Mechanisms of Tetracycline Resistance Development in the Environment as Detected by Real-Time PCR

David Graham
University of Kansas, Lawrence, KS

Abstract

Strong evidence exists suggesting that the use of antibacterial agents in agriculture is increasing the level of antibiotic resistance among microbial pathogens. This presentation focuses on four studies assessing relationships between tetracycline exposure and antibiotic resistance development in the environment. The goal of this research project was to examine the transport pathway from the point of antibiotic use to possible downstream exposure to determine rates and mechanisms by which resistance are gained or lost in different settings. The study used laboratory-, mesocosm-, and field-scale systems (both in rivers and operating feedlot lagoons) and both classical microbiological techniques and quantitative real-time polymerase chain reaction (PCR) for tracking resistant organisms and genes in the systems. Tetracyclines were chosen for study because it and its derivatives are used extensively in clinical or veterinary applications, and the genetic basis of resistance is well established, allowing the use of quantitative real-time PCR for determining quantifying resistance gene numbers in exposed systems.

Two watersheds with variable land use, five feedlots with eight wastewater lagoons, and a series of controlled laboratory and mesocosm experiments were examined to fulfill the goals of this study. In all studies, real-time PCR probe-primer sets for the *tet(O)*, *tet(W)*, *tet(Q)*, *tet(M)*, *tet(A)*, and *tet(B)* resistance genes were used to quantify resistance gene numbers in organisms as a function of antibiotic use patterns and receiving water or other environmental conditions.[1] The full-scale river and feedlot studies showed that elevated resistance genes in receiving waters were seasonal and primarily resulted from the transport of resistant organisms away from the point of veterinary use rather than via *in situ* resistance development.[2,3] Ambient tetracycline levels in receiving waters, however, usually did not correlate with resistance gene numbers; therefore, the *in situ* detection of antibiotics is often a poor marker of environmental exposure.

These studies led to further work assessing how resistance genes move in the environment after release, how long genes were retained after release (and why), and ancillary environmental effects caused by tetracyclines, such as impairment of aquatic plants.[4] Followup laboratory and mesocosm experiments were performed to track key genes and whole-organism resistance in different types of receiving water settings, including light-exposed, dark, high-tetracycline, low-tetracycline, and waters with elevated natural bacterial communities. In general, ambient light supply dominated the fate of both resistant organisms and resistance genes in downstream waters. Resistance gene half-lives were often two to three orders of magnitude greater under dark treatments compared with all other exposures. Some individual resistant bacteria were enriched after initial release into receiving waters, although this was not the norm with most resistance genes and organisms typically dying very rapidly upon release into surface waters. Photodeactivation appeared to be a key mechanism for "resistance" die-off in most systems. *tet(W)* and *tet(M)* had the longest half-lives among all genes tested (in the order of hours), suggesting that these genes might be possible biomarkers for resistance-gene exposure in future work.

Results indicate that tetracycline resistance is developed primarily at the point of use rather than in the environment, although individual resistant organisms can be enriched in some receiving water scenarios. Generally, resistance gene "die-off" is most rapid in light-exposed aquatic systems, which suggests that photodeactivation is important for "resistance" reduction in environmental systems. Work continues on the fate and transfer of resistance genes and organisms in aquatic systems, with the development of a new mathematical receiving-water model being underway to help predict downstream resistance impacts below point and non-point resistance sources.

References

1. Smith M.S., Yang R.K., Knapp C.W., Niu Y., Peak N., Hanfelt M., Galland J.S, and Graham D.W. Quantification of tetracycline resistance genes in feedlot lagoons using real-time PCR. *Appl Environ Microbiol* 2004;70:7372-7377.
2. Peak N., Knapp C.W., Yang R.K., Hanfelt M.M., Smith M.S., Aga D.S., and Graham D.W. Relationships between antibiotic use practices and resistance gene levels in feedlot wastewater lagoons as detected by real-time PCR. *Environ Sci Techno* (submitted, 2005).
3. Knapp C.W., Busch C., Miller J., Hanson M.L., Keen P., Hall K., and Graham D.W. *In situ* resistance development to oxytetracycline in aquatic systems. *Antimicrob Agents Chemother* (submitted, 2005).
4. Hanson M.L., Knapp C.W., and Graham D.W. Field assessment of oxytetracycline exposure to the freshwater macrophytes *Elodea densa* (Planch.) and *Ceratophyllum demersum* L. *Env Poll* (in revision, 2005).

Pharmaceuticals and Personal Care Products as Environmental Contaminants (1): Preliminary Environmental Risk Calculations and Method Development for Analysis in Environmental Media via GC/MS

Kevin Bisceglia, Jim Yu, Ed Bouwer, Mehmet Coelhan, and A. Lynn Roberts
Johns Hopkins University, Baltimore, MD

Abstract

The purpose of this research is to: (1) conduct a preliminary environmental risk assessment (ERA) by compiling data on pharmaceutical usage, occurrence, and potential (eco)toxic risk; and (2) refine analytical methods for quantifying selected pharmaceuticals in sewage and drinking water samples.

A detailed usage, occurrence, and ecotoxicity database was developed for the highest volume ("top 200") pharmaceuticals in the brand name, generic, over-the-counter, and hospital categories for the years 1999-2002. Usage data were used to compute expected introductory concentrations (EICs) according to U.S. Food and Drug Administration (FDA) guidelines; results were compared to measured concentrations whenever possible. As very few pharmaceuticals appear in the U.S. Environmental Protection Agency's (EPA) ECOTOX database, emphasis was placed on toxicity estimates obtained from EPA's Ecological Structure Activity Relationships (ECOSAR) program. The results were used to select an analyte suite of environmentally relevant compounds potentially amenable to analysis via gas chromatography/mass spectrometry. In developing analytical methods, choice of derivatization agent, solvent, and reaction conditions were systematically optimized, as were necessary sample clean-up procedures. Solid phase extraction (SPE) was explored as a preconcentration step, and the selection of sorbent media, sample pH, and elution solvent identity was investigated systematically as well.

The preliminary ERA yielded no correlation between EIC and pharmaceutical sales ranking, indicating that many more pharmaceuticals could be of potential concern than were identified in this study. The majority (greater than 80%) of pharmaceuticals that are potentially present at measurable concentrations (i.e., > 10 ng/L) have not yet been sought by environmental researchers. EICs appear useful in providing order-of-magnitude estimates of concentrations encountered in sewage treatment plant influents. Preliminary calculations (using experimental and ECOSAR-derived toxicity data) indicate that as many as 10 percent of the pharmaceuticals considered in this study are likely to be of environmental concern. The results suggest that the current FDA "trigger" of 1 μg/L for performing risk assessments on new pharmaceuticals may be insufficiently conservative, as some compounds exert toxicity below that threshold.

This project has developed two new multiresidue methods for a total of 52 acidic, neutral, and basic pharmaceuticals and personal care products employing either pentafluorobenzyl bromide or a combination of *N, N*-bis(trimethylsilyl)trifluoroacetamide and chlorotrimethylsilane. The method for the acidic compounds is particularly robust and performs best in water:organic solvent mixtures, thus eliminating the need for drying prior to derivatization or after SPE. The SPE method has proved to be reproducible and exhibits minimal interference when extracting target compounds from waters high in natural organic matter. These methods have been demonstrated to perform well in highly complex matrices, such as raw and finished wastewater, as indicated by high recoveries of isotopically labeled surrogates or laboratory fortified field matrices.

The preliminary ERA performed in this study is the most detailed that has been performed in the United States to date. It demonstrates that EICs can be useful tools in predicting pharmaceutical loading into sewage treatment plants, and as such should be useful to environmental scientists in prioritizing future occurrence and ecotoxicity investigations. The analytical methods developed as part of this study employ techniques that can be adopted easily by other laboratories. Together, the methods are capable of analyzing one of the largest and most diverse suite of biologically active chemicals employed in the United States, and thus should prove useful in identifying the nature and scope of pharmaceutical contamination in U.S. waters.

Pharmaceuticals and Personal Care Products as Environmental Contaminants (2): Biodegradability Studies and Occurrence in Sewage Treatment Plant Influent and Effluent

Jim Yu, Kevin Bisceglia, Ed Bouwer, Mehmet Coelhan, and A. Lynn Roberts
Johns Hopkins University, Baltimore, MD

Abstract

The two major objectives for this research project are to: (1) examine the biodegradability of selected pharmaceuticals and antiseptics; and (2) study the occurrence and removal of selected pharmaceuticals and antiseptics in wastewater influents and effluents.

For examining the biodegradability of acidic pharmaceuticals and personal care products (PPCPs), batch biodegradation experiments have been conducted. Different electron acceptors, microbial inocula, and PPCP concentrations were investigated to elucidate the biodegradability of PPCPs under different environmental conditions.

The occurrence and removal of these PPCPs has been investigated in different sewage treatment plants (STPs) located in the Northeastern seaboard of the United States. These STPs employ different treatment processes and operational characteristics (e.g., different solid residence times and different nutrient removal processes). Quadruplicate 24-hour composite influent and effluent samples were obtained at each site, and U.S. Environmental Protection Agency Method 526 guidelines were followed for quality assurance and quality control in analyzing wastewater samples.

For aerobic biodegradation experiments, 13 of the 18 PPCPs tested underwent extensive (> 80%) biotransformation after 50 days of incubation. Anaerobic conditions (nitrate reducing and iron reducing) seemed to be less favorable for biodegradation of pharmaceuticals. All antiseptics were biotransformed under all testing conditions, whereas phenytoin, 5-fluorouracil, and diclofenac did not biodegrade readily. In general, aerobic conditions were more favorable for biotransformation than anaerobic conditions, and iron-reducing conditions were the least favorable. Experiments with different microbial inocula generally showed little difference in the aerobic biotransformation of different PPCPs, even though the inocula comprised very different microbial communities. Anaerobic microbial communities (nitrate vs. iron reducing) revealed some difference in biodegradability for a few compounds. Experiments with varying initial concentration showed little influence on the extent of biotransformation. By understanding the biodegradability of the target PPCPs, human and ecosystem risks resulting from the presence of these compounds in aquatic systems can be established further.

The majority of the target analytes were detected in the STP influents. Influent concentrations of PPCPs varied between geographic locations, potentially reflecting regional differences in drug usage. The removal efficiencies for different PPCPs also varied from treatment plant to treatment plant, depending on the specific treatment processes and operational characteristics, although incomplete removals were observed for many compounds. Overall, the majority of the target analytes were detected in both the influent and effluent STP streams at ng/L levels, although some PPCPs (e.g., naproxen and ibuprofen) were encountered at μg/L levels.

After reviewing all occurrence and removal data from STPs, a plant will be chosen for further detailed study. This research project will encompass a mass balance study on this single wastewater treatment plant to determine the removal efficiencies of each unit process. Samples will be taken at the influent and effluent of each unit processes to provide further insight on the removal of PPCPs in STPs.

Endocrine Effects of Selective Serotonin Reuptake Inhibitors (SSRIs) on Aquatic Organisms

Marsha C. Black[1], Emily D. Rogers[1], and Theodore B. Henry[2]
[1]University of Georgia, Athens, GA; [2]University of Tennessee, Knoxville, TN

Abstract

Pharmaceutical products can contaminate surface waters after their prescribed medical use and have the potential to negatively affect aquatic organisms. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) used to treat clinical depression. Fluoxetine has been detected in surface waters at subparts-per-billion concentrations and also has been found to accumulate in tissues of fish from effluent dominated streams. Serotonin is an important neurotransmitter, and increased endogenous levels of serotonin resulting from exposure to SSRIs may affect a number of endocrine-mediated processes, including spawning, reproduction, and thyroid-mediated development. Experiments designed to detect endocrine effects of fluoxetine exposure were conducted on two model aquatic species: a fish (western mosquitofish, *Gambusia affinis*) and an amphibian (*Xenopus laevis*). In acute and chronic experiments, neonate western mosquitofish were exposed to aqueous fluoxetine (0.06-6,000 ppb) and effects on survival, sex ratio, and time to sexual maturity were evaluated. Fluoxetine was acutely toxic, and the concentration estimated to kill 50 percent of exposed neonates within 7 days was 614 ppb. Chronic exposure to concentrations less than or equal to 60 ppb did not significantly affect survival or sex ratio. Development of adult sexual morphology, however, was significantly delayed at this concentration by 2-3 weeks relative to control fish. Similar indicators of delayed development were observed in preliminary experiments with the African clawed frog, *X. laevis*. Time to metamorphosis was significantly delayed relative to controls following chronic exposure of tadpoles to 29.5 ppb fluoxetine. Upon reaching metamorphosis, frogs were significantly smaller at all exposure concentrations including 0.059 ppb, which is an environmentally relevant concentration. These experiments show the potential for fluoxetine to disrupt endocrine processes in fish and frogs and demonstrate the need for more targeted studies with fluoxetine and other SSRIs that measure endocrine biomarkers (e.g., hormones, histopathology of gonads and thyroid glands) on the exposed organisms.

Hazards From Waste Pharmaceuticals: Determining Toxicity and Chemical Analytical Methods for Detection

Overview of a Framework for Assessing the Hazards of Human Pharmaceuticals in the Environment From a SETAC Pellston Workshop

Gerald T. Ankley[1], Marsha C. Black[2], Jeanne Garric[3], Thomas H. Hutchinson[4], and Taisen Iguchi[5]
[1]National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN; [2]University of Georgia, Athens, GA; [3]Laboratory for Ecotoxicology, Lyon, France; [4]Astra-Zenica, Brixham, UK; [5]Okazaki National Research Institute, Okazaki, Japan

Abstract

To cost-effectively address the aquatic ecotoxicity of human pharmaceuticals, research is needed to identify which methods from the wide range available are best suited for use in regulatory environmental risk assessments. In some cases, the highly specific, receptor-based pharmacology of drugs may require chronic reproductive testing with fish (e.g., for some endocrine-active pharmaceuticals), whereas other classes of compounds with less specific modes of action (e.g., anaesthetics and analgesics) may be more cost-effectively addressed with short-term, sublethal tests with algae, crustaceans, and/or fish. To try to prevent an inefficient and untargeted approach to test every chemical with every method, there is a need for a focused research program to better define the most suitable species, life stages, and endpoints for an optimized scheme of pharmaceutical testing. This research could be achieved through strategic testing of a set of pharmaceutical materials representative of different key modes/mechanisms of action. This presentation will introduce a conceptual framework and list of reference pharmaceuticals that could be used to determine future testing priorities for the regulatory assessment of pharmaceutical products and complex mixtures containing drugs, their metabolites, and other substances.

Overview of ORD's Aquatic Toxicology Research on Endocrine-Active Chemicals

Joseph E. Tietge
National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, MN

Abstract

Office of Research and Development laboratories are engaged in a variety of research projects with aquatic organisms that are relevant to the assessment of pharmaceuticals in the environment, specifically as they pertain to endocrine pathways. The broad objectives of these projects are to assess effects of chemicals on endocrine pathways in fish and amphibian species in laboratory studies and to evaluate exposure to endocrine-active chemicals in the environment using field studies.

There are three major approaches being used to reach these objectives. In the first approach, two short-term screening assays are being developed to identify chemicals that affect hormone (i.e., estrogen, androgen, and thyroid hormone) regulation and function using a variety of organismal, biochemical, and histological endpoints. One such assay utilizes the fathead minnow (*Pimephales promelas*) in a 21-day protocol to evaluate the effect of test chemicals on estrogen and androgen pathways, as determined by reproductive success, circulating sex steroid concentrations, gonadal histology, secondary sexual characteristics, and other biochemical endpoints. The basic protocol for this assay is complete, and validation studies are currently underway. The other screening assay utilizes the metamorphic phase of the African clawed frog (*Xenopus laevis*) in a 14-to-21-day assay to evaluate the effects of test chemicals on thyroid function. Metamorphosis is a thyroid hormone-dependent event, and perturbations of normal thyroid hormone homeostasis alter developmental rate as well as thyroid histology. This screening assay also is in the process of validation. In the second approach, partial and full life cycle tests are being developed to evaluate the effect of chemicals on reproductive and developmental endpoints in a fish, the Japanese medaka (*Oryzias latipes*), and in an amphibian (*Xenopus tropicalis*). The intent of this research is to determine if endocrine-disrupting chemicals result in transgenerational effects that are not detected in the preceding short-term screening assays. Furthermore, these tests, when fully developed and validated, may be used to better understand population level effects. In the third approach, selected endpoints that were developed in both the screening and testing protocols are being used in field studies wherein naive fish are exposed to surface water discharges, such as effluents emanating from confined animal feeding operations and municipal wastewater treatment plants. These studies demonstrate that androgenic and estrogenic activities can be detected in surface water discharges using well-defined methods. Taken together, these projects have advanced the basic understanding of endocrine disruption in aquatic organisms, help to meet Agency screening and testing requirements mandated through the Food Quality Protection and Safe Drinking Water Acts, and provide the Agency with tools to assess potential endocrine disruption in aquatic species in the environment.

Future activities will focus on the use of genomic, proteomic, and metabonomic endpoints in endocrine-disruption research. The objectives of applying these endpoints are to improve the diagnostic capabilities of the assays, abbreviate the screening assays by using early responding endpoints that are related to later outcomes, provide data that will aid in predictive modeling of endocrine disruption, and establish a molecular basis for interspecies extrapolation.

An Informatic Approach to Estimating Ecological Risks Posed by Pharmaceutical Use

Mitchell Kostich, James Lazorchak, and Gregory Toth
National Exposure Research Laboratory, Office of Research and Development,
U.S. Environmental Protection Agency, Cincinnati, OH

Abstract

Pharmaceuticals administered to humans and other animals are often excreted from treated organisms as intact drug or as active metabolites. Some of these active materials have been shown to make their way into the environment. The environmental concentrations of the vast majority of pharmaceuticals and their metabolites, however, are not known. The sensitivity of native organisms to chronic exposure to the doses of active material likely to be found in the environment also is typically not known. Direct determination of these important parameters is too expensive to perform on the entire pharmacopoeia, and a rational way of prioritizing individual drugs for more detailed study is needed.

For many drugs, sufficient information is available in the scientific literature and within regulatory filings to estimate the upper bound of environmental concentrations and most probable mechanisms of environmental toxicity. Although these estimates involve large confidence intervals, they are quickly and cheaply produced and are probably the best available criteria for prioritizing drugs for more expensive direct tests of environmental impact.

This research project describes a new method similar to that employed by the European Union and by the U.S. Food and Drug Administration for estimating risks of human prescription pharmaceuticals based on information found in regulatory filings, as well as scientific and trade literature. Available data on usage patterns, metabolic transformation, and physical/chemical properties are fed into models of dilution, degradation, partition between matrices, and bioavailability to estimate effective environmental concentrations. Available data on mechanisms of action and modes of toxicity are considered together with cursory phylogenetic analysis to estimate the sensitivity of select native organisms to the estimated highest likely environmental concentrations of each drug.

Scores produced by this procedure will be used to prioritize pharmaceuticals for more detailed analytical and toxicological followup. The potential application of modified versions of this method to over-the-counter pharmaceuticals and veterinary pharmaceuticals also is described.

Identifying Chemical Compounds from Wastewater Discharges

Susan T. Glassmeyer[1], Edward T. Furlong[2], Dana W. Kolpin[3], Jeffery D. Cahill[2], Steven D. Zaugg[2], Stephen L. Werner[2], Michael T. Meyer[4], and David D. Kryak[5]
[1]Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH; [2]U.S. Geological Survey, Denver, CO; [3]U.S. Geological Survey, Iowa City, IA; [4]U.S. Geological Survey, Lawrence, KS; [5]Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC

Abstract

The quality of drinking and recreational water is currently ascertained using indicator bacteria. The tests to analyze for these bacteria require a considerable length of time to complete and do not discriminate between human and animal fecal material sources. To shorten the time needed to test water quality and distinguish the fecal pollution source, chemicals found in human wastewater, such as pharmaceuticals, surfactants, and fecal sterols, were evaluated as tracers of human fecal pollution. These chemicals would have the advantage of requiring shorter analysis times than traditional microbiological techniques and can be selected to be human specific. At 10 locations, water samples were collected upstream and at 2 successive points downstream from a wastewater treatment plant. A treated effluent sample also was collected at each location. This longitudinal sampling scheme was used to estimate the persistence of the target compounds in streams. The water samples were extracted using either solid phase or liquid-liquid extraction and were analyzed using either liquid chromatography/mass spectrometry or gas chromatography/mass spectrometry. Of the 110 chemical analytes investigated in this project, 78 were found in at least one sample. Seventeen of the 110 compounds were classified as either a prescription or nonprescription pharmaceutical. Of these, 9 were found in at least 50 percent of the samples, and 13 were found in at least 10 percent of the samples. Although most concentrations of all of the compounds were in the range of 0.1 to 1.0 µg/L, in some of the more highly contaminated samples concentrations were in the range of 5-20 µg/L. The concentrations of the majority of the chemical compounds present in the samples generally followed an expected trend; they: (1) were non-existent or at only trace levels in the upstream samples; (2) had their maximum values in the wastewater effluent samples; and (3) declined in the two downstream samples. This research project indicates that these chemical analytes do have utility as tracers of human wastewater discharge. The correlation between the concentration of wastewater chemicals and incidence of illness is being investigated via a recreational water epidemiological study. The results of this study will determine if wastewater chemicals can be used as indicators of water quality.

Environmental Stewardship for Pharmaceuticals in the Environment

Conveying Risk and Progress: Communicating With the Public About Pharmaceuticals in Drinking Water

J.C. Davis
Southern Nevada Water Authority, Las Vegas, NV

Abstract

The public's reaction to the notion of pharmaceuticals in their drinking water is understandably visceral and negative for two reasons. First, most people have difficulty comprehending concentrations in the parts per trillion, so they use a "present" or "absent" litmus test when it comes to contaminants. Second, unlike other contaminants, the presence of pharmaceuticals reinforces the relationship between treated wastewater and the source water supply.

When a topic such as pharmaceuticals and personal care products (PPCPs) receives scientific attention, mainstream media interest is bound to follow. Municipal water agencies are placed in the unenviable position of explaining an issue about which there are far fewer answers than questions. To minimize public anxiety, utilities need to be able to convey two key concepts: relative exposure and progress.

On the issue of exposure, there are many natural sources that contain far greater concentrations of endocrine disrupting compounds than drinking water. For instance, a person might be exposed to more phytoestrogen from a dash of soy sauce than by a year's worth of drinking water. The problem is that such an approach does not dispel the concern but merely shifts it to another target. More appropriate is to identify a commonly used pharmaceutical product such as ibuprofen, compare concentrations in water to a single dosage, and use that context to explain that people would have to consume thousands (perhaps millions) of glasses of water per day to get the equivalent of one Advil®. Given the ability of instruments to detect contaminants at previously inconceivable levels, the industry must begin to shift its regulatory emphasis from detection to health effects.

Progress speaks to the question, "What are you doing about it?" Regardless of whether or not something appears to be a legitimate health risk, the public inherently wants their utility to be progressive and take whatever measures are necessary to address the perceived problem. Fortunately, in the case of PPCPs, progress has been exceptionally fast. Scientists have a good understanding of how to find these compounds and what processes remove them, so the remaining question is whether removing these compounds justifies the costs to ratepayers. It is critical in conveying this concept that utilities refer to ratepayer or the public's money when discussing the value of additional treatment-related expenditures. Being frugal with public funds is commendable; doing so with the utility's money is not viewed as such. By conveying that treatment technologies are available but that investing in them may not be necessary, the utility is reducing the perceived health risk.

Wastewater Treatment Plant Perspectives: Preliminary Data Suggesting Endocrine Disruptor Effects of Wastewater Discharge into the Pacific Ocean

Jeffrey L. Armstrong
Orange County Sanitation District, Fountain Valley, CA

Abstract

The presence and effect of endocrine disrupting chemicals (EDCs) in the environment has become a major focus of scientific research and public concern. Research conducted in the United Kingdom and elsewhere has shown that the release of wastewater (municipal and industrial) is a source of EDCs that can adversely affect fish populations. The Orange County Sanitation District (sanitation district) is a publicly owned treatment works (POTW) that serves 2.3 million residents and almost 1,000 permitted industries in a service area of 470 square miles. The sanitation district treats an average of 243 million gallons per day, most of which is discharged through a submarine outfall pipe extending 8 km offshore of Huntington Beach, California. In 1999, the sanitation district began collaborating with local university researchers and others to determine if the release of the treated wastewater was eliciting an endocrine response in flatfish in the monitoring area. This was assessed using a suite of biomarkers and tests, including vitellogenin (vtg) induction, cortisol stress response, CYP1A induction, and DNA damage. Population- and community-level effects were assessed using diversity indices, such as species richness, total abundance, Shannon-Wiener Diversity, and species evenness. The initial approach was to facilitate the work of university researchers, who had the expertise that sanitation district staff lacked, to begin to address the EDC issue. The sanitation district provided scientific staff time and in-kind services, such as vessel use and field crew time for sample collection; provided some supplies; and paid page costs for journal publication of results. Demersal flatfish species were used as target organisms because of their life history characteristics (mode of feeding and site fidelity). The findings of the studies to date have found elevated levels of vtg in both male and female fish at the outfall compared to farfield reference stations, the inhibition of cortisol induction in fish collected at the outfall compared to reference stations, and greater concentrations of EDCs in sediment collected near the outfall compared to stations away from the outfall. The results of the pilot projects indicate that endocrine function is compromised in fish collected near the outfall as measured by estrogenic activity and stress responses. There is no indication, however, of population-level effects at this time. In fact, the population of hornyhead turbot (*Pleuronichthys verticalis*) near the outfall is slightly masculinized, even though males have high vtg levels. In addition to researching potential environmental degradation, the sanitation district also is focused on studying the potential for human health effects that could result from water reuse projects. Based on the results of these pilot projects, subsequent studies are narrowing the focus of endpoints and attention is moving into the treatment plant and to attempt cause-effect relationships with specific responses and chemicals. To better address these issues, the sanitation district is increasing the level of effort both internally and in its collaborations with university researchers and other POTWs. Presently, the sanitation district is entering multi-year, multi-agency projects to better characterize the effect from the release of treated wastewater effluent into coastal southern California waters.

Perspectives From the DEA on What Can and Cannot Be Done Via Drug Take-Back Programs

Vickie Seeger
Drug Enforcement Administration, Washington, DC

Abstract

The Drug Enforcement Administration's (DEA) mission with respect to illicit controlled substances, such as heroin and ecstasy, is to eliminate them outright. The DEA's role, however, is much more complex when it comes to licit controlled pharmaceuticals. On one hand, the DEA prevents, detects, and eliminates the diversion of controlled pharmaceuticals from legitimate channels to illegal use, while at the same time ensuring their availability for legitimate medical and scientific purposes. To facilitate these goals, the Controlled Substances Act (CSA) established a closed system of controlled substance distribution encompassing manufacturers, distributors, pharmacies, and physicians. Components of this closed system include scheduling of all controlled substances; registration of all controlled substance handlers; and recordkeeping for accountability, security, and manufacturing quotas, all under the DEA's oversight.

The CSA does not require patients (ultimate users) to register with DEA. Therefore, once the controlled substances are dispensed to the patient, the medications are outside of the closed system of distribution and not subject to CSA recordkeeping requirements. Currently, there is no regulatory allowance for unused patient medications to be routinely returned to a DEA registrant for disposal or reuse.

Environmental Stewardship of Waste Pharmaceuticals From a Hospital Perspective

Charlotte A. Smith
PharmEcology Associates, LLC, Brookfield, WI

Abstract

Common practices of pharmaceutical waste disposal in hospitals involve the sewering or landfilling of virtually all discarded pharmaceuticals except chemotherapy agents, which are often sent to a regulated medical waste incinerator. These practices are out of sync with both the emerging concerns about pharmaceuticals in the environment and with the requirements of the Resource Conservation and Recovery Act (RCRA) to identify, segregate, and properly manage drugs that are defined as hazardous waste.

U.S. Environmental Protection Agency (EPA) RCRA regulations define a number of common drugs such as epinephrine, warfarin, lindane, and nine chemotherapy agents as hazardous waste when discarded. Approximately 5 percent of the current products on the market are RCRA hazardous waste if discarded. Until recently, EPA and state authorities had not been enforcing RCRA in hospitals. Enforcement efforts by EPA Region 2 and Minnesota, Florida, California, and Washington State have begun to focus the attention of hospitals on pharmaceutical waste management. Despite these enforcement efforts, only a minority of health care facilities is successfully complying with RCRA.

The RCRA regulations have not been significantly updated since 1976 and have not kept up with drug development. More than 100 toxic chemotherapeutic agents can legally be sewered or landfilled under the federal regulations. Fortunately, many health care professionals are aware of the hazardous nature of these drugs and some higher level of treatment is usually provided, although not always the most environmentally sound.

When all RCRA drugs and all chemotherapy agents are destroyed at an RCRA-permitted incinerator, approximately 85 percent of the drug market is still routinely sewered or landfilled, including endocrine disruptors, antihypertensives, antidepressants, anticholesteremics, and antibiotics. Based on an increasing body of research, it is apparent that the continuous introduction of these agents into aquatic environments may have negative consequences on fish and other aquatic species. The impact on human health is not yet known.

In most states, the issue of how pharmaceutical waste is being disposed in hospitals and other health care organizations, such as long-term care facilities and clinics, barely has been considered by either the regulatory community or health care providers. Only the states of California and Washington have required the incineration of all drug waste that cannot meet stringent toxicity limits. Minnesota has extended hazardous waste regulations to include approximately 15 percent of drugs on the market and has encouraged strongly the incineration of the remaining 85 percent.

EPA Region 1 has funded two relevant grants to Hospitals for a Healthy Environment. The first is to develop a national blueprint for pharmaceutical waste disposal and includes Dartmouth-Hitchcock Medical Center and PharmEcology Associates. The second grant involves the training of surveyors for the Joint Commission on the Accreditation of Healthcare Organizations (JCAHO) on the importance of compliance with hazardous waste regulations and other pollution prevention practices. As JCAHO accreditation is of vital importance to many hospitals, this attention will stimulate compliance activity.

An additional barrier to preventing the entry of potent pharmaceuticals into aquatic systems is the requirement by the Drug Enforcement Administration that all unused controlled substances (drugs of abuse) be rendered nonrecoverable and that process be double-witnessed by two health care professionals. With the closing of most hospital incinerators, the most efficient and cost-effective way to render these waste drugs

nonrecoverable is through sewering. Although the search for alternative methods is ongoing, consideration should be given to possible regulatory changes in this area.

Barriers to compliance, such as the ill fit of an industrial hazardous waste model to a health care setting and the lack of interpretive guidance by EPA headquarters has created a nonrational and extremely confusing regulatory environment. It simply is unrealistic to expect every nurse on the floor of a hospital to know which of the 2,000 to 4,000 drugs administered are RCRA hazardous wastes when discarded. In the short term, given the complexity of waste segregation and management challenges inherent in health care settings, every effort should be made by EPA to uniformly clarify regulatory requirements. In the long term, an adaptation of current regulations for health care organizations should strongly be considered by EPA to bring clarity and rational decisionmaking into this significant source of environmental contamination. By utilizing its convening powers, EPA is in a position to bring together the pharmaceutical supply chain and other relevant stakeholders to explore the use of voluntary, collaborative approaches to pharmaceutical waste identification, management, and minimization.

Hospitals are crisis-oriented organizations with multiple priorities constantly vying for scarce resources. Attention should be given to those leaders that have made the investment in time and money to manage their pharmaceutical waste in an appropriate and compliant manner. Encouragement, both through education and the motivation of possible enforcement of existing hazardous waste regulations, should be employed to encourage other hospitals to develop a more compliant and environmentally sound pharmaceutical waste management system.

Managing Emerging Contaminants: A Practical Approach

Al Alwan
Region 5, U.S. Environmental Protection Agency, Chicago, IL

Abstract

This approach for managing emerging contaminants, including pharmaceuticals, is a new way to view the issue. Our regional prospective led us to identify different Regional and U.S. Environmental Protection Agency (EPA) roles in addressing this issue. This presentation will provide an overview of the emerging contaminant issue. The new approach includes the development of five tools to address any new contaminants. These tools are: analytical methods, new biological end points, monitoring and assessments, chemical profile matrix, and collaborations and outreach. Emerging contaminants are national and international concerns. EPA has worked and continues to work on this issue. Environmental laws exist regarding the screening of endocrine disruptor chemicals in drinking water and food. Those involved in this area of research know that there is no easy solution. The major challenge is selecting the best practices to manage this demanding issue. We believe a special new approach is needed to address the problem of emerging contaminants. This new approach should utilize all available tools and result in a solution with multiple options. Collaboration with stakeholders is recommended to define a common goal and explore the best options to manage contaminants. The effort should focus on understanding the contaminant issue and recommending and adopting the best solution.

The Metropolitan Water Reclamation District of Greater Chicago's Efforts To Reduce Pharmaceuticals That Enter the Water Reclamation Plants

Catherine O'Connor, Thomas Granato, and Richard Lanyon
Metropolitan Water Reclamation District of Greater Chicago, Cicero, IL

Abstract

The Metropolitan Water Reclamation District of Greater Chicago (District) is aware of the potential threat to the water environment associated with discharge of pharmaceutical materials to the sanitary sewer system. The District has taken action in a number of ways regarding this emerging issue.

This presentation will review the efforts the District has taken to reduce pharmaceuticals that enter our collection system. The District has issued press releases regarding this matter to alert the public about the potential threat posed by improperly disposed antibiotics and other pharmaceuticals. The District also has contacted the American Medical Association, drug store chains, and independent drug store associations requesting that they issue advisory letters to medical practitioners. The District also is a co-sponsor of household hazardous waste collection events held periodically throughout the metropolitan area at which residents are encouraged to discard their unused prescriptions.

Given the fact that this is an emerging issue, attempting to quantify the types of compounds and their range of concentrations has been part of the District's efforts, working with the U.S. Environmental Protection Agency and in cooperation with universities. The District has cooperated with sampling of water reclamation plant influent, wastewater processing streams, and effluent to understand the concentration and fate of certain classes of chemicals. This presentation also will include the results of all work completed to date.

Collecting Unwanted Medications for Appropriate Disposal

Lynn Rubinstein
Northeast Recycling Council, Inc., Brattleboro, VT

Abstract

Through funding from the U.S. Environmental Protection Agency and the U.S. Department of Agriculture Rural Utilities Service, the Northeast Recycling Council, Inc., has been researching legal strategies and best management practices for collecting and disposing of unwanted medications from the general public. These strategies have been tested through pilot collection events. This presentation addresses the lessons learned, necessary strategies, and the detailed logistics for conducting legal and safe unwanted medication collection events.

The best management practices and legal requirements include: accepting prescription and over-the-counter medications, the physical presence and participation of law enforcement officials and a pharmacist, and disposal of nonregulated medications as a hazardous waste.

Five pilot collections have been held to date: in pharmacy, two as part of scheduled activities for seniors, a stand-alone event, and as part of a household hazardous waste collection. Data from these events and lessons learned also will be discussed.

More events are scheduled, and data will continue to be collected and analyzed.

Maine: First U.S. Legislation for Unused Pharmaceutical Returns

Stevan Gressitt
Maine Association of Psychiatric Physicians, Manchester, ME

Abstract

This presentation focuses on a history of the origin, development, advisors, and complications leading to the first state legislation addressing unused, private citizen (non-Drug Enforcement Administration registrant) medication. Rationale for the legislation will be given as four points. The various interested parties will be identified, their contributions will be provided, and the outline of the legislative coalition will be described. Specific barriers, real, political, or assumed will be made clear. Legislative outcome will be explained, Legislative Study Committee developments will be outlined, and the latest legislative update also will be illustrated. The current status of the final legislation will be given along with a fiscal note. Political and organizational developments subsequent and parallel will be reviewed. Original research for the process and the status of that research will be summarized. Ramifications of one known dataset on returns will be discussed. Known barriers to further progress will be noted from a medical perspective. Pharmacoeconomic aspects of the legislative outcome will be identified, potential for pharmacovigilance and pharmacosurveillance will be noted, and implications for law enforcement and homeland security will be addressed. A taxonomy of return programs will be proposed with comments on the experience from other countries and geo-political areas. Finally, product surety aspects will be noted, and implications for clinical practice will be proposed.