Pharmaceutical Waste Analysis

There has been an increase in Polluted Places nomination sites associated with adverse impacts due to pharmaceutical process waste. The Technical Advisory Board requested additional information concerning pharmaceutical waste to better evaluate potential cleanup sites. The primary problem concerns generalized waste characterization as each facility manufactures different types of drugs and/or health-related equipment with different potential health and environmental impacts. However, there are several waste characteristics that appear throughout the industry – organic loading (BOD and COD), volatile organic compounds (VOCs) including organic solvents, nitrogen compounds, salts, and metals.

1. EPA Wastewater Analysis and Standards

The US Environmental Protection Agency (EPA) has established specific effluent limitations for wastewater generated by the pharmaceutical manufacturing industry (see Technical Guidance Document at http://www.epa.gov/waterscience/guide/pharm.html). EPA established sub-categories for the industry, including:

- Fermentation Operations (A);
- Biological and Natural Extraction Operations (B);
- Chemical Synthesis Operations (C), and,
- Mixing, Compounding or Formulating Operations (D).

Attachment 1 contains excerpts from the EPA technical guidance document describing the various pharmaceutical manufacturing processes and expected wastewater characteristics. The vast majority of US pharmaceutical manufacturing plants utilize mixing, compounding and formulating operations. A table is also included listing the various wastewater treatment processes used by the industry.

Attachment 2 contains a table listing the pollutants present in pharmaceutical wastewater based on industry response to EPA (Table 6-1 from the EPA guidance document). The EPA evaluated each pollutant and excluded from consideration for regulation pollutants discharged in relatively small amounts, those with low toxicity, those without effective treatment technologies, those discharged from a small number of sources, and those that cannot be analyzed by established methods. The resultant effluent standards are listed in Tables 17-4, 17-5 and 17-6 (Attachment 2). The EPA combined the wastewater discharge regulations for subcategories A (fermentation) and C (chemical synthesis) as well as subcategories B (biological and natural extraction) and D (formulating).

2. Other Wastes

The pharmaceutical manufacturing industry produces other waste streams. These include air emissions (Searle was fined $95,000 in 2002 for VOC emissions from solvent storage tanks and associated valves and pumps), wastewater treatment sludge (from chemical/physical treatment processes), and waste drugs (solid/hazardous waste and in the wastewater). **Attachment 3** includes an EPA listing of acutely hazardous and toxic compounds known to exist in pharmaceutical wastes (I suspect that many of these compounds cannot be tested by standard laboratory methods and that specialized, and costly, laboratory services would be required). An emerging area of interest is widespread contamination of water supplies (surface water and ground water) by pharmaceutical and personal care products entering the environment through septic and sewer systems (see *Environmental Health Perspectives* article in Attachment 3).

As an aside, I attended a meeting this week with a first-responder to hemorrhagic fever epidemics. During her discussion, she stated that she spent time in Bosnia during the recent war. One of the big environmental problems was the disposal of tons of expired medicines that had been shipped there as part of the world-wide relief efforts. The drugs were eventually encased in concrete and buried.

3. Wastewater Treatment

Several articles were reviewed concerning pharmaceutical wastewater treatment processes. Use of bio-reactors and oxidation seem to represent state-of-the-art technologies. The following links are available for further information. Of particular interest are the articles on a Polish facility (EMCENTRE link) as the article was peer-reviewed by the Environmental Management Centre in Mumbai, India and the 2006 abstract regarding pharmaceutical wastewater treatment in China (ASCE web site). The contacts in Eastern Europe, India and China may prove to be of value.

- http://www.emcentre.com/unepweb/tec_case/chemical_24/process/p25.htm
- http://www.pubs.asce.org/WWWdisplay.cgi?0600103
- http://www.wyeth.com/ehs/performance/wastewater.asp
- http://www.zenon.com/markets/wastewater/pharmaceutical.shtml
- http://www.hydroxyl.com/industries/pharmaceutical.html and http://www.zenon.com/resources/case_studies/wastewater/ScinoPharm.shtml
- http://www.usfilter.com/en/Product+Lines/Zimpro_Products/Technical+Papers/ (scroll down to the two pharmaceutical case studies)

4. Blacksmith Institute Study

Blacksmith Institute conducted wastewater sampling at a pharmaceutical plant in Meerut, India. Samples were returned to the US for laboratory analysis of Metals, VOCs, and Semi-Volatile Organic Compounds (SVOCs). Based on the EPA data and wastewater treatment information, these are the primary compounds of concern aside from BOD, COD, TSS, salts and nitrogen compounds. The VOC data from this case study are considered suspect due to sample handling and preservation problems. Metals were detected in the wastewater; however, the concentrations were below EPA drinking water standards (for those metals with a standard). The only VOC detected was acetone, a common laboratory artifact. Two SVOCs were detected in one sample: Di-n-Butyl Phthalate (12 µg/L) and 4-Chloro-3-Methyl Phenol (630 µg/L). Di-n-Butyl Phthalate is typically present in water that has been in contact with plastic. The compound is ubiquitous world wide in air, water and food, usually in low concentrations. The EPA recommends that levels of the compound be less than 34,000 µg/L in water (see http://www.eco-usa.net/toxics/dinbutph.shtml).

The compound 4-Chloro-3-Methyl Phenol is a general biocide with the majority used in metal working fluids. The other major use is as a pharmaceutical preservative. For example, hand and body creams contain the compound to prevent degradation by micro-organisms. One information web site (http://www.ukmarinesac.org.uk/activities/water-quality/wq8_31.htm) states that there are little data concerning the fate and toxicity of the compound. A limit of 200 µg/L is mentioned. The compound has many synonyms and is an EPA-listed U (toxic) waste and one of the 128 EPA Priority Pollutants. The EPA web site provides more information and also mentions a 200 µg/L threshold above which skin irritation may occur (see http://www.epa.gov/fedrgstr/EPA-WATER/2004/April/Day-06/w7780.htm).

5. Sampling Procedures

The USGS has developed sampling procedures for pharmaceutical wastewater. The protocol is included as **Attachment 4** (http://water.usgs.gov/owq/FieldManual/ Chapter A5). The USGS study referenced detected pharmaceutical-related waste in surface water throughout the US.

6. Site Visit Recommendations

Once a location has been selected by Blacksmith for a site visit, the following information/protocol will be helpful in completing an assessment:

- Obtain as much information as possible concerning the specific pharmaceutical manufacturing process, products, waste handling, etc.
- Obtain information/evidence of health impacts, particularly if an ailment or incidence can be traced to a specific or probable source.
- Based on this information, determine the appropriate analytical program. If facility-specific information is not available, collect samples for analysis of metals, VOCs, and SVOCs. It is assumed that if the primary pollutants of concern are BOD, COD, nitrogen compounds and salts, Blacksmith would not be very interested in the project. Specialized testing may also be appropriate (specific drugs, for example) if funding is available.
- Select sampling locations based on overall site information.
- Adhere to sampling protocol and sample handling procedures.

This analysis is meant to provide sufficient background information for the TAB evaluation of possible Blacksmith pharmaceutical sites. Please advise regarding inaccuracies or omissions. If additional information is needed on a specific subject, please let me know and I will do some more research.

3.4.2 General Process Descriptions

General process descriptions for each type of process operation are described in the following subsections. The specific processing steps on individual process lines may differ from these general descriptions as process operations will be tailored to the specific product being produced.

3.4.2.1 Fermentation

Most antibiotics and steroids are produced by the fermentation process, which involves three basic steps: inoculum and seed preparation, fermentation, and product recovery. Production of a fermentation pharmaceutical begins in the seed preparation step with spores from the plant master stock. The spores are activated with water, nutrients, and warmth; they are then propagated through the use of agar plates, test tubes, and flasks until enough mass is produced for transfer to the seed tank. In some fermentations, a single seed tank may provide inoculum for several fermentations. In this type of operation, the seed tank is never emptied completely, so the remaining seed serves as the inoculum for the next batch. The seed tank is emptied, sterilized, and reinoculated only when contamination occurs.

Fermentation is conventionally a large-scale batch process. The fermentation step begins with a water wash and steam sterilization of the fermenter vessel. Sterilized nutrient raw materials in water are then charged to the fermenter. Microorganisms grown from seed to aid in the fermentation process are transferred to the fermenter from the seed tank and fermentation begins. During fermentation, air is sparged into the batch and temperature is carefully controlled. After a period that may last from 12 hours to one week, the fermenter batch whole broth is ready for filtration. Filtration removes mycelia (i.e., remains of the microorganisms), leaving the filtered aqueous broth containing product and residual nutrients that are ready to enter the product recovery phase.

There are three common methods of product recovery: solvent extraction, direct precipitation, and ion exchange or adsorption. Solvent extraction is a recovery process in which an organic solvent is used to remove the pharmaceutical product from the aqueous broth and form a more

concentrated solution. With subsequent extractions, the product is separated from any contaminants. Further removal of the product from the solvent can be done by either precipitation, solvent evaporation, or further extraction processes. Normally, solvents used for product recovery are recovered and reused. However, small portions left in the aqueous phase during the solvent "cut" can appear in the plant's wastewater stream. Based on information from the Detailed Questionnaire, the solvents most often used in fermentation operations are acetone, methanol, isopropanol, ethanol, amyl alcohol, and MIBK. Table 3-5 lists solvents used in fermentation operations.

Direct precipitation using heavy metal precipitating agents is another common method of product recovery. The method involves first precipitating the product as a metal salt from the aqueous broth, then filtering the broth, and finally extracting the product from the solid residues. Copper and zinc are priority pollutant metals known to be used in the precipitation process.(2)

Ion exchange or adsorption involves removal of the product from the broth, using solid materials such as ion exchange resin, adsorptive resin, or activated carbon. The product is recovered from the solid phase using a solvent, then recovered from the solvent by evaporation.

Occasionally, a fermentation batch becomes infested with a phage, a virus that attacks microorganisms necessary to the fermentation process. Phage infection is rare in a well-operated plant, but when it occurs, the plant may discharge very large amounts of wastewater in a short period of time because of the decontamination process. Typically, the infested batch is discharged early, and its nutrient pollutant concentration is higher than that of spent broth.

Steam is the major sterilizing medium for most equipment. However, detergents and disinfectants, to the extent that they are used, can contribute to waste loads. An example of a commonly used chemical disinfectant is phenol, a priority pollutant. Air pollution control equipment sometimes installed to clean fermentation waste off-gas is another wastewater source. The air and gas vented from the fermenters usually contain odoriferous substances (e.g., oxides of nitrogen and sulfur) and large quantities of carbon dioxide. Treatment is often necessary to deodorize the gas before release to the atmosphere. Some plants use incineration methods; others

use liquid scrubbers. The blowdown from scrubbers may contain absorbed chemicals, soluble organic compounds, and insoluble organic oils and waxes.

Spent fermentation broth contributes pollutants to wastewater from the food materials contained in the broth, such as sugars, starches, protein, nitrogen, phosphate, and other nutrients. Fermentation wastes are very amenable to biological treatment. The spent broth can be satisfactorily handled by biological treatment systems in a concentrated form. Equalizing the broth prior to treatment helps avoid system upsets that may occur if the biota receive too high feed concentrations at one time.

Data from the Detailed Questionnaire generally show that process wastewater from fermentation plants is characterized by high BOD_5, COD, and TSS concentrations; relatively large flows; and a pH range of approximately 4.0 to 8.0.

3.4.2.2 Biological and Natural Extraction

Many materials used as pharmaceuticals are derived from such natural sources as the roots and leaves of plants, animal glands, and parasitic fungi. These products have numerous and diverse pharmaceutical applications, ranging from tranquilizers and allergy-relief medications to insulin and morphine. Also included in this group is blood fractionation, which involves the production of plasma and its derivatives.

Despite their diversity, all extractive pharmaceuticals have a common characteristic: they are too complex to synthesize commercially. They are either very large molecules, and/or their synthesis results in the production of several stereoisomers, only one of which has pharmacological value. Extraction is an expensive manufacturing process which requires collecting and processing large volumes of specialized plant or animal matter to produce small quantities of products. Facilities utilize extraction when there are no other reasonable alternatives for producing a desired active ingredient.

The extraction process consists of a series of operating steps beginning with the processing of a large quantity of natural or biological material containing the desired active ingredient. After almost every step, the volume of material being handled is reduced significantly. In some processes, reductions may be in orders of magnitude, and complex final purification operations may be conducted on quantities of materials only a few thousandths of the volume handled in earlier steps. Neither continuous processing methods nor conventional batch methods are suitable for extraction processing. Therefore, a unique assembly-line, small-scale batch processing method is used. Material is transported in portable containers through the plant in 75- to 100-gallon batches. A continuous line of containers is sent past a series of operating stations. At each station, operators perform specific tasks on each batch in turn. As the volume of material being handled decreases, individual batches are continually combined to maintain reasonable operating volumes, and the line moves more slowly. When the volume is reduced to a very small quantity, the containers also become smaller, with laboratory-size equipment used in many cases. An extraction plant may produce one product for a few weeks; then, by changing the logistical movement of containers and redefining tasks to be conducted at each station, the plant can convert to the manufacture of a different product.

Residual wastes from an extraction plant essentially will be equal to the weight of raw material, since the active ingredients extracted are generally present in the raw materials at very low levels. Solid wastes are the greatest source of the pollutant load; however, solvents used in the processing steps can cause both air and water pollution. Detergents and disinfectants used in equipment cleaning operations are normally found in the wastewater.

Priority pollutants, including methylene chloride, toluene, chloroform, 1,2-dichloroethane, and phenol, were identified as being used in the manufacturing of extractive pharmaceuticals in the Detailed Questionnaire. The cations of lead and zinc are known to be used as precipitating agents. Phenol was identified as a disinfecting chemical. The other priority pollutants found were used as processing solvents. The Detailed Questionnaire identified nonconventional pollutants most often used in the extractive manufacturing process as ethanol, methanol, n-amyl acetate, isopropanol, and acetone. These nonconventional pollutants may be used as processing solvents. Table 3-6 lists solvents used in biological or natural extraction operations.

Solvents are used in two ways in extraction operations. Some solvents are used to remove fats and oils that would contaminate the products. These "defatting" extractions use an organic liquid that dissolves the fat but not the product material. Solvents are also used to extract the product itself. For example, when plant alkaloids are treated with a base, they become soluble in such selected organic solvents as benzene, chloroform, and 1,2-dichloroethane.

Ammonia is used in many extraction operations because it is necessary to control the pH of water solutions from both animal and plant sources to separate valuable components from waste materials. Ammonium salts are used as buffering chemicals, and aqueous or anhydrous ammonia is used as an alkalinizing reagent. The high degree of water solubility of ammonium salts prevents unwanted precipitation of salt, and they do not react chemically with animal or plant tissue. Such basic materials as hydroxides and carbonates of alkali metals do not have these advantages.

The principal sources of wastewater from biological/natural extraction operations are: 1) spent raw materials (e.g., waste plasma fractions, spent media broth, plant residues); 2) floor and equipment wash water; 3) chemical wastes (e.g., spent solvents); and 4) cleanup of spills.

Wastewater from extraction plants is generally characterized by low BOD_5, COD, and TSS concentrations; small flows; and pH values of approximately 6.0 to 8.0.

3.4.2.3 Chemical Synthesis

Most of the active ingredients marketed and sold as drugs are manufactured by chemical synthesis. Chemical synthesis is the process of manufacturing pharmaceuticals using organic and inorganic chemical reactions. Since most of these compounds are produced in batch operations, the conventional batch reaction vessel is the major piece of equipment used on the process line.

The reaction vessel is one of the most standardized equipment designs in the industry. Generally, it is made of either stainless steel or glass-lined carbon-steel, and it contains a carbon-steel outer shell suitable for either cooling water or steam. Inside the vessel is a motor-driven agitator and a

baffle. Vessels of this type are made in many different sizes, with capacities ranging from 0.02 to 11.0 m^3 or more.

The basic vessels may be fitted with different attachments depending on the process needs of the product to be manufactured. Baffles usually contain sensors to measure the temperature of the reactor contents. Dip tubes may be used to introduce reagents into the vessels below the liquid surface. The vessel's agitators may be powered by two-speed motors or by variable-speed motor drives. The reactor may be mounted on load cells to accurately weigh the reactor contents. The batch reactors are typically installed with only the top heads extending above the plant operating floor to provide the operator with easy access for loading and cleaning. Also, one of the top nozzles may be fitted with a floodlight and another with a glass cover to enable an operator to observe the reactor contents.

The reactors can be modified for additional uses. By using heating or refrigeration devices, the chemicals may be boiled or chilled in them, according to process needs. By adding reflux condensation equipment, the vessel may perform complete reflux operations (i.e., recycling of condensed vapors). The vessels can also become evaporators if vacuum is applied. The reactors may also be used to perform solvent extraction operations and, by operating the agitator at a slow speed, the vessels can serve as crystallizers.

Synthetic pharmaceutical manufacture consists of using one or more of these reactor vessels to perform, in a step-by-step fashion, the various operations necessary to make the product. Following a definite recipe, the operator (or, increasingly, a programmed computer) adds reagents; increases or decreases the flow rate of cooling water, chilled water, or steam; and starts and stops pumps which transfer the reactor contents to another vessel. At appropriate steps in the process, solutions are pumped either through filters or centrifuges, or into solvent recovery headers or waste sewers.

The reactor vessels with an assembly of auxiliary equipment are usually arranged into independent process units, which are suitable for the complete or partial manufacture of many different pharmaceutical compounds. Only with the highest volume products is the process unit

"dedicated" to manufacturing only one product. Large pharmaceutical plants may have many such units, while smaller plants may have only one or two.

Each pharmaceutical product is usually manufactured in a "campaign," in which one or more process units are used for a few weeks or months to manufacture enough compound to satisfy the projected sales demand. Campaigns are usually tightly scheduled, with detailed coordination extending from procurement of raw materials to packaging and labeling of the product. For a variable period of time, a process unit actively manufactures a specific compound. At the end of the campaign for one product, another is scheduled to follow. After equipment cleaning, the same equipment is then used to make a completely different product, using different raw materials, executing a different recipe, and creating different wastes.

A variety of priority pollutants are used as reaction and purification solvents during chemical synthesis. According to the Detailed Questionnaire, priority pollutants used by facilities during the chemical synthesis process include benzene, chlorobenzene, chloroform, chloromethane, o-dichlorobenzene, 1,2-dichloroethane, methylene chloride, phenol, toluene, and cyanide.

The Detailed Questionnaire identified the top five nonconventional pollutants associated with chemical synthesis as methanol, acetone, isopropanol, ethyl acetate, and ethanol. Six-member ring compounds, such as xylene, pyridine, and toluene, are also widely used organic solvents because they are stable compounds that do not easily take part in chemical reactions. These compounds are used either in the manufacture of synthesized pharmaceuticals or are produced as the result of unwanted side reactions. Table 3-7 lists solvents used in chemical synthesis operations.

Solvents are used in chemical synthesis processes to dissolve gaseous, solid, or viscous reactants in order to bring all the reactants into close molecular proximity. Solvents also serve to transmit heat to or from the reacting molecules. By physically separating molecules from each other, solvents slow down some reactions that would otherwise take place too rapidly, resulting in unwanted side reactions and excessive temperature increases.

Some solvents are also used to control the reaction temperature. It is common practice in a batch-type synthesis to select a solvent which is compatible with the reaction and which has a boiling point the same as the desired reaction temperature. Heat is then applied to the reaction mass at a rate sufficient to keep the mixture boiling continuously. Vapors that rise from the reaction vessel are condensed, and the liquefied solvent is allowed to drain back into the reaction vessel. This refluxing prevents both overheating and overcooling of the reactor contents, and can automatically compensate for variations in the rate of release or absorption of chemical energy.

Many plants operate solvent recovery units that purify contaminated solvents for reuse. These units usually contain distillation columns, and may also include solvent/solvent extraction operations in which a second solvent is used to separate impurities. These operations may result in aqueous wastes that contain residues fully or partially saturated with residual solvent. Wastewater is generally produced with each chemical modification that requires filling and emptying the batch reactors. This wastewater can contain unreacted raw materials, as well as some solvents, along with a large number of compounds that differ due to the varied chemical reactions performed (e.g., nitration, amination, halogenation, sulfonation, alkylation). Chemical synthesis effluent generally has a high BOD_5 and COD waste load. The pollutants in chemical synthesis wastewater vary with respect to toxicity and biodegradability. The production steps may generate acids, bases, cyanides, metals, and other pollutants, while the waste process solutions and vessel wash water may contain residual organic solvents. Occasionally, chemical synthesis wastewater is incompatible with biological treatment systems because it is too concentrated or too toxic for the biomass in the treatment system. Thus, it may be necessary to equalize and/or chemically pretreat some chemical synthesis wastewater prior to biological treatment.

Primary sources of wastewater from chemical synthesis operations are: 1) process wastes such as spent solvents, filtrates, and concentrates; 2) floor and equipment wash water; 3) pump seal water; 4) wet scrubber wastewater; and 5) spills. Wastewater from chemical synthesis plants can be characterized as having high BOD_5, COD, and TSS concentrations; large flows; and extremely variable pH values, ranging from 1.0 to 11.0.

3.4.2.4 Mixing, Compounding, or Formulating

Pharmaceutically active ingredients are generally produced by batch processes in bulk form and must be converted to dosage form for consumer use. Common dosage forms for the consumer market are tablets, capsules, liquids, and ointments. In addition, active ingredients can also be incorporated into patches and time release capsules.

Tablets are formed in a tablet press machine by blending the active ingredient, filler, and binder. The filler (e.g., starch, sugar) is required to dilute the active medicinal ingredient to the proper concentration, and a binder (e.g., corn syrup or starch) is necessary to bind the tablet particles together. A lubricant (e.g., magnesium stearate) may be added for proper tablet machine operation. The dust generated during the mixing and tableting operation is collected and usually recycled directly to the same batch, while broken tablets generally are collected and recycled to the granulation operation in a subsequent lot. Some tablets are coated by tumbling with a coating material and then dried. After the tablets have been coated and dried, they are sent to the packaging unit where they are bottled. Tablet-coating operations can be a significant source of air emissions of solvents if solvent-based coatings are used, and can contribute solvents to the plant wastewater if certain types of air pollution control equipment (wet scrubbers or activated carbon) are used to capture solvent vapors from tablet-coating operations. Wastewater from the wet scrubber is likely to be sewered as is the condensate from the steam used to regenerate the activated carbon.

The first step in capsule production is to form a hard gelatine shell. The shells are produced by machines that dip rows of rounded metal dowels into a molten gelatine solution, and then strip the capsules from the dowels after the capsules have cooled and solidified. Imperfect capsules are remelted and reused, if possible, or sold for glue manufacture. Most pharmaceutical companies purchase empty capsules from a few specialty producers. The active ingredient and filler are mixed before being poured by machine into the empty gelatine capsules. The filled capsules are bottled and packaged. As in tablet production, some dust is generated, which is recycled to the production line. Liquid preparations are formulated for injection or oral use. In both cases, the liquid active ingredient is first weighed and then dissolved in water. Injectable solutions are

bulk-sterilized by heat or filtration and then poured into sterilized bottles. Oral liquid preparations can be bottled directly without the sterilization steps. Wastewater is generated by general cleanup operations, spills, and breakage.

Ointments are produced by blending an active ingredient(s) with an ointment base such as polyethylene glycol. The blended product is then poured into tubes by machine and packaged. Wastewater generated from these operations are all from equipment cleaning operations.

The primary objective of mixing, compounding, or formulating operations is to convert the manufactured products into a final, usable form. The necessary production steps typically have small wastewater flows because very few of the unit operations generate wastewater. The primary use of water is in the actual formulating process, where it is used for cooling and for equipment and floor washing.

Wastewater sources from mixing, compounding, or formulating operations are: 1) floor and equipment wash water, 2) wet scrubbers, and 3) spills. The use of water to clean out mixing tanks can periodically flush dilute wastewaters of unusual composition into the plant sewer system. The washouts from mixing tanks may be used to prepare the master batches of the pharmaceutical compounds and may contain inorganic salts, sugars, and syrup. Other sources of contaminated wastewater are dust and fumes from scrubbers, either in building ventilation systems or on specific equipment. In general, this wastewater is readily treatable by biological treatment systems.

An analysis of the pollutant information in the pharmaceutical manufacturing database shows that wastewater from mixing, compounding, or formulating plants normally has low BOD_5, COD, and TSS concentrations; relatively small flows; and pH values of 6.0 to 8.0.

3.4.3 Pharmaceutical Manufacturing Process Variability

The wastewater effluent flow and composition from a typical pharmaceutical manufacturing facility can be highly variable. Factors contributing to such variability are:

- Campaigning;
- Batch processing; and
- Wastewater commingling.

Because many pharmaceutical products are manufactured in campaigns, most wastewater is generated during product changeover. The process equipment must be cleaned out to avoid product contamination. The composition of the wastewater will vary according to the products that were manufactured on that process line.

Pharmaceuticals are manufactured by batch and continuous manufacturing operations. Batch-type production is by far the most common manufacturing technique, as presented in the production operation breakdown in Table 3-8. Many pharmaceutical facilities conduct multiple batch operations, some in series and some concurrently. Often several of the required batch processes are performed at the same time in separate reactors, each with its own schedule. Each batch may have unique waste stream characteristics. In fermentation operations, it can take a few days to several weeks to complete the ferment, during which little or no wastewater is generated. However, during product recovery operations, high-volume, high-strength wastewaters are generated.

It is also common practice in the pharmaceutical manufacturing industry to commingle organic-contaminated wastewaters. In many cases commingling is necessary to collect sufficient wastewater volume to properly operate an economically sized treatment unit such as a steam stripper. Commingled wastes may be added to the treatment unit feed tank on a variable schedule, thus altering the feed composition on a real-time basis. In other cases, segregating for purposes of recovery and treatment may be appropriate and cost effective.

A variety of solvents are used in the pharmaceutical manufacturing industry and end up in the industry's wastewater. Many solvents are process-specific and cannot be interchanged in other pharmaceutical processes. In addition, solvents must be approved by the FDA for each process. FDA regulations require that before a change can be made to an approved process, industry must meet the requirements of product purity and product efficacy as specified in the FDA approval.

Consequently, simplification of wastestream composition by chemical substitution to a common solvent may not be possible or desirable. Nonetheless, EPA has worked with the Food and Drug Administration (FDA) to encourage pollution prevention in the final guidelines and standards. See 7.2.1.2 for a more detailed discussion of EPA and FDA efforts towards pollution prevention in the pharmaceutical industry.

Table 3-5

Solvents Used in Fermentation Operations

Acetone	n-Heptane
Acetonitrile	n-Hexane
Ammonia (aqueous)	Isopropanol
n-Amyl acetate	Isopropyl acetate
Amyl alcohol	Methanol
n-Butyl acetate	Methyl cellosolve
n-Butyl alcohol	Methylene chloride
Chloroform	Methyl isobutyl ketone (MIBK)
N,N-Dimethylformamide	Petroleum naphtha
Ethanol	Phenol
Ethyl acetate	Toluene
Formaldehyde	Triethylamine

Table 3-6

Solvents Used in Biological or Natural Extraction Operations

Acetone	Ethylene glycol
Acetonitrile	Formaldehyde
Ammonia (aqueous)	n-Heptane
n-Amyl acetate	n-Hexane
Amyl alcohol	Isopropanol
n-Butyl alcohol	Isopropyl acetate
Chloroform	Isopropyl ether
1,2-Dichloroethane	Methanol
Diethylmine	Methylene chloride
Diethyl ether	Petroleum naphtha
N,N-Dimethylformamide	Phenol
Dimethyl sulfoxide	n-Propanol
1,4-Dioxane	Pyridine
Ethanol	Tetrahydrofuran
Ethyl acetate	Toluene

Table 3-7

Solvents Used in Chemical Synthesis Operations

Acetone	Formaldehyde
Acetonitrile	Formamide
Ammonia (aqueous)	Furfural
n-Amyl acetate	n-Heptane
Amyl alcohol	n-Hexane
Aniline	Isobutyraldehyde
Benzene	Isopropanol
2-Butanone (MEK)	Isopropyl acetate
n-Butyl acetate	Isopropyl ether
n-Butyl alcohol	Methanol
Chlorobenzene	Methylamine
Chloroform	Methyl cellosolve
Chloromethane	Methylene chloride
Cyclohexane	Methyl formate
o-Dichlorobenzene (1,2-Dichlorobenzene)	Methyl isobutyl ketone (MIBK)
1,2-Dichloroethane	2-Methylpyridine
Diethylamine	Petroleum naphtha
Diethyl Ether	Phenol
N,N-Dimethyl acetamide	Polyethylene glycol 600
Dimethylamine	n-Propanol
N,N-Dimethylaniline	Pyridine
N,N-Dimethylformamide	Tetrahydrofuran
Dimethyl sulfoxide	Toluene
1,4-Dioxane	Trichlorofluoromethane
Ethanol	Triethlyamine
Ethyl acetate	Xylenes
Ethylene glycol	

Table 3-8

Production Operation Breakdown(a)

Type of Operation	Number of Operations					Percent of Total Operation
	Manufacturing Processes				Total	
	Fermentation	Biological Extraction	Chemical Synthesis	Mixing/ Compounding/ Formulating		
Batch	309	189	1,059	3,675	5,232	99
Continuous	16	1	16	8	41	1
Total Number of Operations	325	190	1,075	3,683	5,273	100
Percent of Total Operations	6	4	20	70	100	
Percent of Subcategory Operations which are Batch	95	99	99	100	99	

(a) Production data obtained from 244 facilities responding to the Detailed Questionnaire.

Table 3-9

Trends in Treatment Technologies Used at Pharmaceutical Manufacturing Facilities(a)

Treatment Technology	Percentage of Facilities Using This Type of Treatment Technology Prior to 1986	Percentage of Facilities Using This Type of Treatment Technology in 1989/1990
Neutralization	26.0	44.3
Equalization	20.1	28.6
Activated sludge	16.9	20.5
Settleable solids removal	13.3	NA
Primary sedimentation	12.0	NA
Aerated lagoon	7.5	4.9
Primary clarification	3.9	9.8
Chlorination	3.6	2.5
Polishing ponds	3.2	NA
Waste stabilization pond	2.9	2.5
Trickling filter	2.9	2.0
Multimedia filtration	2.3	6.1
Steam stripping	1.9	5.7
Evaporation	1.9	NA
Secondary clarification	1.6	20.9
Granular activated carbon	1.3	3.3
Oxidation	1.0	2.0
Dissolved air flotation	1.0	NA
pH adjustment	NA	50.0
Phase separation	NA	12.3

The total of the percentages is not 100 because any one facility may have multiple treatment technologies and some facilities do not have treatment in place.

NA - Not available.

(a) Data obtained from reference 22 and the responses to the Detailed Questionnaire.

ATTACHMENT 2

Table 6-1

Pollutants Which May be Present in Pharmaceutical Industry Wastewater

Conventional Pollutants		**Nonconventional Pollutants**
BOD_5	Oil & Grease	Acetaldehyde
pH	Fecal Coliform	Acetic acid
TSS		Acetone
		Acetonitrile
Priority Pollutants		Acetophenone
		Allyl chloride
Acrolein		4-Aminobiphenyl
Acrylonitrile		Ammonia
Benzene		n-Amyl acetate
Benzidine		Amyl alcohol
Bromoform		Aniline
Bromomethane		Benzaldehyde
Chlorobenzene		Benzotrichloride
Chloroethane		Benzyl alcohol
Chloroform		Benzyl chloride
Chloromethane		Benzyl bromide
Cyanide		Biphenyl
1,1-Dichloroethane		Bis(chloromethyl)ether
1,2-Dichloroethane		2-Bromo-Propanoylbromide
1,1-Dichloroethene		2-Butanone (MEK)
1,2-Dichloropropane		n-Butyl acetate
Ethylbenzene		n-Butyl alcohol
Hexachlorocyclopentadiene		tert-Butyl alcohol
Hexachloroethane		sec-Butyl alcohol
Methylene chloride		n-Butylamine
Nitrobenzene		Carbon disulfide
2-Nitrophenol		Catechol
4-Nitrophenol		Chloroacetic acid
o-Dichlorobenzene		2-Chloroacetophenone
p-Dichlorobenzene		3-Chloro-4-Fluoroaniline
Phenol		Chloromethyl methyl ether
1,1,2,2-Tetrachloroethane		COD (Chemical Oxygen Demand)
Tetrachloromethane		
Toluene		Cresol (Mixed)
1,1,1-Trichloroethane		Cumene
Trichloroethylene		Cyclohexane
1,1,2-Trichloroethane		Cyclohexanone
Vinyl chloride		Cyclopentanone

Table 6-1 (Continued)

Nonconventional Pollutants (Continued)	
Cyclohexylamine	Iodomethane
1,2-Dibromoethane	Isobutyraldehyde
1,2-trans-Dichloroethene	Isopropyl ether
Diethylaniline	Isopropanol
Diethyl ether	Isopropyl acetate
Diethylamine	Isobutyl alcohol
Diethyl carbonate	Methanol
Diethyl-ortho formate	Methyl cellosolve
Dimethylamine	Methyl amine
1,1-Dimethylhydrazine	Methyl formate
N,N-Dimethylacetamide	2-Methyl pyridine
N,N-Dimethylformamide	2-Methoxyaniline
N,N-Dimethylaniline	Methyl methacrylate
Dimethylcarbamyl chloride	Methyl-t-butyl-ether
Dimethyl sulfoxide	Methylal
1,4-Dioxane	Methyl isobutyl ketone (MIBK)
N-Dipropylamine	N-Nitrosomorpholine
Epichlorohydrin	n-Octane
Ethanol	n-Pentane
Ethylene oxide	Petroleum naphtha
Ethylamine	Polethylene glycol 600
Ethyl bromide	1,3-Propane sulfone
Ethyl cellosolve	n-Propanol
Ethyl acetate	B-Propiolactone
Ethylene glycol	Propionaldehyde
Ethyl cyanide	1,2-Propyleneimine
Formaldehyde	Propylene oxide
Formamide	Pyridine
Formic acid	Styrene
Furfural	Tetrachloroethene
Glycol ethers	Tetrahydrofuran
n-Heptane	Trichlorofluoromethane
2-Hexanone	2,4,5-Trichlorophenol
n-Hexane	Triethylamine
Hydrazine	Vinyl acetate
Iodoethane	Xylenes

Table 17-4

Pollutants to be Regulated Under PSES and PSNS

Pollutant	Subcategories A and C	Subcategories B and D
Priority Pollutants		
Cyanide (a)	X	
Benzene	X	
Chlorobenzene	X	
Chloroform	X	
o-Dichlorobenzene (1,2-Dichlorobenzene)	X	
1,2-Dichloroethane	X	
Methylene Chloride	X	X
Toluene	X	
Non-Conventional Pollutants		
Acetone	X	X
Ammonia as N (b)	X	
n-Amyl Acetane	X	X
n-Butyl Acetate	X	
Diethylamine	X	
Ethyl Acetate	X	X
n-Heptane	X	
n-Hexane	X	
Isobutraldehyde	X	
Isopropyl Acetate	X	X
Isopropyl Ether	X	
Methyl Cellosolve Methyl Formate	X	
Methyl isobutyl ketone (MIBK)	X	
Tetrahydrofuran	X	
Thethylamine	X	
Xylenes	X	

(a) EPA is only clarifying the monitoring point on the existing regulation.
(b) Ammonia is only regulated for indirect dischargers that discharge to non-nitrifying POTWs.

Table 17-5

PSES and PSNS Effluent Limitations for Subcategory A and C Operations

Pollutant or Pollutant Property	PSES/PSNS for In-Plant Monitoring Points	
	Maximum for any 1 day mg/L	Monthly Average mg/L
Cyanide (1)	33.5	9.4

(1) Cyanide effluent limit established in the 1983 final rule, applies to Subcategory A and C operations only.

Pollutant or Pollutant Property	PSES Effluent Limitations End-of-Pipe Monitoring Points	
	Maximum for any 1 day mg/L	Monthly Average mg/L
Acetone	20.7	8.2
Ammonia as N (2)	84.1	29.4
n-Amyl Acetate	20.7	8.2
Benzene	3.0	0.6
n-Butyl Acetate	20.7	8.2
Chlorobenzene	3.0	0.7
Chloroform	0.1	0.03
o-Dichlorobenzene	20.7	8.2
1,2-Dichloroethane	20.7	8.2
Diethylamine	255.0	100.0
Ethyl Acetate	20.7	8.2
n-Heptane	3.0	0.7
n-Hexane	3.0	0.7
Isobutyraldehyde	20.7	8.2
Isopropyl Acetate	20.7	8.2
Isopropyl Ether	20.7	8.2
Methyl Cellosolve	275.0	59.7
Methylene Chloride	3.0	0.7
Methyl Formate	20.7	8.2
MIBK	20.7	8.2
Tetrahydrofuran	9.2	3.4
Toluene	0.3	0.1
Triethylamine	255.0	100.0
Xylenes	3.0	0.7

(2) Ammonia is only regulated for indirect dischargers that discharge to non-nitrifying POTWs.

Table 17-6

PSES and PSNS Effluent Limitations for Subcategory B and D Operations

Pollutant or Pollutant Property	PSES Effluent Limitations End-of-Pipe Monitoring Point	
	Maximum for any 1 day mg/L	Monthly Average mg/L
Acetone	20.7	8.2
n-Amyl Acetate	20.7	8.2
Ethyl Acetate	20.7	8.2
Isopropyl Acetate	20.7	8.2
Methylene Chloride	3.0	0.7

ATTACHMENT 3

LISTED WASTES

There are two types of listed pharmaceutical hazardous wastes. These are known as acutely hazardous **(P-listed)** and toxic **(U-listed)**.

P-listed Pharmaceutical Wastes – These wastes are known as **acutely hazardous.**

NAME	HW#
Epinephrine (Adrenaline)	P042
Nicotine	P075
Nitroglycerine	P081
Physostigmine	P204
Physostigmine salicylate	P188
Sodium Azide	P105
Strychnine	P108
Warfarin >.3%	P001

U-listed Pharmaceutical Wastes – These wastes are known as **toxic**.

NAME	HW#
Acetone	U002
Chlorambucil	U035
Chloroform	U044
Cyclophosphamide	U058
Daunomycin	U059
Dichlorodifluromethane	U075
Diethylstilbestrol	U089
Formaldehyde	U122
Hexachlorophene	U132
Lindane	U129
Melphalan	U150
Mercury	U151
Mitomycin C	U010
Paraldehyde	U182
Phenacetin	U187
Phenol	U188
Reserpine	U200
Resorcinol	U201
Saccharin	U202
Selenium sulfide	U205
Streptozotocin	U206
Trichloromonofluromethane	U121
Uracil mustard	U237
Warfarin <.3%. (Coumadin)	U248

NOTE: These are not comprehensive lists of "P" and "U" listed chemicals. For a complete list, refer to: 40CFR§261.33. The Code of Federal Regulations is available online at http://www.access.gpo.gov/nara/cfr/, or you can obtain information by calling the U.S. Environmental Protection Agency's RCRA hotline at (800) 424-9346.

We should no longer think of water as a gift of nature but an industry which needs investment.
Thawat Vichaidiji, Thai water official quoted in the *Bangkok Post*, 17 March 1991

WATER POLLUTION

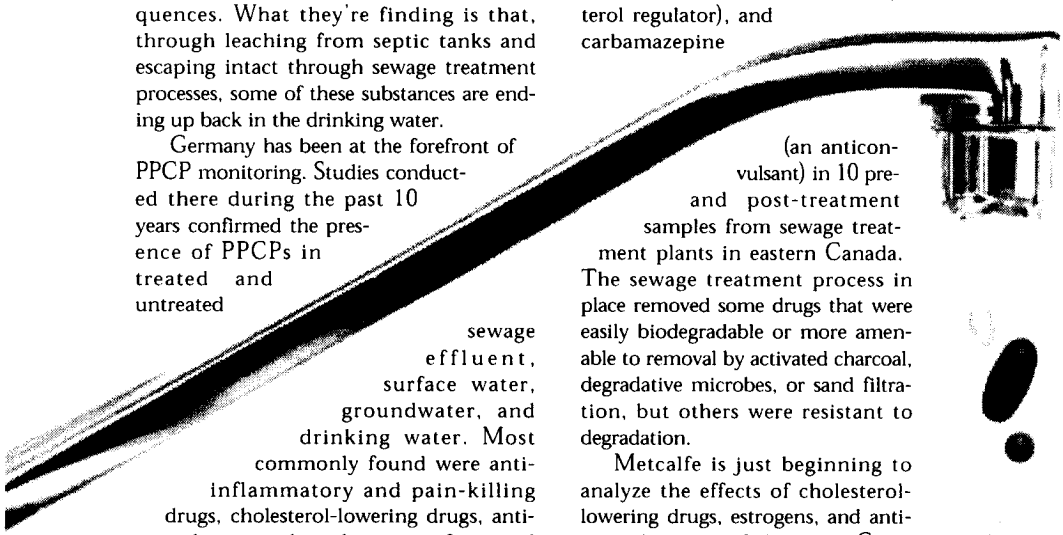
Drugged Drinking Water

Drugs and personal care products that are excreted from or washed off the body naturally end up in the sewage that flows into sewer systems and septic tanks, but where do they go from there? Scientists are beginning to monitor the extent of pharmaceutical and personal care products (PPCPs) in the aquatic environment and their consequences. What they're finding is that, through leaching from septic tanks and escaping intact through sewage treatment processes, some of these substances are ending up back in the drinking water.

Germany has been at the forefront of PPCP monitoring. Studies conducted there during the past 10 years confirmed the presence of PPCPs in treated and untreated sewage effluent, surface water, groundwater, and drinking water. Most commonly found were anti-inflammatory and pain-killing drugs, cholesterol-lowering drugs, anticonvulsants, and sex hormones from oral contraceptives. Samples from 40 German rivers and streams turned up residues of 31 different PPCPs, according to a report presented at the March 2000 American Chemical Society meeting in San Francisco, California, by Thomas Ternes, a chemist at the Institute for Water Research and Water Technology in Wiesbaden.

Researchers worldwide have discovered more than 60 different PPCPs in water sources, according to Christian Daughton, chief of the Environmental Chemistry Branch of the U.S. Environmental Protection Agency (EPA) Environmental Sciences Division in Las Vegas, Nevada. In addition to the drugs noted above, the list includes antineoplastics, beta-blockers, bronchodilators, lipid regulators, hypnotics, antibiotics, antiseptics, X-ray contrast agents, sunscreen agents, caffeine, and fragrances such as synthetic musks. Most PPCPs are detected at concentrations ranging from parts per trillion to parts per billion, and originate in treated and untreated sewage, says Daughton, who coauthored an article on PPCPs in the December 1999 issue of *EHP Supplements*.

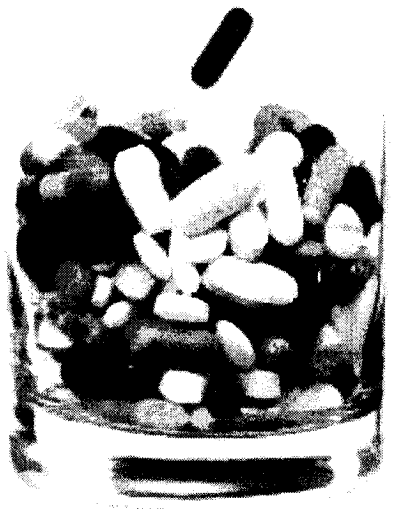
North American researchers are just beginning to look at the issue of PPCPs. Studies presented at the June 2000 Emerging Issues Conference sponsored by the National Ground Water Association, held in Minneapolis, Minnesota, indicate that the problem exists here, too. For example, environmental scientist Chris Metcalfe of Trent University in Peterborough, Ontario, detected the drugs aspirin, ibuprofen, indomethacin, bezafibrate (a cholesterol regulator), and carbamazepine (an anticonvulsant) in 10 pre- and post-treatment samples from sewage treatment plants in eastern Canada. The sewage treatment process in place removed some drugs that were easily biodegradable or more amenable to removal by activated charcoal, degradative microbes, or sand filtration, but others were resistant to degradation.



Metcalfe is just beginning to analyze the effects of cholesterol-lowering drugs, estrogens, and anticonvulsants on fish in the Great Lakes. All three drug types can potentially interfere with normal reproduction and development in fish living downstream from sewage treatment plants. His laboratory studies show that estrogen compounds at parts-per-trillion exposures feminize male fish and disrupt the development of the circulatory system, eyes, and bladder. He says it's too soon to know whether PPCPs adversely affect wild fish populations.

In one of the first studies in the United States to report the occurrence of drugs in drinking water, environmental engineer Glen Boyd had his students at Tulane University in New Orleans, Louisiana, sample water from the Mississippi River, a local lake, and city tap water. Their preliminary experiment targeted the pain reliever naproxen, the sex hormone estrone, and clofibric acid, a major bioactive metabolite from certain anticholesterol drugs. All three were detected at varying concentrations in most of the samples. "The big unknown," says Boyd, "[is whether PPCPs] present a health concern now or in the future." He notes that, although the number of peer-reviewed papers on the topic is limited, government agencies concerned with water quality in the United States and professional organizations serving the water and wastewater communities are beginning to acknowledge PPCPs as an emerging environmental issue.

The long-term outcome of humans ingesting subtherapeutic doses of numerous drugs as well as any dose at all of substances not meant to be ingested remains a major unaddressed issue. "In areas of water scarcity, we'll see more and more reuse of treated sewage to meet drinking water needs," predicts Daughton, thereby increasing the likelihood that PPCPs will end up in drinking water. Extensive monitoring of the occurrence of PPCPs and their concentration trends over time is required to ensure safe water supplies in the future. Then toxicologists need to determine if the kinds and amounts of PPCPs that occur affect people and other living creatures. This subject will require collaboration between the Food and Drug Administration and the EPA, says Daughton, since the former usually does not address environmental concerns and the latter generally does not deal with drug issues.
–Carol Potera



ATTACHMENT 4

WASTEWATER, PHARMACEUTICAL, AND ANTIBIOTIC COMPOUNDS 5.6.1.F

M.E. Lewis and S.D. Zaugg

The USGS differentiates between samples collected for analysis of wastewater compounds and those collected for analysis of pharmaceutical and antibiotic compounds, based on the analytical schedule for the laboratory method.[1] Currently, only the wastewater laboratory method for field-filtered samples (SH1433) is an approved, routine (production) method. (The unfiltered wastewater method LC 8033 also is available but requires a proposal for custom analysis.) At this time, analysis of samples for pharmaceutical and antibiotic compounds is confined to research studies and is available only on a custom basis.

To collect and process surface-water and ground-water samples that will be analyzed for concentrations of wastewater, pharmaceutical, and antibiotic compounds, the standard USGS procedures for collecting and filtering organic compounds are used (see NFM 4, 5.1, 5.2.2). However, special considerations related to personal safety and to sample contamination are required.

Samples collected for analysis of these compounds may be collected directly from sources of raw or treated wastewater. Sources of wastewater include treated and untreated domestic sewage, leaking septic systems or sewer lines, sanitary sewer overflows, and runoff from animal feeding operations. **Handling of such samples can expose personnel to pathogenic microorganisms, and therefore requires strict adherence to safety protocols.**

[1]The compounds included in a given laboratory schedule are subject to change and should be checked when planning a sampling activity that involves collecting data on wastewater, pharmaceutical, or antibiotic compounds.

Samples collected for analysis of wastewater, pharmaceutical, and antibiotic compounds are susceptible to contamination because they are ubiquitous in daily use. **To ensure sample integrity, field personnel should avoid contact with or consumption of products that contain the compounds targeted for analysis, and must adhere scrupulously to equipment-cleaning, equipment-handling, sample-processing, and sample-handling protocols**.

CAUTION: Raw or treated wastewater samples can contain microorganisms harmful to human health—use safe handling protocols.

- **Adhere to safety protocols.** The following precautions must be followed by field personnel when collecting, processing, or handling raw or treated wastewater samples for analysis of wastewater, pharmaceutical, or antibiotic compounds:
  - Be familiar with the basic procedures to minimize exposure to and effects from contaminated water, as described in NFM 9.7.
  - Receive proper immunizations before engaging in field activities and consult with your safety officer on this issue.
  - Avoid direct contact with sewage and other types of wastewater and with equipment still contaminated through contact with the sample or source water.
  - Avoid breathing in sewage and wastewater fumes or mist.
  - Do not use workspace surfaces or equipment that have come into contact with polluted water until they have been decontaminated. Use only those decontamination procedures that are described below under "Prevent sample contamination."

► **Prevent sample contamination:**

— On the day of sampling activities, avoid contact with or consumption of the products listed below. Where contact with or consumption of these products is unavoidable, the collection of field blanks is strongly recommended.

Wastewater compounds

- Soaps and detergents, including antibacterial cleansers
- DEET (active ingredient in most insect repellents)
- Fragrances (cologne, aftershave, perfume)
- Sunscreen
- Animal or human urine or excrement
- Caffeine (coffee, tea, colas)
- Tobacco

Pharmaceutical compounds

- Prescription drugs, medications, and hormonal substances
- Over-the-counter medications
- Selected human antibiotics

Antibiotics

- Human antibiotics
- Veterinary antibiotics

— Wear powderless nitrile laboratory gloves during sampling and processing. Change to clean gloves with each change in activity or potential glove contamination.

— Avoid breathing directly over open samples/equipment.

— Avoid direct contact between yourself (including clothing) and the sample, sampling device, and processing equipment. Clothing is a source of detergents, fragrances, and fire retardants.

- Thoroughly field rinse and seal in a plastic bag all reusable equipment that comes in contact with sewage until the equipment can be properly decontaminated and disinfected (NFM 3.2.2).
- Clean scrupulously all workspace surfaces that come into contact with sewage—use a non-antibacterial soap[2] and water, followed by wiping all potentially contaminated sufaces with a clean, disposable isopropyl alcohol (70-90 percent) pad.
- Avoid any actions at the field site that result in the disposal or release of wastewater and pharmaceutical substances.

► **Implement quality control.** Quality-control samples are a required, integral part of water-quality investigations. As previously noted, samples for analysis of wastewater, pharmaceutical, and antibiotic compounds are vulnerable to contamination.

- Check your quality-control plan for instructions on the collection of field blanks and replicates for these sample types. Although the specific type, number, and distribution of quality-control samples are determined by the design and data-quality requirements of the study (NFM 4.3), field blanks are processed more frequently for these samples than for most other sample types.
- When using a a custom analysis, consult with the laboratory analysts for quality-control recommendations.
- Process an initial field blank to evaluate the potential for contamination associated with the field methods, materials used, and sampling environment. Distribute subsequent field blanks areally and temporally to meet data-quality requirements of the project.
- Use either pesticide- or VOC-grade blank water as the source solution for the field blanks.

[2]The laboratory analysis of wastewater includes triclosan, an active ingredient in most antibacterial soaps. Triclosan is also commonly found in some deodorants, toothpastes, mouthwashes, skin creams, lotions, laundry detergents, and dish soaps.

— The analytical methods used for pharmaceuticals and antibiotics are currently unapproved and are available only on a cutsom basis for research projects. It is reasonable to assume that blank water tested and certified contaminant free for approved organic constituent methods is an appropirate source solution for field blanks for these unapproved methods; however, a more rigorous approach would be to include a source solution blank as an integral part of the project quality-control plan.

— Process the field blanks in the same manner and under the same environmental conditions as the environmental sample (NFM 4.3.1.B).

Wastewater, pharmaceutical, and antibiotic samples are vulnerable to contamination and thus require rigorous quality control, usually by processing field and source-solution blanks.

► **When planning sampling activities that will result in a request for a custom analysis:**

— Contact the NWQL in Lakewood, Colorado, (http://nwql.usgs.gov/) before collecting any samples for unfiltered wastewater-compound analysis or pharmaceutical-compound analysis.

— Check One-Stop Shopping for supplies for USGS projects.

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— Contact the Organic Geochemistry Laboratory in Lawrence, Kansas, (http://ks.water.usgs.gov/Kansas/reslab/) before collecting any samples for antibiotics that will be analyzed using this laboratory's methods.

Replacement page, 11/19/2004

To collect and process samples:

1. Order and assemble all equipment. Select sampling and processing equipment made of fluorocarbon polymers, glass, aluminum, or stainless steel. Avoid equipment made of Tygon®, polyethylene, or other plastics.

2. Clean equipment thoroughly before use, following the general protocols for organic-compound samples described in NFM 3.2.2, but with the following caveats:

   - Use non-antibacterial detergents.
   - Take extra care to ensure that equipment is copiously rinsed with deionized water (DIW) after the detergent wash—detergents are a source of interference in the analysis of pharmaceutical compounds and may include a target analyte (triclosan) of the wastewater analytical method.
   - Follow the DIW rinse with a methanol rinse. Collect the used methanol solution into an appropriate container for disposal.
   - Do not clean or field-rinse the baked-glass sample bottles obtained from OWQRL or another laboratory.

3. Collect and process the samples using methods appropriate for organic compounds, as described in NFM 4 and 5.2.2. Use laboratory-baked, brown (amber) glass sample bottles.

   - For wastewater and pharmaceuticals being shipped to the NWQL for analysis, use 1-L GCC bottles.
   - For antibiotic samples being shipped to the OWQRL for analysis, use 1-L GCC bottles.
   - For antibiotic samples being shipped to the Lawrence Organic Geochemistry Laboratory for analysis, use two 125-mL baked-glass bottles with Teflon® caps per sample.

4. Label bottles with site ID (identifier), date, time, sample type ("filtered" or "unfiltered"), and laboratory code or schedule number.

   a. For samples being shipped to the NWQL:

      - Wastewater, field-filtered – Label sample bottle "GCC–-SH 1433"
      - Wastewater, raw – Label sample bottle "GCC–LC 8033" (add this laboratory code to the Analytical Services Request (ASR) form: **currently this is a custom analysis**)
      - Pharmaceuticals – Label sample bottle "LC9003"

   b. For antibiotic-analysis samples being shipped to the OWQRL – Label sample bottle "Antibiotics"

   c. For antibiotic-analysis samples being shipped to Lawrence, Kansas – Label sample bottles "LC-AN"

5. If collecting wholewater samples for custom wastewater analysis – Fill the 1-L GCC bottle to the shoulder.

6. When collecting filtered samples for wastewater, pharmaceutical, or antibiotic analyses – Filter the samples at the field site.

   - Pass samples through a 0.7-µm nominal pore-size glass microfiber plate filter (GF/F grade), following the procedures for organic compounds described in NFM 5.2.2.A.
   - Fill the 1-L GCC or two 125-mL baked-glass bottles to the shoulder.

7. For samples collected from polluted water, decontaminate the exterior of bottles:

   a. Rinse bottles with copious amounts of water.

   b. Wipe each bottle with a clean, disposable isopropyl alcohol (70-90 percent) pad.

   c. Rinse off each bottle with water.

8. Keep the samples chilled to 4°C or less until they are prepared for shipping.

9. After sample collection and processing, use a non-antibacterial soap and water to thoroughly clean any workspace surfaces that have come into contact with polluted water. Follow the cleaning by decontaminating the workspace with isopropyl alcohol. **Do not use a bleach solution for decontamination of surfaces that come in contact with samples, since any bleach residue will degrade target analytes**.

To ship the sample(s):

1. For raw or treated wastewater, use the following precautions when packing and shipping, as these samples can pose a health hazard to field and laboratory personnel.

   a. Decontaminate any sample bottles containing raw or treated wastewater as described in step 7 above.

   b. Check bottle cap to ensure a tight closure.

   c. Place each bottle inside a foam sleeve and then place bottles into a ziplock bag along with four 3M high-capacity chemical sorbent pads (pads are available from Lab Safety and Supply at www.labsafety.com). Seal the bag.

   d. Place the sealed bag inside two additional ziplock bags (a total of three bags) and seal each bag.

   e. Clearly note in the "Login Comments" section of the ASR form "SAMPLE CONTAINS RAW OR TREATED SEWAGE. HANDLE WITH CARE." Sampling staff or shipping staff should notify the laboratory login staff by telephone or e-mail of the expected delivery of these samples, in advance of their arrival at the lab.

2. For all other samples,

   a. Check that sample bottles are securely capped.

   b. Check that sample bottles are correctly labeled and match the information shown on the ASR form.

   c. Pack all samples in ice to maintain a temperature of or below 4°C without freezing, using standard procedures (NFM 5.5.3 - 5.5.4). Check that the ASR form has been properly sealed in doubled plastic bags and included with the samples.

3. Ship all samples to the laboratory via overnight delivery.