平成１７年度　厚生労働科学研究「医薬品の環境影響評価法に関する研究」班
第二回班会議　記録

○日　時　平成１７年１０月８日（土）　１４：００～１６：５０
○場　所　飯野ビル　キャッスル（千代田区内幸町２－１－１）
○出席者　（分担研究者）井上　達、岩根泰蔵、大野泰雄、菅谷芳雄、関澤　純、
中杉修身、西村哲治、長谷川隆一、山本裕史、吉岡義正、
若林明子
（オブザーバー）東　泰好、粟野勝也、佐神文郎
（事務局）勝　紀子　　以上 15 名

１．山本班員の"US EPA Pharmaceuticals in the Environment" ワークショップ出席報告を聞き、質疑応答を行った。

２．東班員の製薬協有志による予備アンケートの結果の報告を聞き、本アンケートへの参考意見を聞いた。

３．各班員の準備したドラフトを中心に相互に意見を出し合って、今後の文書取りまとめの方向性を検討した。

４．今後の作業予定

（１）報告書原案について、当班会議で討議された方向性に修正したものを
11 月中（遅くも 12 月第 1 週まで）に、主任研究者あてにファイルでお届け願う。

（２）（１）をもとに、
・ 主任研究者は、来年度の継続申請のための、研究計画書を作成する
・ 主任研究者は、研究報告書を作成する
・ 製薬協は、第一次フィードバックを行う（研究報告書に取り入れる）

（３）外国人招へい
・ 関澤先生：FDA の担当官 Dr. Nancy B. Sager に関する履歴等を用意する。
・ アストラゼネカの Tom Hutchinson を 7 月 3-5 日のトキシコロジー学会に呼べないか？
＝＝＞時期が早いのが問題？主任研究者より公定書協会の担当者に、継続研究の立場からの可能性について相談する。
これが行き詰まった際は見送り。

（４）国外出張
・5 月ストックホルムにおける Joint DIA/HESI/SAPS Conference への参加。
＝＝＞主任研究者より、Dr. Holsapple に、日本から発表の用意があるとの手紙を書く。（招へいがあれば、吉岡先生に行って戴く。）
＝＝＞西村先生の参加の可能性も探る（e.g. 来年度の班研究費での派遣の方

向。ただし、金次第。）

（５）トキシコロジー学会への提案
＝＝＞佐神先生より上野先生に co-organize を問合せて戴く

（６）翻訳の件
・EMEA の改訂版がもうじき出る
・Dr. Daughton から渡された資料よりピックアップする。
（いずれも、班員のすすめに従って実行する）

以上

資　料　－　Ⅱ　　目　次

(資料　3)　EPA 「環境中の医薬品類に関するワークショップ」参加報告
徳島大学　総合科学部　山本　裕史

(資料　4)　AGENDA “The U.S. Environmental Protection Agency Meeting on Pharmaceuticals in the Environment”

(資料　5)　Workshop Abstracts
U.S.EPA Meeting on Pharmaceuticals in the Environment

EPA 「環境中の医薬品類に関するワークショップ」参加報告

徳島大学　総合科学部　山本　裕史

平成 17 年 8 月 23 日から 25 日まで、USEPA（米国環境保護庁）の主催によってネバダ州ラスベガス市にある EPA 国立曝露研究試験場で環境中の医薬品類に関するワークショップが開催された。そのワークショップに参加し情報収集・交換を行う（プログラムは別紙資料 A アブストラクトは同 B として配布）とともに、ワークショップ後には同研究試験場に所属し環境中の医薬品類に関して警鐘を鳴らした論文で有名で、その分野の権威である Christian Daughton 博士他の研究者と情報交換を行った。

ワークショップでは、初日 23 日には、主催者の EPA 側からの「環境中の医薬品類」を取り巻く現在の研究助成や法規制の概略に関する報告が 3 件あった後、同じく EPA の Christian Daughton 博士によって環境中の医薬品類についてのこれまでの経緯と現在におけるその問題点の概略等をまとめた報告があった。その後夕方までは、EPA の競争的研究助成システムの一つ STAR (Science To Achieve Results) プログラムにおいて、「環境中の医薬品類の挙動」に関する研究として採択された 6 グループ 10 件の研究報告があった（別紙資料 C に研究報告書の概要：http://epa.gov/nerlesd1/chemistry/pharma/star.htm より）。

研究内容は、下水や河川試料からの医薬品類の分析方法の開発から、地下水や河川、港湾内での医薬品類濃度のモニタリング、環境中での底質の吸着や光分解、生分解等の動態、さらには魚類や両生類を使った水棲生物への影響評価にいたるまで様々な分野にわたり、どの研究もまだ学術誌には掲載されていない最新の成果で非常に興味深いものもあった。

2 日目の 24 日の午前中は環境中の医薬品の有害性評価ということで、従来の毒性試験系と化学分析方法について SETAC（国際環境毒性化学会）、USGS（米国地質研究所）、USEPA、そして USFDA（米国食品医薬品局）の代表らによる発表があった。午前中の後半は分科会で化学分析と毒性試験の 2 つに分かれ、EPA の各支部における医薬品研究における問題点を討議した。比較的新しい化学物質である医薬品類を化学的に分析したり、様々な曝露・毒性試験を行ったりする際に、EPA によって確立し標準化された方法が提供されていないという問題点があると同時に、米国国内で 10 ある各支部ごとにその必要とされる方法が異なることが改めて浮き彫りになった。

24 日の午後から 25 日の午前中にかけては、医薬品類に関わる Environmental Stewardship（環境管理）に関して様々な立場からの発表が行われた。発表者は AWWA（米国水道協会）や地方の上下水道等の水資源管理担当者から、薬物の法規制を行う USDEA（米国薬物取締局）、製薬会社の環境管理担当者、医薬品廃棄物回収を進める NGO 関係者や医師、地方自治体関係者など非常に広範囲に及んだ。24 日夕方には既存の毒

性試験を用いる際の問題点と環境への医薬品排出を防ぐ方法の２つの分科会に分かれて討議を行い、25日の最後には環境管理に関わるパネルディスカッションも開催され、不必要になった医薬品の回収方法を中心に活発な議論がなされた。

翌日の 26 日には EPA 国立曝露試験場の Christian Daughton 博士に面会し、当試験場の科学者 Jones-Lepp 博士らと情報交換を行った。環境中の医薬品類に関する日本の状況、我々の研究グループの概略を説明し、米国での研究助成の状況等に関する質問をした。　ワークショップで発表した６つの研究グループに対する研究助成額は総額およそ300万ドル程度で、2001年から2004年まで3年間の研究期間で、その後は「環境中の医薬品類」に特化した研究助成は行われていないということであった。また、医薬品に関する大きなプロジェクトとしては、AWWA によってラスベガス近くのミード湖にあるネバダ州南部水管理協会の Snyder 博士らに研究助成が行われているなど数件あるほか、USGS でも同様の研究助成があるということであった（http://epa.gov/nerlesd1/chemistry/pharma/grants.htm)。なお、Christian Daughton 博士には報告に用いたスライド（別紙資料 D および E)、論文別刷（別紙資料 F-L）等もいただいているので別紙資料に示す。

以上

The U.S. Environmental Protection Agency
Meeting on Pharmaceuticals in the Environment

U.S. EPA National Exposure Research Laboratory
944 East Harmon Avenue
Las Vegas, NV 89119
August 23-25, 2005

AGENDA

OVERALL GOALS FOR THE WORKSHOP:

- Provide an opportunity for STAR grantees to present the results of their research. (Summaries of the grantees' projects can be accessed at: http://epa.gov/nerlesd1/chemistry/pharma/star.htm)
- Identify research "gaps" important to addressing decisions and/or policy making issues associated with pharmaceuticals in the environment.
- Provide an opportunity for information sharing among scientists and policy-makers from EPA's program offices, regions, and the Office of Research and Development, as well as from States, local agencies, research entities and stakeholders, about the state-of-the science regarding the presence, fate, and effects of pharmaceuticals in the environment and techniques and tools for Regions' and/or States' monitoring programs.
- To the extent there is a problem, identify ways that EPA can be part of the solution by improving the understanding of "institutional barriers" and discussing programmatic approaches (headquarters and Regions).
- Explore the use of voluntary, collaborative approaches to reducing pharmaceuticals in the environment that include Regions and Program Offices, States, Tribes, other federal agencies, and stakeholders.

Tuesday, August 23, 2005

INTRODUCTION & OVERVIEW

7:30 – 8:00 a.m.	Registration
8:00 – 9:45 a.m.	Moderator: **Angela Page**, U.S. EPA, Office of Research and Development
8:00 – 8:15 a.m.	Welcome **Christian Daughton**, U.S. EPA, Office of Research and Development
8:15 – 8:30 a.m.	Overview of the U.S. EPA's Office of Research and Development and the Science To Achieve Results (STAR) Program **Angela Page**, U.S. EPA, Office of Research and Development
8:30 – 8:50 a.m.	Overview Presentation from the U.S. EPA's Office of Water **Octavia Conerly**, U.S. EPA, Office of Water
8:50 – 9:15 a.m.	The EPA Regional Perspective – Why Pharmaceuticals in the Environment Are an Emerging Science Issue to EPA's Regions **Bobbye Smith**, U.S. EPA Region 9, National Regional Science Council
9:15 – 9:45 a.m.	Overview of Science Involved with Pharmaceuticals: A Perspective from the U.S. EPA **Christian Daughton**, U.S. EPA, Office of Research and Development
9:45 – 10:05 a.m.	**BREAK**

FATE, EFFECTS AND OCCURRENCE OF PHARMACEUTICALS IN THE ENVIRONMENT- Session A

10:05 - 11:55 a.m. Moderator: **Angela Page**, U.S. EPA, Office of Research and Development

10:05 - 10:30 a.m. Occurrence, Environmental Fate and Exposure Assessment of Selective Serotonin Reuptake Inhibitors (SSRIs) in Aquatic Environments
Presented by **Kevin Armbrust**, Office of the State Chemist, Mississippi State Chemical Laboratory, Mississippi State, MS (STAR Grant)

10:30 - 10:55 a.m. Occurrence and Fate of Antibiotics and Other Pharmaceutically Active Compounds During Transport To and During Drinking Water Treatment
Presented by **Howard Weinberg**, University of North Carolina, NC (STAR Grant)

10:55 - 11:20 a.m. Occurrence and Fate of High Volume Pharmaceuticals in Wastewater Impacted Environments
Grant Recipient: Bruce Brownawell. Presented by **Mark Benotti**, State University of New York at Stony Brook, NY (STAR Grant)

11:20 - 11:55 a.m. Detection and Fate of Environmental Estrogens in Wastewater Impacted Surface and Groundwaters
Presented by **Bruce Brownawell**, State University of New York at Stony Brook, NY (STAR Grant)

11:55 - 1:30 p.m. **LUNCH**

FATE, EFFECTS AND OCCURRENCE OF PHARMACEUTICALS IN THE ENVIRONMENT – Session B

1:30 - 4:20 p.m. Moderator: **Cynthia Nolt-Helms**, U.S. EPA, Office of Research and Development

1:30 - 1:55 p.m. Mechanisms of Tetracycline Resistance Development in the Environment as Detected by Real-Time PCR
Presented by **David Graham**, University of Kansas, Lawrence, KS (STAR Grant)

1:55 - 2:20 p.m. Fate, Attenutation, and Effects of Fluoroquinolone Antibacterials in Aquatic Systems
Presented by **Charles Knapp**, University of Kansas, Lawrence, KS (STAR Grant)

2:20 - 2:45 p.m. Adsorption of Beta-Blocker Anti-Hypertensive Pharmaceuticals to a Range of Mineral Surfaces
Presented by **Tohren Kibbey**, University of Oklahoma, Norman, OK (STAR Grant)

2:45 - 3:10 p.m. Pharmaceuticals and Personal Care Products as Environmental Contaminants: Preliminary Environmental Risk Calculations and Method Development for Analysis in Environmental Media via GC/MS
Research conducted by **Lynn Roberts**; presented by **Kevin Bisceglia**, The John Hopkins University, Baltimore, MD (STAR Grant)

3:10 - 3:30 p.m. **BREAK**

3:30 - 3:55 p.m. Pharmaceuticals and Personal Care Products as Environmental Contaminants: Biodegradability Studies and Occurrence in Sewage Treatment Plant Influent and Effluent
Research conducted by **Lynn Roberts**; presented by **Jim Yu**, The John Hopkins University, Baltimore, MD (STAR Grant)

3:55 - 4:20 p.m. Endocrine Effects of Selective Serotonin Reuptake Inhibitors (SSRIs) on Aquatic Organisms
Presented by **Marsha Black**, University of Georgia, Athens, GA (STAR Grant)

4:20 - 5:30 p.m. **Poster Session**
Investigators will be available to discuss their posters highlighting research on pharmaceuticals in the environment.

5:30 p.m. **Adjourn**

Wednesday, August 24, 2005

HAZARDS FROM WASTE PHARMACUETICALS: DETERMINING TOXICITY AND CHEMICAL ANALYTICAL METHODS FOR DETECTION

8:00 – 11:50 a.m. Moderator: **Tammy Jones-Lepp**, U.S. EPA, Office of Research and Development

8:00 – 8:25 a.m. Overview of a Framework for Assessing the Hazards of Human Pharmaceuticals in the Environment from a SETAC Pellston Workshop
Presented by **Marsha Black**, University of Georgia, Athens, GA

8:25 – 8:50 a.m. Overview of ORD's Aquatic Toxicology Research on Endocrine-Active Pharmaceuticals
Presented by **Joseph Tietge**, U.S. EPA, Office of Research and Development

8:50 – 9:15 a.m. Analytical Methods for Measurement of Pharmaceuticals in Drinking Water and in the Environment
Presented by **Mike Myer**, U.S. Geological Survey

9:15 – 9:40 a.m. Environmental Risk Assessment of Pharmaceuticals in the Environment
Presented by Chuck Eirkson, U.S. Food and Drug Administration

9:40 – 10:05 a.m. An Informatic Approach to Estimating Ecological Risks Posed by Pharmaceutical Use
Presented by **Mitchell Kostich**, U.S. EPA, Office of Research and Development

10:05 – 10:25 a.m. **BREAK**

10:25 – 10:50 a.m. Identifying Chemical Compounds from Wastewater Discharges
Presented by **Susan Glassmeyer**, U.S. EPA, Office of Research and Development

10:50 – 11:35 a.m. **Research and Regional Needs Breakout Sessions**

BREAKOUT SESSION I: What Chemical Methods Are Needed for Monitoring Pharmaceuticals in the Environment? What Are the Barriers in Using Existing Chemical Methods for Monitoring Pharmaceuticals in the Environment?
Moderator: **Al Alwan**, U.S. EPA, Region 5

Breakout Questions:
Can EPA's process for developing new chemical methods be modified to address the new pharmaceuticals?
Should we evaluate other options outside of EPA? If so, which ones?
What pharmaceuticals concentration would EPA use for baseline and target monitoring?
Which pharmaceuticals should the Regions focus on monitoring?
What can we do until there are EPA approved methods for detection of pharmaceuticals and personal care products?
How do we find out the relative mass of unused versus excreted drugs that enter into wastewater treatment plants?

BREAKOUT SESSION II: What Environmental Exposure and Effects Methods Are Needed for Ecologic Receptors?
Moderator: **Bobbye Smith**, U.S. EPA, Region 9

Breakout Questions:
What new bioassays can the Regions and Programs use with existing resources?
What pharmaceuticals should the Regions focus monitoring resources on?
What EPA bioassay methods can be used?
Are there other agency or stakeholder-sponsored assessment methods that would be useful?
What are the method gaps?

11:35 – 11:50 a.m. **Breakout Sessions Reports to Participants**

11:50 – 1:00 p.m. **LUNCH**

ENVIRONMENTAL STEWARDSHIP FOR PHARMACEUTICALS IN THE ENVIRONMENT – Session A

1:00 – 5:00 p.m. Moderator: **Octavia Conerly**, U.S. EPA, Office of Water

1:00 – 1:25 p.m. Research Needs and Gaps from the Perspective of the Major Water Societies
Djanette Khiari, AWWA Research Foundation

1:25 – 1:50 p.m. Perspectives from Drinking Water Suppliers
J.C. Davis, Southern Nevada Water Authority

1:50 – 2:15 p.m. Wastewater Treatment Plant Perspectives: Preliminary Data Suggesting Endocrine Disruptor Effects of Wastewater Discharge into the Pacific Ocean
Jeffrey Armstrong, Orange County California Sanitation District

2:15 – 2:40 p.m. **BREAK**

2:40 – 3:05 p.m. Perspectives from the Drug Enforcement Administration on What Can and Cannot Be Done via Drug Take-Back Programs
Vickie Seeger, U.S. Drug Enforcement Administration, Office of Diversion Control

3:05 – 3:30 p.m. Perspectives from the Pharmaceutical Industry
Mary Buzby, Merck & Co., Inc., representing the Pharmaceutical Research and Manufacturers of America (PhRMA) PIE Task Force

3:30 – 3:55 p.m. Environmental Stewardship of Waste Pharmaceuticals from a Hospital Perspective
Charlotte Smith, PHARMECOLOGY

3:55 – 4:20 p.m. What Are the Barriers to Reducing Pharmaceuticals in the Environment?
(Panel of Rapporteurs from Days 1 and 2 will synthesize the "learnings")

4:20 – 5:10 p.m. **Breakout Sessions on Identifying the Barriers**

BREAKOUT SESSION I: What Are the Barriers to Using Existing Bioassay Tools to Assess Exposure and Effects of Pharmaceuticals in the Environment?
Moderator: **Bobbye Smith**, U.S. EPA, Region 9

What can we do until there are EPA approved methods for detection of pharmaceuticals and personal care products?

BREAKOUT SESSION II: What Are the Programmatic and/or Regulatory Constraints to Reducing Pharmaceuticals in the Environment?
Moderator: **Chen Wen**, U.S. EPA, Office of Pollution Prevention and Toxics

What social and/or economic research needs to be performed to support action by EPA Regions or Programs?
What agencies and stakeholders would have social and/or economic research that would assist Regions and Programs in developing educational materials?
What are the key agencies and stakeholders to include in pollution prevention discussions?

5:10 – 5:30 p.m. **Breakout Sessions Reports Shared Across Participants**

5:30 p.m. **Adjourn**

Thursday, August 25, 2005

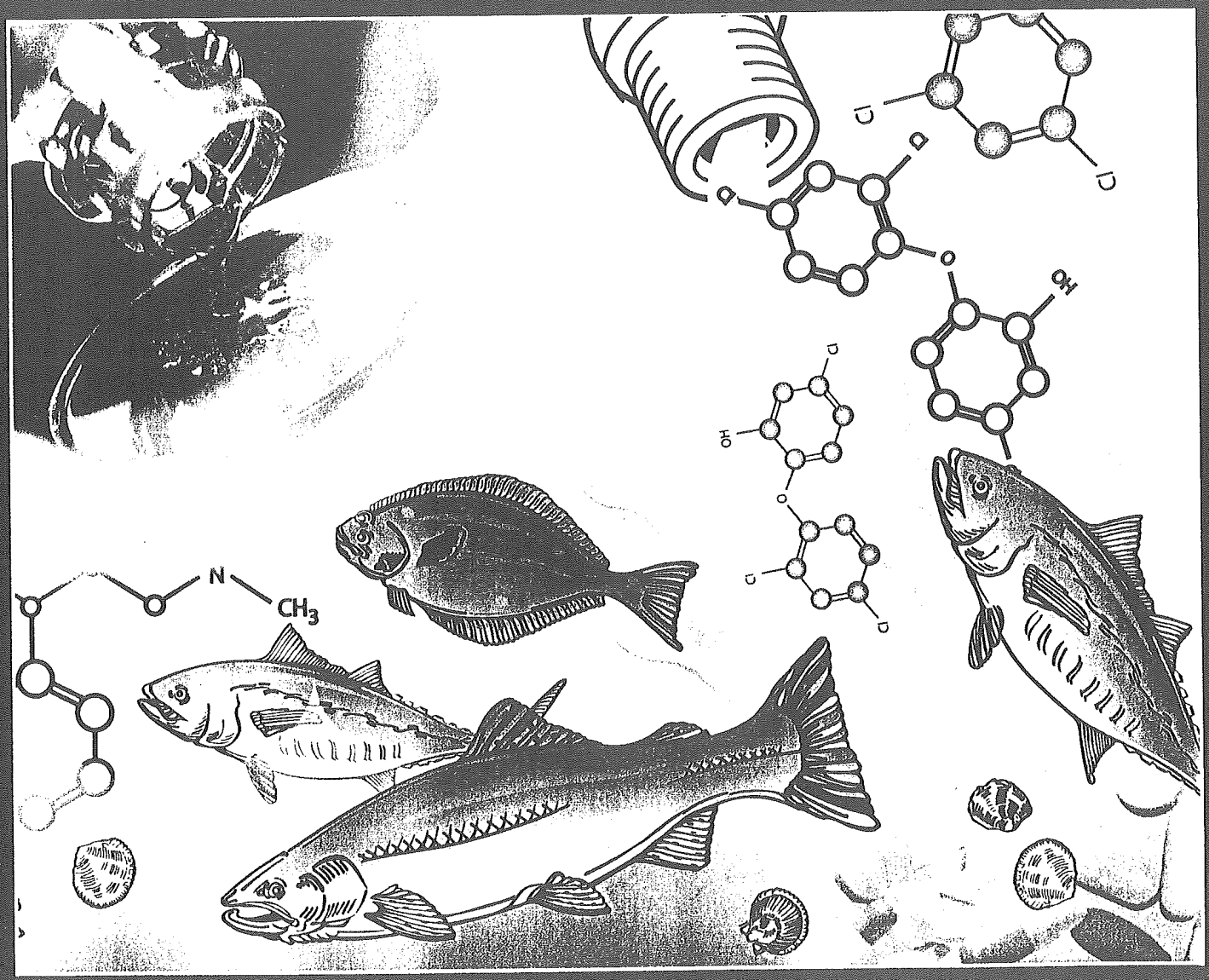
ENVIRONMENTAL STEWARDSHIP FOR PHARMACEUTICALS IN THE ENVIRONMENT – Session B

8:00 – 11:45 a.m.	Moderator: **Mary Dever**, U.S. EPA, Region 1
8:00 – 8:25 a.m.	Managing Emerging Contaminants: A Practical Approach **Al Alwan**, U.S. EPA, Region 5
8:25 – 8:50 a.m.	The Metropolitan Water Reclamation District of Greater Chicago's Efforts to Reduce Pharmaceuticals that Enter the Water Reclamation Plants **Catherine O'Connor**, Metropolitan Water Reclamation District of Greater Chicago
8:50 – 9:15 a.m.	Collecting Unwanted Medications for Appropriate Disposal **Lynn Rubinstein**, Northeast Recycling Council
9:15 – 9:40 a.m.	Maine: First U.S. Legislation for Unused Pharmaceutical Returns **Stevan Gressitt**, Maine Association of Psychiatric Physicians, Medical Director of Northeast Occupational Exchange, Acting Secretary of the Maine Benzodiazepine Study Group
9:40 – 10:05 a.m.	Washington State and King County's Perspective on Pharmaceutical Stewardship **David Galvin**, King County Washington
10:05 – 11:30 a.m.	**Facilitated Discussion: What Are the Next Steps to Address the Issue of Pharmaceuticals in the Environment? How Can EPA Be Part of the Solution?** ***Moderator:* To Be Determined (Multi-Media Pollution Prevention Office and/or Office of Waste Water Management?)** **Facilitator: Bobbye Smith**, U.S. EPA, Region 9 **Note Taker: To Be Determined** **Rapporteur: To Be Determined** Panel of stakeholders, researchers, agencies initiate discussion with audience. **Initial Discussion Questions:** What can we do until there are EPA approved methods for detection of pharmaceuticals and personal care products? What are the key agencies and stakeholders to include in pollution prevention discussions? What internal EPA vetting needs to be done to develop a champion? Are there existing "solutions" that could be piloted in other states, business sectors? Are there cleanup and/or destruction (management) options that could be developed in cooperation with industry sectors? What are the "tools" or approaches? Is there an "energy star"-like opportunity? Product stewardship – how can those principals be made into business opportunities? How to "green" the supply chain and/or reduce the waste stream volume? How to engage with individuals to influence behavior (e.g., don't flush drugs)? How to address "distributed" health care (e.g., nursing homes, hospice, drug rehabilitation centers)? How to find out the relative mass of unused versus excreted drugs that enter into wastewater treatment plants? What will Regions, States, and Tribes do to monitor for pharmaceuticals? Who will be the Headquarters and/or Regional "champions" to work for solutions?
11:30 – 11:45 a.m.	**Wrap-Up and Next Steps**
11:45 a.m.	**Adjourn**

Workshop Abstracts

U.S. EPA Meeting on Pharmaceuticals in the Environment

August 23-25, 2005
Las Vegas, Nevada



Presentation Abstracts

Fate, Effects, and Occurrence of Pharmaceuticals in the Environment

Occurrence, Environmental Fate, and Exposure Assessment of Selective Serotonin Reuptake Inhibitors (SSRIs) in Aquatic Environments

Kevin L. Armbrust and Jeong Wook-Kwon
State Chemical Laboratory of Mississippi, Mississippi State, MS

Abstract

Pharmaceuticals can enter aquatic environments after their prescribed use and lead to negative effects on aquatic organisms. Ideally, information on the environmental fate and effects of these chemicals would be useful prior to their registration so that exposure assessments, and ultimately risk assessments, could be conducted proactively. The Subdivision N battery of tests required by the U.S. Environmental Protection Agency for pesticide registration provide a convenient model to use for pharmaceutical products, as environmental degradation processes are not discriminatory to a chemical's product-use pattern. Selective serotonin reuptake inhibitors (SSRIs) are among the most heavily prescribed drugs. They are biologically active, low concentrations have been shown to affect aquatic organisms, and evidence indicates that they can be present in effluents from wastewater treatment plants. The physical and chemical properties and rates of degradation of five SSRIs (citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline) were measured in investigations modeled after those used for pesticide registration. Additionally, their occurrence in raw wastewater, treated effluent, and downstream receiving waters also was measured. The salts of SSRIs had high water solubilities ranging from 3,022-15,460 mg/L and relatively low octanol-water partition coefficients (log K_{ow}) ranging from 1.12-1.39. Two sediments and three soils with organic matter contents ranging from 0.16 to 1.77 percent and pH ranging between 5.6 and 7.8 were used to measure adsorption coefficients. Values of K_f, K_d, and K_{oc} ranged from 39 to 18,342, from 60 to 42,579, and from 2,256 to 1,053,380, respectively. No significant hydrolytic degradation was detected for any drug in any aqueous solution.

Photolysis was a potential route of degradation for several SSRIs. Paroxetine and fluvoxamine rapidly photodegraded, with half-lives of 0.5-0.7 days and 3.6-6.0 days, respectively. Fluoxetine and citalopram were stable to photolysis at all pH values tested. Most SSRIs except fluvoxamine did not show enhanced photodegradation in synthetic humic water and natural water, possibly because of light attenuation by natural water materials. Several degradation products for each SSRI were detected and identified by liquid chromatography-electrospray ionization-mass spectrometry (LC/ESI/MS). Hydroxyl radical rate constants, measured by competition kinetic methods, ranged from 1.41×10^{12} to 1.99×10^{13} M^{-1} h^{-1}. All SSRIs treated to irradiated water/sediment systems dissipated rapidly, in part as a result of photolysis but mostly because of adsorption to sediment. Nearly constant SSRI residues over time indicated that SSRIs are resistant to microbial metabolism in sediments. No degradation was observed for any drug over a 28-day exposure period in ready biodegradability investigations using activated sludge from a wastewater plant. Methods that employ solid-phase extraction and LC/MS/MS, using ESI in positive ion mode for the determination of five SSRIs and their metabolites in surface water samples, have been developed. The limits of quantitation ranged from 0.4-2.4 ng/L. Samples of influent, effluent, upstream, and downstream water were collected monthly and analyzed. Fluoxetine and sertraline were detected in all samples, ranging in concentration from 0.006-0.076 µg/L and 0.007-0.061 µg/L, respectively. Citalopram also was detected at a concentration of 0.006-0.064 µg/L in selected samples. Laboratory data indicate that the SSRIs as a general class resist most forms of degradation in environmental systems and would partition to sediment where residues of these compounds would persist.

Occurrence and Fate of Antibiotics and Other Pharmaceutically Active Compounds During Transport to and During Drinking Water Treatment

Howard S. Weinberg, Mark D. Sobsey, Philip C. Singer, Embrey L. Bronstad, Vanessa J. Pereira, Katherine M. Stauffenberg, Zhengqi Ye, and Joshua D. Huneycutt
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Abstract

This research project incorporates a multipronged approach to evaluate the fate and transport of pharmaceutically active chemicals in the aquatic environment. Because of their high end-use, antibiotics were singled out for a major investigation that includes isolating them from surface waters impacted by wastewater treatment plant effluent, determining whether antimicrobial resistance traits in bacteria found in those waters are correlated to environmental levels of those compounds, and the fate of such compounds during transport to and through drinking water treatment plants. Appropriate disinfectant quenching—solid phase extraction followed by analysis with liquid chromatography and tandem mass spectrometric methods—was applied to determine occurrence levels for 25 antibiotics, including tetracyclines, sulfonamides, macrolides, quinolones, fluoroquinolones, trimethoprim, and lincomycin in source and finished drinking waters. The method detection limits of the target analytes are generally below 10 ng/L in source water and below 5 ng/L in finished water, but because co-extracted natural organic matter in sample matrices caused signal suppression for most of the analytes, the method of standard addition was used for quantitation. A preliminary occurrence study revealed the presence of a variety of antibiotics in source drinking waters at ng/L levels whose concentrations were somewhat reduced during treatment but in some cases were found, albeit at lower levels, in the finished drinking waters. A kinetic study revealed major differences in the potential for reaction and the fate of antibiotics with disinfectants. Furthermore, it appears that an increase in the levels of antibiotic resistance among naturally occurring microbes downstream of wastewater treatment plant discharges is correlated to the levels of antibiotics in that discharge. Once these chemicals enter "natural systems," they can partition into sediments or undergo some degree of photodegradation with resulting byproducts that might be missed by the techniques used to target individual compounds at environmental levels. A case study with sulfamethoxazole is presented.

It appears that some of the chemical species targeted in this study survive conventional wastewater treatment depositing levels, sometimes exceeding 1μg/L in the receiving stream. This study also has shown that ultraviolet (UV) treatment, which is a technology with increasing deployment for both wastewater disinfection and remediation of water supplies, can remove a variety of compounds including iohexol (a widely used X-ray contrast medium), carbamazepine (an antiepileptic), and analgesics. The effect of using UV together with hydrogen peroxide to enhance oxidation also is demonstrated. Some pharmaceuticals, such as ketoprofen and ciprofloxacin, were significantly removed from synthetic water by direct photolysis using a UV energy that is typically used during drinking water treatment (100 mJ/cm^2), and these results were replicated in contaminated surface waters.

Occurrence and Fate of High-Volume Pharmaceuticals in Wastewater-Impacted Environments

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Abstract

Pharmaceutically active compounds (PhACs) are readily detected in surface waters that receive municipal wastewaters. To understand possible human or ecological exposure risk, a greater understanding of the processes controlling environmental fate of these compounds needs to be developed. In this work, the occurrence and fate of a selection of water-soluble PhACs are compared in wastewater-impacted groundwater and estuarine surface water environments. This research project also has been aimed at determining whether selected PhACs may be useful as tracers of wastewater and other wastewater-derived contaminants in surface and groundwater environments.

Analytical methods employed in this research project were modified from those developed for high volume pharmaceuticals by the U.S. Geological Survey. High performance liquid-chromatography is coupled with time-of-flight mass-spectrometry (HPLC-ToF-MS), a novel MS approach offering unique advantages for this study. The full-spectral sensitivity of the instrument allows for the analyses of non-target compounds and provides indications of analytical interferences or false positive detections of PhACs. The moderately high resolution of ToF-MS and its ability to estimate analyte accurate mass provide enhanced analyte confirmation, determination of elemental formula, and identification of interferences with the same nominal mass.

Studies on the occurrence and fate of PhACs in groundwater focused on field measurements in the upper glacial aquifer of Suffolk County, New York; a region where approximately 75 percent of municipal wastewater is discharged to ground, mostly from cesspools and septic tanks. Groundwater concentrations of detected PhACs were generally low (low to mid ng/L range) with the highest concentrations being immediately adjacent to known point source wastewater introduction. Migration from one treatment plant was followed in more detail. The extent of transport of individual PhACs in the plume tended to be inversely related to their tendency to adsorb to aquifer material. Microbial transformation of some poorly sorbed pharmaceuticals (e.g., metformin) was inferred from their lack of transport. The tendency of PhACs to be detected in groundwater is a function of source strength, analytical sensitivity, and mobility in subsurface environments. The PhACs that were most readily detected included caffeine, paraxanthine (a caffeine metabolite), carbamazepine, and to a lesser extent, sulfamethoxazole and codeine. The transport of caffeine appears to depend on poorly understood biogeochemical transformations. Whereas caffeine distributions were consistent with conservative transport in an oxic wastewater plume at one site, it was rapidly removed within another plume emanating from a septic tank, particularly in an oxidized zone proximate to the main anaerobic plume. A role of abiotic oxidation reactions has been hypothesized to account for this observation and is part of an ongoing doctoral dissertation study.

A wider range of PhACs is readily detected and typically in higher concentrations (low ng/L to low µg/L range) in surface waters of Jamaica Bay, an estuary in New York City highly impacted by municipal wastewaters. The persistence of pharmaceuticals in Jamaica Bay (residence time of a couple of weeks) was demonstrated by both laboratory die-away experiments and by relating chemical concentrations to salinity (freshwater inputs controlled by wastewater discharges to the bay). Of the 12 pharmaceuticals detected throughout the entire salinity range of Jamaica Bay, only nicotine degraded much faster than the residence time of the bay. Diltiazem and, to a lesser extent, trimethoprim were PhACs that were persistent in Jamaica Bay but not transported well in groundwater because of sorption. Of the PhACs measured in Jamaica Bay, caffeine, paraxanthine, nicotine, and cotinine (a nicotine metabolite) had the highest analytical signals and have the potential to serve as the best tracers of wastewater inputs in estuaries.

Detection and Fate of Environmental Estrogens in Wastewater-Impacted Surface and Groundwaters

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Abstract

As a result of numerous reports of feminized male and sexually immature fish from wastewater-impacted water bodies, there has been great interest in the environmental occurrence and fate of steroid estrogens and other contaminants that can interact strongly (e.g., ethynyl-estradiol) or weakly (e.g., several alkylphenols) with estrogen receptor sites. Prior work showed that a portion of steroid estrogens and other estrogen mimicks pass through sewage treatment plants with potentially relevant ecological exposure. Observations in groundwaters, although few, suggested that steroid estrogens were not significantly mobile in porous media environments, perhaps because of the tendency of these moderately hydrophobic chemicals to sorb to geological media. Many pharmacologically active xenobiotic compounds, including steroid estrogens, however, are excreted in human urine primarily as water-soluble glucuronide and sulfate conjugates that can dissociate later releasing the active forms. We hypothesized that under some conditions soluble estrogen conjugates could act as a source of estrogens to aquifers or to groundwater-fed streams and focused our attention on developing methods to analyze for them in environmental samples. A sensitive method to measure steroid estrogen conjugates in matrix-rich sewage influents and effluents (method detection limits ranged from 0.04 to 0.28 ng/L) has been developed using high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) with electrospray ionization. The method employs extensive sample purification by selective extraction from an Oasis HLB solid-phase cartridge followed by separation by anion exchange chromatography. This purification scheme, combined with a stable isotope dilution approach, was used to overcome problems of matrix suppression of ionization and permitted selective and sensitive detection of 10 target conjugates. It was found that the conjugates (especially the glucuronides) are rapidly degraded in sewer pipes prior to reaching sewage treatment plants, within sewage treatment plants (that remove most of the sulfate conjugates), and in septic tanks prior to leaching into groundwater systems. Preservation of these labile compounds was ineffective with common bacterial poisons and required de-activation of extracellular enzymes that hydrolyze the conjugates.

This research project suggests that steroid estrogen conjugates are unlikely to represent a "missing" source of estrogens to receiving waters. Estrogens and xenoestrogens are typically not persistent or mobile in groundwaters. Over short distances (22 feet), however, alkylphenol ethoxylate metabolites, estrogenic fluorescent whitening agents, and free estrone and β-estradiol were mobile in an anerobic septic tank plume. Each of these compound classes, however, were rapidly (less than days) transformed in the portion of the plume that was oxidized and in contact with the water table. Reliable methods also were developed for measuring sediment/soil sorbed estrogens that involve normal phase HPLC extract purification prior to immunoaffinity extraction purification of extracts and analysis by HPLC-time-of-flight-MS. The method has been applied to sewage impacted sediments from Jamaica Bay (New York City). Results indicate that steroid hormones (at a few ng/g) may be preserved with little degradation and that the estrogenic potency (determined with *in vitro* assays of extracts) of steroid hormones was very similar to that of nonylphenol in these sediments. Future work will test whether poor migration of steroid estrogens (and other xeno-estrogens) in aquifers is the result of sorption or transformation reactions.

Fate, Attenuation, and Effects of Fluoroquinolone Antibacterials in Aquatic Systems

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Abstract

The objective of this research project was to assess the fate, attenuation, and ecotoxicity of selected fluoroquinolone (FQ) antibiotics on surface water quality, under both laboratory- and field-scale conditions. Further, emphasis was placed on developing new methods for quantifying FQs and degradation products and developing new molecular techniques for monitoring antibacterial resistance to FQs in microorganisms.

Early work focused on quantifying key FQs, including ciprofloxacin (cipro), enrofloxacin (enro), and their breakdown products at low environmental concentrations, using C-18 solid phase extraction followed by LC-MS analysis.[1,2] The structure of 12 FQ photodegradation and other degradation products were elucidated, including products not previously reported in the literature.

These methods were used for monitoring of cipro and enro within the context of controlled experiments aimed at determining factors that affect their fate in the natural environment. Laboratory results indicate that particulate organic matter (POC) and light intensity and wavelength influence FQ disappearance rate.[3] Photodegradation reactions quickly destroy both cipro and enro under natural sunlight and ambient water conditions. Additionally, both FQs readily adsorb onto POC in solution, although the final fate of the FQs after adsorption has not been fully defined.

Further, fate and effects studies using outdoor 11.3 m^3 aquatic mesocosms verified the laboratory data that POC and light conditions were centrally important to FQ fate in surface waters. The data also indicated that cipro was a significant degradation product of enro, especially under lower light aquatic conditions (~ 25% full light exposure [FLE]), and residual enro could be detected more than 30 days after release when light levels were low (0.5% FLE), suggesting that FQ residuals can accumulate in surface waters under low light conditions.

Associated with FQ fate work, new molecular biological methods for tracking FQ antibacterial resistance in exposed organisms were developed.[4] These methods use density gradient gel electrophoresis for tracking mutations in *gyrA* gene sequences in the region known to confer FQ resistance in bacteria (the quinolone resistance-determining region [QRDR]). Field exposure experiments indicate, however, that at the highest enro exposure levels tested (25 ppb), no major changes in QRDR region were noted. Small changes in microbial community diversity were noted, although it is not possible to state whether such variations resulted from exposure or were a consequence of natural dynamic variability in the microbial communities.

Parallel to the above, toxicity tests were performed on five aquatic organisms with seven FQs (cipro, enro, lomefloxacin, ofloxacin, levofloxacin, clinafloxacin, and flumequine).[5] *Microcystis aeruginosa*, *Lemna minor*, and *Pseudokirchnerialla subcapitata* were sensitive to FQ, whereas no observed effects were found at 10 mg/L with *Daphnia magna* and fathead minnows.

The results generally indicate that FQ antibacterials are short-lived in aquatic water columns; however, they can last for much longer periods in zones where light exposure is minimal. Even in those regions, however, FQs bind to POC matter, which appears to reduce the bioavailability of the compounds.

Future work on FQs should focus more on their possible transmission through the food supply and the transmission of previously resistant organisms of environmental and public health significance. Data indicate that FQ resistance does not readily develop *in situ*, and that the key issue is the migration of resistant pathogens away from the point of resistance development. Work on the quantitative refinement of molecular

biological FQ resistance testing methods is still warranted, however, because this will allow the better tracking of resistance in all settings, including hospitals.

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Adsorption of Beta-Blocker Anti-Hypertensive Pharmaceuticals to a Range of Mineral Surfaces

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Abstract

A wide range of pharmaceutical compounds have been identified in the environment, and their presence has been a matter of growing concern both for human and ecological health. The ultimate fate of pharmaceutical compounds in the environment can be strongly influenced by their adsorption to soils and sediments, because adsorption can slow the migration of chemicals, potentially increasing the time for degradation and reducing the potential for exposure. Adsorption behavior varies from compound to compound and can be difficult to predict for pharmaceutical compounds because their behavior is often controlled by interactions with specific functional groups or complex pH-dependent speciation. To date, only a fraction of pharmaceutical compound classes have seen extensive study of their adsorption behaviors in the presence of natural soils or sediments.

Beta-blocker anti-hypertensive pharmaceuticals are important pharmaceutical compounds for which little adsorption data exists. Beta-blockers are pharmaceuticals that are known to affect the heart and circulatory system (arteries and veins) and are used to treat hypertension. They lower the rate at which the heart beats and in turn reduce blood pressure. Beta-blockers exhibit moderately high solubilities in water and have been detected in groundwater and surface waters at μg/L levels.

The research project examines the adsorption of three beta-blockers (i.e., nadolol, propranolol, and metoprolol) to a wide range of mineral surfaces. Whole soils and sediments often are comprised of a substantial number of different minerals or mineral coatings. Adsorption to whole soils and sediments results from interactions between pharmaceutical compounds and each of the individual mineral surfaces in the whole soil, and the resulting adsorption behavior is a composite of the adsorption to each of the surfaces. The presence of multiple surface types in complex sorbents (e.g., whole soils) is one cause of adsorption nonlinearity and can result in differences in behavior as a function of concentration. A detailed understanding of adsorption requires quantitative knowledge of interactions with all of the specific mineral surfaces present. Adsorption to Canadian River Alluvium (CRA), a material collected from the alluvial channel of the Canadian River in Norman, Oklahoma, has been studied. CRA is predominantly quartz sand, with a large number of additional minerals present in small quantities. To better understand adsorption to CRA, studies were conducted on the adsorption to a high purity natural quartz sand, magnetite, hematite, ilmenite, cordierite, tourmaline, and two types of feldspars, all mineral components identified in analyses of CRA.

Results indicate that the magnitude of adsorption of each compound varies considerably from mineral to mineral on a surface-area-normalized basis. In addition, the relative magnitude of adsorption between the three compounds varies from surface to surface. This presentation will discuss the results of adsorption measurements, and implications for modeling adsorption to complex mineral surfaces. In addition, ongoing work examining the effect of surfactants on beta-blocker adsorption will be discussed.