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C61427B

The following report presents results of a study conducted by a contract laboratory for the National Toxicology Program (NTP). The report may not have been peer reviewed. The findings and conclusions for this study should not be construed to represent the view of NTP or the U.S. Government.

NTP

2-Year Inhalation Toxicity and Carcinogenicity Study of Vanadium Pentoxide in F344 Rats and B6C3F1 Mice

[(Vanadium Pentoxide, V_2O_5, CAS# 1314-62-1, C61427B)]+A

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Method Performance Evaluation and Prestart Report for Toxicokinetic Studies

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Determination of Vanadium in Blood

Prestart Report
June 28, 1996

Battelle
Pacific Northwest National Laboratories
Toxicokinetics and Bioanalytical Research Group
Richland, Washington

+A Added 10/25/96 by Amendment A

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NTP
2-Year Inhalation Toxicity and Carcinogenicity Study of
Vanadium Pentoxide in F344 Rats and B6C3F1 Mice
Method Performance Evaluation and Prestart Report for Toxicokinetic Studies
Determination of Vanadium in Blood

[redacted] _____ 11/11/96

Staff Scientist/Group Leader Date

[redacted] _____ 11/11/96

Chemistry Specialist Date

Quality Assurance Statement

Listed below are the phases and/or procedures included in the work described in this report which were reviewed by the Quality Assurance Unit and the dates the reviews were performed and findings reported to management. (All findings were reported to the study director or designee at the time of the review.)

Phase or Procedure Reviewed	Review Dates	Date of Written Report to Study Director and Management
Data	8/5,22/96	11/2/96
Final Study Report	8/22,23/96	11/2/96
Amendments	10/28/96	11/2/96

[redacted]

Quality Assurance

11/5/96

Date

I. Introduction

Battelle will conduct a chronic inhalation toxicity study of vanadium pentoxide (V_2O_5) aerosol in F344 rats and B6C3F1 mice. The chronic toxicity study incorporates a toxicokinetic study in which samples of blood and lung will be collected from rats and mice at 3, 6, 12, and 18 months, and analyzed for vanadium concentrations. The design of the toxicokinetic studies is discussed in more detail in Section II of this report.

For toxicokinetic studies, samples of lungs and blood will be obtained from rats and mice exposed to V_2O_5 for the determination of vanadium concentrations by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). This report discusses the sample handling and method performance evaluation for the measurement of vanadium in blood from exposed rats and mice via ICP-AES.

The performance characteristics and limitations of the proposed analytical method were evaluated over the range of vanadium concentrations expected in actual study samples to assess sensitivity, selectivity, recovery, linearity, precision, and accuracy. Because vanadium is expected to be stable in frozen blood, and blood samples will be analyzed within ~1 month of sample collection, sample stability was not included in the evaluation.

The approach for completing the method performance evaluation entailed several steps. Vanadium blood concentrations measured during prechronic studies were used to calculate the expected range of blood concentrations for rats and mice during the chronic study. Concentration ranges for the blood assay methods for rats and mice were selected based on these calculations. Subsequently, the method performance evaluation consisted of preparing and analyzing solvent standards and standards prepared by spiking vanadium into rat and mouse blood over the concentration ranges selected for each species. Battelle also conducted ICP-AES studies to determine limits of detection and quantitation for the method.

Calibration curves derived from the analysis of solvent standards and spiked blood standards were compared, and the accuracy and linearity of the calibration curves derived from these standards were evaluated. Precision was estimated through analysis of replicate spiked blood standards at several vanadium concentrations over the selected concentration range for each species. Recovery was determined as the ratio of instrument response in spiked blood standards to that measured in solvent standards containing identical vanadium concentrations.

II. Toxicokinetic Study Design

A. Study Design

Blood samples will be collected from 5 male and 5 female rats each from the 1 and 2 mg V_2O_5/m^3 exposure groups, and from 5 female rats only in the 0.5 mg V_2O_5/m^3 exposure group. In addition, blood will be collected from 5 male and 5 female mice each from the 1, 2, and 4 mg V_2O_5/m^3 exposure groups. The present study design does not include analysis of vanadium in blood from control animals.

B. Collection and Storage of Samples

Blood will be collected from the designated animals in each exposure group (excluding controls) during the scheduled sacrifices. Each animal will be wiped down with a dampened towel to remove test chemical from the fur prior to leaving the exposure room. The animals will be transported to the necropsy laboratory, injected intraperitoneally with Nembutal™ and weighed. Two sets of surgical instruments will be used during tissue removal, one set to open the animal and a second set to remove lungs. This greatly reduces the risk of contamination to the internal organs with test chemical.

Once an animal is anesthetized, its ventral side will be wiped with 70% alcohol and the chest cavity opened. Using a hypodermic syringe and needle, blood will be obtained from the exposed heart in rats and from the abdominal aorta in mice. The blood will be placed in a vacutainer tube with EDTA anticoagulant, transferred directly to a labeled polyethylene tube, and stored at ~-70° C until analyzed.

Lungs and associated tissues will be removed from the thoracic cavity. The mainstem bronchi, heart and mediastinal tissues will be trimmed off at the point of attachment to lung tissue, and the lungs weighed. After weighing, tissues will be placed into labeled tubes, kept on ice until all animals have been sacrificed, and then stored frozen at ~-70° C until analyzed.

III. Materials and Methods

A. Selected Concentration Range for the Analytical Method

Battelle conducted a prechronic toxicity study in which rats and mice were exposed to vanadium pentoxide. Results of rat and mouse blood and serum assays are included in Appendix A of this report.

Immediately after the 14-day study was terminated, samples of blood, serum, and lungs were collected from 5 female rats in the 0, 1, and 2 mg V_2O_5/m^3 exposure groups and from 5 female mice from the 0, 2, and 4 mg V_2O_5/m^3 exposure groups. The blood samples were analyzed for vanadium via ICP-AES using methods similar to those detailed in this report. To estimate blood concentrations expected in the chronic study, Battelle linearly scaled blood concentrations measured at the end of the 14-day study to the exposure concentrations planned for the chronic study. These estimates assumed that blood concentrations will be at steady state after ~2 weeks on exposure. Based on this assumption, vanadium concentrations in blood at all timepoints during the chronic study are presented for rats and mice in Tables 1 and 2, respectively.

The calculations presented in Tables 1 and 2 indicate a range of blood concentrations from ~0.1 to ~0.2 µg/g of vanadium in rats and mice. However, blood concentrations in mice at 1, 4, and 8 days postexposure were somewhat higher than those shown in Table 2, with some concentrations going as high as ~0.9 µg V/g (see data and discussion in Appendix A). Based on this information, the method performance evaluation was designed to encompass blood concentrations from 0.05 to 0.50 µg V/g of blood for rats and from 0.10 to 1.0 µg V/g of blood for mice. For rats, the method evaluation encompassed blood concentrations of 0.05, 0.10, 0.15, 0.20, 0.25, and 0.50 µg of vanadium per gram of blood, assuming sample weight of 1 gram for rat blood. The method evaluation for mice encompassed blood concentrations of 0.10, 0.20, 0.30, 0.40, 0.50, and 1.0 µg of vanadium per gram of blood, assuming a sample weight of 0.5 g for mouse blood.

B. Materials Cleaning and Contamination Abatement Procedures

To avoid sample contamination, it was important to thoroughly clean all sample containers. Teflon microwave digestion vessels and caps were soaked in ~2% Micro® cleaning solution for ~24 hours, rinsed with deionized water, soaked for ~24 hours in aqua regia, and rinsed again with deionized water. Subsequently, they were stored in a mixture of 10% each of HNO_3 and HCl, and then rinsed repeatedly with deionized water just prior to use.

Volumetric glassware was soaked for ~24 hours in a solution containing ~2% HNO_3 and rinsed repeatedly with deionized water. After cleaning, labware was filled with deionized water and stored capped until used. All containers were used as soon as possible after cleaning.

High purity concentrated nitric acid (~70% HNO_3, Trace Metal Grade, Manufactured by Seastar Chemical Inc. for Fisher Scientific, Pittsburgh, PA) was used for preparing standards and for digesting blood samples for analysis. Reagent grade concentrated HNO_3 (~70% HNO_3, Fisher Scientific, Fair Lawn, NJ) was used to prepare solutions for cleaning labware. All dilutions were performed using high purity, deionized water with an electrical resistance of ~18 $M\Omega[\cdot cm]^{+A}$ obtained from a Barnstead Nanopure II deionization system (Barnstead/Thermolyne Corp., Dubuque, IA).

C. Preparation of Blood

1. Method Performance Evaluation

Approximately 100 mL of F344 rat blood and ~100 mL of B6C3F1 mouse blood for preparing spiked blood standards were purchased from Taconic Farms (Germantown, NY) and stored frozen at ~-70° C upon receipt.

Prior to preparing spiked blood standards, the pooled[,]$^{-A}$ blood from each species was thawed and mixed thoroughly. The blood was homogenized in acid-leached 50 mL polypropylene centrifuge tubes. The centrifuge tubes were capped and the homogenized blood was stored at ~-70° C until used. Prior to use, the blood was thawed, mixed thoroughly, and aliquots were weighed directly into microwave digestion vessels used for sample preparation.

2. Study Samples

Blood samples will be collected from designated animals in each exposure group during the scheduled sacrifices and stored in polypropylene tubes at ~-70° C until analyzed. Samples will be thawed prior to analysis, mixed well and approximately 1 g of rat blood or 0.5 g of mouse blood will be weighed directly into microwave digestion vessel liners used for sample preparation.

$^{+A}$ Added 10/25/96 by Amendment A
$^{-A}$ Deleted 10/25/96 by Amendment A

D. Sample Digestion and Preparation for ICP-AES Analysis

Sample preparation procedures used for the method performance evaluation are summarized in Figure 1. Approximately 1 g aliquots of homogenized rat blood or ~0.5 g aliquots of homogenized mouse blood were weighed into clean Teflon liners for microwave digestion (100 mL, Teflon PFA® liners, CEM Corp., Matthews, NC), and 1 mL of Ultrex concentrated HNO_3 was added. For spiked blood standards, aliquots from 0.125 to 0.750 mL of vanadium spiking solutions (see Section III.E.1) were added, and the contents of the vessels were diluted to a solution volume of ~2 mL with deionized water.

The digestion vessels were assembled and the contents digested in a CEM MDS-2000 Microwave Sample Preparation Station (CEM Corp., Matthews, NC) using the temperature and pressure program presented in Table 3. Digestions were prepared in batches of 12 samples, with a total digestion time of ~30 minutes per batch.

After cooling, each vessel was opened and 0.112 mL of an internal standard solution containing yttrium (Y; 624 µg/mL) was added. The digests were quantitatively transferred to plastic vials and diluted to ~14 mL with deionized water. The final acid strength was ~5% HNO_3 and the internal standard concentration following the final dilution was 5 µg Y/mL.

Study samples will be treated in the same manner as described above, except that no aliquots of spiking solutions will be added.

E. Preparation of Solvent Standards, Spiked Blood Standards, and Blanks

1. Solution Preparation

Two independently prepared stock solutions were prepared by serial dilution of two separate vanadium solutions (Standard Reference Materials, SRMs) obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, SRM #3165, 5 mg V/mL in 10% HNO_3). Solutions used in preparing solvent standards and spiked blood standards were prepared by further dilution of these stock solutions in 5% HNO_3. A yttrium solution for use as an internal standard was prepared by serial dilution of a NIST SRM for yttrium (NIST, Gaithersburg, MD, SRM #3167a, 10 mg Y/mL in 10% HNO_3) with 5% HNO_3. These vanadium and yttrium solutions were used for preparing the solvent standards and spiked blood standards described below.

2. Preparation of Solvent Standards

For the analysis of vanadium in rat and mouse blood, solvent standards were prepared by diluting solution aliquots containing 0.05, 0.10, 0.15, 0.20, 0.25, and 0.50 µg of vanadium from alternate, independently prepared stock solutions. Aliquots containing these masses of vanadium were added to individual 15 mL centrifuge tubes, an aliquot of internal standard solution was added, and the samples were diluted to ~14 mL with 5% HNO_3 to give an internal standard concentration of 5 µg Y/mL. Solvent blanks consisted of 5% HNO_3 with an internal standard concentration of 5 µg Y/mL.

As discussed below, aliquots of ~1 g of rat blood or ~0.5 g of mouse blood were used for spiked blood standards. Accordingly, the solvent standards described above contained concentrations equivalent to 0.05, 0.10, 0.15, 0.20, 0.25, and 0.50 µg vanadium per gram of rat blood and 0.10, 0.20, 0.30, 0.40, 0.50, and 1.0 µg vanadium per gram of mouse blood.

3. Preparation of Spiked Blood Standards

Aliquots of ~1 g of rat blood or ~0.5 g of mouse blood were weighed into[ ][-A] 100 mL, Teflon PFA microwave digestion vessel liners and 1 mL of Ultrex concentrated HNO_3 was added. Aliquots of rat and mouse blood were spiked to contain 0.05, 0.10, 0.15, 0.20, 0.25, and 0.50 µg of vanadium by alternately adding aliquots from the same independently-prepared stock solutions used to prepare solvent standards. Sufficient deionized water was added to each vessel to bring the total liquid volume to ~2 mL. The vessels were assembled and the contents were digested and prepared for analysis as described in Section III.D. Given that the rat blood mass used in spiked blood standards was ~1 g, spiked rat blood standards contained vanadium concentrations of 0.05, 0.10, 0.15, 0.20, 0.25, and 0.50 µg vanadium per gram. Spiked mouse blood standards containing ~0.5 g of blood contained vanadium concentrations of 0.10, 0.20, 0.30, 0.40, 0.50 and 1.0 µg vanadium per gram. Triplicate spiked rat and mouse blood standards were prepared at each concentration.

[-A] Deleted 10/25/96 by Amendment A

4. Blanks

Two types of acid blanks were prepared and assayed during the analysis for method performance evaluation. Solvent blanks were prepared by diluting internal standard solution to volume with solvent (5% HNO_3). System blanks were prepared by adding 1 mL of 70% HNO_3 and 1 mL deionized water to clean microwave digestion vessel liners. These vessels were placed in the microwave sample preparation system and the contents were prepared for analysis using procedures identical to those used for blood digestions (see Section III.D).

To determine the background concentration of vanadium in blood, Battelle analyzed several digestions of ~1 g of rat blood or ~0.5 g of mouse blood. Samples were digested using the same procedures outlined in Section III.D and assayed as described in Section III.F.

F. Sample Analysis

1. Instrumental Method

Samples were analyzed with an ARL Model 3410 ICP-AES with Minitorch® (Applied Research Laboratories, Inc., Valencia, CA) used with a CETAC U5000-AT Ultrasonic Nebulizer (CETAC Technologies, Inc., Omaha, NE). Vanadium was quantitated via the V ion emission at 309.311 nm. The internal standard was measured at the ion emission for yttrium at 371.030 nm. Instrument parameters are summarized in Table 4.

2. Calibration and Calibration Verification

The ICP-AES was calibrated against solution standards containing vanadium concentrations of [~~0, 0.01, and 0.05 µg V/mL in the solvent~~ 0, 0.14, and 0.70 mg V/g for rats and 0, 0.28, and 1.4 mg V/g for mice][&A] with internal standard concentrations of 5 µg Y/mL. A calibration check standard was prepared at a concentration of [~~0.025 µg V/mL~~ 0.35 µg V/g for rats and 0.70 µg V/g for mice][&A] with 5 µg Y/mL. Acid blanks and check standards were analyzed after calibration, after approximately every 10 samples, and at the end of analysis. Calibration check standards were maintained within ±10% of the prepared value throughout the analysis.

[&A] Changed 10/25/96 by Amendment A

3. Instrument Response

Following calibration of the ICP-AES, the vanadium concentration was measured in each of the solvent standards, spiked blood standards, acid blanks, system blanks, and blood blanks, and the instrument responses for V and Y were determined.

Instrument response was measured as kilocounts from the photomultiplier. Analyte and internal standard responses were automatically corrected by the instrument for the contribution from the solvent blank, based on analysis of the solvent blanks (with and without internal standard) analyzed with the calibration standards. Internal standard corrections were calculated as

$$R_V/R_Y$$

where R_V is the acid blank corrected response for vanadium in each standard and R_Y is the acid blank corrected response for yttrium in each standard.

All response factors were corrected for internal standard response as described above. No significant vanadium response was observed in solvent blanks or system blanks, however, there was a significant vanadium response in the blood blanks. The response for spiked blood standards was corrected for the vanadium concentration in the blank blood by subtracting the average blank blood response per gram multiplied by the spiked blood weight in grams.

IV. Results and Discussion

A. Absolute Recovery

Percent recovery was calculated as the ratio of corrected response values for spiked blood standards to corrected response values for solvent standards (x100%). Calculated recoveries for spiked rat and mouse blood standards are presented in Table 5 and Table 6, respectively.

Mean recoveries from spiked rat blood ranged from 95% to 117% with relative standard deviations ranging from 4.7 to 32%. Relative standard deviations ranged from 4.7% to 16% at concentrations ≥0.1 µg V/g.

Mean recoveries from spiked mouse blood ranged from 96% to 129% and relative standard deviations from 1.5% to 30%. Recovery was unexpectedly high at 0.2 μg V/g of blood (129%). Relative standard deviations were <10% at all concentrations ≥0.2 μg V/g.

B. Calibration Curves

Calibration curves derived from spiked rat blood standards and associated solvent standards are presented in Figures 2 and 3, respectively. Figures 2 and 3 were derived using linear least-squares fits of the form $y = mx + b$, where y is blank and internal standard corrected response, x is concentration, m is the slope, and b is the intercept. These curves also employed a weighted least-squares fit, using $1/y^2$ as the weighting factor, where y is the blank and internal standard corrected instrument response. For the curves in Figures 2 and 3, solvent standards and spiked rat blood standards were both assumed to contain 1 g of blood. The range covered for these calibration curves was from 0.05 to 0.50 μg V/g.

The curves in Figures 2 and 3 are linear with correlation coefficients ≥0.99. The slope for the solvent standards was ~15% lower than that observed for the spiked blood standards. This difference is discussed further in Section IV.F.

Calibration curves derived from spiked mouse blood standards and associated solvent standards are presented in Figures 4 and 5, respectively. Figures 4 and 5 were derived as described above, using linear least-squares fits weighted by $1/y^2$, where y is the blank and internal standard corrected instrument response. For the curves in Figures 4 and 5, solvent standards and spiked mouse blood standards were both assumed to contain 0.5 g of blood. The range covered for these calibration curves was from 0.10 to 1.0 μg V/g.

The curves in Figures 4 and 5 are also linear with correlation coefficients ≥0.99. The slope for the solvent standards was ~5% lower than that observed for the spiked mouse blood standards. This difference is discussed further in Section IV.F.

C. Calculated Concentrations and Percent Residual Error

Vanadium concentrations in spiked rat blood and associated solvent standards were calculated using the calibration curves in Figures 2 and 3. Vanadium concentrations in spiked mouse blood and associated solvent standards were calculated using the calibration curves illustrated in Figures 4 and 5. Concentrations were calculated for each standard as $x=(y-b)/m$, where x is the vanadium concentration (μg/g), y is the ICP-AES response corrected for the blank and internal standard, b is the y-intercept, and m

is the slope of the calibration curve. For each concentration, the percent residual error was calculated as the relative difference between the calculated concentration and the prepared concentration. Calculated vanadium concentrations and percent residual error values for spiked rat blood standards and associated solvent standards are shown in Tables 7 and 8, respectively. Calculated vanadium concentrations and percent residual error values for spiked mouse blood standards and associated solvent standards are listed in Tables 9 and 10, respectively.

As shown in Table 7, errors in individual spiked rat blood standards ranged from -13.5 to + 22.8%. Mean errors over the range of concentrations ranged from -8% to +5.9%. Solvent standards analyzed with spiked rat blood standards (Table 8) gave considerably better performance, with errors ranging from -3.9% to +4.8%. The poorer accuracy in spiked rat blood standards compared to solvent standards is most likely due to the high and variable endogenous blood vanadium concentrations in blank rat blood.

Errors in individual spiked mouse blood standards (Table 9) ranged from -20.2 to + 37.4%. Mean errors over the range of concentrations ranged from -9.4% to +14.9%. Solvent standards analyzed with spiked mouse blood standards (Table 10) gave better performance, with errors ranging from -11.9% to +10.1%. As mentioned above, the poorer accuracy in spiked mouse blood standards compared to solvent standards is most likely due to the high and variable endogenous blood vanadium concentrations in blank mouse blood.

D. Precision and Accuracy

Mean errors and relative standard deviations in spiked rat blood standards were within 20% at all concentrations ≥0.05 μg V/g (Table 7). However, some of the individual replicates at concentrations ≤0.1 μg V/g produced errors near 20%. Mean recoveries from spiked rat blood were within the range of 95% to 117% over the range of concentrations studies (Table 5). Precision in the recovery values was ~32% at 0.05 μg V/g, but was within 20% at concentrations ≥0.1 μg V/g. Errors in solvent standards were significantly less than those observed in spiked rat blood standards (Table 8). The poorer performance at the lower concentrations in spiked blood standards compared to the solvent standards is probably due to matrix effects.

Mean errors in spiked mouse blood standards were within 20% at all concentrations ≥0.1 μg V/g (Table 9). However, the individual replicates at 0.1 μg V/g all produced absolute errors >20% and the relative standard deviation in the errors at this concentration was 31%. Mean recoveries from spiked mouse blood were within the range of 96 to 129% over the range of concentrations studied (Table 6). Precision in the recovery values was ~30% at 0.1 μg V/g, but was within 20% at concentrations ≥0.2 μg

V/g. Errors in solvent standards were significantly less than those observed in spiked mouse blood standards (Table 10). The poorer performance at the lower concentrations in spiked blood standards compared to the solvent standards is probably due to matrix effects.

E. Detection and Quantitation Limits

The limit of detection (LOD) was determined as the 3 times the standard deviation of the vanadium concentration in blood blanks. The limit of quantitation (LOQ) was determined as 10 times the standard deviation of the blood blanks. Values of LOD and LOQ for vanadium in rat and mouse blood are summarized in Table 11. Detection and quantitation limits are reported in units of concentration (μg V/g) and mass (μg V). [Vanadium concentrations measured in blank rat and mouse blood are tabulated in Table 12.][+A]

The experimental limit of quantitation (ELOQ) was defined as the concentration of the lowest spiked blood standard which produced a precision and accuracy within ±20%. ELOQ values for rats and mice were 0.10 and 0.20 μg V/g respectively (Table 11).

Measured concentrations of vanadium in solvent and system blanks were below the LOD. The average concentration of vanadium in the blood blanks was greater than the ELOQ values specified in Table 11.

F. Selectivity

Possible interferences in this method result from direct overlap of atomic emissions from other elements with the analytical wavelengths for V (309.311 nm) and Y (371.030 nm). Possible interferences for V can arise from atomic emissions from Cr (309.349 nm), Al (309.271, 309.284), and Mg (309.299). Possible interferences for the internal standard (Y, 371.01 nm) can arise from emissions due to V (371.11 nm) and Ti (371.02 nm). Of these possible interferences, Cr and Ti are not expected to occur in significant concentrations in biological samples, and the emissions of these elements are weak at the wavelengths indicated. The emissions for V at 371.12 nm is weak, and is sufficiently different from the yttrium analytical wavelength that it is not expected to present a significant problem (Winge et al., 1985). The monochromator in the ARL 3410 ICP-AES employed for this analysis has a resolution of 0.01 nm, and is capable of resolving the interfering wavelengths from the wavelengths used for V and Y.

In this study, no systematic increase was observed for the internal standard (yttrium) response with increasing concentrations of vanadium. Increases in the response for vanadium were seen in spiked blood standards relative to solvent standards. This indicates that there is a bias introduced by the blood

[+A] Added 10/25/96 by Amendment A

matrix. This can be seen in the slopes of calibration curves derived from spiked blood standards which are higher than those derived from solvent standards (by approximately 5% and 15%). Less bias (~5%) was observed for the mouse blood standards compared to those for the rats (~15%) probably because the mouse blood standards employ less blood (~0.5 g) than spiked rat blood standards (~1 g).

G. Sample Handling and Analysis

For sample analysis, we propose calibrating the ICP-AES using spiked blood standards prepared to contain 0, 0.10, 0.25, and 0.50 µg V/g for rats or 0, 0.20, 0.50, and 1.0 µg V/g for mice in 5% HNO_3 with an internal standard concentration of 5 µg Y/mL. One set of standards will be prepared with each set of digestions.

During study sample preparation, a spiked blood quality control standard will be prepared with each batch of samples. Quality control standards will be prepared to contain 0.25 µg V/g for rats or 0.50 µg V/g for mice, and at least one of these will be prepared with each batch of 12 samples. A quality control standard will be analyzed after calibration, after approximately 10 samples, and at the end of the analysis. The analyzed value of the quality control standards will be required to be within ±20% or the instrument will be recalibrated and samples after the last acceptable quality control standard will be reanalyzed.

Each time point during the study will provide a batch of 25 rat and 30 mouse blood samples. Blood from each species and time point will be prepared separately in four microwave digestion batches of 12. For rat blood, each batch will include 4, 5 or 8 study samples, 1 or 2 QC samples, and 1 or 2 blood blanks. For mouse blood, each batch will include 5 or 10 study samples, 1 or 2 QC samples, and 0 or 1 blood blank. Digestions from each species and time point will be analyzed in two analytical runs.

During sample analysis, Battelle will use the instrument software to automatically generate calibration curves corrected based on calibration blank and internal standard corrected responses. Samples which exhibit a concentration greater than [~~0.0357 µg V/mL~~ 0.5 µg V/g for rats or 1.0 µg V/g for mice][&A] (high calibration standard) will be diluted into the calibration range of the ICP-AES with blank digested blood and reanalyzed.

[&A] Changed 10/25/96 by Amendment A

V. References

Winge, R. K., V. A. Fassel, V. J. Peterson, and M. A. Floyd (1985), *Inductively Coupled Plasma-Atomic Emission Spectroscopy*, Elsevier, New York, 584 pages.

Table 1. Expected Rat Blood Concentrations During a Chronic Two-Year V_2O_5 Exposure[a]

	Exposure Concentration (mg V_2O_5/m^3)		
	0.5	1.0	2.0
Months on Study	Expected Whole Blood Concentration (µg V/g)		
3	0.091	0.18	0.19
6	0.091	0.18	0.19
12	0.091	0.18	0.19
18	0.091	0.18	0.19

[a] Values shown are estimated through calculation, and are rounded to 2-3 significant figures. See text for explanation of methods for estimation.

Table 2. Expected Mouse Blood Concentrations During a Chronic Two-Year V_2O_5 Exposure[a]

	Exposure Concentration (mg V_2O_5/m^3)		
	1.0	2.0	4.0
Months on Study	Expected Whole Blood Concentration (µg V/g)		
3	0.087	0.17	0.17
6	0.087	0.17	0.17
12	0.087	0.17	0.17
18	0.087	0.17	0.17

[a] Values shown are estimated through calculation, and are rounded to 2-3 significant figures. See text for explanation of methods for estimation.

Table 3. CEM MDS-2000 Microwave Digestion Parameters

Stage:	(1)	(2)	(3)	(4)	(5)
Power[a]:	100%	100%	100%	0%	0%
Pressure (lbs/in^2):	0020	0040	0085	0020	0020
Run Time (minutes):	15:00	15:00	15:00	00:00	00:00
Time at Pressure:	05:00	05:00	05:00	00:00	00:00
Temperature Setting[b]:	Off	Off	Off	Off	Off
Fan Speed:	100%	100%	100%	100%	100%
Number of Vessels:	12[c]				
Volume per Vessel:	2 mL[c]				
Sample Weight:	1.0 g[c]				
Acid:	Nitric (~35% w/w)[c]				

a Maximum power output is 650 Watts.

b Temperature not monitored or used as part of the digestion program.

c These parameters are for general user information only and do not affect the microwave digestion program if changed.

Table 4. ICP-AES Instrument Parameters for Analysis of Vanadium in Blood

Parameter	Description
Instrument	ARL 3410 with Minitorch (USN)
Spectrometer Vacuum	120 millitorr
Carrier Argon Pressure/Flow	24 psig
Plasma Argon Pressure/Flow	25 psig
Coolant Argon Pressure/Flow	24 psig
Reflected Power	~7 Watts
Incident Power	~644 Watts
Plate Volts x 10	~303
Plate Current	~561 mA
Grid Current	~103 mA
Drive Volts x 10	~368 V
Wavelength Calibration Line	Argon 355.431 nm
Vanadium Analysis Line	309.311 nm (1st Order, ion line)
Yttrium Analysis Line	371.030 nm (1st Order, ion line)
Integration Time (Iterations)	1 second (3) for V, 1.0 second (3) for Y
Nebulizer	CETAC U5000-AT Ultrasonic Nebulizer 150°C heated stage/-5°C cooled stage
Sample Flow Rate	~2.7 mL/min
Internal Standard (Concentration)	Yttrium (5 µg/mL)
Standard Concentrations	~~[0, 0.01, 0.05 µg V/mL~~ 0, 0.14, 0.70 µg V/g for rats; 0, 0.28, 1.4 µg V/g for mice][&A]
Drift Correction Standards [~~(If Applicable)~~	~~0.01 and 0.05 µg V/mL~~ 0.14 and 0.70 µg V/g for rats; 0.28 and 1.4 µg V/g for mice][&A]
Check Standard	~~[0.025 µg V/mL~~ 0.35 µg V/g for rats 0.70 µg V/g for mice][&A]
Matrix	5% HNO_3

&A Changed 10/25/96 by Amendment A

Table 5. Recovery of Vanadium from Spiked Rat Blood

Spiked Concentration (µg V/g)	n	Mean % Recovery (N=3)[a]	%RSD
0.05	3	95	31.9
0.10	3	111	16.3
0.15	3	107	4.7
0.20	3	112	6.5
0.25	3	117	7.6
0.50	3	108	6.6

a % Recovery = $\frac{\text{Spiked Blood Standard Response}}{\text{Solvent Standard Response}} \times 100$

Table 6. Recovery of Vanadium from Spiked Mouse Blood

Spiked Concentration (µg V/g)	n	Mean % Recovery (N=3)[a]	%RSD
0.10	3	112	30.2
0.20	3	129	9.3
0.30	3	96	1.5
0.40	3	98	2.9
0.50	3	97	5.7
1.00	3	109	2.5

a % Recovery = $\frac{\text{Spiked Blood Standard Response}}{\text{Solvent Standard Response}} \times 100$

Table 7. Calculated Concentrations and Percent Residual Error in Spiked Rat Blood Standards

Vanadium Concentration (µg V/g Blood)	Response Factor[a]	Calculated µg V/g	Calculated µg V/g Blood (mean ±% RSD)	% Residual Error	% Residual Error (mean ± SD)
0.05	0.0084	0.045		-9.1	
0.05	0.0106	0.050		0.8	
0.05	0.0155	0.061	0.052 ±15.6	22.8	4.9 ±16.3
0.10	0.0415	0.120		19.9	
0.10	0.0321	0.099		-1.2	
0.10	0.0313	0.097	0.105 ±12.1	-3.0	5.2 ±12.8
0.15	0.0555	0.151		0.9	
0.15	0.0559	0.152		1.5	
0.15	0.0604	0.162	0.155 ± 3.9	8.3	3.6 ± 4.1
0.20	0.0770	0.200		-0.1	
0.20	0.0825	0.212		6.1	
0.20	0.0877	0.224	0.212 ± 5.7	11.9	5.9 ± 6.0
0.25	0.1067	0.267		6.6	
0.25	0.1020	0.256		2.4	
0.25	0.0919	0.233	0.252 ±6.8	-6.7	0.8 ± 6.8
0.50	0.2059	0.490		-2.1	
0.50	0.1804	0.432		-13.5	
0.50	0.1920	0.458	0.460 ± 6.2	-8.3	-8.0 ± 5.7

[a] RF = $\frac{\text{Spiked Blood Standard V Kcounts - Calibration Blank V Kcounts}}{\text{Spiked Blood Standard Y Kcounts - Reference Blank Y Kcounts}}$ – [Mean Blank Blood RF/g x Sample Weight (g)]

RF = Response Factor

Table 8. Calculated Concentrations and Percent Residual Error in Solvent Standards Analyzed with Spiked Rat Blood Standards

Vanadium Concentration (µg V/g Blood)	Response Factor[a]	Calculated µg/g	% Residual Error
0.05	0.0121	0.050	-0.8
0.10	0.0316	0.100	0.2
0.15	0.0536	0.157	4.8
0.20	0.0736	0.209	4.6
0.25	0.0860	0.241	-3.5
0.50	0.1782	0.480	-3.9

[a] Response Factor = $\frac{\text{Spiked Blood Standard V Kcounts - Calibration Blank V Kcounts}}{\text{Spiked Blood Standard Y Kcounts - Reference Blank Y Kcounts}}$

Table 9. Calculated Concentrations and Percent Residual Error in Spiked Mouse Blood Standards

Vanadium Concentration (µg V/g Blood)	Response Factor[a]	Calculated µg V/g	Calculated µg V/g Blood (mean ±% RSD)	% Residual Error	% Residual Error (mean ± SD)
0.10	0.0223	0.128		27.6	
0.10	0.0242	0.137		37.4	
0.10	0.0130	0.080	0.115 ± 26.8	-20.2	14.9 ± 30.8
0.20	0.0355	0.196		-2.2	
0.20	0.0411	0.224		12.2	
0.20	0.0425	0.232	0.217 ± 8.8	15.8	8.6 ± 9.5
0.30	0.0507	0.274		-8.7	
0.30	0.0509	0.275		-8.4	
0.30	0.0494	0.267	0.272 ± 1.5	-11.0	-9.4 ±1.4
0.40	0.0759	0.403		0.9	
0.40	0.0761	0.404		1.1	
0.40	0.0722	0.384	0.397 ± 2.8	-3.9	-0.6 ± 2.8
0.50	0.0922	0.487		-2.5	
0.50	0.0971	0.513		2.5	
0.50	0.1033	0.544	0.515 ± 5.6	8.9	3.0 ± 5.7
1.00	0.1987	1.035		3.5	
1.00	0.1955	1.019		1.9	
1.00	0.2054	1.070	1.041 ± 2.5	7.0	4.1 ± 2.6

[a] $RF = \frac{\text{Spiked Blood Standard V Kcounts - Calibration Blank V Kcounts}}{\text{Spiked Blood Standard Y Kcounts - Reference Blank Y Kcounts}} - [\text{Mean Blank Blood RF/g x Sample Weight (g)}]$

RF = Response Factor

Table 10. Calculated Concentrations and Percent Residual Error in Solvent Standards Analyzed with Spiked Mouse Blood Standards

Vanadium Concentration (µg V/g Whole Blood)	Response Factor[a]	Calculated µg/g	% Residual Error
0.10	0.0178	0.106	6.0
0.20	0.0308	0.176	-11.9
0.30	0.0523	0.292	-2.5
0.40	0.0763	0.422	5.5
0.50	0.1001	0.551	10.1
1.00	0.1826	0.996	-0.4

[a] RF = $\frac{\text{Spiked Blood Standard V Kcounts - Calibration Blank V Kcounts}}{\text{Spiked Blood Standard Y Kcounts - Reference Blank Y Kcounts}}$

Table 11. Limit of Detection, Limit of Quantitation, and Experimental Limit of Quantitation for Vanadium in Rat and Mouse Blood

Parameters	Rat Blood	Mouse Blood
LOD (µg V/g)	0.043	0.11
LOD (µg V)	0.043	0.056
LOQ (µg V/g)	0.14	0.37
LOQ (µg V)	0.14	0.19
ELOQ (µg V/g)	0.10	0.20
ELOQ (µg V)	0.10	0.10

Table 12. Vanadium Concentrations in Blank Rat and Mouse Blood +A

Replicate #	Mice (µg V/g)	Rats (µg V/g)
1	0.405	0.265
2	0.461	0.279
3	0.394	0.261
4	0.368	0.262
5	0.343	0.297
6	0.345	0.281
7	0.356	0.280
8	0.369	0.279
9	0.349	0.254
Mean ± SD	0.377 ± 0.038	0.273 ± 0.014

+A Added 10/25/96 by Amendment A

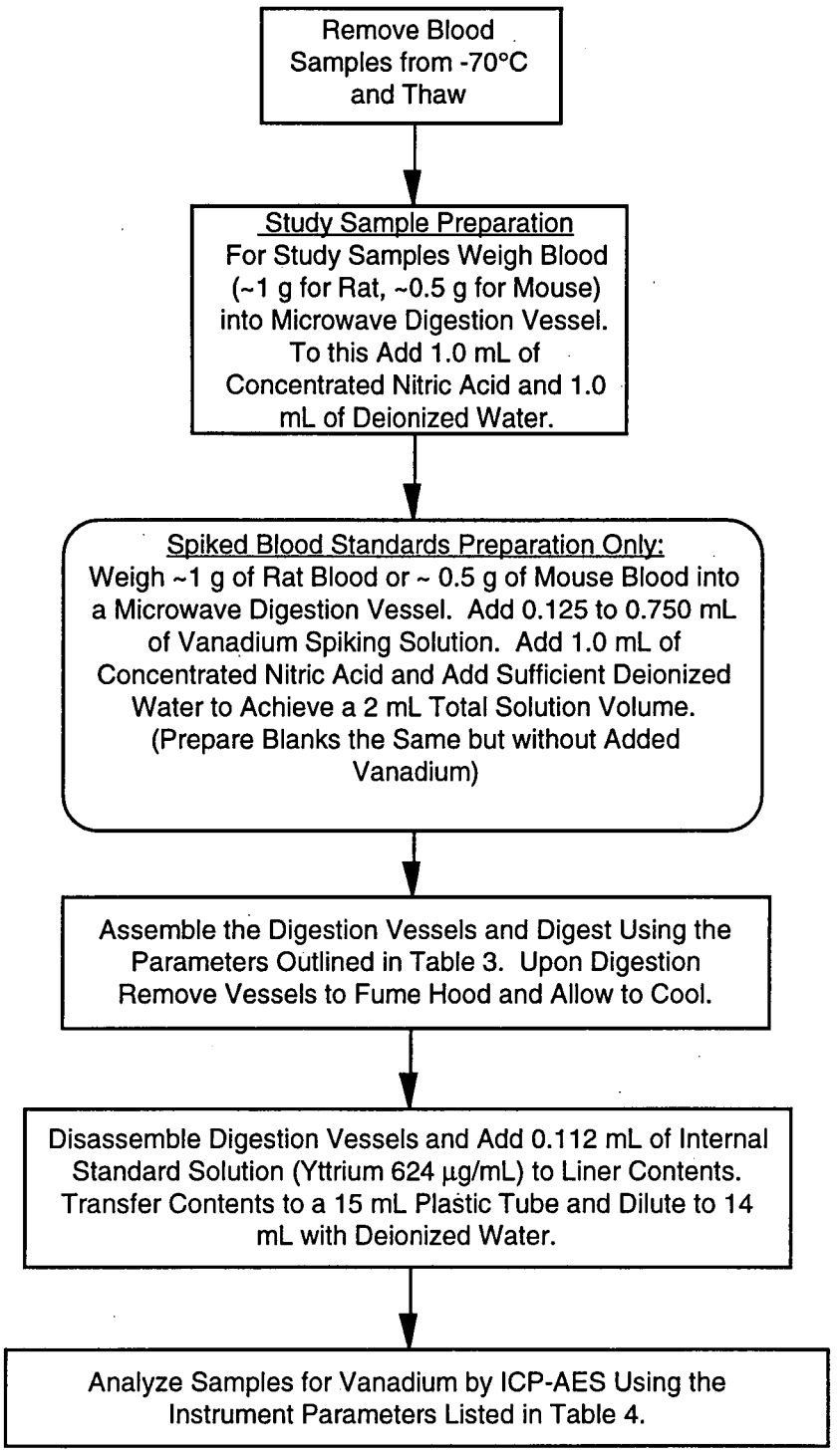

Figure 1. Sample Preparation Scheme for Analysis of Vanadium in Blood

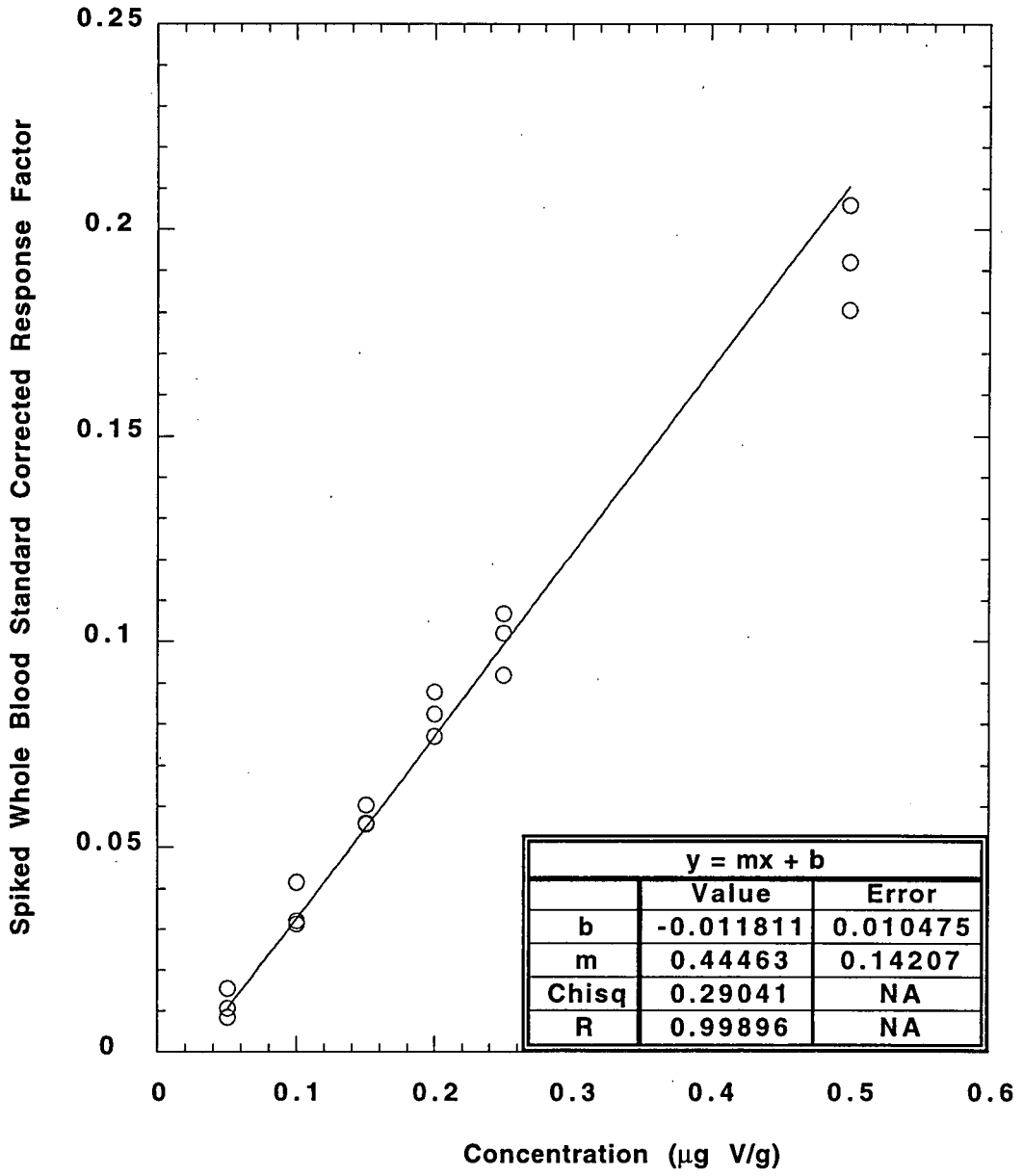

Figure 2. ICP-AES Calibration Curve for Vanadium in Spiked Rat Blood Standards

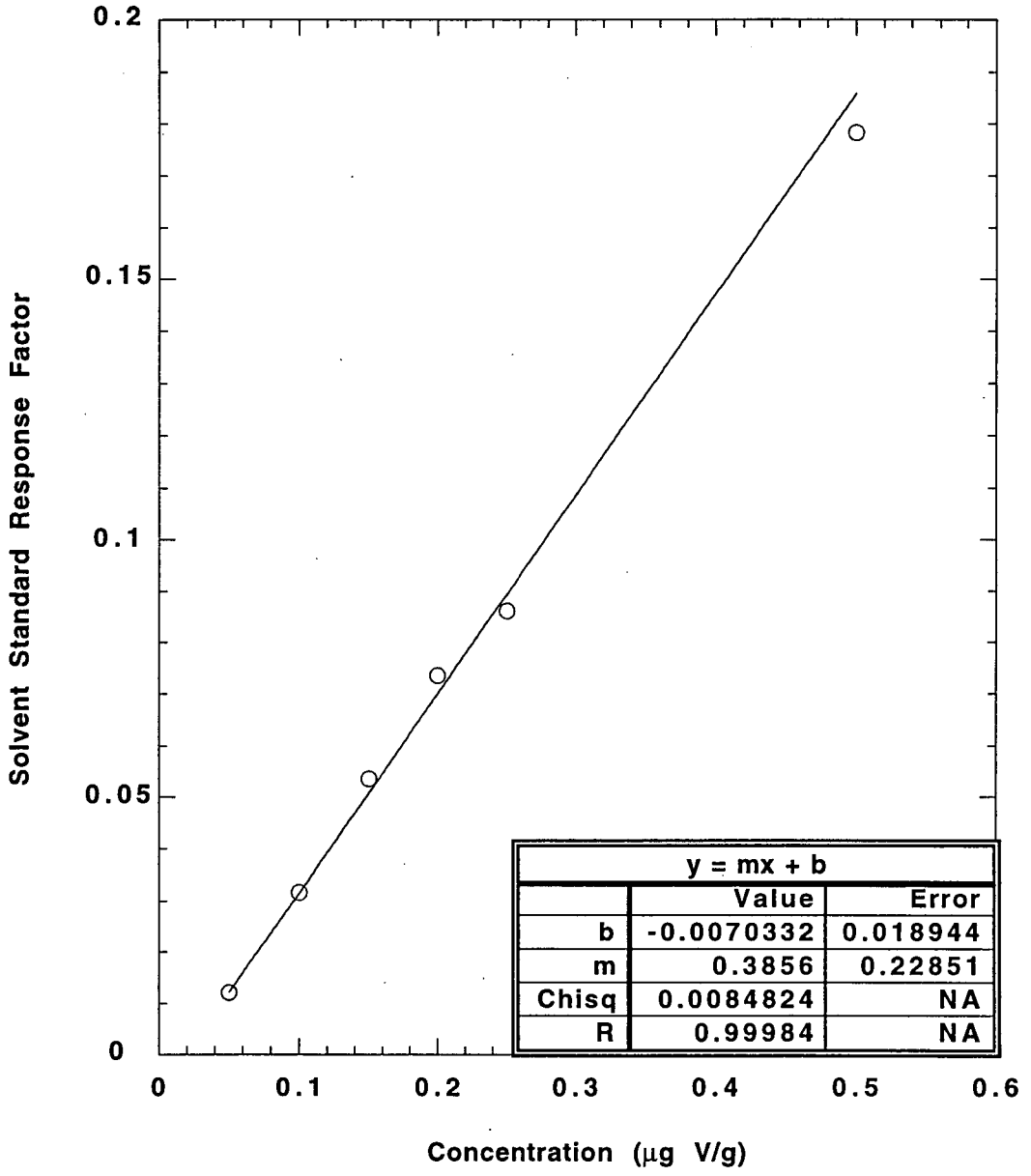

Figure 3. ICP-AES Calibration Curve for Vanadium in Solvent Standards Analyzed with Spiked Rat [~~Whole~~ Blood][&A] Standards

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&A Changed 10/25/96 by Amendment A

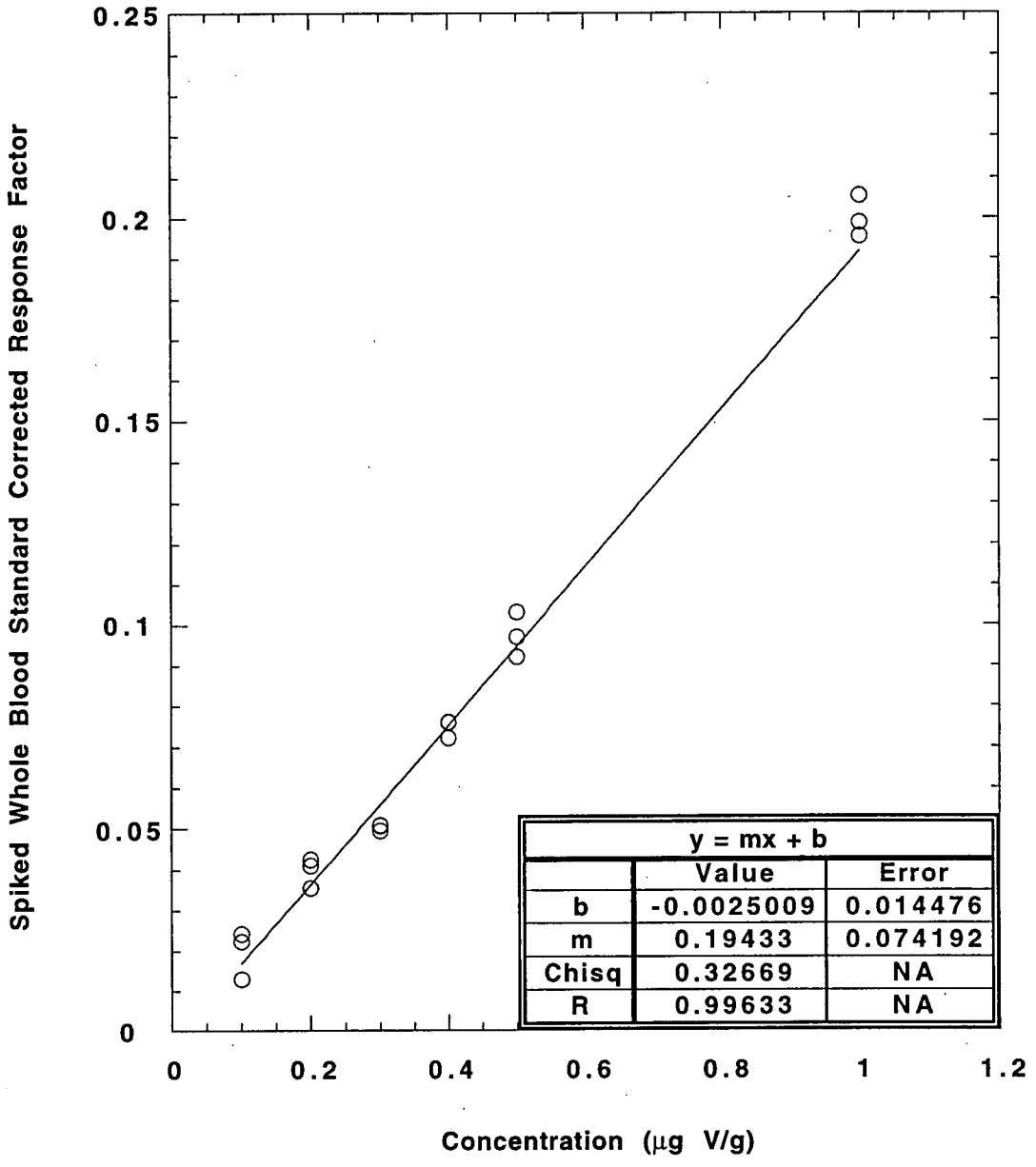

Figure 4. ICP-AES Calibration Curve for Vanadium in Spiked Mouse Blood Standards

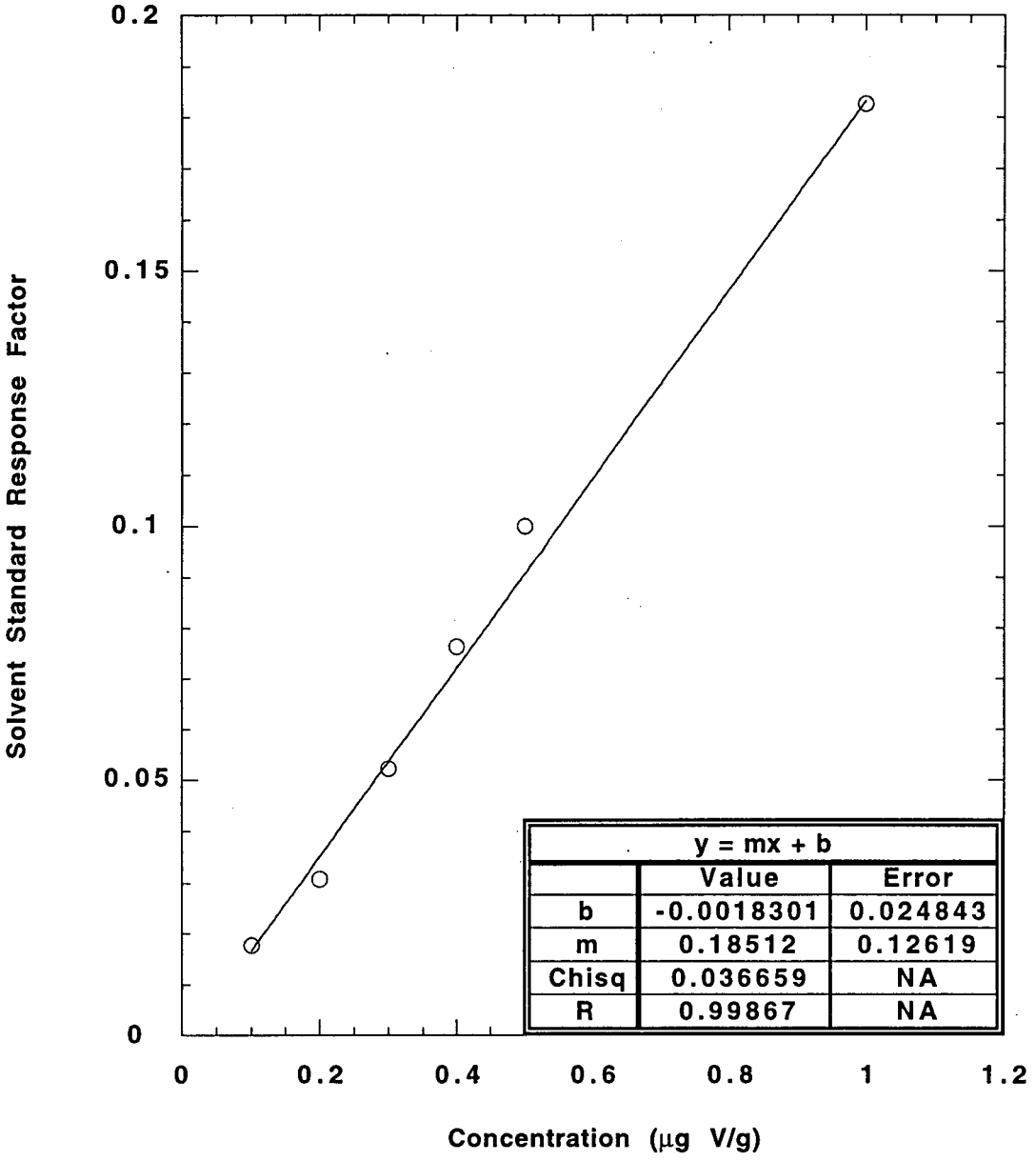

Figure 5. ICP-AES Calibration Curve for Vanadium in Solvent Standards Analyzed with Spiked Mouse Blood Standards

2-Year Inhalation Toxicity and Carcinogenicity Study of Vanadium Pentoxide
in F344 Rats and B6C3F1 Mice
Method Performance Evaluation and Prestart Report for Toxicokinetic Studies

Report Changes and/or Revisions

10/25/96 Amendment A

Cover Page

Add: "Vanadium Pentoxide, V_2O_5, CAS# 1313-62-1, C61427B"

Page i

Add: "Table 12. Vanadium Concentrations in Blank Rat and Mouse Blood ... 21"

Page ii

Add: "REPORT AMENDMENTS ... 26a"

Page iv

Add: Quality Assurance Statement

Reason: Sponsor request
Effective Date: 10/25/96

Page 4

Add: "·cm"
Delete: Comma following "pooled."

Page 6

Delete: Comma following "into"

Page 7

Change: "0, 0.01, and 0.05 µg V/mL in the solvent" to "0, 0.14, and 0.70 mg V/g for rats and 0, 0.28, and 1.4 mg V/g for mice"

Change: "0.025 µg V/mL" to "0.35 µg V/g for rats and 0.70 µg V/g for mice"

Page 11

Add: "Vanadium concentrations measured in blank rat and mouse blood are tabulated in Table 12."

Page12

Change: "0.0357 µg V/mL" to "0.5 µg V/g for rats or 1.0 µg V/g for mice"

Page 16, Table 4

Change: "0, 0.01, 0.05 µg V/mL" to "0, 0.14, 0.70 µg V/g for rats; 0, 0.28, 1.4 µg V/g for mice"

Change: "(If Applicable) 0.01 and 0.05 µg V/mL" to "0.14 and 0.70 µg V/g for rats; 0.28 and 1.4 µg V/g for mice"

Change: "0.025 µg V/mL" to "0.35 µg V/g for rats; 0.70 µg V/g for mice"

Page 21

Add: Table 12

Page 24

Change: "Whole" to "Blood"

Reason: Report preparation errors
Effective Date: 6/28/96

Page 26 a & b

Add: List of Report Changes and/or Revisions

Reason: To summarize changes in the report
Effective Date: 10/25/96

[redacted]
Group Leader

11/10/96
Date