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Agency

Targeted National Sewage Sludge Survey

Sampling and Analysis Technical Report

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Acknowledgments and Disclaimer

The Targeted National Sewage Sludge Survey was made possible by the assistance and cooperation of numerous staff working at each of the sewage treatment facilities involved. The staffs of the facilities contacted during the course of this survey were, without exception, knowledgeable, friendly, helpful, and deservedly proud of their efforts to protect the environment and serve their local constituencies.

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Executive Summary

This Sampling and Analysis Technical Report ("Technical Report") describes the sampling and analysis activities conducted by EPA in support of the Targeted National Sewage Sludge Survey (TNSSS). The TNSSS was designed to: 1) obtain updated occurrence information on nine analytes of potential concern, and 2) obtain occurrence information on a number of contaminants of emerging interest identified by EPA and the National Research Council (NRC). The objective of the survey was to obtain national estimates of the concentrations of these pollutants in sewage sludge for use in assessing if exposures may be occurring and whether those levels may be of concern.

Final sewage sludge is defined as the liquid, solid, or semi-solid residue generated during the treatment of domestic sewage, receiving secondary treatment or better, in a treatment works, which may include sewage sludge processed to meet land application standards. The publicly owned treatment works (POTWs) included in the survey were selected without consideration of their sewage sludge use or disposal practices.

For this survey, EPA focused its efforts on POTWs that treat more than one million gallons of wastewater per day (MGD). This group of facilities collectively generates approximately 94 percent of the wastewater flow in the nation. To be eligible for the survey, EPA also required that a POTW be located in the contiguous United States and employ secondary treatment or better. From the 3,337 POTWs that met the criteria, EPA statistically selected 74 facilities in 35 states for the survey and collected biosolids samples from those facilities. Whether the facility recycles the sewage sludge to land or disposes of it via incineration or surface disposal was not a consideration for selecting a facility for inclusion in the survey. By using statistical methods, the concentration measurements can be extrapolated to the entire population of 3,337 POTWs.

EPA collected samples between August 2006 and March 2007. EPA collected 84 samples of sewage sludge from 74 facilities, one from each of 64 POTWs, as well as two samples at the remaining ten facilities (either because the facility had more than one treatment system and produced two types of final sewage sludge, or for quality assurance purposes. EPA conducted analysis of sewage sludge samples for 145 analytes, including four anions (nitrite/nitrate, fluoride, water-extractable phosphorus), 28 metals, four polycyclic aromatic hydrocarbons, two semi-volatiles, 11 flame retardants, 72 pharmaceuticals, and 25 steroids and hormones.

The survey used both well-established multi-laboratory validated EPA procedures as well as three analytical methods that were developed or updated for the survey. The two new methods are single-lab validated methods for pharmaceuticals (EPA Method 1694), and steroids and hormones (EPA Method 1698). The updated multi-lab validated method is for flame retardants (EPA Method 1614).

EPA took steps to ensure that the results were comparable across all of the facilities sampled. The percent solids in the various sewage sludge samples range from 0.14 to 94.9. To ensure comparability of results, all sample results are reported on a dry-weight basis.

EPA subjected all of the analytical results generated by the laboratories to data review procedures. These procedures used review protocols to ensure that the results met EPA's objectives for data quality.

This Technical Report includes the number of samples in which each analyte was reported, along with minimum and maximum measurements. Reported concentrations and frequency of detects are limited by the sensitivity of the analytical methods used. Some analytes were found in all 84 samples, while others were found in none or only a few of the sewage sludge samples. The minimum concentration is the lowest value reported as present in any sample. EPA did not report a minimum or maximum value for those analytes that were not detected (i.e., a situation that occurred for some of the pharmaceuticals, steroids and hormones). For these situations, EPA used "ND" to indicate that the minimum and maximum values were "not detected." The maximum concentration is the highest value reported as present in any sample.

Briefly, the survey found:

- The four anions were found in every sample.
- 27 metals were found in virtually every sample, with one metal (antimony) found in no less than 72 samples.
- Of the six semivolatile organics and polycyclic aromatic hydrocarbons, four were found in at least 72 samples, one was found in 63 samples, and one was found in 39 samples.
- Of the 72 pharmaceuticals, three (i.e., cyprofloxacin, diphenhydramine, and triclocarban) were found in all 84 samples and nine were found in at least 80 of the samples. However, 15 pharmaceuticals were not found in any sample and 29 were found in fewer than three samples.
- Of the 25 steroids and hormones, three steroids (i.e., campesterol, cholestanol, and coprostanol) were found in all 84 samples and six steroids were found in at least 80 of the samples. One hormone (i.e., 17α-ethynyl estradiol) was not found in any sample and five hormones were found in fewer than six samples.
- All of the flame retardants except one (BDE-138) were found every sample or all but one sample.

It is not appropriate to speculate on the significance of the results until a proper evaluation has been completed and reviewed. EPA plans to evaluate the pollutants identified by the survey as being present in sewage sludge. As its first priority, using the survey information, EPA has begun assessing the nine pollutants identified from the 2003 biennial review as needing updated concentration information and molybdenum to determine whether additional action may be necessary. Later this year, EPA expects to initiate evaluations of other pollutants in the survey that may warrant further consideration. The evaluations will depend on the availability of data needed to conduct the evaluations.

Section 1
Background and Organization

1.1 Regulatory and Surveys History

Sewage sludge is the solid, semisolid, or liquid organic material that results from the treatment of domestic wastewater by municipal wastewater treatment plants, also known as publicly owned treatment works (POTWs). The U.S. Environmental Protection Agency (EPA) uses the terms sewage sludge and biosolids interchangeably, but others often refer to biosolids as sewage sludge that has had additional processing for land application.

Section 405(d) of the Clean Water Act (CWA) requires that the EPA establish requirements for the use or disposal of sewage sludge. The Standards for the Use or Disposal of Sewage Sludge are found at Part 503 of Section 40 of the Code of Federal Regulations (40 CFR 503, hereafter simply "Part 503").

These regulations establish numeric limits, management practices, and operational standards to protect public health and the environment. Sewage sludge is typically used by land applying to fertilize crops or reclaim mined lands, or disposed either by landfilling or surface disposing, or by incinerating. States may adopt additional or more stringent regulations for the use or disposal of biosolids.

Additionally, Section 405(d) of the CWA requires EPA to review existing sewage sludge regulations at least every two years (i.e., biennial review). The purpose of such reviews is to identify additional toxic pollutants, and promulgate regulations, if needed, for those pollutants consistent with the requirements set forth in the CWA.

1.2 Targeted National Sewage Sludge Survey

The Agency periodically conducts surveys to determine what may be present in sewage sludge. EPA has conducted three previous sewage sludge surveys: 1) a 40-city survey in 1982 to develop information on the fate and effects of priority pollutants in wastewater treatment plants and estimates of pollutant concentrations in sewage sludge; 2) a National Sewage Sludge Survey in 1988-1989 to gather information on sewage sludge use or disposal practices and to obtain updated information on the concentration of over 400 pollutants in the Nation's sewage sludge; and 3) a National Sewage Sludge Survey in 2001 to obtain updated national estimates of dioxins and dioxin-like compounds in sewage sludge managed by land application.

In conducting the 2003 biennial review (68 *FR* 75531), EPA identified 15 analytes that needed further evaluation. EPA subsequently reduced the list of analytes to nine based on a biosolids exposure and hazard assessment. EPA also determined it needed updated concentration data for more refined risk assessment and risk characterization of these nine pollutants (see Table 1).

Table 1. 2003 Biennial Review Analytes Requiring Additional Information

Analyte	Type
Nitrate	Anion
Nitrite	Anion
Barium	Metal
Beryllium	Metal
Manganese	Metal
Silver	Metal
Fluoranthene	PAH
Pyrene	PAH
4-Chloroaniline	Semivolatile organic

Inclusion of pollutants in the TNSSS does not reflect a determination that their presence in sewage sludge adversely affects human health or the environment. Rather, EPA decided that updated or new concentration data were needed to assess exposure and help in evaluating whether levels of these pollutants in sewage sludge present environmental or human health concerns.

Given the national scope of the survey, EPA expanded the list of analytes to reflect the Agency's interest in collecting concentration data for other pollutants. The expanded list included 24 additional metals that could be analyzed at little extra cost at the same time as the four metals (barium, beryllium, manganese, and silver) included in the list of nine pollutants above; molybdenum because of the Agency's interest in determining the need for a revised numeric standard for it in land-applied biosolids; and other analytes because of their widespread incidence and use, as well as emerging concern. The latter category included:

- benzo(*a*)pyrene (found in coal tar, automobile exhaust fumes, tobacco and wood smoke, charbroiled food, and burnt toast);
- 2-methylnaphthalene (found in nonstructural caulking compounds and sealants, synthetic resins, rubber adhesives, and wall coverings);
- bis (2-ethylhexyl) phthalate (widely used as a plasticizer in manufacturing of items such as cosmetics, toys, tools, and laboratory equipment);
- fluoride (used in topical and systemic therapy for preventing tooth decay, as well as many other uses);
- water-extractable phosphorus (correlated with phosphorus concentration in runoff from soils amended with manure and biosolids and an effective indicator of loss that may contribute to algae buildup in surface waters);
- 11 polybrominated diphenyl ethers (PBDEs). Four of the PBDEs were of most interest because of available human health information that may be useful for future risk evaluation efforts. PBDEs are used as flame retardants in a wide array of products, including building materials, electronics, furnishings, motor vehicles, plastics, polyurethane foams, and textiles; and
- 97 pharmaceuticals, steroids, and hormones because of broader emerging interest in these analytes.

EPA began sampling in August 2006, using the procedures described in Section 3, and completed sampling in March 2007. Analyses of survey samples were conducted as described in Section 4.

1.3 Content of this Report

This report describes the design, sampling, and analysis activities for the TNSSS. The report addresses the following topics:

- Survey Objective and Design
- Sample Collection
- Sample Analyses
- Data Review Procedures
- Survey Results
- References
- Appendices

Section 2
Survey Objective and Design

2.1 Survey Objective

The TNSSS was designed to: 1) obtain updated occurrence information on nine analytes of potential concern, and 2) obtain occurrence information on a number of contaminants of emerging interest identified by EPA and the National Research Council (NRC) that may be present in sewage sludge generated by POTWs.

2.2 Target Population

For this survey, EPA focused its efforts on POTWs that treat more than one million gallons of wastewater per day (MGD). This group of facilities collectively generates approximately 94 percent of the wastewater flow in the nation. To be eligible for the survey, EPA also required that a POTW be located in the contiguous United States and employ secondary treatment or better. EPA selected POTWs meeting the criteria from information in the 2004 Clean Water Needs Survey and the 2002 version of the Permit Compliance System. From the 3,337 POTWs that met the criteria in either data source, EPA used a stratified random sampling design to statistically select 74 facilities in 35 states for the survey and collected biosolids samples from those facilities. Whether the facility land applies the sewage sludge or disposes of it via incineration or surface disposal was not a consideration for selecting a facility for inclusion in the survey. By using statistical methods, the concentration measurements can be extrapolated to the entire population of 3,337 POTWs.

2.3 Stratification

EPA selected POTWs for inclusion in the survey using a random sampling design stratified for flow. EPA divided the 3,337 facilities in the sample population into three categories, based on their design flow:

- Flow rate of 1 to 10 MGD;
- Flow rate of 10 to 100 MGD; and
- Flow rate of greater than 100 MGD

EPA then selected a proportionate number of POTWs from each of the above stratum at random.

POTWs with flow rates less than 1 MGD were not included in the survey. However, the combined flows of all such facilities represent less than 6% of the total flow of all POTWs nationwide.

2.4 Facility Selections

EPA invited 80 POTWs to voluntarily participate in the survey. The initial written invitation was followed by a telephone call. These communications outlined the nature of the survey, the analytes of interest, and the timeframe for completion. EPA also assured each facility that samples sent to the laboratories for analysis would be submitted as "blind" samples, such that the results from any given sample could not be associated with a particular facility.

These communications identified facilities that did not meet the criteria for POTW selection in the peer-reviewed survey design and outlined above. Some POTWs provided only partial treatment of their wastewater, while others employed wastewater lagoons which do not typically produce sewage sludge, as defined, on a routine basis. Ultimately, EPA eliminated eleven POTWs and found five replacement POTWs. As a result, EPA selected 74 POTWs that met the stated criteria. The rationale for each replacement POTW is included in Table 2.

Table 2. POTWs Selected for Sampling

Facility Name and Flow Group	Flow Stratum	City	ST	Status
Sugar Creek WWTP	1 <MGD <10	Alexander City	AL	Replacement for Coley Creek; Sugar Creek is on the same system and completes the processing of Coley Creek's partially-treated sewage sludge
Aldridge Creek WWTP	1 <MGD <10	Huntsville	AL	Original selection
Phoenix WWTP	10 <MGD <100	Phoenix	AZ	Original selection
Valley Sanitary District STP	1 <MGD <10	Indio	CA	Original selection
San Francisco	> 100 MGD	San Francisco	CA	Original selection
El Estero WWTP	1 <MGD <10	Santa Barbara	CA	Original selection
Santa Rosa	1 <MGD <10	Santa Rosa	CA	Original selection
Stockton Water Quality Plant	> 100 MGD	Stockton	CA	Original selection
Los Angeles County Sanitation District	10 <MGD <100	Whittier	CA	Original selection
Boulder WWTP	1 <MGD <10	Boulder	CO	Original selection
South Windsor	1 <MGD <10	South Windsor	CT	Original selection
Three Oaks WWTF	1 <MGD <10	Estero	FL	Original selection
Orange County Northwest WRF	1 <MGD <10	Orlando	FL	Original selection
Tampa	1 <MGD <10	Tampa	FL	Original selection
Albany	10 <MGD <100	Albany	GA	Original selection
Americus-Mill Creek	1 <MGD <10	Americus	GA	Original selection
Boone STP	1 <MGD <10	Boone	IA	Original selection
Calumet Water Reclamation Plant	> 100 MGD	Chicago	IL	Replacement for MWRDGC North Side WWTP; Calumet is on the same system and completes the processing of North Side's partially-treated sewage sludge
Plainfield WWTP	1 <MGD <10	Plainfield	IL	Original selection
Lake County DPW, New Century STP	1 <MGD <10	Vernon Hills	IL	Original selection
Dupage County-Knollwood STP	1 <MGD <10	Wheaton	IL	Original selection
Blucher Poole WWTP	1 <MGD <10	Bloomington	IN	Original selection
William Ross Edwin WWTP	10 <MGD <100	Richmond	IN	Original selection
Parsons	1 <MGD <10	Parsons	KS	Original selection
Topeka	10 <MGD <100	Topeka	KS	Original selection
Mayfield WWTP	1 <MGD <10	Mayfield	KY	Original selection
Eunice	1 <MGD <10	Eunice	LA	Original selection
Jefferson Parish East Bank WWTP	1 <MGD <10	Marrero	LA	Original selection
Nantucket	1 <MGD <10	Nantucket	MA	Original selection
Salisbury	1 <MGD <10	Salisbury	MD	Original selection
Mechanic Falls Treatment Plant	1 <MGD <10	Mechanic Falls	ME	Original selection
Benton Harbor-St. Joseph WWTP	1 <MGD <10	St. Joseph	MI	Original selection
Wixom WTP	1 <MGD <10	Wixom	MI	Original selection
Festus Crystal City STP	1 <MGD <10	Crystal City	MO	Original selection
Elizabeth City WWTP	1 <MGD <10	Elizabeth City	NC	Original selection
Hillsborough WWTP	1 <MGD <10	Hillsborough	NC	Original selection

Table 2. POTWs Selected for Sampling

Facility Name and Flow Group	Flow Stratum	City	ST	Status
Beatrice	1 <MGD <10	Beatrice	NE	Original selection
Wildwood Lower WTF	10 <MGD <100	Cape May Court House	NJ	Original selection
Middlesex County Utility Authority WRC	> 100 MGD	Sayreville	NJ	Original selection
Verona TWP DPW	1 <MGD <10	Verona	NJ	Original selection
Buffalo	> 100 MGD	Buffalo	NY	Original selection
Canajoharie WWTP	1 <MGD <10	Canajoharie	NY	Original selection
Geneva A-C Marsh Creek STP	1 <MGD <10	Geneva	NY	Original selection
NYC DEP - Jamaica WPCP	10 <MGD <100	New York City	NY	Original selection
North Tonawanda STP	1 <MGD <10	North Tonawanda	NY	Original selection
Clermont County Commissioners	1 <MGD <10	Batavia	OH	Original selection
Bedford	1 <MGD <10	Bedford	OH	Original selection
Metropolitan Sewer District Little Miami WWTP	10 <MGD <100	Cincinnati	OH	Original selection
Northeast Ohio Regional Sewerage District Southerly WWTP	> 100 MGD	Cleveland	OH	Replacement for Easterly; Southerly is on the same system and completes the processing of Easterly's partially-treated sewage sludge
Delaware County Alum Creek WWTP	1 <MGD <10	Delaware	OH	Original selection
Mingo Junction STP	1 <MGD <10	Mingo Junction	OH	Original selection
Duncan Public Utilities Authority	1 <MGD <10	Duncan	OK	Original selection
City of Klamath Falls WWTF	1 <MGD <10	Klamath Falls	OR	Replacement for South Suburban
Western Westmoreland Municipal Authority	1 <MGD <10	Irwin	PA	Original selection
Allegheny County Sanitary Authority	1 <MGD <10	Pittsburgh	PA	Original selection
Greater Pottsville Area Sewer Authority	1 <MGD <10	Pottsville	PA	Original selection
Punxsutawney	1 <MGD <10	Punxsutawney	PA	Original selection
South Kingstown WWTF	1 <MGD <10	Narragansett	RI	Original selection
Plum Island WWTP	10 <MGD <100	Charleston	SC	Original selection
Lawson Fork WTP	1 <MGD <10	Spartanburg	SC	Original selection
Elizabethton	1 <MGD <10	Elizabethton	TN	Original selection
Amarillo	10 <MGD <100	Amarillo	TX	Original selection
Dallas Southside WWTP	> 100 MGD	Dallas	TX	Replacement for Dallas Central; Southside is on the same system and completes the processing of Dallas Central partially-treated sewage sludge
Trinity River Authority of Texas	1 <MGD <10	Ellis County	TX	Original selection
Fredericksburg	1 <MGD <10	Fredericksburg	TX	Original selection
Odo J. Riedel Regional WWTP	1 <MGD <10	Schertz	TX	Original selection
Wagner Creek WWTP	1 <MGD <10	Texarkana	TX	Original selection
Tyler Southside WTP	1 <MGD <10	Tyler	TX	Original selection
Spanish Fork City Corporation	1 <MGD <10	Spanish Fork	UT	Original selection
Buena Vista	1 <MGD <10	Buena Vista	VA	Original selection
Everett City SVC Center MVD	10 <MGD <100	Everett	WA	Original selection; lagoon was sampled during dredging operations
Beaver Dam	1 <MGD <10	Beaver Dam	WI	Original selection
Elkins WWTP	1 <MGD <10	Elkins	WV	Original selection
Huntington	10 <MGD <100	Huntington	WV	Original selection
Facilities originally selected but not sampled				
Coley Creek WWTP		Alexander City	AL	Partial treatment – replaced by Sugar Creek
City of Peoria, Beardsley WWTF		Peoria	AZ	Partial treatment – dropped
Osceola		Osceola	AR	Lagoon – dropped
Red Bluff WWTP		Red Bluff	CA	Lagoon – dropped
MWRDGC North Side WRP		Chicago	IL	Partial treatment – replaced by Calumet
Water Valley		Water Valley	MS	Lagoon – dropped
Northeast Ohio Regional S D Easterly WWTP		Cleveland	OH	Partial treatment – replaced by Southerly
South Suburban Sanitary District		Klamath Falls	OR	Lagoon – replaced by City of Klamath Falls
Saluda		Hampton	SC	Lagoon - dropped
Dallas Central WWTP		Dallas	TX	Partial treatment – replaced by Southside
Moses Lake WWTP		Moses Lake	WA	Lagoon - dropped

Section 3
Sample Collection

EPA collected samples of the final treated sewage sludge at each of the 74 POTWs that ultimately participated in the TNSSS. EPA developed a sampling and analysis plan that was peer-reviewed and describes the sample collection procedures in detail. EPA revised the plan periodically during the survey to address the changing list of facilities and to add updated contact information for the laboratories that performed the analyses. As noted in Section 1.2, EPA sampled between August 2006 and March 2007.

3.1 Training

Prior to the start of sampling, the biosolids samplers were trained by the contractor. The contractor, with assistance from the Alexandria Sanitation Authority, Alexandria, VA, provided the samplers with instructions on sampling techniques, sample point selection, required paperwork, sample packing, and shipping techniques. The samplers also toured the Alexandria Sanitation Authority to become familiar with typical sewage treatment processes. The tour included demonstrations and hands-on training in the collection of sewage sludge. Demonstrations included how to collect a range of samples, from liquid to dewatered sewage sludge.

3.2 Sample Collection

EPA began the sample collection process by identifying the number and nature of the types of sewage sludge produced at each facility. This effort took place during telephone conversations with the plant staff well in advance of sampling. Details were confirmed with plant staff upon gaining access to the final treated sewage sludge. Access to the treated sewage sludge was generally not difficult. However, in several instances, the samplers worked with plant staff to obtain samples from difficult locations where there might be safety concerns. Two facilities required that their personnel collect the actual samples. These instances are described in Section 3.3.

Grab samples were collected using sampling equipment appropriate to the type of sewage sludge (liquid or solid) and the analytes of interest. To avoid or minimize contamination from sampling equipment, plastic equipment was used to collect samples for analyses of metals and anions, and stainless steel equipment was used to collect samples for analyses of all the organics.

Liquid samples were collected as free-flowing materials from storage tanks, transfer lines, taps, and hoses. After purging any lines used to collect samples, liquid samples were collected directly into the final sample containers shown in Table 3. Where possible, plant staff turned on mixing equipment in any storage tanks prior to sampling so that the collected liquids were representative of the bulk sewage sludge.

Solid samples included dewatered sewage sludge. These samples were collected from a belt press, filter press, drying bed, centrifuge, compost pile, or other source on site. The sampler collected small grab samples from multiple areas of any large piles, or multiple grabs from any continuous processes (e.g., belt press). Small grabs were composited in a large pre-cleaned

container of appropriate construction, mixed well, and the mixed sample was transferred into the final sample containers (see Table 3). Several kilograms of material were collected for each type of treated sewage sludge and mixed. Any mixed material that remained after all the sample containers were filled was returned to the sewage sludge process for disposal.

Grabs of solid samples for anions and metals analyses were collected with a large pre-cleaned plastic serving spoon and mixed in a pre-cleaned plastic wastebasket. Grab solid samples for organics analyses were collected using a pre-cleaned stainless steel scoop and mixed in a pre-cleaned stainless steel bowl (8 to 12 quarts). Separate sampling equipment was used for each facility and all equipment was cleaned prior to shipment to the facility.

Sample containers were purchased from commercial suppliers who provided certificates of analysis for common contaminants of interest (e.g., metals, semivolatile organics, pesticides, PCBs). The cleaning procedures applied by the vendors were presumed to be sufficient for the other analytes in the survey for which routine testing by the vendor was not performed. At least two containers of each type were used to prepare equipment blanks as an overall check on possible contamination (see the discussion of equipment blanks later in Section 3.11). Both the high density polyethylene (HDPE) and glass containers were wide-mouth designs, sealed with screw caps containing a polytetrafluorothylene (PTFE) lid liner.

Table 3. Sample Containers for Solid and Liquid Sewage Sludge, by Analysis Fraction

Analysis Fraction	Solid Sample Container	Liquid Sample Container
Metals	500-mL wide-mouth HDPE	500-mL wide-mouth HDPE
Polycyclic Aromatic Hydrocarbons (PAHs) and Semivolatiles (as one analytical fraction)	500-mL wide-mouth glass	1000-mL wide-mouth glass
Inorganic Anions	500-mL wide-mouth HDPE	500-mL wide-mouth HDPE
Polybrominated Diphenyl Ether Congeners	500-mL wide-mouth glass	1000-mL wide-mouth glass
Antibiotics and Drugs	500-mL wide-mouth glass	1000-mL wide-mouth glass
Steroids and Hormones	500-mL wide-mouth glass	1000-mL wide-mouth glass
Archive Samples - for use in the event of breakage, lab accident, or for future EPA studies	2 500-mL wide-mouth HDPE and 4 500-mL wide-mouth glass	2 500-mL wide-mouth HDPE and 4 1000-mL wide-mouth glass
Total Containers per Sampling Point	12	12

3.3 Site-specific Deviations

Site-specific conditions at two facilities required modifications to the equipment protocols. At one facility, the sewage sludge was discharged from a two-story tower with a belt press directly into a dump truck parked below. Access to the bed of the truck was not practical, even if the discharge was stopped temporarily. Therefore, the staff at this facility routinely collected samples in a polyethylene container mounted on the end of a length of PVC pipe. In the presence of the EPA-contractor sampler, the facility staff inserted the device into the discharge from an opening in the tower, collected a small grab sample, and pulled the device back through the opening. Successive grab samples were collected through that opening and quickly placed into either the stainless steel or plastic compositing containers held by the EPA-contactor sampler. The sampler carried the containers down from the tower, mixed the samples in the compositing containers as described above, and then placed the samples into the appropriate final containers. The total contact time of each grab sample with the polyethylene sampling device was on the order of 30 seconds.

At a second facility, sewage sludge was discharged into a roll-off dumpster body in a narrow building which allowed limited access. In the presence of the EPA sampler, one of the facility staff collected the sample in a 5-gallon plastic bucket lined with a trash bag. A stainless steel scoop was used to remove aliquots of sewage sludge from the center of the bucket and transfer the material to a stainless steel bowl. Once that portion was removed, a plastic spoon was used to transfer the sewage sludge to a plastic bowl.

3.4 Representative Samples

The TNSSS was designed to collect sewage sludge samples that were representative of various types of sewage sludge. For bulk sewage sludge, collecting representative samples presented a challenge at some facilities. For example, at one facility that composted its final sewage sludge, samples were collected from one of the long piles of sewage sludge mixed with woods chips. The piles were upwards of 50 feet long and over 6 feet high, with sides sloping up at roughly a 45 degree angle. Samples were collected from the oldest sections of the rows at the facility to represent the length of the typical composting period at the facility, which ranges from one to six months, depending on the season.

Samples of biosolids materials were taken by digging into the side of the compost pile at roughly six points along its length, on both sides of the pile, a foot or more off the ground to avoid materials in contact with the concrete substrate. Materials removed from the pile often contained large chunks of wood or small branches. Because these materials would not fit into the sample containers, they were removed from the compositing containers before mixing the bulk sample. Once the bulk sample was well mixed, the samples were transferred to the final sample containers. This procedure was repeated twice: 1) for samples for the organic parameters, using stainless steel equipment and glass containers, and 2) for the metals and anions, using plastic equipment and containers.

At another facility which produced liquid sewage sludge, samples were collected from a catwalk atop a 1-million gallon storage tank. Sewage sludge was introduced into the tank by water cannon with a 4-inch diameter discharge nozzle. Plant personnel turned on the water cannon and throttled back the flow to a relative trickle and the sampler held each sample container in the edge of the stream until it was full. The containers were capped once they were full and wiped down before packing. Neither of these situations means that the samples were not representative or that the Agency can not rely on the results obtained. It simply points out the complexities and challenges with sampling sewage sludge generated by the variety of treatment processes and management options available nationally.

3.5 Field Duplicates

The sampling plan called for collection of field duplicate samples at 10% of the facilities. A field duplicate sample is a second sample collected at the facility using similar procedures and equipment as the original sample for quality control purposes. The results of the field duplicate sample can be compared to the results of the original sample as a means of assessing the overall precision of the sampling and analysis processes.

Note: The 10% frequency for field duplicates is a common, but arbitrary, choice designed to balance the cost of the additional samples against the desire to assess the precision of the sampling and analysis processes.

Eight facilities were originally selected for collection of field duplicates. This number was ultimately reduced to six because two of the facilities at which field duplicates were to be collected were dropped from the survey and not replaced (for reasons described in Section 2).

Separate EPA sample numbers were assigned to the field duplicates so they were not identifiable by the laboratories as duplicates. The results of the field duplicate analyses are discussed in Section 6.

3.6 Sample Labeling and Tracking

The EPA contract samplers labeled each container with a preprinted EPA Sample Number. An example label is shown at the right. The EPA sample number was specific to each sewage sludge sample at the facility.

EPA Sample No. **68408**

POTW Sewage sludge

Date collected _____

Sampler Initials _____

In addition to labeling each container, the samplers prepared an EPA Traffic Report that documented the origin of the samples. The sampler recorded the name of the facility, date of sampling, sampler's name, and shipping airbill number on the traffic report. The numbers of sample containers of each type (e.g., four plastic and eight glass) that were collected were recorded. The traffic report prepared at the site allowed EPA to track shipments of samples to the EPA Sample Repository at Microbac Laboratories in Baltimore, MD.

3.7 Packing and Shipping Samples to the Repository

The sample containers were packed for shipping using procedures described in the peer-reviewed sampling and analysis plan. Each sample container was either encased in bubblewrap bag or layers of bubblewrap sheeting to prevent its movement during shipping. Samples were packed into sturdy plastic ice chests. All of the samples from a single site could be packed, with ice and bubblewrap, in one 48-quart ice chest, or two 28-quart ice chests, depending on availability.

The samplers purchased ice near each facility, or the POTW provided ice, and packaged it in one-gallon self-sealing plastic bags. Approximately one pound of ice was used for each sample container (e.g., four bags containing two pounds of ice each were used to cool eight samples in a 28-quart ice chest). To prevent leakage during shipping, each ice chest was lined with bubble wrap and two trash bags. Samples and ice were packed into the inner bag, and then tied or sealed shut with tape. Additional packing materials were placed around the inner bag, if needed, and the outer bag sealed shut. The completed traffic report was placed in a plastic bag and affixed to the underside of the lid of the ice chest with either tape or a plastic airbill pouch.

Each cooler was shut with a layer of duct tape placed horizontally across the seam between the ice chest and its lid. Packaging tape or filament tape was applied to the cooler vertically, one band near each end of the ice chest, to secure the lid.

Ice chests were shipped overnight from full-service FedEx offices to the EPA Sample Repository. Samples collected from multiple facilities in a given day were shipped at the end of the day, or depending on the logistics, sent separately from different FedEx locations. Each sample shipment was tracked through the carrier's web site and EPA confirmed receipt at the repository.

Over the course of the survey, samples from two facilities were hand carried to the repository in Baltimore, because the facilities were located nearby. These samples were packed in a similar fashion as those sent by FedEx, except that no airbills were prepared.

3.8 Storage and Shipments to Laboratories

When samples arrived at the sample repository, the staff inspected the ice chests for external damage or leakage (none occurred) and placed them in one of two walk-in freezers dedicated to EPA samples and maintained at -11°C. Freezing at - 11°C reduces microbiological activity and the rates of any chemical reactions that might lead to changes in the sample.

To streamline the shipping logistics and manage both shipping and analytical costs, EPA shipped batches of 15 to 20 samples from the repository to the contract laboratories for analyses. EPA prepared new traffic reports that listed the samples in each shipment. Additional shipments were sent to the laboratories as more facilities were sampled. In all, six shipments were sent to the laboratory performing the analyses of metals, anions, and organics, with the last shipment being the two samples collected at the last facility. For the PBDEs, pharmaceuticals, and steroids and hormones analyses, more samples had been collected and stored at the repository by the time those analyses began. Ultimately, three shipments were made to the laboratory performing the PBDE analyses and three shipments were made to the laboratory performing the pharmaceuticals, steroids, and hormones analyses.

During the packing process prior to shipment, EPA examined the samples for signs of breakage, including cracked glass jars and cracked lids. Although some cracking and breakage did occur in jars or lids, as described below, it was observed that neither cross-contamination occurred due to frozen conditions nor were any samples lost or not available for analysis.

Only three incidents of breakage were observed and these containers were not shipped to laboratories, but were packaged in separate plastic bags and returned to the freezer. The jars may have cracked during freezing, as the cracked jars were all of the 1000-mL size used for liquid samples. While the samplers took care not to fill any of the jars to more than 90% of their capacity, the high water content of the liquid sewage sludge samples could lead to greater expansion during freezing. This may have resulted in a greater risk of breaking the 1000-mL glass jars than their 500-mL counterparts used for solid samples.

All samples sent from the repository were shipped frozen, with large quantities of ice added to all coolers. The laboratories inspected the samples on receipt and reported that all the coolers still contained ice. The laboratories also reported that a small number of samples (<15) were received with cracked lids or jars, which may have cracked during shipping. In each case, the laboratory transferred the sample to a suitable clean container or otherwise protected the contents, such that no samples were lost as a result of shipping. The laboratories stored the

samples frozen until analysis. Each laboratory returned a copy of the traffic report, with the date and time of sample receipt documented on the form.

Methods for the analysis of pharmaceuticals, steroids, and hormones in sewage sludge were not available when sample collection began in August 2006. Therefore, samples were stored frozen (-11°C) until methods for these analytes were available. Except for the two samples collected from one plant in March 2007, all of the samples analyzed for pharmaceuticals, steroids, and hormones were stored frozen for 11 to 15 months.

For the purpose of this survey, EPA assumed that all the analytes of interest were stable in sewage sludge samples stored at -11°C. Because the analyses for metals involved the "total recoverable" concentrations (e.g., all of the metal that can be recovered during digestion with a strong acid), holding frozen samples for extended periods is not a concern because the metals will be recovered even if there were any residual microbiological or chemical activity in the frozen samples. For the anions and semivolatile organics, EPA also did not anticipate any substantive changes in the concentrations of these analytes during storage due to the relatively low storage temperature.

The samples analyzed for pharmaceuticals, steroids, and hormones were held in storage longer than any of the other samples. The stabilities of these analytes have not been studied by EPA. However, EPA began a holding time study of pharmaceuticals in aqueous samples and sewage sludge in late 2008 that may provide some data to assess the freezer storage stability of these analytes in the near future.

3.9 Shipping Issues

EPA tracked each sample shipment through the carrier's web site, or confirmed receipt by contacting the laboratory directly. Only two shipping problems occurred, a surprisingly small number, given the number of shipments involved. In one instance, the laboratory performing the metals and anions analyses was sent the incorrect number of sample containers, which was resolved. In a second instance, the airbills applied to two similar ice chests containing samples for different laboratories were switched and the wrong types of containers were sent to each laboratory. The error was discovered when the samples were received at each laboratory the next morning. Both laboratories kept the samples frozen until they were able to ship them to the intended recipient.

3.10 Sampling Summary

The overall scope of the sample collection effort is summarized in Table 4 below.

Table 4. Summary Sampling Statistics

People	Travel	Shipping
A total of 12 samplers visited 74 plants and collected 84 samples. This included 6 field duplicates and additional samples at 4 plants that produced 2 types of final sewage sludge.	46 one-way airline flights	More than 150 containers of sampling supplies and equipment were shipped to field locations during the survey. Over 3,100 pounds samples and ice packed in 108 coolers were shipped to the EPA Sample Repository in Baltimore, MD, via FedEx. An additional 4 coolers were hand delivered to the repository.
Each sampler visited between 2 and 14 plants and collected 12 jars of sewage sludge per plant, for a total of 1,002 jars.	12 samplers spent 107 days on the road and drove over 19,000 miles	EPA shipped 427 jars to commercial labs for the analyses of metals, anions, organics, PBDEs, pharmaceuticals, steroids, and hormones. Samples from each site remain in an archive at the repository for possible future analyses.

3.11 Equipment Blanks

All of the equipment that came into contact with samples during the collection process was made of stainless steel (for organics) or plastic (for metals and anions) and was used for only one facility to avoid potential cross-contamination between sites. Prior to sending equipment to the field, all of the stainless steel and plastic scoops, spoons, and bowls were washed thoroughly with a non-phosphate detergent, rinsed three times with tap water, rinsed once with reagent water, inverted and air dried. Once dry, stainless steel equipment was wrapped in aluminum foil and plastic equipment was sealed in plastic bags. No field cleaning of equipment was performed during the survey. Liquid samples were placed directly into appropriate containers, while solids samples were placed in appropriate containers using scoops and spoons.

There is no relevant clean solid "reference matrix" for sewage sludge that could be easily used to prepare equipment blanks for the variety of analytes in this survey. Therefore, equipment blanks were created as follows. Two sets of the relevant equipment were sent to each laboratory performing analyses of anions, metals, semivolatiles organics, and PBDEs (e.g., two stainless steel compositing bowls, two stainless steel scoops, and two glass jars for the organics). For the semivolatile organics and PBDEs, there were two styles of bowls, based on availability, and two sizes of glass jars (500-mL and 1000-mL wide-mouths). Because the glass jars were purchased with certificates of analysis for common organics, EPA did not anticipate any effect due to the jar size. Therefore, EPA assembled one blank using each style of stainless steel bowl and included one jar size with one style bowl and the other jar size with the other bowl. For the plastic equipment, EPA used only one style of plastic compositing bowl and one size high density polyethylene resin (HDPE) jar, so four identical sets of equipment were sent to the laboratory performing the metals and anions analyses (two sets for each class of analytes).

Each laboratory used the equipment to prepare the equipment blank by rinsing each piece with the solvents or solutions used to prepare field samples. For organics, including PBDEs, the laboratories poured the same volumes of extraction solvents used for samples over the scoop into the bowl. The analyst carefully swirled the solvent in the bowl to contact the majority of the inner surface, and then poured it into the glass jar. For metals, the laboratory used the acidic sample digestion solution to contact all of the plastic materials. For the anions, the laboratory used reagent water to "extract" the samples in a similar fashion. The laboratory treated the

solvent or solution as if it came from a nominal size field sample and the reported the results accordingly, on a dry-weight basis.

The results for these equipment blanks, or rinsates, represent worst-case estimates of the potential contributions of the equipment to the final sample results. The worst-case nature of the estimate reflects the fact that the samples were solids, and even with vigorous mixing during the compositing steps, not all of the surface area of the sample contacted the entire surface of the bowl. The samples themselves did not contain solvents, or acidic solutions, so the potential transfer of contaminants would be expected to be much less than using the sample preparation solutions. Finally, any contaminants that were transferred from the equipment would be associated with the bulk composite and the final sample sent to the laboratory was only a portion of the total material in the bowl. In practice, 6 to 8 liters of solid sewage sludge were composited to provide material to fill eight 500-mL glass jars, or partially fill eight 1000-mL jars, for the organics analyses. For the metals and anions, only four plastic 500-mL jars were filled. The remainder of the composited material was returned to the sewage sludge disposal process.

The results of the equipment blank analyses for the anions, metals, and semivolatile organics are discussed in detail in Section 6.6. Based on EPA's experience with the semivolatile organic analytes of interest in the survey, and given the fact that EPA had exhausted the supply of one of the types of glass containers used to collect samples, EPA opted not to submit equipment blanks to the laboratory analyzing the pharmaceuticals, steroids, and hormones. There was no issue with organic compounds in general regarding contamination of equipment (e.g., through ambient air, equipment supplier, or laboratory contamination). Thus, the Agency does not believe that contamination with pharmaceuticals, steroids and hormones would have been an issue.

Section 4
Sample Analyses

4.1 Analytes of Interest

The TNSSS was designed to do two things: 1) obtain updated occurrence information on nine pollutants of potential concern, and 2) obtain occurrence information on a number of contaminants of emerging interest identified by EPA and the National Research Council (NRC).

As discussed in Section 1, EPA identified nine pollutants (shown in bold in Table 5) for further evaluation of occurrence in sewage sludge. This evaluation was based on an assessment of chemical pollutants for which EPA had adequate data (e.g., human health benchmark values, and information on fate and transport in the environment).

Given the national scope of the survey, EPA expanded the list of analytes to reflect the Agency's interest in collecting concentration data for other chemicals (see Tables 5 and 6). The expanded list included 24 additional metals that could be analyzed at little extra cost at the same time as the four metals (barium, beryllium, manganese, and silver) included in the list of nine pollutants above; molybdenum because of the Agency's interest in determining the need for a revised numeric standard for it in land-applied biosolids; and other analytes because of their widespread incidence and use and emerging concern. The latter category included:

- benzo(*a*)pyrene (found in coal tar, automobile exhaust fumes, tobacco and wood smoke, charbroiled food, and burnt toast);
- 2-methylnaphthalene (found in nonstructural caulking compounds and sealants, synthetic resins, rubber adhesives, and wall coverings);
- bis (2-ethylhexyl) phthalate (widely used as a plasticizer in manufacturing of items such as cosmetics, toys, tools, and laboratory equipment);
- fluoride (used in topical and systemic therapy for preventing tooth decay, as well as many other uses);
- water-extractable phosphorus (correlated with phosphorus concentration in runoff from soils amended with manure and biosolids and an indicator of loss that may contribute to algae buildup in surface waters);
- 11 polybrominated diphenyl ethers (PBDEs). Four of the PBDEs were of most interest because of available human health information that may be useful for future risk evaluation efforts. PBDEs are used as flame retardants in a wide array of products, including building materials, electronics, furnishings, motor vehicles, plastics, polyurethane foams, and textiles; and
- 97 pharmaceuticals, steroids, and hormones because of broader emerging interest in these analytes.

Table 5. Primary Target Analytes for the TNSSS, by Analyte Class

Analyte Class	Analyte	
Metals	Aluminum	**Manganese**
	Antimony	Mercury*
	Arsenic*	Molybdenum*
	Barium	Nickel*
	Beryllium	Phosphorus
	Boron	Selenium*
	Cadmium*	**Silver**
	Calcium	Sodium
	Chromium*	Thallium
	Cobalt	Tin
	Copper*	Titanium
	Iron	Vanadium
	Lead*	Yttrium
	Magnesium	Zinc*
Polycyclic aromatic hydrocarbons (PAHs)	Benzo(a)pyrene	2-Methylnaphthalene
	Fluoranthene	**Pyrene**
Other semivolatile organics	bis (2-Ethylhexyl) phthalate	**4-Chloroaniline**
Inorganic anions	Fluoride	Water-extractable phosphorus
	Nitrate	**Nitrite**
Polybrominated diphenyl ethers (PBDEs), including the Tetra, Hexa, Penta, and Deca congeners	2,4,4'-TrBDE (BDE-28)	2,2',3,4,4',5'-HxBDE (BDE-138)
	2,2',4,4'-TeBDE (BDE-47)	2,2',4,4',5,5'-HxBDE (BDE-153)
	2,3',4,4'-TeBDE (BDE-66)	2,2',4,4',5',6-HxBDE (BDE-154)
	2,2',3,4,4'-PeBDE (BDE-85)	2,2',3,4,4',5',6-HpBDE (BDE-183)
	2,2',4,4',5-PeBDE (BDE-99)	2,2',3,3',4,4',5,5',6,6'-DeBDE (BDE-209)
	2,2',4,4',6-PeBDE (BDE-100)	

The 9 pollutants in **bold** are those selected in the December 2003 Biennial Review
* Metals currently regulated at 40 CFR 503

Among the other "new and emerging contaminants" of concern in the NRC report were various pharmaceuticals, steroids, and hormones for which several EPA organizations were developing methods at the time that the TNSSS was being planned. EPA included certain pharmaceuticals, steroids and hormones in the TNSSS for which analytical methods were developed. Given the time required to develop and test new methods, EPA proceeded with the sample collection effort for the TNSSS as described in Section 3, and stored samples for the analyses of these analytes of interest until such time as the new methods for these classes of compounds were more fully developed. The drugs, antibiotics, steroids, and hormones added to the TNSSS are shown in Table 6.

Table 6. Pharmaceuticals, Steroids, and Hormones Included in the TNSSS

Analyte Class	Analyte	
Antibiotics and their degradation products, disinfectants, and other antimicrobials	Anhydrochlortetracycline	Ofloxacin
	Anhydrotetracycline	Ormetoprim
	Azithromycin	Oxacillin
	Carbadox	Oxolinic acid
	Cefotaxime	Oxytetracycline
	Chlortetracycline	Penicillin G
	Ciprofloxacin	Penicillin V
	Clarithromycin	Roxithromycin
	Clinafloxacin	Sarafloxacin
	Cloxacillin	Sulfachloropyridazine
	Demeclocycline	Sulfadiazine
	Doxycycline	Sulfadimethoxine
	Enrofloxacin	Sulfamerazine
	4-Epianhydrochlortetracycline	Sulfamethazine
	4-Epianhydrotetracycline	Sulfamethizole
	4-Epichlortetracycline	Sulfamethoxazole
	4-Epioxytetracycline	Sulfanilamide
	4-Epitetracycline	Sulfathiazole
	Erythromycin	Tetracycline
	Flumequine	Triclocarban
	Isochlortetracycline	Triclosan
	Lincomycin	Trimethoprim
	Lomefloxacin	Tylosin
	Minocycline	Virginiamycin
	Norfloxacin	
Other drugs	1,7-Dimethylxanthine	Diphenhydramine
	Acetaminophen	Fluoxetine
	Albuterol	Gemfibrozil
	Caffeine	Ibuprofen
	Carbamazepine	Metformin
	Cimetidine	Miconazole
	Codeine	Naproxen
	Cotinine	Norgestimate
	Dehydronifedipine	Ranitidine
	Digoxigenin	Thiabendazole
	Digoxin	Warfarin
	Diltiazem	
Steroids	Campesterol	Epi-coprostanol
	Cholestanol	Ergosterol
	Cholesterol	β-Sitosterol
	Coprostanol	β-Stigmastanol
	Desmosterol	Stigmasterol
Hormones	Androstenedione	Estriol
	Androsterone	Estrone
	17α-Dihydroequilin	17α-Ethynyl estradiol
	Equilenin	Norethindrone
	Equilin	Norgestrel
	17α-Estradiol	Progesterone
	17β-Estradiol	Testosterone
	β-Estradiol-3-benzoate	

4.2 Analytical Techniques

Table 7 presents the analytical techniques applied to samples in the TNSSS. The target reporting limits in the table are based on a consensus of what might be achievable in a sewage sludge sample. The actual reporting limits achieved are discussed in Section 6.5.

Table 7. Analytical Methods or Techniques

Analyte Class	Method or Technique	Target Reporting Limit (dry weight)
28 Metals, including mercury	ICP/AES, ICP/MS, and CVAA (EPA Methods 200.7, 200.8, and 245.1)	3 to 4 mg/kg
4 Polycyclic aromatic hydrocarbons (PAHs) and 2 semivolatiles (as one analytical fraction)	GC/MS, with selected ion monitoring (SIM), after solvent extraction and gel permeation chromatography (GPC) cleanup (EPA SW-846 Method 8270C)	100 to 300 µg/kg
4 Inorganic anions, including water-extractable phosphorus (WEP)	EPA Methods 340.2, 353.2, and 365.3, after leaching of the solid sample with reagent water with a study-specific protocol	2 to 8 mg/kg
11 PBDE Congeners*	High resolution GC/MS, draft EPA Method 1614	5 to 200 ng/kg
72 Pharmaceuticals	High performance liquid chromatography (HPLC) with tandem MS/MS detection, using an early draft of EPA Method 1694†	Not specified
25 Steroids and hormones	High resolution GC/MS, using an early draft of EPA Method 1698†	Not specified

* The list of target PBDE analytes was limited to the following 11 PBDE congeners: 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, and 209, which include those identified in the method as being of potential environmental or public health significance. There are some differences between the specifics of the method and the procedures used for the TNSSS (see Section 4.4.4 of this report).

† The laboratory solicitation and contract were issued prior to the December 2007 formal release of EPA Methods 1694 and 1698 and as a result, there are some differences between the specifics of those methods and the procedures used for the TNSSS (see Sections 4.4.5 and 4.4.6 of this report).

mg/kg = milligrams per kilogram µg/kg = micrograms per kilogram ng/kg = nanograms per kilogram

As indicated, the survey used both well-established multi-laboratory validated EPA procedures as well as three analytical methods that were developed or updated for the survey. The two new methods are single-lab validated methods for pharmaceuticals (EPA Method 1694), and steroids and hormones (EPA Method 1698). The updated multi-lab validated method is for flame retardants (EPA Method 1614). These three methods have not yet been promulgated at 40 CFR Part 136 for compliance monitoring in CWA programs, including the analysis of sewage sludge.

4.3 Laboratories

EPA awarded competitive-bid analytical contracts to the following commercial laboratories:

- Columbia Analytical Services for the analyses of the metals, anions, and PAHs and semivolatile organics
- Severn Trent Laboratories (now part of Test America) for the analyses of the PBDEs
- Axys Analytical Services for the analyses of pharmaceuticals, steroids, and hormones.

4.4 Method Modifications

From an analytical standpoint, sewage sludge is a challenging matrix. The concentrations of pollutants present in samples vary depending on the nature of the inputs to the treatment plant. In addition to the pollutants of interest, sewage sludge contains a number of other components that are potential interferences in the analyses of the pollutants of interest. These components include lipids and other naturally occurring materials, as well as materials that may be added to the sewage during processing (e.g., surfactants, ferric chloride, polymeric colloids, or lime). These components can manifest themselves as interferences at all stages of the analytical process, from sample preparation through the determinative analysis.

Another analytical challenge with a national survey of sewage sludge is that the various treatment process and disposal practices used nationwide lead to differences in the moisture content of the final sewage sludge sent for use or disposal. Some of the facilities from which samples were obtained in the TNSSS produce liquid final sewage sludge, while others produce solid sewage sludge. Among the sewage sludge that were pourable liquids, the percent solids (hereafter percent solids) content ranged from less than 1% to about 4%, across treatment plants. For the solids, the percent solids content ranged from 5% to 99%. These differences in the form (liquid vs. solid) of the sewage sludge and the range of moisture or solid contents have direct effects on the analyses of the samples. The differences also affect how the data for the survey can be interpreted.

Recognizing these challenges, EPA structured the laboratory subcontracts for the various analyses to achieve the most uniform results across facilities as practical. These modifications are described below.

4.4.1 Ensuring Consistent Method Sensitivity

Many analytical methods applicable to biosolids instruct the laboratory to prepare a specific known weight of a solid material for analysis (e.g., some methods for organics specify using 30 g of sample). However, that sample aliquot may contain significant amounts of moisture. Those same methods may treat samples that are pourable liquids as if they contain little or no solids, and specify using a known volume, such as 1 L, for the analysis, although that volume may contain measurable solids as well. These differences in how liquid and solid samples are prepared and analyzed, as well as the differences in the amount of solids or moisture in the two types of samples, mean that any measure of method sensitivity (e.g., a reporting limit or a detection limit) will depend on the initial mass or volume chosen for analysis and its moisture content.

EPA considered these effects on sensitivity and comparability when it planned the TNSSS. EPA minimized the potential sensitivity differences by instructing the laboratories to determine the percentage of solids (percent solids) of each sample first, and then use that information to select a portion of the sample for the analysis that contains the method-specified sample weight or volume on a dry-weight basis. In addition, even when the laboratories prepared liquid samples using procedures designed for aqueous samples (e.g., liquid-liquid extraction with an organic solvent), they were instructed to report the results in weight/weight units (e.g., ng/kg, μg/kg, or mg/kg) appropriate for the class of analyte, adjusted for the moisture content of the sample (e.g., 100% dry sample).

The laboratory that performed the analyses of the metals, anions, and PAH and semivolatile organics was instructed to determine the percent solids separately for each class of analytes. EPA examined the percent solids data from each class for the first 35 samples and determined that there was no statistically significant difference between the three measurements on each EPA sample (see Section 6.7.7). Nevertheless, in order to ensure the most consistent sensitivity across the survey, EPA instructed the laboratory to continue to determine percent solids on each sample for each class for the remainder of the project.

4.4.2 Anions in Sewage Sludge

Methods for determining anions (e.g., nutrients) in sewage sludge samples require that the anions be dissolved in water and separated from the solid material. In planning the TNSSS, EPA considered several approaches to preparing the sewage sludge samples for the analysis of anions, including:

- EPA Method 1685, Nitrate/Nitrite-N in Water and Biosolids by Automated Photometry, Draft January 2001;
- EPA Method 1688, Total Kjeldahl Nitrogen in Water and Biosolids by Automated Colorimetry with Preliminary Semi-automatic Digestion, Draft January 2001;
- A water extraction (or leaching) procedure developed at the Pennsylvania State University (Vadas, P. A. and Kleinman, P. J. A., 2006).

The two draft EPA methods cited above have only been validated in a single lab. Neither of these methods has been promulgated at 40 CFR Part 136 for compliance monitoring in CWA programs, nor for the analysis of sewage sludge samples. As a result, few, if any, laboratories routinely run samples using those draft methods.

The method developed at Pennsylvania State University was published in the literature and has been used by several mid-western states that regulate the application of manure and biosolids to agricultural lands. However, the authors only used that procedure for the analysis of phosphorus, and not the other anions.

After reviewing the Vadas and Kleinman leaching procedure, EPA concluded that the leachate was amenable to analyses of the anions of interest in the TNSSS by existing commonly used EPA methods that are approved at 40 CFR 136. Therefore, EPA decided to use the leaching procedure to prepare all of the sewage sludge samples and have the anion analyses performed using the following EPA methods:

- Method 340.2, Fluoride, Potentiometric, Ion Selective Electrode, March 1983
- Method 353.2, Nitrate-Nitrite, Colorimetric, Manual Cadmium Reduction, March 1983
- Method 365.3, Phosphorus, All Forms, Colorimetric, Ascorbic Acid, Two Reagent, March 1983

Appendix A of this report provides the TNSSS-specific directions for the leaching procedure. Briefly, the procedure involves:

- Determining the percent solids of the original sample using standard procedures
- Weighing a sample aliquot equal to 0.5 g (dry weight) into a plastic bottle
- Adding 100 mL of reagent water
- Shaking the bottle on a shaker table for 60 minutes at 70 RPM
- Centrifuging the mixture for 10 minutes at 2000 RPM
- Filtering the sample by gravity through a Whatman #2 filter
- Preserving the aqueous leachate sample by adding H_2SO_4 to pH <2
- Analyzing the leachate sample within 48 hours (the holding time for nitrate/nitrite)
- Reporting all results in mg/kg, based on the original 0.5-g sewage sludge sample weight.

The leaching procedure includes steps that will preserve the relative proportions of nitrate and nitrite *after* the sewage sludge sample is leached (e.g., acid preservation and analysis within 48 hours). However, it is not possible to determine whether or not the leaching process itself resulted in oxidation of nitrite to nitrate, or vice versa. Therefore, EPA decided that nitrate and nitrite would be analyzed as the combined parameter nitrate/nitrite, using Method 353.2. This is approach is acceptable for determining better exposure scenarios for nitrate/nitrite during land applicable of biosolids.

In addition, the analytical approach for the survey included determining the element phosphorus (P) as part of the suite of metals. The water-extractable phosphorus (WEP) determined using the leaching procedure above is a useful predictor of the concentrations of phosphorus that might be available for runoff from land to which sewage sludge has been applied. The ratio of the two forms of phosphorus (WEP/P) is an indication of the proportion of the total phosphorus applied that may contribute to runoff. That ratio may be of interest to those states that regulate land application of sewage sludge.

Note: The reader is cautioned about making comparisons between the anion results from this survey and data from other sources that may have used different procedures to leach the anions from the sample. EPA's use of dry-weight reporting units may facilitate such comparisons, but other differences among leaching procedures may still influence the results.

4.4.3 Modified GC/MS Procedures for Semivolatile Organics

Gas chromatography, coupled with mass spectrometric detection (GC/MS), is the backbone of the analytical methods for many organic pollutants, including the PAHs and semivolatile organics of interest in the TNSSS. The most common form of GC/MS analysis is known as "full scan" GC/MS and involves examining all of the mass fragments in a wide mass range that exit the GC column and reach the MS detector. A typical GC/MS method will scan a mass range from 35 to 450 atomic mass units (amu) once every second. The masses of any materials that exit the GC column in that range will be recorded and used to identify the pollutants of interest.

Some EPA GC/MS methods include several hundred target analytes, and while full-scan GC/MS is a powerful technique, it involves tradeoffs in sensitivity and selectivity in order to be applied to large number of analytes simultaneously. Full-scan GC/MS can also be subject to interferences from other materials in the sample that are not of interest.

Some of these "co-extracted" interferences include biolipids that can be removed from the sample extracts using established cleanup procedures. One such procedure is gel permeation chromatography (GPC), which segregates the relatively small pollutant molecules from the larger lipids and other interferences on the basis of molecular size. Based on our experience in two previous sewage sludge surveys, EPA required every sample extract analyzed for the PAHs and semivolatile organics be subjected to GPC cleanup before analysis.

When a GC/MS instrument is operated in full-scan mode, it scans the entire mass range very quickly and there is relatively little time to observe the results at any given mass within that range. This places some practical limits on the sensitivity of the procedure. However, those limitations can be overcome by using the technique known as selected ion monitoring (SIM). In SIM, the MS instrument only looks for a small subset of masses (ions) in the overall mass range. These masses are ones associated with the list of target analytes, and any other masses that exit the GC column are simply ignored. The ions that are ignored may be those associated with interferences, including biolipids, or those from analytes in a full-scan method that are not of interest for a given project. Because the MS can spend more time looking for fewer masses, the sensitivity of the instrument for those pollutants with those masses can increase 10-fold or more over that of a full-scan procedure.

EPA examined the results for the first 50 samples that were analyzed by full-scan GC/MS and found that for many of those samples, the reporting limits achieved by the laboratory were much higher than anticipated. In other words, analytes were reported as "not detected" at concentrations greater than the reporting limits desired for the survey. Many of the increased reporting limits were due to large amounts of interferences that remained in the sample extract even after GPC cleanup, which required dilution of the extract. The laboratory also diluted a smaller number of samples in order to get one or two target analytes (usually bis [2-Ethylhexyl] phthalate) within the instrument calibration range, resulting in a loss of sensitivity for other analytes present at much lower concentrations.

In response to these early findings, EPA required that the laboratory reanalyze the extracts for about 35 samples using a SIM procedure. That procedure included masses that represented the four PAHs and two semivolatile organics that are target analytes in the survey, as well as the surrogate compounds and internal standards used in the full-scan method. The masses used in the SIM procedure are shown in Table 8. Based on successful analyses of these extracts, the SIM procedure was the only GC/MS procedure employed for the analysis of the remaining samples in the survey.

Table 8. Selected Ion Monitoring Parameters for Organic Analytes

Type	Analyte	Quantitation Mass	Approximate Retention Time (min)*
Target Analytes	4-Chloroaniline	127	9.71
	2-Methylnaphthalene	142	10.49
	Fluoranthene	202	15.04
	Pyrene	202	15.32
	bis (2-Ethylhexyl) phthalate	149	16.61

Table 8. Selected Ion Monitoring Parameters for Organic Analytes

Type	Analyte	Quantitation Mass	Approximate Retention Time (min)*
	Benzo(a)pyrene	252	18.12
Surrogates	Nitrobenzene-d_5	82	8.67
	2-Fluorobiphenyl	172	10.94
	p-Terphenyl-d_{14}	244	15.49
Internal Standards	1,4-Dichlorobenzene-d_4	152	7.90
	Naphthalene-d_8	136	9.59
	Acenaphthene-d_{10}	164	11.77
	Phenanthrene-d_{10}	188	13.57
	Chyrsene-d_{12}	240	16.63
	Perylene-d_{12}	264	18.18

*Retention times are specific to the GC column and operating conditions used for these samples. Retentions times on other columns or instruments will differ. These data are presented solely to illustrate the relationships among the target analytes, surrogates, and internal standards.

4.4.4 PBDE Analyses

The original plan for the survey was to analyze for all 209 of the PBDE congeners using the latest draft of EPA Method 1614, a high resolution GC/MS isotope dilution procedure. Method 1614 is a highly sensitive procedure that can determine PBDEs at the part per trillion (ng/kg) levels in solid samples. It employs at least five cleanup techniques, including GPC, to remove interferences from sample extracts. The method employs isotope dilution quantitation, in which PBDE congeners synthesized using only carbon 13 (^{13}C), a stable (nonradioactive) isotope of carbon, are added to the sample prior to extract. These isotopically labeled congeners do not occur in nature and are used as internal standards to quantify the unlabeled PBDE congeners. Because the labeled congeners are carried through the entire sample preparation, cleanup, and analysis process, they can be used to correct for any loss of analytes during the overall analysis, providing a more accurate result for each unlabeled target analyte. For this reason, EPA uses isotope dilution in many of the 1600-series methods, including those for dioxins, furans, PCBs, and PBDEs.

Shortly after work began on the first batch of survey samples, the laboratory and EPA realized that additional efforts would be required to adequately determine PBDEs in sewage sludge. Ultimately, EPA agreed to permit the use of a range of modifications to the original plan to overcome as many analytical difficulties as practical in the time permitted for the TNSSS. Appendix B contains a detailed list of those modifications. The most significant modifications were:

- Due to the high levels of some congeners and some interferences, the initial sample size was reduced from 10 g to 2 g for extraction, and ultimately to 0.2 g (dry weight).
- Samples were not spiked with labeled congeners prior to extraction.
- The sample extracts were concentrated to a final volume of 10 mL after extraction.
- A 1-mL aliquot of the extract was removed for spiking and cleanup.
- Labeled congeners were spiked into the 1-mL extract, which was then carried through the following cleanup steps described in the method: silica gel, GPC, and alumina. The remaining 9 mL of extract were retained in case dilutions or additional cleanups were required.
- After cleanup, extracts were analyzed by HRGC/HRMS.

- The list of target analytes was limited to the following 11 PBDE congeners: 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, and 209, which includes those identified in the method as being of potential environmental or public health significance.
- Data were examined to determine if a larger sample size (greater than 0.2 g) was required to achieve the desired sensitivity, or if the extract required dilution to keep analytes within the calibration range. The latter was much more common.
- If congeners were present above the upper limit of the calibration range, the laboratory evaluated whether or not the peaks had saturated the detector. If saturation did not occur, the results were reported, but flagged in the database as exceeding the calibration range. This approach reduced the dilutions that had to be analyzed for each sample to a practical number.
- Because the labeled congeners are not spiked into the samples before extraction, matrix spike and matrix spike duplicate samples were prepared and analyzed periodically to provide an estimate of extraction efficiency.
- Results for the 11 congeners were corrected for any losses of analytes that occur during the many cleanup steps, but this is not equivalent to the true isotope dilution quantitation procedure in the original method because it does not correct for the efficiency of the sample extraction procedures.

These steps were successful in overcoming challenges inherent in sewage sludge analyses, and produced useful data for the purposes of the TNSSS. However, these steps involved some trade-offs, most notably the loss of true recovery correction of isotope dilution quantitation and the degree to which the very small sample size represents the bulk material.

4.4.5 Pharmaceutical Analyses

The analysis of the pharmaceuticals was performed using an early draft of EPA Method 1694. EPA Method 1694 employs high performance liquid chromatography (HPLC) to separate the analytes of interest and tandem mass spectrometry (MS/MS) to detect them. HPLC (or simply LC) is a technique that allows the analysis of polar compounds in polar solvents. It has advantages over gas chromatographic (GC) methods for the pharmaceuticals because GC methods involve introducing the analytes into the instrumentation in a gaseous form and many of the pharmaceuticals are not easily volatilized. Some have boiling points that are above the operating temperatures of a GC system and others will break down when heated (i.e., they are "labile").

Tandem mass spectrometry involves the use of two quadrapole mass spectrometers in series, with a collision cell between them, such that selected ions produced in the first MS unit are directed into the collision cell and further fragmented before being sent to the second MS for detection. The only ions passed through the collision cell are those selected by the instrument as representing the analytes of interest. These fragments, or "product" ions, are characteristic of the "precursor" compound and are used to positively identify the analyte in the presence of other analytes and potential interferences. The MS/MS detector can be operated in an ionization mode that produces positive ions from the analytes of interest, or in a mode that produces negative ions. The method for pharmaceuticals involves four analytical fractions, three of which operate in the positive ionization mode and one of which uses the negative ionization mode. As employed in this method, the tandem MS unit is operated at unit mass resolution (e.g., it can distinguish between masses that differ by one atomic mass unit).

The pharmaceuticals method employs solvent extraction procedures to isolate the analytes of interest from the sewage sludge samples. Many of the analytes are weak acids or weak bases that ionize in aqueous solutions, losing or gaining a proton from a water molecule. The extraction procedures in the pharmaceutical method involve adjusting the pH of the sample to provide more favorable conditions for isolating the analyte from the sample matrix.

An aliquot of each sewage sludge sample containing a consistent dry weight of solids is mixed with a phosphate buffer solution with a pH of 2. This pH adjustment causes the ionized acid forms of the analytes of interest to gain protons and become neutrally charged molecules that are less soluble in water than their ionized forms and more soluble in a polar organic solvent.

A suite of stable isotopically labeled standards (forms of the analytes that do not occur naturally) is spiked into the sample and the sample is further mixed. The analyst adds acetonitrile to the buffered sample, ultrasonically agitates the mixture for 30 minutes, and centrifuges the solution to separate the solvent extract from the solids. After decanting and collecting the acetonitrile, the analyst performs a second extraction of the solids with fresh aqueous buffered acetonitrile, and a third extraction using acetonitrile alone. All three acid extracts are combined for cleanup.

The base extraction is conducted in a similar fashion, using a second aliquot of the original sample, but the pH of the sample is adjusted to 10 with an ammonium hydroxide solution. Three extractions are performed, two with aqueous buffered acetonitrile and a third extraction with acetonitrile alone. All three base extracts are combined for cleanup.

The combined acid extract is concentrated to remove the acetonitrile and prepared for cleanup by adding disodium EDTA and diluting the solution to 200 mL with reagent water. The aqueous solution is processed through a solid-phase extraction (SPE) cartridge, which traps the analytes of interest. Potential interferences are removed by eluting the cartridge with reagent water and discarding that eluant. The analytes of interest are eluted from the cartridge with methanol, followed by 1:1 acetone:methanol. The eluant is evaporated to near dryness, reconstituted in methanol, spiked with the method-specified internal standards, and brought to a final volume of 4 mL with a 0.1% formic acid buffer solution.

The base extract is subjected to a similar SPE cleanup procedure, but the cartridge is eluted with methanol, followed by 2% formic acid in methanol. The combined eluant is evaporated to near dryness, reconstituted in methanol, spiked with the method-specified internal standards, and brought to a final volume of 4 mL with a 0.1% formic acid buffer solution.

Four separate LC/MS/MS analyses are performed for the pharmaceuticals: three on the acid extract and one on the base extract. Separate chromatographic conditions are associated with each of the four fractions. The LC/MS/MS instrument is operated in the multiple reaction monitoring (MRM) mode, which monitors a series of precursor/product ion transitions that are characteristic of each target analyte.

The labeled analytes spiked into the sample prior to extraction are used to perform isotope dilution quantitation for all of the target analytes that have labeled analogs. For those target analytes for which labeled analogs are not readily available, quantitation is performed using the labeled analog of a similar compound in that fraction. As a result, all of the target

analyte concentrations are corrected for the recovery of the labeled analogs, thus accounting for extraction efficiencies and losses during cleanup.

The approach to ensuring more consistent method sensitivity described in Section 4.4.1 of this report was applied to the pharmaceutical analyses as well.

4.4.6 Steroid and Hormone Analyses

The analysis of the steroids and hormones was performed using an early draft of EPA Method 1698. EPA Method 1698 employs GC to separate the analytes of interest and high resolution mass spectrometry (HRMS) to detect them. As employed in this method, the mass spectrometer achieves a resolution of at least 5,000. The target analytes in the method have molecular weights that range from about 100 to 500, such that the MS can distinguish between analytes with molecular weights that differ by 0.02 to 0.1 atomic mass units, and identify them in the presence of potential interferences.

The steroids and hormones method also employs solvent extraction procedures to isolate the analytes from the sewage sludge samples. An aliquot of each solid sample containing a consistent dry weight of solids is spiked with a suite of stable isotopically labeled standards. The sewage sludge samples that are firm solids are extracted in a Soxhlet extractor with 60:40 acetone:hexane. The extract is split into two portions, with 1/25th of the extract used for steroids analysis and the other 24/25th used for the hormones analysis. This split ratio is used to compensate for the fact that the steroids and their interferences are present at higher levels in the samples than the hormones.

The sewage sludge samples that are pourable liquids are extracted using a liquid-liquid solvent extraction with methylene chloride. The extracts of these samples also are split into two portions, with a split ratio of 1:99, for the steroids and hormones respectively.

For both forms of sewage sludge (liquid and solid), the separate extract portions are subjected to cleanup using a layered alumina-Florisil (LAF) column. Following cleanup, each sample extract is concentrated to approximately 0.1 mL and the extract solvent is exchanged to pyridine.

In order to be amenable to GC analysis, the steroids and hormones are converted to compounds that are more volatile than their native forms. Both the steroids and hormones are derivatized to their trimethyl-silyl ethers using N,O-bis(trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (BSTFA:TMCS). The derivatized extracts are concentrated and spiked with the method-specified internal standards and the final extract volume is adjusted to 500 µL.

Separate GC/HRMS analyses are performed for the steroids and for the hormones, using the same chromatographic conditions for each analytical fraction, but running the two fractions at different dilutions. The GC/HRMS instrument is operated at a mass resolution of at least 5,000, and at least two exact masses are monitored for each target analyte.

As with the pharmaceuticals method, the labeled analytes spiked into the sample prior to extraction are used to perform isotope dilution quantitation for all of the target analytes that have labeled analogs. For those target analytes for which labeled analogs are not readily available,

quantitation is performed using the labeled analog of a similar compound in that fraction. As a result, all of the target analyte concentrations are corrected for the recovery of the labeled analogs, thus accounting for extraction efficiencies and losses during cleanup.

EPA worked with the laboratory to adjust the procedures for the steroids and hormones to address problems encountered early in the survey. In particular, the laboratory modified one of the cleanup procedures. As written, the steroids and hormones method involves solvent extraction of the sample and processes that extract through a layered alumina-Florisil (LAF) cleanup column before splitting the extract into two unequal portions (1/25 and 24/25) for analyses of steroids and hormones, respectively. However, some of the steroids and hormones are present in both extract portions and during the early part of the survey, the high levels of steroids were creating analytical challenges during the analysis of the hormones fraction.

The laboratory proposed a solution to the problem that involved modifying the order in which the extract was subjected to cleanup and split into two portions. Instead of running the entire extract through the LAF column and then splitting it into two unequal portions, the raw extracts were split first and then run through separate LAF cleanups, one for steroids and one for hormones. The LAF cleanup for the steroids was run as described in the original procedure.

The laboratory modified the LAF procedure for the hormones to remove most of the steroids from the hormone fraction. The steroids were eluted from the LAF column with methylene chloride and that eluant was discarded. The hormones then were eluted from the column with methanol, as described in the original procedure.

The laboratory tested the method modifications prior to starting analyses of the survey samples by extracting large samples of sewage sludge from another source (e.g., not a TNSSS sample), spiking the extracts with the hormones of interest to ensure that they were present, and splitting each extract into two portions. One of the portions was run through the LAF column as described in the method. The other portion was subjected to the modified LAF procedure. Both extract portions were analyzed for hormones by GC/HRMS and the results were compared. The change to the LAF procedure dramatically reduced the levels of steroids that remained in the hormone fraction, and improved the results for the hormones.

As noted in Section 4.2, the target analyte list for the TNSSS was based on a variety of factors, but was *not* driven by lists of analytes in any individual methods. EPA had included Mestranol as a target analyte in early versions of the survey plan for the TNSSS. However, Desogestrel was never listed as a target analyte for the survey. Therefore, this modification to the LAF cleanup procedure sacrificed one hormone in favor of improvements for all the other target hormones. Given the difficulties inherent in sewage sludge analyses, EPA judged this to be a reasonable compromise.

4.4.7 Focusing Quality Control on the Survey-specific Analytes of Interest

Because the analytes of interest for the TNSSS often are a very limited subset of the total number of analytes listed in some of the relevant methods, EPA did not require the laboratories to consider those other analytes in either preparing or evaluating the quality control operations associated with these samples. For example, EPA only required the laboratory to spike the analytes of interest for this survey into such QC samples as matrix spike/matrix spike duplicates and ongoing precision and recovery (OPR) samples, not all the analytes that may be listed in a given method. Alternatively, if the laboratory chose to use spiking solutions that contained additional analytes beyond those in Tables 5 and 6, EPA did not require them to assess the results for those non-survey target analytes or take corrective actions if those non-survey analytes failed to meet the acceptance criteria.

This approach allowed the laboratories to focus their efforts on the survey-specific analytes of interest in the face of the analytical challenges presented by sewage sludge matrices. It also allowed EPA to control the costs for the analyses to some degree by eliminating reanalyses related to potential QC failures associated with non-target analytes. It also reduced the costs for EPA's data review efforts described in Section 5.

Section 5
Data Review Procedures

5.1 General Review Procedures

EPA assessed the results for all of the samples analyzed during the survey using well-established procedures described in this section. The analysis involved in the TNSSS was complex and a number of analytical challenges were faced. Biosolids is one of the most challenging environmental matrices known due to the high solids content and matrix interferences present. When conducting analyses in sewage sludge matrices it is expected that some results will have to be qualified to accurately reflect the uncertainty of the values.

EPA subjected all laboratory results to a comprehensive review for completeness and compliance with project and method specifications to ensure that the data met the objectives of the survey. A multi-stage review process was used and designed to identify and correct data deficiencies as early as possible and maximize the amount of usable data generated.

Trained staff reviewed the data using established review process designed to identify and correct data deficiencies as early as possible and maximize the amount of usable data generated during the TNSSS. EPA encoded the data quality information gathered during the review in the final results database using a series of qualifiers and reasons. EPA did not exclude data unless a result was flawed such that no reasonable use could be made of it. The four stages of the review process are described below.

In the first stage of the data review process, EPA performed a data completeness check. Specifically, EPA evaluated elements in the laboratory submission to verify that results for specified samples were provided, that data were reported in the correct format, and that relevant information, such as preparation and analysis logs, was included in the data package. EPA initiated corrective action procedures to resolve any deficiencies identified.

The second stage of the data review process focused on an instrument performance check. EPA verified that calibrations, calibration verifications, standards, and calibration blanks were analyzed at the appropriate frequency and met method or survey performance specifications. Corrective action procedures were initiated to resolve any deficiencies identified.

The third stage of the data review process focused on a laboratory performance check. EPA verified that the laboratory correctly performed the required analytical procedures and was able to demonstrate a high level of precision and accuracy. During this stage, EPA evaluated quality control (QC) elements such as the ongoing precision and recovery (OPR) tests, method blanks, and other QC operations. Again, corrective action procedures were initiated to resolve any deficiencies identified.

In the fourth stage of the data review process, EPA examined method/matrix performance data to discern whether any QC failures resulted from laboratory performance or difficulties with the method or sample matrix. EPA evaluated labeled compound and surrogate spike results and other performance data. The reviewers also verified that proper sample dilutions were performed and that necessary sample cleanup steps were taken. As with previous steps, corrective action procedures were initiated with the laboratory to resolve any deficiencies identified.

The objective of the data review process was to document the quality of all of the data in the TNSSS and identify any limitations that might affect their end use. The EPA database on the mainframe contains data qualifiers applied to results from the TNSSS, individually, and by analyte class.

5.2 QC Acceptance Criteria

As noted in Section 4, the analytes of interest for this survey were subject to quality control. Appendix C presents a summary of the QC acceptance criteria for all of the analyte classes.

The laboratory performing the analyses for the anions, metals, PAHs and semivolatiles used its own in-house limits routinely applied to soil samples (as opposed to aqueous samples), except as noted in Appendix C. The laboratory prepared liquid sewage sludge samples using procedures applicable to aqueous samples (e.g., a liquid-liquid solvent extraction procedure for the organics) and the laboratory ran an aqueous laboratory control sample (LCS) and applied acceptance limits appropriate to that set of procedures. The laboratory derived their acceptance limits for solid samples from historical data for sewage sludge samples. Given the difficulties evident in the analyses of the sewage sludge samples, one might expect that statistically derived acceptance criteria for sewage sludge samples would differ from those for soil samples. Therefore, recoveries falling slightly outside of the limits for soil are not necessarily fatal flaws in these analyses. However, for the sake of transparency, EPA noted all recovery problems in the database.

The laboratory performing the analysis of the PBDEs using draft EPA Method 1614 employed the default acceptance limits in the draft method.

The methods used for the pharmaceutical, steroid, and hormone analyses include QC acceptance criteria. The acceptance criteria include statistically derived limits that are based on data from a single laboratory. As a result, the acceptance limits for some QC operations are fairly wide (e.g., 5 – 200% for labeled compound recoveries). The laboratory that performed the pharmaceutical, steroid, and hormone analyses for the TNSSS was also the laboratory that helped EPA develop these methods. Therefore, EPA believes that the use of the single-laboratory specifications is a reasonable approach.

5.3 Data Qualifiers and Database

After reviewing each data package, the reviewers and the database development staff created an analytical database in MS Access that contained all field sample results from the survey. Appendix D of this report contains a table with the data applied to the results during the review process for TNSSS pollutants and entered into the database.

At intervals during development and upon completion of the database, EPA performed various checks to verify the accuracy of the database, including checks for consistent analyte names, CAS numbers, and data qualifier flags. After completing the review of the results for each analytical fraction, EPA uploaded the data from an Access database to the EPA mainframe using standardized procedures. The final database for the survey includes all of the qualifiers that were applied to the individual sample results.

Note: Except for the "exclude" qualifier in the database, the presence of data qualifiers is not intended to suggest that data are not useable. Rather, the qualifiers are designed to caution the user about an aspect of the data that does not meet the acceptance criteria originally established for the project.

5.4 Data Review Findings

The data review process was crucial in identifying the sensitivity issues associated with the full-scan GC/MS analyses discussed in Section 4. By examining the first sets of sample results shortly after they were delivered by the laboratory, EPA was able to take corrective action and instituted the use of selected ion monitoring for all PAH analyses in the survey to achieve the needed analytical sensitivity.

The data review process uncovered only a few other issues with data quality. Typical issues included:

- Reporting unit issues
- Calculation errors for specific samples
- Blank contamination
- Extract dilution issues
- Matrix spike issues
- Interference issues
- Recovery issues

Specific examples of these issues are presented in Section 5.4.1 to 5.4.7.

5.4.1 Reporting Limits

As expected, some samples with low percent solids were prepared and analyzed as aqueous samples. However, when reporting the results for the earliest such samples, the laboratory performing the metals analyses provided results in the weight/volume units typically applied to aqueous samples. When contacted by the data reviewers regarding this error, the laboratory revised these results and reported them on the basis of the dry weight of solids in the samples, as originally requested.

The laboratory analyzing the pharmaceuticals, steroids, and hormones reported all the sample results on a dry-weight basis, as required, including those samples that were pourable liquids. However, the laboratory used reporting units of nanograms per gram (ng/g), instead of the method-specified units of nanograms per kilogram (ng/kg). This error was discovered during the early stages of the review of these data. However, because the units of ng/g are equivalent to µg/kg, after consulting the laboratory and considering the concentrations of the analytes in the samples, EPA decided to accept the numerical results in ng/g (e.g., EPA kept the numbers reported by the lab), but changed the units in the EPA database to µg/kg (i.e., 100 ng/g is equal to 100 µg/kg).

5.4.2 Calculation Errors

EPA noted that the fluoride results for two sewage sludge samples appeared to be much higher than any other results for this analyte. EPA contacted the laboratory and asked them to check these two results. The laboratory reported that the raw data for these two results were off by two orders of magnitude, due to a transcription error. The laboratory corrected the error and resubmitted the results for those two samples. The corrected results were included in the survey database.

5.4.3 Blank Contamination

EPA examined the results for each analyte in every sample and compared the results to the concentrations of analytes found in each of the method blanks associated with each batch of samples. For all of the analyte classes, the concentrations of the analytes found in the method blanks were generally well below the concentrations in the field samples.

In some cases, there were low levels of analytes in some method blanks. For all of the analytes in the TNSSS, EPA used a common approach to evaluating blank contamination known simply as "the 5x and 10x rules." Under routine circumstances, EPA qualified a field sample result if the concentration of an analyte was not at least 10 times the amount found in the blank. The rationale for the 10x rule is that under the worst of circumstances, in which the material found in the blank is coming from a source within the laboratory, the amount in the blank would only represent 10% of the amount in the field sample, and that small contribution is likely within the overall measurement error. If the amount in the sample is between 5x and 10x the amount in the blank, EPA normally will qualify the sample result as a maximum value because the potential contribution from the laboratory could be as high as 20%. Below 5x the amount in the blank, EPA considered the field sample result to be a non-detect at the nominal reporting limit.

The method blanks for the steroids and hormones presented greater challenges. Three of the steroid and hormone analytes, Cholesterol, Stigmasterol, and β-Sitosterol, were frequently found in the method blanks, and a fourth analyte, Progesterone, was occasionally found in those blanks. During the course of the survey, the laboratory determined that the steroids were adhering to the glassware used to prepare samples, perhaps originating from an earlier sewage sludge sample prepared in the same glassware. Although the laboratory took steps to improve its glassware cleaning procedures to prevent such "carryover," they were not able to completely eliminate the occurrence of some target analytes in the method blanks.

Therefore, EPA flagged the steroids and hormones results, where applicable. Normally, EPA evaluates results for samples that have been diluted for analysis by multiplying the blank result (which is usually not diluted) by the dilution factor for the sample. For example, if a sample extract is diluted by a factor of 50 to bring one or more target analytes within the calibration range of the instrument, EPA will multiply the concentration of an analyte found in the blank by that dilution factor of 50 before comparing the blank and the field sample results. That approach represents a worst-case assumption that the contaminant may be present in the solvent used to extract the samples, thus using more of that solvent to dilute the extract would add more of the contaminant.

In the case of the steroids and hormones, the laboratory checked its reagent water and extraction solvents and found no such contamination. As noted above, the laboratory surmised that the material in the blanks was being transferred from the surface of the glassware used to prepare the blank, but it originated from an earlier sample processed with the same glassware. Thus, the amount, or concentration, found in the blank may represent the material that the sample extract might pick up from similar glassware, but there would be no likely contribution from the solvents themselves, and thus no need to consider the dilution factors of the samples. The dilution factors for samples in thus survey often were quite high.

Even after instituting this minor change to the data review procedures, there were still 24 instances in which the levels in the blank led EPA to consider the results for one or more of the steroids to be a non-detect. These 24 instances represented 14 field samples, with 1 to 3 analytes affected in each of those samples. In many cases, the original result for the field sample was not only less than 5x the blank results it was actually 2x to 3x lower than the amount found in the blank. Of the 14 affected samples, 5 were in the first batch of 12 samples analyzed and reported. The 6 subsequent batches of samples had lower levels of these analytes in the blanks, with 2 batches having no samples set to non-detects and 2 more batches with only 1 difficult analyte. These improved blank results are evidence of the effectiveness of the revised glassware cleaning procedures instituted by the laboratory.

5.4.4 Extract Dilution

Surrogate compounds were added to each sample for the semivolatile and PAH analysis as a measure of sample extraction efficiency. However, if the sample contained large amounts of the target analytes, the laboratory often had to dilute the sample extract to bring the results for that analyte within the instrument's calibration range. If the dilution factor was high enough, the surrogates may have been present in the diluted extract at such low levels that they could not be detected. Without measurable results for the surrogate, EPA could not evaluate the efficiency of the extraction procedures for that sample.

An important part of the data review process was to ensure that results for a given sample included those where the surrogates could be measured as well as results where all of the analytes were within the calibration range. This often requires that the laboratory provide results from multiple analyses of the sample at different dilution factors. EPA worked with the laboratory analyzing the semivolatiles and PAHs to obtain data from as many analyses as practical and minimize situations in which only the most dilute analysis (without observable surrogates) was provided. However, the exceptionally high levels of bis (2-Ethylhexyl)

phthalate in some samples made the situation more difficult. EPA flagged in the database any samples in which the surrogates were diluted out.

5.4.5 Matrix Spikes

Due to the often high and variable levels of many analytes in the samples, some of the MS/MSD samples were spiked at levels too low to provide useful recovery data. This issue occurred frequently for the anions, metals, semivolatile organics and PAHs. It also was an issue for the PBDE analyses when EPA added matrix spike analyses to those analyses to make up for the method modifications that eliminated the use of true isotope dilution quantitation (see Section 4.4.4). Matrix spike samples were not required for the pharmaceutical, steroid, and hormone analyses because those methods use isotope dilution quantitation and therefore provide a sample-specific recovery correction of every analyte.

The difficulty in generating useful matrix spike results was exacerbated by the different approaches to calculating MS/MSD recovery provided in different methods. EPA initially qualified the results for samples associated with "under spiked" MS/MSD samples as estimates. However, EPA recalculated the recoveries of the spiked analytes using an alternative equation designed to address this issue (see Section 6.7) and the bias and precision of the recalculated results were further evaluated.

5.4.6 Interferences

All of the methods used in the TNSSS included some form of identification criteria for the analytes of interest. These criteria included absorption or emission wavelengths, retention times, and mass spectrometric criteria. The GC/HRMS method for the steroids and hormones involves monitoring two characteristic ions for each analyte. As with other many EPA GC/HRMS methods (e.g., Method 1613B, 1614, and 1668A), the responses for both of those ions are used to quantify the concentration of the analyte in the sample, using an approach called dual-ion quantitation.

The method for the steroids and hormones stipulates that the ratio of the abundances of the two ions must be within a certain percentage of the theoretical ratio for the analyte. The theoretical ratio is determined from natural abundances of the exact masses of all of the component elements that make up both of the ions monitored for the analyte. The acceptance criterion in the method for the steroids and hormones is a ± 30% window around the theoretical ion abundance ratio for the analyte.

The purpose of this criterion is to ensure that the analytes of interest are adequately separated from one another and from any potential interfering compounds. As described in the method, if the ion abundance ratio is not met, the analyte cannot be positively identified. The method instructs the analyst to note any instances where both ions from the analyte are present, but the ion abundance ratio acceptance criterion is not met. This approach to reporting results allow the data users to decide how best to use the data.

The most likely reason that the ion abundance ratio does not meet the acceptance criterion is that there is a positive interference for one of the two ions monitored. That positive interference increases the response for that ion, affecting the ion abundance ratio (IAR). Because the areas (abundances) for the peaks representing the two ions are added together to calculate the concentration of the analyte, the positive interference translates into a higher reported concentration for the analyte of interest. Thus, the reported result is an estimated maximum possible concentration (EMPC) for the analyte.

There were 129 instances of EMPCs in the steroid and hormone results out of the total of 2100 results, or about 6% of all steroid and hormone results. In all, 76 of the 84 survey samples had one or more analytes qualified as an EMPC, and 17 of the 25 analytes were affected. The frequencies at which EMPCs were reported are shown in Table 9. In some instances, the observed ion abundance ratio was only marginally outside of the method acceptance limits, and in other instances, the differences were much greater.

Table 9. Frequency of Estimated Maximum Possible Concentrations (EMPCs)

Analyte	# of EMPCs Reported	Analyte	# of EMPCs Reported
Campesterol	50	Androstenedione	3
Estrone	21	β-Stigmastanol	3
Testosterone	11	Equilin	3
Stigmasterol	10	17 α-Dihydroequilin	1
Ergosterol	6	Androsterone	1
Desmosterol	5	β-Estradiol 3-benzoate	1
β-Sitosterol	4	Equilenin	1
Norethindrone	4	Progesterone	1
Norgestrel	4		

The affected analytes include both steroids and hormones, and therefore represent separate analytical runs in the method.

The degree to which the reported results for an EMPC exceed the actual concentration cannot be determined exactly because a number of factors are involved, including the effect of the interferences and the fact that the results are quantified by isotope dilution. In order to place some bounds on the likely effects, EPA examined the survey results for Testosterone in detail. The 11 EMPCs for Testosterone have reported concentrations that range from 34.8 to 2040 µg/kg. The reported ion abundance ratios for these 11 Testosterone results ranged from 0.01 to 2.34, with the laboratory's QC acceptance limits for the ratio being 2.38 to 4.42. The lowest ion abundance ratio (0.01) was associated with the highest reported result (2040 µg/kg), but there is no apparent relationship between the other ratios and results.

Although the actual areas of the two peaks monitored for each analyte are presented in the hard copy raw data provided by the laboratory, these peak areas are not readily accessible in the electronic data. Rather than retrieving several hundred peak areas (2 peaks for each of 129 EMPCs) from the raw data, entering them into a spreadsheet, and checking for data entry errors, EPA took an alternative approach.

Although the actual peak areas were not in an accessible electronic format, EPA had the ion abundance ratios themselves in the database. In order to investigate the likely effects of the interferences, EPA arbitrarily assigned the area of the first peak a value of 10,000 area counts.

Using the reported ion abundance ratios, we calculated the corresponding area of the second peak. As described in the method, the concentration of an analyte is calculated from the sum of the areas of both peaks, so we summed the two "dummy" areas for each sample result. EPA also calculated the area of the second peak using the theoretical ion abundance ratio for the analyte and summed those two areas. Since the sample concentration is proportional to the sum of the areas, we compared the two calculated sums to determine a factor that could be used to convert the reported result to the result that might have been calculated without the positive interferences that affected the ion abundance ratio. The results for the 11 EMPCs for Testosterone are shown in Table 10.

Table 10. Potential Effects of Ion Abundance Ratio on Reported Concentrations of Testosterone

Sample	Dummy Abundance Mass 1	Reported IAR	Dummy Abundance Mass 2	Sum of Dummy Masses	Ratio of Sum from Reported IAR to that from Theoretical IAR	Reported Conc.	Adjusted Conc.
1	10,000	0.01	1,000,000	1,010,000	0.012813046	2040	26.1
2	10,000	0.27	37,037	47,037	0.275127374	97.9	26.9
3	10,000	0.54	18,519	28,519	0.453781513	238	108
4	10,000	0.55	18,182	28,182	0.459203036	701	322
5	10,000	0.68	14,706	24,706	0.523809524	46.9	24.6
6	10,000	1.2	8,333	18,333	0.705882353	65.7	46.4
7	10,000	1.22	8,197	18,197	0.711181770	42.3	30.1
8	10,000	1.4	7,143	17,143	0.754901961	122	92.1
9	10,000	1.99	5,025	15,025	0.861302380	238	205
10	10,000	2.04	4,902	14,902	0.868421053	34.8	30.2
11	10,000	2.34	4,274	14,274	0.906657274	67.3	61.0

The results in Table 10 are sorted by the reported ion abundance ratios. For ease of discussion, the samples addressed in this table were numbered 1 to 11, in order of reported ion abundance ratio. The sample numbers have no other significance. As can be seen for Sample 1, the potential implications of the ion abundance ratio could be dramatic. However, for many of the other samples, the effects are far less apparent. The reported concentrations already include the recovery correction inherent in isotope dilution, so the adjusted concentrations include the same degree of correction.

EPA performed similar calculations for the 50 reported EMPCs for Campesterol. Because none of the observed ion abundance ratios were as far from the theoretical ratio for this analyte, the overall effects on the reported results were smaller. The applicable conversion factors for Campesterol range from 0.173 to 0.907, meaning that the results for this analyte might be reduced by factors from 1.1 to 6. Given the time required, EPA did not perform these calculations for all the other analytes in which EMPC results were reported by the laboratory.

Note: The adjusted concentrations shown in Table 10 illustrate the *potential* change to the reported results. However, the calculations shown assume that the ion abundance ratio problem is the result of a simple positive interference in one of the two ions for the analyte. The actual cause may be more complicated. Therefore, EPA included the original results reported by the laboratory in the survey database, not the adjusted concentrations described above. EPA flagged each result in the database that did not meet the method-specified ion abundance ratio as an "EMPC."

5.4.7 Recovery Issues

Three other analytical issues were noted that had significant potential effects on the survey data for the pharmaceuticals, steroids, and hormones. EPA identified each such instance in the EPA database with the qualifier EXCLUDE to prevent those results from being used to determine the national estimates of the concentrations of pollutants in biosolids.

Two of those issues involved recoveries of the isotopically labeled analytes used to perform isotope dilution quantitation. The third issue involved the "internal standards" added to the sample extracts immediately prior to instrumental analyses and used to measure the recoveries of the labeled analytes.

There were 27 instances where EPA excluded pharmaceutical results from the database, involving 15 analytes. For 13 of those analytes, EPA excluded the results for 3 samples because the labeled compounds for those analytes were not recovered from the samples. These were not simply instances of lower than expected recoveries of the labeled compounds, but rather, no recovery at all (zero). The inability to recover the labeled compound spiked into the sample suggests a significant analytical problem beyond the routine analytical challenges presented by sewage sludge samples. All three samples were extracted on the same date and analyzed together several days later.

There were two instances where the results for the analyte Fluoxetine were excluded because the labeled compound for this analyte was not recovered from those samples.

The remaining 12 results were excluded for the analyte Minocycline. All 12 instances came from the same extraction batch and were excluded because the laboratory did not recover the native (unlabeled) analyte in the laboratory control sample (LCS, also known as an ongoing precision and recovery sample, or OPR) that was prepared at the same time as this batch of 12 samples. The laboratory did not report numerical results for Minocycline in these 12 samples and flagged all of the Minocycline results with an "NQ" flag in the database, indicating that the laboratory was not able to quantify the analyte.

There were 65 instances of steroid and hormone results being excluded from the database, involving 14 analytes. In 42 of those instances, involving 7 analytes, the data were excluded because the laboratory found only trace levels (e.g., extremely low) of the internal standard, Pyrene-d_{10}, added to the sample extract immediately before analysis and used to measure the recovery of the labeled compounds added to the sample before extraction. Trace levels of the internal standard occurred in 6 samples analyzed in the same batch, suggesting a possible problem with the addition of the internal standard (e.g., the automated injection of the internal standard may have failed or been incomplete).

The remaining 23 excluded results involved 7 other analytes where there was no recovery of the labeled compound in the same 6 samples. In these cases, the issue was not simply that the recovery could not be measured because of the very low level of the internal standard present, but rather that there was no measurable signal for the labeled compound itself.

5.5 Revised Results for BDE-209

In March 2008, the laboratory that performed the PBDE analyses in 2007 prepared a new set of calibration standards for the PBDEs, compared the responses of the new standards to data from the standards used for the TNSSS analyses, and found that the results for one of the target congeners (BDE-209) were markedly different. The laboratory also compared the results to a standard from a second source and found similar differences, ultimately tracing the difference to a vial of the original BDE-209 standard that was mislabeled by the manufacturer.

After discussions with the laboratory, EPA decided that the BDE-209 results could be recalculated based on the responses in the single-point calibration verification standard analyzed with each batch of samples and that this recalculation would mathematically adjust the results to a more accurate value. This verification standard was prepared from a separate source from the initial calibration standards and was not affected by labeling error. The laboratory recalculated the BDE-209 results for all 84 field samples and the 2 equipment blanks and resubmitted the corrected data in late May 2008. The resubmitted BDE-209 results were reviewed and uploaded to the survey database on the EPA mainframe.

Section 6
Survey Results

6.1 Summary Results

Table 11 provides a summary of the results for all 84 samples in the first phase (i.e., all analytes excluding pharmaceuticals, steroids, and hormones) of the survey, listing the number of samples in which each analyte was reported, along with the minimum and maximum concentrations. Tables 12 and 13 provide the results for the pharmaceuticals and steroids and hormones, respectively. All sample results are reported on a dry-weight basis, based on the percent solids in the original sample. The percent solids in the various sewage sludge samples range from 0.14 to 94.9. The units for pollutants vary with the class of analyte, as shown. This summary includes the results for the six field duplicate samples and the four POTWs that generate more than one type of sewage sludge. The minimum concentration is the lowest value reported as present in any sample. EPA did not report a minimum or maximum value for those analytes that were not detected. That situation only occurred for some of the pharmaceuticals, steroids and hormones, and EPA used "NA" to indicate that the minimum and maximum values were "not applicable."

Table 11. Summary of Results for Metals, Anions, Organics, and PBDEs

Class	Analyte	Units	# Detects	Observed Dry-weight Concentration	
				Minimum	Maximum
Solids	Percent Solids	%	84	0.43	93.5
Anions	Fluoride	mg/kg	84	7.6	234
	Nitrate/Nitrite		84	1.6	6,120
	Water-extractable phosphorus		84	11.0	9,550
	WEP ratio	unitless	84	0.00065	0.33920
Metals	Aluminum	mg/kg	84	1400	57,300
	Antimony		72	0.45	26.6
	Arsenic*		84	1.18	49.2
	Barium		84	75.1	3,460
	Beryllium		83	0.04	2.3
	Boron		80	5.70	204.0
	Cadmium*		84	0.21	11.8
	Calcium		84	9,480	311,000
	Chromium*		84	6.74	1160
	Cobalt		84	0.87	290
	Copper*		84	115	2,580
	Iron		84	1,575	299,000
	Lead*		84	5.81	450
	Magnesium		84	696	18,400
	Manganese		84	34.8	14,900
	Mercury*		84	0.17	8.3
	Molybdenum*		84	2.51	132
	Nickel		84	7.44	526
	Phosphorus		84	2,620	118,000
	Selenium*		84	1.10	24.7
	Silver		84	1.94	856
	Sodium		84	154	26,600
	Thallium		80	0.02	1.7
	Tin		78	7.50	522
	Titanium		83	18.50	7,020
	Vanadium		84	2.04	617
	Yttrium		84	0.70	26.3
	Zinc*		84	216	8,550

Table 11. Summary of Results for Metals, Anions, Organics, and PBDEs

Class	Analyte	Units	# Detects	Observed Dry-weight Concentration	
				Minimum	Maximum
Organics (PAHs and Semi-volatiles)	4-Chloroaniline	µg/kg	63	51	5,900
	2-Methylnaphthalene		39	10	4,600
	Fluoranthene		77	45	12,000
	Pyrene		72	44	14,000
	bis (2-Ethylhexyl) phthalate		84	657	310,000
	Benzo(*a*)pyrene		64	63	4,500
PBDEs	BDE-28	ng/kg	84	2,200	160,000
	BDE-47		84	73,000	5,000,000
	BDE-66		84	1,800	110,000
	BDE-85		84	3,200	150,000
	BDE-99		84	64,000	4,000,000
	BDE-100		84	13,000	1,100,000
	BDE-138		56	1,900	40,000
	BDE-153		84	9,100	410,000
	BDE-154		84	7,700	440,000
	BDE-183		84	2,100	120,000
	BDE-209		83	150,000	17,000,000

* Metals currently regulated at 40 CFR 503

Table 12. Summary of Results for Pharmaceuticals

Analyte	Units	# Detects	Observed Dry-weight Concentration	
			Minimum	Maximum
Percent Solids	%	84	0.14	94.9
Acetaminophen	µg/kg	2	1,120	1,300
Albuterol		1	23.2	23.2
Anhydrochlortetracycline		1	125	125
Anhydrotetracycline		52	94.3	1,960
Azithromycin		80	10.2	6,530
Caffeine		39	65.1	1,110
Carbadox		0	NA	NA
Carbamazepine		80	8.74	6,030
Cefotaxime		0	NA	NA
Chlortetracycline		1	1,010	1,010
Cimetidine		74	7.59	9,780
Ciprofloxacin		84	74.5	47,500
Clarithromycin		45	8.68	617
Clinafloxacin		0	NA	NA
Cloxacillin		0	NA	NA
Codeine		20	9.59	328
Cotinine		39	11.4	690
Dehydronifedipine		19	3.48	24.6
Demeclocycline		3	96	200
Digoxigenin		0	NA	NA
Digoxin		0	NA	NA
1,7-Dimethylxanthine		4	1,130	9,580
Diltiazem		69	1.39	225
Diphenhydramine		84	36.7	5,730
Doxycycline		76	50.8	5,090
Enrofloxacin		14	12.1	66
4-Epianhydrochlortetracycline		0	NA	NA
4-Epianhydrotetracycline		31	126	2,160
4-Epichlortetracycline		1	974	974
4-Epioxytetracycline		8	35.7	54.9
4-Epitetracycline		80	47.2	4,380
Erythromycin-total		77	3.1	180
Flumequine		0	NA	NA
Fluoxetine		79	12.4	3,130
Gemfibrozil	µg/kg	76	12.1	2,650

Table 12. Summary of Results for Pharmaceuticals

Analyte	Units	# Detects	Observed Dry-weight Concentration	
			Minimum	Maximum
Ibuprofen		54	99.5	11,900
Isochlortetracycline		1	3,140	3,140
Lincomycin		3	13.9	33.4
Lomefloxacin		2	33.3	39.8
Metformin		6	550	1,160
Miconazole		80	14.2	9,210
Minocycline		32	351	8,650
Naproxen		44	20.9	1,020
Norfloxacin		29	99.3	1,290
Norgestimate		0	NA	NA
Ofloxacin		83	73.9	58,100
Ormetoprim		1	5.91	5.91
Oxacillin		0	NA	NA
Oxolinic Acid		1	39.4	39.4
Oxytetracycline		29	18.6	467
Penicillin G		0	NA	NA
Penicillin V		0	NA	NA
Ranitidine		46	3.83	2,250
Roxithromycin		3	14.3	22.8
Sarafloxacin		2	179	1,980
Sulfachloropyridazine		2	35.9	58.7
Sulfadiazine		3	22.9	140
Sulfadimethoxine		5	3.58	62.2
Sulfamerazine		1	5.61	5.61
Sulfamethazine		2	21.5	23.2
Sulfamethizole		0	NA	NA
Sulfamethoxazole		30	3.91	651
Sulfanilamide		8	191	15,600
Sulfathiazole		1	21	21
Tetracycline		81	38.3	5,270
Thiabendazole		58	8.42	239
Triclocarban		84	187	441,000
Triclosan		79	430	133,000
Trimethoprim		24	12.4	204
Tylosin		0	NA	NA
Virginiamycin		15	43.5	469
Warfarin		0	NA	NA

NA = Not applicable, because the analyte was not reported in any sample

Table 13. Summary of Results for Steroids and Hormones

Analyte	Units	# Detects	Observed Dry-weight Concentration	
			Minimum	Maximum
Percent Solids	%	84	0.14	94.9
Androstenedione	µg/kg	32	108	1,520
Androsterone		50	21.3	1,030
Campesterol		84	2,840	524,000
Cholestanol		84	3,860	4,590,000
Cholesterol		81	18,700	5,390,000
Coprostanol		84	7,720	43,700,000
Desmosterol		58	2,730	94,400
17 α-Dihydroequilin		1	98.4	98.4
Epicoprostanol		83	868	6,030,000
Equilenin		1	60.6	60.6
Equilin		15	22.3	107
Ergosterol	µg/kg	53	4,530	91,900
17 α-Estradiol		5	16.1	48.8

Table 13. Summary of Results for Steroids and Hormones

Analyte	Units	# Detects	Observed Dry-weight Concentration	
			Minimum	Maximum
17 β-Estradiol		11	22	355
β-Estradiol 3-benzoate		18	30.2	1850
17 α-Ethinyl-estradiol		0	NA	NA
Estriol		18	7.56	232
Estrone		60	26.7	965
Norethindrone		5	21	1,360
Norgestrel		4	43.8	1,300
Progesterone		19	143	1,290
β-Sitosterol		73	24,400	1,640,000
β-Stigmastanol		83	3,440	1,330,000
Stigmasterol		76	11,000	806,000
Testosterone		17	30.8	2,040

NA = Not applicable, because the analyte was not reported in any sample

6.2 Investigation of Results for Metals

After compiling the results from the first phase of the survey, EPA investigated the potential causes for the maximum results for calcium, iron, phosphorus, and silver. By reviewing the sampler's field notes and ultimately contacting the POTWs by telephone, EPA found that:

- The maximum result for calcium (311,000 mg/kg) was for a sample of Class A sewage sludge produced by a process known as advanced alkaline stabilization with subsequent drying. The alkaline stabilization process involves addition of large amounts of lime (calcium carbonate) to the material. The final sewage sludge is sold as a soil amendment.

- The maximum concentrations of iron (299,000 mg/kg) and elemental phosphorus (118,000 mg/kg) occurred in the same sample. The facility from which this sample was collected adds ferric chloride during its wastewater treatment process to reduce the level of phosphorus in its effluent discharge. This treatment step results in high levels of iron and phosphorus in the sewage sludge from this plant.

- The maximum result for silver (856 mg/kg) occurred in a sewage sludge sample from a POTW that employs a "complete mix activated sewage sludge process" and disposes of its sewage sludge by incineration. There are two major industrial dischargers to this POTW, but neither employs silver. The plant is not aware of any other instances of high silver results in their sewage sludge. There were no apparent calculation or transcription errors. The laboratory noted that this result was determined by ICP/MS, and when the laboratory re-examined its result for the ICP/AES analysis of the same sample, silver was present in that analysis at about 900 mg/kg, thus seemingly confirming the ICP/MS results.

6.3 Comparison of Metals Results to Current Standards

As noted in Section 1 of this report, the sewage sludge regulations at Part 503 include standards for land application of nine metals. These standards are based on the dry-weight concentrations. Table 14 illustrates the maximum results from this survey for the nine metals.

Table 14. Comparison of Survey Maximums to Existing Regulatory Limits

Pollutant	Dry-Weight Concentration in mg/kg		Number of TNSSS Results Over Ceiling
	Land application ceiling	Survey Maximum	
Arsenic	75	49.2	0
Cadmium	85	11.8	0
Copper	4,300	2,580	0
Lead	840	450	0
Mercury	57	8.3	0
Molybdenum	75	**132**	2
Nickel	420	**526**	3
Selenium	100	24.7	0
Zinc	7,500	**8,550**	1

Maximum results that exceed the land application ceiling are shown in **bold.**

Note: It is critical to note that the selection of facilities to be sampled in this survey was *not* based on whether they managed their sewage sludge by land application, nor was a goal of this survey to assess compliance. In fact, a number of the facilities disposed of their sewage sludge by incineration or placement in a landfill, and those facilities need not meet the ceiling concentrations shown in Table 14.

As shown in **bold** in Table 14, three metals had observed concentrations in this survey that exceeded the land application ceiling concentrations (molybdenum, nickel, and zinc). The maximum observed concentrations for all other regulated metals were below the land application ceiling concentrations.

Five samples in this survey, collected from four facilities, contained metals that exceeded the land application limits (two of those samples were a pair of field duplicates collected from one facility). One sample exceeded the limits for both molybdenum and nickel. Of the four facilities involved, one incinerates its sewage sludge on site and the other three send their sewage sludge to landfills. None of the facilities that actually dispose of their sewage sludge by land application exceeded the limits.

6.4 Analytical Completeness

"Completeness" is a quality assurance measure of the number of samples collected compared to the number of useable results produced. Although the laboratories experienced a number of difficulties with the samples from this survey and not all of the results met all of the acceptance criteria in the applicable analytical methods, laboratories made acceptable efforts to overcome these challenges and adequately document any QC issues encountered. In all cases laboratories provided acceptable documentation for every sample in the survey.

6.5 Analytical Sensitivity

As noted earlier in this report, the two previous national sewage sludge surveys experienced analytical sensitivity challenges for some analytes. This was largely due to the co-extracted interferences present in the challenging matrix and the wide variation in the solids content of treated sewage sludge disposed of nationwide. EPA designed the TNSSS for practical analytical sensitivity, taking the steps outlined in Section 4 to ensure that the results were directly comparable across facilities in the survey.

Table 7 lists the target reporting limits for all classes of analytes in the survey. Those targets were based on EPA's decision of what was practical in sewage sludge. The anions were found in every sample and all but six metals were found in the 100 percent of the survey samples (see Table 11). Thus, sensitivity is not a concern. Therefore, the focus of analytical sensitivity is on the actual reporting limits for the other analyte classes and on the metals that were *not* found in every sample (i.e., antimony, beryllium, boron, thallium, tin, and titanium). Table 15 provides a comparison of those target limits with the actual reporting limits.

Table 15. Analytical Sensitivity

Analyte Class	Target Reporting Limit (dry weight)	Actual Reporting Limit for Non Detects	
Metals	3 to 4 mg/kg	Antimony	0.05 mg/kg
		Beryllium	0.02 mg/kg
		Boron	5 mg/kg
		Thallium	0.02 mg/kg
		Tin	5 mg/kg
		Titanium	2 mg/kg
PAHs and semivolatiles	100 to 300 µg/kg	4-Chloroaniline	10 µg/kg (by SIM)
		2-Methylnaphthalene	
		Fluoranthene	
		Pyrene	
		Benzo(a)pyrene	
		bis (2-Ethylhexyl) phthalate	NA
PBDEs	5 to 200 ng/kg	BDE 138	5000 ng/kg
Anions	2 to 8 mg/kg	All anions detected in all samples	NA

NA = Not applicable, analyte reported in all samples in the survey

As can be seen in Table 15, the laboratories not only met, but far exceeded, the majority of the sensitivity targets. The reporting limits for boron and tin were only slightly higher than the target range. Four of the six samples in which tin was not reported include situations where the results for tin in the samples and their associated method blank differed by less than a factor of five. As part of the data review process described in Section 5, the tin results were reset in those four samples to non-detects at the nominal reporting level.

Bis (2-Ethylhexyl) phthalate is a common laboratory contaminant and EPA reviewed the results for all of the method blanks to ensure that the laboratory was not the source of the bis (2-Ethylhexyl) phthalate in the samples. Although the laboratory reported bis (2-Ethylhexyl) phthalate in many of the method blanks for organics, the levels in the samples are two to four orders of magnitude higher than the levels in the blanks, indicating that sensitivity was not an issue.

The reporting limits for the other five organics were well below the target range using the selected ion monitoring modifications described in Section 4. The full-scan GC/MS results

included in the database are for samples in which the majority of the analytes were present above the original target reporting limits in Table 11, thus sensitivity was not an issue for those samples.

All of the PBDE congeners except BDE-138 and BDE-209 were detected in all of the survey samples, often at high levels relative to the calibration range of the method. There was only one non-detect for BDE-209. BDE-138 was not detected in 30 of the survey samples with the reporting limit of 5,000 ng/kg. That reporting limit is significantly higher than the original target, but reflects the need to adjust the sample size and extract dilution to accommodate the very high levels of the other congeners that the laboratory reported in the survey samples, which were often 10 to 20 times higher than the BDE-138 concentrations. Therefore, sensitivity was not a significant issue for the PBDE analyses.

Because the methods for the pharmaceuticals, steroids, and hormones were under development at the time the TNSSS began, EPA did not set any goals for sensitivity for these analytes. The reporting limits are based on the "Minimum Levels" in the methods, which were not optimized for the analysis of sewage sludge samples, but apply to all solid matrices. The Minimum Level is the concentration in the sample that is equivalent to the concentration of the lowest calibration standard analyzed by the laboratory. When used as a reporting limit, that value is adjusted for the nominal sample size and the moisture content of the sample (i.e., it is a dry-weight concentration). The advantage of the approach used for the TNSSS to ensure consistent sensitivity is that the reporting limit for each analyte was the essentially the same for all the samples, regardless of the moisture content of the original sample.

The Minimum Levels for the 72 pharmaceuticals vary by analyte, and range from 2 µg/kg for analytes such as Albuterol, Digoxin, and Erythromycin, to 1,000 µg/kg for 1,7-Dimethyl-xanthine. The Minimum Levels for the steroids and hormones ranged from 21 µg/kg for Testosterone, to 2,500 µg/kg for Cholesterol. Table 16 presents the Minimum Levels for the pharmaceuticals and Table 17 presents the Minimum Levels for the steroids and hormones.

Table 16. Minimum Levels for the Pharmaceuticals

Minimum Level µg/kg (dry-weight)			
2	**4**	**10**	**20**
Albuterol	Cimetidine	Azithromycin	Cloxacillin
Digoxin	Dehydronifedipine	Carbadox	Codeine
Diltiazem	Diphenhydramine	Carbamazepine	Enrofloxacin
Erythromycin-total	Ormetoprim	Clarithromycin	Lincomycin
Roxithromycin	Oxolinic Acid	Cotinine	Lomefloxacin
Sulfadimethoxine	Ranitidine	Flumequine	Naproxen
	Sulfamerazine	Fluoxetine	Norgestimate
	Sulfamethazine	Gemfibrozil	Oxacillin
	Sulfamethizole	Miconazole	Penicillin G
	Sulfamethoxazole	Ofloxacin	Triclocarban
		Sulfachloropyridazine	
		Sulfadiazine	
		Sulfathiazole	
		Thiabendazole	
		Trimethoprim	
		Warfarin	
Minimum Level µg/kg (dry-weight)			
40	**100**	**400**	**1,000**
Ciprofloxacin*	Sarafloxacin†	Metformin▲	1,7-Dimethylxanthine
4-Epioxytetracycline	4-Epianhydrotetracycline	4-Epianhydrochlortetracycline	
4-Epitetracycline	4-Epichlortetracycline	Acetaminophen	
Cefotaxime	Anhydrochlortetracycline	Minocycline	
Chlortetracycline	Anhydrotetracycline	Triclosan	
Clinafloxacin	Caffeine		
Doxycycline	Demeclocycline		
Isochlortetracycline	Digoxigenin		
Oxytetracycline	Ibuprofen		
Penicillin V	Norfloxacin		
Tetracycline	Sulfanilamide		
Tylosin			

There were 3 analytes with unique MLs. They were grouped with other analytes in this table for simplicity. The actual MLs are shown below.

* Actual ML = 35 µg/kg

† Actual ML = 91 µg/kg

▲ Actual ML = 200 µg/kg

Table 17. Minimum Levels for the Steroids and Hormones

Minimum Level µg/kg (dry-weight)		
21	**42**	**104**
17 α-Dihydroequilin	Norgestrel	Androstenedione
17 α-Estradiol		Progesterone
17 α-Ethinyl-estradiol		
17 β-Estradiol		
Androsterone		
Equilenin		
Equilin		
Estriol		
Estrone		
Norethindrone		
Testosterone		
β-Estradiol 3-benzoate		
Minimum Level µg/kg (dry-weight)		
500	**1,500**	**2,500**
Campesterol	β-Sitosterol	Cholesterol
Cholestanol	β-Stigmastanol	Desmosterol
Coprostanol		Ergosterol
Epicoprostanol		
Stigmasterol		

There were 15 pharmaceuticals and 1 hormone that were not detected in any sample. EPA examined the supporting QC data, such as the OPR samples and labeled compound recoveries, looking for indications of methodological issues.

There were several pharmaceutical analytes that exhibited occasional low recoveries in an OPR aliquot, no recoveries in an OPR aliquot, or low labeled compound recoveries in a sample. For example, 1,7-Dimethylxanthine was only found in 4 samples, and there were 12 samples (prepared in one batch) that were associated with an OPR aliquot with low recovery of this analyte. The other 72 samples were analyzed in 6 other batches associated with acceptable OPR recoveries for 1,7-Dimethylxanthine.

There were 13 results for Warfarin where the sample exhibited low labeled compound recovery and EPA qualified the non-detect results for the analyte. There were no issues with labeled compound recoveries or OPR recoveries for Warfarin in the other 71 samples in the survey. Therefore, EPA does not believe that the fact that Warfarin was not detected in any sample is an indication of a methodological challenge (i.e., that the method did not work). Rather, it may be related to method sensitivity or breakdown of this analyte during sewage treatment.

For the steroids and hormones, 17 α-Ethinyl-Estradiol was the one analyte that was not reported in any sample. EPA noted four instances where the labeled compound associated with this analyte was not recovered at all, leading EPA to exclude those four non-detect results from the survey database. In three other instances, EPA noted that the recovery of the labeled compound was low, but there was some recovery. However, EPA did not note OPR issues for this analyte, which suggests that in those specific samples there may be issues associated with the specific biosolids samples being analyzed rather than pervasive analytical problems.

6.6 Equipment Blank Evaluation

Equipment blanks were prepared as described in Section 3.11 in each of the laboratories that analyzed sewage sludge samples in the first phase of the TNSSS. Equipment blanks were prepared for anions, metals, semivolatile organics and PAHs, and PBDEs. Equipment blanks were not prepared for the pharmaceuticals, steroids, and hormones in the second phase of the TNSSS.

EPA evaluated the results for the equipment blanks by comparing them to their associated method blanks and to the sample results. This was done to determine if any analytes of concern were present at levels that might affect EPA's use of the data.

6.6.1 Semivolatile Organics and PAHs

For semivolatile organics and PAHs, the laboratory reported only one target analyte in either equipment blank, BEHP, at a concentration of 10 μg/kg, based on a nominal 10-g sample weight. However, BEHP is a common laboratory contaminant and the method blank associated with both equipment blanks was reported to contain 11 μg/kg. In addition, all of the field sample results for BEHP were orders of magnitude higher than either the method blank or equipment blank results, ranging from 657 to 310,000 μg/kg. Therefore, there is no evidence that the compositing equipment contributed BEHP to any of the field samples.

6.6.2 Metals

The laboratory reported low concentrations of eight elements in the two equipment blanks. Table 18 presents the results for the two equipment blanks. The reported concentrations are based on the nominal 1-g dry-weight aliquot used for the field samples. The laboratory reported all other metals as non-detects in the two equipment blanks.

The concentrations of some of the metals in the equipment blanks exceeded the concentrations in the associated method blank, although in some instances only marginally. For example, the laboratory reported nickel in the method blank at 0.05 mg/kg, and at 0.1 mg/kg in one equipment blank, but nickel was not detected in the other equipment blank. The laboratory reported lead in one equipment blank at 0.03 mg/kg, which is only marginally above the laboratory's reported detection limit of 0.02 mg/kg for lead, and lead was not detected in the other equipment blank. Therefore, the equipment blank results are not unexpected, or of significant concern.

Table 18. Equipment Blank Metal Results

Analyte	Concentration (mg/kg)	
	Equipment Blank 1	Equipment Blank 2
Barium	27.2	27.9
Calcium	26.9	22.3
Copper	1.33	1.56
Lead	0.03	ND
Magnesium	2.8	2.1
Manganese	0.07	ND
Nickel	0.1	ND
Zinc	3.3	6.7

ND = not detected

The concentrations in the field samples generally were several orders of magnitude higher than those in the equipment blanks (see Table 11). However, EPA took a conservative approach to evaluating the potential impact of the equipment blanks. EPA compared the results for each analyte in each of the solid samples against the results for both equipment blanks and flagged in the database any solid sample result that was not at least five times higher than the result in either of the two equipment blanks.

Barium was the only metal analyte affected, with the results in four solid sewage sludge samples less than five times the results in the equipment blanks (27.2 and 27.9 mg/kg, respectively). Those results are shown in Table 19. The value of 5 is a multiplier that is often used for the evaluation of blanks. It indicates that the amount of contaminant in the equipment blank might account for 20% or more of a given field sample result.

Table 19. Comparison of Sample Results and Equipment Blank Results for Barium

Sample	Result (mg/kg	Ratio of Sample Result to Equipment Blank Result	
		Sample/Equipment Blank 1	Sample/Equipment Blank 2
68320	128	4.7	4.6
68343	75.1	2.8	2.7
68344	78.6	2.9	2.8
68393	134	4.9	4.8

The fact that the results for these four samples are less than five times the equipment blank results is not clear evidence that any portion of the barium in the samples was actually derived from the sampling equipment. As noted in Section 3, equipment blanks for solids are conceptually different than those for aqueous samples and sampling equipment. In the 2001 NSSS, equipment blanks were prepared by placing wet sand in the compositing bowls used for collecting samples. Aliquots of the sand had been analyzed for dioxins, furans, and PCBs and the sand was found to be free of these analytes, so it could be mixed with reagent water to simulate wet sewage sludge. Equipment blanks prepared in that manner in the 2001 survey demonstrated that the stainless steel equipment used to collect samples did not contribute any dioxins, furans, or PCBs.

In this survey, sand could not be used for equipment blanks because it contains metals. No readily available solid reference material is free of metals and resembles sewage sludge, so it was not practical to prepare equipment blanks for solids that mimic the way that the sample comes in contact with the sampling equipment.

6.6.3 PBDEs

The laboratory did not detect any of the PBDE congeners in the two equipment blanks above their nominal reporting limits. The reporting limits for these blanks ranged from about 100 ng/kg to 20,000 ng/kg, for the various congeners, based on a nominal sample size of 10 g. However, because the sample size for the field samples was reduced to 0.2 g, EPA examined the equipment blank in greater detail. In addition to using reporting limits that were based on the low-point of the instrument calibration, the laboratory estimated the signal-to-noise based detection limits for each analyte in each sample. Those estimated detection limits were markedly lower than the nominal reporting limits (e.g., 1 ng/kg versus 100 ng/kg). The low levels of PBDEs detected in the equipment blanks were similar to those reported in the method blanks, indicating that the sampling equipment did not contribute additional PBDEs to the samples.

6.6.4 Anions

The equipment blanks for the anions analysis contained low levels of fluoride and nitrate/nitrite. The results for both analytes in both equipment blanks were below the laboratory's reporting limits, but above their detection limits, and the laboratory reported the results as "estimated." Neither analyte was detected in the method blanks associated with the equipment blanks or the field samples. Table 20 presents the results for the anions in the two equipment blanks.

Table 20. Equipment Blank Anion Results

Analyte	Concentration (mg/kg)	
	Equipment Blank 1	Equipment Blank 2
Nitrate/Nitrite	5.2	1.9
Fluoride	11	18

The equipment blank results for water-extractable nitrate/nitrite are two to five times lower than the laboratory's nominal reporting limit. The concentration of water-extractable nitrate/nitrite in the field samples ranges from 1.6 mg/kg to more than 6,100 mg/kg. Of 41 sewage sludge samples that contained less than 10 mg/kg of nitrate/nitrite, 29 of those are solid samples that came in contact with the plastic sampling equipment. However, the other 12 samples with less than 10 mg/kg nitrate/nitrite are liquid sewage sludge that was never in contact with the equipment. Because of this, EPA concluded that nitrate/nitrite levels observed in blanks were acceptable for the purposes of this report.

Fluoride is added to drinking water by many municipal water systems for its dental decay prevention benefits. It also is present in toothpastes and mouthwashes that are rinsed down the drain after use. Fluoride is soluble in water, which is, in fact, the basis of the analytical results for this survey, as the samples were leached with reagent water as described in Appendix A, and the leachate was analyzed for all of the anions. Figure 2 is a plot of the fluoride concentrations versus the percent solids in each survey sample.

Water is removed from sewage sludge by a variety of means, including several types of presses, centrifugation, and air drying. Given the solubility of fluoride, one would expect that water removed by mechanical means such as presses and centrifugation would take some portion of fluoride with it, leaving the solids with lower concentrations then at the start of the water removal process.

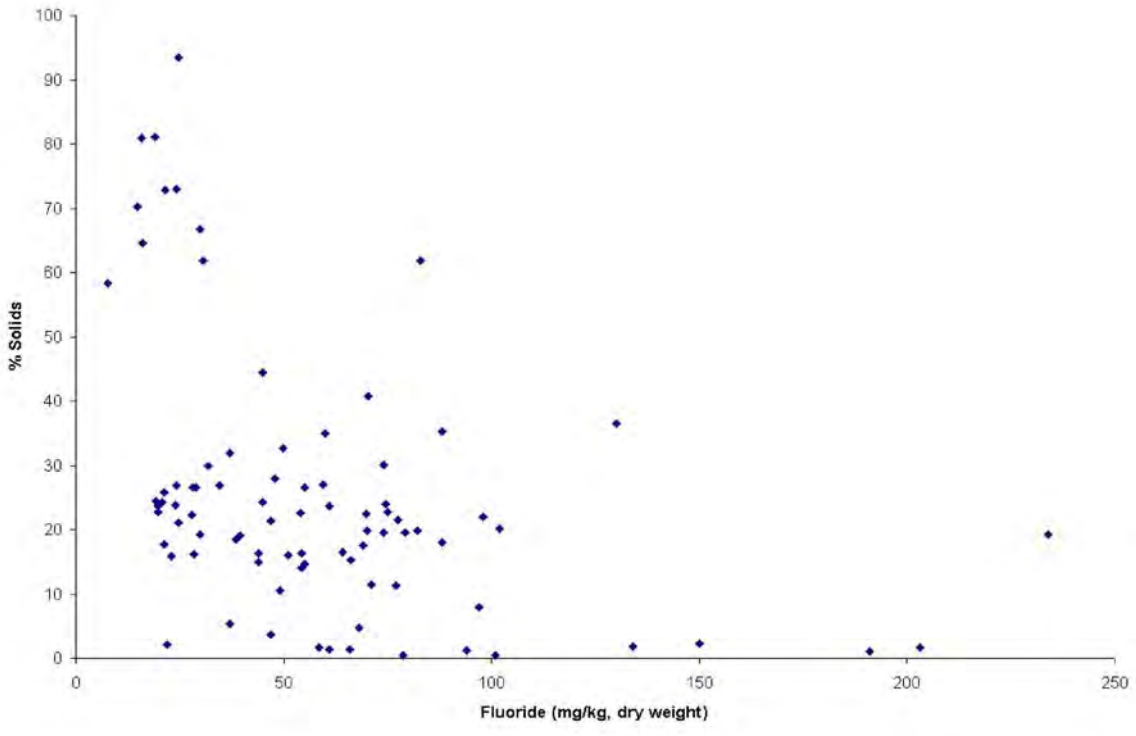

Figure 2. Plot of Fluoride Concentration versus Percent Solids

As can be seen in Figure 2, there is an inverse relationship between solids and fluoride in these samples, with most of the highest fluoride concentrations in the samples with the lowest solids contents.

Note: All of the fluoride results are reported on a dry-weight basis in this survey, so the results are directly comparable across samples with different percent solids.

The presence of fluoride in the two equipment blanks may indicate that the reagent water rinse did not remove some of the fluoride in the tap water used to wash the equipment. As with the nitrate/nitrite results, the amounts of fluoride reported in the two equipment blanks are 2 to 4 times lower than the laboratory's reporting limits. EPA treated the fluoride results in the field samples in the same fashion described above for nitrate/nitrite, flagging any results in solid samples less than five times the higher equipment blank result as an estimate, but retained the data in the database. A total of 62 fluoride results for solid samples were flagged in the database. However, there also were 12 liquid samples with fluoride concentrations in that same range of up to five times the higher equipment blank result. These samples never contacted the sampling equipment, so the fluoride in them cannot be attributed to the equipment.

As noted in Section 3, the sampler mixed a large quantity of sewage sludge in each plastic bowl, and only placed a portion of that material in jars for analysis. The moisture in the original sewage sludge samples was not free flowing for the solid samples, thus it would not contact the surfaces of the equipment in the same way that the reagent water used to prepare the anions equipment blank did. EPA does not believe that the qualifiers applied to these results significantly compromise data usability.

6.7 Field Duplicate Results

As part of the quality assurance effort, EPA collected field duplicate samples to assess the overall precision of the sampling and analysis approach for the survey. Of the 80 facilities originally selected for sampling, 8 were chosen at random for collection of a field duplicate. As described in Section 3, two of those facilities were not sampled and not replaced, and one other field duplicate was collected at a different plant than originally planned.

The tables in this section present the relative percent differences (RPDs) between the two results in each of the six pairs of field duplicate samples, for each analytical class. The RPD is used as the measure of precision because both results from the pair are measured concentrations and there is no "true" concentration to be used in the comparison. The formula for RPD is shown below where Result 1 and Result 2 represent the concentrations reported in the two samples in each pair. The vertical bars in the numerator indicate it is the absolute value of the difference, and the factor of 100 converts the value to a percentage.

$$\mathrm{RPD} = \frac{|\mathrm{Result\,1} - \mathrm{Result\,2}|}{\frac{(\mathrm{Result\,1} + \mathrm{Result\,2})}{2}} \times 100$$

In cases where both of the results in a field duplicate pair were non-detects, there was little point in comparing the sample-specific reporting limits. EPA indicated these instances with ND, for "not detected." When only one of the results in a field duplicate pair was a non-detect, EPA did not calculate the RPD and indicated these instances with NC, for "not calculated."

EPA did not establish a formal acceptance limit for the RPD of field duplicates in this project. However, 50% is often used as a default limit that reflects the sum of the anticipated analytical variability and the variability in the sample collection process.

6.7.1 Anions

Table 21 summarizes the field duplicate results for the anions. The majority of the RPD values for the anions are less than 20%, and are well within the expected variability of *laboratory* duplicate analyses. Using the default limit of 50% for *field* duplicates, the 16 of 24 observed RPD values in Table 21 that are below 20% indicate better than anticipated results for these QC samples.

Table 21. Comparison of Field Duplicate Results for Anions

	Relative Percent Difference (%)					
Analyte	**Pair 1**	**Pair 2**	**Pair 3**	**Pair 4**	**Pair 5**	**Pair 6**
Fluoride	11.9	18.3	8.0	35.1	8.1	3.6
Nitrate/Nitrite	19.0	17.1	16.1	15.7	2.2	36.2
Water-Extractable Phosphorus	84.3	26.0	33.4	39.5	9.0	20.5
Total Solids	8.1	0.2	0.8	58.7	0.9	1.0

The RPD values above 20% occur primarily for the water-extractable phosphorus (WEP) and most of the analytes in Pair 4. The WEP results are more variable than those for fluoride or nitrate/nitrite. All three analytes are extracted from the sewage sludge at the same time using the same leaching procedure. The WEP differences between some of the duplicate pairs may simply be due to variability.

Field duplicate Pair 4 exhibited large RPD values for the anions and all other analytes (see Tables 21 – 26). EPA examined the sample collection information and found that this was a liquid sewage sludge collected from a large storage tank. Because of safety concerns at the facility, the sampler observed as one of the facility staff opened a series of valves, flushed liquid sewage sludge through the piping, and collected each aliquot of the first sample, then collected the second sample. The percent solids results for these two samples are 1.85% and 1.01% in the aliquots use for the anions analyses. However, the percent solids in the aliquots used for the metals analyses are 4.27% and 1.01%, while the aliquots for the organics are 0.61% and 3.2% respectively. These data for total solids suggest that liquid sewage sludge was not particularly homogeneous and the sampling procedures used for this facility did not result in true duplicate samples.

6.7.2 Metals

Table 22 presents the field duplicate results for the metals. The majority of the RPD values for the metals (125 of 174) are less than 20%, and 135 RPD values are less than 30%. As with the anions, results for field duplicate Pair 4 are markedly higher than the other five pairs.

Table 22. Comparison of Field Duplicate Results for Metals

Analyte	Relative Percent Difference (%)					
	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6
Aluminum	0.0	1.3	4.2	100.5	7.7	0.7
Antimony	ND	3.6	3.0	97.5	19.3	2.3
Arsenic	6.2	0.0	4.5	109.2	30.8	7.3
Barium	4.1	59.8	4.6	100.7	10.8	1.5
Beryllium	0.0	3.6	0.0	83.3	25.0	7.4
Boron	13.7	2.9	1.6	115.1	12.6	0.1
Cadmium	7.1	1.7	3.7	106.1	31.5	7.3
Calcium	4.3	1.9	2.5	112.2	11.2	1.5
Chromium	7.5	6.8	27.9	120.3	29.4	0.9
Cobalt	2.7	0.2	4.6	110.4	22.4	9.1
Copper	3.4	1.7	5.4	100.7	10.3	2.9
Iron	4.7	3.6	2.5	106.5	9.6	0.6
Lead	7.1	0.6	3.9	105.4	30.5	8.5
Magnesium	10.5	0.7	4.9	112.5	8.5	1.2
Manganese	4.8	5.4	3.1	105.8	12.1	1.0
Mercury	25.6	95.3	2.7	89.5	11.8	7.1
Molybdenum	9.1	5.0	0.5	112.6	31.2	8.4
Nickel	5.1	3.3	4.3	107.9	27.7	11.4
Phosphorus	4.7	0.6	4.3	106.1	11.0	0.6
Selenium	2.7	1.1	12.5	118.7	31.3	9.8
Silver	5.0	1.8	10.2	95.4	8.8	16.9
Sodium	1.6	41.8	4.2	121.6	5.2	3.2
Thallium	6.1	3.1	0.0	112.5	41.4	0.0
Tin	6.6	0.5	ND	94.1	9.3	23.5
Titanium	9.9	3.9	27.2	96.8	4.6	16.2
Vanadium	8.3	4.1	3.6	100.7	28.6	8.7
Yttrium	8.1	1.6	4.3	107.5	28.0	8.2
Zinc	4.6	1.6	5.4	106.5	10.7	1.5
Total Solids	0.4	1.2	0.4	123.5	2.7	1.0

ND = not detected in either sample in the duplicate pair

6.7.3 Semivolatile Organics and PAHs

Table 23 presents the field duplicate results for the semivolatile organics and PAHs. A total of 29 of 42 RPDs are less than 20% and 31 of 42 RPDs are less than 50%. The issues with Pair 4 are apparent in the organics data as well (see Section 6.7.7).

Table 23. Comparison of Field Duplicate Results for Semivolatile Organics and PAHs

Analyte	Relative Percent Difference (%)					
	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6
2-Methylnaphthalene	12.7	36.6	ND	ND	ND	17.1
4-Chloroaniline	NC	7.4	8.0	41.8	12.7	5.0
Benzo(a)pyrene	2.4	3.1	ND	143.9	8.7	17.0
bis (2-Ethylhexyl) phthalate	16.7	3.6	0.0	157.8	15.9	10.8
Fluoranthene	16.7	0.0	0.0	144.3	2.1	14.2
Pyrene	0.0	6.9	ND	128.9	5.7	8.9
Total Solids	1.2	4.3	1.2	136.0	10.6	1.0

ND = not detected in either sample in the duplicate pair
NC = not detected in one sample in the duplicate pair and therefore RPD was not calculated

6.7.4 PBDEs

Table 24 presents the field duplicate results for the PBDEs. A total of 36 of 72 RPD values are less than 20% and 58 of 72 are less than 40%. Field duplicate Pair 4 exhibits higher RPD values than then other five pairs, but the differences are not as marked as for the other analyte classes. It is possible that the difference may be a reflection of the smaller sample size used for the PBDE analyses compared to the other classes. Due to the concentrations of PBDEs in biosolids, which can interfere with analysis, the laboratory extracted just 0.2 g of the sewage sludge, compared to samples of up to 10 g for other samples and analytes. Extracting a smaller sample eliminated the need for repeated dilution of the sample, resulted in fewer burdens to (i.e., didn't overwhelm) the laboratory equipment, and made quantitation possible.

Table 24. Comparison of Field Duplicate Results for PBDEs

	Relative Percent Difference (%)					
Analyte	**Pair 1**	**Pair 2**	**Pair 3**	**Pair 4**	**Pair 5**	**Pair 6**
BDE-28	19.6	16.7	11.2	42.4	21.9	19.4
BDE-47	16.7	22.2	9.1	35.3	26.3	9.5
BDE-66	28.0	10.0	6.5	25.6	2.7	14.6
BDE-85	45.3	25.6	2.3	40.0	30.8	20.2
BDE-99	17.4	15.1	19.0	48.6	27.8	1.0
BDE-100	14.7	22.2	9.5	48.6	34.2	14.6
BDE-138	NC	34.3	ND	44.6	ND	8.0
BDE-153	18.2	15.4	11.1	45.0	27.9	8.7
BDE-154	21.1	15.0	13.3	45.1	28.6	10.5
BDE-183	16.9	18.2	10.3	46.2	28.6	19.4
BDE-209	33.0	32.7	4.9	44.7	41.9	25.0
Total Solids	4.6	7.1	2.5	38.9	28.2	0.0

ND = not detected in either sample in the duplicate pair
NC = not detected in one sample in the duplicate pair and therefore RPD was not calculated

6.7.5 Pharmaceuticals

Table 25 presents the field duplicate results for the pharmaceuticals. As noted earlier, in cases where both of the results in a field duplicate pair were non-detects, EPA indicated these instances with ND, for "not detected." There are 270 instances of NDs for the pharmaceuticals.

When only one of the results in a field duplicate pair was a non-detect, EPA did not calculate the RPD and indicated these instances with NC, for "not calculated." There were 18 instances of NCs for the pharmaceuticals. In Table 25, **bold** is used to indicate all of the RPD values that exceeded 50%.

Table 25. Comparison of Field Duplicate Results for Pharmaceuticals

	Relative Percent Difference (%)					
Analyte	**Pair 1**	**Pair 2**	**Pair 3**	**Pair 4**	**Pair 5**	**Pair 6**
Acetaminophen	ND	ND	ND	ND	ND	ND
Albuterol	ND	ND	ND	NC	ND	ND
Anhydrochlortetracycline	ND	ND	ND	ND	ND	ND
Anhydrotetracycline	13.9	NC	11.2	31.8	22.6	15.3
Azithromycin	14.8	9.4	9.38	**50.9**	25.6	18.9
Caffeine	ND	ND	3.71	**72.4**	21.4	ND
Carbadox	ND	ND	ND	ND	ND	ND
Carbamazepine	18.4	6.0	9.32	38.2	11.1	19.7
Cefotaxime	ND	ND	ND	ND	ND	ND

Table 25. Comparison of Field Duplicate Results for Pharmaceuticals

Analyte	Relative Percent Difference (%)					
	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6
Chlortetracycline	ND	ND	ND	ND	ND	ND
Cimetidine	3.6	34.4	**73.1**	**116**	24.5	17.0
Ciprofloxacin	38.4	5.5	0.54	**50.7**	2.0	5.5
Clarithromycin	ND	ND	20.6	19.0	ND	6.4
Clinafloxacin	ND	ND	ND	ND	ND	ND
Cloxacillin	ND	ND	ND	ND	ND	ND
Codeine	ND	ND	ND	39.4	NC	ND
Cotinine	ND	10	10.5	NC	27.3	19
Dehydronifedipine	ND	ND	27.8	27.3	ND	ND
Demeclocycline	ND	ND	ND	ND	ND	ND
Digoxigenin	ND	ND	ND	ND	ND	ND
Digoxin	ND	ND	ND	ND	ND	ND
Diltiazem	NC	34.1	**50.5**	32.9	46.4	2.96
1,7-Dimethylxanthine	ND	ND	ND	ND	ND	ND
Diphenhydramine	22.0	0.8	11.9	15.8	29.5	6.23
Doxycycline	48.1	27.4	15.7	17.4	15.8	4.55
Enrofloxacin	ND	ND	ND	18.9	7.2	ND
4-Epianhydrochlortetracycline	ND	ND	ND	ND	ND	ND
4-Epianhydrotetracycline	ND	NC	2.5	36.3	35.0	5.30
4-Epichlortetracycline	ND	ND	ND	ND	ND	ND
4-Epioxytetracycline	ND	ND	ND	ND	ND	NC
4-Epitetracycline	37.6	27.8	24.4	**55.2**	3.24	18.2
Erythromycin-total	**51.7**	44.1	2.25	32.5	**138**	22.2
Flumequine	ND	ND	ND	ND	ND	ND
Fluoxetine	25.5	10.1	40.3	47.9	7.5	18.5
Gemfibrozil	3.4	13.1	7.8	**71.1**	4.7	2.6
Ibuprofen	ND	2.2	14.2	**119.1**	3.0	0
Isochlortetracycline	ND	ND	ND	ND	ND	ND
Lincomycin	ND	ND	ND	ND	NC	ND
Lomefloxacin	ND	ND	ND	ND	ND	ND
Metformin	ND	ND	ND	ND	ND	ND
Miconazole	5.7	1.80	ND	2.3	**187**	33.8
Minocycline	ND	ND	9.2	ND	5.1	6.2
Naproxen	ND	12.0	11.8	**68.5**	15.5	NC
Norfloxacin	ND	ND	18.2	**59.2**	1.8	ND
Norgestimate	ND	ND	ND	ND	ND	ND
Ofloxacin	15.9	16.5	3.9	29.0	11.2	12.3
Ormetoprim	ND	ND	ND	ND	ND	ND
Oxacillin	ND	ND	ND	ND	ND	ND
Oxolinic acid	ND	ND	ND	ND	ND	ND
Oxytetracycline	ND	NC	ND	ND	NC	9.04
Penicillin G	ND	ND	ND	ND	ND	ND
Penicillin V	ND	ND	ND	ND	ND	ND
Ranitidine	ND	ND	NC	**121.5**	2.1	ND
Roxithromycin	ND	ND	4.03	ND	ND	ND
Sarafloxacin	ND	ND	ND	ND	ND	ND
Sulfachloropyridazine	ND	ND	ND	NC	ND	ND
Sulfadiazine	ND	ND	ND	ND	ND	ND
Sulfadimethoxine	ND	ND	ND	NC	ND	ND
Sulfamerazine	ND	ND	ND	ND	ND	ND
Sulfamethazine	ND	ND	ND	ND	ND	ND
Sulfamethizole	ND	ND	ND	ND	ND	ND
Sulfamethoxazole	ND	ND	ND	**56.3**	ND	ND
Sulfanilamide	ND	ND	ND	ND	ND	ND

Table 25. Comparison of Field Duplicate Results for Pharmaceuticals

Analyte	Relative Percent Difference (%)					
	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6
Sulfathiazole	ND	ND	ND	ND	ND	ND
Tetracycline	15.2	1.6	5.09	2.3	0.52	13.1
Thiabendazole	ND	ND	20	0.8	0.30	0.49
Triclocarban	6.1	10.2	0	13.2	7.31	5.90
Triclosan	5.7	12.4	37.3	38.3	26.9	41.0
Trimethoprim	ND	ND	30.8	ND	ND	NC
Tylosin	ND	ND	ND	ND	ND	ND
Virginiamycin	ND	22.1	NC	ND	NC	ND
Warfarin	NC	ND	ND	ND	ND	ND
Total Solids	1.7	4.0	2.84	**88.0**	6.21	3.54

ND = Not detected in both samples in the pair
NC = Not calculated because one of the results was a non-detect
RPD values greater than 50% are shown in **bold**.

EPA calculated 143 RPD values for the pharmaceuticals, not including those for the total solids in each sample. Of the 143 RPDs, 16 exceeded 50%, although some only marginally (e.g., 50.5, 50.7, and 50.9%). Of the 16 values greater than 50%, 11 occurred in Field Duplicate Pair 4. As discussed above, EPA believes that the RPD values in Pair 4 reflect differences in the two samples of liquid sewage sludge collected at that facility, with the RPD for the total solids at 88%.

Many of the 270 instances where an analyte was not detected in either sample in the field duplicate pair are a function of the low frequency at which some of the pharmaceuticals were detected. For example, as shown in Table 12, Acetaminophen was only detected in 2 of the 84 survey samples. Therefore, its frequency of occurrence was only 2.38%. The chance that a field duplicate sample would be collected at any of the 74 POTWs in the survey was 8.1% (6 out of 74 plants). The likelihood of detecting Acetaminophen in both samples in a field duplicate pair is on the order of 0.2% (e.g., 2.38% x 8.1%). Therefore, the fact that Acetaminophen is listed as ND in Table 25 for all 6 field duplicate pairs is not surprising.

In contrast, Ciprofloxacin was reported in all 84 samples from the survey, including the 6 field duplicate samples. Therefore, EPA was able to calculate an RPD value for each field duplicate pair in Table 25. Except for field duplicate Pair 4, the RPDs for Ciprofloxacin indicate good precision (four of the RPD values are less than 6%).

6.7.6 Steroids and Hormones

Table 26 presents the field duplicate results for the steroids and hormones.

Table 26. Comparison of Field Duplicate Results for Steroids and Hormones

Analyte	Relative Percent Difference (%)					
	Pair 1	Pair 2	Pair 3	Pair 4	Pair 5	Pair 6
Androstenedione	ND	9.7		ND	ND	**83.3**
Androsterone	ND	ND	3.4	ND	NC	28.8
Campesterol	0	10.4	5.1	**88.9**	**96.6**	17.1
Cholestanol	11.1	9.3	6.2	**97.0**	32.8	7.2
Cholesterol	0.0	3.4	2.5	**99.4**	48.0	3.7
Coprostanol	13.4	14.5	3.8	**110.4**	39.8	13.6
Desmosterol	20.0	3.2	14.8	**96.7**	9.0	23.1
17 α-Dihydroequilin	ND	ND	ND	NC	ND	ND
Epicoprostanol	17.3	38.4	13.4	**109.7**	32.8	5.6
Equilenin	ND	ND	ND	ND	ND	ND
Equilin	ND	ND	13.4	ND	ND	5.7
Ergosterol	38.4	ND	33.6	**93.8**	24.4	43.0
17 α-Estradiol	ND	ND	ND	ND	NC	ND
17 β-Estradiol	ND	ND	ND	**119.5**	27.5	ND
β-Estradiol 3-benzoate	ND	ND	ND	**88.3**	ND	ND
17 α-Ethinyl-estradiol	ND	ND	ND	ND	ND	ND
Estriol	ND	ND	**90.0**	NC	21.0	NC
Estrone	ND	8.3	3.1		0.6	4.5
Norethindrone	ND	ND	ND	ND	ND	ND
Norgestrel	ND	ND	ND	ND	ND	ND
Progesterone	ND	20.5	ND	ND	ND	**65.9**
β-Sitosterol	38.7	8.8	16.2	**87.4**	44.4	3.2
β-Stigmastanol	39.0	3.8	14.3	**87.0**	33.1	30.6
Stigmasterol	25.5	5.1	34.5	**83.6**	**61.0**	2.5
Testosterone	ND	ND	ND	ND	ND	NC
Total Solids	1.7	4.0	2.8	**88**	6.2	3.5

ND = Not detected in both samples in the pair
NC = Not calculated because one of the results was a non-detect
RPD values greater than 50% are shown in **bold**.

There were 6 instances of NCs for the steroids and hormones. For the steroids and hormones, EPA calculated 74 RPD values, not including those for the total solids in each sample. Of those 74 RPDs, 17 exceeded 50%. There were 70 instances where EPA did not calculate an RPD value because both results for the analyte were non-detects (listed as ND in Table 26). As discussed for the pharmaceuticals, the prevalence of the ND entries in Table 26 is largely a function of the frequency of occurrence of the analytes across all samples. For example, Norgestrel was only reported in 4 of 84 samples from the survey (4.76%), yielding a very low likelihood it would be found in both samples from a field duplicate pair. The occurrence of Cholesterol in 81 of 84 samples is not surprising, given that it is excreted by humans. That high frequency of occurrence enabled us to calculate an RPD value for all 6 field duplicate pairs.

Of the 17 RPD values for steroids and hormones greater than 50%, 12 occurred in Field Duplicate Pair 4. EPA believes that the RPD values reflect differences in the two samples of liquid sewage sludge collected at that facility. The differences are evident in the total solids contents of each sample, where the RPD is 88%.

6.7.7 Results in Liquid Samples

The results for all classes of analytes support EPA's conclusion that the sampling procedures were appropriate and effective in collecting sewage sludge for the TNSSS. The exception was for the facility from which Field Duplicate Pair 4 was collected. EPA believes that the variability of the field duplicate results for Pair 4 is not typical of the variability observed for the other liquid samples in the survey, but may reflect site-specific conditions.

Given the variability of the results for Field Duplicate Pair 4 shown in the tables above, EPA examined the results for those two samples in greater detail, comparing the two samples from that one site to the liquid sewage sludge samples from other sites. EPA examined the percent solids data from the anions, metals, and semivolatile organics analyses for all 19 liquid sewage sludge samples collected during the survey. Because the results for the PBDEs, pharmaceuticals, steroids, and hormones were delivered later in the TNSSS effort, EPA did not include them in this more detailed analysis of liquid sample results.

EPA examined the percent solids results generated during analyses of the anions, metals, and semivolatile organics in all 19 liquid sewage sludge samples (19 samples x 3 classes = 57 measurements in all). Based on the observed distribution of results, the data were transformed by taking the natural log of the results and subjecting them to an F-test. The null hypothesis was that the three aliquots from each of the two samples in Pair 4 did not have significantly different variances from the variances of the three aliquots in each of the other liquid samples, indicating that they came from the same population. Table 27 presents the log-transformed percent solids data for all 19 liquid sewage sludge samples.

Table 27. Log-transformed Percent Solids Data for Liquid Sewage Sludge Samples

Liquid Sample	Natural Log of % Solids			Variance of Logs
	Anions	Metals	Organics	
1	1.284	1.747	0.425	0.450
2	3.795	3.773	3.795	0.000
3	0.560	0.560	1.621	0.376
4	4.126	4.126	4.126	0.000
5	1.543	0.270	1.085	0.416
6	0.482	0.482	1.456	0.316
7 - FD	0.615	1.452	-0.494	0.953
8 - FD	0.010	0.010	1.163	0.443
9	2.434	2.407	2.477	0.001
10	2.425	2.434	2.370	0.001
11	1.677	1.128	0.920	0.153
12	-0.844	-0.693	-0.942	0.016
13	0.293	0.647	0.604	0.037
14	0.815	0.658	0.888	0.014
15	0.182	0.182	0.191	0.000
16	-0.673	-0.844	-0.892	0.013
17	0.270	0.207	0.182	0.002
18	2.072	1.991	1.652	0.049
19	0.788	-0.041	0.775	0.226

FD = Field duplicate pair

Table 28 presents the results of the F-test. The pooled within-sample variance (the standard deviation squared) is markedly greater for the two field duplicate samples, compared to the remaining liquid sewage sludge samples in the survey. The F-ratio in Table 28 is greater than the critical value of F, leading to the conclusion that the variances in the percent solids results for the field duplicate samples are greater than would be expected by chance from samples in a single population.

Table 28. F-test Results for Field Duplicates and Other Liquid Samples	
Pooled Variance (field duplicates)	0.698
Pooled Variance (non-field duplicates)	0.122
F-ratio	5.731
F-critical	2.650
p-value	0.001229

Therefore, the variability exhibited by the field duplicate results for Pair 4 is *not* typical of the variability that is apparent for the other liquid samples in the survey. The results for Pair 4 are not an indication that the sampling procedures used for the survey are inappropriate for liquid samples. Rather, the results for Pair 4 may reflect site-specific conditions. However, both sets of results for all of the field duplicate pairs, including Pair 4, are included in the survey database.

6.8 Matrix Spike and Duplicate Results

Matrix spike (MS) samples and matrix spike duplicate (MSD) samples, or matrix spike samples and unspiked duplicate (DUP) samples, were prepared with batches of field samples analyzed for the anions, metals, semivolatile organics and PAHs, and the PBDEs analyzed as described in Section 4 without isotope dilution quantitation. These QC samples served to demonstrate the applicability of the methods to the matrices in question, e.g., liquid and solid sewage sludge. The use of an MSD versus a DUP is generally called out in the method, with MS/MSD pairs being the norm in methods for organics, and MS and DUP samples being the norm in methods for metals and other inorganic such as anions. The results for those analyses are discussed below by analyte class. Appendix C presents the QC acceptance limits used by the laboratories.

Because the methods used for the pharmaceuticals, steroids, and hormones use isotope dilution quantitation, those methods do not require that separate MS/MSD samples be analyzed. Rather, the recoveries of the isotopically labeled analytes spiked into every sample are monitored and used to correct the results for the target analytes.

The MS/MSD results for the TNSSS are discussed in the subsections that follow, by analyte class. The labeled compound recoveries for the isotope dilution methods are discussed in Section 6.8.8.

6.8.1 Matrix Spike Results for Anions

The laboratory prepared eight sets of matrix spike samples for the anion analyses. Table 29 presents the results in terms of the percent recovery of each analyte spiked into the sample.

Table 29. Matrix Spike Recoveries for Anions in Sewage Sludge

	Matrix Spike Recovery (%)							
Analyte	**MS1**	**MS2**	**MS3**	**MS4**	**MS5**	**MS6**	**MS7**	**MS8**
Fluoride	93	74	66	82	93	60	68	77
Nitrate/Nitrite	98	101	103	95	100	90	101	96
Water-Extractable Phosphorus	-24	88	75	102	90	95	80	95

The laboratory's acceptance limits for matrix spike recoveries were 75 - 125%, and 18 of 24 recoveries in Table 29 met those limits. The laboratory reported the recovery of WEP in MS3 as 74.8%, before rounding. That value is only marginally out of the specification, and rounds to 75%, the lower limit of the laboratory's acceptance range.

The laboratory reported the recovery of WEP in one matrix spike (MS1) as -24%. Negative recoveries are not physically possible and the reported recovery is a function of the manner in which it is calculated. Although most recent published EPA methods explicitly include example calculations for QC parameters such as recovery, some older analytical methods do not. EPA Methods 340.2, 353.2, and 365.3, used for the anion analyses, are among those methods without example calculations. In the absence of project-specific requirements, the laboratory performing the anions analyses relied on formulae from the SW-846 methods manual from EPA's Office of Solid Waste. Chapter One of the manual includes definitions of a number of commonly used terms and provides the equation for the calculation of recovery shown below:

$$\%R = \frac{100\,(x_s - x_u)}{K}$$

where:

- %R = percent recovery
- x_s = measured value for spiked sample
- x_u = measured value for unspiked sample
- K = known value for the spike in the sample

This same basic formula appears in many individual EPA methods. The remainder of this report refers to this equation as the "traditional approach" to calculating recovery. As written, K is often interpreted as the amount of material spiked into the sample. That interpretation ignores any "background" concentration of the analyte in the unspiked sample. In practice, the equation produces recoveries that appear reasonable and generally meet expectations in those samples where the background concentration of the analyte is either very low, or where the amount spiked into the sample is much greater than the background amount. However, the laboratory calculates negative recoveries any time the result in the spiked sample is less than that in the unspiked sample, even if that is a function of inhomogeneity in the original sample. Given the Law of Conservation Mass, whereby matter cannot be created or destroyed, negative recoveries are physically impossible.

Laboratories commonly prepare MS samples without extensive knowledge of the background levels of any target analytes in specific samples. Therefore, a laboratory may blindly spike an amount that is similar to that already in the sample and the equation above will perform poorly.

Given the frequency at which negative and unrealistic recoveries are reported using the equation above, EPA developed an alternative calculation that considers the result found in the matrix spike sample in comparison to what was found in the unspiked sample plus the amount spiked. The alternative equation is as follows:

$$\%R = \frac{C_s}{C_u + C_n} \times 100$$

where:

- %R = percent recovery
- C_s = measured value for spiked sample
- C_u = measured value for unspiked sample
- C_n = nominal spike added to the sample

Eliminating the subtraction operation in the numerator of the equations prevents the occurrence of any negative values. Moving the concentration of the unspiked sample to the denominator more effectively addresses the issue of the "background" concentration.

In the case of the first MS sample in Table 29, the water-extractable phosphorus result in the MS sample (271 mg/kg) was less than the result in the unspiked sample (317 mg/kg), despite adding 189 mg/kg of phosphorus, leading to a negative recovery (-24.3%) using the traditional approach, as follows:

$$(100 \times (271 - 317))/189 = -4600/189 = -24.3\%$$

Using the alternative equation above and the same laboratory results, the recovery in the matrix spike is calculated as:

$$100 \times (271/(317 + 189) = 27100/506 = 53.6\%$$

While the recovery of 53.6% is still below the acceptance limits used by the laboratory, it is a more rational expression of the results in this sample.

The alternative equation increased the recovery of WEP dramatically in this example. However, it did not cause the recoveries that already appear reasonable to exceed the acceptance limits when they did not otherwise do so. For example, Table 29 lists the recovery of nitrate/nitrite in MS3 as 103%. This value is rounded down from 103.3652% determined via the traditional calculation. Using the alternative equation, the recovery is 103.3466%, a trivial difference that is removed when the results are rounded to the nearest whole percentage. Given this trivial difference, EPA did not recalculate every recovery reported by the laboratory, but focused this discussion on the negative recoveries alone.

6.8.2 Duplicate Results for Anions

The laboratory prepared eight sets of duplicate sample analyses for anions. Table 30 presents the results shown in terms of the relative percent difference (RPD) for each analyte in the sample. Although the laboratory used acceptance limits expressed to the nearest whole percentage, Table 30 presents the RPDs to one decimal place to illustrate the small differences between many of the duplicate pair results.

Table 30. Duplicate Precision for Anions in Sewage Sludge

	Relative Percent Difference (%)							
Analyte	**Dup 1**	**Dup 2**	**Dup 3**	**Dup 4**	**Dup 5**	**Dup 6**	**Dup 7**	**Dup 8**
Fluoride	0.9	2.7	4.5	14.6	5.4	12.2	0.4	14.6
Nitrate/Nitrite	3.6	0.0	23.0	17.1	2.2	8.3	6.9	10.7
Water-extractable Phosphorus	6.1	5.2	18.8	4.6	9.4	6.8	4.1	11.1

The laboratory's acceptance limit for precision (RPD) is 20% and all but one result in Table 30 met that limit. The highest RPD value reported by the laboratory was 23.0% for nitrate/nitrite in Duplicate 3. This RPD was only slightly outside of the 20% limit.

Except as discussed above, the recovery data in Table 29 and the precision data in Table 30 demonstrate that the methods were generally accurate and precise when applied to sewage sludge samples.

6.8.3 Matrix Spike Results for Metals

The laboratory prepared 15 sets of matrix spike samples for the metals analyses. Table 31 presents the matrix spike recoveries in three parts (Tables 31A, 31B, and 31C), rounded to one-tenth of a percent, to illustrate some of the smaller differences.

Because calcium and magnesium are common components of soils and occur at levels that vary widely, the laboratory did not spike these two metals into solid samples, and they do not appear in Table 31. However, calcium and magnesium also are not major metals of concern in this survey.

The laboratory analyzed mercury separately from any other metals. Therefore, the results for mercury appear at the bottom of the table because the laboratory prepared only 9 matrix spike samples for mercury and did not necessarily use the same field samples for the mercury matrix spike analyses as for the other metals.

During the survey, as the laboratory gained experience with the sewage sludge samples, they adjusted the amounts of some metals spiked into each matrix spike sample in an effort to account for the background concentrations. For example, for aluminum, the spiking concentrations ranged from about 400 mg/kg to about 3200 mg/kg, while copper spiking levels ranged from about 40 mg/kg to 4000 mg/kg. These adjustments to the spiking levels were not always successful in addressing recovery issues.

Aluminum and iron presented problems with reported recoveries. The laboratory also reported negative recoveries for phosphorus, again a function of the assumption that the background concentrations are much lower than the spiking levels. Therefore, Table 31 includes the recoveries reported by the laboratory using the traditional calculation from the methods (in the MS# columns) along with the alternative calculation described above (in the ALT# columns). Using the alternative calculation, all of the negative recoveries reported by the laboratory become positive values. In addition, many of the very high recoveries (e.g., over 250%) are greatly reduced in magnitude.

Table 31A. Matrix Spike Recoveries for Metals in Sewage Sludge, Calculated in the Traditional Fashion and with an Alternative Equation

	Recovery (%)									
Analyte	**MS1**	**ALT1**	**MS2**	**ALT2**	**MS3**	**ALT3**	**MS4**	**ALT4**	**MS5**	**ALT5**
Aluminum	177.7	102.4	334.5	104.2	68.8	98.5	400	114.9	443.9	107.9
Antimony	44.3	45.7	41.1	43.7	75.7	75.7	41.5	42.0	46.4	47.4
Arsenic	102.0	101.8	102.0	101.7	101.5	101.4	111.1	110.6	101.6	101.5
Barium	99.2	99.7	98.8	99.5	101.9	101.0	96.3	98.2	117.5	105.7
Beryllium	108.7	108.3	99.7	99.7	102.3	102.3	112.4	112.0	101.2	101.2
Boron	105.1	103.5	106.5	105.9	110.6	108.4	110.6	108.9	103.7	103.0
Cadmium	110.7	105.9	117.4	108.6	112.9	111.5	121.8	119.6	110.7	107.9
Chromium	93.9	98.8	113.7	105.0	110.5	106.9	118.8	110.5	118.1	102.4
Cobalt	101.1	101.0	102.9	102.7	107.6	107.3	116.3	115.7	115.7	104.0
Copper	83.2	98.5	59.7	96.7	79.5	97.9	40.0	95.2	173.3	104.7
Iron	-203.0	98.0	1543.8	111.3	-71.4	96.5	265.0	103.4	156.2	100.8
Lead	111.3	104.4	108.3	103.0	111.1	108.6	118.9	113.6	109.5	105.7
Manganese	78.2	97.9	135.9	108.1	91.7	99.7	81.0	95.1	101.3	100.4
Molybdenum	103.0	102.6	108.7	105.7	111.5	110.0	110.3	110.0	107.5	106.9
Nickel	101.7	101.3	105.5	103.8	105.6	105.1	118.3	114.7	102.5	102.2
Phosphorus	-101.5	96.9	-123.5	91.2	-203.9	90.1	-46.0	91.7	-41.8	96.0
Selenium	102.3	102.0	101.4	101.3	102.8	102.6	112.9	112.2	103.5	103.2
Silver	68.0	90.7	93.8	98.2	103.3	101.4	83.1	92.3	101.8	101.1
Thallium	104.8	104.7	106.2	106.2	107.4	107.4	108.9	108.8	108.4	108.4
Tin	96.6	96.8	95.4	95.9	95.6	95.9	91.3	91.6	81.1	82.6
Titanium	47.3	57.7	29.4	43.2	16.3	21.9	55.5	61.1	10.8	14.8
Vanadium	101.4	101.0	105.9	104.3	107.3	106.4	117.7	115.1	101.4	101.3
Yttrium	102.2	101.8	102.4	101.9	106.0	105.6	119.6	115.5	103.7	102.7
Zinc	50.8	97.1	41.2	96.6	99.9	100.0	72.0	94.1	125.3	101.9
Mercury	106.0	101.3	76.0	97.0	110.8	104.1	106.6	103.2	62.5	94.4

Table 31B. Matrix Spike Recoveries for Metals in Sewage Sludge, Calculated in the Traditional Fashion and with an Alternative Equation

	Recovery (%)									
Analyte	**MS6**	**ALT6**	**MS7**	**ALT7**	**MS8**	**ALT8**	**MS9**	**ALT9**	**MS10**	**ALT10**
Aluminum	236.2	104.3	194.9	104.3	653.0	353.7	202.0	103.2	485.0	325.1
Antimony	72.4	72.9	74.8	75.8	69.1	69.4	47.9	49.1	74.3	74.5
Arsenic	103.0	102.8	102.8	102.7	140.3	137.8	100.3	100.2	119.1	118.5
Barium	103.7	101.8	107.8	104.2	119.5	118.8	92.6	96.9	103.0	102.9
Beryllium	93.0	93.1	95.6	95.8	98.7	98.7	117.5	116.7	100.9	100.9
Boron	105.7	104.8	102.6	101.9	119.5	119.1	103.4	102.3	127.0	126.3
Cadmium	110.3	108.8	107.2	106.3	119.9	119.3	111.8	109.0	107.7	107.6
Chromium	93.5	96.7	100.0	100.0	116.9	116.3	88.6	96.6	184.4	171.2
Cobalt	102.4	102.3	102.3	102.2	118.8	118.2	99.6	99.6	110.3	110.2
Copper	111.3	101.1	182.6	103.6	216.4	191.8	38.4	93.6	304.4	247.3
Iron	94.7	99.8	1167.5	103.9	2126	465.7	-1312.7	92.5	250.4	214.3
Lead	107.2	105.7	110.1	108.8	329.8	263.9	131.2	113.6	166.5	157.6
Manganese	98.8	99.5	99.3	99.7	206.4	186.1	10.1	93.7	102.7	102.6
Molybdenum	109.6	108.4	108.2	107.3	101.9	101.8	100.6	100.5	107.1	106.7
Nickel	100.3	100.3	99.8	99.8	150.0	145.0	103.2	102.4	184.9	171.3

Table 31B. Matrix Spike Recoveries for Metals in Sewage Sludge, Calculated in the Traditional Fashion and with an Alternative Equation

	Recovery (%)									
Analyte	**MS6**	**ALT6**	**MS7**	**ALT7**	**MS8**	**ALT8**	**MS9**	**ALT9**	**MS10**	**ALT10**
Phosphorus	-63.0	96.5	243.4	102.0	360.6	263.5	-282.7	91.1	320.6	238.4
Selenium	103.4	103.1	101.7	101.6	111.7	111.3	101.8	101.7	114.4	113.6
Silver	56.8	85.1	33.4	84.0	95.8	96.0	67.7	87.3	114.2	112.1
Thallium	105.9	105.9	105.7	105.7	108.9	108.9	104.7	104.7	103.9	103.9
Tin	92.5	93.1	85.4	86.1	94.0	94.0	99.7	99.7	83.6	83.6
Titanium	13.7	20.7	22.5	29.5	91.0	91.0	85.0	90.8	79.0	79.0
Vanadium	98.9	99.0	115.8	106.7	169.7	162.1	109.6	105.8	139.8	136.8
Yttrium	101.9	101.7	99.7	99.8	140.0	137.4	107.0	105.6	100.1	100.1
Zinc	95.7	99.5	109.4	101.1	230.8	201.3	92.0	92.2	160.4	153.4
Mercury	236.7	145.5	116.2	115.6	124.9	109.0	91.4	94.4	--	--

Table 31C. Matrix Spike Recoveries for Metals in Sewage Sludge, Calculated in the Traditional Fashion and with an Alternative Equation

	Recovery (%)									
Analyte	**MS11**	**ALT11**	**MS12**	**ALT12**	**MS13**	**ALT13**	**MS14**	**ALT14**	**MS15**	**ALT15**
Aluminum	229.9	184.4	500.0	108.6	-395.9	75.8	301.9	107.3	147.5	101.9
Antimony	77.7	77.7	55.9	56.7	75.2	75.6	54.7	56.1	85.1	85.4
Arsenic	101.2	101.2	95.9	96.1	69.7	70.5	-8.2	56.4	105.8	105.6
Barium	107.7	107.6	95.2	98.0	47.7	82.3	115.1	103.5	120.7	108.8
Beryllium	101.4	101.4	100.1	100.1	94.5	94.5	91.9	92.6	109.4	109.2
Boron	119.5	118.4	100.1	100.1	88.7	90.7	99.8	99.8	111.6	106.8
Cadmium	104.4	104.4	108.7	107.4	101.9	101.7	55.5	76.9	114.1	111.9
Chromium	105.3	105.3	102.8	101.6	77.4	87.6	118.9	108.7	130.0	104.7
Cobalt	103.5	103.5	100.9	100.9	95.8	96.0	102.5	102.3	108.2	107.8
Copper	155.8	153.7	62.1	97.8	-204.1	74.7	181.8	104.0	178	105.9
Iron	505.6	224.6	250	102.6	-1573.6	77.4	1209.8	109.4	599.9	103.2
Lead	100.0	100.0	108.9	106.0	62.2	82.6	120.5	107.6	114.9	109.8
Manganese	100.3	100.3	92.1	99.1	0.0	89.0	178.0	104.1	124.0	105.9
Molybdenum	105.3	105.3	108.8	107.2	99.3	99.3	-40.9	50.9	112.9	110.5
Nickel	105.0	105.0	102.8	102.3	91.0	92.3	104.7	103.8	116.2	109.5
Phosphorus	384.0	169.0	-80.2	96.2	-142.0	76.9	105.9	100.6	260.0	102.3
Selenium	100.3	100.3	93.1	93.5	91.8	92.2	-98.2	38.9	107.8	107.3
Silver	90.9	91.8	94.5	97.2	41.0	79.4	79.2	93.5	91.0	97.4
Thallium	99.4	99.4	104.5	104.5	101.0	101.0	95.4	95.6	111.6	111.6
Tin	90.2	90.3	88.9	90.0	91.8	92.2	84.0	84.3	99.4	99.4
Titanium	90.5	90.8	35.7	51.4	23.9	26.8	27.0	28.3	40.8	47.7
Vanadium	100.9	100.9	109.1	108.1	92.6	93.7	102.5	101.9	106.9	105.0
Yttrium	100.0	100.0	107.8	106.9	108.2	106.2	89.1	91.2	104.8	104.4
Zinc	461.3	237.9	86.1	98.5	-106.1	77.7	181.8	104.9	138.0	103.8

Using either calculation, many of the metals exhibited a small positive bias. A total of 12 the 25 spiked metals have mean traditional recoveries between 101% and 115%, while 14 of 25 metals have mean alternative recoveries in the same range. Five metals exhibited a slight negative bias using the traditional calculation, with mean recoveries ranging from 80% to 97%.

Antimony, phosphorus, and titanium exhibited mean traditional recoveries below 70%. The alternative calculation dramatically altered the mean recovery of phosphorus, raising it from 39% to 120%, by virtue of eliminating the large number of negative values in the traditional calculation.

The mean recovery of titanium was only 43% in these 15 matrix spike samples, with only four values in the acceptable range. Similarly, the mean recovery of antimony was 62% in these 15 matrix spike samples, with only five values in the acceptable range. Neither titanium nor antimony is among the nine pollutants initially selected for this survey (see Section 1), nor one of the metals that currently has a regulatory standard in sewage sludge.

6.8.4 Duplicate Results for Metals

Over the course of the survey, the laboratory analyzed 15 samples for metals in duplicate, to assess analytical precision. Table 32 presents the results for those duplicate analyses. The laboratory prepared and analyzed only seven duplicates for mercury, and as with the matrix spike results, not necessarily using the same field samples as for the other metals. The exception is for Duplicate 8, where the results for mercury are from the same sample as for all the other metals. Table 32 is divided into several parts. The vast majority of the RPD values are less than the acceptance limit of 30%. The exceptions are almost exclusively in Duplicates 8, 10, and 11. All three of these samples were liquid sewage sludge.

Table 32A. Duplicate Precision for Metals in Sewage Sludge

	Relative Percent Difference (%)							
Analyte	**Dup1**	**Dup2**	**Dup3**	**Dup4**	**Dup5**	**Dup6**	**Dup7**	**Dup8**
Aluminum	14.4	6.2	0.7	13.6	3.1	8.1	1.4	142.1
Antimony	8.3	4.5	ND	21.9	18.8	10.0	6.8	146.3
Arsenic	2.5	0.0	8.6	6.8	3.2	10.0	4.4	145.0
Barium	2.3	0.8	3.1	1.4	1.7	5.8	1.4	140.3
Beryllium	6.3	0.0	8.0	18.2	3.8	4.4	5.4	141.9
Boron	13.8	2.8	6.5	13.9	5.0	6.1	4.2	141.4
Cadmium	1.3	4.8	3.4	5.2	0.6	3.1	6.4	151.0
Calcium	1.2	3.9	0.7	17.1	1.8	6.1	1.1	139.0
Chromium	0.7	2.2	4.7	13.6	3.8	8.0	5.0	140.0
Cobalt	0.4	2.4	4.3	10.1	4.7	5.3	9.3	144.4
Copper	3.2	3.0	2.6	2.1	0.7	5.5	0.9	138.1
Iron	0.7	5.4	1.5	7.3	1.4	7.5	0.9	140.9
Lead	3.9	4.0	3.7	0.5	0.5	1.6	7.2	150.7
Magnesium	4.3	8.5	0.7	27.3	4.3	6.6	1.4	140.6
Manganese	2.3	6.2	1.8	3.2	1.2	5.6	1.9	141.0
Molybdenum	1.2	4.2	3.3	14.1	0.9	16.2	5.0	134.1
Nickel	1.2	2.3	4.3	2.9	3.7	9.3	5.5	143.6
Phosphorus	1.6	0.0	2.8	2.4	0.6	5.9	1.2	141.1
Selenium	3.6	3.4	2.6	3.3	1.8	9.1	2.8	141.0
Silver	12.4	7.6	2.1	1.7	1.5	8.0	15.9	139.5
Sodium	1.0	10.4	3.3	2.0	2.0	4.4	1.0	134.1
Thallium	0.0	0.0	0.0	22.2	4.0	2.9	4.3	167.0
Tin	1.0	2.3	3.3	9.1	0.7	2.1	5.8	ND
Titanium	51.1	22.5	1.4	18.2	4.3	1.2	1.0	142.5
Vanadium	1.1	3.2	4.1	2.9	6.6	3.1	11.9	148.9
Yttrium	0.0	3.8	3.5	6.1	2.6	3.7	4.3	149.4
Zinc	3.2	0.6	0.8	0.5	0.8	5.9	1.3	141.1
Mercury	17.5	2.5	28.7	14.1	5.8	29.9	--	136.1

Table 32B. Duplicate Precision for Metals in Sewage Sludge

	Relative Percent Difference (%)						
Analyte	**Dup9**	**Dup10**	**Dup11**	**Dup12**	**Dup13**	**Dup14**	**Dup15**
Aluminum	5.0	148.3	94.0	7.4	20.4	2.5	0.1
Antimony	20.5	97.6	30.9	6.3	21.9	11.2	10.7
Arsenic	1.8	89.8	41.7	0.4	7.7	18.8	7.3
Barium	3.2	147.9	43.6	0.2	21.8	3.5	2.3
Beryllium	6.2	81.1	ND	0.0	15.2	30.9	0.0
Boron	8.7	135.6	71.2	8.3	11.5	3.5	0.7
Cadmium	2.5	105.9	38.9	1.2	17.5	33.1	11.6
Calcium	3.1	140.0	82.1	0.4	24.6	2.7	2.4
Chromium	2.9	83.9	43.1	4.3	23.9	1.6	1.9
Cobalt	2.2	98.0	17.8	0.0	27.0	1.8	9.5
Copper	1.9	148.2	44.9	0.3	23.1	1.2	2.1
Iron	3.0	144.6	91.8	4.4	19.8	0.6	1.3
Lead	2.3	89.9	43.6	1.9	28.9	5.0	8.8
Magnesium	3.4	129.8	51.1	3.7	19.1	0.8	1.6
Manganese	2.3	144.9	87.6	0.7	13.5	2.3	2.3
Molybdenum	1.9	91.2	48.0	4.2	6.1	46.0	6.0
Nickel	0.6	89.6	38.2	0.4	32.5	7.9	11.2
Phosphorus	2.4	137.7	63.4	0.9	11.3	1.5	2.0
Selenium	0.0	63.9	50.0	1.3	2.2	45.2	8.6
Silver	2.7	118.2	36.5	0.0	26.8	2.5	4.6
Sodium	1.9	122.4	5.5	1.3	21.3	2.4	1.3
Thallium	2.2	66.7	ND	8.0	6.5	43.7	0.0
Tin	6.2	ND	48.4	1.3	21.0	7.6	9.1
Titanium	12.6	ND	68.5	55.3	11.1	1.0	4.4
Vanadium	7.3	89.8	53.1	2.6	20.4	1.1	10.5
Yttrium	2.4	84.3	46.9	5.7	2.4	32.7	8.2
Zinc	1.7	148.8	94.5	0.1	19.9	2.2	1.6
Mercury	--	--	--	8.6	18.9	--	--

The differences apparent in the three liquid sewage sludge samples in Table 32 (duplicates 8, 10, and 11) cannot be attributed to differences between the percent solids results in different containers, as was suggested for the *field* duplicate samples earlier. Each of these duplicates was prepared at the laboratory from the single 500-mL HDPE container of sewage sludge from the particular POTW.

The laboratory homogenized each sample before removing the two aliquots (the original sample and the duplicate), but those procedures may not have been entirely adequate for samples with very low solids contents, or the samples may have settled between collection of the two aliquots. In addition, the laboratory only measured the percent solids of each sample once, for the original aliquot. Therefore, if the duplicate aliquot used for the metals analysis had slightly different solids content than that of the original sample aliquot, this would not be known or reflected in the dry-weight results in this survey.

EPA does not include laboratory duplicate analyses in a survey database, since that would provide two results for each sample chosen for this laboratory QC test. Therefore, while the RPD values in Table 32 may be useful in diagnosing laboratory issues, they do not influence the survey data EPA may use.

6.8.5 MS/MSD Results for Organics

Organic contaminants such as the PAHs and semivolatiles of interest in this survey are generally less common in environmental samples. Therefore, most methods for organics specify using MS and MSD samples as the means of assessing the applicability of the method to the matrix of interest, rather than a single MS sample and a duplicate sample analysis. The advantage of spiking the analytes into both QC samples is that it avoids the difficulty of comparing non-detect results to assess precision. If the laboratory only analyzes an unspiked duplicate sample and a compound is not found, there is no numerical result that can be compared to the original sample result, which may also be a non-detect. While one can compare reporting limits for non-detects, those limits may differ for legitimate reasons that do not reflect analytical precision.

The laboratory prepared and analyzed seven sets of MS/MSD samples for the organics in this survey. They prepared some of the MS/MSD samples in conjunction with the full-scan GC/MS analyses and prepared others, later in the survey, with the selected ion monitoring (SIM) analyses. Five of the seven MS/MSD pairs were solid samples and two were liquid samples. The laboratory employed separate acceptance limits for samples analyzed as liquids versus those analyzed as solids. The results of all seven sets are summarized in Table 33.

Table 33. MS and MSD Recovery and Precision for Organics

	MS/MSD 1 (solid)			MS/MSD 2 (solid)			MS/MSD 3 (solid)		
Analyte	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD
2-Methylnaphthalene	55	60	9	19	11	56	120	143	18
4-Chloroaniline	89	98	10	-480	-503	5	671	685	2
Benzo(a)pyrene	104	112	7	-641	-589	9	131	115	14
bis (2-Ethylhexyl) phthalate	149	226	41	-8024	-9476	17	-7160	-4296	50
Fluoranthene	62	63	3	-2101	-1980	6	93	105	12
Pyrene	51	61	17	-613	-754	21	105	146	32
	MS/MSD 4 (liquid)			MS/MSD 5 (liquid)			MS/MSD 6 (solid)		
	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD
2-Methylnaphthalene	62	67	9	99	108	8	109	110	1
4-Chloroaniline	66	80	19	97	95	2	75	85	12
Benzo(a)pyrene	67	80	18	169	94	58	117	120	3
bis (2-Ethylhexyl) phthalate	-72	16	314	-1362	-275	133	267	416	44
Fluoranthene	43	44	2	290	119	84	124	135	8
Pyrene	57	66	15	278	100	94	123	141	14
	MS/MSD 7 (solid)								
	MS Rec	MSD Rec	RPD						
2-Methylnaphthalene	92	97	5						
4-Chloroaniline	53	57	7						
Benzo(a)pyrene	83	95	14						
bis (2-Ethylhexyl) phthalate	4622	4785	4						
Fluoranthene	78	97	22						
Pyrene	77	91	17						

The recoveries in Table 33 exhibit some of the same issues as for the anions and metals, including large negative recoveries for some analytes, particularly bis(2-Ethylhexyl) phthalate. The laboratory adjusted the amounts of the analytes spiked into samples over the course of the survey. However, the within-sample variability affected many of the recoveries, resulting in some negative recovery values. Therefore, EPA recalculated the MS/MSD recoveries and RPDs using the alternative recovery equation described earlier. Table 34 presents the recalculated results, rounded to one-tenth of a percent, to illustrate some of the smaller differences.

Table 34. Alternative MS and MSD Recovery and Precision for Organics

	MS/MSD 1 (solid)			MS/MSD 2 (solid)			MS/MSD 3 (solid)		
Analyte	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD
2-Methylnaphthalene	66.6	70.4	5.5	67.6	64.2	5.2	109.7	120.8	9.6
4-Chloroaniline	94.1	99.1	5.2	41.3	38.9	6.0	670.6	685.0	2.1
Benzo(a)pyrene	104.0	111.7	7.1	61.3	64.0	4.3	113.0	106.0	6.4
bis (2-Ethylhexyl) phthalate	112.3	131.6	15.8	71.7	66.7	7.2	82.2	89.2	8.2
Fluoranthene	76.1	77.1	1.3	40.3	43.6	7.9	98.6	101.0	2.4
Pyrene	71.1	76.8	7.7	70.8	65.0	8.5	101.1	110.0	8.4
	MS/MSD 4 (liquid)			MS/MSD 5 (liquid)			MS/MSD 6 (solid)		
	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD	MS Rec	MSD Rec	RPD
2-Methylnaphthalene	63.1	68.5	8.2	99.3	107.6	8.0	108.9	109.9	0.9
4-Chloroaniline	78.5	87.2	10.5	98.3	97.4	0.9	89.6	93.8	4.6
Benzo(a)pyrene	74.8	84.5	12.2	142.1	96.0	38.7	114.8	117.4	2.2
bis (2-Ethylhexyl) phthalate	73.9	87.3	16.6	68.1	91.8	29.6	115.3	129.0	11.2
Fluoranthene	69.1	69.6	0.7	183.3	108.3	51.4	114.1	120.6	5.5
Pyrene	70.3	76.7	8.7	171	99.9	52.5	114.6	126.1	9.6
	MS/MSD 7 (solid)								
	MS Rec	MSD Rec	RPD						
2-Methylnaphthalene	92.5	97.3	5.1						
4-Chloroaniline	91.2	91.9	0.8						
Benzo(a)pyrene	90.0	97.2	7.7						
bis (2-Ethylhexyl) phthalate	526.1	541.5	2.9						
Fluoranthene	88.3	98.6	11.0						
Pyrene	88.0	95.3	8.0						

As Table 34 illustrates, all of the negative recovery values are eliminated and the exceptionally large negative and positive recoveries for bis (2-Ethylhexyl) phthalate were reduced as well. With the notable exceptions of 4-chloroaniline in MS/MSD 3 and bis (2-Ethylhexyl) phthalate in MS/MSD 7, the recoveries range from 39% to 183%.

A total of 74 out of 84 recoveries are less than 125% and 70 of 84 recoveries are in the range of 70% to 130%. Only 4 recoveries are below 50%. A total of 38 of 42 RPD values are less than 20%, with the other 4 RPDs between 30% and 53%. These alternative recovery data demonstrate that the analytical methods employed for this survey exhibit precision and bias within expected norms for the analysis of organics.

6.8.6 MS/MSD Results for PBDEs

The laboratory performed the PBDE analyses using EPA Method 1614, which normally employs isotope dilution quantitation of the analytes of interest. Because every sample is spiked with the labeled compounds and their recoveries are measured in every sample, isotope dilution methods do not require the analysis of MS/MSD aliquots to assess bias. However, as noted in Section 4, the survey involved a number of modifications to the published method in order to overcome analytical challenges presented by the sewage sludge samples. One of these modifications was that the laboratory did not spike the labeled compounds into the sample before extraction, but rather spiked the raw sample extracts. Using this modification, the laboratory was able to successfully analyze the survey samples, but their data on labeled compound recovery does not include an assessment of extraction efficiency. Therefore, in conjunction with that modification, the laboratory agreed to prepare MS/MSD aliquots with each batch of field samples analyzed by the modified procedure. Ultimately, four sets of MS/MSD analyses were performed. Table 35 summarizes the MS/MSD results for the PBDEs.

Table 35. MS/MSD Recovery and Precision for PBDEs

	MS/MSD 1			MS/MSD 2		
Analyte	**MS Rec**	**MSD Rec**	**RPD**	**MS Rec**	**MSD Rec**	**RPD**
BDE-28	91	128	19	61	54	4.5
BDE-47	0	3430	0	0	0	0
BDE-66	0	197	0	26	0	0
BDE-85	102	202	23	44	18	7.1
BDE-99	0	1750	0	0	0	0
BDE-100	146	547	23	0	0	0
BDE-138	144	180	15	104	91	7.4
BDE-153	91	256	22	0	0	0
BDE-154	95	224	20	0	0	0
BDE-183	104	123	8.7	54	35	9.8
BDE-209	171	2910	18	0	0	0
	MS/MSD 3			**MS/MSD 4**		
	MS Rec	**MSD Rec**	**RPD**	**MS Rec**	**MSD Rec**	**RPD**
BDE-28	99	100	1.8	91	60	35
BDE-47	215	121	5.4	298	0	0
BDE-66	98	98	1	107	69	35
BDE-85	102	105	3.2	113	61	41
BDE-99	172	116	3.6	315	0	0
BDE-100	118	98	5.1	128	2.9	51
BDE-138	141	94	20	123	84	34
BDE-153	94	102	4.4	106	36	45
BDE-154	96	101	3.5	105	40	44
BDE-183	106	98	3.8	88	58	34
BDE-209	261	143	8.7	291	179	28

The laboratory reported recoveries as zero (0) any time the calculated recovery was negative, and reported the RPD as 0 when either of the recovery values was reported as 0.

As noted in Table 35, the laboratory's reporting practices included substituting zero (0) for any calculated negative recoveries, as well as for the RPD when either recovery value in the MS/MSD pair is reported as 0. There are 16 negative recoveries reported as 0 for first 2 MS/MSD pairs and only 2 negative recoveries for the last 2 MS/MSD pairs. This is because the laboratory increased their spiking levels for the later analyses. There are also a number of reported recoveries well over 100%, ranging as high as 3430% in one case.

Given these recoveries and RPDs, EPA performed the alternative calculations described in Sections 6.7.1 to 6.7.5 for the PBDE data. Those alternative recoveries and RPDs are shown in Table 36, rounded to one-tenth of a percent, to illustrate some of the smaller differences.

Table 36. Alternative MS/MSD Recovery and Precision for PBDEs

	MS/MSD 1			MS/MSD 2		
Analyte	**MS Rec**	**MSD Rec**	**RPD**	**MS Rec**	**MSD Rec**	**RPD**
BDE-28	95.7	116.1	19.3	81.5	51.7	44.8
BDE-47	96.2	126.0	26.8	89.0	77.3	14.1
BDE-66	72.3	124.7	53.2	82.9	76.5	8.1
BDE-85	100.3	126.4	23.0	88.3	82.1	7.2
BDE-99	94.3	121.2	24.9	84.1	78.0	7.6
BDE-100	103.1	129.9	23.0	83.8	71.7	15.5
BDE-138	127.0	148.4	15.5	101.9	94.6	7.5
BDE-153	98.7	123.8	22.6	84.5	76.8	9.6
BDE-154	98.6	120.5	20.0	85.7	77.6	9.9
BDE-183	101.8	111.5	9.1	78.4	71.4	9.4
BDE-209	99.9	120.4	18.6	94.0	79.5	16.7
	MS/MSD 3			**MS/MSD 4**		
	MS Rec	**MSD Rec**	**RPD**	**MS Rec**	**MSD Rec**	**RPD**
BDE-28	99.5	99.5	0.0	91.8	64.5	35.0
BDE-47	107.2	101.6	5.4	120.8	68.7	55.0
BDE-66	98.5	98.1	0.5	106.2	74.1	35.5
BDE-85	101.3	103.3	2.0	109.0	72.1	40.8
BDE-99	104.1	100.4	3.6	121.3	66.5	58.4
BDE-100	104.2	98.6	5.6	110.0	65.3	51.1
BDE-138	136.7	164.4	18.4	121.1	86.1	33.8
BDE-153	96.8	100.3	3.5	103.0	65.5	44.5
BDE-154	99.3	101.7	2.4	102.2	65.2	44.2
BDE-183	103.8	98.8	5.0	89.3	63.3	34.1
BDE-209	111.9	102.6	8.7	172.4	130.1	27.9

Using the alternative calculations, all 18 of the negative values originally reported by the laboratory as "0" were eliminated. The recalculated recoveries range from 52% to 172%. The laboratory employed acceptance limits of 50-150% for MS/MSD recoveries, and all but the one recalculated recovery of 172.4% fall within that range. The calculated RPD values range from 0% to 58.4%. Only three recalculated RPD values were above the laboratory's acceptance limit of 50%.

As with the other analytical classes, these alternative recovery data demonstrate that the analytical methods employed for this survey exhibit precision and bias within expected norms for the analysis of PBDEs.

6.8.7 Qualification of Sample Results based on MS/MSD Results

During review of the sample results, EPA qualified any MS/MSD results reported by the laboratory that fell outside of the relevant acceptance limits. Those data qualifiers were carried over into the results database. However, the results of the alternative calculations shown in Section 6.7 for all classes of analytes demonstrate that the shortcomings in MS/MSD recoveries and precision are largely a function of the calculations in many EPA methods.

Therefore, while retaining the data qualifiers that indicate that the recoveries and/or RPD reported by the laboratories fell outside of the acceptance limits, EPA also added a qualifier to affected samples to indicate that the alternative calculations suggest that method performance in the sewage sludge matrix is not an immediate concern. This new qualifier is "ACAP," for "Alternative Calculation indicates Acceptable Performance," as stated in the database.

6.8.8 Labeled Compound Recoveries for Isotope Dilution Methods

As noted elsewhere in this report, EPA's isotope dilution methods spike labeled analogs of the target analytes into each sample and monitor the recoveries of those labeled compounds as a measure of method performance. Therefore, EPA's isotope dilution methods do not require that the laboratory prepare separate matrix spike samples. Rather than assessing extraction efficiency and other aspects of method performance on 5% of the samples (i.e., 1 out of every 20 samples is used to create an MS/MSD pair), the isotope dilution methods generate extraction efficiency data on 100% of the samples, and use those data to correct the final results for each analyte for the recovery of its labeled compound.

During the data review process, EPA checked the recovery of every labeled compound in every field sample and QC samples against the method acceptance criteria for that analyte. When the labeled recovery fell outside of the acceptance criteria, EPA flagged in the database the sample results for the associated unlabeled analytes.

Section 7
References

NRC, 2002. *Biosolids Applied To Land: Advancing Standards And Practices*, National Research Council of the National Academies. July 2002.

USEPA, 2003. United States Federal Register. 68 *FR* 75531. Wednesday December 31, 2003, pp. 75531-75552.

Vadas, P. A. and Kleinman, P. J. A., 2006. *Effect of Methodology in Estimating and Interpreting Water-Extractable Phosphorus in Animal Manures,* J. Environ. Qual., 35: 1151-159.

Appendix A
Solids Leaching Procedure for Anions

1. Using the percent solids determined by drying an aliquot of the sample of known weight overnight at 103 - 105°C and reweighing, weigh out a sample aliquot equal to 0.5 g dry weight in a 200- or 250-mL wide-mouth (screw-top) plastic bottle.

2. Add reagent water until the total mixture mass is 100.5 g. The resultant solids:solution ratio is 0.5 g solids:100-mL solution, or 1:200.

3. Seal bottles with screw top caps and place in a standard laboratory shaker set at 70 revolutions per minute for 60 minutes.

4. Upon completion of agitation, centrifuge sealed bottles at 2000 rpm for 10 minutes.

5. Gravity filter the concentrate from step 4 using Whitman #2 filter paper. (Suggest 150-mm circular filters folded and placed in simple plastic lab funnels.)

6. Adjust the pH of the filtrate to pH<2 with H_2SO_4 to preserve the nitrate/nitrite in the filtrate and store at 4°C until analysis.

7. The holding time for nitrate/nitrite is 48 hours, so samples must be analyzed for all three analytes within 48 hours of preservation.

8. All sample results will be reported in mg/kg on the basis of the original 0.5-g sludge sample.

Adapted from Vadas, P. A. and Kleinman, P. J. A., 2006.

Appendix B
Method Modifications for the PBDE Analyses

The following table summarizes the modifications made to the sample preparation and analysis procedures described in EPA Method 1614 for the analysis of the PBDEs. EPA and the laboratory instituted these modifications to address the interferences present in extracts of the samples, and the relatively high levels of some PBDEs in the samples.

The table provides a reference to the relevant section of the draft method, the original method specification, and the revised approach employed for this survey.

Method Section	Topic	Original Method Specification	Revised Approach
11.5.1	Sample size	10 g dry weight	As little as 0.2 g dry weight
11.5.2	Spiking labeled standards	Spike into sample before extraction	Spike sample extract before cleanup
12.6	Macroconcentration of extract	3 – 4 mL	10 mL
13	Cleanups	May use GPC, silica gel, alumina, or Florisil, if needed.	Must use silica gel, GPC, and alumina, in that order
13.2.3	GPC cleanup	Process 5 mL of extract	Process 1 mL of the 10 mL extract
1.1.1 and 17	Target analytes	All 209 possible BDE congeners	Only 11 congeners: BDE-28 BDE-47 BDE-66 BDE-85 BDE-99 BDE-100 BDE-138 BDE-153 BDE-154 BDE-183 BDE-209
17.1	Isotope dilution quantitation	11 congeners determined by true isotope dilution, and the remaining congeners by internal standard. The internal standards are the labeled congeners for other PBDEs that are added to the samples before extraction.	11 congeners quantified using labeled standards for 8 of those 11 congeners, all spiked into the extract before cleanup. All results are corrected for losses during the cleanup steps, but not for initial extraction efficiency.
17.1	Matrix spike samples	Not used, due to isotope dilution	Added periodic MS/MSD to provide data on extraction efficiency
17.5	Calibration range	All results must be within the calibration range, or must be diluted to bring them within range	Results for some congeners like BDE-209 flagged “E” in the database if above the calibration range but not high enough to saturate the detector system.

Appendix C
QC Acceptance Criteria

QC Acceptance Criteria for the Targeted National Sewage Sludge Survey

Analytical Fraction	QC Parameter	Analyte	Acceptance Limits (%)
Anions	LCS	Fluoride	85-115
		Phosphorus	85-115
		Nitrate/Nitrite	90-110
	MS Recovery	All analytes	75-125
	Duplicate Precision (RPD)	All analytes	20
Metals	LCS for solid samples	Aluminum	58-142
		Antimony	12-223
		Arsenic	77-123
		Barium	82-118
		Beryllium	77-122
		Boron	56-144
		Cadmium	80-121
		Calcium	79-121
		Chromium	78-121
		Cobalt	80-120
		Copper	82-118
		Iron	50-150
		Lead	79-121
		Magnesium	77-123
		Manganese	80-120
		Mercury	60-123
		Molybdenum	72-128
		Nickel	81-119
		Phosphorus	NA
		Selenium	76-124
		Silver	61-139
		Sodium	56-145
		Thallium	76-124
		Tin	NA
		Titanium	40-160
		Vanadium	76-124
		Yttrium	NA
		Zinc	79-120
	LCS for liquid samples	All metals	85 -115
	MS/MSD Recovery	All metals, except as noted below	70-130
		Mercury	60-128
		Tin	50-150
		Titanium	50-150
	MS/MSD Precision (RPD)	All metals	30
Organics	LCS	4-Chloroaniline	39-95
		2-Methylnaphthalene	12-159
		Fluoranthene	47-139
		Pyrene	52-129
		bis (2-Ethylhexyl) phthalate	39-174
		Benzo(a)pyrene	49-144
	MS/MSD Recovery for solid samples	4-Chloroaniline	10-104
		2-Methylnaphthalene	39-116
		Fluoranthene	49-130
		Pyrene	58-110
		bis (2-Ethylhexyl) phthalate	53-172
		Benzo(a)pyrene	50-126

NA = Not applicable. The commercial reference material used for the LCS does not have certified values for these analytes.

QC Acceptance Criteria for the 2006–2007 Targeted National Sewage Sludge Survey

Analytical Fraction	QC Parameter	Analyte	Acceptance Limits (%)
Organics	MS/MSD Recovery for liquid samples	4-Chloroaniline	10-62
		2-Methylnaphthalene	10-109
		Fluoranthene	10-150
		Pyrene	10-136
		bis (2-Ethylhexyl) phthalate	10-150
		Benzo(a)pyrene	10-152
	MS/MSD Precision (RPD)	All analytes, all matrix types	40
	Surrogate Recovery	Nitrobenzene-d_5	35-128
		2-Fluorobiphenyl	43-133
		p-Terphenyl-d_{14}	49-137
PBDEs	LCS	All analytes	25-150
	Labeled Compound Recovery	All, except as shown below	25-150
		^{13}C-BDE-209	20-200
	MS/MSD Recovery	All analytes	50-150
	MS/MSD Precision (RPD)	All analytes	50

QC Acceptance Criteria for Pharmaceuticals for the Targeted National Sewage Sludge Survey

Analyte	VER (%)	OPR (%)
Acid-Extractable Fraction - Positive Electrospray Ionization		
Acetaminophen	70 -130	50 -120
Azithromycin	70 -130	33 -120
Caffeine	70 -130	50 -124
Carbadox	70 -130	33 - 144
Carbamazepine	70 -130	21 - 137
Cefotaxime	70 -130	8 - 186
Ciprofloxacin	70 -130	50 - 120
Clarithromycin	70 -130	8 - 154
Clinafloxacin	70 -130	5 - 200
Cloxacillin	70 -130	5 - 200
Codeine	70 -130	34 - 129
Cotinine	70 -130	50 - 124
Dehydronifedipine	70 -130	42 - 120
Digoxigenin	70 -130	8 - 183
Digoxin	70 -130	5 - 148
Diltiazem	70 -130	11 - 120
1,7-Dimethylxanthine	70 -130	50 - 138
Diphenhydramine	70 -130	48 - 120
Enrofloxacin	70 -130	50 - 125
Erythromycin	70 -130	50 - 158
Flumequine	70 -130	36 - 200
Fluoxetine	70 -130	49 - 125
Lincomycin	70 -130	5 - 120
Lomefloxacin	70 -130	17 - 120
Miconazole	70 -130	27 - 120
Norfloxacin	70 -130	50 - 135
Norgestimate	70 -130	36 - 120
Ofloxacin	70 -130	50 - 200
Ormetoprim	70 -130	50 - 120
Oxacillin	70 -130	5 - 200

QC Acceptance Criteria for Pharmaceuticals for the Targeted National Sewage Sludge Survey

Analyte	VER (%)	OPR (%)
Oxolinic Acid	70 -130	42 - 124
Penicillin G	70 -130	5 - 200
Penicillin V	70 -130	5 - 200
Roxithromycin	70 -130	38 - 120
Sarafloxacin	70 -130	17 - 200
Sulfachloropyridazine	70 -130	50 - 200
Sulfadiazine	70 -130	5 - 200
Sulfadimethoxine	70 -130	50 - 120
Sulfamerazine	70 -130	50 - 148
Sulfamethazine	70 -130	50 - 142
Sulfamethizole	70 -130	50 - 120
Sulfamethoxazole	70 -130	50 - 120
Sulfanilamide	70 -130	5 - 189
Sulfathiazole	70 -130	41 - 120
Thiabendazole	70 -130	50 - 120
Trimethoprim	70 -130	50 - 126
Tylosin	70 -130	16 - 149
Virginiamycin	70 -130	5 - 189
Tetracyclines		
Anhydrochlortetracycline	70 -130	50 - 135
Anhydrotetracycline	70 -130	7 - 141
Chlortetracycline	70 -130	45 - 172
Demeclocycline	70 -130	5 - 200
Doxycycline	70 -130	22 - 166
4-Epianhydrochlortetracycline	70 -130	18 - 120
4-Epianhydrotetracycline	70 -130	5 - 200
4-Epichlortetracycline	70 -130	40 - 150
4-Epioxytetracycline	70 -130	50 - 142
4-Epitetracycline	70 -130	50 - 173
Isochlortetracycline	70 -130	5 - 200
Minocycline	70 -130	5 - 176
Oxytetracyclin	70 -130	50 - 183
Tetracycline	70 -130	50 - 155
Acid-Extractable Fraction - Negative Electrospray Ionization		
Gemfibrozil	70 -130	50 - 120
Ibuprofen	70 -130	50 - 120
Naproxen	70 -130	50 - 120
Triclocarban	70 -130	50 - 120
Triclosan	70 -130	50 - 120
Warfarin	70 -130	50 - 120
Base-Extractable Fraction - Positive Electrospray Ionization		
Albuterol	70 -130	50 - 133
Cimetidine	70 -130	5 - 120
Metformin	70 -130	50 - 149
Ranitidine	70 -130	24 - 160

QC Acceptance Criteria for Pharmaceutical Labeled Compounds for the Targeted National Sewage Sludge Survey

Analyte	VER (%)	OPR (%)	Recovery in Samples (%)
Acid-Extractable Fraction - Positive Electrospray Ionization			
$^{13}C_2$-^{15}N-Acetaminophen	70 -130	5 - 200	19 - 200
$^{13}C_3$-Caffeine	70 -130	5 - 200	31 - 200
$^{13}C_3$-^{15}N-Ciprofloxacin	70 -130	5 - 200	37 - 181
Cotinine-d_3	70 -130	5 - 120	5 - 145
$^{13}C_2$-Erythromycin	70 -130	50 - 120	23 - 120
Fluoxetine-d_5	70 -130	50 - 126	40 - 148
$^{13}C_6$-Sulfamethazine	70 -130	5 - 157	12 - 120
$^{13}C_6$-Sulfamethoxazole	70 -130	50 - 146	40 - 129
Thiabendazole-d_6 [A]	70 -130	50 - 146	32 - 140
$^{13}C_3$-Trimethoprim	70 -130	50 - 177	50 - 172
Tetracyclines			
Thiabendazole-d_6 [A]	70 -130	50 - 120	30 - 132
Acid-Extractable Fraction - Negative Electrospray Ionization			
Gemfibrozil-d_6	70 -130	38 - 122	21 - 123
$^{13}C_3$-Ibuprofen	70 -130	28 - 122	29 - 127
^{13}C-Naproxen-d_3	70 -130	34 - 131	14 - 132
$^{13}C_6$-Triclocarban	70 -130	5 - 172	5 - 147
$^{13}C_{12}$-Triclosan	70 -130	5 - 168	5 - 153
Warfarin-d_5	70 -130	50 - 177	50 - 200
Base-Extractable Fraction - Positive Electrospray Ionization			
Albuterol-d_3	70 -130	35 - 121	39 - 141
Metformin-d_6	70 -130	5 - 141	5 - 200

[A] Thiabendazole-d_6 is used as a labeled analog in both the tetracyclines and the acid extractable-positive electrospray fractions, with separate acceptance criteria.

QC Acceptance Criteria for Steroids and Hormones for the Targeted National Sewage Sludge Survey

Analyte	VER (%)	OPR (%)
Androstenedione	70 - 130	5 - 200
Androsterone	70 - 130	50 - 121
Campesterol	70 - 130	40 - 200
Cholestanol	70 - 130	50 - 164
Cholesterol	70 - 130	5 - 200
Coprostanol	70 - 130	34 - 200
Desmosterol	70 - 130	5 - 200
17α-Dihydroequilin	70 - 130	45 - 151
Epicoprostanol	70 - 130	50 - 197
Equilenin	70 - 130	5 - 200
Equilin	65 - 135	5 - 200
Ergosterol	50 - 150	5 - 200
17α-Estradiol	70 - 130	50 - 120
17α-Ethinyl estradiol	70 - 130	50 - 123
17β-Estradiol	70 - 130	50 - 176
β-Estradiol-3-benzoate	70 - 130	5 - 189
Estriol	70 - 130	5 - 193
Estrone	70 - 130	50 - 173
Norethindrone	70 - 130	45 - 200
Norgestrel	70 - 130	46 - 200
Progesterone	70 - 130	5 - 200
β-Sitosterol	70 - 130	5 - 200
β-Stigmastanol	70 - 130	29 - 200
Stigmasterol	70 - 130	50 - 200
Testosterone	70 - 130	50 - 136

QC Acceptance Criteria for Steroid and Hormone Labeled Compounds for the Targeted National Sewage Sludge Survey

Analyte	VER (%)	OPR (%)	Recovery in Samples (%)
Cholesterol-d_7	70 - 130	50 - 120	50 - 120
17α-Ethinyl estradiol-d_4	70 - 130	50 - 120	50 - 120
17β-Estradiol-d_4	70 - 130	50 - 120	29 - 132
Norethindrone-d_6	70 - 130	37 - 120	12 - 120
Norgestrel-d_6	70 - 130	36 - 120	7 - 120
Progesterone-d_9	70 - 130	5 - 200	5 - 200