Arsenic Species in Frozen Human Urine

Human exposure to arsenic comes primary from two sources -- drinking water and food. Depending on the source, arsenic may be present in several chemical forms that differ greatly in toxicity. The Centers for Disease Control and Prevention (CDC) has established a network of laboratories to monitor arsenic exposure throughout the U.S. by measuring arsenic species in urine. To ensure accuracy of these measurements, CDC is collaborating with NIST to develop a new frozen urine Standard Reference Material (SRM) to be certified for arsenic species.

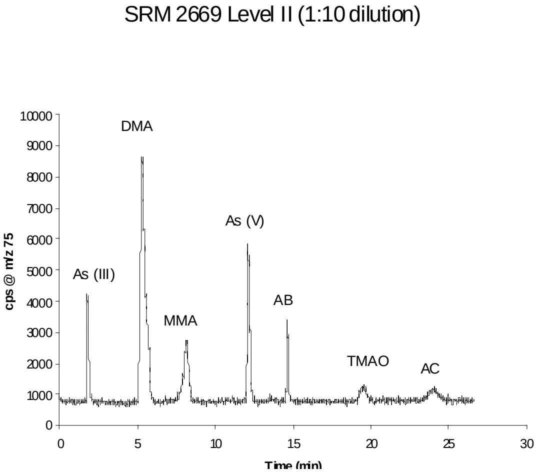
L.L. Yu, T.A. Butler, W.C. Davis, S.A. Rabb, R.L. Zeisler, G.C. Turk (Div. 839), and M.P. Cronise (Div. 232)

Work is progressing on a three-year project in which NIST and CDC are collaborating to produce SRM 2669 Arsenic Species in Frozen Human Urine, which is the first frozen urine SRM and the first SRM to be certified for multiple arsenic species. Each unit of the SRM will consist of two different urine samples containing arsenic species that appear in human urine following ingestion of foods with naturally occurring arsenic compounds and of arsenic-containing drinking water. The two urine pools were formulated by mixing analyzed sub-lots of urine to produce mixtures with the desired species and levels. The candidate SRM has been bottled and frozen and certification measurements are presently underway. In response to earlier studies that indicated that the presence of dissolved oxygen could adversely affect the stability of some arsenic species, the urine mixtures were sparged with nitrogen to remove oxygen, and the SRMs were bottled in nitrogen-containing glove boxes. The candidate SRM units are packaged in sealed gas-impermeable Mylar bags that contain an oxygen absorber.

Concentrations of seven arsenic species (arsonite, arsonate, monomethyl-arsonate, dimethylarsinate, trimethylarsine oxide, arsenobetaine, and arsenocholine) and total arsenic will be certified in the urine. Each analyte will be present at a normal level and an elevated level near the 95 percentile as determined by CDC population studies. The elevated levels are not all contained in the same sample. Certified values for the arsenic species will be based on measurements performed at NIST (both in Gaithersburg and at NIST's facility at the Hollings Marine Laboratory in Charleston) and measurements performed by expert laboratories including the CDC, the Armed Forces Institute of Pathology, Rutgers University, and Brock University (Canada). The chromatogram shown in the figure was obtained using a method that was developed for this project; species are separated using ion chromatography with element-specific detection of arsenic by inductively coupled plasma mass spectrometry (ICP-MS). The figure shows that each of the seven arsenic species is detected with baseline separation in the Level II urine.

SRM 2669 will be the first frozen urine reference material with values assigned for all important arsenic species. An earlier Certified Reference Material produced by the National Institute for Environmental Studies in Japan is based on freeze-dried urine and is not certified for the As^{+3} and As^{+5} species, which are the most toxic arsenic species and, therefore, critical for detection of arsenic poisoning. The frozen urine matrix of SRM 2669 is better suited for the intended purpose to validate the methods and measurements for urine analysis. Release of the new SRM is planned for early 2008.

SRM 2669 Arsenic Species in Frozen Human Urine will provide the accuracy base for biomonitoring of arsenic exposure. Concentrations of seven arsenic species indicative of different sources of arsenic exposure will be certified.



Development of a New Beryllium Oxide Standard Reference Material

Beryllium and compounds containing beryllium are used in the production of high-performance alloys that are relied upon in the aerospace and defense industries. These chemicals pose a significant health risk for workers in these fields because exposure to Be can cause beryllium disease, a serious, chronic, and sometimes fatal illness in susceptible individuals. Beryllium workers must be carefully monitored for potential exposure. These monitoring systems require properly validated analytical methods for the determination of Be-containing particles collected on swipes and monitoring filters. Validation of these methods is hampered by the absence of suitable reference materials. This is especially the case for BeO and other Be compounds that are extremely difficult to dissolve. The U.S. Department of Energy (DOE), the National Institute for Occupational Safety and Health (NIOSH), and NIST have entered into an agreement to develop a new BeO SRM.

G.C. Turk, M.R. Winchester, and T.A. Butler (Div. 839)

Given the significant health concerns associated with BeO, a somewhat unconventional approach to the production and certification of an SRM has been initiated. Specifically, the sample preparation leading up to the ICP-OES analyses are being performed by four DOE and NIOSH laboratories with extensive experience working with Be materials. NIST has developed the complete experimental design and is requiring these laboratories to comply with it. An Excel spreadsheet was developed at NIST and provided to the labs to guide them through the sample preparation process and to ensure that all necessary sample preparation data are recorded. In addition to the spreadsheet, NIST has provided each of the labs with a sample preparation kit. This kit contains all chemicals needed to perform preparations, including the bottled BeO candidate SRM, the high-purity, well-characterized Be metal, an internal standard stock solution, and acid. It also contains all bottles, labels, and packaging needed to ship the digested solutions back to NIST for analysis. Finally, the kit contains small pieces of gold wire having mass values that are traceable to mass standards maintained by NIST. These wires are being used by the labs to establish traceability of their mass measurements.

Measurement of Be in the BeO SRM will be performed using high-performance inductively-coupled plasma optical emission spectrometry (ICP-OES). The ICP-OES instrument will be calibrated with solutions prepared from high-purity, well-characterized Be metal. The SRM will be released in 2007 and will be used to validate analytical methods used for Be determinations. It may also be used to set up a proficiency testing program to implement the requirements of CFR850 (Chronic Beryllium Disease Prevention Program), as specified in 10 CFR Part 850.24, Section (f) (Periodic Exposure Monitoring Analysis), preventing exposure to Be that leads to chronic beryllium disease.

A worker is preparing to sample BeO. The new BeO SRM will enable the validation of analytical methods for the determination of Be.

Photo courtesy of Dr. Gregory Day, NIOSH

Development of SRMs for Botanical Dietary Supplements

As part of a multi-year interagency agreement among the National Institute of Standards and Technology (NIST), the National Institutes of Health's Office of Dietary Supplements (NIH/ODS), and the Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) and the FDA Center for Drug Evaluation and Research (CDER), NIST is developing Standard Reference Materials (SRMs) for botanical dietary supplements. Taxonomically authentic botanical reference materials with assigned values for concentrations of active and/or marker compounds, pesticides, and heavy metals are being produced to assist in the verification of manufacturers' label claims and for use in quality control during the manufacturing process.

L.C. Sander, K.E. Sharpless, and S.A. Wise (Div. 839)

Dietary supplements are products containing vitamins, minerals, herbs or other botanicals, amino acids, etc. that are consumed to increase total daily intake and/or for perceived health benefits. Many people believe that botanical supplements will improve their health and that these "natural" remedies are both effective and free from the side effects that may occur with other medications. There are occasional reports of inaccurate labeling, adulteration, contamination (with pesticides, heavy metals, or toxic botanicals), and drug interactions.

Despite questions about the quality and safety of dietary supplements (including vitamins), about 75% of the US population continues to use them. The US consumer spends more than $20 B on these supplements per year, with expected spending growth of 3% to 5% each year.

Congress recognized the lack of publicly available, validated analytical methods for dietary supplements – and a lack of reference materials for validation of analytical methods – in 1994 when the Dietary Supplement Health and Education Act (DSHEA) was enacted. As part of DSHEA, NIH/ODS was directed to fund development of analytical methods and reference materials for dietary supplements.

Shown here are materials that are currently available or for which Certificates of Analysis are in review. Also shown are the constituents for which values are assigned. The constituents in all of these materials have been determined by multiple independent methods with measurements performed by NIST and collaborating laboratories. The methods utilized different sample extraction and cleanup steps in addition to different instrumental analytical techniques and approaches to quantification.

SRM	Name	Constituents
1588b	Organics in Cod Liver Oil	Fatty acids and organic contaminants
3240	*Ephedra sinica* Stapf Aerial Parts	Ephedrine alkaloids, toxic elements
3241	*Ephedra sinica* Stapf Native Extract	Ephedrine alkaloids, toxic elements
3242	*Ephedra sinica* Stapf Commercial Extract	Ephedrine alkaloids, toxic elements
3243	Ephedra-Containing Solid Oral Dosage Form	Ephedrine alkaloids, caffeine, toxic elements
3244	Ephedra-Containing Protein Powder	Ephedrine alkaloids, caffeine, toxic elements, nutrients
3245	Ephedra Dietary Supplement Suite	(Two bottles of each of the ephedra materials listed above; see analytes listed above)
3246	*Ginkgo biloba* (Leaves)	Flavonoids, terpene lactones (ginkgolides), toxic elements
3247	*Ginkgo biloba* Extract	Flavonoids, terpene lactones (ginkgolides), toxic elements
3248	Ginkgo-Containing Tablets	Flavonoids, terpene lactones (ginkgolides), toxic elements
3249	Ginkgo Dietary Supplement Suite	(Two bottles of each of the ginkgo materials listed above; see analytes listed above)
3276	Carrot Extract in Oil	Carotenoids, tocopherols, fatty acids

These materials are provided primarily for use in method development and as control materials to support the measurement of these constituents in other similar products. These materials will help manufacturers of dietary supplement products to characterize raw materials to prevent the use of materials that are contaminated or adulterated. In addition, the SRMs will assist self-assessment of consistency and quality in finished products, and provide a foundation to which label information can be linked. The goal of this ongoing effort is to provide the dietary supplement industry and measurement communities with tools that will lead to improved quality of dietary supplements, and ultimately reduce public health risks that could potentially be associated with these products.

Future Plans: The development of botanical dietary supplement SRMs is an ongoing effort. Materials based on saw palmetto, bitter orange, green tea, black cohosh, berries of the genus *Vaccinium*, soy, kudzu, and red clover are currently in progress. Oils extracted from a number of plants (perilla, flaxseed, evening primrose, borage), and a mixture of vegetable oils with values assigned for tocopherols and tocotrienols are also being prepared as SRMs.

Application of Specimen Bank Resources to Investigate Tributyltin in the Alaskan Arctic Marine Ecosystem

Among the different compounds in formulations of antifouling paint, tributyltin (TBT) is the most active and toxic. TBT is known to be an endocrine disruptor at very low concentrations in some marine animals. Biological impacts have been reported in confined environments such as harbors or industrialized bays. An ultra-sensitive analytical method for the determination of TBT and its two metabolites (mono- and dibutyltin) was used for analysis of cryogenically archived specimens from NIST's Marine Environmental Specimen Bank. These results are the first report of the presence of this class of compounds in the Arctic marine environment.

D. Point, P.R. Becker, S.J. Christopher, R.S. Pugh, M.B. Ellisor, A. Moors, and S.A. Wise (Div. 839)

Use of tributyltin (TBT) in antifouling paint began in the 1950s. TBT-based paints were widely used until 1988, when they were banned for use on all boats less than 25 meters long. Tankers and cargo-type vessels are still allowed to use this class of paints, although a complete ban is proposed for 2008. The presence of TBT and its two metabolites (monobutyltin [MBT] and dibutyltin [DBT]) has been reported in several species of marine mammals and pelagic fish throughout the world, thus suggesting a global contamination pattern for this class of compounds.

An ultra-sensitive analytical method recently developed at NIST was used to analyze samples from the Marine Environmental Specimen Bank (Marine ESB) at the Hollings Marine Laboratory to determine if TBT, DBT, and MBT can be detected in the Arctic marine environment. This method, speciated isotope dilution-gas chromatography-inductively coupled plasma mass spectrometry (SID-GC/ICP-MS), was developed to quantify medium to trace concentrations of butyltins in cryogenically homogenized natural biological materials. Analyses of the banked samples documents, for the first time, background concentration levels of butyltin species in different compartments of the Arctic marine ecosystem. The spatial distribution of butyltins was investigated using banked murre eggs (*Uria aalge* and *U. lomvia*) collected in Alaska by the Seabird Tissue Archival and Monitoring Project (STAMP). Temporal trends were investigated by analyzing ringed seal (*Phoca hispida*) and beluga whale (*Delphinapterus leucas*) liver samples collected over a 10-yeard period near Barrow, Alaska, and banked by the Alaska Marine Mammal Tissue Archival Project (AMMTAP).

Butyltin species were found in the majority of the samples analyzed. The analysis of more recent samples (1999) compared to older samples (1988) revealed recent exposure to butyltins even 10 years after the initiation of environmental regulations.

Cryogenically stored samples are best for butyltin impact studies because degradation of thermolabile species is minimized.

Tissue samples from upper trophic level Arctic species, such as murres and beluga whales, are particularly appropriate for monitoring butyltin compounds.

Photos: NMFS National Marine Mammal Laboratory

The main source of TBT in this region is unknown. One possibility is that it could be related to shipping traffic activity, mainly characterized by cargo transits and icebreaker activity for which no regulation of TBT antifouling paints is in place. However one should be cautious when considering sources because very little is known regarding the physiochemical behavior of these compounds in the environment, transport mechanisms, and the role of biota in their transformation and transfer through the food webs.

Future Plans: Additional analyses will be performed on more recently collected samples (2002) to further evaluate the temporal trends.

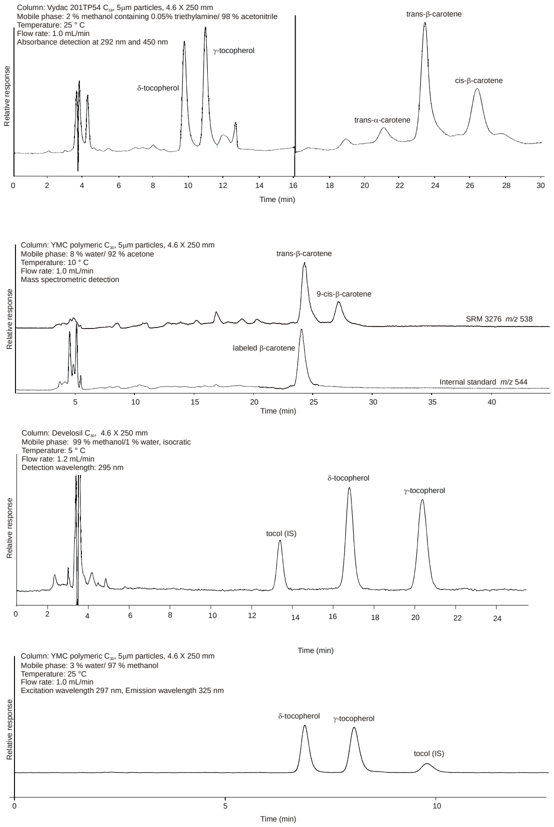
Carrot Extract Standard Reference Material

NIST is working in collaboration with the National Institutes of Health Office of Dietary Supplements (NIH-ODS), and Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER) and Center for Food Safety and Applied Nutrition (CFSAN) to develop a number of Standard Reference Materials (SRMs) for use in validating analytical methods and for quality assurance for constituents of dietary supplements. A carrot extract in oilwith values assigned for carotenoids, tocopherols, and fatty acids was recently completed.

C.A. Rimmer, J.B. Thomas, K. Putzbach, D.L. Duewer, L.C. Sander, M.M. Schantz, K.E. Sharpless, S.A. Wise, and T. Yarita (Div. 839)

Although some of the "newer" dietary supplements do not have recommended daily intakes, beta-carotene has been used in foods and dietary supplements for many years. Vitamin A, produced from beta-carotene, is one analyte for which label information is required on both foods and dietary supplements. Thus, Standard Reference Material (SRM) 3276 Carrot Extract in Oil has been produced to assist the dietary supplement, food, nutrition, and also clinical communities in method development and validation as well as quality assurance. For carotenoid and tocopherol analyses, the material requires no sample preparation, other than dilution with an appropriate solvent, for liquid chromatographic (LC) analysis. This is in contrast to other carotenoid-containing SRMs available from NIST – SRM 968c Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum and SRM 2383 Baby Food Composite – which require that carotenoids be extracted prior to analysis.

The Certificate of Analysis for this material will provide certified values for *trans*- and total beta-carotene as well as delta- and gamma-tocopherol. Reference values are assigned for 9-*cis*-beta-carotene and *trans*-alpha-carotene. Both NIST and laboratories participating in the NIST Micronutrients Measurement Quality Assurance Program provided data used for value assignment. Carotenoid and tocopherol values were assigned using results from several independent chromatographic separation and detection techniques at NIST (see Figure). Carotenoids were measured using polymeric C_{18} and C_{30} columns, both of which can separate *trans*-beta-carotene from *cis* isomers; absorbance detection was used with the C_{18} column and mass spectrometric detection with the C_{30} column. LC on the same C_{18} column, with absorbance detection, was used for measurement of the tocopherols; two different C_{30} columns with fluorescence and mass spectrometric detection were also used. The C_{30} columns are able to separate gamma- and beta-tocopherol whereas most C_{18} columns cannot; no beta-tocopherol was detected in SRM 3276.



Certified values for 12 fatty acids are also provided in this material. Fatty acids analyses were conducted at NIST and involved the use of two sample preparation methods, gas chromatographic separation, and flame ionization or mass spectrometric detection.

The carrot extract SRM can be used by the dietary supplement, food, nutrition, and clinical communities for method development, method validation, and quality assurance.

This material is intended for use in method development and as a control material to support analytical methods for the determination of beta-carotene, tocopherols, and fatty acids.

CCQM Activities in the Gas Analysis Working Group

Since 1998, the Analytical Chemistry Division (ACD) has participated in 25 of 38 studies of the Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM) Gas Analysis Working Group (GAWG). The CCQM conducts international comparisons to establish equivalence among measurements made by national metrology institutes (NMIs). Intercomparisons in 2006 include studies involving carbon dioxide, oxygen, n*-hexane, nitric oxide, and hydrogen sulfide in gas cylinders.*

F.R. Guenther, W.R. Miller, G.C Rhoderick, and W.J. Thorn (Div. 839)

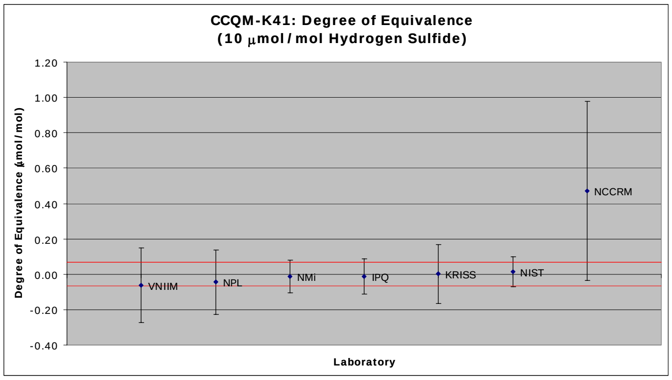
Studies in the GAWG typically take one of two forms. In measurement-capability studies, cylinders are distributed to laboratories for analysis and the agreement of results is assessed. In preparative-capability studies, participants prepare a cylinder to contain a certain concentration of the study gases, and the coordinating laboratory analyzes the samples under high-repeatability conditions to see whether the target was met. ACD participated in the following CCQM comparisons within the GAWG during the past year: CCQM-K52 360 µmol/mol Carbon Dioxide, CCQM-K53 100 µmol/mol Oxygen Preparative, CCQM-K54 100 µmol/mol *n*-Hexane in Methane Preparative, and CCQM-P73 50 µmol/mol to 70 µmol/mol Nitric Oxide Preparative Study. In addition, ACD completed the final reporting for the CCQM-K41 10 µmol/mol Hydrogen Sulfide.

Results are not yet available for two of these studies, CCQM-K52 and K53. CCQM-52 is a study in which capabilities are assessed for measurement of atmospheric-concentration carbon dioxide in air at concentrations of 350 µmol/mol and higher. A total of 18 NMIs participated in this study. CCQM-K53 is a primary standard preparative study in which NMIs were instructed to prepare a primary standard containing oxygen at an amount-of-substance fraction of 100 µmol/mol. This study is intended to underpin the NMIs' preparative capabilities, and also to validate purity assessment. A total of 12 NMIs are participating in the study.

A Draft A report was provided at the Fall 2006 meeting of the GAWG for CCQM-K54 *n*-Hexane in Methane. This study was designed to test preparative capabilities for the syringe injection technique of preparing volatile organic compound (VOC) gas standards. A total of 9 NMIs participated in this study. The results from the NIST-prepared cylinder show excellent agreement with the regression line calculated from the results.

Preliminary results were available for CCQM-P73 Nitric Oxide in Nitrogen Preparative Study. NIST prepared primary standards at nitric acid mole fractions of 43 µmol/mol and 67 µmol/mol in nitrogen. The results from the NIST-prepared cylinders showed excellent agreement with the target values.

In 2005, ACD coordinated CCQM-K41 10 µmol/mol Hydrogen Sulfide, and results were reported to the CCQM GAWG this past year. This key comparison was intended to compare the capabilities for the preparation and value assignment of gas standards for hydrogen sulfide in nitrogen. The nominal amount-of-substance fraction of the comparison standard was 10 µmol/mol, which is close to regulatory levels in most countries. Participants in this study were Russia (VNIIM), England (NPL), the Netherlands (NMi), Portugal (IPQ), Korea (KRISS), China (NCCRM), and NIST. The Key Comparison Reference Value was calculated from the participants' data and thus represents a consensus value. (Cylinders could not be prepared gravimetrically because hydrogen sulfide may be absorbed onto the walls of the cylinder and has been shown to be unstable in cylinders at the comparison concentration.) Gas mixtures were procured from a vendor, analyzed by NIST to determine stability, and then those determined to be stable were shipped to the participants for analysis. After analysis, the cylinders were returned to NIST for reanalysis. Through this process, NIST could accurately track the stability of the gas mixtures. In parallel, NIST also analyzed a stable reference, a hydrogen sulfide gas mixture that has been shown to be stable over many years. Using the stability data, the participants' analytical results, and NIST's analysis of the stable reference, a consensus value could be calculated which represents the concentration of the stable reference. The key comparison demonstrated that the results of the laboratories agreed within 0.5 % relative to the consensus value. A subset of 6 participants' results agreed with the consensus value to better than 0.25 %.



Impact: The results from these studies will be used to benchmark the appropriate Comparability and Measurement Claims (CMCs) of the participating NMIs.

Future Plans: Key comparisons planned for the coming year include: CCQM-K46 40 µmol/mol Ammonia in Nitrogen, CCQM-K51 5 µmol/mol Carbon Monoxide in Nitrogen, and CCQM-QM-K1 Ozone Reference Photometer Ongoing Comparison.

CCQM Activities in the Inorganic and Electrochemical Analysis Working Groups

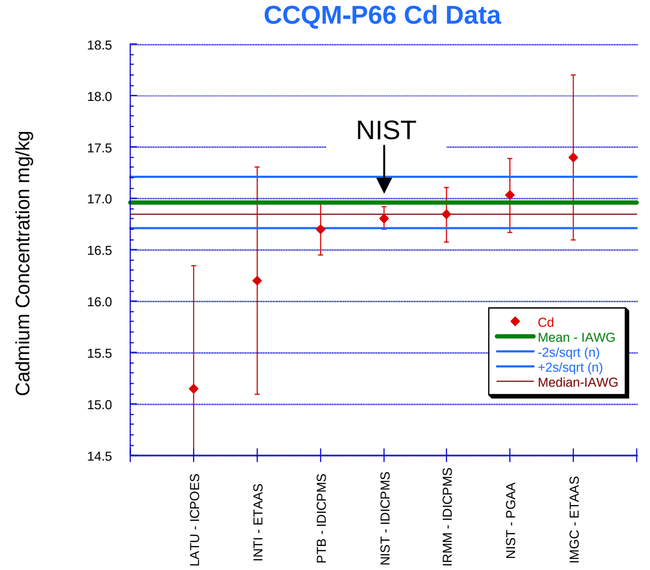
Since 1997, the Analytical Chemistry Division (ACD) has participated in 34 of 53 studies of the Consultative Committee for Amount of Substance (CCQM) organized by the Inorganic Analysis Working Group (IAWG) and 11 of 15 studies organized by the Electrochemical Analysis Working Group (EAWG). The CCQM conducts international comparisons to establish equivalence among measurements made by national metrology institutes (NMIs). Studies conducted during the past year included measurement of a wide variety of elements in materials related to the *environment, food and nutrition, metals and purity analysis, geological and advanced materials, and pH..*

R.R. Greenberg, G.C. Turk, K.W. Pratt, R. Zeisler, K.E. Murphy, S.E. Long, L.J. Wood, L.L. Yu, J.R. Sieber, A.F. Marlow, S.J. Christopher, W.C. Davis, W.R. Kelly, J. Mann, and E.A. Mackey (Div. 839)

During the past few years, ACD participated in CCQM studies in areas related to the environment, food and nutrition, metals and purity analysis, as well as geological and advanced materials. ACD coordinated the following CCQM studies within the IAWG during 2006: CCQM-P66 Metals in Fertilizer and CCQM-K49/P85 Essential and Toxic Elements in Bovine Liver.

Pilot Study CCQM-P66 was the first study of a fertilizer-based material, and proved to be quite challenging. Seven NMIs and one expert laboratory participated in this study. The data for Cd, shown in the figure, demonstrated the best agreement for any of the elements studied. Only NIST and the Institute for Reference Materials and Measurements (IRMM) provided data for all five elements in CCQM-P66. It is not clear at this time whether or not there will be a follow-up key comparison.

Key comparison CCQM-K49 and an associated pilot study, CCQM-P85, are currently in progress, with a deadline for data reporting in February 2007. The studies include seven toxic and essential elements that present varying degrees of difficulty in their determination, from relatively easy (Fe and Zn), to moderately difficult (Se, Pb and Cd) to very difficult (As and Cr). In addition, CCQM-K49/P85 represents a first for the IAWG in that all NMIs with inorganic analysis capabilities were requested to participate in either the key comparison or the pilot study for at least two designated elements, iron and zinc. In view of the large number of studies undertaken each year, participation by NMIs in any one particular study has dramatically decreased, limiting the ability to compare measurement capabilities. It has been decided that one study per year will be designated as an exemplary study, in an attempt to maximize participation in a common study to enable a direct comparison of NMI capabilities.



Ten NMIs and 5 expert laboratories participated in CCQM-P86 Total Se and Se Speciation Analysis of Pharmaceutical Supplements. NIST submitted results for both total Se and selenomethionine, which appeared to be in good agreement with other results submitted (no formal report has been made available to date). A Key Comparison is planned to follow up on this study, but at lower Se levels. Wheat flour containing a total Se content of about 1 mg/kg (total Se) was proposed.

In addition, we recently applied a double spike technique developed here at NIST (Mann and Kelly, 2005) in a CCQM pilot study (CCQM P75) to determine the δ^{34}S of the essential amino acid methionine. We had previously improved a specialized high-accuracy mass spectrometric method to precisely measure the isotopic composition and concentration of sulfur in small samples (<1 µmole S). The uncertainty for our results in the CCQM study (reported as combined uncertainties) was smaller then that reported by the five other laboratories that participated and on half the amount of sample (100 µg). Additionally, two other measurements in which we used a factor of 10 less sample (10 µg) also fall within in our uncertainty reported, again showing the capability of the technique for measuring small sample sizes.

NIST participated in several additional pilot studies and key comparisons with good results including CCQM-K42 Constituents of an Aluminum Alloy, CCQM-P64 Trace Elements in Soybean Powder, and CCQM-P62 Purity Analysis of Nickel Based on 6 Metallic Elements.

Under the auspices of the Electrochemical Analysis Working Group, NIST participated in three Key Comparisions: two in the area of pH and one in the area of electrolytic conductivity: CCQM-K18 Carbonate Buffer, pH 10; CCQM-K19 Borate Buffer, pH 9.2; and CCQM-K36a/K36b Electrolytic Conductivity, 0.5 S/m and 5 mS/m. In addition, results for CCQM-K34 Potassium Acid Phthalate, Amount Content of Weak Acid, conducted in 2004, were approved by the BIPM for the Key Comparison Database.

Impact: The results from these and future studies will be used to benchmark the appropriate Comparability and Measurement Claims (CMCs) of the participating NMIs.

Future Plans: NIST plans to participate in the following IAWG and EAWG comparisons in FY07: CCQM-K49 Toxic and Essential Elements in Bovine Liver; CCQM-K56 Elements in Soybean Powder; CCQM-K57 Elements in Clay; CCQM-K58 Nitrogen and Trace Elements in Silicon Nitride; CCQM-K43.1 (Subsequent) Methylmercury in Swordfish;, CCQM-P97 Cadmium and Lead in Herbs; CCQM-K59 Nitrate Calibration Solution; CCQM-K48 Potassium Chloride; CCQM-K20 Tetroxalate Buffer, pH 2, organized by NIST; and CCQM-P83 Electrolytic Conductivity, 0.5 mS/m.

Reference: Mann, J. L. and Kelly, W. R. (2005) Measurement of sulfur isotope composition ($\delta^{34}S$) by multiple-collector thermal ionization mass spectrometry using a ^{33}S-^{36}S double spike. *Rapid Commun. Mass Spectrom.*, **19**: 3429–3441.

CCQM Activities in the Organic Analysis Working Group

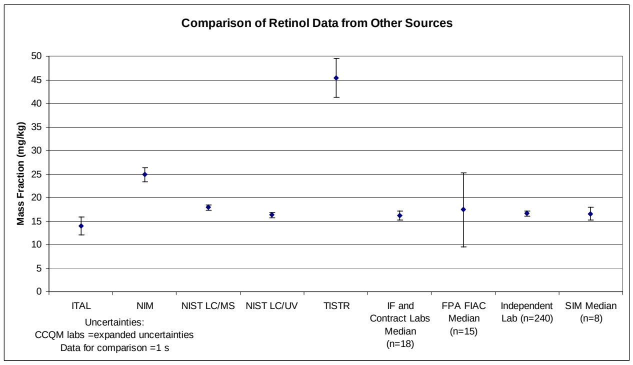
The Analytical Chemistry Division (ACD) has participated in 40 of 55 Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM) Organic Analysis Working Group (OAWG) studies since 1997, and coordinated a number of studies during the past year. The CCQM conducts international comparisons to establish equivalence among measurements made by national metrology institutes (NMIs). OAWG studies during the past two year included measurement of vitamins in infant/adult formula, hormones in human serum, volatile organic compounds in methanol, ethanol in water, and polycyclic aromatic hydrocarbons in soil and particulate matter.

M.M. Schantz, D.L. Duewer, K.E. Sharpless, S.S-C. Tai, and S.A. Wise (Div. 839)

In past years, ACD has coordinated or participated in CCQM studies in areas related to the environment and clinical chemistry, including studies in which purity of various compounds was assessed. ACD coordinated the following CCQM studies within the OAWG during 2006: CCQM-P77a and b Cortisol and Progesterone in Serum, CCQM-P78 Nutrients in Adult/Infant Formula – the first food nutrition study, CCQM-K27.2 Ethanol in Water, and CCQM-K47 Volatile Organic Compounds (VOCs) in Solvent. A particulate matter sample that meets the homogeneity requirements for use in the planned CCQM-K50 for PAHs in soil and particulate matter was characterized.

Six NMIs or their designees participated in the pilot study CCQM-P77 Cortisol and Progesterone in Serum with one of the NMIs returning data for cortisol only. With the exception of one of the designated laboratories that used an immunoassay method, the data agreed well among the remaining five laboratories. For the cases were there were minor discrepancies, potential sources of error have been identified, and NMIs will investigate and report their findings in April 2007. A key comparison is planned for 2007.

Five NMIs or their designees participated in the pilot study CCQM-P78 Nutrients in Infant/Adult Formula, in which vitamin A, folic acid, and niacin were measured; one of the NMIs returned data for only one of the three analytes. Since the same sample had been used in other interlaboratory studies, the data among the NMIs could be compared even though a limted number of NMIs participated (see Figure). A key comparison is planned following the April 2007 OAWG meeting.



Key Comparison CCQM-K27.2 was the second subsequent study for Ethanol in Aqueous Matrix. Five laboratories participated, with the data from four of those laboratories agreeing within 2%. The data for the four laboratories are comparable to the data from the previous two key comparisons so this study will be finalized.

Key Comparison CCQM-K47 Volatile Organic Compounds in Solvent and the subsequent pilot study CCQM-P61.1 run concurrently were co-coordinated by NIST and CENAM in Mexico. There were five participants in CCQM-P61.1 and eight participants in CCQM-K47. Because there are discrepancies between the participant results and the gravimetric concentrations from the preparation of the solutions, the National Meterology Institute of the Netherlands also analyzed the study samples against their gas standards. The data confirmed the gravimetric concentrations for three of the four analytes of interest. Further discussions will be held at the April 2007 meeting to determine the Key Comparison Reference Values (KCRVs) for CCQM-K47.

Four NMIs or their designees participated in the pilot study CCQM-P68 19-Norandrosterone in Human Urine, which was coordinated by the National Measurement Institute of Australia (NMIA). In order to participate in this intercomparison, NIST developed a new LC/MS/MS method for 19-norandrosterone. The results from the four NMIs were in excellent agreement, with a relative standard deviation (RSD) of only 1.7%.

Impact: The results from these studies will be used to benchmark the appropriate Comparibility and Measurement Claims (CMCs) of the participating NMIs.

Future Plans: The materials identified for key comparison CCQM-K50 will be distributed early in 2007 for discuss at the October 2007 meeting. Key comparisons are planned for nutrients in infant formula and for cortisol and progesterone in serum.

CCQM Activities in the Organic Analysis Working Group

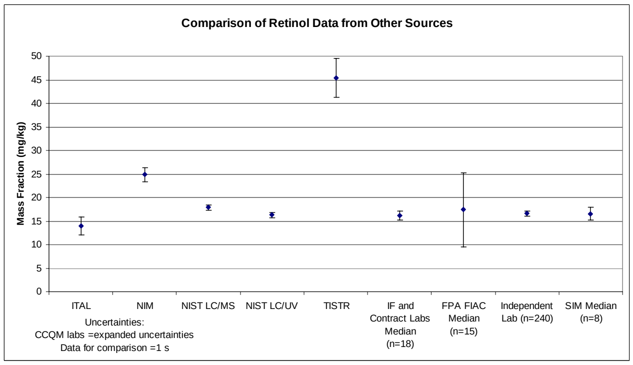
The Analytical Chemistry Division (ACD) has participated in 40 of 55 Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM) Organic Analysis Working Group (OAWG) studies since 1997, and coordinated a number of studies during the past year. The CCQM conducts international comparisons to establish equivalence among measurements made by national metrology institutes (NMIs). OAWG studies during the past two year included measurement of vitamins in infant/adult formula, hormones in human serum, volatile organic compounds in methanol, ethanol in water, and polycyclic aromatic hydrocarbons in soil and particulate matter.

M.M. Schantz, D.L. Duewer, K.E. Sharpless, S.S-C. Tai, and S.A. Wise (Div. 839)

In past years, ACD has coordinated or participated in CCQM studies in areas related to forensics, the environment, and clinical chemistry, including studies in which purity of various compounds was assessed. ACD coordinated the following CCQM studies within the OAWG during 2006: CCQM-P77a and b Cortisol and Progesterone in Serum, CCQM-P78 Nutrients in Adult/Infant Formula – the first food nutrition study, CCQM-K27.2 Ethanol in Water, and CCQM-K47 Volatile Organic Compounds (VOCs) in Solvent. A particulate matter sample that meets the homogeneity requirements for use in the planned CCQM-K50 for PAHs in soil and particulate matter was characterized.

Six NMIs or their designees participated in the pilot study CCQM-P77 Cortisol and Progesterone in Serum with one of the NMIs returning data for cortisol only. With the exception of one of the designated laboratories that used an immunoassay method, the data agreed well among the remaining five laboratories. For the cases were there were minor discrepancies, potential sources of error have been identified, and NMIs will investigate and report their findings in April 2007. A key comparison is planned for 2007.

Five NMIs or their designees participated in the pilot study CCQM-P78 Nutrients in Infant/Adult Formula, in which vitamin A, folic acid, and niacin were measured; one of the NMIs returned data for only one of the three analytes. Since the same sample had been used in other interlaboratory studies, the data among the NMIs could be compared even though a limted number of NMIs participated (see Figure). A key comparison is planned following the April 2007 OAWG meeting.



Key Comparison CCQM-K27.2 was the second subsequent study for Ethanol in Aqueous Matrix. Five laboratories participated, with the data from four of those laboratories agreeing within 2%. The data for the four laboratories are comparable to the data from the previous two key comparisons so this study will be finalized.

Key Comparison CCQM-K47 Volatile Organic Compounds in Solvent and the subsequent pilot study CCQM-P61.1 run concurrently were co-coordinated by NIST and CENAM in Mexico. There were five participants in CCQM-P61.1 and eight participants in CCQM-K47. Because there are discrepancies between the participant results and the gravimetric concentrations from the preparation of the solutions, the National Meterology Institute of the Netherlands also analyzed the study samples against their gas standards. The data confirmed the gravimetric concentrations for three of the four analytes of interest. Further discussions will be held at the April 2007 meeting to determine the Key Comparison Reference Values (KCRVs) for CCQM-K47.

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Impact: The results from these studies are used to benchmark the appropriate Compariblity and Measurement Claims (CMCs) of the participating NMIs.

Future Plans: In 2007, ACD will participate in key comparisons for nutrients in infant formula. cortisol and progesterone in serum, and PAHs in soil and particulates.

References:

Tai, S.-C., Xu, B., and Welch, M. J., "Determination of 19-Norandrosterone in CCQM-P68. NIST Results Using an Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry Method," sumitted to *Accreditation and Quality Assurance.*

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M.M. Schantz, D.L. Duewer, K.E. Sharpless, S.S. Tai, and S.A. Wise (Div. 839)

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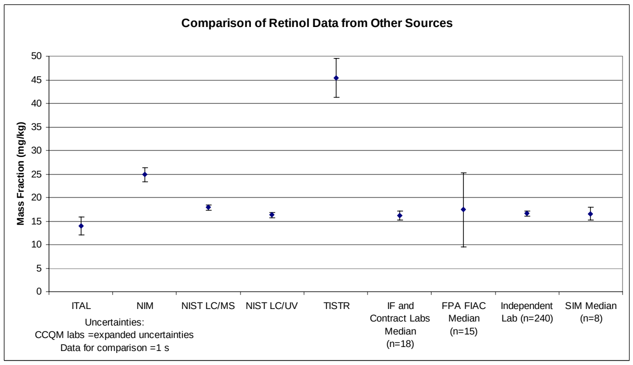

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Impact: The results from these studies will be used to benchmark the appropriate Comparibility and Measurement Claims (CMCs) of the participating NMIs.

Future Plans: The materials identified for key comparison CCQM-K50 will be distributed early in 2007 for discuss at the October 2007 meeting. Key comparisons are planned for nutrients in infant formula and for cortisol and progesterone in serum.

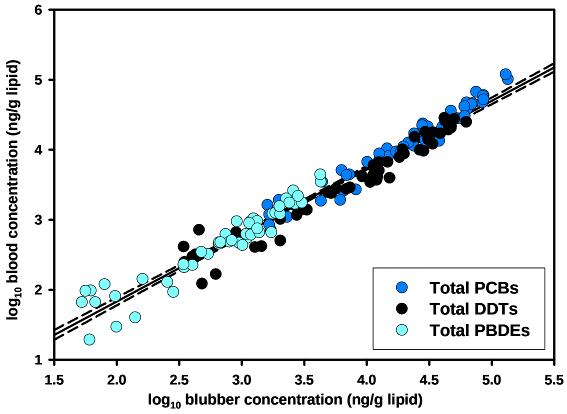
NIST Assists NOAA with Bottlenose Dolphin Health Assessments

NIST continues to provide its expertise to the National Oceanic and Atmospheric Administration (NOAA) in certain aspects of NOAA's management of protected marine mammals. One element of this partnership is NIST's contribution to NOAA's long-term bottlenose dolphin health assessments. NIST has assisted in 20 dolphin health assessments and will be involved in two planned for 2007. The data and expertise provided by NIST are vital to federal and academic partners who interpret toxicological and health-related information collected on these important coastal animals.

J.R. Kucklick, S.J. Christopher, P.R. Becker, R.S. Pugh, M. B. Ellisor, J.M. Keller, C.E. Bryan, J.E. Yordy, D.L. Zdankiewicz, M.M. Schantz, G.C. Turk, and S.A. Wise (Div. 839)

Bottlenose dolphins are considered indicators of environmental condition. They accumulate persistent organic pollutants, such as polychlorinated biphenyls (PCBs), DDTs, and brominated flame retardants, as well as mercury from their local environment to some of the highest levels observed in wildlife. The toxic effects of persistent pollutants have been implicated in very high (> 50%) mortality of dolphin calves born to first-time mothers and the occurrence of dolphin mass mortality events. Since 2000, NOAA has studied the health of bottlenose dolphins by collecting data and samples from animals captured and then released at several locations along the U.S. Atlantic and Gulf Coasts. In 2002, NOAA asked NIST to provide technical assistance for the project by (1) designing a protocol for use at all east coast sampling locations for collecting, handling, and storing blood, blubber, and skin to be used for organic contaminant and/or trace element analyses; (2) permanently banking blood and blubber samples for future studies; (3) analyzing samples collected during dolphin health assessments for persistent organic pollutants and trace elements; and (4) providing technical assistance in the field during sample collection. To date, samples have been collected from over 300 dolphins. Subsamples of plasma and blubber from most of these collections are banked in the NIST Marine Environmental Specimen Bank at the Hollings Marine Laboratory, Charleston, SC. Samples have been analyzed for a suite of legacy pollutants, such as PCBs, mercury and other trace elements, and a number of compounds of emerging interest such as brominated flame retardants. Two studies have been performed by NIST in which means of assessing persistent organic pollutants and trace elements in dolphins using minimally invasive methods were examined. Remote biopsy techniques that do not require capturing the animal, which is stressful to wild dolphins, are available for collection of skin and blubber. To validate the remote biopsy technique for trace elements, paired skin and blood samples were collected from captured dolphins. In addition, a body location study was performed on a deceased dolphin to examine the variability of mercury and trace elements in skin taken from different locations. The data show variation in mercury content in skin on the order of 15%, whereas other elements such as lead and cadmium exhibit greater than 50% variability across an individual animal. Mercury in skin was also highly correlated to blood mercury concentrations in blood. These data imply that skin obtained from a remote dart biopsy may be used to assess mercury contamination in blood.

A similar study was performed using paired blood and blubber samples from captured dolphins. Samples were analyzed for persistent organic pollutants. Data from this study show that concentrations in blubber and blood are highly correlated (see figure). Although dolphins and many other marine mammals carry large burdens in their blubber (nearly 1 g in some animals), the pollutants are not compartmentalized in blubber as previously believed, but are in equilibrium with blood. Thus for mercury and organic contaminants, remote dart biopsies can be used to assess contamination in blood, blood being the main vehicle of organ exposure to these toxicants.



Impact: NOAA has an extensive remote biopsy collection program for many different marine mammal species. Results from NIST's investigations indicate, at least for bottlenose dolphins, that samples collected in this manner can provide useful data on the exposure of dolphins to mercury and persistent organic pollutants such as PCBs, DDTs, and brominated flame retardants.

An Estimation of Illicit Drugs from Chemical Measurements in Municipal Wastewater

Over the last decade, municipal wastewater has been recognized as a substantial reservoir for pharmaceutical and personal care products (PPCPs) and their bioactive metabolites that are excreted from human usage. As originally proposed by Daughton and Ternes [1], illicit drug usage at both the community and metropolitan level may potentially be estimated from chemical measurements within municipal wastewater systems. In this context, NIST has developed a robust analytical methodology for the quantification of drugs of abuse and their respective metabolites in wastewater matrices. This method is intended for the determination of the chemical profiles of cocaine and its major metabolites in wastewater matrices and for the characterization of the major component removal processes in sewer systems. Ultimately, deterministic/stochastic models that include uncertainty analysis will be developed to describe component transport (and determine origin) within municipal wastewater systems and allow for the estimation of drug usage within a selected municipality and its population.

R.M. Heltsley, P.R. Becker, J.R. Kucklick, A.M. Peck, M.M. Schantz, and K.A. Lippa (Div. 839)

According to the 2005 National Survey on Drug Use and Health (NSDUH) [2], approximately 22 million Americans aged 12 or older were classified with a substance or drug dependency. Surveys coupled with crime statistics are typically used to estimate drug consumption rates but may under represent the magnitude of the problem as they are based primarily on voluntarily provided information. Thus, development of real-time analytical methodologies for the analysis of sewage treatment facilities influent waters have been proposed as a means to indirectly and non-intrusively monitor the drug usage within a population. By developing rigorous analytical methods that reliably and accurately determine the concentrations of drugs and their respective metabolites in wastewater, NIST is facilitating an objective, scientific-based approach to the assessment of community-wide drug use.

After a review of the pertinent pharmacology and forensic science literature, we have compiled a list of probable cocaine metabolites (including unique metabolites from pyrolysis or "smoking" activities) that may enter municipal wastewater through human urine input. A comprehensive study to characterize the concentration of cocaine and its metabolites in human urine was recently conducted by Paul *et al.* [3]; these data collected from 17 living individuals were used to generate an average for the relative amounts of each cocaine metabolite present in urine and were used as an initial estimate of the input profile of these components into the sewer system. These data are illustrated in Figure 1.

NIST has developed an isotope dilution liquid chromatography-tandem mass spectrometry (LC/MS/MS) method to quantify concentration levels of cocaine and its major metabolites [cocaine, cocaethylene, benzoylecgonine, norcocaine, norcocaethylene, norbenzoylecgonine, ecgonine, ecgonine methyl ester, ecgonine ethyl ester, *m*-hydroxybenzoylecgonine, anhydroecgonine (ecgonidine), and anhydroecgonine methyl ester (methyl ecgonidine)] in wastewater and surface water. Method development has been conducted by team members located at the Hollings Marine Lab in Charleston, SC, and the method was applied to various municipal wastewater samples obtained from the Plum Island Wastewater Treatment Plant in Charleston. A typical molar fraction profile of cocaine and its major metabolites in wastewater are compared with the urine profiles reported by Paul *et al.* in Figure 1. The appreciably different chemical profiles between the urine input and the wastewater samples suggest that significant transformation processes are occurring within the sewer system.

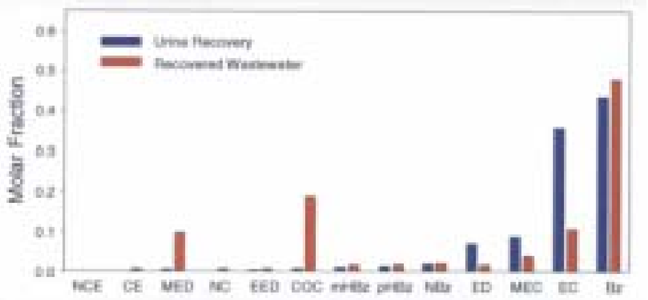


Figure 1. Typical profiles of cocaine (COC) and its major metabolites in the dissolved phase of wastewater using the developed methods (in red) in contrast with urine profiles determined for living individuals (in blue) [3].

Impact: The new analytical method represents the first step in developing comprehensive environmental measurement protocols to monitor and predict the usage of illicit drugs. At-risk communities could be readily identified with such monitoring approaches, thus facilitating social, community, and education-based programs intended to reduce and ultimately prevent drug use within our population.

Future Plans: We are in the process of adapting methods for the measurement of illicit drugs in the solid material component of wastewater. The potential environmental fate of cocaine and its metabolites (i.e., hydrolysis, mineral catalysis, sorption, microbial degradation) are presently being characterized through laboratory kinetic reactor experiments; these data are expected to elucidate the potential sinks and sources for cocaine and its metabolites within the sewer environment.

References:

[1] Daughton, C.G.; Ternes, T.A. *Environ. Health Perspect*. **1999**, *107* (suppl 6), 907-938.

[2] Substance Abuse and Mental Health Services Administration. (2006). *Results from the 2005 National Survey on Drug Use and Health: National Findings* (Office of Applied Studies, NSDUH Series H-30, DHHS Publication No. SMA 06-4194). Rockville, MD.

[3] Paul, B.D., Lalani, S., Bosy, T., Jacobs, A.J., Huestis, M.A. *Biomedical Chromatography* 19: 677–688. 2005.

Development of a New Multi-Nutrient Fertilizer Standard Reference Material

In the past, the fertilizer industry was responsible for validating only the nutrient content of their products until an imported material used to manufacture fertilizers was contaminated with cadmium (Cd) at the percent level. This discovery resulted in many states enacting new regulations limiting the amounts of contaminant metals in fertilizers. In response, the fertilizer industry requested that NIST develop reference materials to support the development and validation of new analytical methods that will be required to demonstrate compliance with the new and proposed regulations.

E.A. Mackey, J.R. Sieber, A.F. Marlow, S.E. Long, K.E. Murphy, R.O. Spatz, M.S. Rearick, L.J. Wood, and L.L. Yu (Div. 839)

The fertilizer industry and its regulators need a well-characterized multi-nutrient blended fertilizer refernce material for use in method development and validation. A candidate material was obtained from the industry to determine the best method for preparing a homogenous material, and to develop an appropriate analytical approach.

A US fertilizer company then provided 187 kg of coarse-ground, blended fertilizer comprised of ammonium phosphate, different forms of urea, potassium nitrate, potash, borax, and various metal oxy-sulfates. Preliminary analyses of sieved portions indicated that the material should be further ground to improve homogeneity. The material was jet-milled, blended, and bottled to create SRM 695 Trace Elements in Multi-Nutrient Fertilizer. Subsamples of this bottled material were subjected to x-ray fluorescence spectrometry (XRF) and prompt gamma-ray activation analysis (PGAA) to determine material homogeneity. Material homogeneity was acceptable. Additional analyses using XRF, instrumental neutron activation analysis (INAA), PGAA, and inductively coupled plasma optical emission spectrometry (ICP-OES), isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS), and cold vapor ICP-MS were performed to determine element content. Results were combined to provide certified or reference values for the elements of interest.

Certified or reference values were provided for all regulated elements including As, Cd, Co, Cr, Hg, Mo, Ni, Pb, Se, and Zn; nutrient elements B, Ca, Cl, Cu, Fe, K, Mg, Mn, N, and P; and four additional non-nutrient elements, Al, Na, Ti, and V.

The new SRM 695 Trace Elements in Multi-Nutrient Fertilizer will allow the fertilizer industry and state and local laboratories to develop and validate methods and verify critical analyses needed for regulatory compliance.

Impact: **About a dozen US states now have fertilizer regulations in place and several other states have proposed regulations that are pending approval. In addition, other countries have proposed limits on metals for these materials and these new regulations will impact global trade. Use of this new SRM will enable compliance with national and international regulations for metal content of fertilizer materials.**

Future Plans: NIST will continue to work with fertilizer producers and state regulatory agencies to assess the impact of this SRM and needs for additional reference materials.

Standards Development and Measurements to Support Global Climate Change Studies

Gaseous primary standard mixtures (PSMs) have been developed by NIST for several atmospheric gases that can contribute to changes in the radiative environment of the earth. These greenhouse gases have been monitored by the atmospheric-environment community worldwide for many years, and have gained importance since the Kyoto protocol was implemented to promote reduced emissions of greenhouse gases.

G.C. Rhoderick (Div. 839)

NIST had previously developed gaseous primary standard mixtures (PSMs) for methane (CH_4), carbon dioxide (CO_2), tetrafluoromethane (CF_4), nitrous oxide (N_2O), dichlorodifluoromethane (CCl_2F_2), trichlorofluoromethane (CCl_3F), sulfur hexafluoride (SF_6), 1,1,1,2-tetrafluoroethane ($C_2F_4H_2$), carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), trichlorotrifluoroethane ($C_2Cl_3F_3$), 1,1,1-trichloroethane ($C_2H_3Cl_3$), and trichloroethylene (C_2HCl_3). These standards were developed to support the in-house standards base for SRM certification; international intercomparison programs between national metrology institutes (NMIs) to determine equivalence; the NIST Fourier transform infrared (FTIR) spectral database project; and the International Halocarbon Experiment (IHALACE) program sponsored by the World Meteorological Organization (WMO), the National Oceanographic and Atmospheric Administration (NOAA), and the National Aeronautics and Space Administration (NASA). More than 20 gravimetrically-prepared PSMs now exist and are used to define the NIST primary calibration scales for these key atmospheric species and in the analysis of samples in various international comparisons.

IHALACE Program:

Twenty laboratories around the world involved in atmospheric measurements participated in the IHALACE program to assess the agreement of measurements; the ultimate goal was to determine whether a single traceable source of standards was necessary. NIST used PSMs to analyze one set of three atmospheric gas samples passed among 10 of the 20 labs. NIST analyzed and assigned values for CH_4, N_2O, CCl_2F_2, CCl_3F, CCl_4, $CHCl_3$, $C_2Cl_3F_3$, $C_2H_3Cl_3$, and C_2HCl_3 in the low pmol/mol concentration region. Results were sent to the IHALACE referrers for compilation with other participants' data. At this time, the IHALACE samples are in their final cycle of analysis. Compilation of results will be completed in 2007.

CCQM GAWG Activities with WMO's Global Atmospheric Watch:

As part of a subcommittee of the Gas Analysis Working Group (GAWG) of the Consultative Committee for Amount of Substance – Metrology in Chemistry (CCQM), NIST is participating in the development of a VOC standard for the WMO Global Atmospheric Watch (GAW) volatile organic compounds (VOCs) program operated by the Institute for Metrology and Climate Research (IMK-IFU) in Garmisch- Partenkrichen, Germany. Representatives from several NMIs and the IMK-IFU met in Germany in 2006 to begin discussions on the standards needed.

AIGER:

Standards were also developed to support the American Industry/Government Emissions Research (AIGER) program (a consortium of the major automotive producers) in the measurement of exhaust species related to climate change and to help them meet regulations for emissions requirements. Individual standards containing methanol, ethanol, HFC-134a, N_2O, and toluene were developed and certified.

Development of PSMs for these programs will both underpin the SRM and NIST Traceable Reference Materials (NTRM) programs, and serve to establish equivalency between NMIs and atmospheric research measurement laboratories.

Future Plans:

In 2007, standards development will begin for hydrocarbon (VOC) standards in conjunction with other NMIs to help support the WMO/GAW-VOC program. In addition, research will begin on the feasibility of gas mixtures containing key terpenes, also in support of the WMO/GAW-VOC program. NIST will participate in a EUROMET comparison of VOC standards, which will begin in March 2007.

Gas Standards in Support of NASA's Space Shuttle Monitoring Program

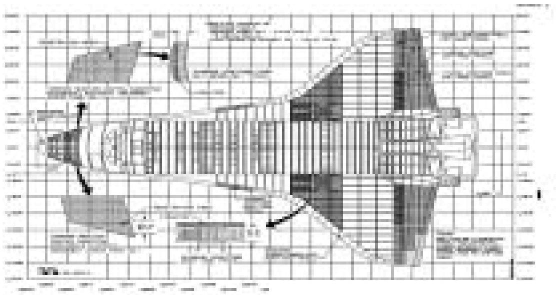
The National Aeronautics and Space Administration (NASA) Kennedy Space Center needs gas mixtures containing argon, helium, hydrogen, and oxygen to calibrate mass spectrometer sensors in and around the space shuttle launch area. NIST obtained gravimetrically prepared gas mixtures at three different concentration ranges from a specialty gas company, certified these gas mixtures, and returned them to NASA. They are now being used to calibrate the monitors during shuttle launches.

G.C. Rhoderick, W.R. Miller, and W.J. Thorn III (Div. 839)

Hydrogen or oxygen leaks can cause a space shuttle mission to be scrubbed, or worse. Mass spectrometers are used to "sniff" connections within the shuttle and the launch platform to detect gas leaking out. They are also used to sniff nitrogen-filled areas for argon to see whether air is leaking in. This leak detection goes on prior to a launch, up until the shuttle leaves the launch pad.

In 1990 NIST developed 20 primary standards for NASA and used them to analyze 20 cylinders containing two similar four-component blends in a balance of nitrogen. NIST successfully assigned concentration values for each component in all 20 mixtures with an expanded uncertainty of 0.8 % to 2.0 % relative. NASA used these standards until 2005 in a quality assurance program to verify vendor-supplied gas standards that they use to calibrate their sensor system.

In 2005 the Kennedy Space Center in Florida needed three new five-component gas standards to calibrate the mass spectrometers that they use for leak detection. A specialty gas vendor gravimetrically blended 15 cylinders of each of three gas mixtures (Mixtures 1, 2, and 3) containing varying levels of argon, helium, hydrogen, and oxygen in nitrogen. Mixture 3 represents a new concentration level that was lower than the materials that NIST provided in 1990. Mixture 3 also had a NASA uncertainty requirement of ± 3 %. NASA asked NIST to analyze and certify the concentration of each component in all 45 cylinders and meet new measurement uncertainty targets at the 95 % confidence level.



Schematic of Space Shut-

Using high-capacity, high-sensitivity cylinder balances, NIST prepared 22 new gravimetric primary standards to uncertainties of $\pm \leq 0.2$ % relative. A total of five gravimetrically prepared high-concentration binary gravimetric mixtures were blended and verified. These binary mixtures were then combined to produce five-component blends that spanned the requested concentrations specified by NASA for Mixtures 1, 2, and 3. Each of the new primary standards was analytically verified using gas chromatography. Existing primary standards from NIST's 1990 NASA standards effort combined with the 22 new primary standards allowed concentrations to be assigned to the 45 mixtures.

In 2006 NIST successfully certified each component in the three different gas mixture sets. Mixtures 1 and 2, with concentrations in the percent and high µmol/mol concentration levels, were certified with expanded uncertainties (95 % confidence interval) of 0.5 % to 1.0 % relative. Mixture 3 represented a much lower concentration level, 25 µmol/mol, than had previously been supplied to NASA by NIST. Expanded uncertainties of 1.0 % to 2.2 % relative were assigned to the concentration values, uncertainties which surpassed NASA's specifications. These certified gas mixtures were placed into service by NASA for the July 4, 2006 space shuttle launch of Discovery STS-121. NASA now has a sufficient supply of NIST-certified gas standards for use through the remaining lifetime of the shuttle fleet.

Future Plans: New gas mixtures may be requested by the NASA Stennis Space Center. They have discussed the need for standards to monitor and study effects of certain gases on shuttle engines during the burn stages of the launch.

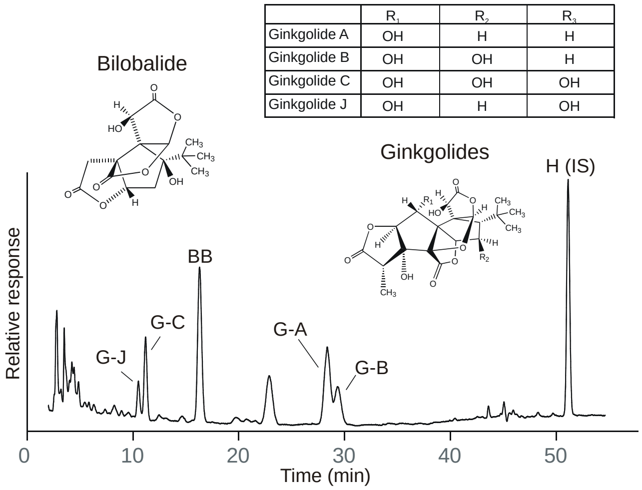
Ginkgo biloba Dietary Supplement Standard Reference Materials

NIST is working in collaboration with the National Institutes of Health Office of Dietary Supplements (NIH-ODS), and Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER) and Center for Food Safety and Applied Nutrition (CFSAN) to develop Standard Reference Materials (SRMs) to support the development of analytical methods and to provide quality assurance for constituents in dietary supplements. A suite of three ginkgo-containing reference materials will be the next SRMs to be issued as part of this program.

K.E. Sharpless, R.R. Greenberg, S.B. Howerton, S.E. Long, K.E. Murphy, B.J. Porter, K. Putzbach, M.S. Rearick, C.A. Rimmer, L.C. Sander, G.C. Turk, S.A. Wise, and L.J. Wood, R.L. Zeisler (Div. 839), and D.K. Hancock (Div. 831)

The enactment of the Dietary Supplement Health and Education Act (DSHEA) in 1994 by the U. S. Congress has promoted growth in the nutritional supplement industry, due in part to the way in which dietary supplements are regulated. DSHEA provides a legal definition of dietary supplements that classifies these materials separately from food additives and pharmaceutical drugs. Requirements for product labeling are less stringent than for drug substances, and the burden of proof for the safety of dietary supplements is placed on the FDA. Reference materials are needed for use in method validation and as controls to support the analysis of dietary supplements and related botanical materials. Potential applications include: 1) verification of product label claims; 2) quality assurance in product manufacturing; and 3) support of measurements associated with clinical trials.

A suite of three ginkgo-containing SRMs has been developed: SRM 3246 *Ginkgo biloba* (Leaves), SRM 3247 *Ginkgo biloba* Extract, and SRM 3248 Ginkgo-Containing Tablets, representing a variety of natural, extracted, and processed sample matrices that provide different analytical challenges. In addition to the three individual SRMs, all three ginkgo-containing SRMs will be available packaged together, two bottles of each, as SRM 3249. The Certificates of Analysis for these materials will provide certified values for five terpene lactones, three flavonoid aglycones, and four potentially toxic trace elements (arsenic, cadmium, lead, and mercury). The concentrations of these constituents have been determined by multiple independent methods with measurements performed by NIST and collaborating laboratories. The methods utilized different sample extraction and cleanup steps in addition to different instrumental analytical techniques and approaches to quantification. Flavonoid aglycones were determined at NIST by liquid chromatography (LC) with absorbance and mass spectrometric (MS) detection. Terpene lactones (ginkgolides) were determined by two different LC/MS methods. The total ion chromatogram for one of these methods is shown below. Toxic elements were determined at NIST by isotope dilution – inductively coupled plasma mass spectrometry (Cd, Pb, and Hg) and instrumental neutron activation analysis (As).



	R_1	R_2	R_3
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide J	OH	H	OH

Ginkgo-containing products represent a large share of the dietary supplement market and are widely used to improve cognitive function.

These materials are provided primarily for use in method development and as control materials to support analytical methods for the determination of these constituents. The SRM suites will assist manufacturers of dietary supplements to characterize raw materials, to prevent the use of materials that are contaminated or adulterated. In addition, the SRMs will assist self-assessment of consistency and quality in finished products, and to provide a foundation to which accuracy of label information can be linked. The goal of this ongoing effort is to provide tools to the dietary supplement industry and measurement communities that will lead to improved quality of dietary supplements, and ultimately reduce public health risks that could potentially be associated with these products.

Development of Reference Methods and Standard Reference Materials for Clinical Diagnostic Markers

For nearly four decades NIST has been involved in the development of reference methods and SRMs to support accuracy in health-related measurements. In the 1960s NIST developed pure compound standards for use as calibrants. During the 1970s the focus was on the development of highly accurate and precise isotope dilution mass spectrometry-based "definitive methods" for 12 health status markers (i.e., Ca, Cl, Li, Mg, K, Na, cholesterol, creatinine, glucose, triglycerides, urea, and uric acid) in human serum. The first human serum-based SRMs certified for metabolites and electrolytes (e.g., SRM 909 Human Serum) were introduced in the 1980s. During the 1990s new serum-based SRMs were issued as frozen rather than lyophilized materials, and new efforts were focused on proteins as health markers as well as toxic metals. By the beginning of the 21st century, these efforts were directed toward the development of reference measurement procedures and reference materials for new health status markers to address the European Union directive regarding in-vitro diagnostic medical devices (IVD MD) which requires that **"…..the traceability of values assigned to calibrators and control materials must be assured through available reference measurement procedures and/or reference materials of a higher order ...".**

K.W. Phinney, M.J. Welch, D.M. Bunk, N. Dodder, S.E. Long, B.C. Nelson, M. Satterfield, L.T. Sniegoski (Guest Scientist), S.S-C. Tai, and L.L. Yu (Div. 839)

Since 2000 NIST has developed reference measurement procedures (the preferred term, replacing definitive methods) and reference materials for several new health markers. New methods based on liquid chromatography/mass spectrometry (LC/MS) or tandem mass spectrometry (LC/MS/MS) have been developed for homocysteine, folate, estradiol-17β, cortisol, progesterone, testosterone, thyroxine (T4), and triiodothyronine (T3).

With the completion of the reference measurement procedures for these new markers, several new SRMs have been added to the suite of materials for clinical laboratory measurements. SRM 1955 Homocysteine and Folate in Human Serum was completed in 2006. Homocysteine is a risk factor for heart disease and other conditions associated with oxidative stress; folate counteracts effects of homocysteine and has also been shown to reduce the risk of neural tube defects in fetuses. This SRM is a three-level frozen serum material with certified concentrations for homocysteine and 5-methyltetrahydrofolic acid, the principal form of folic acid in blood and an important antioxidant. The development of SRM 1955 was a collaborative effort between NIST and the Centers for Disease Control and Prevention (CDC), both of which provided measurements for certification. SRM 971 Hormones in Human Serum is another new reference material and is intended to support measurements of the following hormones: estradiol-17β, cortisol, progesterone, testosterone, T3, and T4. The SRM consists of two serum pools, one from normal adult males and one from normal, premenopausal adult females. Measurements of cortisol and progesterone are completed, and the NIST results will be combined with results from two collaborating laboratories to assign certified values for these two hormones. Measurements of the remaining hormones will be completed in 2007.

The incidence of kidney disease is rising rapidly in the U.S. Early detection and treatment can prevent kidney failure, but early detection depends on better measurements of kidney function than are currently available. Serum creatinine is the preferred measurement, but existing methods provide varying results. NIST collaborated with the National Kidney Disease Education Program (NKDEP) and the College of American Pathologists (CAP) to develop a new reference material, SRM 967 Creatinine in Frozen Human Serum, to address this measurement problem. As part of this work, NIST has developed a new, rapid, isotope dilution liquid chromatography/mass spectrometry (ID-LC/MS) method for serum creatinine to replace the tedious isotope dilution gas chromatography/mass spectrometry (ID-GC/MS) definitive method used previously at NIST.

NIST also initiated development of a series of serum-based SRMs for assessment of nutritional status, including vitamin D, vitamin B_6, and vitamin B_{12}. The reliability of clinical laboratory measurements for these analytes has been called into question, and individuals suffering from nutritional deficiencies may not be properly identified and treated. NIST is working with the National Institutes of Health (NIH) to develop reference materials to address measurement variability in this area.

Metabolomics is an emerging discipline that seeks to characterize the complex profile of metabolites in cells, tissues, and other biological samples. Because metabolite levels can provide insight into the response of an organism to disease, metabolomics research may lead to the discovery of new clinical markers for disease diagnosis and treatment. NIST is developing SRM 1950 Metabolites in Human Plasma to support the development of new measurement technology in metabolomics. SRM 1950 will consist of a plasma pool collected from 100 men and women (equal numbers of each) and with a racial distribution that reflects the U.S. population. The initial value assignment phase for this SRM will target approximately 50 metabolites for which NIST has existing methods (e.g., cholesterol, electrolytes, glucose, hormones).

> These new reference methods and the associated SRMs will provide critical traceability to the IVD industry and will help improve the reliability of routine clinical measurements. Better clinical measurements lead to better diagnoses, enabling earlier and more cost-effective treatments.

Much of the focus in the field of proteomics is also directed toward identification of biomarkers for particular diseases. The success of this effort depends upon validated approaches to protein identification and quantification. NIST is continuing research to develop reference methods for clinically relevant proteins. One of the most promising approaches is to break the protein down into specific peptides and measure the concentration of the peptides. This "bottom-up" approach relies on quantitative digestion of the proteins with enzymes such as trypsin. NIST has been investigating the effects of experimental factors on trypsin digests of proteins and exploring the quantitative nature of tryptic digestion. Additionally, using *in vitro* methods for protein production, isotopically labeled proteins are being prepared for use as internal standards in quantitative methodology.

To address needs in healthcare and public safety, CDC has established a network of laboratories to monitor arsenic poisoning throughout the U.S. by measuring arsenic species in urine. To ensure accuracy of these measurements, CDC collaborated with NIST to produce SRM 2669 Arsenic Species in Frozen Human Urine. SRM 2669 will provide the accuracy base for biomonitoring of arsenic exposure. Concentrations of seven arsenic species (arsonite, arsonate, monomethylarsonate, dimethylarsinate, trimethylarsine oxide, arsenobetaine, and arsenocholine) indicative of different sources of arsenic exposure will be certified.

Recent state-of-the-art measurements of cadmium, mercury, and methylmercury in SRM 966 Toxic Metals in Bovine Blood were used to upgrade the certification status of the material. This work considerably enhances the quality of the SRM and its usefulness to the clinical measurement community, especially for speciated mercury measurements. The updated Certificate of Analysis now has certified values for lead, cadmium, and total mercury in both Levels 1 and 2, and reference values for inorganic mercury and methylmercury in Level 2. Prior to this work, methylmercury was only listed on the Certificate as an information value.

In addition to its public safety role, CDC collects data on the trace element content of human body fluids and tissues as part of the National Health and Nutrition Examination Survey (NHANES). NIST has characterized two biological materials for elemental composition: SRM 1598a Animal Serum and SRM 1577c Bovine Liver. Certified or reference values are provided for all of the elements that CDC monitors as part of NHANES.

Future plans: NIST is collaborating with the CDC to develop a new SRM to meet the expanding needs for the measurement of trace elements in human urine. This new SRM, which will be frozen urine, will include both a natural and a fortified level, and will be an improvement over the existing SRM 2670a Toxic Elements in Freeze-Dried Urine in terms of commutability and elemental composition.

Standard Reference Materials for Hexavalent Chromium in Contaminated Soil

During the last century, chromate chemical production facilities processed considerable quantities of chromite ore for industrial manufacturing. The waste material from these operations, known as chromite ore processing residue (COPR,) ended up in waste sites across the country, and as fill material for building foundations, road construction, and other civil engineering applications. In New Jersey alone, over 160 waste sites have been identified that are contaminated with COPR. This waste material consists of a mixture of chromite and chromate, otherwise known as hexavalent chromium, which is now widely recognized as a human carcinogen. To remediate these sites, there is a need to make high-quality chemical measurements of the hexavalent chromium in waste samples. To support these efforts, NIST, in cooperation with the New Jersey Department of Environmental Protection (NJDEP), and the U.S. Environmental Protection Agency (EPA), is developing a Standard Reference Material (SRM)to provide traceability for measurements of hexavalent chromium in soil and contaminated waste. A suitable collection site has been identified and the material processed for production of the SRM.

S.E. Long, K.E. Murphy, T.W. Vetter and J.R. Sieber (Div. 839)

The environmental community, including those responsible for promulgating analytical methods, developing policy, enforcing regulations, and interpreting laboratory data, has long known that transition metal chemistry in non-aqueous media involves the actual species present, and not just the total elemental composition. The risk to humans is often dependent upon the form of the metal in the soil or sediment. Chromium (Cr) in the environment exists in two principal oxidation states – Cr-III, a micronutrient, and Cr-VI, a known carcinogen. In the presence of inorganic and/or organic matrix components such as sulfide, iron, manganese, and organic carbon, the two species can interconvert from one form to the other. The use of some analytical methods is also known to alter the species distribution, thereby compromising test data designed to evaluate risk assessment. Species-based environmental regulation is now a reality despite the uncertainties in the underpinning measurement science. There is therefore a need for a high-quality reference material to improve the quality of these measurements.

To respond to this need, NIST has worked jointly with NJDEP and the EPA to collect and prepare SRM 2701 Hexavalent Chromium in Contaminated Soil. This material, a soil contaminated with COPR, was collected from a waste site in New Jersey and was milled, blended, radiation sterilized, and bottled into 80 g units by the United States Geological Survey (USGS) in Colorado. The material is now undergoing a six-month stability assessment study by a group of eight expert laboratories using approved analytical methods. If it is demonstrated that the material has acceptable stability, certification measurements will commence at NIST. The intention is to provide certified values for both total chromium and hexavalent chromium and other species of interest such as mercury. Additional reference measurements are planned for other critical components including sulfide, manganese, iron, and total organic carbon. The properties of the SRM (pH and eH) will also be characterized to provide the user with additional stability mapping information.

Impact: Hexavalent chromium litigation and waste-site remediation activities are now common, with an associated need for high-quality chemical information on the chromium species present. The use of this new SRM material will provide traceability for environmental measurements in compliance with national regulations.

Future Plans: The remaining material collected from the site will be used to generate additional QC materials for use by the EPA. This will considerably enhance the effectiveness of the EPA-approved test methods for hexavalent chromium. The QC materials will be prepared by custom blending SRM 2701 stock material gravimetrically with quartz sand to produce traceable mixtures of known hexavalent chromium concentration and uncertainty.

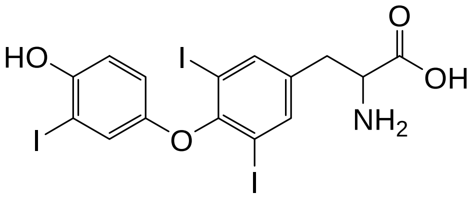
Development of Reference Methods and Materials for the Determination of Hormones in Human Serum

Many life functions are regulated by hormones. When hormone levels deviate from the norm, serious health consequences may result. Timely and effective treatments require accurate diagnoses of hormone levels. New reference methods and reference materials are being developed to support accuracy and traceability for clinical laboratory measurements of non-steroid hormones related to thyroid function and other metabolic processes

K.W. Phinney, S.S-C. Tai, and M.J. Welch (Div. 839)

NIST is developing reference methods and Standard Reference Materials (SRMs) to support accuracy and traceability for hormone assays. A suite of reference methods, based on liquid chromatography and mass spectrometry (LC/MS and LC/MS/MS), has now been developed for estradiol-17β [1], testosterone, cortisol, progesterone, thyroxine (T4), and triiodothyronine (T3).

T3 and T4 are hormones produced by the thyroid gland that regulate many cellular functions including carbohydrate metabolism and protein synthesis. Both elevated and decreased thyroid output of these hormones are relatively common conditions. Reference methods for measurement of these hormones in serum have been developed at NIST [2,3] and will be utilized for certification of SRM 971.



T3

A reference method for cortisol has also been developed and published [4]. Cortisol is a hormone produced by the adrenal gland. Measurement of cortisol levels in serum can be used to diagnose problems in the adrenal or pituitary gland, as well as other disorders. Previous experience at NIST and at other laboratories indicated that routine clinical measurements overestimate the cortisol present in serum by 25% to 50% when compared to isotope dilution (ID) MS methods.

Reference method development was recently completed and the method published for progesterone [5]. Progesterone is found at low levels in premenopausal adult women and very low levels in adult men, but levels in women rise significantly during pregnancy. The accuracy of the new isotope dilution LC/MS/MS method for progesterone was established by comparing the results obtained by LC/MS/MS for a Certified Reference Material (CRM) to those obtained by a gas chromatography/mass spectrometry (GC/MS) reference method.

These reference methods are being applied to the certification of a new Standard Reference Material, SRM 971 Hormones in Human Serum. The SRM consists of two serum pools, one from normal adult males and one from normal, premenopausal adult females. Measurements for cortisol and progesterone are completed, and the results will be combined with results from two collaborating laboratories to assign certified values for these two hormones. Measurements for the remaining hormones will be completed in FY07.

Improving the accuracy of clinical assays for hormones will improve diagnoses and result in earlier treatments. The new methods and the SRM will help improve accuracy of these assays and will also provide high-order reference systems for traceability.

Publications:

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2. Tai, S.S-C., Bunk, D.M., White V, E., and Welch, M.J., "Development and Evaluation of a Reference Measurement Procedure for the Determination of Total 3,3',5-Triiodothyronine in Human Serum Using ID-LC/MS/MS," Anal. Chem. 76, 5092-5096 (2004).

3. Tai, S.S-C., Sniegoski, L.T., and Welch, M.J., "Determination of Thyroxine in Human Serum by ID-LC/MS with Electrospray Ionization," Clin. Chem. 48, 637-642 (2002).

4. Tai, S.S-C. and Welch, M.J., "Development and Evaluation of a Candidate Reference Method for the Determination of Total Cortisol in Human Serum Using ID-LC/MS and LC/MS/MS," Anal. Chem. 76, 1008-1014 (2004).

5. Tai, S.S.-C., Xu, B., and Welch, M.J., "Development and Evaluation of a Candidate Reference Measurement Procedure for the Determination of Progesterone in Human Serum Using ID-LC/MS/MS," Anal. Chem. 78, 6628-6633 (2006).

New SRM for Organic Contaminants in House Dust

House dust is a repository for pesticides, flame retardants, and other chemicals used indoors and tracked into the house from outdoors. Once indoors where they are protected from environmental degradation, pollutants associated with dust persist for long periods, particularly if the dust is embedded in carpets. NIST has produced two house dust SRMs certified for these contaminants.

M.M.Schantz, J.M. Keller, J.R. Kucklick, A.M. Peck, D.L.Poster, H.M. Stapleton, S. Vander Pol, and S.A. Wise (Div. 839)

A variety of chemical contaminants reside in house dust. Pesticides become associated with house dust primarily through interior use of pest control formulations, intrusion of vapors from foundation and crawl space treatments, and track-in of lawn and garden chemicals. Polycyclic aromatic hydrocarbons (PAHs) derive from indoor sources such as combustion, cooking, and smoking, as well as track-in of contaminated yard soil or residues from garage floors. Flame retardants, such as polybrominated diphenyl ethers (PBDEs), are commonly applied to many products found within homes (e.g., TVs, computers, furniture, carpets, etc.).

NIST has produced two house dust SRMs for lead and other trace elements (SRM 2583 Trace Elements in Indoor Dust, Nominal 90 mg/kg Lead and SRM 2584 Trace Elements in Indoor Dust, Nominal 1 % Lead). A third new house dust material, SRM 2585 Organic Contaminants in House Dust is intended for use in evaluating analytical methods for the determination of selected PAHs, polychlorinated biphenyl (PCB) congeners, chlorinated pesticides, and PBDE congeners in house dust and similar matrices [1].

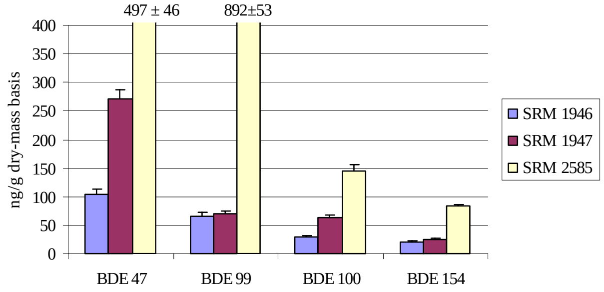
A total of 63 PAHs, 43 PCB congeners, 13 pesticides, and 18 PBDE congeners were value assigned with certified and reference values for the individual compounds using the results from two to six analytical methods. The concentrations of the individual PAHs range from approximately 20 ng/g to 4500 ng/g; concentrations for the PCB congeners range from approximately 2 ng/g to 40 ng/g; concentrations for the pesticides range from approximately 4 ng/g to 300 ng/g ; and concentrations for the PBDE congeners range from approximately 4 ng/g to 2500 ng/g (see figure). The relative uncertainties of the certified and reference concentration values range from <1% to 30% with most of the uncertainties less than 10%.

Impact: SRM 2585 is one of the first matrix reference materials available with values assigned for PBDE congeners, an emerging class of contaminants [2].

The Centers for Disease Control and Prevention (CDC) has noted that the levels of brominated diphenyl ether congeners (PBDEs) in human blood have been increasing over the last decade. This new SRM will aid in the development of new analytical methods for the determination of current and emerging contaminants in the home.

SRM 2585 is intended for use as a control material for the determination of environmental contaminants in house dust and related matrices and may also be useful for the development of analytical methods for emerging contaminants and other classes of compounds, including personal care products [3].

Concentrations of PBDEs in SRM 2585 Organic Contaminants in House Dust compared to those in SRM 1946 Lake Superior Fish Tissue and SRM 1947 Lake MIchigan Fish



Future Plans: The stability of the PBDE congeners in SRM 2585 will be monitored closely, particularly the concentration of the decabrominated congener that may degrade over time.

References:

[1] Poster, D. L., Kucklick, J. R., Schantz, M. M., Vander-Pol, S. S., Leigh, S. D., and Wise, S. A., ***"Development of a House Dust Standard Reference Material for the Determination of Organic Contaminants,"*** submitted to Environ. Sci. Technol. (2006).

[2] Stapleton, H. M., Keller, J. M., Schantz, M. M., Kucklick, J. R., Leigh, S. D., and Wise, S. A., ***"Determination of Polybrominated Diphenyl Ethers (PBDEs) in Environmental Standard Reference Materials,"*** submitted to Anal. Bioanal. Chem., 2006).

[3] Peck, A.M., Kucklick, J.R., Schantz, M.M., "Synthetic musk fragrances in environmental Standard Reference Materials," Anal. Bioanal. Chem., published on-line Aug 2006.

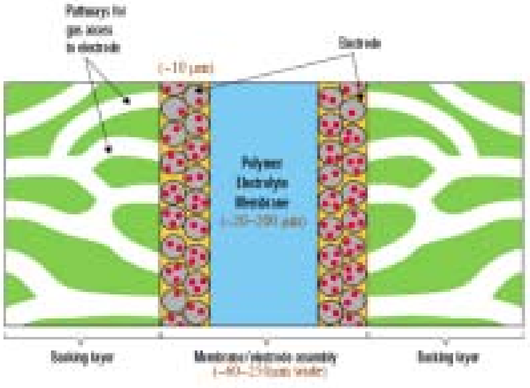
Imaging the Heart of a Cell: Hydrogen Fuel Cells and Nanomedicine

Until recently, there was no instrument that provided the ability to observe the chemical actions operating in a fuel cell or across a single biological cell subjected to certain drug treatments. X-ray techniques were ineffective or destructive. Neutron radiation is gentle enough; however existing neutron detectors could not resolve features much finer than 20 micrometers. To solve this problem, we conceived and designed an imaging technology that could break the neutron detector resolution barrier. Exploratory research suggests that neutron-imaging resolution could be improved by two orders of magnitude – achieving a useful resolution that can address crucial dynamical aspects of both biological cells and fuel cells.

R.G. Downing (Div. 839) and M. Arif (Div. 846)

Hydrogen fuel cell development plays an important role in the national effort to establish a viable hydrogen economy. Currently scientists are striving to improve fuel cell efficiency and longevity. Their progress is impeded by the inability to map the flow characteristics of hydrogen species across the proton exchange membrane (PEM). This thin membrane, only 10 to 200 micrometers thick, divides the cell but more importantly serves as the conduit for charge carriers, hydrogen ions, and associated catalytic impurities that are embedded in the PEM over time.

The problem has been "seeing" through the massive mechanical structure that constitutes the cell, yet resolving the submicrometer distribution of hydrogen as it flows across the membrane. Neutron probes have recently been used to penetrate through a working fuel cell and measure the quantity and distribution of hydrogen compounds *in situ*. However, existing neutron image resolution is limited to 20 to 125 micrometers. The membrane itself is about this thickness. To address the problem, we have conceived and designed an imaging technology that breaks the current neutron detector resolution barrier.



Enlarged cross-section of a membrane/electrode assembly showing structural details.

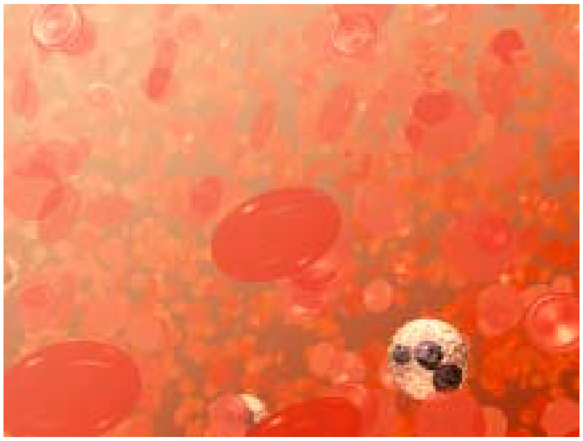
The innovative detector uses specially designed coincident, high-speed, time-of-flight sensors developed for planetary space probes. Intense neutron beams from the NIST Center for Neutron Research (NCNR) shine through the operating fuel cell in much the same fashion as does an x-ray at the doctor's office, however the neutrons shine continuously. The image of the hydrogen flow is projected upon the detector that is carefully aligned beneath the cell. Time-resolved images might be obtained if the neutron fluence is adequate. Multidimensional images can be extracted too.

Initial calculations show that theoretical resolution of the detector is better than 100 nanometers. Biological cell imaging then becomes a possibility with this resolution. Furthermore, cold neutrons are penetrating and have an associated energy of milli-electron volts, a very cold breeze of particles that mostly pass through the sample. Cancer drugs containing boron-, gold-, or gadolinium-loaded nanotubes or liposomes would be readily visible in the cell. The detector would reveal the amount and the location of these compounds and verify if indeed the drugs were reaching the cell nucleus as thought. All this valuable information provides interpretive diagnostics to direct medical research.

The detector design was funded under a CSTL exploratory program. The Neutron Interactions and Dosimetry Division is providing funding to build a prototype detector as part of this collaborative effort.

Impact: Two of the largest burdens on the US economy are medical and energy costs. This instrument has the potential to advance research in both of these major areas. By partnering with industry and academia, new insight can be made into the chemical dynamics of cancer drugs in cells and hydrogen flow in PEMs – all at the nanoscale.

Future Plans: A prototype instrument will be assembled to conduct the proof of concept test. If successful, then plans are to construct a fully functioning device including environment controls for biological specimens and engineering to image working hydrogen fuel cells.



Source: National Cancer Institute, Donald Bliss (Artist)

In vitro Synthesis of Stable Isotopically Labeled Proteins for Use as Internal Standards for Mass Spectrometric Quantitation of Clinical Protein Biomarkers

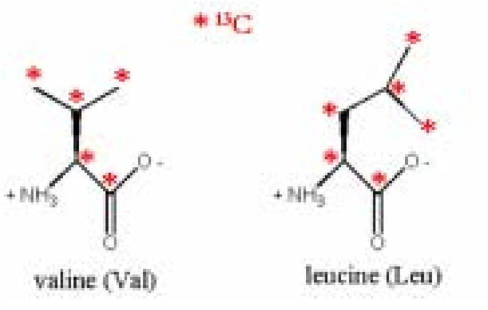
While the use of proteins as clinical biomarkers has become more prevalent, the clinical methods employed to measure these analytes are often based on immunological affinity or enzyme activity. In order for such tests to be traceable to standards of a higher order, reference materials and methods must be established for clinically relevant proteins. Although liquid chromatography/mass spectrometry (LC/MS) methods are increasingly utilized for quantitative protein measurements, the lack of appropriate internal standards hinders the development of standardized analytical methods for clinical proteins. To address this problem, NIST is investigating potential sources of stable-isotope labeled proteins for use as internal standards in quantitative mass spectrometric methods.

J. Camara, D.M. Bunk, N. Dodder (Div. 839), and P. Reddy (Div. 831)

In clinical proteomics, a “bottom up” approach is often used: in order to make full-length proteins more amenable to mass spectrometric detection, protein mixtures are often digested with a proteolytic enzyme, such as trypsin, prior to analysis. Known amounts of stable isotope-labeled peptides are added as internal standards to allow for quantitation by isotope dilution mass spectrometry. While peptide standards are relatively easy to synthesize, they are not ideal. Ideally, the internal standard should mimic the analyte throughout the analytical procedure. However, peptides cannot account for loss of full-length protein or incomplete enzyme digestion that may occur during sample processing. In this work, cell-free *in vitro* protein expression systems were investigated for their ability to produce customized, full-length proteins for use as internal standards. A full-length, stable isotope-labeled protein standard spiked into a sample prior to processing would account for the multiple variables that may affect the final levels of measured peptides.

Traditionally, stable isotope-labeled proteins are expressed in cells cultured in stable isotope-labeling growth media. However, these media are extremely expensive, and the total amount of target protein, as well as the level of stable-isotope incorporation, is inefficient. More recently, cell-free, *in vitro* protein expression systems have become available in which cell extracts contain the components necessary for coupled transcription/translation, resulting in the expression of a protein of interest from a DNA template. In many *in vitro* expression systems, individual amino acids can be completely replaced by stable isotope-labeled versions for high-efficiency incorporation into the target protein.

We have evaluated the protein production efficiency of these expression kits and have engineered a DNA expression template with the necessary elements for synthesis of C-reactive protein (CRP) in an *Escherichia coli*-based system. (CRP is used as a clinical marker of inflammation.) We are also investigating methods such as size-exclusion LC and native gel electrophoresis to characterize CRP pentamer formation, which would be required to mimic native CRP. In addition, we are exploring computer simulations of mass spectrometric ion distributions, which will allow us to characterize the extent to which stable isotopes are incorporated into various target proteins. This knowledge will be required to utilize the labeled proteins as internal standards for quantitative mass spectrometry.



Examples of stable isotope-labeled amino acids

Future plans:

Because there is an ongoing need for reference materials and methods for protein measurements, NIST will continue to evaluate cell-free *in vitro* protein expression systems as a source of stable isotope-labeled proteins for internal standards. We will proceed with the expression and characterization of labeled C-reactive protein as our first clinical candidate and plan to extend the project to include additional clinically relevant proteins.

Development of Standard Reference Material (SRM) 955c Lead in Caprine Blood

The SRM 955 series of lead (Pb) in blood standards has been heavily relied upon for quality assurance of clinical blood Pb measurements. It is estimated that over 300 thousand U.S. children aged 1 to 5 years have blood Pb levels greater than the Centers for Disease Control and Prevention (CDC) recommended level of 10 µg/dL. SRM 955c, the fourth in a series of NIST standards certified for blood Pb concentration, was developed in conjunction with the Trace Elements/Lead Poisoning Laboratory, Wadsworth Center, New York State Department of Health and partially funded by CDC. A unit of SRM 955c consists of four vials of frozen caprine (goat) blood at four concentration levels: a base level and three progressively elevated levels that contain endogenous Pb and other toxic elements of interest. SRM 955c will be used by clinical laboratories and hospitals to evaluate the accuracy of Pb concentration measurements in blood and to validate working or secondary reference materials for Pb in blood analysis.

K.E. Murphy and T.W. Vetter (Div. 839), P.J. Parsons, C. Geraghty, C.D. Palmer, and M.E. Lewis, Jr. (Wadsworth Center, New York State Department of Health)

Lead is neurotoxic and particularly harmful to the developing nervous systems of fetuses and young children. In 1990 the U.S. Department of Health and Human Services established a national goal to eliminate blood Pb levels greater than 25 µg/dL by 2000, and set a new goal to eliminate blood Pb levels greater than 10 µg/dL in children under the age of six by 2010 [1].

In response to the continued need for a blood-based reference material with accurately certified Pb concentrations, NIST developed SRM 955c Lead in Caprine Blood. The composition of this material differs from previous issues of SRM 955. The first SRM 955 was based on heparinized porcine blood obtained from Pb-dosed hogs. SRMs 955a and SRM 955b were based on bovine blood from Pb-dosed cows. SRM 955c is based on caprine blood obtained from Pb-dosed goats. The hemopoietic system of the adult goat is much closer to that of humans, thus making it a better model for assessing proficiency for erythrocyte protoporphyrin, a biomarker of Pb exposure. Adult goats were dosed with lead acetate to produce blood pools containing Pb physiologically bound to red blood cells (erythrocytes). In addition, the blood pools were spiked with inorganic arsenic, cadmium, inorganic mercury, methylmercury, and ethylmercury. Certified values are provided for Pb and are based on analysis using a high-accuracy ID ICP-MS method [2]. Table 1 lists the certified values and expanded uncertainties for Pb in the four levels of SRM 955c. Compared to previous issues of SRM 955, the Pb concentration for Level 1 is an order of magnitude lower and represents a true base level. Expanded uncertainties of less than 0.6 % relative were achieved for Levels 2, 3, and 4.

Table 1. Certified values (µg/dL) and expanded uncertainties for lead in SRM 955c.

Level 1	0.424	±	0.011
Level 2	13.950	±	0.080
Level 3	27.76	±	0.16
Level 4	45.53	±	0.27

Future Plans: Additional analyses will be performed to provide certified or reference values for inorganic arsenic, cadmium, inorganic mercury, methylmercury and ethylmercury.

References:
[1] Surveillance for Elevated Blood Lead Levels, 1997-2001, http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5210a1.htm, accessed December 2006.
[2] Murphy, K.E.; Paulsen, P.J; Fresenius J. Anal. Chem., Vol. 352, pp. 203-208 (1995).

SRM 955c is used to validate the accuracy of blood Pb measurements.

Impact: **Over 2 million blood Pb measurements are performed in the U.S. annually. SRM 955 is heavily relied upon for quality assurance of clinical blood Pb measurements, with over 200 units distributed per year. SRM 955c is the fourth issue of this material. Significant improvements over previous issues have been realized in the material composition, Pb levels, and expanded uncertainties of the certified Pb concentrations.**

Renewal of NIST Benchmark SRMs for Accurate Determinations of Trace Elements in Serum and Liver Tissues

The Centers for Disease Control and Prevention (CDC) collects data on elemental composition of human body fluids and tissues as part of major studies such as the National Health and Nutrition Examination Survey (NHANES). These studies require the analysis of large numbers of samples obtained from study subjects. Accuracy of the analytical data is critical and must be assured with proper quality control materials. NIST supports clinical trace element measurements with several benchmark SRMs. Supplies of two of these materials, SRM 1598 Bovine Serum and SRM 1577b Bovine Liver have been exhausted and the replacement materials, SRM 1598a Animal Serum and SRM 1577c Bovine Liver, are being issued. Certified or reference values are provided for all of CDC's priority elements in these materials.

R. Zeisler, S.J. Christopher, S.E. Long, E.A. Mackey, K.E. Murphy, and R.O. Spatz (Div. 839)

Units of SRM 1598a have been prepared from a serum pool derived from a mixture of serum from healthy bovine and porcine animals. Sera were collected under strict protocols designed to preserve the original composition and to minimize contamination. Briefly, the blood was obtained directly from an incision of the carotid artery and was collected in clean polyethylene pails. It was then immediately (before clotting) distributed among clean polyethylene bottles for further preparation in a clean room facility. After clotting, serum was obtained by centrifugation. These samples were stored at 4 °C. To prepare individual units of SRM 1598a, the serum was then pooled and 5 mL aliquots were dispensed into pre-cleaned polypropylene tubes, which were capped and frozen at –80 °C.

For elemental certification in SRM 1598a, NIST values for Cd and Ni were obtained with isotope dilution mass spectrometry (IDMS); all other elements were determined with at least one method carried out at NIST: instrumental neutron activation analysis (INAA), neutron activation analysis with pre-concentration of selected elements on Chelex 100 columns (PNAA), and inductively coupled plasma mass spectrometry (ICP-MS). Results from these measurements were combined with data provided by collaborating research and clinical laboratories using graphite furnace atomic absorption spectrometry, isotope dilution gas chromatography – mass spectrometry, ICP-MS, ICP atomic emission spectrometry (ICP-AES), INAA, and neutron activation analysis with radiochemical separations (RNAA). The CDC priority elements for which certified values are provided are shown in Table 1.

Table 1. Certified values (µg/L) and expanded uncertainties for CDC priority elements in SRM 1598a. (Ni and V were not certified in SRM 1598.)

Al	2.3	±	0.6
Cr	0.33	±	0.08
Cu	1580	±	90
Mn	1.78	±	0.33
Ni	0.94	±	0.18
Se	134.4	±	5.8
V	1.88	±	0.11
Zn	880	±	24

For SRM 1577c, liver tissue was collected from 31 steers that were slaughtered at Texas A&M University School of Veterinary Medicine. The animals were slaughtered for the purpose of teaching students bovine anatomy and how to butcher. The meat from these animals was prepared for retail under the supervision of a State of Texas meat inspector to ascertain the health of the animals. Each liver was rinsed with HPLC-grade water to remove excess blood, bile, and any other extraneous material. Fresh tissue (120 kg) was harvested from this process, frozen in clean Teflon bags, and shipped to NIST. The tissue was homogenized at NIST with a food processor equipped with titanium blades. The resulting paste was poured into glass trays, frozen, and lyophilized. The dry material was blended in a food processor and then jet-milled. The resulting final product was radiation

sterilized and bottled in 20 g units. Initial analyses by INAA established homogeneity of selected minerals and trace elements in the SRM. Analysis for value assignment will involve INAA, IDMS, ICP-MS, and ICP-AES at NIST with cooperating laboratories contributing results from GFAAS, ICP-MS, and INAA measurements with the aim to certify all CDC priority elements, a number of Environmental Protection Agency priority elements, and other minerals and trace elements.

Impact: The delivery of the two SRMs with an expanded list of elements certified at naturally occurring levels establishes a benchmark for the measurements of critical elements in human and animal body fluids and tissues.

Future Plans: Extension of measurements to the determination of metalloproteins and other metal-containing moieties is foreseen. It is becoming more important to characterize many elements at levels much lower than previously observed. New and improved analytical procedures must be employed to measure contamination-free levels of ultra-trace elements.

New Standards for Next-Generation Low-Emission Vehicles

In May 2006 NIST introduced two new gas-mixture Standard Reference Materials (SRMs) that contain significantly lower concentrations of nitric oxide (NO) in nitrogen than previously available. SRM 2737 contains a nominal NO concentration of 500 nmol/mol (ppb) and SRM 2738 contains nominally 1000 nmol/mol (ppb). Both SRMs are sold in large (30 L water volume) aluminum cylinders. The release of these SRMs concluded five years of development by NIST, with the work partially funded by the American Industry/Government Emissions Research (AIGER) consortium (U.S. Environmental Protection Agency (EPA), the California Air Resources Board (CARB), General Motors, Ford, and Daimler-Chrysler). AIGER members have identified the need for significantly lower concentration NIST gas standards containing carbon monoxide, hydrocarbons, and nitric oxide. SRMs 2737 and 2738 were identified as their highest priority.

W.J. Thorn III (Div. 839)

AIGER members are working together to facilitate the automobile industry meeting more stringent 2003 Federal Tier II and California LEV II emission regulations that go into effect over the next several years. New lower-concentration gas standards are required for emission testing of newer vehicles that produce lower levels of pollutants. The new engines employ fuel injection with air-to-fuel ratios that are optimized by on-board computers. Tailpipe emissions are further reduced by highly efficient catalytic converters.

NIST has had a long-term relationship with the AIGER group that requested that the Analytical Chemistry Division develop two new gaseous SRMs at low NO concentrations. A major roadblock to successfully developing these SRMs had been the inability of NIST's specialty gas contractors to achieve stable NO mixtures at lower SRM concentrations. NIST became proactive in solving the stability problem resulting in a two-step successful treatment:

1) All cylinders are pretreated with an NO_2 mixture.

2) Oxygen contamination levels for dilution nitrogen are qualified by measurement at < 2 nmol/mol.

Using the above treatment, NIST developed ten stable low NO working standards ranging from 500 nmol/mol to 1250 nmol/mol. These cylinders served as the low NO benchmarks until more accurate gravimetric standards were prepared in 2005. These are the benchmarks against which the concentration values for SRMs 2737 and 2738 were assigned and against which the SRMs are being monitored for stability. Each SRM cylinder has been individually certified for NO and NO_x concentration values at an expanded uncertainty of ± 1.5% relative.

To demonstrate that analytical capabilities and results at this low level are comparable to other laboratories', NIST participated with other national metrology institutes in an international intercomparison study (CCQM-K26a NO in Nitrogen [Ambient Level]) in which each laboratory independently measured the nitric oxide concentration of a gas mixture sent by the coordinating laboratory. The Gas Metrology Group at NIST assigned a concentration of (715 ± 7) nmol/mol nitric oxide in nitrogen for the NIST test sample, referencing the suite of low-concentration NO working standards. This result was in excellent agreement with the assigned concentration value determined by the CCQM coordinating laboratory for this test sample.

Gas mixtures have been developed to meet other AIGER needs. NIST completed multiple analyses on scrubbed air and scrubbed nitrogen at the threshold of detection for CO, CO_2, SO_2, and NO_X. AIGER members submitted eight cylinders of air and eight cylinders of nitrogen that will serve as a zero reference during analytical testing. AIGER requires a known zero gas standard to establish a true zero on their analytical monitors to assist in meeting regulations. The cylinders will be shipped back to the AIGER members who ordered them.

Several AIGER members have requested that NIST blend individual gravimetric standards containing toluene, nitrous oxide, refrigerant 134b, methanol, and ammonia at a single point-of-reference concentration of 10 µmol/mol. AIGER members have purchased Innova Photoacoustic analyzers that can be calibrated using these standards. Cars are sealed into "sheds" and subjected to various environmental conditions, such as "baking." The photoacoustic analyzers are used to monitor fugitive emissions (e.g., the "new car smell," outgassing from tires) which are regulated. NIST has completed all of the standards with the exception of ammonia, which will be completed in the coming year.

Environmental Specimen Bank Activities

Environmental specimen banking is the long-term preservation of representative environmental specimens for deferred analysis and evaluation. A systematic well-designed specimen bank program is not only a valuable component of real-time monitoring and basic research, but it also enables investigators to extend their research into the past and provides for future verification of analytical results. Formal environmental specimen banks are recognized internationally as integral parts of long-term environmental monitoring and research.

P.R. Becker, R.S. Pugh, M.B. Ellisor, A.J. Moors, B.J. Porter, J.R. Kucklick, S.J. Christopher, S.S. Vander Pol, R.D. Day, W.C. Davis, J.M. Keller, D. Point, A. Guichard, J.R. Flanary, J.E. Yordy, C.E. Bryan, M.M. Schantz, G.C. Turk, and S.A. Wise (Div. 839)

NIST maintains two facilities for the cryogenic banking of environmental specimens collected as part of ongoing research and monitoring programs of other government agencies. Established in 1979, the National Biomonitoring Specimen Bank (NBSB) is located at the NIST Center for Neutron Research, Gaithersburg, Maryland campus. A second facility, the Marine Environmental Specimen Bank (Marine ESB), was established by NIST in 2002 at the Hollings Marine Laboratory (HML), Charleston, South Carolina. The Marine ESB is devoted to the cryogenic banking of marine and coastal environmental specimens. The major agency supporters of the Marine ESB are the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Department of the Interior (DOI).

The NIST environmental specimen banks include well-developed banking protocols, computerized sample tracking (chain-of-custody) systems, maintenance of many forms of data associated with original specimens, and large investments in state-of-the-art facilities and equipment required to store specimens over relatively long periods of time. Both banks emphasize cryogenic storage using ultra-cold (–80 °C) electric freezers and liquid nitrogen vapor (–150 °C) freezers with continuously monitored security systems.

The National Marine Mammal Tissue Bank, which was established by federal legislation in 1992, is maintained by NIST for the National Marine Fisheries Service and the Fish and Wildlife Service as a component of the NBSB and Marine ESB, with the Marine ESB providing the lead. Tissue samples for banking are collected from individual stranded animals, mass strandings, and animals incidentally taken in commercial fishing operations; as part of unusual mortality event investigations; from Alaska Native subsistence hunts; and as part of on-going live-capture and release studies of marine mammal population health. At present, NIST maintains 3,017 tissue samples collected from 1,075 individuals representing 43 species of marine mammals from throughout U.S. coastal waters, including Alaskan. Since establishing the Marine ESB, NIST has used its banking expertise to develop protocols and to collect and archive blood and blubber samples for NOAA’s ongoing bottlenose dolphin health assessment studies, to collect eggs as part of a DOI environmental monitoring program on Alaska seabird colonies, and to collect eggs and feathers as part of a DOI peregrine falcon monitoring program. To date, blood and blubber have been collected from 364 bottlenose dolphins, 1,031 eggs have been archived from 5 species of arctic seabirds throughout Alaska, and 102 samples of eggs and feathers have been collected from the peregrine falcon program.

From 1985 until 1992, the NBSB provided for the banking of fish tissues, sediments, mussels, and oysters as part of NOAA’s National Status and Trends Program. During this period, 194 sediments, 197 mussels/oysters, and 31 fish tissue specimens from ≈300 U.S. coastal sites were banked. Banking for this program ended in 1992 when NOAA funding support ended. Realizing the value of the banking component of this program, some funding was restored and banking of mussels and oysters began again in 2005 and continued through 2006, with the lead being provided by the Marine ESB. Since 2005, mussel and oyster collections have been banked from 73 sites from throughout the coastal U.S.

The Hollings Marine Laboratory (HML) is operated by the National Centers for Coastal Ocean Science (NCCOS), with a mission to “provide science and biotechnology applications to sustain, protect, and restore coastal ecosystems, with emphasis on links between environmental condition and the health of marine organisms and humans.” HML partnership organizations include NIST, the National Oceanic and Atmospheric Administration (NOAA), the South Carolina Department of Natural Resources, the Medical University of South Carolina, and the College of Charleston.

The primary function of the NIST environmental specimen banks is to provide samples for retrospective analysis. A major effort at the HML involves the identification and investigation of newly emerging contaminants of concern in the marine environment. The banked specimens provide a valuable resource for investigating temporal environmental trends in concentrations of such compounds and for determining patterns of past exposure in marine biota. Geographic and time-trend studies using banked marine mammal tissues and seabird eggs are being conducted for brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs) and hexabromochlorododecane (HBCD), organomercury, butyltin compounds, and perfluorinated compounds. Banked Alaska marine mammal specimens were also accessed in 2006 for stable isotope and fatty acid analyses in a study investigating food web changes that might be associated with climate changes.

Environmental specimen banking was recognized in November 2005 by the International Environmental Specimen Bank Symposium, held in Charleston, South Carolina, and at the Hollings Marine Laboratory. The three-day symposium and half-day workshop attracted 80 participants representing 8 countries (Germany, Japan, Canada, Sweden, France, Finland, South Africa, and the U.S.). Selected papers based on presentations at the symposium were published in a special issue of the *Journal of Environmental Monitoring* (Volume 8, Number 8, August 2006).

Impact: The establishment of the Marine ESB in association with the HML has provided a renewed interest and expansion in specimen banking as part of marine environmental monitoring and health research. Additional specimen types have been added to the bank and additional sponsors have contributed to the expansion of the banking program with primary interest in use of banked specimens for investigating newly emerging environmental contaminants.

Future Plans: NIST will continue to work with HML partners and other federal agencies and partners to expand environmental specimen banking activities. NIST is working with these partners to add serum banking as part of aquatic animal disease investigations. Existing specimens archived in the NBSB and Marine ESB are being identified for future work to measure new contaminants of interest as related to issues on ocean and human health.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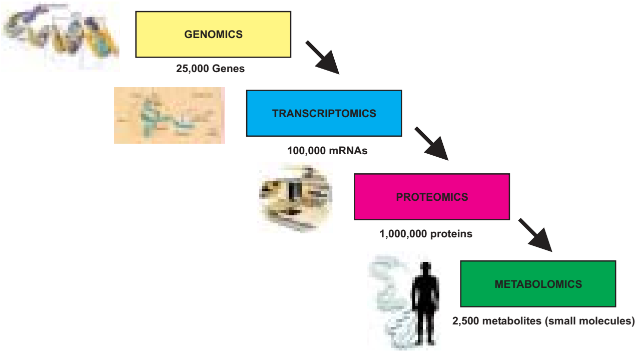
Special Issue – International Environmental Specimen Banking

Development of Standard Reference Material for Metabolites in Plasma

Metabolomics is a relatively new field that builds upon ongoing work in genomics and proteomics. The goal of metabolomics is to identify, measure, and interpret the complex profile of metabolites in cells, tissues, and other biological samples. These measurements may provide insight into the chemical and molecular pathways that are involved in normal function as well as disease. The National Institutes of Health (NIH) Metabolomics Technology Development Initiative reflects the need for new tools to identify and quantify metabolites in human systems. NIST is working with NIH to develop a plasma-based Standard Reference Material (SRM) that can be utilized to evaluate new procedures and equipment for measuring metabolites and improve the reproducibility of measurements by providing a stable standard for comparison.

K.W. Phinney, L.C. Sander, K.E. Sharpless, G.C. Turk, and S.A. Wise (Div. 839)

NIST is developing a plasma-based Standard Reference Material (SRM) to support technology development in the field of metabolomics. The metabolome represents the collection of all metabolites in an organism at a particular moment in time. Because metabolites constitute the end products of gene expression, metabolomics may provide a unique mechanism to examine genotype-phenotype relationships. Metabolomics is increasingly being used in health-related applications, including drug development, and may ultimately lead to better disease diagnosis and treatment.



Metabolomics has been identified in the NIH Roadmap for Medical Research as an important tool in understanding cellular pathways in normal and disease states. The complexity of the metabolome poses significant analytical challenges because hundreds of metabolites may be detected in a single sample. Analytical techniques that are currently used for metabolomics research include chromatography, mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy. At the present time, however, the human metabolome remains poorly characterized, and normal levels have only been established for a limited number of metabolites. As a result, interpretation of the physiological significance of metabolite concentrations remains difficult, and most investigations have been qualitative, rather than quantitative in nature.

NIH Roadmap FOR MEDICAL RESEARCH

The NIH Roadmap Metabolomics Technology Development Initiative is intended to promote the discovery of novel technologies to enhance understanding of metabolic pathways and networks. Discussions among roadmap investigators and between NIH and NIST identified a need for a reference material to support metabolomics technology development. The new SRM 1950 Metabolites in Human Plasma will assist the metabolomics community by providing a material with well-characterized concentrations of up to 100 metabolites at normal physiological levels and was designed in consultation with NIH and the roadmap investigators. The SRM is designed to serve as a tool to benchmark the development of new technology and will facilitate the move toward quantitative technologies for examining changing metabolite concentrations.

Development of SRM 1950, NIST's first plasma-based SRM, is underway. The SRM will consist of a plasma pool collected from an equal number of men and women and with a racial distribution that reflects the U.S. population. The initial value assignment phase for this SRM will include approximately 50 metabolites for which NIST has existing methods (e.g., cholesterol, electrolytes, glucose, hormones). Additional information for value assignment will be provided by collaborating laboratories to which the candidate SRM is distributed. NIST will develop methods for additional high-priority analytes, as identified by NIH, during the second phase of value assignment.

Metallomics: A Multidisciplinary Approach for the Determination of Metalloproteins Used as Health and Disease Markers

There have been estimates that over 30% of known proteins require metal cofactors for proper functionality. Since their discovery, metalloproteins have been the focus of research in biology and medicine because of their various functions in connection with protein folding and neurodegenerative diseases, metalloenzyme activity, and detoxification through transport and storage mechanisms. For example, quantitative determination of Zn, Cu, and Fe in brain proteins is of interest for studying Alzheimer's and Parkinson's diseases. The emerging science of "metallomics" requires versatile measurement tools that allow for trace detection, identification, and quantitation of covalently bound metals in proteins or metal-protein complexes.

W.C. Davis, S.J. Christopher, S.E. Long,
M. Vergne, A. Peck (Div. 839), L. Kilpatrick (Div. 838), and J. Baatz (Medical U. of SC)

It is difficult to identify and quantify a metalloprotein in a complex biological matrix with a single analytical technique. The isolation, quantitation, and structural determination of a metalloprotein requires the use of numerous complementary biological processing, instrumental separation, detection technologies. Protein separation by multidimensional liquid chromatography and/or 2D gel electrophoresis interfaced to inductively coupled plasma mass spectrometry (ICP-MS) may provide for rapid, metal-selective screening of target proteins, their subsequent quantitation at low concentration levels, and finally, structure elucidation with the assistance of organic mass spectrometry.

NIST researchers are working to combine multidimensional separation techniques with mass spectrometry to provide metal-selective screening, quantitation, and structural information for metalloproteins.

We are investigating the use of laser ablation (LA) ICP-MS for the multi-elemental screening of separated proteins on polyacrylamide gels. The analysis is relatively rapid, allowing mapping of whole gels in less than two hours; is amenable to multi-element data collection including the physiologically important elements P and S; and affords detection limits for important trace elements like Cu and Zn at the nanogram of protein level. This technique is useful in identifying areas of elemental localization as a precursor to more in-depth studies using organic mass spectrometry.

To date our research has concentrated on development of methods for separation of proteins and their subsequent transfer onto polyvinylidene fluoride (PVDF) membranes that are amenable to LA-ICP-MS (see example in Figure 1), and assessment of metal response as a function of metalloprotein concentration.

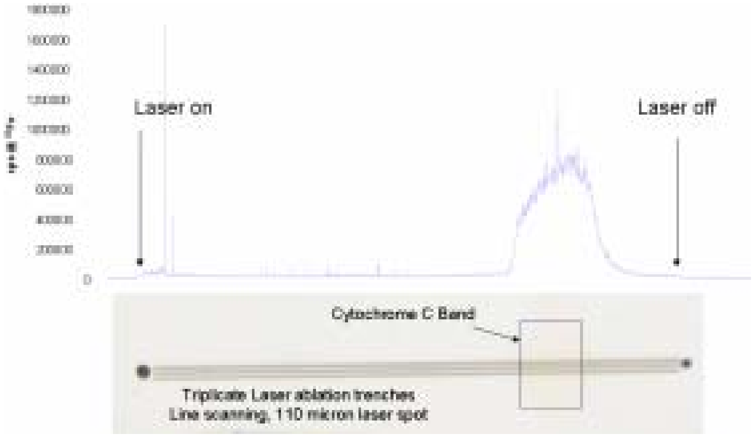
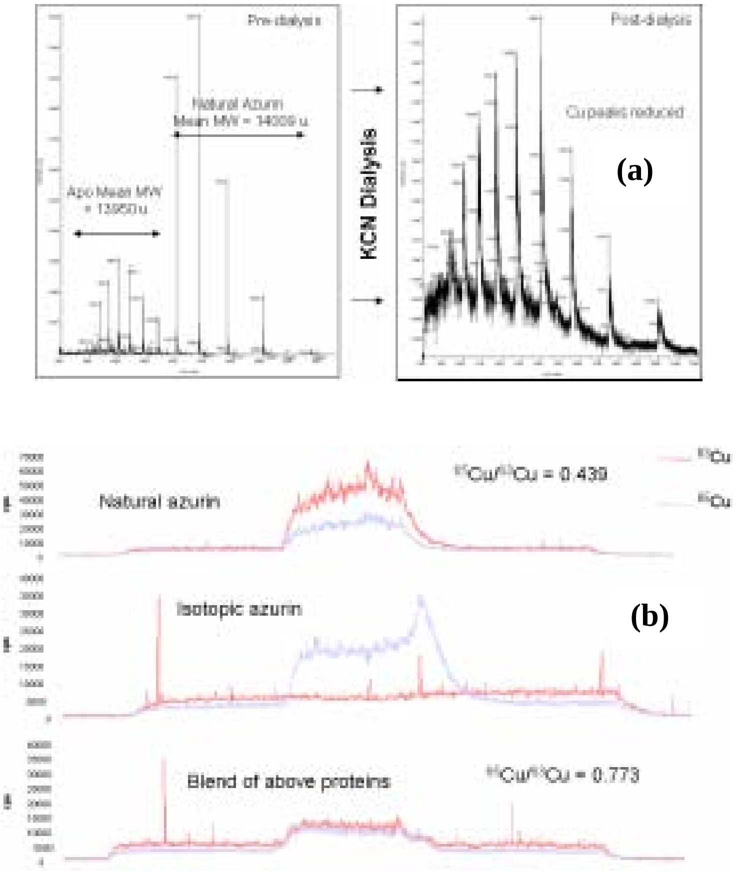


Figure 1.** **Laser ablation trenches and corresponding ICPMS $^{56}Fe^{+}$ response across a cytochrome c protein band separated by SDS-PAGE and transferred to PVDF.

We are developing new methods for quantitation of metalloproteins by measuring the associated metal. To this end, proof-of-principle experiments have been performed to synthesize isotopic metal analogs of metalloproteins through dialytic exchange and employ them as calibrants. This requires monitoring the exchange of the metal using both electrospray and ICP mass spectrometry (Figure 2a and 2b).

Impact: Development of qualitative and quantitative tools for metalloprotein metrology will help benchmark measurements in biological systems and identify new classes of quantifiable biomarkers.

Figure 2. (a) Monitoring the extraction of natural copper from Azurin to form the apoprotein. (b) LA-ICP-MS isotopic tracking of Cu isotopes in a mixture of natural and isotopic Azurin; protein bands are blotted onto a PVDF membrane.



Method Development for the Simultaneous Quantification of Mercury and Tin Species in Sediment and Tissue SRMs

Methods applicable to speciation of mercury and organotin in biological reference materials using speciated isotope dilution-gas chromatography-inductively coupled plasma-mass spectrometry (SID-GC/ICP-MS) have been developed and optimized. These methods were applied to measurement of methylmercury, inorganic mercury, and mono-, di-, and tributyltin species in sediment and tissue Standard Reference Materials (SRMs).

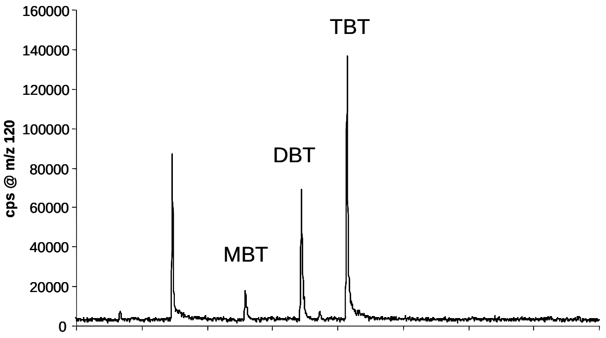
W.C. Davis, D. Point, and S.J. Christopher (Div. 839)

Tributyltin (TBT) and its degradation products, dibutyltin (DBT) and monodibutyltin (MBT), are toxic compounds widely dispersed in the environment. TBT has been used as a biocide in marine antifouling paints since the 1970s. Its release into the marine environment has resulted in extensive damage to organisms such as oysters and mussels. In the case of mercury, the environmental and human health effects are generally related to the environmental transformation of inorganic mercury to toxic methylmercury, and subsequent bioaccumulation of methylmercury in wildlife and humans. In some cases, more than 90% of the total mercury found in edible fish tissue is in the form of methylmercury. The potential for human and environmental impacts calls for improvements in current analytical methods to obtain accurate measurements for specific organometallic species.

Most methods for the analysis of organometallic species are based on hyphenated instrumental techniques in which the species are first separated by gas or liquid chromatography and then detected by ICP-MS. Our recent research has centered on the implementation of advanced calibration procedures that yield accurate results suitable for the certification of SRMs. Despite significant improvements in methods and instrumentation, transformation of mercury species during sample preparation may occur. The transformations can result in significant bias in the measurement of mercury species. For this reason, a double-spike speciated isotope dilution (DS-SID) method has been implemented using a methylmercury ($CH_3{}^{202}Hg$) spike (CRM AE670, Institute of Reference Materials and Measurements) in concert with inorganic mercury ($^{201}Hg^{+2}$ spike, Oak Ridge National Laboratory) for the simultaneous determination of both species. A reaction model has also been developed to correct for the possible transformations affecting methylmercury and inorganic mercury measurements in biological tissues. This method is now being used to measure mercury species in both marine biota and sediment SRMs.

In addition, a SID method was developed for the quantitation of MBT, DBT, and TBT in SRM 1974b Organics in Mussel Tissue (*Mytilus edulis*). These are the first values assigned for organotins in a cryogenic fresh-frozen SRM at low concentrations. Multiple methods were compared using varying extraction conditions to check for potential butyltin species degradation. No statistcally significant differences were obseved between the extraction methods for the individual organotin species.

Future Plans: Measurements of mercury and butyltin species in SRM 1974b will continue to assess the stability of these analytes at storage temperatures of – 80 °C.



GC/ICP-MS determination of mono-, di-, and tributyltin in SRM 1974b Organics in Mussel Tissue (*Mytilus edulis*).

Molecular Simulation of Alkylsilane Stationary Phases in Liquid Chromatography

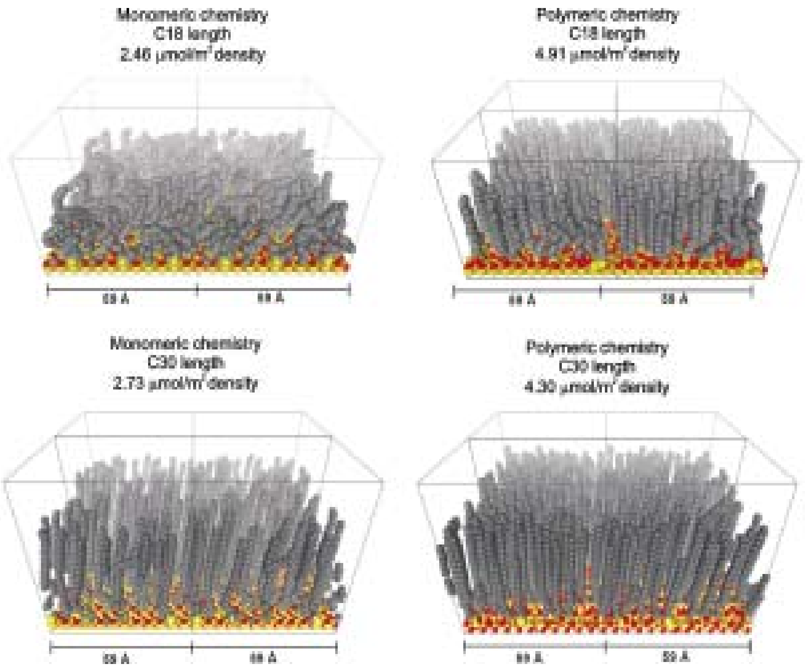
"Shape-selective" polymeric stationary phases are routinely employed over the more common monomeric stationary phases in reversed-phase liquid chromatography (LC) to improve the separation of geometric isomers of constrained solutes, such as polycyclic aromatic hydrocarbons, carotenoids, steroids, and polychlorinated biphenyls. Critical to describing such shape-selectivity processes in LC is the elucidation of the molecular-level structural features of the alkylsilane stationary phase that promote such separations. The evaluation of these potential molecular recognition sites within an alkyl-modified surface can be achieved through the use of computational tools, such as molecular dynamic simulation and surface topography techniques. Results from these modeling investigations are intended to support the elucidation of shape-selective retentive processes.

K.A. Lippa, L.C. Sander (Div. 839), and R.D. Mountain (Div. 838)

NIST researchers have investigated the molecular dynamics (MD) of chromatographic models that represent both monomeric and polymeric stationary phases with alkylsilane surface coverages and bonding chemistries typical of actual materials in an effort to elucidate the molecular-level structural features that control shape-selective separations. The structural characterization of these models is consistent with previous experimental observations of alkyl chain order and disorder. Highly ordered morphological features were characterized (identified) for simulated chromatographic models that represent materials with enhanced shape recognition capability. Deep cavities within the alkyl chains were identified for models representing shape-selective stationary phases. These results provide a more robust physical representation of alkyl-modified surfaces that supports the elucidation of retentive processes, as well as illustrating the potential for such computational techniques to predict the molecular recognition capabilities of novel analyte-specific sorbents.

MD simulations of C_8-, C_{18}-, and C_{30}-modified silicas have been carried out to study changes in stationary phase architecture that may result from variations in chain length, bonding density, synthetic approach, and temperature. Surfaces were modeled using a quartz substrate with vicinal –OH groups (9.8 µmol/m^2), and alkyldimethylsiloxane or alkyldisilanolsiloxane groups were linked via siloxane bonds. Surface densities ranged from 1.6 µmol/m^2 to 5.9 µmol/m^2 for C_8, C_{18}, and C_{30} ligands, and some models were constructed of three oligomer units to approximate bonding in polymeric phases.

Results from these models highlighted several trends in molecular structure. The thickness of the phases increased with increased bonding density as individual chains were more constrained by neighbors. Correspondingly, the *gauche* fraction also decreased with increased density. At the same bonding density, longer chain length phases were more ordered than shorter chain length phases. Finally, increased order was observed at reduced temperature. The resultant model snapshots shown below exemplified the aggregate structural features observed for chromatography phase models of varying bonding chemistry, density, and length. Chain extension and increased *trans* conformations are observed with models of polymeric C_{18} phases. C_{30} stationary phase models exhibit significantly increased *trans* conformations, even with lower surface densities. Figure 1 shows snapshots from molecular dynamic simulations of various monomeric and polymeric C_{18} and C_{30} stationary phases.



Analytical shape computation techniques were applied for the detection of cavities and the calculation of molecular surface properties of isolated cavity features and other ordered formations within these resultant alkyl stationary phase simulation models. Deep cavities 0.8 nm to 1 nm wide (8 Å to 10 Å) within the alkyl chains were identified for C_{18} polymeric models representing shape-selective stationary phases (Figure 2). Similar-structure cavities with significant alkyl chain ordered regions (> 1.1 nm or 11 Å) were isolated from two independent C_{18} models (differing in bonding chemistry, density, and temperature) that represent highly shape-selective materials. The size and depth of these ordered regions increased (up to 2.8 nm or 28 Å) for the extended-length C_{30} alkyl phase models. These results provide a more robust physical representation of alkyl-modified surfaces that supports the elucidation of retentive processes.

Impact: The models that have resulted from this study clearly indicate the extent of molecular order within alkyl-modified surfaces and are consistent with spectroscopic studies (e.g., Raman, ^{13}C NMR, and infrared spectroscopy). The results provide a compelling molecular-level vision of alkyl-modified surfaces that will guide future development of materials for chromatographic and biotechnological applications.

Future Plans: It is our continuing goal to investigate such molecular-level interactions by simulating constrained-shape solute interactions with fully solvated (i.e., water-acetonitrile mixtures) reversed-phase LC models to represent a range of shape-selective chromatographic behavior. In addition, the docking of solutes with various shapes and molecular dimensions from the liquid phase to the alkyl stationary phase will be employed to determine the effectiveness of these morphological features in the shape recognition process.

Publications:

K. A. Lippa, L.C. Sander, R.D. Mountain, **Molecular Dynamics Simulations of Alkylsilane Stationary-Phase Order and Disorder. 1. Effects of Surface Coverage and Bonding Chemistry**, *Anal. Chem.*, 77 (24), 7852 - 7861, 2005.

K.A. Lippa, L.C. Sander, R.D. Mountain, **Molecular Dynamics Simulations of Alkylsilane Stationary-Phase Order and Disorder. 2. Effects of Temperature and Chain Length**, *Anal. Chem.*, 77 (24), 7862 -7871, 2005.

K.A. Lippa, L.C. Sander, **Cavity Formations within Alkyl-Modified Surfaces via Molecular Dynamic Simulations,** *J. Chromatography A*, 1128 (2006) 79-89, 2006.

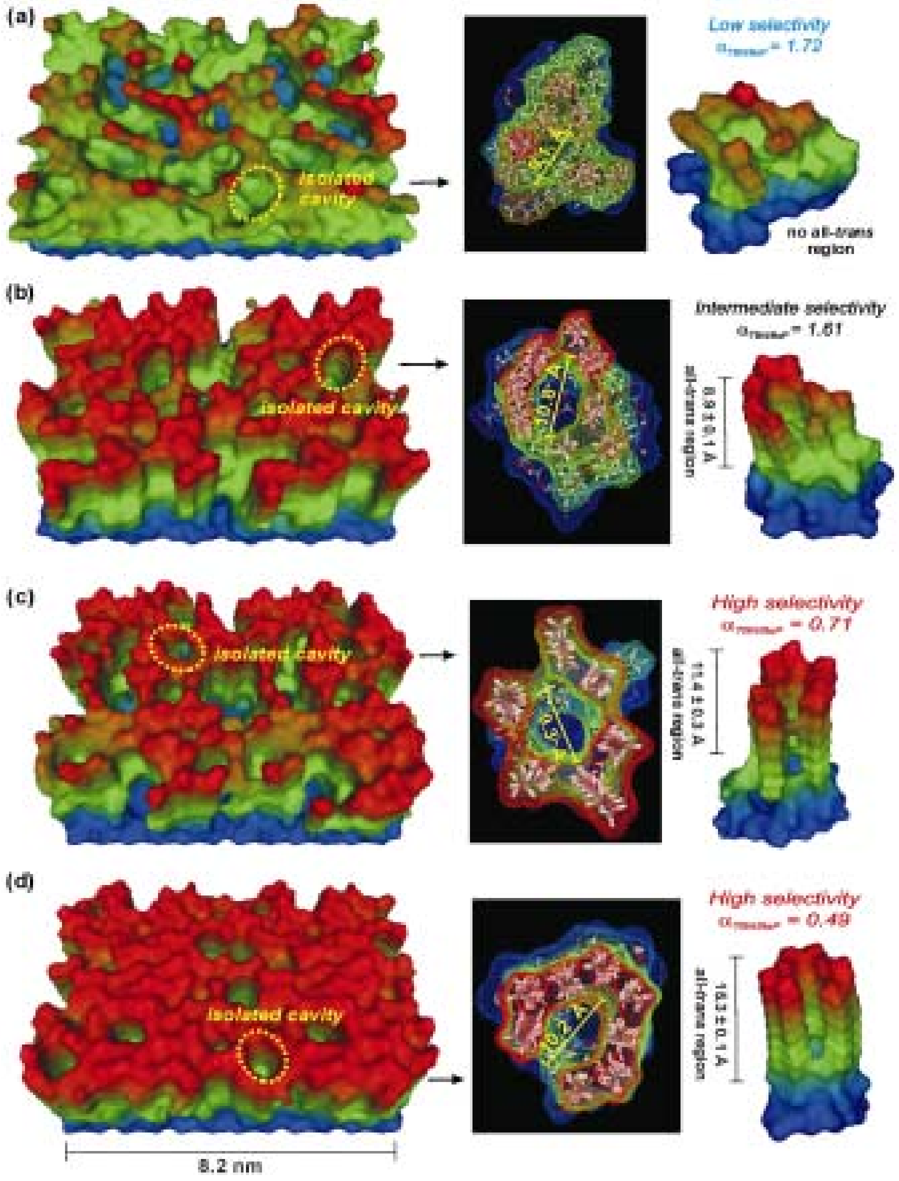


Figure 2 shows molecular surfaces generated for molecular dynamic simulation snapshots of (a) C_{18} monomeric model at 3.28 μmol/m^2 surface coverage representing an actual RPLC phase with low shape selectivity for tetrabenzonaphthalene (TBN) and benzo[a]pyrene (BaP) ($\alpha_{TBN/BaP}$ = 1.72) and three C_{18} polymeric models at (b) 3.89 μmol/m^2, (c) 4.91 μmol/m^2 and (d) 5.94 μmol/m^2 surface coverages also representing actual phases with intermediate to high shape selectivity ($\alpha_{TBN/BaP}$ from 1.61 to 0.49). Surfaces are color-coded according to the height along the z-axis.

Development of a Measurement Quality Assurance Program to Support Biomarker Discovery for Early Cancer Detection

Advances in technology and improvements in the methodologies for protein identification have produced rapid growth in the area of cancer biomarker discovery. Unfortunately, to date, there has been little effort to establish robust validation tools and methods to assure the reliability of protein biomarker discoveries. Subsequently, recent experience has greatly undermined confidence that proteomic measurements can be used to discover biomarkers that are sufficiently accurate to diagnose or predict the occurrence of cancer. NIST is working with the National Cancer Institute (NCI) of the National Institutes of Health (NIH) to establish a measurement quality control program for cancer biomarker discovery and biomarker validation research by providing reference materials and data analysis support.

D. Bunk (Div. 839) and S. Stein (Div. 838)

The idea that a protein present in a biological fluid such as plasma or urine could be measured as a diagnostic marker for cancer goes back to 1847 when Henry Bence-Jones discovered that large amounts of a protein were present in the urine of patients with myeloma. Since this discovery, substantial scientific effort has been placed on the discovery of other unique proteins or changes in the concentration or structure of normally occurring proteins in biological fluids (i.e., biomarkers) that could aid in the early diagnosis of cancer. Rapid advances in technology and improvements in the methodologies for protein identification have extended proteomics into the field of cancer biomarker discovery. Biomarker discovery research is critical to the successful realization of the promise of the rapidly expanding field of proteomics to produce major breakthroughs in disease diagnosis and treatment. Unfortunately, the search for new cancer biomarkers has not been successful. In the 159 years since the discovery of the first protein cancer biomarker, only nine proteins have FDA clinical diagnostic approval for cancer, and nearly all of these were discovered prior to the advance of proteomics into the field of cancer biomarker discovery.

To date, there has been little effort to establish robust measurement quality assurance tools for proteomics that are necessary to validate protein biomarker discoveries. In response to these measurement needs, NIST is working with NCI to establish a measurement quality assurance program for cancer biomarker research.

NIST researchers have provided support for the National Cancer Institute's Clinical Technology Assessment for Cancer program by collaborating on study design, providing study materials, and by providing critical data analysis support.

The aim of NCI's Clinical Proteomic Technology Initiative for Cancer is to refine and standardize the proteomic technologies, reagents, methods, and analysis platforms used in cancer biomarker research, to ensure reliable and reproducible research results. One aspect of this program was the establishment of a collaborative network of five research centers in the United States to evaluate and enhance proteomic measurement capabilities for clinical cancer research through a rigorous evaluation of existing technologies and the development of new technologies. To support this effort, NIST provided the necessary reference materials with which NCI's research centers conducted interlaboratory studies. Study data were analyzed at NIST after overcoming the difficulties of sharing and comparing data in the many proprietary and incompatible data formats that are common in proteomic mass spectrometric data.

Future Plans: **As the initiative progresses in the next five years, NIST will play a key role in overall study design and support the research carried out in the centers by proving advanced proteomic reference materials in the form of well-characterized human plasma and other clinical specimens, standard test data sets, and libraries of tandem mass spectra of peptides derived from human plasma proteins.**

Method Development and Measurements of Polybrominated Diphenyl Ethers (PBDEs) in Tissue, Serum, and Sediment SRMs

Polybrominated diphenyl ethers (PBDEs) are flame retardant compounds that are commonly added to many plastics, resins, and textiles that are then incorporated into products such as TVs, computers, furniture, and carpets. PBDEs can volatilize or leach out of the products in which they are applied and be transported long distances in the environment, due to their physico-chemical properties. Methods have been developed to quantify PBDEs in a variety of environmental-matrix Standard Reference Materials (SRMs), and the predominant PBDE congeners have been quantified in marine tissue, human serum, and sediment. The Centers for Disease Control and Prevention (CDC) also provided data for PBDE congeners in the human serum SRM. Data from these methods have been combined to value assign a variety of NIST SRMs for PBDE concentrations.

D.L.Poster, N.G. Dodder, J.M.Keller, J.R. Kucklick, M.M. Schantz, H.M. Stapleton, and S.A. Wise (Div. 839)

Polybrominated diphenyl ethers (PBDEs) are flame-retardant compounds that are commonly added to many plastics, resins, and textiles, and then incorporated into consumer products. PBDEs can leach out of these products and are now considered ubiquitous environmental contaminants. Much attention has been focused on their transport, uptake, and fate in both the environment and in humans. Presently, there is an increased need for measurements of PBDEs in environmental matrices and human serum. Reference materials certified for PBDEs are needed to ensure the quality of these measurements.

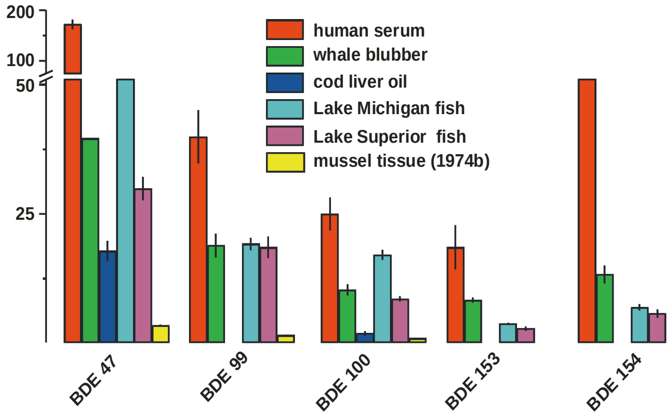
Ten SRMs have been characterized for a suite of 26 PBDE congeners using gas chromatography with mass spectrometric detection operated in both the electron impact and the negative chemical ionization modes. These SRMs include matrices such as marine mammal tissue, fish tissue, mussel tissue, human serum, marine sediment, and house dust (see Figure). The development of gas chromatographic methods using cool on-column injection and appropriate capillary columns has allowed for the measurement of the fully brominated PBDE (2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether; PBDE 209) using an isotopically labeled PBDE 209 as an internal standard for quantification of PBDE 209 in these materials. This congener has often been difficult to measure due to its thermal instability and its degradation at elevated temperatures. PBDE 209 was observed to be the dominant PBDE congener in the sediment and house dust SRMs, and thus, these SRMs will be very useful for laboratories interested in routine measurements for PBDE 209.

Impact:
The concentrations and relative contributions of the PBDE congeners measured in these SRMs are indicative of levels measured in environmental samples, illustrating their use as quality control materials. Additional values are reported for two sediment SRM materials used recently in an interlaboratory comparison exercise and the results support the use of these materials as reference materials for sediment studies.

Legislation has been passed to reduce the use of PBDEs in consumer products, but these compounds have already become ubiquitous in our environment.

Future Plans:
Other flame retardants such as hexa-bromocyclododecane (HBCD) are now being used at higher rates as a replacement for PBDEs in some parts of the world. Therefore, there is interest in the determination of the concentrations of HBCD in environmental samples. Evidence also suggests that PBDEs may be metabolized by some organisms to hydroxylated and methoxylated derivatives. Selected methoxylated PBDEs (MeOBDEs) have been quantified in marine mammal blubber and cod liver oil SRMs.

***PBDE congener composition in human serum (in pg/g) and environmental biota (in ng/g wet mass) SRMs based on certified and reference concentrations*.**



Evaluation of Oleoresin Capsicum (Pepper Spray) Canisters for Chemical Content and Reliability

A program to characterize the chemical compositions and the physical characteristics of pepper spray products has been established in the Analytical Chemistry Division at the request of the NIST Office of Law Enforcement Standards (OLES) and with funding from the National Institute of Justice. A variety of products representing a cross section of those used by law enforcement agencies have been selected for testing. The identities and concentrations of the active ingredients were determined by liquid chromatography/electrospray mass spectrometry and the identities of carriers and propellants by gas chromatography/mass spectrometry. Physical testing includes measurement of the number of one-second bursts in a canister, a range test, a spray pattern test, drop tests, and, for the products that produce fogs, the droplet size. Improved analytical methods have been developed for the determination of the active ingredients.

E. White V, R.G. Christensen (ret.), K.W. Phinney, B.J. Porter, L.C. Sander, R.Q. Thompson (Guest Researcher), J.L. Waddell (Guest Researcher) (Div. 839), and C. Presser (Div. 836)

Pepper spray is used by law enforcement officers to subdue non-cooperative individuals. The active ingredient in most pepper sprays is oleoresin capsicum (OC), an extract of hot peppers. A family of structurally related compounds called capsaicinoids is responsible for the pungency ("heat") of chili peppers, and these sample compounds are found in OC. Pepper spray is effective 85% to 90% of the time, and its use reduces the number of injuries to officers and suspects, as well as the number of use-of-force complaints. The causes of failures have not been established, but may include low concentrations of the active ingredients, poor delivery, and variable subject response. The goal of this study was to determine the identities and concentrations of the ingredients and to examine the mechanical performance of pepper spray units to establish potential modes of failure and hazards in handling.

Eleven commercial pepper spray products were selected for this study, and 100 canisters of each product were obtained for testing. Each of these products was subjected to a series of physical and chemical tests to evaluate product performance characteristics. Multiple canisters of each product were studied to gauge the consistency of the product.

New instrumentation was developed to support this effort. A test chamber was constructed for physical testing. Semi-automated devices were fabricated and tested for determining canister spray capacity, for performing pattern and range tests, and for performing drop tests. Environmental chambers were constructed for storage of canisters under controlled conditions. Phase Doppler interferometry instrumentation was utilized to measure particle sizes near the point of impact within the test chamber.

The determination of product potency was based upon measurement of eight capsaicinoids by liquid chromatography/mass spectrometry (LC/MS). The carrier and propellant in each product were identified by gas chromatography/mass spectrometry (GC/MS). Significant differences in the levels of active ingredient were observed among the products tested. The percent active ingredient ranged from 0.25% to 2.75% for the products included in this study. Products with similar claims of potency did not necessarily contain comparable levels of capsaicinoids, and in some cases, there was considerable variability in capsaicinoid levels among canisters of the same product. Leakage of propellant and/or pepper spray liquid was observed in some instances during physical tests or during storage at elevated temperature. Differences were also noted between the performance characteristics specified by the manufacturer (e.g., range, number of one-second bursts) and the results actually observed during the study.

Impact: A reliable set of quantitative chemical and physical measurements on a representative set of canisters is expected to provide a basis for determining the most likely causes of failures in the field and to provide a benchmark against which manufacturers can assess the performance of their products. As a result, law enforcement agencies will be better able to make informed product selection decisions.

Future Plans: New tasks funded by OLES to continue this effort will focus on 1) improvement of the reproducibility and accuracy of the range and pattern tests, 2) development of a product testing protocol standard for pepper sprays, and 3) preparation of a Standard Reference Material suitable for use in the chemical analysis of commercial pepper sprays.

Development of Proteomics-Based Methods for Protein Quantification

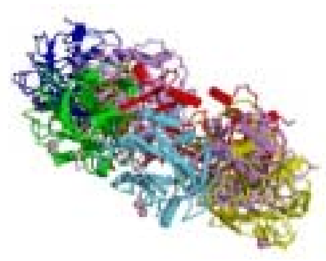
Reference measurement procedures are an important part of clinical measurement standardization. They can be used to directly assess the accuracy of routine methods or to assign or verify the concentrations of controls and calibrators used in routine methods. Reference measurement procedures also provide a means to demonstrate traceability of routine methods and materials to higher-order reference materials. Such traceability is required by the European Directive on in vitro *diagnostics (IVD) for all measurements in laboratory medicine. Lists of such higher-order methods are published by the Joint Committee for Traceability in Laboratory Medicine (JCTLM).*

N. Dodder and D. Bunk (Div. 839)

While there are many reference measurement procedures for clinical inorganic and small organic species, few exist for clinically relevant proteins. A recent survey of clinical reference methods by the JCTLM indicated approximately 25 documented reference methods for serum proteins, most using immunological methodologies or an enzyme activity measurement. Only two reference methods, both for HbA1c, use modern analytical measurement procedures – such as capillary electrophoresis, liquid chromatography, and mass spectrometry – for the sensitive and direct measurement of the clinical analyte. In order to meet the standardization needs for clinically relevant proteins, new reference measurement procedures must be developed.

In this work, an approach typically used in “bottom-up” proteomics is used for the quantification of clinically relevant proteins in serum. Specifically, quantification is achieved through the measurement of peptides generated from the enzymatic digestion of the target protein in serum. Because the analyte (protein) and measurand (peptides) are different, care must be taken to identify potential sources of bias in the enzymatic digestion process that converts analyte to measurand. Therefore, much of the focus in the development of this approach has been in the exploration of the enzymatic digestion process.

Because proteolytic digests, particularly trypsin digests, are at the core of our proteomics-based approach, we have devoted a large effort to understanding the practical nature of trypsin through fundamental studies of how experimental factors affect trypsin digests of analyte proteins. In collaboration with scientists from the national metrology institutes of the United Kingdom (LGC) and Germany (PTB), we have explored the quantitative nature of tryptic digestion. Successful quantitative measurements have been made on serum C-reactive protein using this approach.



C-Reactive Protein
Source: ***Protein Data Bank***

To develop a methodology capable of quantifying proteins present at low concentrations in complex clinical matrices such as plasma or serum, work has been started to incorporate affinity-based extraction techniques, in the form of antibody-coupled magnetic beads, into the measurement approach. Additionally, using *in vitro* methods for protein production, isotopically labeled proteins are being prepared for use as internal standards in this quantitative methodology.

As the field of proteomics matures, it is very likely that more protein biomarkers will be discovered and used for clinical diagnoses. New immunoassays for protein biomarkers will require validation through more metrologically sound approaches.

Accurate and precise protein quantitation may help answer fundamental biological questions regarding protein expression and its relation to the genome and environment. Additionally, the techniques developed for protein quantitation can also be used in the areas of drug discovery and biotechnology.

Future Plans:

Because protein quantification is a critical measurement capability, NIST will continue research in the development of proteomic-based approaches to protein quantification. Further development of the use of affinity reagents and isotopically labeled proteins as internal standards is needed to validate this approach and to apply it to clinically relevant protein measurements.

Developing NIST Reference Materials for Trace Explosives Detection

An important tool to protect against terrorist explosives events is the sensitive and accurate measurement of residues transferred by the handling and fabrication of improvised explosives devices. Currently there are no NIST certified reference materials specifically addressing trace explosives analysis. Commercial solutions of explosives are only available at high concentrations and are not suitable for trace detection evaluations. Solutions cannot be used to test and validate the performance of the entire residue detection protocol, including particle collection and transfer of the characteristic compounds into the instrument prior to the measurement. The goal of this project is to develop two NIST Reference Materials that can be used to test and calibrate both field-deployable detectors, such as those used in transportation security, as well as sophisticated laboratory instruments, such as those used for post-blast forensic investigations. SRM 8106 Trace Solution Explosives will address the low-level calibration of explosives detectors and RM 8105 Trace Particulate Explosives will be used to evaluate the collection and testing of explosives particles.

W. MacCrehan and B. Benner (Div. 839)

The challenge is to create reference materials that will provide calibrated trace amounts of two compounds (TNT and RDX) that are commonly used in plastic and military explosives in a form that is safe to handle. Using these reference materials, customers will be able to test and calibrate a wide variety of trace explosives detection equipment.

This work is part of a larger effort by the Department of Homeland Security to promote standardization for critical measurements. RM development is being done in conjunction with the Analytical Microscopy Group of the Surface and Microanalysis Division of NIST (Div. 837), who are investigating the properties of explosives residues and testing the performance of field-deployable explosives detection systems. This project builds on our previous research in analytical methods that support the forensic investigation of improvised explosive devices prepared using gunpowder, which resulted in the development of RM 8107 Additives in Smokeless Powder.

In the past year, seven candidate materials for the Trace Particulate Explosives material were prepared and evaluated. Candidate materials were selected to provide high thermal stability and appropriate particle size for representing explosives residues. To fabricate the candidates, the active ingredients of the plastic explosive C4 (containing RDX) and TNT were dissolved in organic solvent and coated onto a series of inert substrates. The solvent was removed under vacuum using a rotary evaporator, leaving trace amounts of the explosives coated on the inert substrates. Once prepared, each candidate material was extracted, and the explosives content determined by liquid chromatography. To investigate the stability of each candidate material upon storage, subsamples were stored at five temperatures between –20 °C and 70 °C and analyzed over a two-month period. Candidate materials were further evaluated using the most commonly deployed detection system for trace explosives detection, ion mobility spectrometry. From these experiments, a single "best candidate" substrate has been selected – porous silica beads with a bonded hydrocarbon coating. We are currently preparing 1 kg of the RM candidate.

NIST trace explosives reference materials will provide customers with well-characterized materials to strengthen detection technology: from manufacturers of current technology and their users, to developers of next-generation technology.

Prototype materials for the Trace Solution Explosives SRM have also been prepared and tested. The concentrations of these solutions have been chosen so that a single drop will contain sufficient explosive to provide a minimal positive 'alarm' signal with the most commonly deployed explosive detectors. This solution SRM will support the upcoming ASTM "Standard Practice to Verify Minimum Acceptable Performance of Trace Explosives Detectors." Development and promotion of this work within the ASTM Committee E54 Homeland Security Applications has been a collaborative effort of the Surface and Microanalysis Division, Analytical Chemistry Division, and the NIST Office of Law Enforcement Standards.

Future Plans: The certification of the reference materials for RDX and TNT should be complete by the end of FY07. In future years, we may develop a series of reference materials encompassing an array of ingredients used in the fabrication of improvised explosive devices.

Publications:

MacCrehan, W. "Development of a NIST Trace Particulate Explosives Reference Material to Evaluate IMS Detectors," *International Journal for Ion Mobility Spectrometry*, in press.

Support of Industry Compliance with the EU Directive on Restriction of Certain Hazardous Substances (RoHS)

The Analytical Chemistry Division (ACD) is involved in standards development and reference material certification for restricted substances in materials through participation in international test method development programs, providing Standard Reference Materials (SRMs) for test method validation, and investing in development of new SRMs. ACD involvement with RoHS is based in large measure on feedback obtained during a NIST workshop in October 2005, which resulted in a prioritized list of materials for new SRMs. Current projects include production of SRMs for lead-free solder and polymers containing high levels of restricted substances. Collaborations are underway with the National Institute of Metrology of China (NIM-C) and the Institute for Reference Materials and Measurements (IRMM) of the European Union (EU).

J.R. Sieber, G.C. Turk, M.M. Schantz, D.L. Poster (Div. 839), and Y. Li (NIM-China)

The EU RoHS Directive is the first of a number of regulations from the EU, China, Korea, California, and other governments restricting levels of hexavalent chromium (Cr^{+6}), cadmium (Cd), mercury (Hg), lead (Pb), polybrominated biphenyls (PBB), and polybrominated diphenylether (PBDE) compounds in electronic and electrical products. Recent estimates place the costs of compliance in these industries at nearly $100 billion because the restrictions created expensive consequences throughout the entire supply chain:

1. Manufacturers must either test their products or rely on testing done by components suppliers.
2. Manufacturers must share all declared composition data throughout the supply chain.
3. Manufacturers must replace materials containing high levels of restricted substances, e.g., lead-tin solder, and redesign products for reliable operation.

Manufacturers' in-house laboratories and commercial testing labs worldwide need valid test methods to meet the high demand for testing of alloys, coatings, polymers, resins, glass, and other materials. Certified reference materials (CRMs) are needed to support test methods.

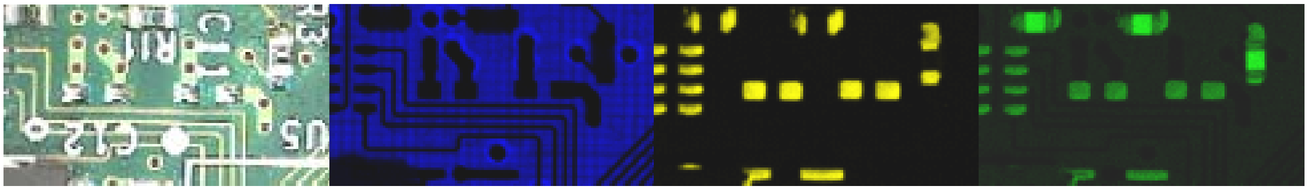
NIST was well-positioned to respond to these needs. In 2005 NIST representatives joined standards development committees of the International Electrotechnical Congress (IEC) and ASTM International. A NIST workshop brought together industry experts and provided prioritization of SRM and method development projects.

***Standards Development Activities*:** In January 2005 ASTM International Committee F40 on Declarable Substances in Materials was organized with NIST as one of over 100 charter members. F40 has published several standards related to restricted substances. Important test methods for solder and polymers are under development.

In early 2006, NIST provided SRMs used in validation studies of the methods for Cd and Pb in aluminum and steel alloys in a draft standard from IEC Technical Committee 111. The methods for steel and aluminum were shown to be valid, but other methods for glass, polymers, and coatings on alloys were not successfully validated, due in part to a lack of CRMs.

***Certified Reference Materials*:** In 2006 ACD initiated projects to develop new SRMs for plastics and lead-free solder containing known concentrations of restricted substances. Progress was made in development of an SRM containing known concentrations of PBDE compounds in solution, and house dust SRM 2585 was certified for the same compounds. Additionally, ACD determined flame retardant concentrations in a polymer CRM under development by IRMM. Three materials for use as SRM 2855 Additive Elements in Polyethylene have been developed in cooperation with ASTM.

***Future Plans*:** Standards committee activities will involve participation in validation studies for IEC TC111 and ASTM F40 method development for coatings, solder, and plastic. SRM development projects for solder and plastic are slated for completion during 2007. A new project is underway to create a candidate material for elements in polyvinylchloride (PVC) in collaboration with NIM-C. Researchers are developing new test methods for analysis of polymers by inductively coupled plasma optical emission spectrometry and X-ray fluorescence spectrometry.



X-ray map of elements in circuit board (2 cm^2 area): (l to r) Photo, Br flame retardant, Sn solder, Pb solder + ceramic.

Absolute, On-Line Measurement of the Total Alkalinity of Seawater: A Tool for Evaluation of Global Climate Change Mediation Technologies for CO_2

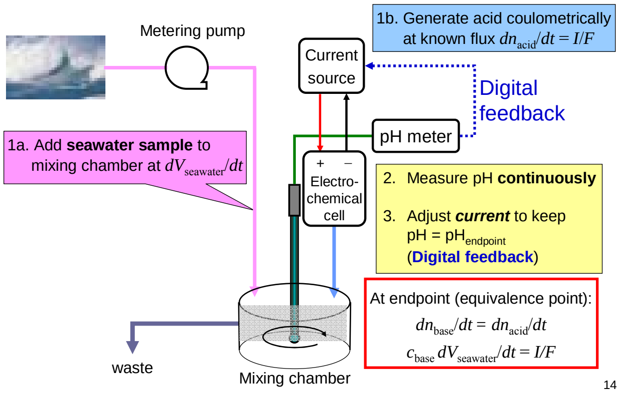
An automated flux titration system was developed for the measurement of total alkalinity (TA) of seawater samples. This system yields absolute TA values without the need for periodic standardization of a standard acid solution. The determination is performed in an entirely closed system, which avoids uptake or loss of carbon dioxide (CO_2) from the sample during the sampling or titration steps. Systematic bias associated with equilibration with the surface atmosphere is thus eliminated.

K.W. Pratt and T.W. Vetter (Div. 839)

NIST is working o provide an absolute, shipboard-compatible measurement system of seawater TA for on-site monitoring. Together with separate pH measurements, the TA defines the capacity of seawater to accept CO_2, e.g., in sequestration of anthropogenic CO_2 to mediate global climate change (GCC).

Research has demonstrated the feasibility of the proposed approach. The principles of flux titration were tested and verified for synthetic seawater samples of known TA value in a closed system using absolute electrochemical (coulometric) generation of standard acid in a separate electrolyte stream. A novel iterative procedure was developed to obtain preliminary, “rough” values for the endpoint pH and approximate TA value. These preliminary values are required for the subsequent high-accuracy TA determination. The iterative procedure takes advantage of the fundamental differential equations, derived for this work, that describe the response time of the system. The unproductive period otherwise occupied in waiting for steady-state response is thereby utilized to provide rapid pre-characterization of the TA value. Fundamental hurdles to the operation of the system have been characterized. Approaches have been outlined to eliminate these hurdles and achieve reliable operation.

Impact: The system under development will yield a rugged, reliable measurement instrument for shipboard measurements of TA of seawater samples retrieved either by continuous or by grab sampling from depth, followed by subsequent sub-sampling at the surface. Results will be directly traceable to the Systèm international d’unités (SI), based on the fundamental chemical laws of electrolysis, stoichiometry, and acid-base titration theory. These results will enhance the understanding of global oceanic dynamics of the CO_2 system. This knowledge will aid worldwide efforts to predict the consequences of perturbations of the oceanic system that result from increases in atmospheric CO_2 levels or from intentional sequestration of CO_2 by deep water injection of anthropogenic CO_2.



High-Accuracy Measurement of SiGe Chips Using ICP-OES

Silicon germanium (SiGe) technology has played a significant role in the development and success of the wireless and computer industries. The fabrication process using this technology is very similar to that for silicon-based chips. Therefore, only small changes in production are required, allowing for lower costs. SiGe devices can also be smaller, with reduced noise and higher frequencies, while maintaining the same low power requirements as Si devices. These favorable qualities make SiGe technology competitive with more expensive counterparts based on group III-V semiconductors, such as gallium arsenide (GaAs) and indium phosphide (InP). As the popularity of SiGe technology continues to advance in today's electronics market, characterization of these semiconductors becomes more vital. The Surface and Microanalysis Science Division (837) of NIST is leading an effort in conjunction with the semiconductor industry to provide chemically characterized SiGe reference materials to be used for validation of analytical methodologies. The Analytical Chemistry Division has participated in this effort by developing and applying an inductively coupled plasma-optical emission spectroscopy (ICP-OES) method that can be used to determine accurately and precisely the Ge atom fractions in SiGe alloys.

S.A. Rabb, M.R. Winchester, and L.L. Yu (Div. 839)

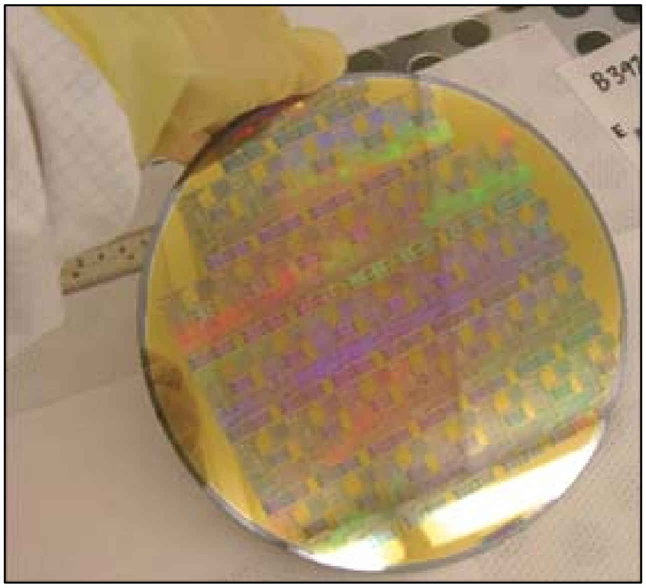
As the market for SiGe technology grows in the semiconductor industry, accurate characterization of SiGe materials is needed to ensure the quality of these materials and protect the economic interests of the semiconductor industry. However, reference materials do not exist to validate the methods used to characterize these materials. As part of an effort to provide SiGe reference materials, an ICP-OES method was developed to enable very accurate and precise determinations of Ge atom fractions in SiGe alloys. This method was then applied to SiGe candidate reference materials provided by the semiconductor industry.

SiGe chips of three different nominal Ge atom fractions (3.5 %, 6.5 %, and 14 %) were analyzed. All chips were digested at ambient temperature in capped polyethylene bottles to prevent the potential loss of Si in the presence of HF. The recoveries of Si and Ge after acid digestion of multiple Si and Ge metal pieces were observed to be 100.0 % ± 0.2 % for both analytes. Analyses were conducted using high-performance ICP-OES (HP-ICP-OES). HP-ICP-OES utilizes multiple measurements of every standard and sample in a randomized sequence. Correction for drift is accomplished by fitting a polynomial equation (up to sixth order) to the data for all standards and samples as a function of time, then applying correction factors derived from the fitted equation. Analyte concentrations in the standards and samples were closely matched to prevent uncertainties caused by nonlinearity and/or a non-zero intercept in the calibration.

The Ge atom fractions were successfully determined using the HP-ICP-OES methodology. Relative expanded uncertainties (95% confidence) were on the order of 0.2 % for all SiGe chips analyzed. The HP-ICP-OES values for these materials will be used in conjunction with INAA for assignment of certified values.

Future Plans: HP-ICP-OES analyses will be performed as needed to assist in the production and certification of additional SiGe reference materials.

Availability of SiGe reference materials will allow the semiconductor industry to validate analytical methods.



SRMs for Contaminants on Air Particulate Matter

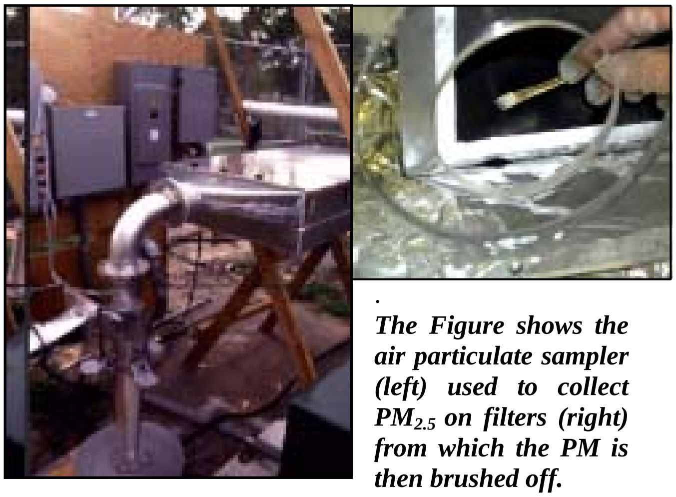
In 1997 the Environmental Protection Agency (EPA) issued new standards for air particulate matter (PM) under the national ambient air quality standards, including new regulations for PM less than 2.5 µm ($PM_{2.5}$), which is the respirable PM fraction, in addition to the existing PM_{10} standards. Research recommendations were made by the National Research Council at the request of Congress and the EPA to focus on evaluating what types of particles cause detrimental health effects. To support compositional analyses and other investigations on the fine PM, quality assurance materials are necessary; however, few appropriate fine particulate materials are currently available to support this research. NIST has been collaborating with the EPA to develop SRMs to support measurements of organic species in fine PM. In addition, research continues on the composition of bulk urban dust with the benchmark SRM 1648 being renewed.

M.M. Schantz, J.R. Kucklick, E.A. Mackey, B.J. Porter, D.L. Poster, J.R. Sieber, R.O. Spatz, S.A. Wise, and R. Zeisler (Div. 839)

NIST and the EPA collaborated in the late 1970s to develop two SRMs for ambient air particulate matter. SRM 1648 Urban Particulate Matter, which was collected in St. Louis, MO, was issued in 1978 with certified concentrations for trace element content. SRM 1649 Urban Dust/Organics, which was collected in Washington, DC, was issued in 1982 with certified values for five polycyclic aromatic hydrocarbons (PAHs) and was reissued in 1998 with values assigned for over 100 organic contaminants. Both of these air particulate SRMs have been widely used in the environmental/atmospheric particulate measurement community for nearly three decades. Supplies of both SRM 1648 and 1649 are nearly exhausted; bulk PM (< 60 µm) from the original late 1970s collections is available and measurements are in progress to reissue these SRMs. The reissuing of these materials, however, does not adesss the need for a more contemporary PM reference material, particularly for $PM_{2.5}$. Recently the EPA and NIST initiated a new collaboration to address reference materials for fine PM.

The recent NIST/EPA collaboration focused on three projects: (1) establishment of an interlaboratory comparison program to assess measurement comparability, (2) development of solution SRMs for organic compounds of interest for PM measurements, and (3) collection of bulk contemporary $PM_{2.5}$ for use as a future SRM. Three interlaboratory comparison exercises have been conducted for the determination of organic contaminants and source markers in PM samples. A series of calibration solution SRMs have been ampouled for aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), nitro-substituted PAHs (nitro-PAHs), hopanes/steranes, ^{13}C-labeled and deuterium-labeled levoglucosan, and acids (alkanoic and resin; deuterium-labeled and non-labeled).

As part of this collaboration, the Organic Speciation Working Group was formed in 2000 to assist in this effort by participating in interlaboratory comparison studies and by providing input for the development of SRMs to support these measurements. This group has participated in three interlaboratory comparison studies for the determination of PAHs, nitro-PAHs, alkanes (including hopanes and cholestanes), sterols, carbonyl compounds (ketones and aldehydes), acids (alkanoic and resin), phenols, and sugars in PM-related samples. Based in part on the results of these studies and input from the Organic Speciation Working Group, priorities for the development of a number of calibration solution SRMs were identified including: aliphatic hydrocarbons, PAHs, nitro-PAHs, hopanes/steranes, ^{13}C-labeled and deuterium-labeled levoglucosan (for use as internal standards), and acids (deuterium-labeled for use internal standards and non-labeled). SRM 1494 Aliphatic Hydrocarbons in *Iso*-Octane was issued in early 2004. SRMs 2260a PAHs in Toluene and 1491a Methyl-Substituted PAHs in Toluene, which are redesigned solutions with values assigned for an expanded list of 53 PAHs and alkyl-substituted PAHs, were issued in 2005. Five additional solutions will be issued in early 2007: SRM 2264 and SRM 2265 Nitro-PAHs I and II, respectively, in Methylene Chloride, SRM 2266 Hopanes and Steranes in 2,2,4-Trimethylpentane, SRM 2267 Deuterated Levoglucosan in Ethyl Acetate, and SRM 2268 Carbon-13 Labeled Levoglucosan in Ethyl Acetate. Three organic acid solutions have been prepared and are being monitored for stability prior to issuing SRMs.



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The Figure shows the air particulate sampler (left) used to collect $PM_{2.5}$ on filters (right) from which the PM is then brushed off.

Two recent collections of 20 g each of ultra fine particulate matter at a site in Baltimore, MD (see figure) have been used to prepare an interim reference material for distribution in the NIST/EPA interlaboratory comparison exercises for the determination of organic compounds. The results from the second interlaboratory study using the interim reference material have been used in conjunction with NIST measurements to assign concentration values for the compounds of interest. This interim reference material is now available to laboratories involved in EPA $PM_{2.5}$ research programs for use as a control or reference material. The second $PM_{2.5}$ material collected in Baltimore was used in the third NIST/EPA intercomparison study along with SRM 1648 Urban Particulate Matter and RM 8785 Air Particulate Matter on Filter Media (A Fine Fraction of SRM 1649a Urban Dust on Quartz-Fiber Filters). The sampler has been adapted to isolate the $PM_{2.5}$ portion from a subsample of SRM 1649a Urban Dust. This $PM_{2.5}$ portion of SRM 1649a has been homogenized and bottled for use in a future intercomparison study.

SRM 1648 is being replaced with SRM 1648a using PM from the original collection. Instrumental neutron activation analysis, X-ray fluorescence spectrometry, and solid-sample graphite furnace atomic absorption spectrometry have established homogeneity of SRM 1648a for a typical sample size of several milligrams and have confirmed the identical composition of SRM 1648 and SRM 1648a. Analyses for value assignment by NIST and collaborating expert laboratories were completed in late 2006, and SRM 1648a will be issued in early 2007. For the replacement of SRM 1649a, measurements for PAHs, nitro-PAHs, polychlorinated biphenyls (PCBs), chlorinated pesticides, and brominated flame retardants are in progress with completion targeted for late 2007.

Impact: The development of SRMs to support measurements for organic species in fine PM will expand quality assurance capabilities to important source markers and species critical to human health. Replenishment of the supply of bulk urban dust will extend NIST's support for inorganic characterization of aerosols for many years. Users can continue to rely on their experience with SRM 1648 and 1649a and can refer to extensive literature resource on both materials.

Future plans: The high-volume sampler will be used to prepare a $PM_{2.5}$ fraction from a collection of contemporary total suspended particulate matter as a means of providing sufficient fine PM for future SRM development.

Publications:

- Pancras, J. P., Ondov, J. M., Zeisler, R., ***"Multielement Electrothermal AAS Determination of Eleven Marker Elements in Fine Ambient Aerosol Slurry Samples Collected with SEAS-II,"*** Analytica Chimica Acta 2005, 538, 303-312.
- Schantz, M.M., Wise, S.A., and Lewtas, J., ***"Intercomparison Program for Organic Speciation in PM2.5 Air Particulate Matter: Description and Results for Trials I and II,"*** NISTIR 7229 (2005).
- Schantz, M., Poster, D., Kucklick, J., Wise, S., McDow, S., and Lewtas, J., ***"Intercomparison Program for Organic Speciation in PM2.5 Air Particulate Matter: Description and Results for Trial III,"*** NISTIR 7303 (2005).

SRMs for Organic Contaminants in Human Serum and Milk

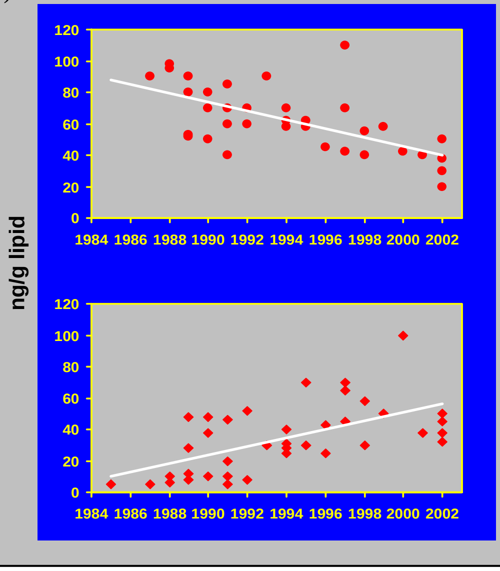
NIST and the Centers for Disease Control and Prevention (CDC) Division of Laboratory Sciences, Analytical Toxicology Branch, are collaborating to develop two new SRMs to meet the expanding needs for the measurement of organic contaminants in human serum; one of these materials, SRM 1957, is a natural level (non-fortified) and the second, SRM 1958, is a fortified material. In addition to the serum samples, CDC and NIST are collaborating to develop two SRMs for organic contaminants in human milk, SRM 1953, a natural level (non-fortified) and SRM 1954 (fortified). Results from measurements made at NIST and CDC as well as data from interlaboratory studies organized by CDC and NIST will be used for value assignment of SRMs 1953, 1954, 1957, and 1958.

M.M. Schantz, R.M. Heltsley, J.M. Keller, J.R. Kucklick, and S.A. Wise (Div. 839)

For the past 25 years NIST has developed a number of serum-matrix Standard Reference Materials (SRMs), primarily for measurements of clinically important analytes (e.g., cholesterol, glucose, and creatinine). The first serum-based SRM for the measurement of organic contaminants was SRM 1589 Polychlorinated Biphenyls (as Aroclor 1260) in Human Serum, which was issued in 1985 with certified concentration for total polychlorinated biphenyl (PCB) concentration expressed as Aroclor 1260. SRM 1589 was prepared by fortifying human serum with Aroclor 1260 prior to freeze-drying. This material was replaced in 2000 with SRM 1589a PCBs, Pesticides, and Dioxin/Furans in Human Serum, which contained only natural levels of these constituents, thereby providing users with a more useful material. SRM 1589a has been used extensively by the CDC in human monitoring studies to validate measurements of contaminants in human serum, particularly to support the NHANES program. The Certificate of Analysis for SRM 1589a was recently updated to include concentration values for brominated flame retardants, e.g., polybrominated diphenyl ethers (PBDEs), as well as values for additional polychlorinated biphenyls (PCBs) and pesticides.

The National Health and Nutrition Examination Survey (NHANES) program, conducted since 1971, is designed to assess the health and nutritional status of Americans. The survey combines personal interviews, physical examinations, diagnostic procedures and laboratory tests for approximately 5000 people each year. In addition to measurements of nutrients, recent NHANES samples of blood and urine have also been analyzed for a variety of contaminants.

In the last decade the concentrations of PCBs, pesticides, and dioxins/furans in the U.S. population have decreased by approximately 50% while the concentrations of the PBDEs have increased by a factor of 4: PCB 153 (top) and PBDE 47 (bottom) concentrations over time. (Sjodin et al., *Retrospective Time-Trend Study of Polybrominated Diphenyl Ether and Polybrominated and Polychlorinated Biphenyl Levels in Human Serum from the United States,* Environ. Health Perspect. 112, 654-658, 2004.)



Because of the changes in contaminant levels in human serum since SRM 1589a was prepared in 1996, a more contemporary serum SRM was requested by CDC. Approximately 200 L of human serum was procured from various locations around the U.S., combined, and homogenized. Half of this serum was dispensed and freeze-dried as SRM 1957. The remaining half was spiked with a solution containing 172 selected chlorinated dioxins and furans, brominated dioxins and furans, pesticides, polychlorinated biphenyls, brominated flame retardants, polychlorinated naphthalenes, halogenated phenols, brominated dioxins/furans, hydroxylated PCBs, and toxaphenes at concentrations approximately 10 times higher than median concentrations found in the US population during the 2003 NHANES study. This fortified serum will be SRM 1958.

CDC and NIST are also collaborating on the development of two human milk SRMs. Approximately 100 L of human milk was procured from a number of milk banks around the U.S., combined, and homogenized. Half of this milk was dispensed and frozen as SRM 1953 while the remaining half was spiked with a solution containing the same suite of 172 contaminants as used for the serum SRM 1958 (above). This fortified milk material will be SRM 1954.

SRM 1957, with natural contaminant levels, will be representative of a serum with concentrations of contaminants currently found in the U.S. population and will replace the currently available SRM 1589a. SRM 1958, spiked at levels 10 times higher than natural levels, will be useful for the development of analytical methods for emerging contaminants and other classes of compounds, demonstrating that analytes of interest have been properly identified.

***Impact*:** SRMs 1953 and 1954 will be the first human milk reference materials available for contaminant monitoring while SRM 1957 and 1958 will be the most characterized serum reference materials developed for contaminant analysis.

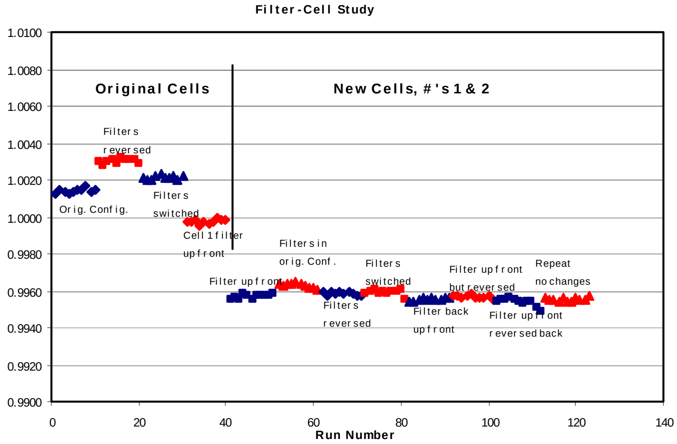
Improving Measurements with NIST's Ozone Reference Standard

The concentration of ozone in the atmosphere continues to be an important global issue both scientifically and politically. Stratospheric ozone protects the Earth from harmful ultraviolet radiation, while tropospheric ozone is a major health concern and contributes to global climate change as a greenhouse gas. The NIST Standard Reference Photometer (SRP), developed jointly with the U.S. Environmental Protection Agency (EPA), has provided an infrastructure for the calibration and traceability of ozone measurements within the U.S. since 1983, and it serves as the highest-level ozone reference standard in the U.S. NIST has provided SRPs to 19 laboratories in 16 countries including several SRPs to the International Bureau of Weights and Measures (BIPM) to serve as an ozone reference standard under the International Committee of Weights and Measures (CIPM).

J.E. Norris and F.R. Guenther (Div. 839)

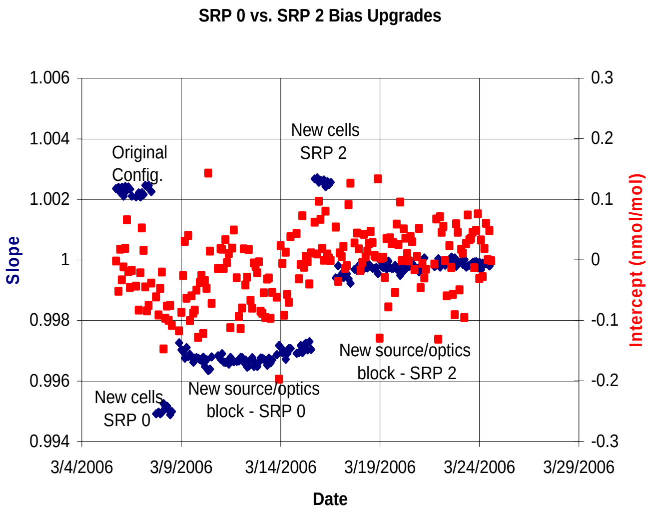
Ozone measurements in the U.S. and many other countries are traceable to the NIST Standard Reference Photometer (SRP). The International Bureau of Weights and Measures (BIPM) and 16 other countries have adopted the SRP as their ozone reference standard. In 2005 the BIPM and NIST completed an in-depth study of systematic biases in the Standard Reference Photometer (SRP) that revealed a temperature measurement bias causing an underestimate of ozone mole fractions by 0.4 %, and an optical measurement bias causing an overestimate of ozone mole fractions by 0.5 %. This resulted in a total estimated measurement bias of 0.1 %. Because all SRPs are essentially identical, these measurement biases exist on all units by varying amounts dependent upon instrument settings, cell window alignment, and laboratory conditions. NIST investigated and developed instrument modifications to reduce or eliminate these biases.

A re-designed source/optics block reduces the temperature gradient through the absorption cells to less than 0.2 °C by thermally isolating the heated-source lamp block. This change and the repositioning of the temperature probe to better monitor the average temperature of the absorption cells has greatly reduce the temperature bias. New absorption cells have been developed with each cell having optically sealed windows positioned at 3° angles. Additionally, collimation of light from the source lamp has been improved to reduce divergence. These design changes help eliminate internal reflections between cell windows and make o-rings to seal the windows to the cell, a potential source of leaks, unnecessary. Two sets of these new cells were tested on one of NIST's two SRPs, SRP 0, with various optical filters and physical positioning to examine the effects on the measurement slope of SRP 0. The results, shown in Figure 1, indicate the effect on the measurement slope due to various positions of the optical filters with the original absorption cells and with the newly designed 3° angled window absorption cells. With the original cells, a maximum 0.16 % change in the slope was seen by changing the position and orientation of the filters, followed by a 0.17 % change from the original filter configuration, to a single filter positioned before the cells. The new cells minimize this effect to a maximum of 0.08 % change in the slope regardless of the filter configuration/orientation. The total optical path-length bias measured on SRP 0 during this study was 0.57 %.



Major Accomplishments: During the past year, NIST has developed an upgrade for the SRP that minimizes the temperature gradient and optical path-length biases and has upgraded NIST's two SRPs, SRP 0 and SRP 2. The results of comparisons between these two SRPs are shown in Figure 2. The upgrades show improved agreement due to the variations in the respective biases. All SRPs worldwide are expected to be upgraded within the next two years, and overall measurement agreement, currently 0.7 %, is expected to improve.

Impact: Worldwide interest in the NIST SRP to serve as a high-level national reference standard for ozone measurement and traceability has continued. Proper maintenance and measurement comparability of all SRPs has a direct effect on global ozone measurements within the troposphere.



Future Plans: In 2007 NIST will participate in a CCQM key comparison on ozone measurements at ambient levels. Plans for several countries' SRP upgrades are already scheduled to take place in 2007, before their participation in the CCQM key comparison. Changes in the ozone mole fraction measurement in an upgraded SRP will be clearly quantified and documented. Also, when an SRP is returned to NIST for upgrade or maintenance, NIST can provide a direct comparison to NIST's primary SRP and submit the data to the BIPM for inclusion in the key comparison.

Sulfur, Mercury, and Chlorine in Fossil Fuel SRMs

The U.S. Environmental Protection Agency (EPA) mandated ultra-low sulfur diesel fuel (ULSD, upper limit 15 µg/g) for on-road use starting June 2006 to make possible more efficient exhaust emission after-treatment technologies. This will result in substantially reduced particulate emissions from diesel engines. The accurate determination of sulfur in ULSD at low levels is a major measurement challenge with enormous economic consequences, mostly in avoided costs, for petroleum refineries and in every link in the distribution system. To meet this challenge in a cost-effective manner and with the least disruption in product availability, the industry must have highly accurate real-matrix sulfur standards. NIST responded in 2005 with the production of three low-level sulfur distillate fuel reference materials. This year we have described a procedure for the blending of distillate fuel SRMs that will enable the petroleum community to prepare standards at any sulfur level and be traceable to NIST values. The implementation of this procedure within the petroleum community will provide greater flexibility in preparing natural-matrix standards at any desired sulfur level and at the same time reduce the number of SRMs that NIST will need to produce.

NIST has a suite of eight coal SRMs that are certified for sulfur and mercury concentrations. Three coal SRMs were also certified for chlorine this past year. The mixing procedure mentioned above can also be used for these standards if they are used in combustion instruments. This will make possible multipoint calibration curves over narrow concentration ranges.

W.R. Kelly, R.D. Vocke, J.L. Mann,
S.E. Long (Div. 839), and B.S. MacDonald (232)

NIST fossil fuel SRMs continue to be in high demand by the petroleum industry and the fossil fuel- based electric power industries as shown in Table 1. The increase in demand for coal SRMs has been about 14 % per year, and last year the demand for coal SRMs was almost half (49 %) of the fossil fuel sales. The demand for diesel SRMs has doubled between FY 2004 and FY 2006 which is probably the effect of more restrictive limits by EPA on sulfur in diesel fuel. The high demand for diesel fuel SRMs in FY 2005 was the result of a large purchase by EPA for an interlaboratory comparison.

Table 1. Comparison of Sales of Coal and Diesel Fuel SRMs as Percentage of Total Fossil Fuel SRM Sales

Sales by Fiscal Year	FY03	FY04	FY05	FY06
Total Fossil Fuels	2305	2578	3734	3243
Coal and Petroleum Coke	1081 47 %	1192 46 %	1387 37 %	1598 49 %
Diesel Fuel	287 17 %	358 15 %	1229 33 %	611 19 %

The U.S. and the European Union (EU) have mandated ULSD fuel for on-road use to reduce particulate emissions from diesel engines. The new U.S. limit is 30 times lower (15 µg/g versus 500 µg/g) than the current regulatory limit, although higher than the EU mandate of 10 µg/g. However, diesel fuel in the US is moved long distances by pipeline, which makes it more susceptible to contamination; therefore the sulfur concentration at refineries must be near 7 µg/g to ensure retail outlets meet regulatory requirements, which is essentially the same concentration level required for EU refineries. The EPA estimates that the health and welfare benefits of this new regulation will be about $150 billion annually to the American public. *Per capita* benefits in the EU should be similar because their consumption of diesel is slightly greater than that of the US and their population is larger.

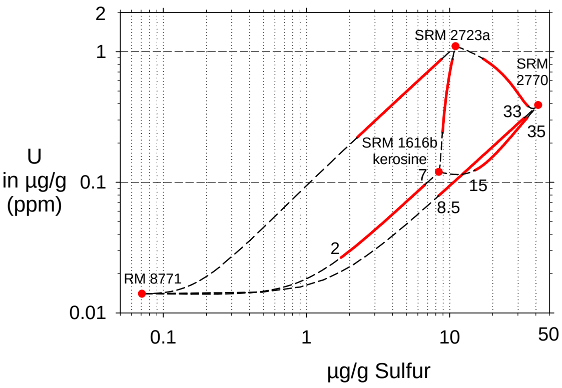


Fig. 1. A log-log graph showing uncertainties and concentration of possible gravimetric mixes of four low-sulfur level distillate fuel oils. The red circles indicate the certified values for the SRMs. The red lines indicate the concentration range possible for binary mixtures assuming 4 to 1 mixing ratios.

Three low-sulfur distillate fuel reference materials were certified last year, SRM 1616b, SRM 2770, and RM 8771. These certified reference materials complement the four existing distillate fuel SRMs at sulfur concentrations of 11 µg/g, 427 µg/g, 1731 µg/g, and 3882 µg/g. The four low-sulfur standards are plotted in Figure 1 and can be used in a binary mixing system to generate concentrations desired by an end-user. The possible mixtures are indicated by the solid red lines. The lines terminate at mixing ratios of 4 to 1 and vice versa. There is very good concentration coverage from 2 µg/g to 35 µg/g with expanded uncertainties less than 1 µg/g at the high end and less than 0.1 µg/g between 2 and 7 µg/g.

NIST has eight coal SRMs all of which are certified for sulfur and mercury. In recent years there has been increasing interest in the chlorine content of coal. The chlorine content of coal determines to a large degree the speciation of inorganic mercury emissions from coal-fired power plants. The fraction of mercury that is emitted as elemental mercury is inversely proportional to the chlorine content. Three coals were certified for chlorine this year and are listed in Table 2 along with the previously determined values for mercury and sulfur. An additional five coals will be certified in 2007. The chlorine was determined by isotope dilution negative thermal ionization mass spectrometry.

Table 2. New Chlorine Values by Isotope Dilution for Three SRM Coals

SRM Coal	**Cl µg/g**	**Hg ng/g**	**S %**
SRM 2692b	**1651 ± 28**	**133.3 ± 4.1**	**1.170 ± 0.020**
SRM 2693	**369.6 ± 5.7**	**37.3 ± 7.7**	**0.4571 ± 0.0067**
SRM 2685b	**517 ± 13**	**146 ± 11**	**4.730 ± 0.068**

Impact: The combined impact of all NIST fossil fuel SRMs certified for sulfur has been studied in detail by Martin *et al.* (2000) in an economic impact report. The lower sulfur regulations in on-road diesel fuel compared to year 2000 logically mean that the environmental impacts are now even greater. The current lower sulfur limit of 15 µg/g compared to the former limit of 500 µg/g means that the petroleum industry must control diesel fuel quality in terms of sulfur content with much smaller tolerances. It will be very difficult and expensive to blend down streams of high sulfur concentration to pass the regulatory specification. For example, to blend a 20 µg/g product to 10 µg/g would require an equal volume of diesel fuel near zero concentration.

If the blending of liquid and solid fossil fuels is accepted widely in the fossil fuel community, this could reduce significantly the number of SRMs that NIST would produce and maintain in the future.

Future Plans: A number of states now require, or will require in the near future, that diesel fuel be sold as a mixture of petroleum diesel and bio-diesel. There is a clear need for a new pure (B100) bio-diesel SRM certified for both inorganic and organic constituents.

There are currently no NIST crude oil SRMs certified for mercury content at levels measurable by the petroleum industry. To address the critical need for this, a crude oil candidate SRM, having a relatively high level of mercury, will be developed in the coming year.

Publications:

Kelly, WR, MacDonald, BS, and Leigh, SD (2006) ***"Determination of Sulfur in Fossil Fuels: User Prepared Standards with Concentrations and Uncertainties Traceable to NIST Values,"*** accepted for publication in Journal of ASTM International.

Mann, JL and Kelly, WR (2005) ***"Measurement of sulfur isotope composition (δ^{34}S) by multiple-collector thermal ionization mass spectrometry using a ^{33}S-^{36}S double spike,"*** Rapid Commun. Mass Spectrom.**19**, 3429-3441.

Martin, SA, Gallaher, MP, and O'Connor, AC (2000) **"Economic Impact of Standard Reference Materials for Sulfur in Fossil Fuels,"** 00-1 Planning Report, NIST.

Simultaneous Determination of Sulfur Isotopic Composition and Concentration in Two High-Elevation Snowpits Using a $^{33}S/^{36}S$ Double Spike Technique

The biogeochemical cycling of sulfur (S) and the formation of sulfate (SO_4^{2-}) aerosol particles in the atmosphere have important consequences for global climate that are not fully quantified. Sulfate aerosols interact "directly" with incoming solar and outgoing terrestrial radiation through scattering and absorption and "indirectly" function as cloud condensation nuclei (CCN) that influence the concentration and size of droplets as well as their surface reflectivity and the radiative properties of clouds. Snow and ice cores provide archives of atmospheric SO_4^{2-} aerosols in many remote regions of the Earth including polar, temperate, and tropical environments. NIST has improved a specialized high-accuracy mass spectrometric method to precisely measure the isotopic composition of sulfur simultaneously with its concentration. Sulfur isotope composition measurements of these aerosols provide information on the degree of influence anthropogenic contributions have on the natural sulfur cycle, and ultimately on global climate.

J.L. Mann and W.R. Kelly (Div. 839)

The variability of sulfur isotope ratios in nature, caused by mass-dependent fractionation during biogeochemical processing, provides a chemical means for tracing the various sources of sulfur aerosols and a useful tool for understanding the sulfur cycle. Snow and ice cores provide archives of the sources, sinks, and processing of sulfur that reflect changes in this cycle through time. These archives can be used to assess the current and historical changes in sulfur source contributions to remote regions of the Earth including polar, temperate, and tropical regions. Typical concentrations of sulfate in these regions are 25 ng/g to 100 ng/g (ppb); thus, 300 g to 4000 g of sample is required to obtain sufficient material (≈ 33 µg S) for traditional isotope ratio mass spectrometric (IRMS) analysis. Global atmospheric sulfur cycling is a dynamic process that varies on short timescales, and these large quantities of sample can mask seasonal changes in sulfur sources. Consequently, an analytical technique that allows for the reduction of the amount of sample needed for analysis is required to minimize masking and increase resolution.

In this study we have focused on the use of multiple-collector thermal ionization mass spectrometry (MC-TIMS) combined with a $^{33}S/^{36}S$ internal standard for simultaneous determination of sulfur isotope composition $\delta^{34}S$ and concentration of small (< 1 µg) sulfate (SO_4^{2-}) samples. The fundamental limitation to accurate and precise isotopic ratio measurements by thermal ionization is that the measured ratio differs from the true ratio in the source as a result of instrumental fractionation during vaporization of the sample from the filament. To address this changing ratio and to improve precision and accuracy in the $^{34}S/^{32}S$, a well-characterized $^{33}S/^{36}S$ internal standard was added to the samples and was used to calculate a fractionation factor (α) that corrects for this changing ratio (instrumental fractionation) to give the true ratio in the source. The $^{32}S/^{33}S$ ratio that has also been corrected for instrumental fractionation was used to calculate the sulfur concentration. This technique has a significant advantage over the IRMS technique that is limited to only isotope composition measurements.

Snowpit samples from the Inilchek Glacier in Kyrgyzstan and Summit, Greenland were analyzed. In the case of the Inilchek samples, because of the high measurement precision, low blank concentrations, and relatively precise determination of the blank composition, uncertainties of approximately ± 0.40‰ (2σ) on the individual $\delta^{34}S$ measurements were obtainable. The isotopic ratios and associated uncertainties are stated in parts-per-thousand (‰) difference from the Vienna Canyon Diablo Troilite (VCDT) isotope-ratio standard, as defined by the formula $\delta^{34}S\ (‰) = [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{VCDTstd} - 1] \times 1000$. These precisions were determined using less meltwater, averaging 205 mL, and on smaller sample sizes, 0.26 µmol to 1.82 µmol S, than are typically used for gas-source analysis and rival the precisions obtained by these techniques for larger sample sizes.

The $\delta^{34}S$ results for the Inilchek and Greenland samples using the new double-spike technique demonstrate it to be a superior method for the determination of sulfur isotope composition of snow samples containing nanogram-per-gram levels of sulfate. The new technique has reduced the sample size required for sulfur isotope analysis by as much as a factor of 10.

The $\delta^{34}S$ measurements for Greenland are the first high-resolution measurements obtained for this location. The amount of meltwater used for sulfur isotope analysis in this portion of the study averaged 272 mL, which is considerably less than the 1 L to 2.5 L used in previous research. The uncertainties determined were quite large, averaging ±

1.5‰ (2σ), due to the increased blank amount and the smaller sample size used for analysis in this portion of the study. If the blank amount is lowered and the uncertainty in the blank composition reduced, estimates of the precisions obtainable, averaging ± 0.75‰ (2σ), show that up to a factor of two to three improvement in the uncertainties on sample sizes ranging from 0.05 μmol to 0.29 μmol S is attainable with the double-spike technique. In this case the precisions obtained would be similar to those of gas-source techniques for snow samples but for much smaller sample sizes, 0.1 μmol S rather than 1 μmol S.

The elemental and sulfur isotope data obtained from the samples showed that the main sulfate contributors to the Inilchek Glacier in Kyrgyzstan were anthropogenic and evaporite dust, while at the Summit, Greenland site these were mostly anthropogenic and marine biogenic in origin. Overall, the results show that anthropogenic inputs tend to dominate, averaging almost 75%, for both sites. The anthropogenic contributor dominates throughout the year in the Inilchek region, while in Greenland the influence of anthropogenic sources wanes during the summer months when the marine biogenic contribution increases.

In addition to being able to measure smaller sample sizes, the double-spike MC-TIMS method offers two additional advantages over gas-source mass spectrometry. First, because it uses an internal standard rather than an external standard, it is intrinsically accurate because only isotope ratios need to be measured; therefore complete recovery of the sample is not required for unbiased results. Also, mass fractionation that may be caused by losses during drying and/or chemical reduction of the sample is accounted for by adding the spike prior to sample processing. This is a considerable advantage for small sample sizes (< 1 μmol S) where losses can result in potentially large biases without the use of an internal standard.

The second advantage is a better evaluation of both the blank amount and composition. It is important to note that these are the first $\delta^{34}S$ measurements that include a correction for blank, and demonstrate that blanks must be measured to assess the accuracy of the $\delta^{34}S$ data. In all previous studies there is no mention of blanks and therefore this potentially significant bias and uncertainty has been ignored.

Publication:

Mann, J. L., Kelly, W. R., Shuman, C. A., and Kreutz, J., "Measurement of Sulfur Isotope Composition in Two High-Elevation Snowpits by Multiple-Collector Thermal Ionization Mass Spectrometry (MC-TIMS) Using a 33S-36S Double Spike," Geochimica et Cosmochimica Acta

Impact and Future Plans: The significant reduction in sample size permitted by the double spike technique may provide the tool required to access the high-resolution temporal record contained in ice cores. Applying this technique to ice cores will extend the high-resolution temporal record back in time to establish a timeline of changes in the atmospheric sulfur cycle and allow for a more detailed reconstruction of the atmospheric sulfur cycle and the understanding of changes in sources, sinks, and processing through time. This becomes particularly important when examining periods in geologic history where abrupt changes (less than a decade) in climate occur. We currently are working with groups from the University of Saskatchewan and the University of Washington to apply this technique to ice samples that record dramatic changes in global climate.

Determination of Traces of Fissionable Materials using Delayed Neutron Activation Analysis

Detection and measurement of traces of fissionable uranium and plutonium can be performed by delayed neutron activation analysis (DNAA). The method is intrinsically specific to nuclear fission, the sensitivity is excellent, and the procedure is simple, rapid, and readily automated for high throughput. Tiny traces of fissionable uranium or plutonium can be left behind whenever these materials are handled or transported. Using neutrons from the NIST research reactor, the delayed neutrons from fission in these traces can be used to detect and quantify U and Pu in swipe samples with excellent speed, sensitivity, and specificity.

R.M. Lindstrom and R.G. Downing (Div. 839)

Delayed neutron activation analysis (DNAA) has been established at NIST for the measurement of small quantities of fissionable nuclides such as ^{235}U and ^{239}Pu. After a brief neutron irradiation, the sample is placed quickly into a neutron detector array and the neutron emission rate measured and compared with that of a standard. The method is well-tested, rapid, specific, matrix-independent, nondestructive, and sensitive. The NIST system can detect less than one nanogram of either of these species, in less than 3 minutes per sample.

The neutron detection consists of ten pressurized ^{3}He proportional counters in a 30 cm x 30 cm cylindrical moderator of polyethylene, lined with 2 cm of lead to absorb gamma radiation. The existing pneumatic rabbit assembly controls the irradiation. After removal from the reactor, the sample is blown rapidly to the neutron detector through a polyethylene flight tube. The neutron emission rate is measured as a function of time, and the fissionable content is determined by comparison with a standard of known uranium content. Tests have shown that the system's response to gamma radiation and interferences from fast-neutron reactions on oxygen and thorium are negligible. Automation of the sample transfer sequence, now under way, will make the analysis more reproducible and less labor intensive.

Impact: With the completion and verification of the DNAA system, NIST has a readily accessible, rapid means of measuring traces of fissionable U and Pu in samples of forensic interest. In addition, the specificity and sensitivity of this method of analysis will be put to use in certifying trace uranium in Standard Reference Materials.

Future plans: It has been demonstrated elsewhere that ^{233}U, ^{235}U, and ^{239}Pu can be distinguished by the relative yields of delayed neutron precursors with different half-lives, and also of several fission products. We plan to add a gamma-ray detector into the neutron moderator to exploit this signature

As shown in the figure, an important tool in nuclear forensics is the collection and analysis of "swipe" samples at sites where materials of interest may be or may have been at one time.

Traceable Calibrations Gases: SRMs, NTRMs, and EPA Protocol Gases

Two NIST programs provide calibration gas standards with NIST analyses and concentration value assignment: the gas mixture Standard Reference Material (SRM) program and the NIST Traceable Reference Material (NTRM) program. These two programs meet the traceability needs of the specialty gas industry. However the demand for traceable gases from end users is too great for NIST to meet, and NIST must rely on the specialty gas industry to extend traceability to these users through the U.S. Environmental Protection Agency (EPA) Protocal Gas Program. The EPA specifies the Protocol Gas program in regulations that apply to measurement of stack gas emissions; the Protocol Gas mixtures extend traceability to end users in the U.S.

W.D. Dorko (Div. 839)

The Gas Mixture SRM and NTRM Programs provide the specialty gas industry with gas standards with defined NIST traceability. These calibration gas standards are mixtures contained in high-pressure compressed-gas metal cylinders. In the SRM program, NIST initiates the production of mixtures after which NIST analyzes every SRM mixture against primary standards and assigns certified concentration values. Standards supplied through the SRM program have the lowest uncertainties, but are not always available and are resource-intensive for NIST to provide. In the NTRM program, specialty gas companies initiate production with NIST concurrence, after which the company analyzes 100% of the cylinders against an SRM; NIST selects 10% for analysis against NIST primary standards. Certified concentration values are assigned by combining the NIST data with the producer's data. The NTRM program provides standards that, by their nature, have greater uncertainties than the SRMs, and are used primarily by specialty gas companies to calibrate production lines of protocol gas mixtures. Both programs require that mixtures be made as a group of cylinders with identical content. These mixtures are specified in the Code of Federal Regulations (CFR) for use in calibration of instruments used to monitor regulated emissions. The NTRM program was developed to augment the SRM program because the SRM program could not provide all of the required standards in a timely manner. Recently the NTRM program was expanded to include the NTRM Prime (NTRM'), in which NIST analyzes every sample, to address the high-accuracy and precision needs of certain customers in the automotive industry.

The EPA Protocol Gas program was initiated in 1980 to meet an even greater demand for standards required by the EPA. In this program, the specialty gas companies follow an EPA protocol to blend and analyze individual mixtures calibrated against SRMs or NTRMs. Protocol mixtures are then sent to end users with no direct involvement of either EPA or NIST. To provide quality assurance (QA) for the Protocol Gases, NIST is now working with EPA on a verification program where Protocol Gases can be sampled and analyzed by NIST to determine compliance with producer certificates.

NIST-traceable calibration gas standards are stipulated in the CFR to be used for the calibration of instruments employed in the monitoring of regulated emissions.

NIST is striving to improve the traceability link so that end users can confidently rely on the specialty gas industry to provide their gas mixtures. At every stage of increased need for calibration gas standards, NIST has been flexible and responsive in development of programs to meet the needs of the EPA and the specialty gas industry.

NIST TRACEABLE GAS STANDARDS:

SRM: **NIST initiates production of the gas mixtures, analyzes each cylinder, and assigns certified concentration values.**
NTRM: **Specialty gas companies initiate mixture production and analyze 100% of the cylinders; NIST selects 10% for NIST analysis.**
NTRM': **Expanded from the NTRM program; NIST analyzes every sample to meet customer needs.**
EPA Protocol Gas: **Specialty gas companies follow an EPA protocol that specifies blending and analysis of protocol mixtures using SRMs or NTRMs for their analyses. NIST is currently working on a quality assurance procedure with EPA.**

Future Plans: To support the needs of the SRM, NTRM, and NTRM' programs, NIST plans to continue its efforts to increase the number and concentration ranges of gas species for which it maintains primary standards, the foundation of these programs**.** NIST will continue with the Protocol Gas verification program. Meetings with interested parties – NTRM producers, regulating bodies, and end users – will be held during FY07 to discuss the NIST programs and to evaluate the results of recent verification analyses.

Establishing Measurement Traceability for Gaseous Mercury Emissions Monitoring

On March 15, 2005 the U.S. Environmental Protection Agency (EPA) issued the Clean Air Mercury Rule (CAMR) that defined a cap on the amount of mercury that could be emitted from coal-fired power plants, and also provided for an emissions trading program for flexibility in attainment. To support this ruling, NIST was tasked by the EPA with providing traceability for measurements of the emitted gas-phase mercury (Hg). Mercury is a neurotoxin that accumulates in the food chain and is therefore a health concern. Concentrations of mercury in the air are of little direct health concern. However, when the mercury in the air re-deposits, it enters the food chain and bioaccumulates. Fish can have mercury levels several orders of magnitude greater than the level found in air.

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NIST was tasked by EPA with providing traceability for measurements of mercury emitted from coal-fired power plants. Initial efforts were focused on developing gas standards for elemental mercury. Two sources for providing standard gas mixtures containing elemental mercury were considered: gas cylinders containing mercury in air and devices that generate elemental mercury gas mixtures. In both cases, the mercury sources have to be calibrated to quantify their mercury output. At NIST, isotope dilution - cold vapor - inductively coupled plasma mass spectrometry (ID-CV-ICP-MS) is used for the analysis of mercury in the gas phase. ID-CV-ICP-MS has been used at NIST for many years for certifying mercury in Standard Reference Materials of both solid and liquid matrices. This technique is a high-accuracy analytical technique, and was modified and evaluated as a method to be used for measuring mercury in the gas phase. Another analytical technique used by NIST for mercury quantification is the gold amalgam trap-and-purge with subsequent detection using a cold vapor atomic absorption spectrometry (CVAAS) system. This instrument must be calibrated with a traceable mercury solution, such as an SRM.

NIST worked with a commercial specialty gas vendor to procure three sets of gas mixtures of elemental mercury (Hg^0) in nitrogen. The nominal concentrations of the Hg^0 in the cylinders are 2 µg/m^3, 5 µg/m^3, and 20 µg/m^3. NIST analyzed and delivered one of the sets of three cylinders to the EPA, with concentrations certified and traceable to the SI (International System of Units) by CVAAS using a gold amalgam trap and ID-CV-ICP-MS. (The cylinders are re-analyzed on a periodic basis to determine whether or not there is any degradation of the Hg^0 concentration.) While seeking traceable standards in the 2% relative uncertainty range, NIST has moved from CVAAS (uncertainties in the 6 % relative range) to ID-CV-ICP-MS (uncertainty about 1 % relative) as the primary reference method for mercury analysis.

The outputs of a single mercury generator from each of five different manufacturers were also quantified by both the ID-CV-ICP-MS and the CVAAS. The better reproducibility of the results of these experiments led to a decision to use generators rather than cylinders as the primary traceability mechanism.

The proposed protocol is for NIST to certify one generator from each of the manufacturers, and then have each manufacturer calibrate generators that they sell by using a continuous mercury analyzer that is calibrated with the NIST-certified generator. Experiments performed at NIST have demonstrated that these calibrations can be accomplished to within 1% precision.

Impact: EPA is using the certified cylinder mixtures in their program to audit mercury-monitoring sites to determine compliance with regulations. These cylinders have been characterized by NIST using ID-CV-ICP-MS and CVAAS to determine concentration and stability of the gas mixture. This project has also provided data on the performance of mercury generation devices, and the data have shown that they are suitable for providing traceability. A protocol is being written by EPA and NIST to establish a traceable linkage to NIST using mercury gas generators and cylinder gas mixtures.

Future Plans: The Traceability Protocol using mercury generators will be finalized in 2007. Work has begun on development of a method for studying oxidized mercury (Hg^{++}) to provide traceability for measurements being made in support of EPA's proposed regulations. Gas standards for Hg^{++} are produced from a generation system that differs from the Hg^0 system. Experiments are ongoing to establish a procedure to employ the ICP-MS to characterize Hg^{++} generation systems. Cylinders containing mercury in air are also being developed to meet the program requirements.

Upgrades for Cold Neutron Beam Facilities

A reconstruction of neutron guide NG7 in the NIST Center for Neutron Research will result in a much more spacious sample area for prompt gamma-ray activation analysis (PGAA). It will greatly reduce background levels of radiation and allow the analysis of bulky samples. The neutron depth profiling (NDP) instrument will also be moved to the same guide.

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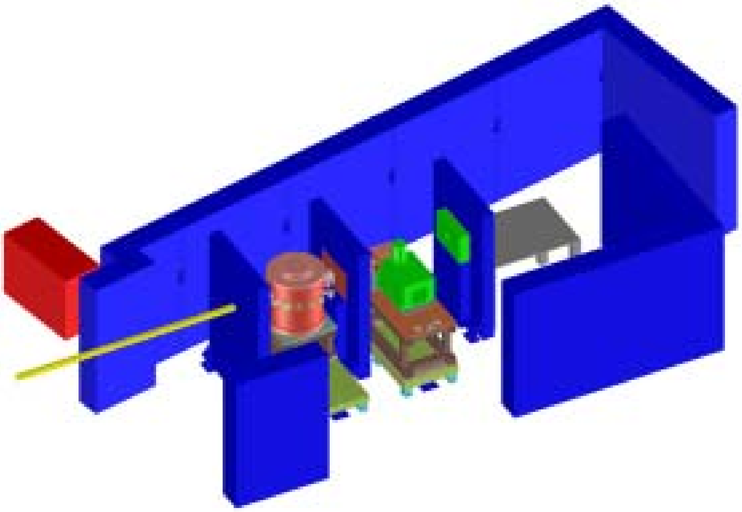
Both the PGAA and the NDP instruments at the NIST Center for Neutron Research, are being reconfigured to operate together on a new beam from a dedicated curved guide, NG7'. The bottom half of the existing NG7 guide will be compressed from 5 cm x 5 cm to 3 cm x 3 cm, bent 10° with ten 50-cm supermirror guide sections, and then made more uniform by passing through an additional 7.5 m of straight guide. According to Monte Carlo modeling, at the exit of the guide, the thermal neutron equivalent fluence rate will be 5 x 10^8 cm^{-2} s^{-1}. As the figure shows, kinematically mounted tripod instrument bases and shielding walls are designed for ready but precise reconfiguration as experimental requirements dictate. The PGAA sample position was previously 3 cm directly underneath the adjacent guide, so the sample size and shape were severely restricted and the background was undesirably high. The new position will give greatly improved access to the sample area, and consequent flexibility with regard to sample size and shape, detector geometry, and sample environment control. A large evacuable sample chamber will have space for positioning equipment, a beam chopper, and a variety of sample holders and containers. A cryostat will replace the normal chamber when sample temperature is important. The sample-detector distance will be adjustable, and space is available for a second Ge detector for gamma-gamma coincidence measurements. The NDP instrument is relocated from guide NG0. In the new position, the beam will contain less gamma radiation, and thus the background will be improved. A multidetector array of charged particle detectors is being constructed for the NDP instrument and will improve the determination of He, N, and Cl profiles.

The new configurations will provide greater sensitivities, better detection limits, and much lower background radiation. Analyses of SRMs and other materials will greatly benefit from these improvements.

Future Plans: The new configuration is scheduled to be installed in mid-2007, and will be followed by a complete facility characterization.

In this illustration, the neutron beam enters from the left, passes through the NDP chamber, and then the PGAA system. On the right side is an optical bench, for installation of ad-hoc experiments requiring a freely accessible neutron beam.



Development of Serum-Based SRMs to Assess Nutritional Status

NIST is working with the National Institutes of Health Office of Dietary Supplements (NIH/ODS) and the Centers for Disease Control and Prevention (CDC) to develop a series of serum-based Standard Reference Materials. The goal of these Standard Reference Materials is to reduce the interlaboratory variability of clinical vitamin measurements and to enhance the accuracy of nutritional status data in the National Health and Nutrition Examination Survey (NHANES).

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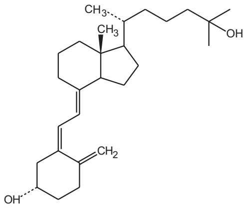
A variety of nutrients are essential for normal functioning of the human body, and deficiencies can lead to serious illnesses. In addition, a number of studies have suggested that certain nutrients may provide some benefit in reducing the risk of cancer and other diseases. Accurate measurement of vitamin levels is therefore essential for identifying individuals affected by nutritional deficiencies and for evaluating the potential health benefits associated with dietary supplementation.

Previous interlaboratory comparisons have indicated that discrepancies can exist between different assays used to assess nutritional status and that there is a significant need for reference materials to reduce this variability. NIST is developing a series of serum-based Standard Reference Materials (SRMs) that can be used as control materials by laboratories measuring vitamins (e.g., folate, D, B_6, B_{12}) in human serum. NIST is also developing higher-order analytical methods (e.g., isotope dilution liquid chromatography with mass spectrometric detection (ID LC/MS)) for use in characterizing these SRMs. Values in these serum-matrix materials will be assigned using a combination of NIST data, including data from higher-order methods, and data provided by collaborating laboratories.

NHANES surveys are designed to assess the health and nutritional status of Americans and have been conducted since 1971. Interviews, physical examinations, diagnostic procedures, and lab tests are conducted on approximately 5000 people each year to provide information on diseases, conditions, health indicators, and risk factors.

SRM 1955 Homocysteine and Folate in Human Serum is currently available and consists of three sera with different analyte concentrations. Homocysteine is a risk factor for heart disease, and folate counteracts effects of homocysteine. Folic acid has also been shown to reduce the risk of neural tube defects in fetuses. Both NIST and CDC provided certification measurements for this SRM, which was issued in 2005.



SRM 972 Vitamin D in Human Serum is currently in development and will consist of four different sera. Vitamin D deficiency is associated with muscle weakness and osteoporosis that can contribute to an increased risk of falls and fractures. Vitamin D status is generally monitored by measuring levels of 25-hydroxyvitamin D (25-OH-D). Because vitamin D has two forms, D_2 and D_3, accurate assessment of vitamin D status should include measurement of both hydroxylated forms (25-OH-D_2 and 25-OH-D_3 [shown]). The levels of both analytes, as well as that of a related compound 3-epi-25-OH-D, will be characterized in the SRM.

SRM 3950 Vitamin B_6 in Human Serum is another vitamin-related SRM that is currently in development and will consist of two sera with different analyte concentrations. Vitamin B_6 is converted to pyridoxal 5′-phosphate (PLP), the analyte of clinical interest when assessing vitamin B_6 dietary status. Although PLP deficiency is believed to be rare, tests for PLP deficiency are not widely performed because of a lack of accepted analytical methodology and because quality control materials are not readily available. In addition, questions remain about the level of PLP that should be anticipated in healthy individuals. There is some evidence that low levels of PLP may be associated with an increased risk for cardiovascular and other diseases.

SRM 3951 Vitamin B_{12} in Human Serum is the final serum-based SRM currently being developed and will consist of three sera with different analyte concentrations. Vitamin B_{12} (cobalamin compounds) is necessary for the formation of healthy red blood cells and in maintenance of the central nervous system. It has been estimated that 10% to 15% of adults over the age of sixty are affected by vitamin B_{12} deficiency, but most of these individuals lack any overt symptoms of deficiency. Previous comparisons of vitamin B_{12} diagnostic kits have shown that different assay techniques often do not provide comparable results.

The serum-based SRMs with values assigned for vitamins are necessary to ensure that vitamin measurements are reliable and that patients are provided with appropriate treatments when necessary.

Development of Multivitamin/Multielement Dietary Supplement SRM

NIST is working with the National Institutes of Health Office of Dietary Supplements (NIH/ODS) and the U.S. Department of Agriculture (USDA) in support of development of an analytically substantiated dietary supplement ingredient database (DSID). The initial DSID focus is characterization of vitamin and mineral supplements. As part of this effort, USDA will contract with analytical laboratories for analyses of dietary supplements available in the marketplace. NIST is producing a Standard Reference Material to be used as an analytical control in this study.

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NIST is producing Standard Reference Material 3280 Multivitamin/Multielement Tablets for use as a control by laboratories making vitamin or element measurements in similar types of dietary supplements. In cases where higher-order analytical methods were not already available at NIST, such methods (e.g., isotope dilution liquid chromatography with mass spectrometric detection (ID LC/MS)) have been developed for use in characterizing this SRM.

Characterization of SRM 3280 Multivitamin/Multielement Tablets is nearing completion. Certified and reference values will be assigned for the 18 elements and 15 vitamins/carotenoids listed on a typical multivitamin/multielement dietary supplement label. Elements to be determined include: boron, calcium, chloride, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorous, potassium, selenium, silicon, tin, vanadium, and zinc. The vitamins and carotenoids to be measured include: vitamin A, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E, vitamin K, vitamin $B_{1,}$ vitamin B_2, niacin, folic acid, biotin, pantothenic acid, ∃-carotene, and lutein.

A combination of results from two independent methods employed at NIST, including higher-order methods, as well as results from USDA and European laboratories expert in the measurement of vitamins will be used for characterization of this material. The elements were determined at NIST using a combination of several methods including x-ray fluorescence spectrometry (XRF), instrumental neutron activation analysis (INAA), prompt gamma activation analysis (PGAA), and inductively coupled plasma-mass spectrometry (ICP-MS) to provide measurements by at least two methods for each element. XRF was used to assess the homogeneity of the SRM for most of the elements.

The vitamins and carotenoids are being measured at NIST using two independent analytical methods (where feasible) based on liquid chromatography (LC) with UV/visible absorbance or fluorescence detection, LC with mass spectrometric detection (LC/MS), and/or LC with tandem mass spectrometry (LC/MS/MS). The SRM's homogeneity with respect to the vitamins will be assessed using these methods.

For both the vitamin/carotenoid and element determinations, samples of the SRM were distributed to other laboratories (e.g., USDA, Food Products Association, European Committee for Standardization (CEN)) to provide additional results to assist in the value assignment of the contents of the SRM.

USDA, NIH, and CDC will use the multi-vitamin SRM as a control material in support of on-going measurements of vitamins and elements in dietary supplements to be included in the DSID. Dietary supplement manufacturers will also use the SRM as a control material for quality assurance of measurements so that label claims for their own products are accurate; other analytical laboratories will use the material for quality assurance and method validation.

As database and product label information become more accurate, researchers will be better able to accurately estimate vitamin and element intakes from dietary supplements.