCs of the resident group (7.13 nmol/L) vs. the control group (8.07 nmol/L) (p = 0.24).

Based on these findings, it can be concluded that the blood chromium levels in residents are the same as those than in a group of individuals with no potential exposure to site-related Cr(VI).

4 Discussion and Conclusions

In the present study, chromium in the blood plasma (indicative of chromium III and Cr(VI) exposure) and erythrocytes (indicative of (Cr(VI) exposure) were measured and compared to determine whether there was a statistically significant difference between the two groups. In this investigation, three blood samples were collected at least a week apart, to account for potential fluctuations of chromium intake from the diet or other non-occupational exposures to chromium. No statistically significant differences were found between the mean blood erythrocyte or plasma chromium levels between the control and case groups. This indicates that residential exposure to chromium is no different than that found in the normal control population. In general, the reference values for chromium in plasma for populations that are not occupationally exposed to chromium ranges from 0.04-0.35 µg/L (Christensen, et al., 1993) with a mean of 0.25 µg/L. The average chromium concentration in plasma of residents was found to be

5.71 nmol/L. This is equivalent to 0.27 µg/L (converting nmol/L to µg/L using a factor of 5.19 x 10^{-2}). This value is within the range reported for non-occupationally exposed reference populations. The average concentration (5.71 nmol/L) reported in this study is also within the range reported by Torra et al. (1999) who studied 243 healthy adults aged 17-71. In the Torra et al. (1999) study the plasma chromium concentration ranged from 0.6 to 6 nmol/L, with a reported 95th percentile of 5 nmol/L.

This finding is not entirely unexpected as the groundwater flow (at least based on preliminary results) appears to be moving away from the residential community (i.e., the community is hydraulically up-gradient of the spill Site). This is further supported by the fact that the majority of monitoring well results obtained from sampling of wells (i.e., wells located up-gradient of the spill location) appear to be within or below the Canadian Drinking Water Standard of 0.05 mg/L (excluding MW-13).

In conclusion, this study shows unequivocally that residents living in the community do not have statistically higher levels of chromium in plasma or red blood cell components.

5 Report Limitations

ChemRisk® Canada, a division of ChemRisk LLC ('ChemRisk') has prepared this document for the exclusive use of Dr. Cristin Muecke, MD, Medical Officer of Health, Dr. Paul Van Buynder, Deputy Chief Medical Officer of Health, Deputy Medical Officers of Health, Central Region, Fredericton, New Brunswick, Canada, in addition to the Office of the Chief Medical Officer of Health to provide advice in evaluating the potential exposure to chromium as a result of residents residing in a community that may have been impacted by a spill of chromic acid. ChemRisk will not be responsible for the use of this document or any opinions expressed herein by any third party, or reliance on or any decision made based on it without the prior written consent of ChemRisk. ChemRisk accepts no responsibility for damages, if any, by any third party as a result of decisions or actions based on this document. Other than by Drs. C. Muecke and P. Van Buynder, and the Office of the Chief Medical Officer of Health, their respective Departments, or the Government of New Brunswick, copying or distribution of this report or use of or reliance on the information contained herein, in whole or in part, is not permitted without the express written permission of ChemRisk. Nothing in this report is intended to constitute or provide a legal or medical opinion. The Medical Officer of Health provided ChemRisk the summary data for chromium levels in plasma and RBC.

6 References

ATSDR. 2008. Toxicological profile from chromium, Draft for Public Comment, Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services. Available online at http://www.atsdr.cdc.gov/toxprofiles/tp7.html#bookmark08. Accessed on December 6, 2009.

Christensen J.M., Holst E., Bonde J.P., and Knudsen L. 1993. Determination of chromium in blood and serum. Evaluation of quality control procedures and estimation of reference values in Danish subjects. Sci Total Environ. 132: 11–25.

Costa M. 1997. Toxicity and carcinogenicty of Cr(VI) in animal models and humans. Crit Rev Toxicol 27(5):431-442.

Finley, B.L., B.D. Kerger, M.W. Katona, M.L. Gargas, G.C. Corbett, and D.J., Paustenbach. 1997. Human ingestion of chromium (VI) in drinking water: Pharmakokinetics following repeated exposure.

Goyer, R.A. (2001) Toxic effects of metals, in Klassen, C.D. (Ed): Casarett and Doull's Toxicology: The basic science of poisons, 6th, New York, pp 811-867.

Kerger B.D., Finley B.L., Corbett G.E., Dodge D.G., and Paustenbach, D.J. 1997. Ingestion of chromium (VI) in drinking water by human volunteers: absorption, distribution, and excretion of single and repeated doses. J Toxicol Environ Health 50:67-95.

Korallus, U. 1986. Chromium compounds: Occupational health, toxicological and biological monitoring aspects. Toxicol. Environ. Chem. 12:47–59.

Lewalter, J., U. Korallus, C. Harzdorf, and H. Weidemann. 1985. Chromium blood detection in isolated erythrocytes: A new principle of biological monitoring of exposure to hexavalent chromium. Int. Arch. Occup. Environ. Health. 55:305-318.

Minoia C., Pietra R., Sabbioni E., Ronchi A., Gatti A., Cavallieri A., and Manzo L. 1992. Trace element reference values in tissues from inhabitants of the European community. III. The control of preanalytical factors in the biomonitoring of trace elements in biological fluids. Sci Total Environ. 120: 63-79.

NRC: Recommended Dietary Allowances, 10th ed. Washington DC: Na- tional Academy Press, 1989, pp 1–285.

Shemin, D., and D. Rittenberg. 1946. The life span of the human red blood cell. The journal of biological chemistry. p 627-636.

Stoecker B.J. (1996) Chromium, in Ziegler EE, Filer LJ (eds): Present Knowledge in Nutrition. Washington, DC: ILSI, 1996, pp 344–352.

Torra, M., M. Rodamilans, J. Corbella, R. Ferrer, and R. Mazzara (1999) Blood chromium determination in assessing reference values in an unexposed Mediterranean population. Biological Trace Element Research. 70: 183-189.

Versieck J., Barbier F., Cornelis R., and Holst J. 1982. Sample contamination as a source of error in trace element analysis of biological samples. Talanta. 29: 973–984

Appendix A:

Location Key – Spill Site

TerrAtlantic Engineering Limited

SITE ASSESSMENT AND REMEDIATION - 85 MELISSA STREET

SCALE 1:1,000

20 metres

Appendix B:

Statistical Analysis Output from SAS/STAT

The SAS System

The UNIVARIATE Procedure
Variable: Resident_Chromium_Result

Moments			
N	70	**Sum Weights**	70
Mean	5.17442857	**Sum Observations**	362.21
Std Deviation	2.94220643	**Variance**	8.65657865
Skewness	5.688349	**Kurtosis**	41.0270969
Uncorrected SS	2471.5337	**Corrected SS**	597.303927
Coeff Variation	56.8605091	**Std Error Mean**	0.35166093

Basic Statistical Measures			
Location		**Variability**	
Mean	5.174429	**Std Deviation**	2.94221
Median	4.810000	**Variance**	8.65658
Mode	4.230000	**Range**	24.78000
		Interquartile Range	1.73000

Tests for Location: Mu0=0				
Test	**Statistic**		**p Value**	
Student's t	**t**	14.71425	**Pr > |t|**	<.0001
Sign	**M**	35	**Pr >= |M|**	<.0001
Signed Rank	**S**	1242.5	**Pr >= |S|**	<.0001

Tests for Normality				
Test	**Statistic**		**p Value**	
Shapiro-Wilk	**W**	0.514105	**Pr < W**	<0.0001
Kolmogorov-Smirnov	**D**	0.241532	**Pr > D**	<0.0100
Cramer-von Mises	**W-Sq**	1.212507	**Pr > W-Sq**	<0.0050
Anderson-Darling	**A-Sq**	7.048162	**Pr > A-Sq**	<0.0050

Quantiles (Definition 5)	
Quantile	**Estimate**
100% Max	26.540
99%	26.540
95%	7.880

The SAS System

The UNIVARIATE Procedure
Variable: Resident_Chromium_Result

Quantiles (Definition 5)	
Quantile	**Estimate**
90%	7.120
75% Q3	5.770
50% Median	4.810
25% Q1	4.040
10%	3.175
5%	2.880
1%	1.760
0% Min	1.760

Extreme Observations			
Lowest		**Highest**	
Value	**Obs**	**Value**	**Obs**
1.76	31	7.50	61
1.92	19	7.88	45
2.12	48	7.88	68
2.88	33	9.23	27
3.08	21	26.54	55

Missing Values			
		Percent Of	
Missing Value	**Count**	**All Obs**	**Missing Obs**
.	29	29.29	100.00

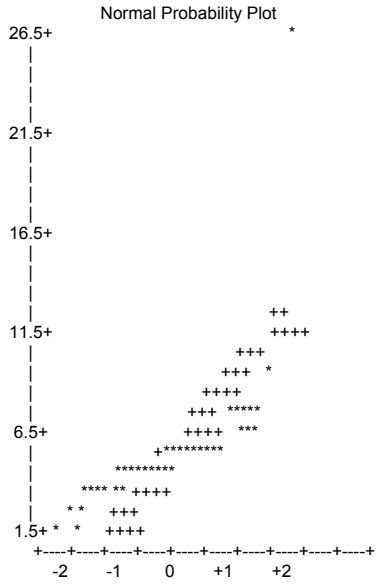
The SAS System

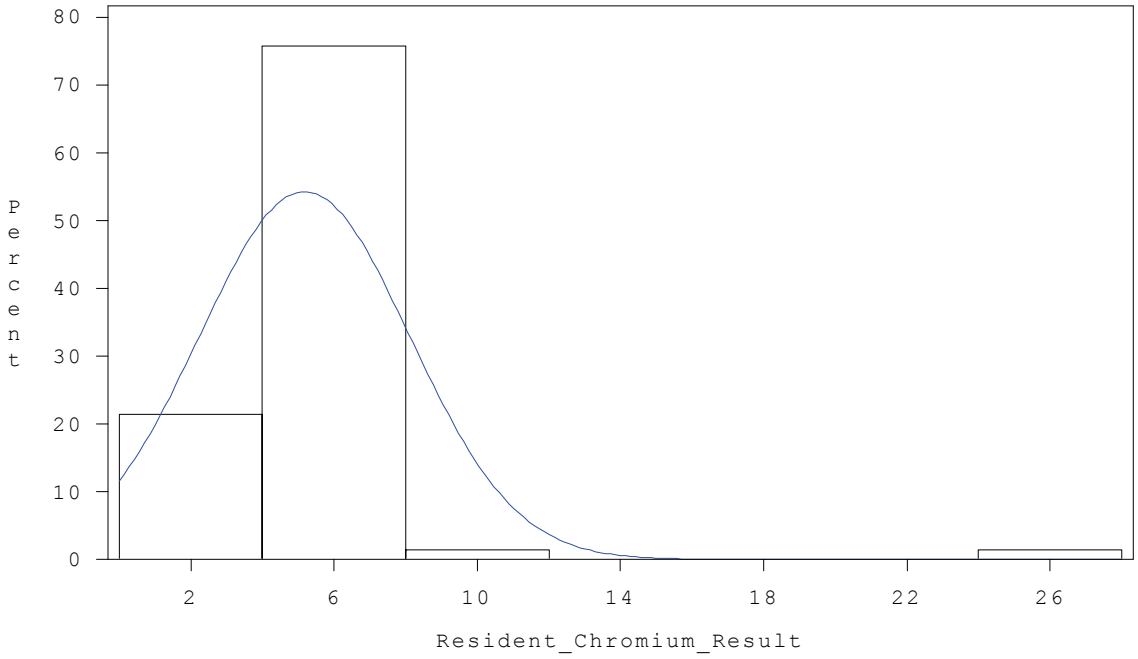
The UNIVARIATE Procedure
Variable: Resident_Chromium_Result

```
Stem Leaf                 #          Boxplot
 26 5                     1          *
 25
 24
 23
 22
 21
 20
 19
 18
 17
 16
 15
 14
 13
 12
 11
 10
  9 2                     1          0
  8
  7 111599                6          |
  6 00224                 5          |
  5 00002222444466888889  20         +--+--+
  4 000002222224444668888 22         *-----*
  3 11135566888           11         |
  2 19                    2          |
  1 89                    2          |
    ----+----+----+----+--
```

The SAS System

The UNIVARIATE Procedure
Variable: Resident_Chromium_Result




80
70
60
50
40
30
20
10
0
Percent
2
6
10
14
18
22
26
Resident_Chromium_Result

The SAS System

The UNIVARIATE Procedure
Fitted Distribution for Resident_Chromium_Result

Parameters for Normal Distribution		
Parameter	**Symbol**	**Estimate**
Mean	Mu	5.174429
Std Dev	Sigma	2.942206

Goodness-of-Fit Tests for Normal Distribution				
Test	**Statistic**		**p Value**	
Kolmogorov-Smirnov	**D**	0.24153247	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	1.21250683	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	7.04816207	**Pr > A-Sq**	<0.005

Quantiles for Normal Distribution		
	Quantile	
Percent	**Observed**	**Estimated**
1.0	1.76000	-1.67017
5.0	2.88000	0.33493
10.0	3.17500	1.40384
25.0	4.04000	3.18994
50.0	4.81000	5.17443
75.0	5.77000	7.15892
90.0	7.12000	8.94502
95.0	7.88000	10.01393
99.0	26.54000	12.01902

The SAS System

The UNIVARIATE Procedure
Variable: Resident_Chromium_Erythrocyte_Re

Moments			
N	70	**Sum Weights**	70
Mean	7.12714286	**Sum Observations**	498.9
Std Deviation	4.80168815	**Variance**	23.0562091
Skewness	1.71748223	**Kurtosis**	3.35513217
Uncorrected SS	5146.61	**Corrected SS**	1590.87843
Coeff Variation	67.3718522	**Std Error Mean**	0.57391151

Basic Statistical Measures			
Location		**Variability**	
Mean	7.127143	**Std Deviation**	4.80169
Median	5.600000	**Variance**	23.05621
Mode	3.100000	**Range**	23.30000
		Interquartile Range	5.10000

Note: The mode displayed is the smallest of 3 modes with a count of 4.

Tests for Location: Mu0=0				
Test	**Statistic**		**p Value**	
Student's t	**t**	12.41854	**Pr > |t|**	<.0001
Sign	**M**	35	**Pr >= |M|**	<.0001
Signed Rank	**S**	1242.5	**Pr >= |S|**	<.0001

Tests for Normality				
Test	**Statistic**		**p Value**	
Shapiro-Wilk	**W**	0.833852	**Pr < W**	<0.0001
Kolmogorov-Smirnov	**D**	0.168384	**Pr > D**	<0.0100
Cramer-von Mises	**W-Sq**	0.536144	**Pr > W-Sq**	<0.0050
Anderson-Darling	**A-Sq**	3.213619	**Pr > A-Sq**	<0.0050

The SAS System

The UNIVARIATE Procedure
Variable: Resident_Chromium_Erythrocyte_Re

Quantiles (Definition 5)	
Quantile	**Estimate**
100% Max	25.2
99%	25.2
95%	15.6
90%	13.6
75% Q3	8.8
50% Median	5.6
25% Q1	3.7
10%	3.0
5%	2.3
1%	1.9
0% Min	1.9

Extreme Observations			
Lowest		**Highest**	
Value	**Obs**	**Value**	**Obs**
1.9	9	15.2	22
2.1	8	15.6	14
2.3	65	20.0	67
2.3	46	22.7	24
2.3	34	25.2	33

Missing Values			
		Percent Of	
Missing Value	**Count**	**All Obs**	**Missing Obs**
.	29	29.29	100.00

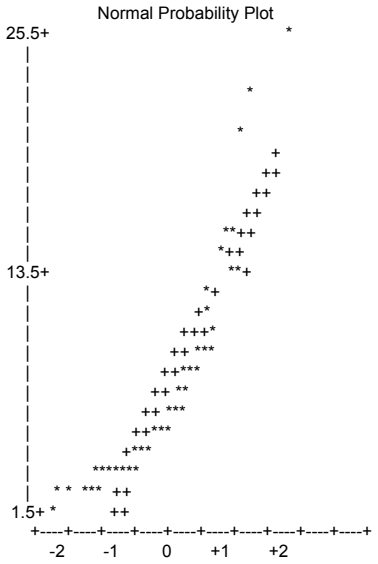
The SAS System

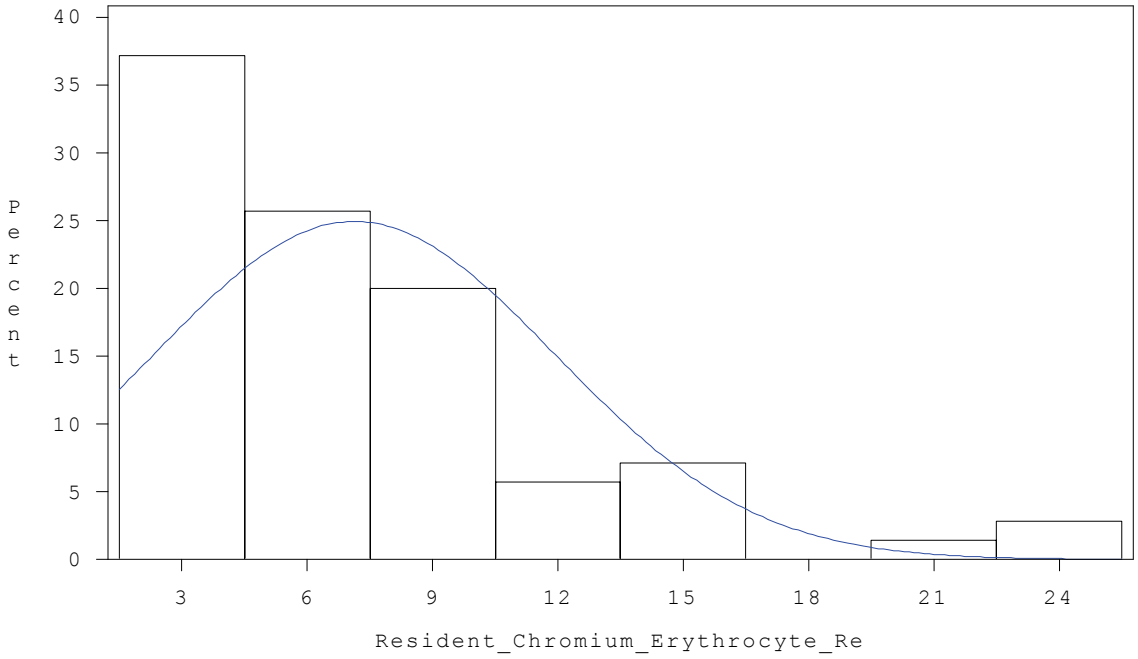
The UNIVARIATE Procedure
Variable: Resident_Chromium_Erythrocyte_Re

```
Stem Leaf              #          Boxplot
 25 2                  1          *
 24
 23
 22 7                  1          0
 21
 20 0                  1          0
 19
 18
 17
 16
 15 26                 2          |
 14 2                  1          |
 13 157                3          |
 12 5                  1          |
 11 02                 2          |
 10 2                  1          |
  9 4468               4          |
  8 1337788            7          +-----+
  7 79                 2          | + |
  6 0222377            7          |   |
  5 000244666          9          *-----*
  4 02222468           8          |   |
  3 1111335555788      13         +-----+
  2 133359             6          |
  1 9                  1          |
    ----+----+----+----+
```

The SAS System

The UNIVARIATE Procedure
Variable: Resident_Chromium_Erythrocyte_Re




Percent
40
35
30
25
20
15
10
5
0
3
6
9
12
15
18
21
24
Resident_Chromium_Erythrocyte_Re

The SAS System

The UNIVARIATE Procedure
Fitted Distribution for Resident_Chromium_Erythrocyte_Re

Parameters for Normal Distribution		
Parameter	**Symbol**	**Estimate**
Mean	Mu	7.127143
Std Dev	Sigma	4.801688

Goodness-of-Fit Tests for Normal Distribution				
Test	**Statistic**		**p Value**	
Kolmogorov-Smirnov	**D**	0.16838377	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	0.53614358	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	3.21361928	**Pr > A-Sq**	<0.005

Quantiles for Normal Distribution		
	Quantile	
Percent	**Observed**	**Estimated**
1.0	1.90000	-4.04325
5.0	2.30000	-0.77093
10.0	3.00000	0.97353
25.0	3.70000	3.88845
50.0	5.60000	7.12714
75.0	8.80000	10.36583
90.0	13.60000	13.28075
95.0	15.60000	15.02522
99.0	25.20000	18.29754

The SAS System

The UNIVARIATE Procedure
Variable: Control_Chromium_Result

Moments			
N	75	**Sum Weights**	75
Mean	5.1696	**Sum Observations**	387.72
Std Deviation	1.53000745	**Variance**	2.34092281
Skewness	0.43101047	**Kurtosis**	0.59288812
Uncorrected SS	2177.5856	**Corrected SS**	173.228288
Coeff Variation	29.5962445	**Std Error Mean**	0.17667004

Basic Statistical Measures			
Location		**Variability**	
Mean	5.169600	**Std Deviation**	1.53001
Median	5.000000	**Variance**	2.34092
Mode	4.810000	**Range**	7.70000
		Interquartile Range	1.73000

Note: The mode displayed is the smallest of 2 modes with a count of 7.

Tests for Location: Mu0=0				
Test	**Statistic**		**p Value**	
Student's t	**t**	29.26133	**Pr > |t|**	<.0001
Sign	**M**	37.5	**Pr >= |M|**	<.0001
Signed Rank	**S**	1425	**Pr >= |S|**	<.0001

Tests for Normality				
Test	**Statistic**		**p Value**	
Shapiro-Wilk	**W**	0.979214	**Pr < W**	0.2545
Kolmogorov-Smirnov	**D**	0.100926	**Pr > D**	0.0579
Cramer-von Mises	**W-Sq**	0.077768	**Pr > W-Sq**	0.2248
Anderson-Darling	**A-Sq**	0.447307	**Pr > A-Sq**	>0.2500

The SAS System

The UNIVARIATE Procedure
Variable: Control_Chromium_Result

Quantiles (Definition 5)	
Quantile	**Estimate**
100% Max	9.62
99%	9.62
95%	7.50
90%	7.31
75% Q3	5.96
50% Median	5.00
25% Q1	4.23
10%	3.08
5%	2.69
1%	1.92
0% Min	1.92

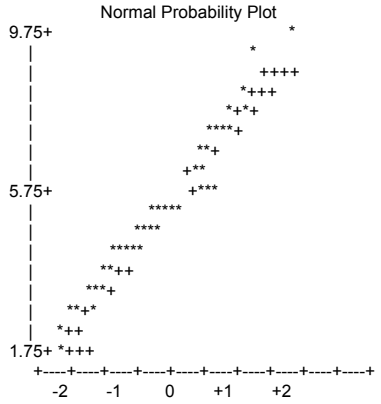
Extreme Observations			
Lowest		**Highest**	
Value	**Obs**	**Value**	**Obs**
1.92	50	7.50	27
2.12	70	7.50	71
2.50	74	8.08	65
2.69	75	9.42	67
2.88	53	9.62	63

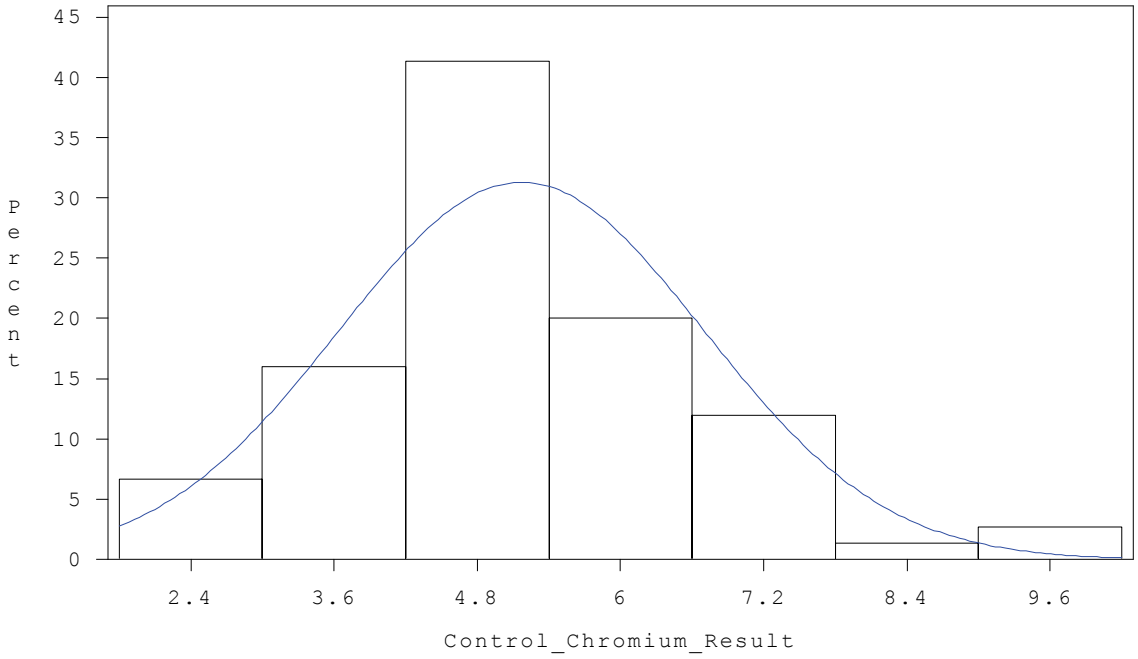
Missing Values			
		Percent Of	
Missing Value	**Count**	**All Obs**	**Missing Obs**
.	24	24.24	100.00

The SAS System

The UNIVARIATE Procedure
Variable: Control_Chromium_Result

```
Stem Leaf                #          Boxplot
   9 6                   1          0
   9 4                   1          0
   8
   8 1                   1          |
   7 55                  2          |
   7 11333               5          |
   6 55577               5          |
   6 000244              6          +-----+
   5 666668              6          |     |
   5 0002224444444       13         *--+--*
   4 66668888888         11         |     |
   4 00002222444         11         +-----+
   3 6668                4          |
   3 1111                4          |
   2 579                 3          |
   2 1                   1          |
   1 9                   1          |
     ----+----+----+----+
```




45
40
35
30
25
20
15
10
5
0
Percent
2.4
3.6
4.8
6
7.2
8.4
9.6
Control_Chromium_Result

The SAS System

The UNIVARIATE Procedure
Fitted Distribution for Control_Chromium_Result

Parameters for Normal Distribution		
Parameter	**Symbol**	**Estimate**
Mean	Mu	5.1696
Std Dev	Sigma	1.530007

Goodness-of-Fit Tests for Normal Distribution				
Test	**Statistic**		**p Value**	
Kolmogorov-Smirnov	**D**	0.10092627	**Pr > D**	0.058
Cramer-von Mises	**W-Sq**	0.07776782	**Pr > W-Sq**	0.225
Anderson-Darling	**A-Sq**	0.44730679	**Pr > A-Sq**	>0.250

Quantiles for Normal Distribution		
	Quantile	
Percent	**Observed**	**Estimated**
1.0	1.92000	1.61027
5.0	2.69000	2.65296
10.0	3.08000	3.20882
25.0	4.23000	4.13763
50.0	5.00000	5.16960
75.0	5.96000	6.20157
90.0	7.31000	7.13038
95.0	7.50000	7.68624
99.0	9.62000	8.72893

The SAS System

The UNIVARIATE Procedure
Variable: Control_Chromium_Erythrocyte_Res

Moments			
N	75	**Sum Weights**	75
Mean	8.07333333	**Sum Observations**	605.5
Std Deviation	4.80543986	**Variance**	23.0922523
Skewness	1.2944724	**Kurtosis**	1.20492366
Uncorrected SS	6597.23	**Corrected SS**	1708.82667
Coeff Variation	59.5223765	**Std Error Mean**	0.5548844

Basic Statistical Measures			
Location		**Variability**	
Mean	8.073333	**Std Deviation**	4.80544
Median	6.700000	**Variance**	23.09225
Mode	3.700000	**Range**	21.00000
		Interquartile Range	5.40000

Note: The mode displayed is the smallest of 2 modes with a count of 5.

Tests for Location: Mu0=0				
Test	**Statistic**		**p Value**	
Student's t	**t**	14.54958	**Pr > |t|**	<.0001
Sign	**M**	37.5	**Pr >= |M|**	<.0001
Signed Rank	**S**	1425	**Pr >= |S|**	<.0001

Tests for Normality				
Test	**Statistic**		**p Value**	
Shapiro-Wilk	**W**	0.873799	**Pr < W**	<0.0001
Kolmogorov-Smirnov	**D**	0.154387	**Pr > D**	<0.0100
Cramer-von Mises	**W-Sq**	0.50305	**Pr > W-Sq**	<0.0050
Anderson-Darling	**A-Sq**	2.987865	**Pr > A-Sq**	<0.0050

The SAS System

The UNIVARIATE Procedure
Variable: Control_Chromium_Erythrocyte_Res

Quantiles (Definition 5)	
Quantile	**Estimate**
100% Max	23.3
99%	23.3
95%	18.8
90%	15.6
75% Q3	10.2
50% Median	6.7
25% Q1	4.8
10%	3.7
5%	2.7
1%	2.3
0% Min	2.3

Extreme Observations			
Lowest		**Highest**	
Value	**Obs**	**Value**	**Obs**
2.3	53	18.1	34
2.5	51	18.8	33
2.7	49	20.4	52
2.7	46	21.0	25
2.9	60	23.3	12

Missing Values			
		Percent Of	
Missing Value	**Count**	**All Obs**	**Missing Obs**
.	24	24.24	100.00

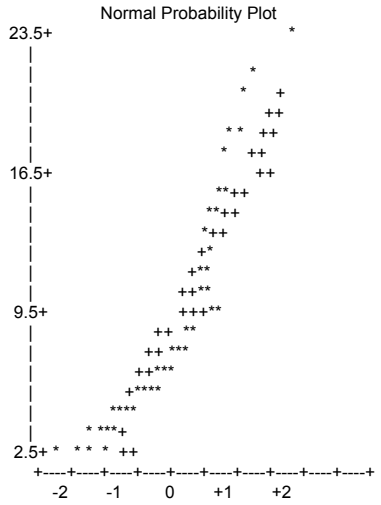
The SAS System

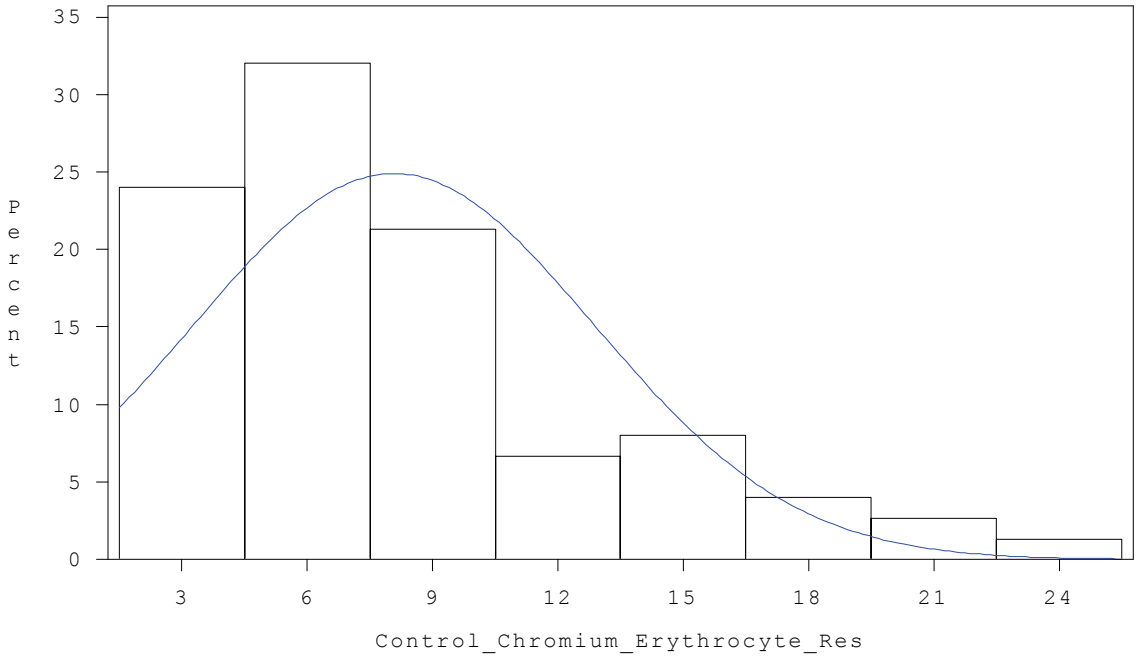
The UNIVARIATE Procedure
Variable: Control_Chromium_Erythrocyte_Res

```
Stem Leaf              #          Boxplot
 23 3                  1          0
 22
 21 0                  1          0
 20 4                  1          0
 19
 18 18                 2          0
 17 3                  1          |
 16                               |
 15 66                 2          |
 14 004                3          |
 13 7                  1          |
 12 3                  1          |
 11 257                3          |
 10 248                3          +-----+
  9 4668               4          |     |
  8 1358               4          |  +  |
  7 333555999          9          |     |
  6 0235577            7          *-----*
  5 002266888          9          |     |
  4 024488888          9          +-----+
  3 177777888          9          |
  2 35779              5          |
    ----+----+----+----+
```

The SAS System

The UNIVARIATE Procedure
Variable: Control_Chromium_Erythrocyte_Res




35
30
25
20
15
10
5
0
Percent
3
6
9
12
15
18
21
24
Control_Chromium_Erythrocyte_Res

The SAS System

The UNIVARIATE Procedure
Fitted Distribution for Control_Chromium_Erythrocyte_Res

Parameters for Normal Distribution		
Parameter	**Symbol**	**Estimate**
Mean	Mu	8.073333
Std Dev	Sigma	4.80544

Goodness-of-Fit Tests for Normal Distribution				
Test	**Statistic**		**p Value**	
Kolmogorov-Smirnov	**D**	0.15438682	**Pr > D**	<0.010
Cramer-von Mises	**W-Sq**	0.50304953	**Pr > W-Sq**	<0.005
Anderson-Darling	**A-Sq**	2.98786458	**Pr > A-Sq**	<0.005

Quantiles for Normal Distribution		
	Quantile	
Percent	**Observed**	**Estimated**
1.0	2.30000	-3.10579
5.0	2.70000	0.16909
10.0	3.70000	1.91491
25.0	4.80000	4.83211
50.0	6.70000	8.07333
75.0	10.20000	11.31455
90.0	15.60000	14.23175
95.0	18.80000	15.97758
99.0	23.30000	19.25246