3/18/08 Bd. Mtg. Item 13
Recycle Water Policy
Deadline: 3/10/08 by 12 p.m.

O.W.L. FOUNDATION
Open Space Water Resource Protection Land Use
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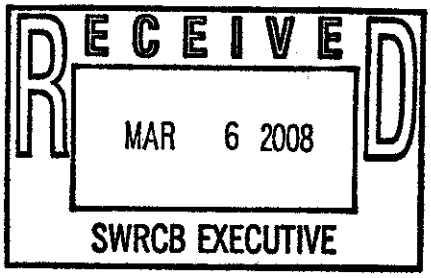
State Water Resources Control Board
via email Ms. Jeanine Townsend
Clerk to the Board
jtownsend@waterboards.ca.gov



Comments on Revisions to DRAFT Recycled Water Policy Staff Report and Attendant Environmental Documents

Dear Board Members;

The O.W.L. Foundation ("Foundation") thanks you for providing this opportunity to comment on the "Draft Staff Report and Certified Regulatory Program Environmental Analysis Recycled Water Policy" ("Report"). We have structured this comment letter with general comments interspersed with specific questions and passages that require clarification from the Board. In order to quickly locate specific questions and passages that require further elucidation, we have marked some paragraphs with an arrow (➔). Note: all bibliographic references are to be considered part of this administrative record in their entirety. We are requesting that the State Water Resources Board ("Board") prepare its responses in a form that corresponds to our letter and that responses consider all text not just that marked with arrows. We assume that the Board will respond fully to each of the following comments in light of the strict requirements of CEQA, as discussed at the end of this letter.

"Recycled" Water is not Fresh water

Given the fact that treated sewage intended for reuse contains a multitude of hazardous substances, including infectious pathogens and other pollutants, the Foundation agrees with this statement on page 2 of the Report: "Water reclamation requirements are issued to protect public health . . . " However, narrowly following " . . . criteria specified in regulations adopted by the California Department of Public Health (CDPH)" does not absolve the State Water Resources Board ("Board") from noting, referencing and suggesting possible remedies for known health risks attendant to the reuse of processed sewage.

The Foundation notes a vast lacuna in the Report with regards to these well known, and widely written about, public health risks. To ignore these very dangerous public health risks simply because the CDPH has not as yet adopted regulations pertaining to them appears to be a clear truancy of the Board's responsibility to protect the public's health. The CDPH may or may not have initiated criteria specified in regulations regarding any number of risks, but it would be remiss for the Board not to bring to the attention of health officials all known risks by detailing them in state policy.

➔We assume that the Board is familiar with the mechanisms of sewage treatment and conversant with the literature generated by specialists in this field. Assuming that the authors of the Report possess this expertise, it is logically inconsistent that they would simultaneously also be ignorant of the dangers to public health represented by these known constituents of "recycled" water. The Report appears mysteriously silent on these essential health issues when the topic obviously needs to be included, regulations notwithstanding.

"Recycled" water at the current level of purity represents a significant health risk

As long ago as 2004, the Water Environment Research Foundation ("WERF") published a study[1] that discovered significant loads of infectious pathogens in recycled water. The real concern with this study, aside from the fact that disease-causing organisms of any kind survive sewage treatment, is that the recycled water where they were discovered satisfied all existing criteria specified in regulations adopted by the state.

Since its publication, the Rose et. al. study has gained considerable notoriety and has circulated widely, not only among member organizations of WERF, but to many other interested parties. It is safe to say that there cannot be but a few people dealing in water issues remaining who are unaware of the fact that

[1] WERF; Water for Reuse; Final Report 2004; Reduction of Pathogens, Indicator Bacteria, and Alternative Indicators by Wastewater Treatment and Reclamation Processes, Joan B. Rose, Ph.D. et. al.; 00-PUM-2T

dangerous pathogens exist in recycled water and that that "existing criteria specified in regulations" have been notoriously delinquent in conforming to reality.

Nevertheless, to ignore a health risk of this magnitude based solely on the inaction of another government bureau would seem to be a regrettable failure to execute specific responsibilities.

➔Are the authors of the Report unaware of the Rose et. al. study? If they are aware of it, then please explain in detail how omitting reference to the fact that recycled water contains infectious pathogens can be justified with California Health and Safety Code 5411 which states: "No person shall discharge sewage or other waste, **or the effluent of treated sewage or other waste**, in any manner which will result in contamination, pollution or a nuisance." [emphasis added]

California Health and Safety Code 5411.5(a) is even more specific in spelling out the necessity to contact health officials, stating: "Any person who, without regard to intent or negligence, causes or permits any sewage or other waste, or the effluent of treated sewage or other waste to be discharged in or on any waters of the state, or discharged in or deposited where it is, or probably will be, discharged in or on any waters of the state, as soon as that person has knowledge of the discharge, ***shall immediately notify the local health officer or the director of environmental health of the discharge***." [emphasis added].

Any knowledge of the dangers of recycled water requires immediate notification of health officials. Simply complying with this law would be an obvious direct route to the CDPH and a valuable first step in securing any criteria in health regulations that the Board discovers lacking.

➔Please explain in detail why the Report does not contain language highlighting the risk of infectious pathogens in recycled water to public health. Also, please expand upon the rationale not to notify health officials that treated sewage effluent (so-called "recycled" water) containing infectious agents is being discharged in public spaces and that these waters can find their way to both waters owned by the state and to privately owned groundwater resources.

Invasive MDR Infection

There is mounting suspicion that multi-drug resistant ("MDR") pathogens escaped the hospital setting because of widespread use of "recycled" water. If the sewage treatment process actually removed everything except the molecule H_2O, then the resulting product would indeed be recycled and sewage would be restored to fresh water status. Sewage cleaned to this level of purity, like that produced by reverse osmosis groundwater replenishment systems, generate clean water that is safe for reuse. But water

containing MDR pathogens, multi-drug resistant genes and other contaminants, represent a significant risk to public health.

In October of 2007, R. Monina Klevans, M.D. published a report[2] in the Journal of the American Medical Association revealing that 19,000 Americans died in 2005 of invasive infections from methicillin-resistant Staphylococcus aureus, or MRSA (*nota bene*: more than 90,000 Americans were infected that year). This study alerted the medical community that MRSA is actually twice as common as previously thought. Indeed, the number of deaths exceeds that due to complications resulting from AIDS. Other studies[3] have demonstrated that multi-drug resistant genetic material can pass though standard sewage treatment plants unscathed and enter drinking water supplies.

These two studies alone provide sufficient justification to begin stringent testing of sewage effluent before it is used as a substitute for fresh water. MDR pathogens and genes erode the efficacy of our entire antibiotic pharmacopoeia and have the potential to render all antibiotics useless. The threat of not denaturing every single gene and every single pathogen from every single source of sewage effluent could trigger a lethality greatly exceeding that of any of the major wars in history.

Indeed it would be difficult to overestimate the handicap of practicing medicine without so-called "miracle" drugs. Without antibiotics bacterial infections would have to be treated by amputation, the way physicians did over 100 years ago. Unfortunately, we are rapidly approaching this grim future as we watch MDR bacteria achieve ever-increasing virulence and prevalence, while at the same time watch antibiotic efficacy continue to plummet.

➔The detection of MDR pathogens and MDR genes in sewage strongly suggest that the reuse of treated sewage water (so-called "recycled" water) plays a sizeable role in this downward spiral. The above mentioned studies provide a credible, theoretical explanation of how MRSA escaped the hospital setting and wound up as an endemic infection in the community. Recycled water containing MDR pathogens and MDR genes spread multi-drug resistance. It is incumbent upon the State Water Resources Control Board to highlight the health risks posed by so-called "recycled" water when crafting a statewide recycled water policy. Please explain why mention of such a formidable, known health risk managed to escape the attention of the Report.

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[2] Invasive Methicillin-Resistant *Staphylococcus aureus* Infections in the United States, Klevens et al. *JAMA*.2007; 298: 1763-1771.

[3] Pruden, A.; Pei, R.; Storteboom, H.; Carlson, K. H Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado. Environ. Sci. Technol.; (Article); 2006; 40(23); 7445-7450

Beyond Infection

As long ago as 2003, The American Academy of Pediatrics recognized phthalates[4] as a potential toxic hazard and has recommended further study of phthalate poisoning and the physical harm they cause. The United States Geological Survey ("USGS") and the Center for Disease Control ("CDC"), among other organizations, have detected phthalates (a family of chemicals that are biologically active in very small doses and act as endocrine disruptors) in rivers, streams, sediment beds, sewage sludge (used widely as "fertilizer"), fish, birds and people. Phthalates commonly enter the waste stream as leachate from plastic materials where phthalates have been added for flexibility. However, phthalates are also found in shampoos, paints, insecticides and numerous other products. Ultimately, waste phthalates wind up in sewage treatment plants but are not removed in the sewage treatment process. The increased use of "recycled" water distributes phthalates widely which helps to account for their surprising ubiquity.

It has long been known that these powerful environmental contaminants affect the development of reproductive organs by interfering with normal endocrine processes. Phthalates disrupt cellular receptors, hormonal pathways and communications of glands. This damage has been observed throughout the world in fish populations, for example male fish carrying egg sacks and other disturbing abnormalities. It is highly significant that many of these deformed fish were discovered near sewage treatment plant outflows. The connection between the dangers of hormone-laden treated sewage water and biological damage is inescapable.

➔We are compelled to ask, what happens when this "treated" effluent is sprayed on schoolyards, public parks and golf courses? Phthalates mimic estrogen, the primary female hormone. Hormonal activity is powerful enough that hormones, and the chemicals that mimic them, can be biologically active in parts per trillion. Until proven otherwise, phthalate contamination would have to be a prime suspect in the rise of certain endometrial and invasive breast cancers; deep vein thrombosis; stroke; myocardial infarction and pulmonary embolism. Until the role of phthalates in these maladies can be ruled out or until all phthalates are removed from "recycled" water, these dangers must be assumed to exist in recycled water. Please explain how mention of this significant health peril long-associated with recycled water was not discussed in the Report.

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[4] AMERICAN ACADEMY OF PEDIATRICS: Katherine M. Shea and Committee on Environmental Health Pediatric Exposure and Potential Toxicity of Phthalate Plasticizers Pediatrics, Jun 2003; 111: 1467 - 1474.

Pharmaceuticals

Sewage treatment has become notorious for its inability to remove virtually any and all pharmaceuticals from processed effluent. It has become routine for police agencies around the world to test rivers, streams, estuaries, or any body of water that receives sewage effluent, for illegal drugs or for the metabolites of those drugs in order to gauge the amount of drug abuse in a given region.

"Recycled" water not only may contain illegal drugs; it may also contain legal ones too. Antibiotics, chemotherapy drugs, hormone supplements, indeed every medication known to medical science will pass through virtually every sewage treatment process intact. Since drugs merge into the resulting effluent they become part of "recycled" water and therefore, if used as a substitute for fresh water, expose the public to a health hazard.

Even if the amount of drugs in "recycled" water is slight, contaminated water remains a public health hazard for two separate and distinct reasons. Firstly, many drugs are known to bioaccumulate, meaning that exposure over time increases the risk of toxicity and damage. Secondly, chemicals not removed from sewage that wind up in "recycled" water interact sometimes producing entirely new toxicants that were never introduced to the waste stream.

One alarming study[5] by Bender et. al. has revealed the futility of trying to predict all of the possible chemical reactions taking place in sewage treatment. The Bender study provides convincing evidence that toxic substances are being created in the sewage process itself. Given the enormous inventory of chemicals that exist in sewage it is obviously impossible to predict every possible dangerous combination or reaction. Nevertheless, the sheer size of this inventory also provides an equally enormous opportunity for other unpredictable reactions to take place, making the production of other new toxic compounds a virtual certainty.

➔Since the chlorination process can react with Acetaminophen to spawn two new toxicants, then what other combinations are taking place in sewage treatment and how much of these resulting reactions are constituents of "recycled" water? Assuming that knowing this number is impossible, please explain why no mention of the widely recognized phenomenon of emerging toxicants from unpredictable reactions in sewage is absent in the report. The choice in crafting sound recycled water policy seems clear: either

[5] Transformation of Acetaminophen by Chlorination Produces the Toxicants 1,4-Benzoquinone and *N*-Acetyl-benzoquinone Imine, Mary Bedner and William A. MacCrehan, *Analytical Chemistry Division, National Institute of Standards and Technology, Mailstop 8392, Gaithersburg, Maryland 20899-8392*

remove all contaminants from sewage, and return the sewage to fresh water, or test this material before it is used as a substitute for fresh water and exposing the public to risk. Please expand in some detail why such a straightforward solution to this danger was not discussed in the Report.

Retention Pools

Surprisingly, the Report contains no mention of exposed retention pools used for storing recycled water. Here in Sonoma County, numerous vast open ponds of treated sewage containing the above-mentioned contaminants have been planned under two separate projects: the Incremental Recycled Water Project ("IRWP") and the North Sonoma County Agricultural Reuse Project ("NSCARP"). Some of these pools are planned directly on top of active earthquake faults. Most are on state-identified groundwater recharge lands with only a "liner" separating the aquifer from the contaminants. (The Sonoma County dump at Mecham Road was recently condemned because the "liner" leaked dump exudates into the aquifer).

➔There are no specifications to enclose open ponds of treated sewage despite the danger of aerosolization[6]. The Foundation was very much looking forward to new recycled water policy that dealt with the dangers of open ponds. If the authors of the study are unaware of these dangers, please consult the bibliographic material in footnote number 6 for a more complete treatment. However, since it is generally common knowledge that a brisk wind will lift open water out of virtually any containment. Please explain why retention ponds used for recycled water was not examined in the Report.

Given the above documented public health hazards inherent in recycled water, it would seem that the Board would have a simple choice regarding recycled water policy: **Either, 1) Remove all contaminants and infectious agents of any description so that treated sewage is restored to fresh water. Or, 2) Test all recycled water before it is dumped into any body of water, used for irrigation, sprayed on to public spaces or in any other way risks exposure to people, animals or plants.**

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[6] Sea Salt Aerosol Production: Mechanisms, Methods, Measurements, and Models - A Critical Review, by Ernie R. Lewis (Author), Stephen E. Schwartz, Amer Geophysical Union (January 2005), ISBN-10: 0875904173 [also has fresh water models]

Anguelova, M., R.P. Barber, Jr., and J. Wu, Spume drops produced by the wind tearing of wave crests, J. Phys. Oceanogr., 29, 1156-1165, 1999.

Koga, M., Direct production of droplets from breaking wind-waves – its observation by a multi-colored overlapping exposure photographic technique, Tellus, 33, 552-563, 1981.

Tegowski J, A laboratory study of breaking waves, OCEANOLOGIA 46 (3): 365-382 2004

Young IR, Banner ML, Donelan MA, et al. An integrated system for the study of wind-wave source terms in finite-depth water JOURNAL OF ATMOSPHERIC AND OCEANIC TECHNOLOGY 22 (7): 814-831 JUL 2005

Wu, J., Spray in the atmospheric surface layer: laboratory study, J. Geophys. Res., 78 (3), 511-519, 1973.

Since numerous sewage treatment facilities around the globe are able to remove all contaminants from raw sewage and produce clean water from it, the technology to do so is well established. The ability to test for public health hazards as itemized above is also perfectly within the grasp and scientific ability of virtually any sewage treatment facility.

➔Please explain the rationale for not including these two reasonable and rather obvious paths of action in the Report.

In conclusion, we note that the statute known as CEQA, its implementing Guidelines and case law interpreting the statute and the Guidelines provide the following: That the evaluation and response to public comments is an essential part of the CEQA process. Failure to comply with that requirement can lead to disapproval of the project. Further, in a final EIR, the lead agency must evaluate and respond to all the relevant comments on the Draft EIR that it receives within the public review period. The responses must describe the disposition of the environmental issues raised in the comments. The lead agency must specifically explain its reasons for rejecting these suggestions, receiving comments and for proceeding with the project and its environmental impacts. There must be a good faith, reasoned analysis and response. Conclusory statements unsupported by factual information will not suffice.

Sincerely,

H.R. Downs, President

Attached exhibits:
OWL_Reader_Pathogens.pdf (6MB)
OWL_Reader_Pollutants.pdf (1.5MB)

AN O.W.L. FOUNDATION READER

A COLLECTION OF ARTICLES CONCERNING THE CHEMICALS LEFT BEHIND IN PARTIALLY TREATED SEWAGE (also known as "tertiary-treated" wastewater) AND THEIR HEALTH IMPLICATIONS

Article published - Sep 6, 2006

More 'Intersex Fish' Found in Potomac

By MATTHEW BARAKAT
Associated Press Writer

Some species of male fish in the Potomac River and its tributaries are developing female sexual traits at a frequency higher than scientists have seen before, raising concerns about pollutants in a waterway that provides drinking water for millions of people.

The so-called "intersex fish," which produce immature eggs in their testes, were discovered in the Potomac rivershed in 2003 and have also been found in other parts of the country.

But the frequency that the U.S. Geological Surveys found last year is much higher than what has been found elsewhere, said fish pathologist Vicki Blazer.

In some Potomac tributaries, nearly all of the male smallmouth bass caught in last year's survey were the abnormal fish. In the Potomac itself, seven of 13 largemouth bass exhibited female characteristics, including three that were producing eggs.

Although the frequency discovered was surprisingly high, Blazer cautioned that the sample size was relatively small, with about 10 male and 10 female fish taken from each of eight locations in Maryland, Virginia and the District of Columbia.

Researchers were reluctant to remove large numbers of bass from the rivers because of conservation concerns, she said.

Female fish caught in the survey did not develop any unusual sex traits, though fish of both sexes exhibited lesions and other pollution-related problems, said Blazer, who coordinated the survey.

Smallmouth bass appear to be more susceptible to intersex development than largemouth bass, Blazer said.

Blazer said researchers are still waiting on data that would help them determine the water quality at the time the fish were caught, but preliminary data taken from the Potomac found a variety of chemical pollutants.

It is not exactly clear what is causing the changes, though it is likely a combination of pollutants, scientists say.

Certain chemicals and pesticides are believed to stimulate estrogen production. Also, estrogen

from birth control pills and human waste can make its way from sewage treatment plants to the waterways.

The Environmental Protection Agency has been studying the issue of so-called "endocrine disruptors" since 1996, but currently does not issue guidelines to water treatment plants for allowable levels of estrogenic compounds.

Jeanne Bailey, a spokeswoman for Fairfax Water, said the findings are a concern.

The water authority, which draws from the Potomac and Occoquan rivers to provide service to roughly 1.5 million people, is working with USGS and other agencies to research and develop ways to improve water treatment to eliminate potentially harmful compounds.

The water treatments used by Fairfax Water, including ozone and activated charcoal, have been shown to reduce levels of estrogenic compounds, she said.

Bailey cautioned against drawing dire conclusions about the impact on human health. She said, "Fish are a great indicator of the health of our waters, but they are not a great indicator of what may translate to humans."

April 17, 2007

Bringing Cancer to the Dinner Table: Breast Cancer Cells Grow Under Influence of Fish Flesh

Tests of river fish indicate their flesh carries enough estrogen-mimicking chemicals to cause breast cancer cells to grow

Many streams, rivers and lakes already bear warning signs that the fish caught within them may contain dangerously high levels of mercury, which can cause brain damage. But, according to a new study, these fish may also be carrying enough chemicals that mimic the female hormone estrogen to cause breast cancer cells to grow. "Fish are really a sentinel, just like canaries in the coal mine 100 years ago," says Conrad Volz, co-director of exposure assessment at the University of Pittsburgh Cancer Institute. "We need to pay attention to chemicals that are estrogenic in nature, because they find their way back into the water we all use."

Volz and colleagues, including biochemist Patricia Eagon, took samples from 21 catfish and six white bass donated by local anglers as part of a study presented at the American Association for Cancer Research meeting in Los Angeles this week. The fish were caught in five places: a relatively unpolluted site 36 miles upstream from Pittsburgh on the Allegheny River; an industrial site on the Monongahela River; an Allegheny site downstream from several industries that release toxic chemicals; and the confluence of the Allegheny and Monongahela rivers, where Pittsburgh dumps much of its treated sewage and sewer outflows. "This is the largest concentration of

combined sewer outflows in the U.S.," Volz notes, about the confluence, known as the Point. The researchers also bought several fish at the store as controls.

Using an organic solvent, the researchers created an extract from the skin, flesh and fat of the various fish. They then bathed a breast cancer cell line—known as MCF-7—in the extract. "We used this cell line because it has estrogen receptors in it, meaning that if estrogens are present it causes this cell line to proliferate," Volz explains. "If you put something on it and it grows, then it must be stimulating the estrogen receptor." In addition to responding to pure estrogen applied as a positive control, the extract from two of the white bass and five of the catfish caused the breast cancer cells to thrive.

The highest response came from fish caught in the industrial section of the Monongahela River. "The Monongahela River area is the area in Pittsburgh that was the site of most of the steel production over the last 100 years," Volz says. "That area is still an industrial beehive." But the broadest response came from where the sewer outflows and sewage treatment plants flow into the rivers from Pittsburgh; three of the four catfish caught here caused the breast cancer cells to proliferate. "Sewage might be more responsible for putting estrogenic chemicals in the water than the industries alone," Volz adds. "All of the hormone replacement products that women use go down the drain, along with birth control pills, antibacterial soaps, and many of the plastics we use, like Bisphenol A, have such effects."

It remains unclear exactly what estrogen-mimicking chemicals were actually present in the fish and what kind

of cancer-causing role they might have. But their effects on the fish themselves were clear: the gender of nine of the fish could not be determined. "Increased estrogenic active substances in the water are changing males so that they are indistinguishable from females," Volz says. "There are eggs in male gonads as well as males are secreting a yolk sac protein. Males aren't supposed to be making egg stuff."

And this estrogen burden is widespread. The store-bought white bass caused breast cancer cells to grow like its river-caught counterparts (as well as containing higher levels of mercury, arsenic and other contaminants) after being trucked to Pittsburgh from Lake Erie. "These fish, again, were in waters that were seeing industrial waste as well as possible combined sewer outflows," Volz notes. "This isn't just happening in Pittsburgh, this is happening everywhere in the industrialized world."

Volz says he and his fellow researchers are launching a broader survey this summer that will entail sampling fish all along the Allegheny River. Efforts will be made to determine if it is industrial waste, sewage or agricultural runoff—or all three—that is responsible for the problem. In the meantime, cooking the fat out of fish may be the best defense. "If you broil fish and let the fats drip out that will take most of the contaminants out," Volz says, though that may not be enough given other exposures to potentially tainted water. "What our study does show us is that there is exposure potential to vast populations that use water from our rivers as their drinking water supply."

from birth control pills and human waste can make its way from sewage treatment plants to the waterways.

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The New York Times
nytimes.com

September 18, 2006

Study: Drugs, Chemicals in Sewage Sludge

By THE ASSOCIATED PRESS
http://www.nytimes.com/aponline/us/AP-Waste-Contamination.html
TACOMA, Wash. (AP) -- Promoted as a great way to dispose of treated waste, the sewage sludge sold to homeowners to spray on their lawns and gardens may also be adding drugs, flame retardants and other chemicals to the landscape, according to a study.

Chad Kinney, an assistant professor of chemistry and biochemistry at Eastern Washington University, found dozens of medicinal, industrial and household compounds in treated sewage sludge, also known as biosolids, that government agencies sell as lawn-and-garden enhancements.

"No matter what biosolid we looked at, there were some of these compounds in it," said Kinney, whose research on the subject was published in online editions of the journal Environmental Science & Technology. The U.S. Geological Survey's Toxic Substance Hydrology Program supported his work, which began while he was a postdoctoral fellow at the U.S. Geological Survey.

Kinney and his team studied nine biosolid products from seven states: Washington, Arizona, Wisconsin, Kansas, Colorado, Texas and Iowa.

The scientists found that it didn't matter what wastewater treatment method was used, 25 compounds were found in each of the samples. They were looking for 87 different compounds and found 55 in one or more of the biosolids and at least 30 in each of the samples. The product with the most compounds had 45.

Although government regulators and health officials said there is no immediate risk to public health, the study's authors called for more research on the long-term impact on the environment.

"We've been using biosolids for over 30 years safety," said Peggy Leonard, biosolids program manager for King County's waste treatment division, which produces GroCo. "As far as I know, there is no risk."

Thomas Burke, a professor of public health policy at Johns Hopkins University in Baltimore, said Kinney's research and other studies should be a wake up call for the U.S. Environmental Protection Agency.

"I don't think people understood before this that they might be applying pharmaceuticals and disinfectants to their front lawns," Burke said.

The EPA has promoted the benefits of biosolids for decades because they contain the same nutrients -- nitrogen and phosphorus -- found in fertilizers.

Rick Stevens, national biosolids coordinator for the EPA, said in an e-mail to The News Tribune

of Tacoma that the agency stands by its existing biosolids regulations. State officials also said they do not think people should worry about exposure to chemicals in biosolids.

In King County, Leonard called Kinney's research a "good start," but said it fails to answer whether the chemicals break down in soils and whether they pose danger.

Dan Thomas, Tacoma's wastewater operations manager, said the issues raised by Kinney's report are not new.

"It's something we need to keep our eye on but we're not super-concerned at this time. We know these constituents are here. There's no reason to believe there's a health threat," Thomas said.

Soil scientists at Cornell University's Waste Management Institute have been asking for more regulatory scrutiny of biosolids.

"I certainly would not use this material on my garden" said Ellen Harrison, director of the Waste Management Institute.

Burke of Johns Hopkins called the EPA regulations out of date, adding that some of the chemicals identified in the study have been shown to disrupt fish reproduction.

"These are things that have biological implications and we have to understand them better," Burke said.

Information from: The News Tribune, http://www.thenewstribune.com

Environ. Sci. Technol., **ASAP Article** 10.1021/es0509073 S0013-936X(05)00907-7
Web Release Date: November 30, 2005

Transformation of Acetaminophen by Chlorination Produces the Toxicants 1,4-Benzoquinone and *N*-Acetyl-*p*-benzoquinone Imine

Mary Bedner* and William A. MacCrehan

Analytical Chemistry Division, National Institute of Standards and Technology, Mailstop 8392, Gaithersburg, Maryland 20899-8392

Received for review May 12, 2005

Revised manuscript received October 18, 2005

Accepted October 25, 2005

Abstract:

The reaction of the common pain reliever acetaminophen (paracetamol, 4-acetamidophenol) with hypochlorite was investigated over time under conditions that simulate wastewater disinfection. Initially, the reaction was studied in pure water at neutral pH (7.0), a range of reaction times (2-90 min), and a molar excess of hypochlorite (2-57 times) relative to the acetaminophen concentration. The reaction was monitored using reversed-phase liquid chromatography (LC) with ultraviolet absorbance, electrochemical, and mass spectrometric detection. At 1 μmol/L (150 ppb) and 10 μmol/L (1.5 ppm) levels, acetaminophen readily reacted to form at least 11 discernible products, all of which exhibited greater LC retention than the parent. Two of the products were unequivocally identified as the toxic compounds 1,4-benzoquinone and *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is the toxicant associated with lethality in acetaminophen overdoses. With a hypochlorite dose of 57 μmol/L (4 ppm as Cl_2), 88% of the acetaminophen (10 μmol/L initial) was transformed in 1 h. The two quinoidal

oxidation products 1,4-benzoquinone and NAPQI accounted for 25% and 1.5% of the initial acetaminophen concentration, respectively, at a 1 h reaction time. Other products that were identified included two ring chlorination products, chloro-4-acetamidophenol and dichloro-4-acetamidophenol, which combined were approximately 7% of the initial acetaminophen concentration at 1 h. The reaction was also studied in wastewater, where similar reactivity was noted. These results demonstrate that acetaminophen is likely to be transformed significantly during wastewater chlorination. The reactivity of the chlorine-transformation products was also studied with sulfite to simulate dechlorination, and 1,4-benzoquinone and NAPQI were completely reduced.

[Full text in html]
[Full text in pdf]

Stay calm everyone, there's Prozac in the drinking water

Mark Townsend
Sunday August 8, 2004
The Observer

It should make us happy, but environmentalists are deeply alarmed: Prozac, the anti-depression drug, is being taken in such large quantities that it can now be found in Britain's drinking water.

Environmentalists are calling for an urgent investigation into the revelations, describing the build-up of the antidepressant as 'hidden mass medication'. The Environment Agency has revealed that Prozac is building up both in river systems and groundwater used for drinking supplies.

The government's chief environment watchdog recently held a series of meetings with the pharmaceutical industry to discuss any repercussions for human health or the ecosystem.

The discovery raises fresh fears that GPs are overprescribing Prozac, Britain's antidepressant of choice. In the decade up to 2001, overall prescriptions of antidepressants rose from nine million to 24 million a year.

A recent report by the Environment Agency concluded Prozac could be potentially toxic in the water table and said the drug was a 'potential concern'.

However, the precise quantity of Prozac in the nation's water supplies remains unknown. The government's Drinking Water Inspectorate (DWI) said Prozac was likely to be found in a considerably 'watered down' form that was unlikely to pose a health risk.

Dr Andy Croxford, the Environment's Agency's policy manager for pesticides, told The Observer: 'We need to determine the effects of this low-level, almost continuous discharge.'

Norman Baker, the Liberal Democrat's environment spokesman, said the revelations exposed a failing by the government on an important public health issue. He added that the public should be told if they were inadvertently taking drugs like Prozac.

'This looks like a case of hidden mass medication upon the unsuspecting public,' Baker said. 'It is alarming that there is no monitoring of levels of Prozac and other pharmacy residues in our drinking water.'

Experts say that Prozac finds its way into rivers and water systems from treated sewage water. Some believe the drugs could affect their

judge's chambers?

History boys swap Broadway for the BBC

Cyclists warned to spare Brighton's blushes

Non-swimmers get two weeks of pool training

Playground fun 'needs more risks'

Oxford targets East Enders

News in Brief

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reproductive ability.

European studies have also expressed disquiet over the impact of pharmaceuticals building up in the environment, warning that an effect on wildlife and human health 'cannot be excluded'.

'It is extremely unlikely that there is a risk, as such drugs are excreted in very low concentrations,' a DWI spokesman said. 'Advanced treatment processes installed for pesticide removal are effective in removing drug residues,' he added.

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May 30 2006

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The Times August 05, 2005

Where rivers run high on cocaine

By Nigel Hawkes

Analysis of waste water in Italy shows a startlingly high level of drug abuse

THE rivers of Italy are flowing with cocaine, say scientists who have adopted a new approach to measuring the extent of drug misuse. The biggest river, the Po, carries the equivalent of about 4kg (8lb 13oz) of the drug a day, with a street value of about £20,000.

Cocaine users among the five million people who live in the Po River basin in northern Italy consume the drug and excrete its metabolic by-product, benzoylecgonine (BE). This goes from sewers into the river. So a team led by Dr Ettore Zuccato, of the Mario Negri Institute for Pharmacological Research in Milan, estimated the use of cocaine by testing the waters of the Po for BE, and for any cocaine that had passed through the body unaltered or reached the sewers in other ways.

What they found surprised them. They calculated that for every 1,000 young adults in the catchment area, about 30 must be taking a daily dose of 100 milligrams of cocaine, which greatly exceeds official national figures for cocaine use.

According to official Italian statistics, 1.1 per cent of people between the ages of 15 and 34 admit to having used cocaine "at least once in the preceding month". Almost all cocaine use occurs in this age group.

Assuming that there are 1.4 million young adults in the Po River basin, the official statistics suggest that there would be 15,000 cocaine-use events per month. But the evidence from the water suggests that the real usage is about 40,000 doses a day, a vastly greater figure.

"The economic impact of trafficking such a large amount of cocaine would be staggering," Dr Zuccato said. "The large amount of cocaine — at least 1,500kg — that our findings suggest is consumed per year in the River Po basin would amount, in fact, to about $150 million in street value, based on an average US street value of $100 per gram."

To confirm their findings, the team also sampled urban waste water from Cagliari in Sardinia, Latina

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in central Italy, and from Cuneo and Varese in the north — all medium-sized cities. The values they obtained from the undiluted waste water were far higher than those in the Po, as would be expected. But when translated into likely local use of the drug, they produced very similar figures — which suggests that the Po region is not exceptional in its cocaine consumption. The results cannot be explained by assuming that some drug trafficker was panicked into dumping his stash down the lavatory. If so, much more pure cocaine would have been found, and much less of its human metabolite, BE. In fact, the ratio of cocaine to BE was consistent throughout all the samples.

If anything, Dr Zuccato said, the method would be expected to underestimate rather than to overestimate cocaine use, because some would be lost or absorbed in sediments. So the real consumption may be even higher.

This method has previously been used by the same team to measure the by-products of widely-used prescription drugs, and has produced results consistent with known prescribing patterns. So it seems to work.

The technique has been developed by the Italian team and is complex, as it needs to be to detect such tiny residues — of the order of billionths of a gram per litre of water.

The scientists say that the method needs to be tested further before being brought into general use, but suggest that it would be a more reliable and much cheaper way of tracking trends in drug use than by using population surveys.

"The approach tested here, which is in principle adaptable to other illicit drugs, could be refined and validated to become a general, rapid method to help estimate drug abuse at the local level," they report in the journal *Environmental Health.*

"With its unique ability to monitor changing habits in real time, it could be helpful to social scientists and authorities for continuously updating the appraisal of drug abuse."

The levels of the drug and the metabolite found in river water are so low that any effect on natural life is very unlikely. But this is not true of all chemicals. Research indicates that chemicals that mimic natural hormones are having an effect on fish in many rivers, including "feminising" many male fish. The sources of these chemicals include hormones excreted by the human body and industrial chemicals that reach the waterways.

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Article published - Mar 5, 2007

Panel looks at safety of chemical in popular plastic

Bisphenol A could cause complications with pregnancy

By TINA HESMAN
ST. LOUIS POST-DISPATCH

The safety of a chemical that's probably in your cell phone, eyeglass lenses, car, computer, baby bottles, microwaveable dishes - and hundreds of other popular products - will face public scrutiny today.

The chemical bisphenol A is used to make lightweight clear plastics and resins used as adhesives and coatings in everyday products.

Critics are concerned that the chemical could harm human health, particularly the development of fetuses and children, because it works like the female sex hormone estrogen. Other chemicals that mimic estrogen, notably the banned pesticide DDT, have been shown to interfere with hormone function and cause abnormalities in wildlife and laboratory animals.

The chemical industry contends that the weight of scientific evidence on bisphenol A doesn't support the claims of harm. But the chemical has been the subject of much controversy recently.

The San Francisco City Council passed a measure to bar bisphenol A and some other components of plastics, from products for children. But the European Union recently increased the level of exposure it considers safe for human health.

Starting toay, a panel of 15 scientists convened by the National Institutes of Health's Center for the Evaluation of Risks to Human Reproduction will hold three days of meetings in Alexandria, Va., to examine the safety of bisphenol A. The panel issued a draft report in December but will finalize the report and release its conclusions and recommendations this week. The

panel has no regulatory authority, and the findings are not binding.
Frederick vom Saal of the University of Missouri-Columbia, one of the leading experts on low-level exposure to bisphenol A, intends to address the scientific panel during a public comment period today.
Like everyone else wishing to comment on the draft report, he will get seven minutes to make his remarks. Scientists who study bisphenol A, such as vom Saal, were not invited to participate on the panel.
Bisphenol A was originally developed in the 1930s as an estrogen for birth control, said vom Saal. It was never used for that purpose because scientists quickly discovered that multiple molecules of bisphenol A could link together to form clear, hard plastics, vom Saal said. More than 6 billion pounds of bisphenol A plastics are made globally each year, vom Saal said.
The chemical is ubiquitous and almost unavoidable, vom Saal said.
Most people carry the chemical around in their bodies at low levels - about 1 part per billion in blood, urine and tissues.
"That seems like a staggeringly small number until you realize that the natural hormone it's acting like works at levels 10,000 times lower than that," vom Saal said.
The increase in bisphenol A production parallels the rise in obesity, vom Saal said. That's no coincidence, he says. His work with mice suggests that exposure to the chemical during pregnancy can lead to obesity in adulthood.
He points to more than 140 government-sponsored studies that have linked exposure to the chemical to breast cancer, prostate cancer, changes in reproductive organs, brain changes, obesity and other indications of harm. No industry-sponsored studies have uncovered evidence of harm from bisphenol A, vom Saal said.
Industry representatives say looking at individual studies is the wrong approach.
"This isn't a basketball game. You don't just count up studies and see what the score is," said Steven Hentges, executive director of the Polycarbonate Bisphenol A Global Group of the American Chemistry Council.
The studies must be considered in aggregate and evaluated for reproducibility, consistency and relevance to human health, Hentges said.
"In every case in which the evidence is evaluated together, the conclusion is bisphenol A is not a risk to human health, particularly at the low levels to which we are exposed," he said.

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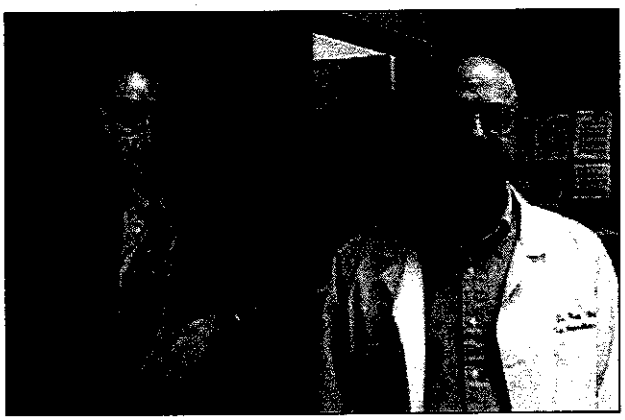
MIZZOU NEWS

Uncovering a Hidden Danger

By Matt McGowan

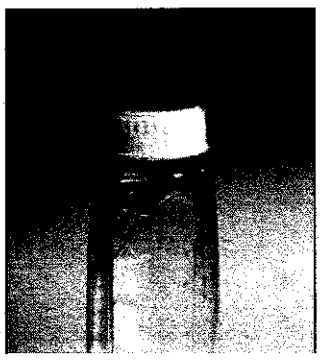


MU researchers Fred vom Saal, left, and Wade Welshons have conducted landmark studies on the potential health effects of chemicals in the home and environment. For more information about their work, go to the Endocrine Disruptors Group web site. Photos courtesy of MU Publications and Alumni Communication and the College of Veterinary Medicine

By nature, Fred vom Saal is not a crusader, but he doesn't want to wait 10 years for a governmental agency to ban a chemical that his research shows harms animals. He doesn't want to wait for thousands of people to show severe abnormalities from years of eating foods packaged in plastic.

Since their landmark findings in 1997 on low-dosage effects of Bisphenol A (BPA) on mice, vom Saal and Wade Welshons, researchers at the University of Missouri-Columbia, have labored to warn the public and government agencies of the dangers associated with the prevalent chemical that is used in many plastic products, including baby bottles, food-storage containers and toys.

In May vom Saal presented new scientific evidence about this chemical at the Toxicology and Risk Assessment Conference, an annual conference sponsored by several governmental agencies, including the U.S. Environmental Protection Agency, to examine the possible dangers of toxic chemicals.



During the conference near Dayton, Ohio, vom Saal argued that scientific findings in more than 35 publications in peer-reviewed scientific journals provide credible evidence that the chemical is harmful to every type of animal that has been studied, and this chemical is thus very likely to produce the same types of abnormalities in humans. These findings are based on independent academic research that has studied the effects of BPA.

"This evidence will ultimately convince federal regulatory

agencies that BPA should be illegal for use in food and beverage containers," vom Saal said. "It's only a matter of time."

Bisphenol A is an artificial estrogen, but it is bonded together in a chain of bisphenol A molecules to create the plastic called polycarbonate as well as resins that are used to line cans and as dental sealants. Each day, consumers use several plastic products that contain BPA, a chemical found in the 1930s by a Nobel-prize winning scientist to act like estrogen. In the 1950s, chemists linked BPA together to create polycarbonate material, and companies began using the chemical in plastics production. Today, BPA, one of the top 50 chemicals in production in the United States, generates billions of dollars for the plastics industry, which produces about 2.5 billion pounds of the chemical per year.

Vom Saal said scientists have known for many years that the polycarbonate bond created by BPA was unstable and that the chemical would eventually leach into food or beverages in contact with the plastic. The obvious concern today is that it may leach into food products, ranging from microwavable dinners to baby formula, that are packaged in polycarbonate plastic.

"The idea that this is a strong, durable product is an illusion," vom Saal said. "The chemists have known that the Bisphenol A chemical is constantly leaching and coming into contact with food or water. It's going to damage your body."

Researchers also have known that supplemental estrogens are harmful to animals and people, especially during fetal development. Vom Saal, Welshons and other scientists were particularly interested in BPA because they knew blood proteins involved in protecting against effects of natural estrogens would not protect against the chemical. Thus, this artificial hormone could travel directly through the blood into cells and damage them.

In 1997, the MU researchers published the first scientific article detailing the effects in animals of very low environmental exposure to BPA. Vom Saal and Welshons performed a prostate and sperm count study on male mice and demonstrated that BPA caused prostate hyperplasia — excessive growth of prostate tissue, a pre-condition of cancer. Since then, other studies, both theirs and those from other academic laboratories have shown that low-level exposure to BPA caused decreased sperm production in males, accelerated rate of growth, sex reversal in frogs, early onset of puberty, chromosome damage in female ovaries and a variety of behavioral changes.

With funding from the National Institutes of Health, Vom Saal and Welshons have shifted their research efforts toward an explanation of how and why BPA has such a powerful effect on an animal's endocrine system and reproductive organs. They have begun the process of identifying the molecular mechanisms at work when the hormone enters an animal's cells.

"There are safe alternatives," vom Saal said of products made with BPA. "There are plastic products that do not have Bisphenol A or other toxic chemicals. They can be made safely and used safely. There is no reason to keep using a chemical that has such a high potential to cause harm."

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Last Update: January 2, 2007

"Gender benders" cause sperm burn out

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12:56 03 July 2002
NewScientist.com news service
Claire Ainsworth, Vienna

The first direct evidence that "gender bender" chemicals affect the fertilising ability of sperm has been revealed - but it is unclear whether this would boost or harm fertility.

Researchers told the European Society for Human Reproduction and Embryology conference in Vienna that chemicals that mimic the effects of the female sex hormone oestrogen can prime sperm into becoming prematurely active, burning out before they have a chance to meet an egg.

"It's certainly very exciting work," says Chris Barratt at the University of Birmingham. "Sperm and the fertilisation process may be much more sensitive to artificial oestrogens than we thought."

Chemicals that mimic action of oestrogen abound in food and the environment. Many occur naturally in plants such as soy and hops, and are eliminated from the body within a few hours. Others take the form of synthetic chemicals such as pesticides and plasticisers, and build up in body tissues.

Research had already suggested a link between environmental oestrogens, testicular problems and low sperm counts, but this is the first time anyone has looked at their effect on sperm function, according to Lynn Fraser at King's College London, who led the new study.

Turn on

Oestrogen in semen and in the vagina is vital for fertilisation because it literally turns sperm on. The hormone stimulates a sperm to swim, and triggers physical changes that prime it for meeting with an egg - a process called capacitation.

Once a primed sperm docks with the egg, the cap on its head ruptures and releases a cocktail of enzymes that help it burrow inside and fertilise the egg. Normally, proteins in semen restrain this process, making sure it does not occur too soon.

If it does, the sperm will be unable to enter the egg. "Once they have undergone this reaction, they cannot fertilise, no matter how much they wiggle," explains Fraser.

Fraser and her team tested the effect of oestrogen and three oestrogen mimics - genistein, found in soya, 8-prenylnaringen, found in hops, and nonylphenol, found in paints, herbicides and pesticides.

They mixed the chemicals with mouse sperm and found all the compounds triggered capacitation and enzyme release. But the oestrogen mimics were far more powerful, triggering capacitation at concentrations a thousand times lower than oestrogen itself.

What's more, when the team tested the compounds on sperm that had already capacitated, they found that the oestrogen mimics triggered the premature release of the enzymes, whereas oestrogen did not.

Doubled fertilisation

However, when researchers mixed sperm with eggs and then treated them with the compounds, the number of eggs fertilised was doubled. This could be a benefit for IVF techniques, said Fraser.

"At first sight, these results might suggest that oestrogens, particularly those found in the environment, could help fertility. However, the responses we have seen could have negative effects over time," said Fraser.

Asked if oestrogen mimics could harm fertility in real life, Fraser said: "The potential answer to that question would be yes." Premature capacitation and enzyme release of sperm might not be a serious problem for normal fertile men, but it could be for men with lower sperm counts.

On the other hand, if the proteins that normally keep sperm under control still do their job, then the extra oestrogen-like activity could actually make sperm more fertile by increasing the numbers primed for fertilisation.

Barratt agrees that the jury is out until more research is done. But he adds that human sperm is known to be more sensitive than mouse sperm to progesterone, a hormone in the same class as oestrogen, meaning that oestrogen mimics could in theory have an even greater effect on humans.

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Chemicals in plastics harming unborn boys

Scientists say chemicals have gender bending effect

Ian Sample, science correspondent
Friday May 27, 2005

Guardian

Scientists in America have found the first evidence that common chemicals used in products as diverse as cosmetics, toys, clingfilm and plastic bags may harm the development of unborn baby boys.

Researchers have long known that high levels of substances called phthalates have gender-bending effects on male animals, making them more feminine and leading to poor sperm quality and infertility. The new study suggests that even normal levels of phthalates, which are ubiquitous, can disrupt the development of male babies' reproductive organs.

The discovery poses a huge problem for the chemical industry, which is already embroiled in a battle with the government over EU proposals on chemical safety.

Several types of phthalates, which are used to make plastics more pliable, and have been around for more than 50 years, have been banned, but many are still produced in vast quantities.

The study was carried out by scientists from centres across the US, including the University of Rochester and the National Centre for Environmental Health.

The researchers measured the levels of nine widely used phthalates in the urine of pregnant women and compared them with standard physiological measurements of their babies.

Tests showed that women with higher levels of four different phthalates were more likely to have baby boys with a range of conditions, from smaller penises and undescended testicles to a shorter perineum, the distance between the genitals and the anus. The differences, say the authors, indicate a feminisation of the boys similar to that seen in animals exposed to the chemicals.

Shanna Swan, an obstetrician at the University of Rochester, and lead scientist on the study, said researchers must now unravel what kinds of products are most to blame. One way that phthalates get into the bloodstream is when they seep into food from plastic packaging.

"It's going to take a while to work out which of these sources is most relevant to human exposure," she said.

Although the observed differences in body measurements were subtle, they indicate that what is generally regarded as the most ubiquitous class of chemicals is having a significant effect on newborns.

"Every aspect of male identity is altered when you see this in male animals," said Fred vom Saal, professor of reproductive biology at the University of Missouri-Columbia. Levels of aggression, parenting behaviour and even learning speeds were affected, he said.

Andreas Kortenkamp, an expert in environmental pollutants at the School of Pharmacy in London, said: "If it's true, it's sensational. This is the first time anyone's shown this effect in humans. It's an indicator that something's gone seriously wrong with development in the womb and that's why it's so serious."

He added: "These are mass chemicals. They are used in any plastic that is pliable, whether it's clingfilm, kidney dialysis tubes, blood bags or toys. Sorting this out is going to be an interesting challenge for industry as well as society."

The work, which is to appear in the journal Environmental Health Perspectives, is due to be presented at the Endocrine Disrupting Chemicals Forum in San Diego on June 3.

Gwynne Lyons, toxics adviser to the WWF, said: "At the moment regulation of the chemicals industry is woefully inadequate."

She added: "Right now the government is looking at how the regulation of hormone disrupting chemicals could be made more effective under new EU chemicals law, but the chemicals industry is lobbying very hard to water down this legislation.

"Political agreement on this legislation is not expected until later this year so it remains to be seen whether the UK government has the guts to stand up to industry lobbying. If they don't, wildlife and baby boys will be the losers."

Breast Cancer and the Environment: Science News from Silent Spring Institute
May 2005

Pollution Hits Home

In 1987, Dr. Ana Soto at Tufts University faced a perplexing problem. She was studying how exposure to estradiol – a natural estrogen – makes estrogen-sensitive human breast cancer cells grow. But, unexpectedly, the unexposed control cells in her lab began to proliferate! It took years to figure out that new plastic test tubes in her laboratory were to blame. The tubes were leaching nonylphenol, a synthetic chemical found in many common products, such as detergents, plastics and pesticides.

With this discovery, Dr. Soto began a new area of research that has led her laboratory and others to identify more than 150 chemicals that mimic estrogen, block androgen, or otherwise affect hormones. These chemicals are known as endocrine disrupting compounds (EDCs). They are found in building materials, furniture, and everyday products – detergents, pesticides, plastics, cosmetics – and in air and water pollution.

Given that natural estrogen and pharmaceutical estrogens, such as HRT, increase breast cancer risk, it makes sense to target estrogen mimics and other EDCs in breast cancer research. If we find links between these chemicals and breast cancer, we will be a big step closer to breast cancer prevention.

In order to study the links between chemicals and breast cancer, we need to first measure and understand how women are exposed. Because many of the EDCs are in consumer products and because all of us, and especially women, spend a lot of time at home, Silent Spring Institute decided to tackle EDCs in a study of exposures in homes.

We tested for 89 EDCs in air and dust in 120 homes on Cape Cod, where we have been studying possible environmental links to breast cancer for the last ten years. Results were published in the scientific journal *Environmental Science and Technology*, which called the study "the most comprehensive assessment to date" of pollutants in homes. For 30 of the chemicals we tested, ours are the first measurements ever reported from indoor environments.

- We found 67 target compounds in all, with an average of about 20 per home. That's a reminder that when we think about effects of chemicals on health, we have to take into account multiple exposures rather than the one-at-a-time approach that is currently used for chemicals regulation.
- The study showed that chemicals break down very slowly indoors. We found DDT, which was banned more than 30 years ago, in about two thirds of the homes. As toxicologist Ruthann Rudel says, "Think about what your furniture would look like if you left it out on the street for thirty years. Now think about it in your living room. Protected from sun, rain, and wind, materials stay pretty much in tact." The lesson here is that we need to be more careful about testing chemicals before we put them into use, because banning them later won't get them out of our homes.
- The most abundant pollutants were phthalates (from plastics and personal care products, such as nail polish and hair spray) and certain phenols from disinfectants, detergents, and adhesives, for example in furnishings.
- We found phthalates in every home. Researchers have found phthalates are associated with androgen-blocking effects in males, including lowered sperm count and certain hormonal birth defects. Their effects on girls and women have not been investigated much yet. Many breast cancer activists have joined the recent effort to remove phthalates from

cosmetics, as the Europeans are doing; and 116 cosmetics manufacturers have agreed. (Go to www.safecosmetics.org to learn more.)

- We found 27 different pesticides in all.
- We found the flame retardant PBDE's at ten times the levels reported in Europe, where these chemicals are not used as much.

_____Julia G. Brody, Ph.D., Silent Spring Institute

For more scientific detail, please visit the Silent Spring Institute web site resources on household exposure: http://library.silentspring.org/news/hesresults.asp.

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Consumer Factsheet on: DI (2-ETHYLHEXYL) PHTHALATE

List of Contaminants

As part of the Drinking Water and Health pages, this fact sheet is part of a larger publication:
National Primary Drinking Water Regulations

This is a factsheet about a chemical that may be found in some public or private drinking water supplies. It may cause health problems if found in amounts greater than the health standard set by the United States Environmental Protection Agency (EPA).

What is DEHP and how is it used?

Di (2-ethylhexyl) Phthalate, or DEHP, is the most commonly used of a group of related chemicals called phthalates or phthalic acid esters. The greatest use of DEHP is as a plasticizer for polyvinylchloride (PVC) and other polymers including rubber, cellulose and styrene. A number of packaging materials and tubings used in the production of foods and beverages are polyvinyl chloride contaminated with phthalic acid esters, primarily DEHP.

The list of trade names given below may help you find out whether you are using this chemical at home or work.

Trade Names and Synonyms:

DEHP
BEHP
Dioctyl phthalate
Pittsburgh PX-138
Platinol AH
RC Plasticizer DOP
Reomol D79P
Sicol 150
Staflex DOP
Truflex DOP
Vestinol AH
Vinicizer 80
Palatinol AH
Hercoflex 260
Kodaflex DOP
Mollan O
Nuoplaz DOP
Octoil
Eviplast 80

Fleximel
Flexol DOP
Good-rite GP264
Hatcol DOP
Ergoplast FDO
DAF 68
Bisoflex 81

Why is DEHP being Regulated?

In 1974, Congress passed the Safe Drinking Water Act. This law requires EPA to determine safe levels of chemicals in drinking water which do or may cause health problems. These non-enforceable levels, based solely on possible health risks and exposure, are called Maximum Contaminant Level Goals.

The MCLG for phthalate has been set at zero because EPA believes this level of protection would not cause any of the potential health problems described below.

Based on this MCLG, EPA has set an enforceable standard called a Maximum Contaminant Level (MCL). MCLs are set as close to the MCLGs as possible, considering the ability of public water systems to detect and remove contaminants using suitable treatment technologies.

The MCL has been set at 6 parts per billion (ppb) because EPA believes, given present technology and resources, this is the lowest level to which water systems can reasonably be required to remove this contaminant should it occur in drinking water.

These drinking water standards and the regulations for ensuring these standards are met, are called National Primary Drinking Water Regulations. All public water supplies must abide by these regulations.

What are the Health Effects?

Short-term: EPA has found phthalate to potentially cause the following health effects when people are exposed to it at levels above the MCL for relatively short periods of time: mild gastrointestinal disturbances, nausea, vertigo.

Long-term: Phthalate has the potential to cause the following effects from a lifetime exposure at levels above the MCL: damage to liver and testes; reproductive effects; cancer.

How much DEHP is produced and released to the environment?

Disposal of polyvinyl chloride and other DEHP-containing materials by incineration, landfill, etc., will result in the release of DEHP into the environment. DEHP has been detected in the effluent of numerous industrial plants.

From 1987 to 1993, according to EPA's Toxic Chemical Release Inventory, DEHP releases to land and water totalled over 500,000 lbs., of which about 95 percent was to land. These releases were primarily from rubber and plastic hose industries. The largest releases occurred in Wisconsin and Tennessee.

What happens to DEHP when it is released to the

environment?

DEHP will adhere to soil, and so will neither evaporate nor leach into groundwater. DEHP has a strong tendency to adsorb to soil and sediments. In water, it will be degraded by microbes in a matter of weeks. DEHP does have a tendency to accumulate in aquatic organisms.

How will DEHP be Detected in and Removed from My Drinking Water?

The regulation for phthalate became effective in 1994. Between 1993 and 1995, EPA required your water supplier to collect water samples every 3 months for one year and analyze them to find out if phthalate is present above 0.6 ppb. If it is present above this level, the system must continue to monitor this contaminant.

If contaminant levels are found to be consistently above the MCL, your water supplier must take steps to reduce the amount of phthalate so that it is consistently below that level. The following treatment methods have been approved by EPA for removing phthalate: Granular activated charcoal.

How will I know if DEHP is in my drinking water?

If the levels of phthalate exceed the MCL, 6 ppb, the system must notify the public via newspapers, radio, TV and other means. Additional actions, such as providing alternative drinking water supplies, may be required to prevent serious risks to public health.

Drinking Water Standards:

Mclg: zero

Mcl: 6 ppb

DEHP Releases to Water and Land, 1987 to 1993 (in pounds):

	Water	Land
TOTALS* (in pounds)	**16,910**	**471,191**
Top Five States*		
WI	500	255,000
TN	3,491	80,419
OH	268	62,982
NJ	3,956	23,139
NY	500	13,284
Major Industries		
Misc rubber products	274	311,900

Rubber, plastic hose	10	80,019
Cyclic crudes, intermed.	3,099	12,200

* Water/Land totals only include facilities with releases greater than 100 lbs.

Learn more about your drinking water!

EPA strongly encourages people to learn more about their drinking water, and to support local efforts to protect and upgrade the supply of safe drinking water. Your water bill or telephone book's government listings are a good starting point.

Your local water supplier can give you a list of the chemicals they test for in your water, as well as how your water is treated.

Your state Department of Health/Environment is also a valuable source of information.

For help in locating these agencies or for information on drinking water in general, call: EPA's Safe Drinking Water Hotline: (800) 426-4791.

For additional information on the uses and releases of chemicals in your state, contact the: Community Right-to-Know Hotline: (800) 424-9346

List of Contaminants

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Last updated on Tuesday, February 28th, 2006
URL: http://www.epa.gov/safewater/dwh/c-soc/phthalat.html

'Gender-bending' chemicals found to 'feminise' boys

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17:17 27 May 2005
NewScientist.com news service
Andy Coghlan

"Gender-bending" chemicals mimicking the female hormone oestrogen can disrupt the development of baby boys, suggests the first evidence linking certain chemicals in everyday plastics to effects in humans.

The chemicals implicated are phthalates, which make plastics more pliable in many cosmetics, toys, baby-feeding bottles and paints and can leak into water and food.

All previous studies suggesting these chemicals blunt the influence of the male hormone testosterone on healthy development of males have been in animals. "This research highlights the need for tougher controls of gender-bending chemicals," says Gwynne Lyons, toxics adviser to the WWF, UK. Otherwise, "wildlife and baby boys will be the losers".

The incriminating findings came from a study of 85 baby boys born to women exposed to everyday levels of phthalates during pregnancy. It was carried out by Shanna Swan at the University of Rochester School of Medicine and Dentistry, New York, US, and colleagues.

As an index of feminisation, she measured the "anogenital distance" (AGD) between the anus and to the base of the penis. She also measured the volume of each boy's penis. Earlier studies have shown that the AGD is twice in boys what it is in girls, mainly because in boys the hormone testosterone extends the length of the perineum separating the anus from the testicles.

Undescended testicles

In animals, AGD is reduced by phthalates - which mimic oestrogen - which keep testosterone from doing its normal job. At higher doses, animals develop more serious abnormalities such as undescended testicles and misplaced openings to the urethra on the penis - a group of symptoms called "phthalate syndrome" in animals.

When Swan's team measured concentrations of nine phthalate metabolites in the urine of pregnant women, they found that four were linked with shorter AGD in sons born to women showing high exposure levels.

Although none of the boys developed abnormal genitals, the quarter of mothers who were exposed to the highest concentrations of phthalates were much more likely to have had boys with short AGDs compared with the quarter of mothers who had the lowest exposures to the chemicals.

And although all the boys had genitals classified as "normal", 21% of the boys with short AGDs had incomplete testicular descent, compared with 8% of other boys. And on average, the smaller the AGD, the smaller the penis.

Changing masculinisation

Swan believes that at higher exposures, boys may suffer from testicular dysgenesis syndrome - the human collection of more serious abnormalities which corresponds to "phthalate syndrome".

"We're not exactly seeing testicular dysgenesis syndrome, but a cluster of endpoints consistent with it," said Swan on at an international conference on Endocrine Disrupting Chemicals in San Diego, US.

"If you see this, you're very likely to see every other aspect of masculinisation changed too," says Fred vom Saal, professor of reproductive biology at the University of Missouri-Columbia, US.

Vom Saal says this could include behavioural changes like those seen in animals, including an aversion to "rough-and-tumble" play and a reduction in aggressiveness.

Criticising methods

Environmentalists say the results strengthen the case for a ban or restriction on some phthalates in baby toys, as has been proposed in Europe and California.

But phthalate manufacturers maintain that the chemicals have been thoroughly tested and are safe. They are also critical of aspects of the study. David Cadogan, director of the European Council for Plasticisers and Intermediates, points out that just one urine sample was taken from each pregnant woman, which cannot rule out drastic variations in exposure over time.

Also, he says that all AGD measurements should have been taken in babies exactly the same age, not in babies ranging from three to 24 months in age as in the study. The disparity in ages meant that complicated mathematical analyses had to be applied which may have made it more difficult to distinguish genuine differences in AGD from differences accounted for by age or weight.

Swan's results will appear in the journal *Environmental Health Perspectives*.

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Europe launches chemical safety crackdown
http://www.newscientist.com/article.ns?id=dn4327

29 October 2003
"Gender benders" cause sperm burn out
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03 July 2002
Boys won't be boys
http://www.newscientist.com/article.ns?id=mg17423495.300

29 June 2002

Weblinks

Shanna Swan, University of Rochester
http://www.fcm.missouri.edu/faculty/swan.html

Endocrine Society Forum on Endocrine Disrupting Chemicals
http://www.endo-society.org/educationevents/annual/2005/disrupting-chemicals.cfm

European Council for Plasticisers and Intermediates
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WWF minisite on endocrine-disrupting chemicals
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Printed on Tue Aug 29 14:38:46 BST 2006

Medscape from WebMD www.medscape.com

Nurses: Taking Precautionary Action on a Pediatric Environmental Exposure: DEHP

Anna Gilmore Hall

Pediatr Nurs. 2006;32(1):91-93. ©2006 Jannetti Publications, Inc.
Posted 04/11/2006

Abstract and Introduction

Abstract

Di(2-ethylhexyl) phthalate, or DEHP, is a chemical used to soften rigid polyvinyl chloride (PVC) plastic. Medical devices made of flexible PVC, such as intravenous (IV) bags and tubing, contain DEHP to make them pliable and soft. Animal studies show that exposure to DEHP can damage the liver, kidneys, lungs, and reproductive system, particularly the developing testes of prenatal and neonatal males. Because of this, the U.S. Food and Drug Administration (FDA) has issued a Public Health Notification on PVC devices containing DEHP, urging health care providers to use DEHP-free devices for certain vulnerable patients. Many hospitals and health systems have successfully transitioned away from use of DEHP, particularly in neonatal intensive care units (NICUs).

Introduction

June 9, 2005, The Los Angeles Times - "A new Harvard study of infants treated in neonatal intensive care units finds that a substance used in medical supplies shows up in babies' bodies. What's more, the substance shows up in those babies in roughly direct proportion to the amount to which the babies are exposed. The substance, dubbed "DEHP," is a chemical used in PVC plastic products that has been shown to cause testicular damage in animal studies."

As a pediatric nurse, are you aware of the health risks associated with DEHP-containing medical devices, and do you know what the alternatives are?

Prenatal and neonatal children are two of the most vulnerable populations to receive health care. Every day, nurses do everything in our power to ensure that our most vulnerable patients are protected from harm. When chemicals and materials used in the delivery of health care are linked to adverse health effects, protecting these ultra-vulnerable populations is of particular concern. Learn about just such an instance, and the safer alternatives that are available, below.

What It Is

Polyvinyl chloride (PVC) is one of the most widely used plastics in medical care, used in everything from blood bags to exam gloves. Because this type of plastic is naturally hard and brittle, chemicals are added to make it soft and flexible. Medical products made of PVC are usually softened with a plasticizer from the family of chemicals known as phthalates (pronounced THAH-lates).

Di(2-ethylhexyl) phthalate, or DEHP, is the specific phthalate commonly used in PVC to provide flexibility and

resistance to temperature fluctuations. DEHP can be found in a wide variety of medical products, such as bags containing blood, plasma, intravenous fluids, and total parenteral nutrition, tubing associated with their administration, nasogastric tubes, enteral feeding tubes, umbilical catheters, extracorporeal membrane oxygenation (ECMO) circuit tubing, hemodialysis tubing, respiratory masks, endotracheal tubes, and examination gloves (Green et al., 2005).

Outside the health care setting, people are exposed to DEHP and other phthalates from a variety of sources, including PVC toys, vinyl shower curtains, car seats, wallpaper, floor coverings, and many other consumer products.

The Problem With DEHP

In addition to the characteristics that make it useful for medical devices, DEHP is also highly lipophilic (fat soluble). When used in PVC plastic, DEHP is loosely chemically bonded to the plastic and readily leaches into blood or other lipid-containing solutions in contact with the plastic. The rate of DEHP leaching depends on many factors. The type of solution in contact with the plastic, temperatures during storage and at the time of use, storage time, and percent DEHP in the plastic product all play a part (Marcel, 1973).

This leaching of DEHP into humans via the solution with which it is in contact increases the risk of certain adverse health outcomes. Animal studies show that exposure to DEHP can damage the liver, kidneys, lungs, and reproductive system, particularly the developing testes of prenatal and neonatal males. The FDA and National Toxicology Program's Center for Evaluation of Risks to Human Reproduction conclude that these animal studies are relevant to people.

As stated in the 2002 *Aggregate Exposures to Phthalates in Humans* report, "[d]eveloping organisms are uniquely vulnerable to phthalate exposures, and in particular, the developing male reproductive tract appears to be the most sensitive organ system. Abnormal development of the testes, penis, and other components of the male reproductive tract occurs at levels of exposure that are hundreds or thousands of times lower than those necessary to cause damage in adults (DiGangi, Schettler, Cobbing, & Rossi, 2002).

More recently, researchers at Harvard School of Public Health have found that babies in neonatal intensive care units have high exposure levels to this reproductive toxicant. For the peer-reviewed study, researchers measured the level of a DEHP metabolite in the urine of neonates treated in the NICUs, and were able to correlate the level of exposure to DEHP-containing products to the level of DEHP metabolite found in the babies' urine. Infants who received intensive treatments with PVC medical devices were exposed, on average, to levels of DEHP that were 25 times higher than levels measured in the general population by the U.S. Centers for Disease Control. As their medical treatments intensified, the sick infants were exposed to progressively higher exposures of DEHP (Green et al., 2005).

Government Actions

Several government agencies have concluded that some patients are likely to be exposed to potentially unsafe amounts of DEHP while receiving medical care. The U.S. Food and Drug Administration (FDA) has issued an FDA Safety Assessment and a Public Health Notification on DEHP-containing PVC devices, urging health care providers to use alternatives for certain vulnerable patients (U.S. Food and Drug Administration, 2001).

According to the FDA Public Health Notification: "Two factors determine the degree of risk posed by exposure to DEHP in a medical setting. The first is the patient's sensitivity to DEHP. Based on the evidence cited above, the male fetus, male neonate, and peripubertal male would appear to be high-risk groups. The second factor is the dose of DEHP received by the patient. This is determined largely by the type of procedure performed, as well as the frequency and duration of these procedures."

FDA identified the following procedures as posing the highest risk of exposure to DEHP:

- Exchange transfusion in neonates
- ECMO in neonates
- Total Parenteral Nutrition (TPN) in neonates (with lipids in PVC bag)
- Multiple procedures in sick neonates (high cumulative exposure)
- Hemodialysis in peripubertal males
- Hemodialysis in pregnant or lactating women

- Enteral nutrition in neonates and adults
- Heart transplantation or coronary artery bypass graft surgery (aggregate dose)
- Massive infusion of blood into trauma patient
- Transfusion in adults undergoing ECMO

The initial FDA safety assessment followed closely on the heels of an October 2000 report by the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction. The expert panel report expressed "serious concern" that exposure to DEHP may adversely affect male reproductive tract development in critically ill infants and "concern" over the levels of DEHP exposure to pregnant women, breast-feeding mothers and healthy infants and toddlers (U.S. Department of Health and Human Services, 2000). In Canada, an expert advisory panel to Health Canada, the Canadian equivalent to the U.S. FDA, has recommended that health care providers not use DEHP-containing devices in the treatment of pregnant women, breastfeeding mothers, infants, males before puberty, and patients undergoing cardiac bypass hemodialysis or heart transplant surgery (Health Canada, 2003).

Alternatives: What's Already Been Done

Cost effective alternatives to DEHP-containing medical devices are available for most uses in health care. With the weight of the evidence suggesting that exposure to this reproductive toxicant should be eliminated where possible, many hospitals and health systems have successfully transitioned away from use of DEHP, particularly in neonatal intensive care units (NICUs).

June 9, 2005, San Francisco Chronicle, Toxic agent found in treated newborns is linked to plastic: "Kaiser Permanente, Alta Bates Summit Medical Center, Catholic Healthcare West and the John Muir Medical Center in Walnut Creek, among others, already are buying mostly DEHP-free plastic devices, and pressuring manufacturers and suppliers to produce safe alternatives, according to representatives."

John Muir Medical Center in Walnut Creek, CA, first considered eliminating DEHP from its NICU when a resident pediatrician brought the issue to the attention of the intensive care nursery (ICN) staff. The information he provided, as well as the 2002 FDA public health notification, prompted the ICN staff to look closely at ways to reduce the potential risks of DEHP. They evaluated the NICU's product list and identified which products contained DEHP and which were DEHP-free. Once this step was accomplished, the hospital's clinical nurse specialist led the DEHP reduction effort, working together with the medical and nursing staff to develop a short- and long-term DEHP reduction plan. John Muir was able to announce a virtually DEHP-free NICU within 6 months of beginning this effort (Health Care Without Harm [HCWH], 2003a).

A similar DEHP elimination story on a much larger scale comes from Kaiser Permanente, the nation's largest nonprofit health plan that operates 29 medical centers in 9 states and the District of Columbia. Beginning in July 2001, after learning of the potential hazards to neonatal patients from DEHP exposure, Kaiser Permanente staff underwent a process to identify DEHP-containing medical devices used in NICUs and to evaluate alternatives. Staff used a risk-management process to target products and began a series of clinical trials to test alternatives. Based on their results, the health system chose to switch to non-DEHP products for three commonly used NICU devices: umbilical vessel catheters, PICC lines, and enteral feeding products (HCWH, 2003b).

At Miller Children's Hospital in Long Beach, CA, a patient safety approach framed their DEHP elimination efforts. Because numerous scientific studies show that TPN bags and tubing pose the highest risk of DEHP exposure to neonates, this was their first priority product to eliminate, followed by IV sets. With help from the pediatric department's medical staff, clinical products committee, central supply products manager, and the hospital's risk management attorney, Miller Children's Hospital attained its target of 100% DEHP elimination in IV and TPN products in December 2002 (HCWH, 2003c).

What You Can Do

One of the first steps to eliminating PVC from health care facilities is to identify which products contain it. Health Care Without Harm has developed a PVC/DEHP Audit Tool for help with this; see www.noharm.org/pvcDehp/reducingPVC.

Once DEHP-containing products have been identified, an evaluation of safer alternatives must be performed. Often it

is beneficial to organize a committee of stakeholders to perform this evaluation and determine a plan for replacing DEHP-containing products with their safer DEHP-free counterparts.

The Environmental Health Hot Topics column focuses on issues, information, and practical guidelines related to environmental health problems, including sources of toxicants and resources for nurses to prevent, minimize, or treat adverse environmental exposures particularly as they relate to children. To suggest topics, obtain author guidelines, or to submit queries or manuscripts, contact Ann Pike-Paris, MS, RN, Section Editor; Pediatric Nursing, East Holly Avenue Box 56; Pitman, NJ 08071-0056; (856) 256-2300 or FAX (856) 256-2345.

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Sidebar: Resources

For More Information

Health Care Without Harm
PVC & DEHP: The Issue
www.noharm.org/PVCdehp/issue
Aggregate Exposures to Phthalates in Humans Report
www.noharm.org/library/docs/Phthalate_Report.pdf

Government Reports and Advisories

U.S. Food and Drug Administration
FDA Public Health Notification: PVC Devices Containing the Plasticizer DEHP
www.fda.gov/cdrh/safety/dehp.html

U.S. Food and Drug Administration
Safety Assessment of DEHP Released from PVC Medical Devices
www.noharm.org/details.cfm?ID=740&type=document

Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, U.S. Department of Health and Human Services
NTP-CERHR Expert Panel Report on Di(2-ethylhexyl) Phthalate
www.noharm.org/details.cfm?type=document&id=744

Health Canada
Draft Position Statement on DEHP in Medical Devices
www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/md-im/dehp_position_draft_ebauche_e.pdf

Tools, Alternatives, and Case Studies

Alternatives to PVC and DEHP Medical Devices
www.noharm.org/pvcDehp/pvcFree

PVC/DEHP Audit Tool
www.noharm.org/details.cfm?type=document&id=741

Replacing DEHP: Hospital Case Studies
www.noharm.org/pvcDehp/reducingPVC#case

A series of fact sheets on reducing exposure to DEHP
www.noharm.org/pvcDehp/reducingPVC#tools

Sidebar: Latest Science Indicates

- Intensive use of DEHP-containing medical devices in NICU infants results in higher exposure to DEHP as reflected by elevated urinary levels of MEHP, the chemical's metabolite. (Harvard study)
- Patients receiving certain intensive medical treatments may receive unsafe amounts of DEHP. (FDA Safety Assessment)

Sources: Green et al. (2005); U.S. Food and Drug Administration (2001).

Sidebar: Hot Box - DEHP

Chemical name: di(2-ethylhexyl) phthalate

Chemical family: phthalates

Use in health care: as a plasticizer in flexible PVC plastics

Exposure risks: damage to liver, kidneys, lungs, and reproductive system

Most vulnerable populations: pregnant women and children, particularly prenatal and neonatal males

Anna Gilmore Hall, RN, is Executive Director, Health Care Without Harm, Arlington, VA. HCWH is an international coalition of 443 organizations in 52 countries working together to transform the health care industry so it is ecologically sustainable. Ms. Hall received her CAE certification in association management from the American Society of Association Executives in 2003.

Groundwater Replenishment System

Studies Conclude Water Produced by Groundwater Replenishment System Will Be Safe, Improve Basin's Quality

Water quality has always been a top priority of the Orange County Water District (OCWD). This commitment – along with the need to ensure enough safe, high-quality water for the future – came to the forefront nearly a decade ago when the sponsoring agencies proposed the Groundwater Replenishment (GWR) System.

The GWR System will provide a new supply of reliable, high-quality water for north and central Orange County by purifying highly treated sewer water through state-of-the-art microfiltration, reverse osmosis and ultraviolet light and hydrogen peroxide treatment. The result will be water of near-distilled quality from the GWR System in 2007.

To underscore its commitment to safety and quality, OCWD commissioned water quality studies on the project in 2000. The studies were intended to provide additional information on the proposed treatment processes along with information on how the GWR System water will be used to replenish the groundwater basin underlying north and central Orange County.

The water quality study tested the proposed treatment processes on the same source water as that for the full-scale GWR System. Real operating data (not a textbook study) was used as the basis for the water quality evaluation.

Published Findings Confirm Safety

In findings released in 2001, the studies concluded that the water produced by this system would be safe for consumers and actually improve the groundwater basin's overall quality. The findings were published in a report called the "Groundwater Replenishment System Water Quality Evaluation – Risk Assessment" (EOA, Inc., November 2000).

The Groundwater Replenishment System "will produce very safe, high-quality water through a process similar to that used by bottled water companies."

Sandra Smoley, R.N.
Former Agency Secretary, California Health and Welfare Agency

OCWD and Orange County Sanitation District (OCSD) also appointed an independent advisory committee to provide an additional level of expertise and review of the studies. The advisory committee concurred with the report's findings. The advisory committee was composed of recognized experts in the fields of public health, microbiology, environmental engineering, toxicology and risk assessment, including professors from University of California, Davis; University of California, Berkeley; and the University of North Carolina.

Even before the water quality studies were completed, the GWR System underwent rigorous scrutiny by interested citizens, water experts and local, state and federal officials. The first extensive study, an environmental impact report/statement prepared in 1998-99, found the project will have no significant adverse environmental impacts and noted that "...the quality of the recycled water is expected to be better than that of alternative water supplies" available to Orange County.

Following the environmental review, OCWD decided to further confirm these findings and took the additional step of conducting the water quality studies.

Conducting the Studies

The purpose of the studies was to compare different water sources representing two alternatives. Under one option ("No Action"), the groundwater basin would continue to receive water from the Santa Ana River and the county's two imported supplies, the Colorado River and Northern California, just as it does today. Under the other alternative ("Proposed Action"), water produced by the GWR System would be added to the existing blend of Santa Ana River and imported water from the two imported supplies.

The experts who conducted the studies employed methods consistent with the U.S. Environmental Protection Agency's guidance for risk assessment. They used estimates of the relative risks to human health associated with each alternate water source. They analyzed samples from the three sources (Santa Ana River, imported water from Northern California and Colorado River) and identified constituents of potential concern in each.

"The Groundwater Replenishment System will greatly reduce Orange County's reliance on imported water by rejuvenating valuable water lost to the ocean as well as provide a creative, new, safe and reliable water supply to meet our growing demand for high-quality water."

Hoag Memorial Hospital Presbyterian

Conclusions Concerning Public Health

In keeping with standard practices in such analyses, the studies divided the possible health risks associated with the three water supplies into three categories: non-carcinogenic, carcinogenic and microbiological contaminants. In brief, the conclusions drawn about the three categories follow:

Risks associated with non-carcinogenic health effects

Water from any of the three sources should not cause significant non-carcinogenic risk to public health. Indeed, the potential risk posed by GWR System water is lower than the other two sources.

Risks associated with carcinogenic health effects

The carcinogenic risks associated with direct consumption of water from the GWR System should be lower than that associated with either Santa Ana River or imported (purchased) supplies from the Colorado River and Northern California.

Arsenic is the constituent that accounts for the majority of the risk in both alternatives ("No Action" and "Proposed Action.") The levels of arsenic in all three water sources, however, are below the existing regulatory minimum levels for public safety.

N-nitrosodimethylamine (NDMA) and 1,4 dioxane – which are used primarily as commercial chemicals – present more carcinogenic risk than any other constituent identified in GWR System water. At the time this study was performed, the California Department of Health Services had not established regulations regarding maximum levels of NDMA or 1,4 dioxane in drinking water. It should be noted, however, that the membrane technologies – microfiltration and thin-film composite reverse osmosis and ultraviolet light and hydrogen peroxide – will remove emerging compounds such as NDMA and 1,4 dioxane. All of these technologies will be used on 100 percent of the water purified by the GWR System.

Risks associated with microbiological contaminant health effects

GWR System water is "...projected to pose much less risk than Santa Ana River or imported water supplies from bacteria, parasites and viruses, provided that all processes in the system treatment facility are operating fully and properly," the report said. It is important to note that for purposes of the studies, the experts assumed that each supply was consumed directly, before being used to recharge the groundwater basin. In fact, GWR System product water will be percolated into the groundwater basin where it will remain for at least one year. This will allow the GWR System water to undergo a natural filtering process while blending with water from the Santa Ana River, Northern California and the Colorado River.

Recommendations Concerning Operations

The conclusions about public health risks assume that the full-scale GWR System produces water of a quality similar to that evaluated in the studies. To ensure such production, the study concludes that the system should incorporate a detailed monitoring program to ensure ongoing, reliable operations in both treatment and pipeline conveyance. The program should include a plan to dispose of water that does not meet standards, the study said (the GWR System will include constant monitoring programs).

"The project will produce the safest and highest quality water available from any source – imported or local."

Taiwanese Medical and Dental Association of Orange County

Conclusion of Independent Advisory Committee

The independent advisory committee reviewed the report and summarized its findings. The committee agreed with the report's findings and concluded that "...the health risk associated with the quality of recharge water expected under the 'Proposed Action' (GWR System) will be less than or equal to that associated..." with the existing water supplies.

Preparation of Risk Assessment

EOA Inc., an environmental and public health engineering firm based in Oakland, Calif., conducted the risk assessment studies. In addition, OCWD organized the independent advisory committee. The committee members were:

- Robert C. Cooper, Ph.D., professor at University of California, Berkeley (microbiology, virology, public health)
- George Tchobanoglous, Ph.D., P.E., professor at University of California, Davis (environmental engineering)
- Eddie Wei, Ph.D., professor at University of California, Berkeley (toxicology)
- Douglas Crawford-Brown, Ph.D., professor at University of North Carolina (environmental science)
- Margie Nellor, M.S., Los Angeles County Sanitation District (health effects)

OCWD also assembled a group of six ex-officio advisors to ensure that local stakeholders and staff from the appropriate health and regulatory agencies understood and accepted the assessment. The advisors represented the California Department of Health Services, the Santa Ana Regional Water Quality Control Board, the City of Anaheim and also included a congressional fellow.

To see a copy of the Executive Summary of the report, please contact the Orange County Water District public affairs department at 714-378-3206. Copies of the full report are in the OCWD Technical Library.

The Groundwater Replenishment System "...will provide a long-term public health benefit to Orange County residents."

John Balbus, M.D., M.P.H.
Director, Environmental Health Program
Environmental Defense

How the System Works

The Groundwater Replenishment System, a joint project of the Orange County Water District and the Orange County Sanitation District, will use state-of-the-art membrane technology and ultraviolet light to produce water of near-distilled quality that exceeds state and federal drinking water standards. The water will then be used as another source to replenish Orange County's groundwater basin, along with water from the Colorado River, Northern California and the Santa Ana River.

The process will begin with highly treated sewer water from OCSD's Fountain Valley facility. This water will undergo several additional treatment steps, also referred to as an "integrated treatment process," that includes microfiltration, thin-film composite reverse osmosis and ultraviolet light and hydrogen peroxide treatment.

After undergoing this additional treatment, the water will be used to replenish the groundwater basin underlying north and central Orange County. The purified water will be pumped to spreading basins and travel the same natural filtering path that rainwater takes as it moves underground. It also will be used to expand the Seawater Intrusion Barrier that keeps the Pacific Ocean out of the groundwater basin.

Once in the basin, the purified water will blend with other groundwater from the Santa Ana River and imported sources.

What Other Public Health, Medical Professionals and Scientists Have Said About the Groundwater Replenishment System

"I am confident that on balance, the System will make a substantial contribution to the prevention of disease transmission and maintenance of overall public health within the county."

Sanford Brown, M.P.H., Ph.D.
Professor Emeritus of Health Science, California State University, Fresno

The Groundwater Replenishment System "will be approved and monitored by the California Department of Health Services, the Regional Water Quality Control Board and Orange County Health Agency…this should provide adequate oversight and public health protection."

Christine L. Moe, Ph.D.
Associate Professor, Rollins School of Public Health, Emory University

"I am confident that the advanced treating technologies that will be applied to previously treated wastewater – microfiltration, reverse osmosis and ultraviolet light and hydrogen peroxide – will produce output flows of clean, high-purity water."

H. John Blossom, M.D.
Director, California Area Health Education Center, University of California, San Francisco-Fresno

"The Groundwater Replenishment System will provide ample protection from waterborne disease for Orange County residents."

Ralph Morris, M.D., M.P.H.
Public Health Physician

"Hospitals in Orange County are significant users of water..." The Groundwater Replenishment System "will help assure the availability of this valuable resource in the future."

Hospital Association of Southern California

"Having completed my assessment, I wish to commend the Orange County Water District and Orange County Sanitation District for moving forward with this project."

Kellogg J. Schwab, Ph.D.
The Center for Water and Health, Johns Hopkins Bloomberg School of Public Health

http://www.sciencedaily.com/releases/2007/05/070507071807.htm

Premature Births Peak Seasonally When Pesticides And Nitrates In Surface Water Are Highest

Science Daily — The growing premature birth rate in the United States appears to be strongly associated with increased use of pesticides and nitrates, according to work conducted by Paul Winchester, M.D., professor of clinical pediatrics at the Indiana University School of Medicine. He reports his findings May 7 at the Pediatric Academic Societies' annual meeting, a combined gathering of the American Pediatric Society, the Society for Pediatric Research, the Ambulatory Pediatric Association and the American Academy of Pediatrics.

Dr. Winchester and colleagues found that preterm birth rates peaked when pesticides and nitrates measurements in surface water were highest (April-July) and were lowest when nitrates and pesticides were lowest (Aug.-Sept.).

More than 27 million U.S. live births were studied from 1996-2002. Preterm birth varied from a high of 12.03% in June to a low of 10.44% in September. The highest rate of prematurity occurred in May-June (11.91%) and the lowest for Aug-Sept (10.79%) regardless of maternal age, race, education, marital status, alcohol or cigarette use, or whether the mother was an urban, suburban or rural resident. Pesticide and nitrate levels in surface water were also highest in May-June and lowest in August -- September, according to the U.S. Geological Survey.

For the past four years, Dr. Winchester and colleagues have focused attention on the outcomes of pregnancy in Indiana and the United States in relation to environmental pesticides and nitrates in surface and drinking water. Last year at the Pediatric Academic Societies' annual meeting, Dr Winchester reported that birth defects peak in Indiana and in the United States as a whole during April through July, the same months as pesticides and nitrates reach their maximum concentrations in surface water. This year's presentation expands upon that work.

"A growing body of evidence suggests that the consequence of prenatal exposure to pesticides and nitrates as well as to other environmental contaminants is detrimental to many outcomes of pregnancy. As a neonatologist, I am seeing a growing number of birth defects, and preterm births, and I think we need to face up to environmental causes," said Dr Winchester, who is also director of Newborn Intensive Care Services at St. Francis Hospital in Indianapolis.

"Preterm births in the United States vary month to month in a recurrent and seasonal manner. Pesticides and nitrates similarly vary seasonally in surface water throughout the U.S. Nitrates and pesticides can disrupt endocrine hormones and nitric oxide pathways in the developing fetus," he said.

"I believe this work may lay the foundation for some of the most important basic and clinical research, and public health initiatives of our time. To recognize that what we put into our environment has potential pandemic effects on pregnancy outcome and possibly on child development is a momentous observation, which hopefully will help transform the way humanity cares for its world," said James Lemons, M.D., Hugh McK. Landon Professor of Pediatrics at the IU School of Medicine. Dr. Lemons is director of the section of neonatal-perinatal medicine at the IU School of Medicine and heads the Riley Hospital for Children of Clarian Health's section of neonatal-perinatal medicine.

Collaborating with Dr. Winchester on this study were Akosua Boadiwaa Adu-Boahene and Sarah L. Kosten of the IU School of Medicine, Alex K Williamson of the U.S. Geological Survey, and Ying Jun,

Ph.D. of the University of Cincinnati. The work was funded by the Division of Neonatology, Department of Pediatrics of the IU School of Medicine.

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AMERICAN ACADEMY OF PEDIATRICS

TECHNICAL REPORT

Katherine M. Shea, MD, MPH, and the Committee on Environmental Health

Pediatric Exposure and Potential Toxicity of Phthalate Plasticizers

ABSTRACT. Phthalates are plasticizers that are added to polyvinyl chloride (PVC) products to impart flexibility and durability. They are produced in high volume and generate extensive though poorly defined human exposures and unique childhood exposures. Phthalates are animal carcinogens and can cause fetal death, malformations, and reproductive toxicity in laboratory animals. Toxicity profiles and potency vary by specific phthalate. The extent of these toxicities and their applicability to humans remains incompletely characterized and controversial. Two phthalates, diethylhexyl phthalate (DEHP) and diisononyl phthalate (DINP), have received considerable attention recently because of specific concerns about pediatric exposures. Like all phthalates, DEHP and DINP are ubiquitous contaminants in food, indoor air, soils, and sediments. DEHP is used in toys and medical devices. DINP is a major plasticizer used in children's toys.

Scientific panels, advocacy groups, and industry groups have analyzed the literature on DEHP and DINP and have come to different conclusions about their safety. The controversy exists because risk to humans must be extrapolated from animal data that demonstrate differences in toxicity by species, route of exposure, and age at exposure and because of persistent uncertainties in human exposure data. This report addresses sensitive endpoints of reproductive and developmental toxicity and the unique aspects of pediatric exposures to phthalates that generate concern. DEHP and DINP are used as specific examples to illustrate the controversy.

ABBREVIATIONS. PVC, polyvinyl chloride; DEHP, diethylhexyl phthalate; DINP, diisononyl phthalate; MEHP, monoethylhexyl phthalate; ECMO, extracorporeal membrane oxygenation; NOAEL, no observable adverse effect level; LOAEL, lowest observable adverse effect level; CERHR, Center for the Evaluation of Reproductive Risks to Humans; DBP, dibutyl phthalate.

BACKGROUND INFORMATION

Sources, Uses

Phthalates are plasticizers that impart flexibility and durability to polyvinyl chloride (PVC) products, including building materials, food packaging, clothing, toys, children's products, blood bags, intravenous fluid bags and infusion sets, and other medical devices.[1] They are also used in solvents, lubricating oils, fixatives, and detergents and in products such as cosmetics and wood finishes.[2] Phthalates are not covalently bound to the plastic matrix and leach out of PVC when they come in contact with lipophilic substances. In addition, they are released directly into the environment during production and use and after disposal of PVC and other phthalate-containing products. Phthalates bioaccumulate in invertebrates, fish, and plants but do not biomagnify, because higher animals efficiently metabolize and excrete phthalates. They are ubiquitous contaminants in food, indoor air, soils, and sediments.[3]

Human Exposure

Levels of human exposure are estimated on the basis of annual production volumes and usage patterns of phthalate-containing products as well as environmental monitoring data, dietary surveys, and mathematical modeling of human activity patterns. These exposure estimates are imprecise and subject to error. Environmental monitoring data are best for diethylhexyl phthalate (DEHP), which is produced in volumes approaching 2 million tons per year.[2] In the general population, the major source of human exposure is food contaminated during growth, production, processing, or packaging. Food surveys have documented the highest levels in fatty foods, such as dairy (including infant formulas), fish, meat, and oils. These surveys vary significantly among nations and over time because of differences in food production and consumption patterns, but the most recent analyses of infant formulas show significant decreases in contamination with DEHP and all other phthalates tested.[2,4,5] The second highest source of exposure is indoor air, where DEHP adheres strongly to aerosol particles. Because of its low water solubility and low vapor pressure, little DEHP is found in outdoor air or water. It is estimated that exposure to DEHP in the general population (excluding occupational exposure, medical exposures, and nondietary ingestions in children) is in the range of 3 to 30 μg/kg of body weight per day.[1,6,7] Exposures to other phthalates, including diisononyl phthalate (DINP), are usually assumed to be lower primarily because production volumes are lower.

Pediatric Exposure

Phthalates have been shown in animal studies to cross the placenta and pass into breast milk,[8–10] so

The guidance in this report does not indicate an exclusive course of treatment or serve as a standard of medical care. Variations, taking into account individual circumstances, may be appropriate.

PEDIATRICS (ISSN 0031 4005).

prenatal exposure and exposure from breastfeeding may occur in humans. Infants and young children consume more calories per kilogram of body weight, consume relatively more dairy and other fatty foods, and have higher minute ventilation than do adults, so dietary exposures and exposure from indoor air would be expected to be higher in infants and young children.[11] It is estimated that the total intake of DEHP, excluding nondietary ingestion, is higher in all children younger than 19 years than in adults.[7] Highest estimated intakes are in children 0.5 to 4 years old (Table 1).

Nondietary ingestion of phthalates can occur when children mouth, suck, or chew on phthalate-containing toys or other objects.[12–14] This source of exposure is difficult to quantify directly. Estimates are made by combining data on the amount of time children mouth nonfood items[15,16] and leaching rates of DEHP and DINP from phthalate-containing objects in mouthing studies performed in adults. The phthalate content of a product does not correlate with leaching rates in mouthing studies, so simple extrapolation of exposure from phthalate content is not possible.[17] Nondietary ingestion can be expected to increase total exposure by an order of magnitude or more.[15,17–19] In the United States and Canada, this uncertainty in predicting exposure levels, especially in very young children and infants, has led to the removal of all phthalates from infant bottle nipples, pacifiers, teethers, and infant toys intended for mouthing.[2] DINP has been substituted for the more toxic DEHP in many other toys intended for older children.[17]

Pediatric Medical Exposures

Neonates can have high exposures to DEHP and its toxic monoester metabolite, monoethylhexyl phthalate (MEHP), when undergoing replacement of blood products, exchange transfusion, extracorporeal membrane oxygenation (ECMO), and other life-saving procedures. DEHP is the only phthalate currently used in medical devices.[2]

PVC medical devices contain, on average, 20% to 40% DEHP by weight. DEHP imparts important qualities to PVC products, such as flexibility, strength, broad-range temperature tolerance, stability during sterilization, resistance to kinking, and optical clarity. It has been known since the early 1970s that DEHP and MEHP are infused with blood products[20–23] and during hemodialysis.[24–26] Beginning in the 1980s, investigators measured DEHP and MEHP delivered during neonatal exchange transfusions.[27–29] More recently, large exposures have been documented during ECMO[30,31] and cardiac surgery.[32] Preliminary data also show possible exposure during mechanical ventilation if PVC circuitry is used.[33,34] PVC infusion lines for lipid-containing parenteral nutrition may also deliver large amounts of DEHP to neonates.[35,36] Empirical data have shown that neonatal medical exposure can be 3 orders of magnitude or more above exposures in the general population. For example, Sjoberg[27] has documented neonatal exposure to DEHP of up to 3300 μg/kg (3.3 mg/kg) per exchange transfusion. The same investigator measured MEHP exposures and found that MEHP could be infused at 100 and 360 μg/kg (0.1 and 0.36 mg/kg) per exchange transfusion.[27,28] Because very ill neonates receive multiple medical interventions, it is likely that total exposures to DEHP and MEHP could be even higher.[2,37]

Toxicology of Phthalates

Phthalates have not been shown to be acutely toxic. Chronic toxicity has been studied only in laboratory animals. A few occupational studies in humans have suggested some excess risk of adverse health effects with chronic exposure.[38–40] A single case-control study found higher serum levels of several phthalates in girls with premature thelarche compared with girls in a control group.[41] No short- or long-term follow-up studies have evaluated possible phthalate toxicity in medically exposed infants. Because human toxicity has not been well studied, animal toxicology data must be examined for relevance to human exposures. The toxicity of each phthalate ester depends on conversion of the parent compound to a toxic metabolite. The amount of conversion varies with route of exposure (ingestion, dermal absorption, inhalation, or intravenous exposure), the animal species studied, and age at which animals are exposed. These differences in toxicokinetics are well demonstrated by the data available on DEHP.

Route of Exposure

The toxicokinetics of DEHP via all exposure routes have been studied in rodents.[2] When DEHP is administered orally, it is rapidly metabolized by pancreatic lipases in the lumen of the gut to the toxic metabolite MEHP. MEHP, not DEHP, is readily absorbed across the intestine. Dermal absorption of DEHP is poor. Inhaled DEHP is absorbed as the parent compound and metabolized to MEHP, and both are broadly distributed throughout tissues in experimental animals. In rats, DEHP administered parenterally is converted to MEHP much less efficiently than is DEHP administered orally, and higher

TABLE 1. Estimated Daily Intake of DEHP (μg/kg of Body Weight per Day)[7]

Substrate/Medium	Age (Years)				
	0.0–0.5	0.5–4	5–11	12–19	20–70
Ambient air: Great Lakes region	0.00003–0.0003	0.00003–0.0003	0.00004–0.0004	0.00003–0.0003	0.00003–0.0003
Indoor air	0.86	0.99	1.2	0.95	0.85
Drinking water	0.13–0.38	0.06–0.18	0.03–0.10	0.02–0.07	0.02–0.06
Food	7.9	18	13	7.2	4.9
Soil	0.000064	0.000042	0.000014	0.000004	0.000003
Total estimated intake	8.9–9.1	19	14	8.2	5.8

doses are required to produce toxicity by the parenteral route.

Species Differences

The differences of most interest among species are those between rodents (for which the most toxicity data exist) and primates. Data on primates are limited but do illustrate important differences. Rodents have more intestinal lipase than primates do, so for any given oral dose, more toxic metabolite is likely to be absorbed in rodents than in primates. Metabolism and excretion pathways for MEHP are different in rodents than in primates, so the half-life of the toxic metabolite may differ. Absorption through rat skin, although poor, is better than through human cadaver skin; absorption of phthalates through the skin of premature infants has not been studied.[2]

Age at Exposure

The toxicokinetics of DEHP are potentially quite different in very young and premature infants. In mature humans, like in rodents, DEHP is metabolized to MEHP by pancreatic lipase and absorbed through the gut. It is then glucuronidated and excreted, resulting in little or no tissue accumulation.[2] In infants, pancreatic lipase systems are not fully mature until 6 to 12 months of age,[42–44] suggesting a possible protective effect of immaturity by decreasing the creation and absorption of MEHP from oral DEHP exposures. Breast milk, salivary, and gastric lipases may, however, compensate and allow conversion of orally acquired DEHP to MEHP.[45,46] Neither premature nor full-term infants have mature glucuronidation until about 3 months of age.[47] Thus, this important clearance mechanism for MEHP is not fully available to neonates and young infants, and MEHP may have a longer half-life in the body. Levels of DEHP in plasma of children undergoing ECMO (parenteral exposure) are higher early in the course of treatment than they are toward the end, but it is not known whether this represents increased metabolism, improved elimination, or redistribution into the tissues.[31] DEHP levels are higher at necropsy in premature neonates who have received varying levels of blood products compared with infants who have not received blood products.[48] The toxicokinetics of MEHP have not been well studied in humans.

Although less well studied, DINP and the other phthalates are likely to demonstrate similar toxicokinetic differences by route, species, and age at exposure.[49]

General Toxicity (Toxicity in Mature Animals)

In mature animals, each phthalate has a different toxicity profile. The liver, kidneys, thyroid, and testes are common targets for general toxicity from oral exposures. Much of the concern about phthalates arises from reports beginning in the 1980s showing several to be carcinogens in rodents. DEHP causes liver cancers[50] and DINP causes kidney and liver cancers in rodents.[49] The mechanism of liver neoplasia caused by DEHP is believed to be attributable to peroxisome proliferation and a cascade of cellular events that do not occur in the human liver,[51] but this theory remains to be confirmed.[52] The mechanism of carcinogenesis of DINP in rodent liver is not fully understood but may also involve peroxisome proliferation. The development of kidney neoplasms in rodents caused by DINP may also be mediated through a mechanism that is not relevant in humans.[53] No studies exist that evaluate perinatal phthalate exposure as a risk factor for adult cancers in humans. Nonetheless, research indicates that carcinogenic risk to humans from at least some of the phthalates may be lower than that to laboratory animals, and focus has shifted to other toxic endpoints.

Developmental and Reproductive Toxicity

Developmental (teratogenic) and reproductive toxicity are studied in laboratory animal systems by exposing adult males and females to chemicals before mating, during some or all of gestation and lactation, or continuously for multiple generations. Different conclusions can be drawn about developmental and reproductive toxicities depending on doses tested, route of administration, timing of exposure, and endpoints studied. Some studies use only high doses of a chemical to determine if any hazard exists. Other study designs use finer dose increments to establish the dose-response relationship to determine if there is a dose that is not associated with any adverse effect, also know as "no observable adverse effect level" (NOAEL). Some studies look at endpoints, such as gross malformations and fetal demise, and others examine tissues for histologic or biochemical abnormalities. Conclusions about human toxicity must be extrapolated from animal studies after considering the extent and strength of existing data sets, assessing the uncertainties remaining, and making judgments about the similarity of animal systems to human systems. As new studies accumulate, these conclusions are continuously revised.[54]

Phthalates can produce fetal death, malformations, and reproductive toxicity with different profiles for each chemical.[55] The different phthalates can also have quite different potencies. The extent of these toxicities and their applicability to humans remain incompletely characterized and controversial. Brief summaries of these animal data on DEHP and DINP follow.

Animal Data on DEHP

DEHP causes skeletal, cardiovascular, and eye abnormalities; neural tube defects; intrauterine death; increased postnatal death; and decreased intrauterine and postnatal growth in rodent pups whose dams received DEHP in feed or by gavage during pregnancy. A "lowest observable adverse effect level" (LOAEL) is observed with fetal toxicity occurring at the same dose or a lower dose than that causing mild maternal toxicity. Thus, fetal toxicity could occur without evidence of maternal toxicity after oral exposure.[2,56–58]

The most sensitive system is the reproductive tract of immature males. Pathologic changes in the testes and decreased sperm numbers are consistent effects across studies. Changes in weight of the testes, vac-

uolization of Sertoli cells, and atrophy of the seminiferous tubules have been observed in rodent pups exposed to DEHP in utero via dietary exposure of dams (LOAEL, 38–141 mg/kg per day; NOAEL, 3.7–14 mg/kg per day).[59,60] In a multigenerational study in which rodent pups of both sexes were exposed throughout prenatal and postnatal life and then mated, complete infertility was observed in females, and decreased fertility was observed in males.[60] A rodent study of intravenous exposure found histologic abnormalities in Sertoli cell endoplasmic reticulum and changes in spermatocyte structure (LOAEL, 250 mg/kg per day; NOAEL, 25 mg/kg per day).[61] In vitro studies demonstrated that the Sertoli cell is the primary cellular target and that MEHP is the toxic metabolite.[62] Evidence suggests that the mechanism of reproductive toxicity in rodents is different from the mechanism of carcinogenesis.[63]

Animal Data on DINP

The evidence on the toxicity of DINP is not as complete as that on the toxicity of DEHP. In general, DINP shows similar patterns of developmental toxicity, but at higher exposure levels. DINP has not been shown to cause reproductive toxicity. DINP causes skeletal and genitourinary abnormalities when rodent pups are exposed in utero at maternal oral doses of 500 to 1000 mg/kg per day (LOAEL), and as with DEHP, fetal toxicity can be seen at lower doses than can maternal toxicity.[49] The single reported reproductive toxicity study in rodents found normal reproductive system structure and function at very high exposure levels but did not evaluate the full range of endpoints tested for DEHP.[49]

Statement of Problem—Extrapolation to Risks to Humans

Expert panels, advocacy groups, and industry groups have analyzed the literature on DEHP and DINP and have come to different conclusions. The European Parliament has recommended bans on certain uses of phthalates,[64] and in the United States, manufacturers have voluntarily changed patterns of use.[2] The controversy exists because risk to humans must be extrapolated from data on laboratory animals for chemicals that demonstrate differences in toxicity by species and route of exposure. Also, experimental exposures often differ from human exposure patterns in terms of dose (high versus low) and timing (acute versus chronic). It is not surprising that consensus has not been achieved.

The most intense disagreement surrounds DEHP and exposures from medical uses. The American Council on Science and Health[a] (the "Koop report") concluded "that DEHP, as used in medical devices, is unlikely to pose a health risk to even highly exposed humans."[65(p25)] The report stressed the benefits of DEHP in successful medical interventions, many of which are life saving. Citing decreased conversion of DEHP to its toxic metabolite, MEHP, in primates versus rodents, lack of evidence in humans of DEHP toxicity, and the fact that medical exposures are intravenous in contrast to the oral exposures in most animal studies, the Koop report concluded that carcinogenesis and developmental and reproductive toxicity from DEHP are not likely at anticipated exposure levels. In contrast, the Lowell Center for Sustainable Production[b] released a report based on a different interpretation of the same literature.[66] This report concluded "the weight of the evidence indicates a significant potential for serious adverse effects on human health from DEHP-containing medical devices."[66(p53)] Stressing the data showing that liver cancer is caused by a different mechanism than are other toxicities and that intravenous exposures to DEHP often involve concomitant exposure to the toxic metabolite MEHP, uncertainties in exposure estimates, and unknowns about metabolism of DEHP in infants, the Lowell report assumed a precautionary stance and called for minimizing human exposure to DEHP from medical devices, including using available alternative medical devices that do not contain DEHP.[67]

Controversy also surrounds childhood exposures to DINP. The Koop report concludes that DINP is unlikely to pose a health risk for children on the basis of wide differences between estimated exposure doses in children and the much higher doses required to cause adverse effects in laboratory animals. A risk assessment by Wilkinson[c] reaches a similar conclusion, but Fiala[d] recommends removal of DINP (and DEHP) from children's toys because exposure may be high enough to cause concern.[18,68] The European Union has banned certain uses of phthalates in response to ongoing assessment of their expert committee and public concern.[64] For DINP, which is acknowledged to be less toxic to laboratory animals than is DEHP, the controversy centers around uncertainties about the magnitude of human exposures, particularly from nondietary ingestion by infants and toddlers.[15,17,19,69]

Recent Reports

In July 2000, the first expert panel convened by the Center for the Evaluation of Reproductive Risks to Humans[2] (CERHR) under the direction of the National Toxicology Program, funded by the National Institutes of Health, and housed at the National Institute of Environmental Health Sciences completed a 15-month analysis of the developmental and repro-

[a]"The American Council on Science and Health, Inc, is a consumer education consortium concerned with issues related to food, nutrition, chemicals, pharmaceuticals, lifestyle, the environment, and health." It is a nongovernmental, nonprofit organization partially supported by industry. Information is available online at: http://www.acsh.org/about/index.htm.

[b]"The Lowell Center of Sustainable Production develops, studies and promotes environmentally sound systems of production, health work environments, and economically viable work organizations." It is composed of faculty and staff at the University of Massachusetts Lowell and can be accessed online at: http://www.uml.edu/centers/lcsp.

[c]Authors of this evaluation cite funding by Jellinek, Schwartz & Connolly Inc, of Arlington, Virginia.

[d]Authors of this evaluation work for the Consumer Council, Austrian Standards Institute and the Institute of Food and Chemistry and Food Technology at Vienna University of Technology.

ductive risks to humans of 7 phthalate esters, including DINP and DEHP.[55]

For DEHP, the CERHR expert panel[e] expressed minimal concern over the exposure to the general adult population. The panel expressed concern that infants and young toddlers, because of their dietary preferences and mouthing behaviors, might have higher exposures to DEHP at a time when the male reproductive tract is still developing and potentially vulnerable.[70] Of similar concern was the possibility that pregnant and lactating women might deliver higher levels of DEHP and MEHP to their infants via placental transfer and breast milk than is estimated for the general population, which is potentially more dangerous to males with developing reproductive tracts. Pointing out that levels of documented single-source intravenous exposures in newborn humans can exceed NOAELs in rodents and approach toxic intravenous doses in rodents, the CERHR expert panel expressed "serious concern"[2(p101)] that critically ill boys undergoing intense medical or surgical treatment might receive doses of DEHP and MEHP that could damage the reproductive tract. The panel acknowledged that the benefits of such intense therapies outweigh the risks of these exposures. It stressed the need for more precise human exposure data, particularly for multiple simultaneous medical exposures, and for better data on primate toxicity and toxicokinetics to evaluate more precisely the risks and benefits of medical exposures. The US Food and Drug Administration and Health Canada recently issued reports that reiterate the concern that some subpopulations of medically exposed individuals, including highly exposed male infants, could be at risk of testicular toxicity from exposure to DEHP.[71,72]

For DINP, the CERHR panel expressed minimal concern for exposures via food consumed by pregnant women. Models of mouthing behavior suggest that young children may experience higher exposures than the general population if they chew or suck on toys or products containing DINP. This uncertainty was enough to raise the concern from minimal to low with respect to DINP toxicity in young children. Some manufacturers are voluntarily decreasing DINP content in toys in response to consumer concerns.[73]

The National Toxicology Program also sponsored a study assessing biomarkers of several phthalates, which indicates that exposures to some other phthalates may be higher than previously assumed relative to both DEHP and DINP.[74] Dibutyl phthalate (DBP) is also teratogenic and toxic to the testes in laboratory animals, though less potent than DEHP.[75] Using 289 nonrandom urine samples collected for the *Third National Health and Nutritional Examination Survey*, the authors found that the monoester metabolite of DBP (one of the phthalates used in cosmetics), was higher than anticipated. The levels of MEHP and the toxic metabolite of DINP were lower than expected compared with the monoester metabolite of DBP, raising questions about the accuracy of previous exposure estimates and assumptions about human metabolism, excretion, and tissue sequestration for these phthalates. As with DEHP and DINP, the toxicity of DBP to humans depends on level of exposure and efficiency of conversion to the toxic metabolite, coupled with the potency and toxicity of the toxic metabolite. The discrepancy between exposures estimated from secondary data and presumed use patterns and those inferred from this small, initial study of specific biomarkers of exposure in a human population highlights the need for better exposure data for all phthalates.

This work has been extended by the Centers for Disease Control and Prevention, the National Toxicology Program, and the National Institute of Environmental Health Sciences in the *Second National Report of Human Exposure to Environmental Chemicals*,[76] which includes analysis of urinary metabolites for the same 7 phthalates as in the Blount study.[74] This larger study used a representative random sample of 2541 US residents and included urinary samples from 328 children from 6 to 11 years of age and 752 children from 12 to 19 years of age. Urinary concentrations of the monoester metabolites were similar to or slightly lower than those found in the smaller previous study, but significant differences were found in concentrations depending on age and sex. For 3 of the phthalate esters, DEHP, DBP, and monobenzyl phthalate, monoester metabolite concentrations were highest in the youngest age category and decreased significantly with increasing age. Females tended to have higher concentrations than did males. This is strong evidence of the importance of performing thorough investigation of exposures through the entire pediatric age spectrum.

CONCLUSIONS

The 1990s began a period of increased attention to the special vulnerabilities of children to environmental hazards. The conflicting conclusions on the safety of phthalates under current exposure conditions provide important illustrations of the subtlety and complexity of the science and policy components required to protect children from environmental hazards. Pediatricians are well positioned to provide leadership in advocating for child-protective standards and policy on phthalates and all areas of children's environmental health. Conclusions about health risks specific to DEHP and DINP can be generalized to many environmental toxicants and aide the development of research priorities and policy decisions that will promote and protect children's environmental health.

1. Phthalates are important components of PVC and other consumer products and are widely distributed environmental contaminants. DEHP and DINP are phthalates of particular concern because of their known toxicities and the potential for significant exposure in sensitive populations.
2. Human exposure to phthalates is universal. Levels of exposure in the general population are estimated to be on the order of tens of μg per kg

[e]Katherine Shea, MD, MPH, lead author of this technical report, was a member of the CERHR Expert Panel on Phthalates.

per day. Food is considered to be the major source of exposure to DEHP and DINP, excluding occupational exposure, nondietary ingestions, and for DEHP only, medical exposures.

3. Human data on exposure to phthalates are very limited. In particular, data on the magnitude and distribution of exposures in sensitive subpopulations, such as women of childbearing age, neonates, infants, and toddlers in the general population and medically exposed fetuses, premature infants, neonates, young children, and adolescents, are lacking. New biomarker data from the Centers for Disease Control and Prevention cast doubt on the accuracy of previous estimates of human exposure, which have been used for risk assessment to date.
4. DEHP and DINP are animal carcinogens, but most recent information suggests that the mechanisms of carcinogenesis may not be relevant to human systems. DEHP is a reproductive toxicant, and DEHP and DINP are developmental toxicants in animals. The most sensitive system is the immature male reproductive tract. The mechanisms of reproductive toxicity are distinct from the mechanism of carcinogenesis.
5. No studies have been performed to evaluate human toxicity from exposure to these compounds.
6. As with many environmental toxicants, children may be at higher risk of adverse effects of phthalates because of anticipated higher exposures during a time of developmental and physiologic immaturity. In response to this theoretical concern, measures to decrease possible exposure through nondietary ingestion are underway. In the United States and Canada, all phthalates have been removed from infant bottle nipples, teethers, and toys intended for mouthing. Manufacturers have voluntarily begun to substitute the less toxic DINP for DEHP in other toys.
7. Pediatric medical exposures to DEHP are of concern. DEHP has been documented to be toxic to the male reproductive tract in laboratory animals at doses near those resulting from intensive medical procedures in humans.[2] Although some of the species and route differences suggest a lower risk to human infants of testicular damage from DEHP exposure, some medical exposures involve concomitant exposure to MEHP, the toxic metabolite. Sertoli cells continue to increase in number through puberty; therefore, medical exposures beyond the newborn period may also be of concern.[71] There are no studies that have evaluated the effect of medical exposures to DEHP and MEHP on testicular function in humans.[67]
8. In light of recent toxicology and exposure evidence and the concern of the CERHR expert panel for the medically exposed infant, medical institutions, including neonatal and pediatric intensive care units and dialysis units, may find it necessary to look at the risk-benefit relationship between DEHP-containing medical devices and their alternatives. Interventions designed to minimize DEHP exposure in the medical setting could be designed. DEHP has important characteristics that improve the function of medical devices. Any substitutes must be shown to be toxicologically safer and functionally equivalent. Publication of a comprehensive comparison of developmental and reproductive toxicities between DEHP and proposed alternatives would be useful. In addition, studies designed to evaluate total DEHP and MEHP exposure from multiple concurrent medical procedures could be very valuable in resolving this controversy.
9. Improved data on pediatric exposures to phthalate esters, including transplacental, breast milk, medical, and nondietary ingestion, would significantly facilitate accurate risk assessments.
10. Improved understanding of the toxicokinetics of phthalates, including creation, distribution, and excretion of the toxic metabolites in subhuman primates or exposed humans, would enable more accurate evaluation of acceptable exposure levels. Determination of the toxicokinetics of phthalates in sensitive subpopulations, including pregnant and lactating women, premature infants, full-term infants, and small children, is also needed.

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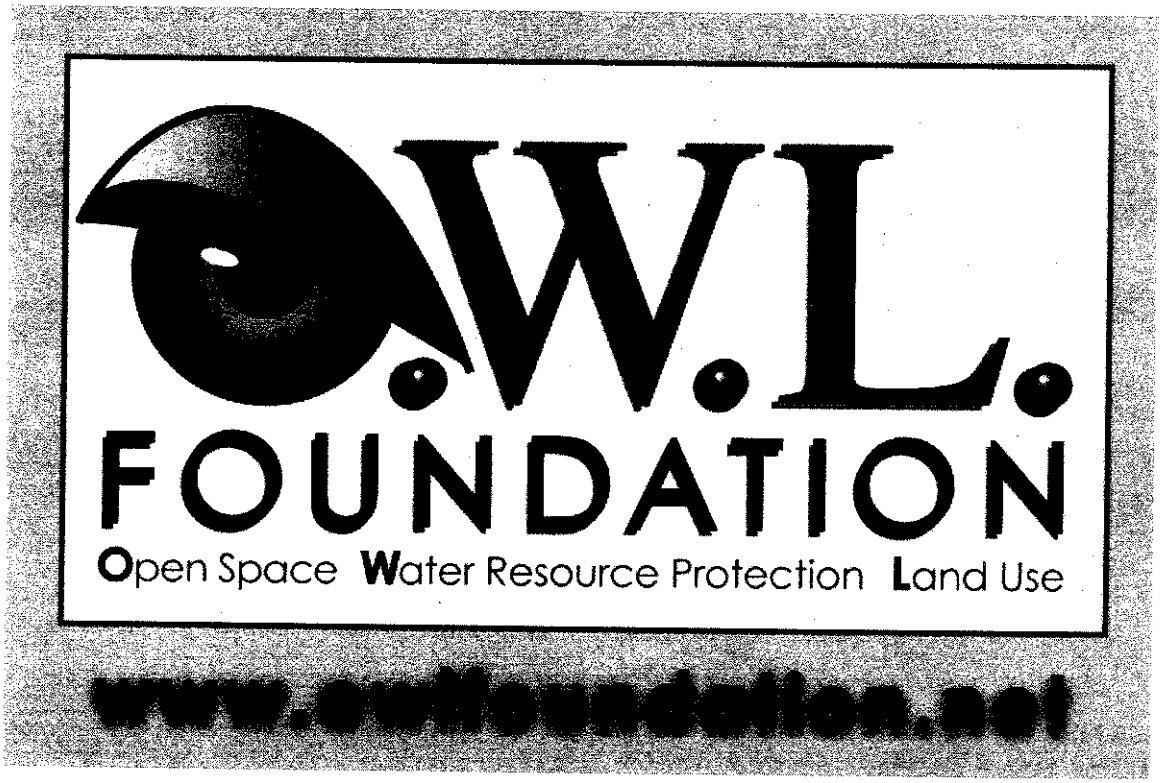

A COLLECTION OF ARTICLES CONCERNING
PATHOGENS THAT EMERGE FROM TREATED
SEWAGE AND THEIR HEALTH IMPLICATIONS

Environ. Sci. Technol. 2006, 40, 7445–7450

Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado†

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This study explores antibiotic resistance genes (ARGs) as emerging environmental contaminants. The purpose of this study was to investigate the occurrence of ARGs in various environmental compartments in northern Colorado, including Cache La Poudre (Poudre) River sediments, irrigation ditches, dairy lagoons, and the effluents of wastewater recycling and drinking water treatment plants. Additionally, ARG concentrations in the Poudre River sediments were analyzed at three time points at five sites with varying levels of urban/agricultural impact and compared with two previously published time points. It was expected that ARG concentrations would be significantly higher in environments directly impacted by urban/agricultural activity than in pristine and lesser-impacted environments. Polymerase chain reaction (PCR) detection assays were applied to detect the presence/absence of several tetracycline and sulfonamide ARGs. Quantitative real-time PCR was used to further quantify two tetracycline ARGs (tet(W) and tet(O)) and two sulfonamide ARGs (sul(I) and sul(II)). The following trend was observed with respect to ARG concentrations (normalized to eubacterial 16S rRNA genes): dairy lagoon water > irrigation ditch water > urban/agriculturally impacted river sediments ($p < 0.0001$), except for sul(II), which was absent in ditch water. It was noted that tet(W) and tet(O) were also present in treated drinking water and recycled wastewater, suggesting that these are potential pathways for the spread of ARGs to and from humans. On the basis of this study, there is a need for environmental scientists and engineers to help address the issue of the spread of ARGs in the environment.

Introduction

The spread of antibiotic-resistant pathogens is a growing problem in the U. S. and around the world. Recently a 2000 World Health Organization (WHO) report (*1*) focused on antibiotic resistance as one of the most critical human health challenges of the next century and heralded the need for "a global strategy to contain resistance". According to the report, more than two million Americans are infected each year with resistant pathogens and 14 000 die as a result. The rapid growth of the problem emphasizes the need for intervention. For example, vancomycin is currently considered to be the most powerful antibiotic of "last resort", yet within 10 years the incidence of vancomycin-resistant enterococci (VRE) increased in the United States from 0% to 25% (*2, 3*). Resistance to penicillin, the antibiotic that originally revolutionized human health 50 years ago, is now as high as 79% in *Staphylococcus pneumoniae* isolates in South Africa (*4, 5*). Alarmingly, diseases that were once considered to be eradicated, such as tuberculosis, are now beginning to make a comeback because of antimicrobial resistance (*1, 6, 7*). As with other dangerous pollutants that spread in the environment and threaten human health, there is a need for environmental scientists and engineers to help address the critical problem of microbial resistance to antibiotics.

The rise of antibiotic resistance is considered to be closely linked with the widespread use of antibiotic pharmaceuticals in humans and animals. In particular, more than one-half of the antibiotics used in the U. S. are administered to livestock for purposes of growth promotion or infection treatment (*8, 9*). In both animals and humans, up to 95% of antibiotics can be excreted in an unaltered state (*10, 11*). Some removal has been observed in wastewater treatment plants (WWTPs); however, as is true with the larger problem of pharmaceutical compounds, WWTPs are not designed for the removal of micropollutants (*12–14*). Residual antibiotics thus are released into the environment where they may exert selection pressure on microorganisms. While overprescribing or other improper use/disposal of antibiotics in humans is generally considered to contribute to the problem, several studies have also linked agricultural antibiotic use with antibiotic-resistant infections in humans (*15–23*). For example, avoparcin, an antibiotic growth-promoter used in poultry, was recently banned in Europe because of its association with the development of vancomycin-resistant enterococci (*24*).

Because of the direct selection pressure that antibiotics exert on organisms carrying antibiotic resistance genes (ARGs), the transport pathways of antibiotic-resistant microorganisms and the ARGs that they carry are expected to be similar to the pathways of antibiotic pharmaceuticals. In fact, it is likely that ARGs persist further in the pathway, considering that in many cases they are maintained in the microbial populations even after the antibiotic selection pressure has been removed (*25–28*). Also, horizontal gene transfer (HGT) is a major mechanism for sharing ARGs between microbes and has been documented to occur between nonpathogens, pathogens, and even distantly related organisms, such as Gram-positive and Gram-negative bacteria (*25, 29–31*). In many cases, ARGs have been discovered to occur as part of multiple antibiotic resistant (MAR) superintegrons, which may contain over 100 ARG cassettes (*32*). These MAR superintegrons cause multiple-drug resistance in organisms, meaning that even when very different antibiotics are used, one antibiotic may coselect for resistance to other antibiotics (*5, 33*). MAR gene cassettes and ARGs are notorious for being associated with plasmids and/or transposons that facilitate HGT. Finally, even if cells carrying ARGs have been killed, DNA released to the environment has been observed to persist, to be protected from DNAse, especially by certain soil/clay compositions, and to be eventually transformed into other cells (*34–36*). For all of these reasons, ARGs in and of themselves can be considered to be emerging "contaminants" for which mitigation strategies are needed to prevent their widespread dissemination.

The purpose of this study was to document the occurrence of tetracycline and sulfonamide ARGs in various environmental compartments in northern Colorado. These two ARG

† This article is part of the Emerging Contaminants Special Issue.

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10.1021/es060413l CCC: $33.50
Published on Web 08/15/2006

groups were chosen because sulfonamide and tetracycline antibiotics have been previously characterized in Poudre River sediments and shown to relate to urban/agricultural activity (*37*). The breadth of the study included Cache La Poudre (Poudre) River sediments, dairy lagoon water, irrigation ditch water, a wastewater recycling plant (WRP), and two drinking water treatment plants (DWTPs). The hypothesis was that environmental compartments most directly impacted by urban/agricultural activity would have significantly higher concentrations of ARGs than less impacted and pristine environments. Irrigation ditch waters, which were directly adjacent to farms, were investigated as a potential pathway of ARGs from farms to the Poudre River, while the WRP and the DWTPs were explored as potential routes of human environmental input and consumption. The presence/absence of several ribosomal protection factor tetracycline ARGs and folic acid pathway sulfonamide ARGs was determined using a polymerase chain reaction (PCR) detection assay, and four commonly occurring ARGs were further quantified by quantitative real-time PCR (Q-PCR). Documenting the baseline occurrence of ARGs in a cross-section of environmental compartments will take a step toward understanding and modeling the fate and transport phenomena associated with these emerging contaminants.

Experimental Section

Poudre River Sediment Sampling. Because of its pristine origins and zonation corresponding to land use, the Poudre River has served as a good model for relating human and agricultural activities with the occurrence of antibiotic pharmaceuticals (*37*) and ARGs (*38*). Five sampling sites were the focus of this study, numbered sequentially in the direction of flow from west to east, with the following characteristics: site 1, pristine location at the river origin in the Rocky Mountains; site 2, light-agriculture-influenced area; site 3, urban-influenced area at the outlet of the Fort Collins Drake WWTP; site 4, heavy-agriculture-influenced area between Fort Collins and Greeley; and site 5, heavy-agriculture- and urban-influenced area just east of Greeley, which is a major center for the meat-packing industry. Over 90 confined animal feeding operations (CAFOs), dairies, and ranches are located between sites 3 and 5. Further attributes of the Poudre River watershed that contribute to its suitability for investigating the impacts of urban and agricultural activity on antibiotics and ARGs have been described previously (*37*, *38*).

Sediment samples were collected along the Poudre River at the five sites on August 18, 2005, October 27, 2005, and February 17, 2006. The flow rates on these three dates were 1.04, 14.19, and 0.14 $m^3 s^{-1}$, respectively (U. S. Geological Survey station number 06752260, Fort Collins, CO). Sampling at three points in time provided insight into potential temporal variations in ARG concentrations, and the February 17th date is exactly 1 year later than a previously published sampling date (*38*). The upper sediments (about 5 cm) from the middle and two sides of a cross-section at each site were sampled and composited. Samples were collected using a shovel and mixed well in sterilized centrifuge tubes. Fifty-five grams of mixed sample at each site were stored at −80 °C for subsequent molecular analysis.

Bulk Water Sampling. Irrigation ditch waters were investigated as a potential pathway of ARGs from farms to the Poudre River. Grab samples of bulk water were collected in sterile containers from irrigation ditches on August 18, 2005, corresponding to the August sampling date of the Poudre River sediments. All irrigation ditches were located between site 4 and site 5 on the Poudre River within a 3.5 km × 2 km zone north of the river, and a total of ten locations were sampled. To investigate a potential source of ARGs within this zone, a microaerophillic dairy lagoon (~1 mg/L dissolved oxygen in the upper 1 m) and an anaerobic dairy lagoon (0 mg/L dissolved oxygen) from an anonymous farm located 8 km from site 5 were sampled on October 20, 2005. Finally, source water, and pre-chlorinated, and post-chlorinated bulk water were collected from two anonymous DWTPs and an anonymous WRP in northern Colorado in February, 2005. The DWTP was studied as a potential direct route of ARGs to consumers, and the WRP was considered a potential human input into the environment. To collect fine particulates from the dilute ditch water, DWTP, and WRP samples for subsequent analysis, 500 mL of well-mixed sample was filtered using a 0.45 μm glass fiber filter (Whatman). This concentration step was not required for dairy lagoon samples.

DNA Extraction. DNA was extracted from 0.5 g of composited sediment using the FastDNA Spin Kit for Soil (MP Biomedicals) and from 1.8 mL of dairy lagoon water using the Ultraclean Microbial DNA Kit (MoBio Laboratories, Inc.) according to manufacturer protocol. Both approaches employ a bead-beating procedure. For fine particulates collected on filters from bulk water, the filters were cut into small pieces and added directly to the extraction tubes. Extraction yield and the quality of the DNA were verified by agarose gel electrophoresis and spectrophotometry.

Detection and Quantification of ARGs. Polymerase chain reaction detection assays were used for broad-scale screening of the presence/absence of five ribosomal protection factor tetracycline ARGs (tet(BP), tet(O), tet(S), tet(T), and tet(W)) (*39*) and four folic acid pathway sulfonamide ARGs (sul(I), sul(II), sul(III), and sul(A)). Development and validation of sul primers was described in Pei et al. (*38*). Positive controls consisted of cloned and sequenced PCR amplicons obtained from Poudre River sediments. Both positive and negative controls were included in every run, and negative signals were confirmed by spiking positive control template into the sample to verify a signal. Forty cycles were used to improve chances of product formation from low initial template concentrations. Further details on reaction mixes and temperature programs are available in Pei et al. (*38*); note that annealing temperatures for tet primers vary from Aminov et al. (*39*). Two tetracycline ARGs (tet(W) and tet(O)) and two sulfonamide ARGs (sul(I) and sul(II)) that were commonly occurring according to the PCR presence/absence assays were further quantified by Q-PCR using a SybrGreen approach. For further details on Q-PCR methods, see Pei et al. (*38*). Eubacterial 16S rRNA genes were quantified according to the TaqMan Q-PCR method described by Suzuki et al. (*40*) so that ARGs could be normalized to the total bacterial community. This provided a means to correct for potential variations in extraction efficiencies. By quantification of 16S rRNA genes, it was also possible to compare ARGs proportionally between samples of different overall population sizes. Matrix effects associated with extraction of DNA from environmental samples were corrected for by performing spiked matrix control tests and determining template suppression factors as described in Pei et al. (*38*). All Q-PCR analyses were performed using a Cepheid SmartCycler (Sunnyvale, CA).

Statistics. The influences of the environment (sites, ditch water, and dairy lagoons) on the normalized and non-normalized copies of ARGs were analyzed using the Mixed Procedure, which fits a variety of mixed linear models to data. This provides the flexibility of simultaneously modeling means, variances, and covariances (*41*−*44*). Through the use of this test, it was thus possible to comprehensively compare overall differences between different environmental compartments with respect to ARG concentrations. For comparison of the five Poudre River sites, multiple sampling time points were treated as replicates. Mixed Procedures were conducted using SAS 9.0 (SAS Institute Inc., Cary, NC). A

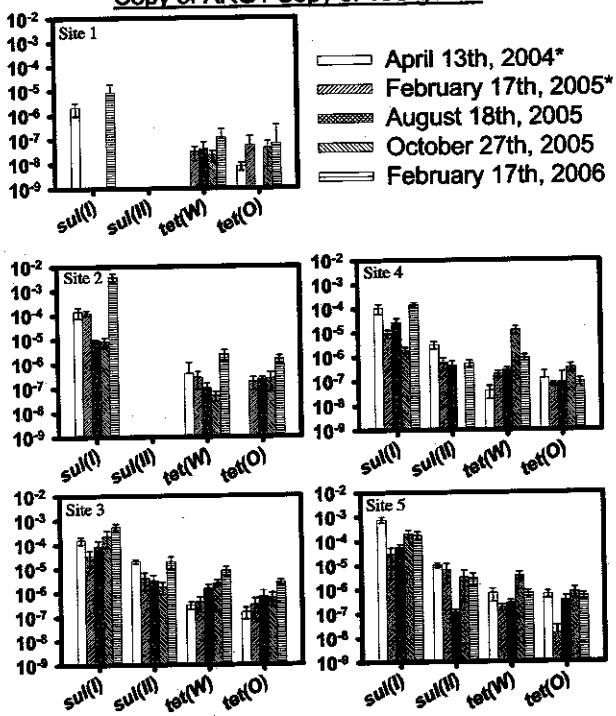

FIGURE 1. Distribution of four ARGs (sul(I), sul(II), tet(O), and tet-(W)) in Poudre River sediments on three sampling dates, compared to two previously published sampling dates (April 13, 2004, and February 17, 2005 (*38*)), as determined by Q-PCR: site 1, pristine site; site 2, light agricultural activity; site 3, heavy urban activity; site 4, heavy agricultural activity; site 5, heavy urban and agricultural activity. Error bars represent the standard deviation of six measurements from three independent Q-PCR runs analyzing DNA extract from composite samples.

p-value <0.05 was considered to indicate significance. Averages and standard deviations of all data were determined using Microsoft Excel, 2003.

Results and Discussion

Occurrence of ARGs in Northern Colorado. Figure 1 summarizes the Q-PCR data obtained for the four ARGs at the five Poudre River sites, while Figure 2 summarizes the same analyses for the ditch waters and dairy lagoon water. When August 2005 data for the Poudre River sediments are compared with the dairy lagoon and ditch water, the following trend is observed with respect to ARG concentrations: dairy lagoon water > ditch water > river sediments ($p < 0.0001$), for all ARGs except sul(II), which was absent from the ditch waters. This is based on pooling of all 10 ditch water sites, the two dairy lagoons, and sites 4 and 5, which were directly adjacent to the ditch water sampling locations. Within each of these three pools, there was no statistical difference observed among the samples. Therefore, it was observed as expected that environmental compartments most directly impacted by human/agricultural activity showed higher concentrations of ARGs. This trend is even stronger in considering absolute quantities of ARGs (not normalized to 16S rRNA genes), because the concentration of cells in the dairy lagoon water was orders of magnitude higher than that of the ditch water or the sediments.

In developing a hypothetical pathway for ARGs, a trend is not as clear. The overall trend in terms of ARG concentrations of dairy lagoon water > ditch water > river sediments suggests that on-farm compartments, such as lagoons may be the source of ARGs, which are subsequently attenuated in ditch water before reaching Poudre River sediments.

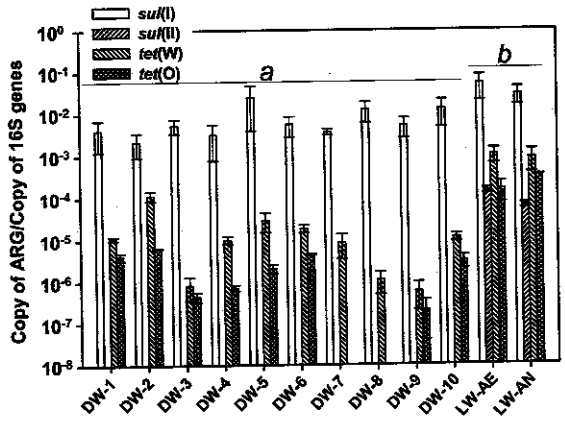

FIGURE 2. Distribution of four ARGs (sul(I), sul(II), tet(O), and tet-(W)) at 10 sampling points of irrigation ditch water (DW-1−DW-10) located between site 4 and site 5 compared with that of a microaerophillic dairy lagoon (LW-AE) and an anaerobic dairy lagoon (LW-AN). DW samples were concentrated from 500 mL, and LW samples were extracted directly from 1.8 mL. All samples were normalized to the total 16S rRNA genes. Error bars represent three independent Q-PCR runs in duplicate. The labels a and b indicate that the data sets fell into two statistically different groups, according to the Mixed Procedure.

However, this trend is not supported in terms of sul(II), which is entirely absent from the ditch water and therefore cannot be the source of what is observed in the Poudre River sediments. An alternative source of the sul(II) that appears at sites 4 and 5 could instead be human inputs. This is supported by the data presented in Figure 1, in which it is observed that sul(II) is consistently present at high levels on average at site 3, which is at the point of discharge of the Drake WWTP, while consistently lower (comparing each date sampled) at site 4 (entirely absent for the October event) and equivalent or lower at site 5, which has mixed human/agricultural inputs. Because sul(II) is present in the dairy lagoon waters, it must also have agricultural sources, but it may attenuate too quickly to be transported to the ditches and subsequently to the river sediments. On the basis of this study and a previous study (*38*), it is appears that of the four ARGs quantified sul(II) is the most sensitive indicator of human/agricultural impact, and thus it is suggested that it attenuates quickly in the absence of direct inputs. The other ARGs in the Poudre River sediments at sites 4 and 5 may be of either/both human and agricultural origin, since they followed a decreasing trend from the dairy lagoon through the ditch water but were also present at site 3.

In addition to having higher concentrations of three out of four of the ARGs, the dairy lagoon water was also observed to have more different kinds of ARGs present than the irrigation ditch water according to the PCR assay (Table 1). Together with the Q-PCR results, these data further support the concept that there is some attenuation of ARGs between any linkages that may connect dairy lagoon water and irrigation ditch water. Future work should implement ARG fingerprinting/source tracking to fully characterize the potential pathways.

Temporal Variations of ARG in Poudre River Sediments. As observed in a previous study that compared a high-flow sampling point (6.8 $m^3\ s^{-1}$, April 2004) with a low-flow sampling point (0.6 $m^3\ s^{-1}$, February 2005), the ARG concentrations in the Poudre River sediments are variable with time (*38*). To better understand temporal variations in ARG concentrations, the Poudre River sediments were sampled at three additional time points and compared with the two previously published time points. The February sampling point in this study took place exactly 1 year after

TABLE 1. PCR Presence/Absence Assay of Various ARGs in Ditch (DW)[a] and Dairy Lagoon (LW) Water[b]

ARG	DW-1	DW-2	DW-3	DW-4	DW-5	DW-6	DW-7	DW-8	DW-9	DW-10	LW-AE	LW-AN	+ control
tet(BP)	−	−	−	−	−	−	−	−	−	−	−	−	+
tet(O)	+	+	+	+	+	+	−	−	+	+	+	+	+
tet(S)	−	−	−	−	−	−	−	−	−	−	−	−	+
tet(T)	−	−	−	−	−	−	−	−	−	−	+	+	+
tet(W)	+	+	+	+	+	+	+	+	+	+	+	+	+
sul(I)	+	+	+	+	+	+	+	+	+	+	+	+	+
sul(II)	−	−	−	−	−	−	−	−	−	−	+	+	+
sul(III)	−	−	+	+	+	−	−	−	−	−	+	+	+
sul(A)	−	−	−	−	−	−	−	−	−	−	−	−	+

[a] Collected August 18, 2005. [b] Collected October 20, 2005.

the previous February event. In support of the relationship between ARG concentration and relative environment impact observed above, the pristine site (site 1) consistently had the lowest average concentrations of ARGs with time, with sul(II) completely absent and no individual ARG consistently present at all five sampling times (Figure 1). When presence/absence of ARGs are compared, site 2 appears to be the next lowest in terms of overall impacts. For example, sul(II) is consistently absent at site 2, and tet(O) was absent in one of the five sampling events, whereas these genes were consistently present at sites 3, 4, and 5. In terms of ARG concentrations, tet(W) and tet(O) at site 2 were equal or less than site 3; however, these two genes were sometimes higher and sometimes lower than at sites 4 and 5. On the basis of ARG averages and presence/absence of ARGs, sites 1 and 2 were the least impacted, as expected.

When the Mixed Procedure was applied to the data, in which the time points were pooled as replicates, it was found that there was no statistical difference between the five sites for the 16S normalized data, except in the case of sul(II) ($p = 0.0117$). However, when the same test was performed with non-normalized data, it was found that sites 1 and 2 were statistically lower than sites 3, 4, and 5 in terms of sul(I) ($p = 0.00296$), sul(II) ($p = 0.0199$), and tet(O) ($p = 0.0102$). Though normalizing to 16S genes provides a comparison of ARGs as a proportion of the total population, arguably it may be the absolute quantities of ARGs that are more critical.

While spatial variations in ARGs could be fairly well-characterized, it is difficult to identify clear temporal patterns. Comparison of the two February sampling dates that were exactly a year apart provides some insight. All four genes were either the same on average for both events (tet(O) for sites 1 and 4 and sul(II) for sites 4 and 5) or higher in the 2006 event (all other genes, except sul(II) at sites 1 and 2, where it was not present) (Figure 1). This suggests the possibility that all ARGs are increasing in concentration with time. However, the trends in between these two dates do not support this. Only tet(W) and tet(O) at site 3 increase consistently with time. All remaining ARGs at the five sites either decrease before increasing (e.g., tet(W) at site 2 and sul(II) at site 3), are constant and then increase (e.g., tet(O) at site 2 and tet(W) at site 1), or increase and then decrease (e.g., tet(W) at sites 4 and 5) (Figure 1). Therefore, no clear trend was identified with time.

It was also attempted to analyze trends in the data with respect to river flow rate. This was of interest because flow rate directly relates to runoff and nonpoint source inputs, which were hypothesized in the previous study to play a role in the observed increase in the number of kinds of ARGs detected in Poudre River sediments (*38*). The October 2005 sampling date provided a second sampling date at high flow (14.9 $m^3 s^{-1}$), compared to the previously published April 2004 high-flow sampling date (6.8 $m^3 s^{-1}$). (All other dates were at or below 1.0 $m^3 s^{-1}$.) Interestingly, all four ARGs increased on average at site 5 in comparing the high-flow

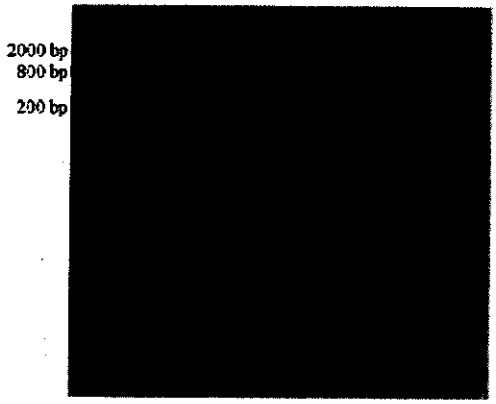

FIGURE 3. Agarose gel analysis of PCR presence/absence (in duplicate) of two ARG families, tet(W) and tet(O): + = positive control; − = negative control. The presence of a band at the same molecular weight as + indicates the presence of an ARG: 1 = WRP effluent; 2 = WRP chlorinated effluent; 3 = DWTP a influent; 4 = DWTP a treated water pre-chlorination; 5 = DWTP a treated water post-chlorination; 6 = DWTP b influent water; 7 = DWTP b treated water pre-chlorination; 8 = DWTP b treated water post-chlorination. The band appearing below 200 bp is consistent with a primer dimer.

October event with the immediately previous low-flow event in August (Figure 1). At site 4, tet(W) and tet(O) increased, but sul(II) stayed the same, and sul(I) decreased. There was no effect at all at site 3, which is affected primarily by point discharge rather than runoff, site 2, or site 1. However, attempts to plot ARG concentrations versus flow rate did not reveal any clear trend. Thus, it is still not possible to make a conclusive judgment on the effect of flow rate on ARG concentrations, though the role of nonpoint source inputs merits further investigation. To accomplish this, it would be necessary to gather more data with time/flow or monitor a much more controlled and smaller-scale system.

Wastewater Recycling Plant and Drinking Water Treatment Plants. A PCR presence/absence assay was conducted on the influent, intermediate effluent, and final effluent of two drinking water treatment plants (DWTP "a" and DWTP "b") and the pre-chlorinated and chlorinated effluent of a WRP. It was observed that both tet(W) and tet(O) were present at detectable levels in all samples except the source water for DWTP "a" (Figure 3). This indicates that the same two genes that were common in various environmental compartments in northern Colorado are also present in treated recycled wastewater and bulk drinking water. These two genes also showed a response to the level of impact; e.g., they were highest in dairy lagoon water and ditch water and lowest on average at the pristine site. On the basis of the intensity of the signal, they were also higher in the recycled wastewater than in the drinking water, as would be expected. Though these two ARGs are not directly associated with any known human pathogens, they may be indicators of links

between human/agricultural activity and ARGs in drinking water. Considering that drinking water is a direct route to human consumers, this emphasizes the need to better understand the pathways by which ARGs are spread in the environment and potential ways that the spread of ARGs may be reduced. For example, vancomycin resistance genes were found in drinking water biofilms in a recent study (*45*). Considering that vancomycin is typically the antibiotic of last resort when all else fails, this underscores the need to address this issue before it is too late. One possibility may be to make simple modifications to wastewater and drinking water treatment plants to reduce the spread of ARGs.

ARGs as Emerging Contaminants. On the basis of this study it is clear that ARGs are present in various environmental compartments, including river sediments, irrigation ditch water, dairy lagoon water, DWTPs, and a WRP. Furthermore, quantitative techniques incorporating Q-PCR provide a means to compare the concentrations of ARGs associated with the known urban and agricultural impacts, which provides a more direct measure than previous culture-based methods. On the basis of this occurrence survey, it is argued that ARGs are emerging contaminants that need to be further studied in the paradigm of environmental science and engineering. The concept of ARGs as "pollutants" has also been suggested by Rysz and Alvarez (*46*).

It should be noted that besides the tetracycline and sulfonamide ARGs that were the focus of this study, there are numerous other ARGs that have been described in the literature and likely even more that have not yet been discovered, each potentially with its own unique properties. Thus, each ARG may have different behaviors with respect to fate and transport and response to physical, chemical, and/or biological treatment. In terms of defining fate and transport characteristics of ARGs in general, it is expected that their behavior will be distinct in comparison to "typical" contaminants. For example, ARGs may be sequestered with bacteria, which are themselves transported, or they may be present as naked DNA bound to clay particles (*47*). Furthermore, ARGs may actually amplify in the environment under some conditions. This is indeed a unique contaminant property. Considering the significance of the problem of the spread of antibiotic resistance, further effort by environmental researchers to better understand these emerging contaminants is well-warranted. This is especially true as the rate of discovery and development of new antibiotics is continually declining (*48*), while the corresponding development and spread of resistance is occurring at a rapid pace. On the basis of this study, understanding ARGs as emerging contaminants can add a new and important angle to helping to approach this important problem.

Acknowledgments

Funding for this study was provided by U. S. Department of Agriculture (USDA) NRI Watersheds program, the USDA Agricultural Experiment Station at Colorado State University, and the National Science Foundation CAREER program. The authors thank Jessica Davis, Kathy Doesken, and Sung-Chul Kim for coordinating collection of ditch and dairy lagoon water samples as well as the anonymous providers of the drinking water and recycling plant samples.

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Received for review February 20, 2006. Revised manuscript received July 10, 2006. Accepted July 17, 2006.

ES060413L

Antibiotic Resistance from Wastewater Oxidation Ponds

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Source: Water Environment Research, Volume 77, Number 7, November 2005 , pp. 2996-3002(7)

Publisher: Water Environment Federation

Abstract:

In an extensive, multiyear study of antibiotic resistance from wastewater oxidation ponds, five mobile home park wastewater oxidation ponds in Clarke and Oconee counties were shown to be discharging high numbers of antibiotic-resistant bacteria into the waterways of North Georgia. This effluent contributed to higher nitrogen, phosphorus, and fecal coliform levels in creeks downstream from the ponds. A survey of residents revealed that many people did not complete their antibiotic prescriptions, and the majority flushed leftover antibiotic medications down the toilet. In the pond discharges, resistance was found to eighteen antibiotics: amikacin, amoxicillin/clavulanic acid, ampicillin, apramycin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, kanamycin, naladixic acid, streptomycin, sulphamethoxazole, trimethoprim/sulphamethoxazole, and tetracycline.

The discharged bacteria contained both integrons and plasmids, the latter being transferable to a laboratory strain of Escherichia coli (E. coli). A turtle was found living at a pond discharge site with multiply-antibiotic-resistant bacteria in its feces. Last year, RNA fingerprinting conclusively documented the survival of three multiply-resistant important pathogenic bacteria. Ceftriaxone-resistant Stenotrophomonas maltophilia and Pseudomonas aerogenosa and a ciprofloxacin-resistant E. coli were traced through oxidation pond stages and into the discharge, thus documenting that the pathogens survived the treatment process. In addition, a potential pathogen, a serotype group D Salmonella *spp.*, was found in the discharge.

In this study, tetracycline-resistance genes C and G were detected in the first and second stages of the oxidation pond and the discharge went directly into the environment. These genes are generally found in intestinal bacteria, so it can be inferred that they are from a human source. Antimicrobial residue from the beta-lactam family of antibiotics was found in all oxidation pond stages and in the creek above the pond. Tetracycline residue was found in the first and second stages of the pond. In addition to the antibiotics, genes coding for antibiotic resistance and the antibiotics themselves were documented to survive oxidation pond treatment. Tetracycline-resistant

genes were identified in the oxidation pond stages and in the discharge going into the environment.

A model was also developed to study oxidation pond function in the laboratory. A biofilm was created using a highly antibiotic-resistant Salmonella typhimurium *3/97*, and pond water was added. The biofilm was processed via a rotating disk bioreactor specifically designed to study biofilms in nature, but with conditions that were more favorable to bacterial inhibition than those in nature. Cultures revealed that, under these optimal conditions, S. typhimurium *3/97* was still present in this *in vitro* system. Thus, the competitive inhibition process that helps to remove bacteria in oxidation ponds did not effectively remove an important bacterium, S. typhimurium 3/97, in this mock oxidation pond.

The bioreactor model developed in this study can be used to further investigate discharges from oxidation ponds. From this data, it is apparent that the problem is two-fold. A cost-effective technique must be developed that inactivates antibiotic-resistant bacteria in oxidation pond discharges and also removes the antibiotics. A public awareness campaign was initiated by the author to encourage proper use and disposal of antibiotics, as flushing them is a common practice in the United States.

Keywords: wastewater; oxidation pond; antibiotic resistance; wastewater; biofilm; human waste lagoon
Document Type: Research article
DOI: 10.2175/106143005X73875

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For Immediate Release
Tuesday, October 24, 2006

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Colorado State University
Knowledge to Go Places

COLORADO STATE RESEARCHER URGES SCIENTIFIC COMMUNITY TO TREAT ANTIBIOTIC RESISTANCE GENES AS WATER CONTAMINANTS

FORT COLLINS - Antibiotic resistance genes - a growing health problem around the world - are present in water systems in northern Colorado and should be viewed more seriously as contaminants by the scientific community, a Colorado State University civil engineering professor urges in an upcoming issue of the American Chemical Society's Environmental Science and Technology journal.

Amy Pruden, an assistant professor of civil and environmental engineering, and her team looked for the occurrence of tetracycline and sulfonamide antibiotic resistance genes in five sampling sites along the Poudre River. As expected, they found higher concentrations in more populated or heavily farmed areas, but still detected antibiotic resistance genes in all sampling areas.

Tetracycline and sulfonamide are commonly used antibiotics in people and animals.

Pruden found that treated water and wastewater also carried the genes.

"Microbes carrying these antibiotic resistance genes are not effectively killed by antibiotics, and the presence of these drugs in the environment may stimulate them to proliferate," Pruden said.

Pruden co-authored the paper with Ken Carlson, a civil engineering professor, and two graduate students, Ruoting Pei and Heather Storteboom. Funding for the research was provided through a $400,000, five-year CAREER grant awarded this year from the National Science Foundation and from the U.S. Department of Agriculture NRI Watersheds program and the USDA Agricultural Experiment Station at Colorado State.

Pruden notes that even if cells carrying the genes have been killed, the DNA still winds up in the environment and may get transferred to other cells. She stressed that testing so far only covers two classes of antibiotic resistance genes - others may also be present in the environment and have varied responses to environmental conditions.

"Antibiotic resistance genes in and of themselves can be considered to be emerging 'contaminants' for which mitigation strategies are needed to prevent their widespread dissemination," Pruden said. "This is especially true as the rate of discovery and development of new antibiotics is continually declining while the corresponding development and spread of resistance is occurring at a rapid pace."

More than 2 million Americans are infected each year with resistant pathogens; 14,000 die, according to the World Health Organization. The WHO considers antibiotic resistance to be one of the most pressing health challenges of the next century.

"This is a new class of contaminant that can have a measurable impact on human and environmental health," Pruden said. "I want to know how they're spread and develop models and find out how to treat them."

The next step of her research is to follow the path of the genes through the watershed and more closely identify the sources. At the same time, she will investigate basic modifications to water treatment to destroy the genes' DNA.

"We have some evidence of how they spread in the environment, but we need to study it in more detail to confirm the pathways," she said. "Where is it coming from, where does it end up, how does it get there?

"With environmental problems, it's often when you reach a point of no return that people start thinking about doing something," she said. "We're hoping to avoid that."

Pruden is teaching an experimental course in molecular biology for engineers this fall. The course is now a permanent part of the engineering curriculum and will help equip engineering students to tackle complex bioenvironmental problems.

She is also working with Carlson to test an early-warning security system designed to alert city utility officials when major pollutants are detected in water supplies. If installed, the real-time monitoring system, integrated by ST-Infonox of California, would help city officials respond quickly to foreign substances in the water distribution system, helping to combat any potential terrorist or natural threats.

ST-Infonox officials are working with city officials in Loveland and Fort Collins to test the technology on municipal water systems.

Pruden joined Colorado State University in 2002. She obtained her bachelor's and her doctoral degree from the University of Cincinnati.

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ASM General Meeting : 106th ASM General Meeting Press Kit : Press Releases : Monday, May 22, 2006

The Importance of Municipal Sewage Treatment in the Spread of Antibiotic Resistance

106th General Meeting of the American Society for Microbiology
May 21-25, 2006, Orlando, Florida
For more information on any presentation at the 106th General Meeting of the ASM contact Jim Sliwa, ASM Office of Communications at jsliwa@asmusa.org
EMBARGOED UNTIL: **Monday, May 22, 9:00 a.m. EDT**
(Session 041/Q, Paper Q-032)

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Our study determined that substantial numbers of antibiotic-resistant bacteria were present in municipal wastewater, and that the existing treatment infrastructure did not adequately prevent release of antibioticresistant bacteria into the environment. Many of the bacteria found in the wastewater treatment plant and in the plant effluent were tentatively identified as potential pathogens and were also resistant to multiple antibiotics, raising public health concerns. We believe that wastewater treatment plants could be modified to further prevent the release of resistant bacteria to the environment.

Sara Firl and Leslie Onan performed this study under the supervision of principal investigator Dr. Timothy LaPara at the University of Minnesota, Department of Civil Engineering. Funding was provided by the Center for Urban and Regional Affairs at the University of Minnesota and Geomatrix Consultants, Inc. The work is being presented as a poster at the 106th General Meeting of the American Society for Microbiology in Orlando on May 22.

The spread of antibiotic-resistant bacteria is a major public health concern. Infections previously treatable are increasingly resistant to antibiotics. Scientists believe that the spread of antibiotic resistance results from both misuse of antibiotics and transfer of resistance between bacteria. A potentially large reservoir for antibiotic-resistant bacteria is municipal wastewater. People release resistant bacteria with fecal matter into the wastewater stream, which is collected and treated at municipal treatment facilities before release to the environment. The objective of this study was to investigate how many resistant bacteria were present at municipal wastewater plants and if the existing infrastructure of waste treatment was adequate to remove resistant bacteria before discharge.

In our study, the effect of effluent treatment (clarification and disinfection) and biosolids treatment (sludge digestion) on the removal of antibioticresistant bacteria was investigated at three wastewater treatment facilities.

We found substantial numbers of resistant bacteria at the wastewater treatment facilities and that, although effluent treatment reduced the numbers of bacteria, large quantities of resistant bacteria were discharged. Numerous bacteria isolated from the effluent stream were resistant to multiple antibiotics and closely related to potentially pathogenic bacteria. Our research suggests that the existing wastewater treatment infrastructure should be modified to better prevent release of these potentially dangerous bacteria to the environment.

USGS at Society of Environmental Toxicology and Chemistry: From Pharmaceuticals in Groundwater to Mercury in High U.S. Mountains

Released: 11/6/2006 12:12:41 PM

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The 27th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) is being held November 5-9, 2006, at the Palais de Congrès in Montréal, Québec, Canada. Additional information about the conference can be found online at http://montreal.setac.org/home.asp While the conference is occurring, please contact Catherine Puckett for information about specific presentations.

A Happy Medium? Antidepressants in Aquatic Systems: Wastewater treatment plants do a remarkable job at removing the bulk of chemicals from the waste stream. But recent USGS studies have shown that a wide range of pharmaceuticals and other human-caused waste compounds remain despite wastewater treatment and are discharged to receiving waters across North America. Antidepressants are a commonly used class of pharmaceuticals whose pharmacological effects may extend beyond humans to aquatic organisms present in surface water systems that receive treated wastewater discharge. Yet few methods exist to detect antidepressants in the environment, and their effects on aquatic organisms are only beginning to be understood. Recently, USGS researchers developed a method to study the distribution and fate of antidepressants and their breakdown products in aquatic environments, including municipal wastewater and surface water. Venlafaxine (Effexor) was the predominant antidepressant researchers found in wastewater and river-water samples from Colorado, Iowa, and Minnesota, though other antidepressants were found as well. Typical concentrations of individual antidepressants ranged from a few nanograms per liter to thousands of nanograms per liter (for Venalfaxine) in wastewater. This indicates that wastewater is a point source of antidepressants into the environment, at concentrations that may impact aquatic life. **For more information, please contact Edward T. Furlong, USGS, at efurlong@usgs.gov or 303-236-3941; or Melissa Schultz, College of Wooster, Wooster, OH at mschultz@wooster.edu or 330-263-2645. SETAC presentation is Monday, Nov. 6, 8:20 a.m., Room 517A, Palais de Congrès.**

Pharmaceuticals in Long Island's Groundwater: Pharmaceuticals can infiltrate groundwater systems in areas susceptible to wastewater contamination. In studies by Stony Brook University and the U.S. Geological Survey of ground-water wells in Suffolk County, Long Island, NY, near permitted wastewater treatment facilities discharging to ground water, scientists detected pharmaceuticals in concentrations generally 1-200 ng/L (parts per trillion).These vanishingly small concentrations are several orders of magnitude below the concentrations where any effects have been observed or predicted for the compounds measured in this study. Acetaminophen, caffeine, carbamazepine (anti-epileptic), cotinine (human metabolite of nicotine), paraxanthine (human metabolite of caffeine), and sulfamethoxazole (antibiotic) were found most often in both studies. However compounds were more frequently detected in the shallower wells. These occurrences, and laboratory studies, suggest that of these compounds, caffeine, carbamazepine, paraxanthine, and sulfamethoxazole are more persistent in groundwater and have the most potential for transport in the subsurface. **For more information, contact Mark J. Benotti at mbenotti@usgs.gov or 631-736-0783 x126. SETAC presentation is Thursday, Nov. 9, 4:50 p.m., Room 516AB, Palais de Congrès.**

After the Hurricanes - The Contaminants Left Behind in New Orleans: USGS researchers measured numerous semivolatile organic compounds in street floodwater mud and Lake Pontchartrain sediment samples collected in September and October 2005 after the levee breaches caused by Hurricane Katrina and subsequent flooding from Hurricane Rita. These compounds include compounds organochlorine pesticides, PCBs, PAHs, current-use pesticides, anthropogenic indicator compounds (AICs), and pharmaceuticals (in the mud only). Contaminant concentrations in street mud varied substantially and for some - including PAHs, some AICs, and four termiticides - were highest at several sites near downtown New Orleans when compared with other locations (Chalmette, Ninth Ward, Slidell, Rigolets). USGS researchers found that the highest concentrations of urban-related compounds (such as chlordane and PAHs) in lakebed sediments exceeded average concentrations in U.S. urban lakes and sediment quality guidelines, but were not markedly dissimilar to historical values or to those reported from other urban areas. The highest concentrations were limited to within a few hundred meters of the 17th Street Canal outlet into the lake. This research suggests that the impacts of the hurricanes on the sediment history of Lake Pontchartrain are most likely transitory and confined to a relatively small geographic region. **For more information, contact W. T. Foreman at wforeman@usgs.gov or 303-236-3942. SETAC presentation is on Thursday, Nov. 9, 10:40 a.m., Room 516C, Palais de Congrès.**

Rocky Mountain High -- Mercury in Cold Environments of the Western United States: Atmospheric deposition of mercury in remote areas

in the Western United States is sufficient to pose a risk to human and ecosystem health at sites favorable for methylation, a process in which mercury in the environment is converted into a highly toxic form that accumulates in organisms and is amplified up the food chain. USGS researchers and partners measured mercury in snowpack samples during 2003-2005 as part of the National Park Service Western Airborne Contaminants Assessment Project. Eight high-altitude, high-latitude sites were selected for study in or near national parks in Colorado, Montana, California, Oregon, and Alaska. Mercury levels were lowest in the North Cascades, highest in the Rocky Mountains, and were related to the amount of particulate carbon in the snow, with both found at higher levels in forested sites than in open meadows. Seasonal variations were lowest in Denali National Park and highest in Olympic National Park. Mercury concentrations were higher during the warm season than the snow season. Total annual fluxes of mercury were as high as 10 mg m-2 at some sites in the Rocky Mountains, which receive mercury deposition equal to that in the Upper Midwest or Northeast. Global and regional sources of mercury emissions contribute to its deposition, with regional sources likely contributing more in the Rocky Mountains, where there are more upwind sources of emissions. **For more information, contact Don Campbell at Donald.Campbell@usgs.gov or 303-236-4882, ext. 298. SETAC presentation is on Thursday, Nov. 9, 8 a.m.- 7 p.m., Exhibit Hall, Palais de Congrès. Poster #934.**

Contaminants Lower Reproductive Health of Gila River Fish: Downstream of Phoenix, southern Arizona's Gila River is primarily recharged by irrigation return water, storm water, and wastewater treatment plant effluent, and fish and aquatic invertebrate habitats are degraded. Largemouth bass, common carp and channel catfish from the Gila had elevated levels of organochlorine pesticides, many of which have been associated with estrogen-like effects in fish. Reproductive biomarkers, including gonad size and hormone concentrations, were notably different in fish from the Gila River when compared to fish from the Colorado River, indicating that organochlorine contaminants may be affecting the reproductive health of fish populations in the Gila River downstream of Phoenix. **For more information, contact Jo Ellen Hinck at jhink@usgs.gov or 573-876-1808. SETAC presentation is on Thursday, Nov. 9, 2:30 p.m., Room 517B, Palais de Congrès.**

Aquatic Herbicides May Benefit Invasive Aquatic Species: Aquatic plants are frequently exposed to low-levels of agricultural herbicides at concentrations less than those known to cause adverse effects in the laboratory. Laboratory studies have shown that low levels of herbicide exposure can actually increase growth rates of aquatic plants due to physiological stress adaptations. Scientists from the U.S. Geological Survey and the University of Manitoba studied whether low levels of herbicides in aquatic systems may actually aid non-native invasive aquatic plants, allowing them to out-compete or displace more desirable native aquatic plant species. Researchers studied the effects of atrazine herbicide on both native and non-native, invasive aquatic plants in experimental ponds over a period of 42 days. Results confirmed that lower levels of atrazine could actually stimulate growth of some invasive species. Some invasive aquatic plant species were less sensitive to atrazine than a common native aquatic plant species. Although the invasive species did not totally displace the native species, the results indicate that some herbicides may have the unintended consequence of benefiting non-native invasive species that may compete with native aquatic plant species. Aquatic plants are critical components of aquatic ecosystems by providing habitat and energy sources for many fish and invertebrates. **For more information, contact James F. Fairchild at jfairchild@usgs.gov or 573-876-1871. SETAC presentation is on Wednesday, Nov. 8, 10:20 a.m., Room 516AB, Palais de Congrès.**

Wastewater Issues Get Wormy: Recent research indicates that earthworms may be an important initial step by which organic contaminants could enter the terrestrial food web. Wastewater treatment plants process millions of gallons of mixed solid and liquid human waste daily, returning treated effluent to surface and ground water and disposing of the residual sludge. Roughly half of the many thousands of dry tons of treated sludge (usually referred to as biosolids) generated annually in the U.S. are applied to agricultural soils as a nutrient-rich soil amendment. Recent USGS research has identified a wide variety of organic contaminants (such as disinfectants, pharmaceuticals, synthetic fragrances, and plasticizers) that can be present in biosolids, often in concentrations tens to thousands of times higher than found in treated liquid waste. One concern related to the practice of land application of biosolids is whether any of these organic contaminants find their way into soil-dwelling organisms. To address this concern, USGS and Eastern Washington University scientists collaborated on a study of earthworms collected from agricultural soils in the Midwest and Western United States that had been exposed to land-applied biosolids. The samples were analyzed for a diverse array of pharmaceuticals and other organic contaminants (77 target compounds were measured). Soil and earthworm samples were collected from select agricultural fields early and late in the growing season. Thirty-one compounds including triclosan (household disinfectant), several fragrances, caffeine, and fluoxetine (the antidepressant Prozac) were detected in earthworms from biosolid-applied fields, with tissue concentrations ranging from 100's to 1000's of micrograms per kilogram (parts per billion). These results demonstrate that earthworms can accumulate a range of these chemically diverse organic contaminants within their tissues, and may be an important initial step by which these compounds could enter the terrestrial food web. **For more information contact Ed Furlong, USGS, at efurlong@usgs.gov or 303-236-3941, and Chad Kinney, Eastern Washington University, at ckinney@mail.ewu.edu or 509-359-7932. SETAC presentation is on Thursday, Nov. 9, 4:10 p.m., Room 516 AB, Palais de Congrès.**

Toxicity Tests for Endangered Mussels: The United States is home to more mussel species than any other country in the world. Despite the diversity of mussels found in the country, no other widespread group of animals in North America is as imperiled or has faced as many extinctions. The abundance and variety of mussels have declined sharply over the past century, but the cause of mussel decline is not well understood. Researchers at the USGS-Columbia Environmental Research Center, in cooperation with other government agencies, academia, and private industry, are developing the first standardized toxicity tests using several life stages of freshwater mussels to assess the effects that pollution may have on these declines. Mussels are filter feeders that readily accumulate toxins. Results of this ground-breaking work indicate

that water quality criteria for individual chemicals established for the protection of aquatic organisms may not be adequately protective of sensitive stages of freshwater mussels. **For more information, contact Ning Wang, nwang@usgs.gov or 573-441-2946. SETAC presentation is on Thursday, Nov. 9, 8 a.m.-7 p.m., Exhibit Hall, Palais de Congrès. Poster # 1056**

Toxic Tango: Interactions of Mercury and Selenium on Bird Embryos: Mercury and selenium are common environmental contaminants that sometimes occur together at elevated levels in bird eggs. Both have been associated with reproductive impairment in birds, in particular by embryonic death and deformities. Although a lot is known about the toxicity of these two contaminants by themselves in eggs, little is known about potential toxic interactions when they occur in the same egg. USGS research indicates that combining the contaminants had a worse effect on mallard embryos than either one did separately. Follow-up studies, however, revealed that combined effects of these two contaminants may vary by species and exposure amount. **For more information, contact Gary Heinz at gary_heinz@usgs.gov or 301-497-5711. The SETAC presentation is on Wednesday, Nov. 8, 10 a.m., Room 511AD, Palais de Congrès.**

Contaminants Affect Over-Winter Survival of Swallows: The effects of chronic contaminant exposure on over-winter survival of birds are largely unknown. These studies are difficult to carry out because suitable bird species may not occur in contaminated locations, there may be insufficient number of breeding birds, they may be difficult to capture, or the species may to too long-lived to study within a reasonable time. Tree swallows overcome many of these research problems. Large numbers of breeding birds can be attracted to a site because they will readily nest in man-made nest boxes. They are also relatively easy to capture, return to the same breeding site year after year, and are short lived. The Housatonic River in western Massachusetts is extremely contaminated with polychlorinated biphenyls (PCBs) and PCB concentrations in swallow eggs are associated with decreased reproductive success. USGS researchers studied the effect on over-winter survival of chronic exposure to PCBs for 5 years on the Housatonic River to determine if adult swallow survival was reduced in this highly contaminated environment. Researchers found that annual over-winter survival was reduced significantly by about 5 percent in females that nest at the most contaminated sites. **For more information contact Christine M. Custer, ccuster@usgs.gov or 608-781-6247. SETAC presentation is on Monday, Nov. 6, 2:10 p.m., Room 517 B, Palais de Congrès.**

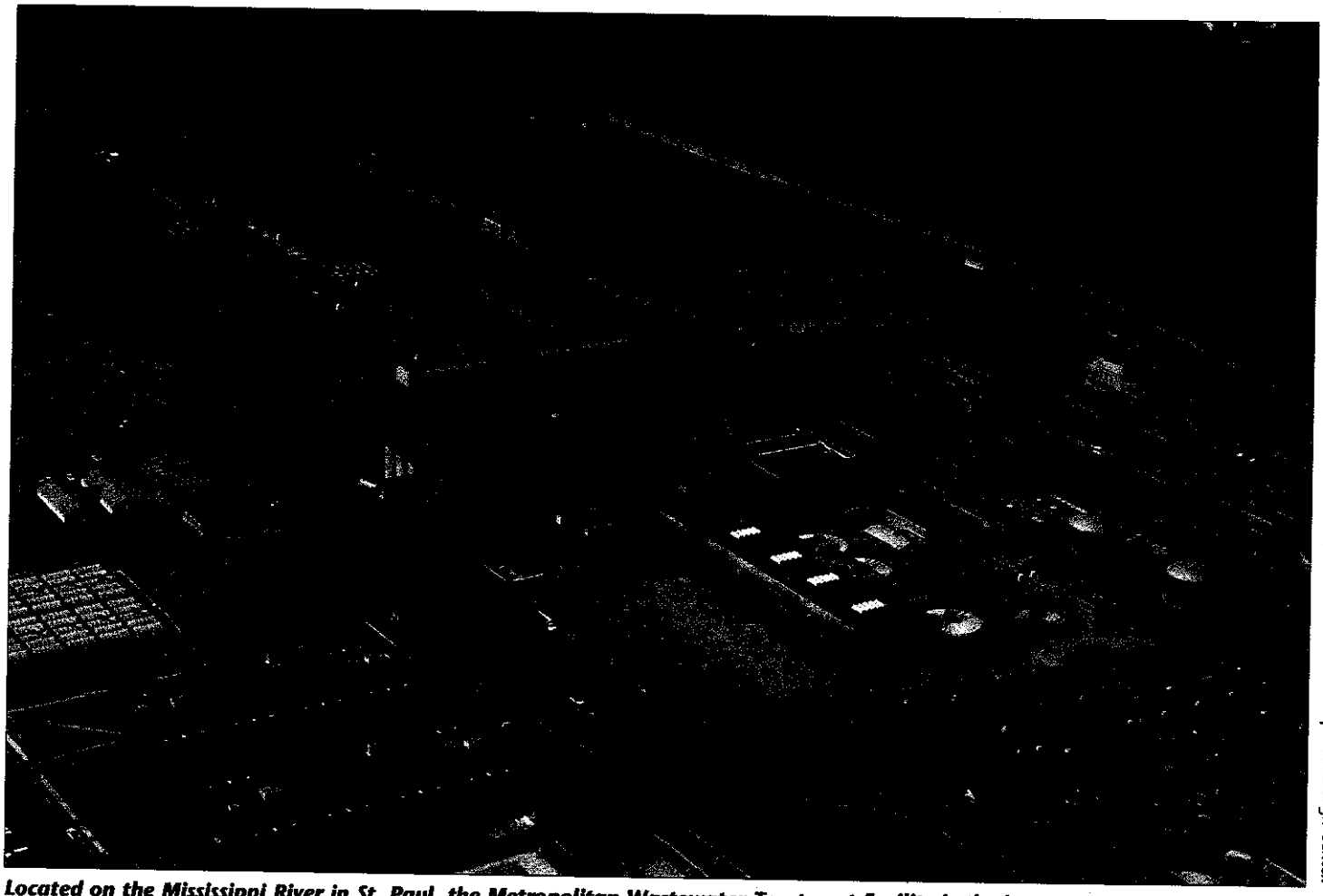
Fungicides: Analysis, Fate, and Toxicity: The recent spread of Asian soybean rust to North America has increased interest in fungicides to combat this scourge. Despite decades of agricultural and urban use, relatively little data are available on the fate and effects of fungicides in the aquatic environment. One of the most used fungicides in the United States, chlorothalonil, has been used for over 50 years for a variety of applications. Other fungicides (azoxystrobin, myclobutanil, propiconazole, pyraclostrobin, and tebuconazole) have been recently registered for treatment of soybean rust and are rapidly increasing in use. Some of these fungicides are highly toxic to fish and other aquatic life. Fungicides are often not included in monitoring programs, although fungicides and their degradates have been detected in water, sediments, air and rainfall at concentrations that can cause adverse effects to aquatic organisms. Effective monitoring of fungicide concentrations is required to understand if increasing use will result in increasing stream concentrations. The focus of this session will range from older fungicides such as chlorothalonil to newer fungicides such as the triazoles and strobilurins. Topics will include analysis of fungicides and their degradates, environmental occurrence, degradation pathways, modes of action, and toxicity to aquatic organisms. **For more information, contact Kathryn Kuivila at kkuivila@usgs.gov or 916-278-3054. The symposium, which is sponsored by the U.S. Geological Survey and Mississippi State Chemical Laboratory, will take place on Wednesday, Nov. 8, in room 510 BD, Palais de Congrès, from 8:00 - 11:40 a.m.**

How Much is Too Much? Mercury Thresholds for Common Loon Eggs: Assessing the ecological risk of mercury exposure to fish-eating wildlife is a priority issue for federal and state resource management agencies. Atmospheric mercury deposition has increased due to industrial activities exposing fish-eating wildlife populations in New England, coastal Atlantic states, the Southeast and the Upper Midwest to elevated mercury in their prey. The USGS, Wisconsin Department of Natural Resources, and the University of Wisconsin have conducted research to generate a scientifically defensible common loon/mercury risk assessment model. The work focused on the common loon because this species is sensitive to the toxic effects of mercury and has the greatest risk of mercury exposure among wildlife species on inland (non-marine) North American aquatic systems. A critical component of the model is determining the level of mercury in loon eggs that poses a population level risk. In 2005 and 2006, researchers conducted a study to better characterize methylmercury exposure in eggs of Wisconsin common loons and to determine the level of exposure in eggs that reduces fitness and survival of loon embryos and resultant chicks. Blood mercury levels in a sample of Wisconsin loon chicks indicated mercury exposure in some chicks rivaled that of adult birds during the breeding season. Blood mercury concentrations rapidly declined in growing chicks, such that by six weeks of age blood mercury levels were about 6 percent of levels at hatch. Reduced embryo survival was evident at an egg content concentration of mercury that is representative of what is often found on low pH lakes in northern Wisconsin, although sample sizes are small. **For more information, contact Kevin Kenow at kkenow@usgs.gov or 608-781-6278. SETAC presentation is on Wednesday Nov. 8 at 8:40 a.m., Room 511 AD, Palais de Congrès.**

Links and contacts within this release are valid at the time of publication.

Municipal Wastewater Treatment: A Novel Opportunity to Slow the Proliferation of Antibiotic-Resistant Bacteria?

by Timothy M. LaPara, Sara J. Firl, Leslie J. Onan, Sudeshna Ghosh, Tao Yan, and Michael J. Sadowsky



Located on the Mississippi River in St. Paul, the Metropolitan Wastewater Treatment Facility is the largest treatment plant in Minnesota, averaging 180 million gallons of wastewater daily from 62 communities and 800 industries.

The discovery of antibiotics and their subsequent application to clinical medicine is one of the outstanding scientific achievements of the twentieth century. The tale of how antibiotics were discovered is one of scientific legend: Sir Alexander Fleming astutely recognized that a contaminated Petri dish actually contained a bacteria-killing mold. For his discovery of penicillin, Fleming shared the 1945 Nobel Prize in physiology/medicine with Sir Howard Florey and Ernst B. Chain.

The unique feature of penicillin (and other antibiotics) is not merely that it kills bacteria—there are many compounds that have such a capability—but that it specifically affects bacteria. This key feature is absolutely critical for the medical application of antibiotic therapy. Antibiotics administered to humans are lethal to disease-causing bacteria but do not impact the patient. This is possible because antibiotics act on features of the bacterial cell that are absent in humans. For example, penicillin prevents the formation of new bacterial wall materials; human cells do not even contain a cell wall.

During the last half-century, antibiotics have become pervasive in human medicine. Since the discovery of penicillin, a plethora of new antibiotics, semi-synthetic antibiotics, and synthetic antibiotics (antibacterials) have been discovered or developed (Table 1). These new drugs target different features of bacterial physiology, thus expanding the range of bacterial species that can be successfully treated with antibiotics. Antibiotics are also used extensively in agriculture and for other non-medical purposes. Low doses of antibiotics are often included in animal feed to promote growth and increase weight gain, as well as prevent the onset of

Table 1. Major Classes of Antibiotics and Antibacterials, and Representative Drugs in Each Class

Class	Representative Drug(s)
β-lactams	Penicillin, Amoxicillin, Methicillin
Macrolides	Tylosin, Erythromycin
Tetracycline	Tetracycline, Oxytetracycline
Ansamycins	Rifampin
Quinolones/fluoroquinolones	Nalidixic acid, Ciprofloxacin

disease. Although reliable estimates are difficult to obtain, most scientists believe that approximately 70% of all antibiotics are used for agricultural purposes.

In this article, we report on a research project that investigated the role of municipal wastewater treatment facilities in the spread or control of antibiotic-resistant bacteria. The project was supported by a grant from CURA's Faculty Interactive Research Program, as well as grants from the Undergraduate Research Opportunity Program at the University of Minnesota. We hypothesized that the disinfection processes most treatment facilities use would adequately inactivate antibiotic-resistant bacteria in wastewater. However, our research suggests that treatment facilities, which are primarily designed to protect water quality, do not adequately prevent resistant bacteria from being released into the environment. We conclude that relatively simple changes in the design, operation, and regulation of municipal wastewater treatment facilities could substantially reduce the release of these bacteria and, we hope, slow the proliferation of antibiotic resistance among bacteria appearing in clinical patients.

A Brief History of Antibiotic Resistance

Antibiotic-resistant bacteria were discovered soon after the medical use of penicillin began. At the time, the development of resistant bacteria was largely viewed as inconsequential. If a patient had an infection that a resistant bacterium caused, then an alternative antibiotic was always available for effective treatment. However, some foresighted scientists warned of the pending problem of antibiotic resistance. In his Nobel acceptance speech, Alexander Fleming himself cautioned doctors about the danger of giving an "underdosage" of penicillin, noting: "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body . . . Moral: If you use penicillin, use enough."

The pioneering work of Stuart Levy in the 1970s was also informative. Levy was concerned that antibiotic use in agriculture at subtherapeutic concentrations could lead to the proliferation of antibiotic resistance. His research demonstrated that tetracycline-resistant bacteria were present in the droppings of chickens within one week after tetracycline was included in their feed. More alarming, however, the bacteria in chickens that were fed only tetracycline became resistant to multiple antibiotics within two weeks. Finally, multiple-antibiotic-resistant bacteria dominated the fecal material of farmers working with these chickens within five months, even though the farmers had received no antibiotics during the study.

It was not until the 1980s, however, when a multiple-drug-resistant form of tuberculosis emerged, that scientists became concerned about antibiotic resistance. Multiple-drug resistance soon appeared among other pathogens, particularly among nosocomial (hospital-acquired) infections. Today, 40% to 60% of nosocomial *Staphylococcus aureus* infections are methicillin resistant. The problem of antibiotic-resistant bacteria is particularly significant for immunodeficient patients, who are susceptible to a broader array of pathogens, many of which are multiple-drug resistant.

Many believe that the problem is linked to excessive antibiotic use in hospitals, making them a "hot spot" for resistant bacteria. Unfortunately, mounting evidence refutes this perspective. Community-acquired methicillin-resistant *Staphylococcus aureus* is becoming far more prevalent, particularly at public gymnasiums, where insufficiently sanitized towels are prevalent.

The Development of Antibiotic Resistance in Bacteria

The simplest method by which bacteria become resistant to antibiotics is via a *point mutation* of the deoxyribonucleic acid (DNA) within their genome. Point mutations are typically lethal to the bacterium or have no effect, but on rare occasions these mutations are beneficial (from the bacterium's perspective) and allow the organism to become resistant to antibiotics. Point mutations, however, are not the major concern with respect to antibiotic resistance. This form of bacterial evolution is slow and random, and it is unlikely that bacteria could rapidly achieve resistance to multiple antibiotics via point mutations alone.

Ultimately, the proliferation of antibiotic resistance is caused by the propagation of specific genes that allow bacteria to defy the lethal effects of antibiotics. These *antibiotic resistance genes* are probably not new, but likely result from millions of years of evolution, during which time bacteria have developed many mechanisms to survive the dangers that the world thrusts upon them. Certainly, many of these genes were specifically developed to counteract antibiotics, which are, after all, naturally occurring compounds. Many antibiotic resistance genes, however, likely are subtle adaptations of genes that provide protection against other toxic compounds. For example, there is a strong correlation between genes that encode for resistance to heavy metals and antibiotic resistance genes.

The existence of antibiotic resistance genes, however, is insufficient to explain the global proliferation of resistance. Bacteria also harbor other genes that are specifically designed to help bacteria rapidly evolve—genes designated as *evolution genes* by 1978 Nobel Prize winner Werner Arber. Evolution genes allow bacteria to rapidly develop new genes (usually by manipulating preexisting genes) and to spread them

throughout the bacteria population. The evolution genes that allow *lateral gene transfer* are perhaps the most important class of evolution genes with respect to antibiotic resistance. Lateral gene transfer is the exchange of genetic material between different bacteria; it allows bacteria to share their abilities to resist antibiotics. This is believed to be the principal mechanism by which similar resistance genes are found throughout the world among many different species of bacteria.

During the last 20 years, scientists have also recognized the importance of *integrons*, another type of evolution gene. Integrons are responsible for integrating resistance genes into the genomes of bacteria, and then controlling the expression of these resistance genes. Because of this unique ability, integrons can be viewed as a genetic "luggage rack" in which different genes can be kept until they are needed. Integrons are a key component in the development of multiple-antibiotic-resistant bacteria because they allow bacteria to easily accumulate numerous genes.

Responding to Antibiotic Resistance

Although scientists have known about antibiotic-resistant bacteria for almost as long as they have known about antibiotics, the assumption was that new antibiotics would be discovered or developed faster than bacteria could become resistant. The discovery of new antibiotics, however, has slowed substantially since the 1960s. In fact, most "new" antibiotics are merely subtle modifications of previously existing ones and have little impact on bacteria that are already resistant.

During the last decade, therefore, there has been a considerable effort to restrict antibiotic use to only those applications where antibiotics are appropriate. Physicians are now reminded to avoid prescribing antibiotics for viral infections such as influenza and the common cold. Likewise, patients are carefully instructed to follow prescription guidelines so that enough of the drug is administered to limit the development of resistant bacteria. There is also increasing pressure to limit or eliminate non-medical use of antibiotics and antibacterials. As noted above, a substantial fraction of all antibiotics are used in agriculture at subtherapeutic concentrations. Although the United States appears to be far from prohibiting this practice, the European Union is banning subtherapeutic antibiotic use in agriculture in 2006. Although more controversial, many scientists—led by the Alliance for the Prudent Use of Antibiotics—are recommending the elimination of triclosan and other antibacterials from liquid hand soap, toothpaste, and other common household items.

A New Paradigm: Resistance Control?

The current situation with respect to antibiotic resistance is bad and the future is bleak. The discovery of new drugs has slowed to a trickle—a problem that will only worsen as pharmaceutical companies devote a greater fraction of their research and development budgets to less essential drugs (e.g., Botox, Viagra). Simultaneously, the ever-increasing use and misuse of antibacterials in common household products can only exacerbate the problem.

From our perspective, current efforts to reduce the spread of antibiotic resistance are an excellent first step. Certainly, our historically indiscriminate use of antibiotics needs to end. The more important issue is to identify novel approaches to limit the spread of antibiotic resistance. Our intention in undertaking this research, therefore, was to take a different approach to solving the problem of antibiotic resistance. We started by asking some simple yet fundamental questions about the proliferation of antibiotic-resistant bacteria.

First, where do the majority of antibiotic-resistant bacteria originate? Certainly, many bacteria are naturally resistant, but the majority of antibiotic-resistant bacteria result from antibiotic use. Therefore, people and animals taking antibiotics are most likely the primary source of antibiotic-resistant bacteria.

Second, how do resistant bacteria spread throughout the world after they originate inside a person? Humans actually contain about 10 times more bacterial cells in their bodies than they do human cells. The overwhelming majority of these bacterial cells reside in our gastrointestinal tracts, and most are released from the body during defecation.

Having asked and answered these two simple questions, we then inferred that municipal wastewater treatment plants, which handle virtually all human toilet waste in large municipalities (in rural areas, septic systems are more commonly used), would be critical in reducing the spread of antibiotic resistance. We hypothesized that municipal wastewater treatment facilities could adequately control the release of antibiotic-resistant bacteria to the world.

Municipal Wastewater Treatment Facilities: How Do They Work?

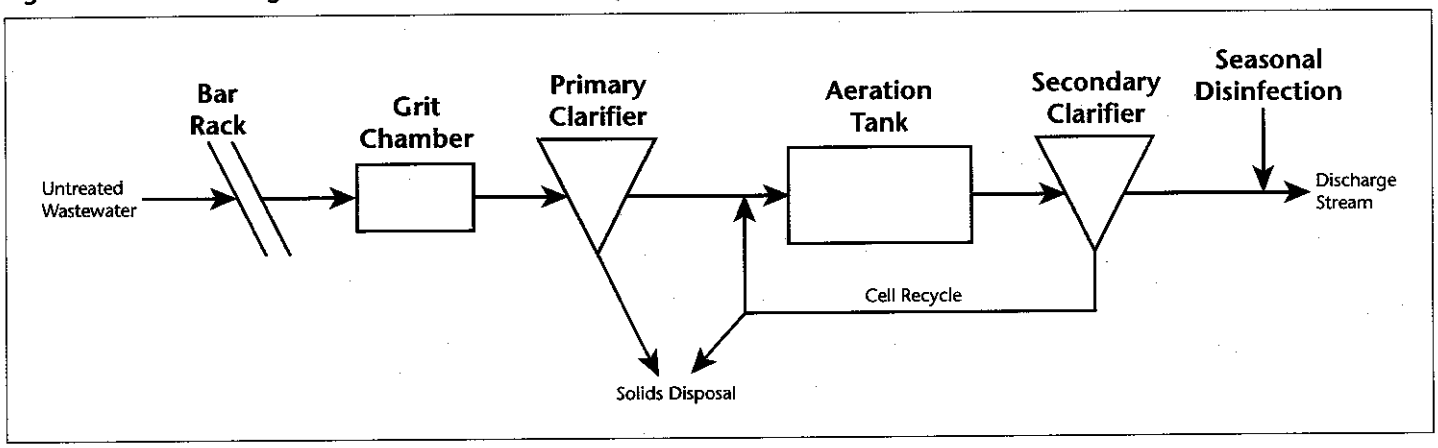
Municipal wastewater treatment facilities are primarily designed and operated to protect the environment. Municipal wastewater treatment facilities remove readily biodegradable compounds from sewage. Although there is relatively little in human sewage that is toxic, these biodegradable compounds are of environmental concern because if they were released untreated, they would biodegrade in the environment, resulting in oxygen depletion leading to septic conditions. Municipal wastewater treatment facilities, therefore, allow surface waters to maintain high dissolved oxygen levels, improving their aesthetic and recreational use value, as well as their ability to support healthy populations of fish and other aquatic fauna.

Although all municipal wastewater treatment facilities are unique, most are similar in design and involve a common series of unit operations (Figure 1). The first few unit operations, called primary treatment, are designed to remove particles from the wastewater. The bar rack removes large particles (greater than 1 inch), whereas the grit chamber removes sand and other dense, rapid-settling particles. The primary clarifier is a quiescent settling zone that allows organic particles to settle or float so that they can be removed. These primary treatment operations account for about 50% of the treatment that occurs.

The next unit operation, the aeration tank, is designed to remove dissolved organic compounds (which are readily biodegradable) from the wastewater by creating conditions favorable for the growth of bacteria. The tank works by bubbling air through the wastewater, allowing bacteria to metabolize pollutants that are present. Because these bacteria grow in excessive quantities, they must be removed from the wastewater. This is accomplished by the next unit operation, which is a quiescent settling chamber called the secondary clarifier. The combination of the aeration tank and the secondary clarifier is called the activated sludge process, which is the most common technology for the secondary treatment of wastewater.

Following primary and secondary treatment, the quality of municipal wastewater is quite good—not yet potable (i.e., safe to drink), but often

Figure 1. Schematic Diagram of a Conventional Municipal Wastewater Treatment Process



Note: Individual unit operations are labeled in bold lettering.

as good as or better than the quality of many lakes and rivers. This treated wastewater, however, still contains pathogenic bacteria that could make people sick if they accidentally ingested the water. Municipal wastewater treatment facilities, therefore, perform a final treatment step in which the treated wastewater is disinfected to help reduce the number of disease-causing microbes. Disinfection is required only when recreational use of the receiving stream is a reasonable expectation. In Minnesota, for example, wastewater treatment facilities usually disinfect their wastewater only from April to November.

In addition to treating the wastewater, municipal wastewater treatment facilities must deal with the solid residues that the primary and secondary clarifiers collect. These solid residues are readily biodegradable organic materials that are most commonly treated by a process called *anaerobic digestion*. The conventional anaerobic digestion process, which largely mimics our gastrointestinal tracts (hence the "digestion" nomenclature), is kept free of oxygen and operated at 98.6°F. Following digestion,

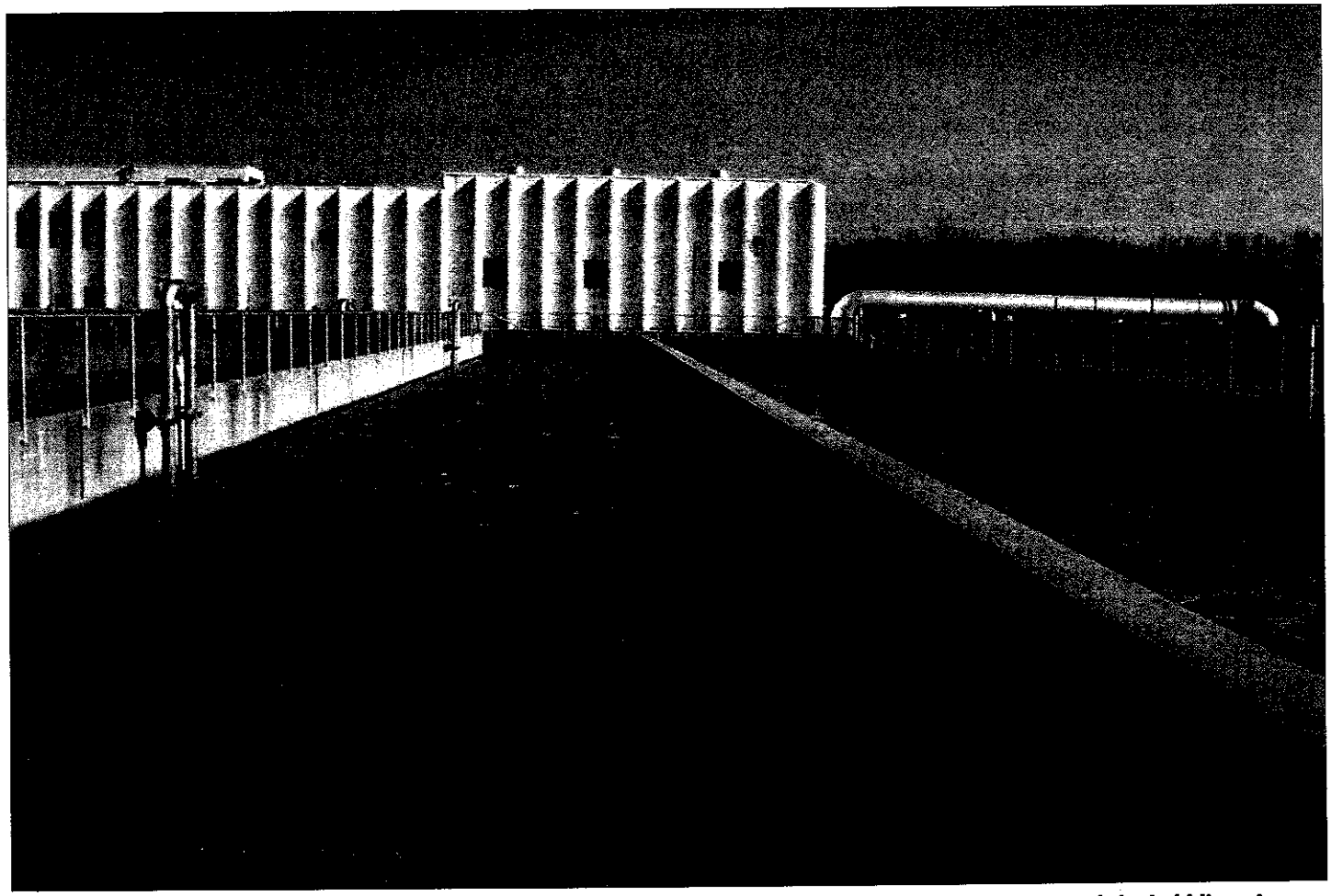


Photo courtesy of Timothy LaPara

An aeration tank at a municipal wastewater treatment plant. The tank removes dissolved organic compounds by bubbling air through the wastewater, creating favorable conditions for the growth of bacteria that are capable of metabolizing pollutants.

the treated wastewater solids are either applied to farmland as a fertilizer and soil conditioner, or sent to a landfill for disposal. The former alternative is preferred as a "sustainable" practice, whereas landfill space is finite.

Because anaerobic digestors operate at conditions similar to the human body, they are not particularly good at eliminating human pathogens. Numerous alternative treatment technologies, therefore, have been developed to better treat wastewater solids. All of these alternative treatment technologies are more expensive, however, and thus municipalities do not frequently use them. Perhaps the most attractive treatment alternative is thermophilic anaerobic digestion, which operates almost identically to conventional anaerobic digestion, except that it operates at sufficiently high temperatures (greater than 110°F) to kill most human pathogens.

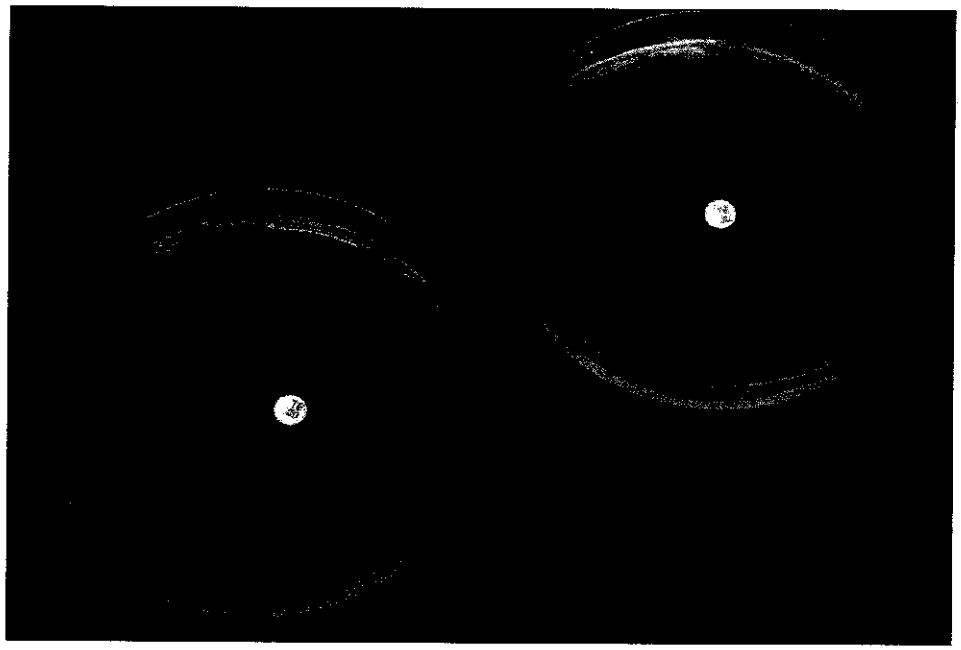


Photo © Steve Schneider, 2006

Bacteria growing on petri dishes that include disks treated with the antibiotic tetracycline. The bacteria growing on the left petri dish were obtained from treated wastewater and are resistant to tetracycline, as shown by the ability of the microbes to grow near the white disk. The bacteria growing on the right petri dish are a tetracycline-sensitive strain of* E. coli. *The circular ring around the disk shows that these microbes cannot grow in the presence of tetracycline.

Methodology and Analysis

The first goal of our project was to determine the extent to which municipal wastewater treatment facilities prevent the release of antibiotic-resistant bacteria. There are two potential paths by which antibiotic-resistant bacteria can escape a municipal wastewater treatment facility. The most obvious is in the treated wastewater. Our research, therefore, investigated the importance of secondary clarification and disinfection in preventing the release of antibiotic-resistant bacteria from the aeration tank. Resistant bacteria could also be released in the solids collected during primary treatment and from the secondary clarifier. Our research, therefore, compared the effectiveness of two variations of conventional anaerobic digestion and thermophilic anaerobic digestion at destroying resistant bacteria.

We investigated the efficacy of wastewater disinfection at the Metropolitan Wastewater Treatment Facility in St. Paul. This facility is very large, treating an average of 180 million gallons of sewage each day. Typically, the quality of treatment from the Metropolitan plant is top-notch, and the facility regularly wins state and national awards for operational excellence. Throughout the year, we quantified about 100,000 (10^5) tetracycline-resistant bacteria per milliliter of water in the aeration tanks at the Metropolitan plant. From the treated wastewater, we quantified about 300 tetracycline-resistant bacteria per milliliter in the winter (i.e., when disinfection was not performed) and about 30 tetracycline-resistant bacteria per milliliter during the summer (i.e., during the disinfection period). That is, about 99.6% and 99.97% of the resistant bacteria in the aeration tanks are removed in the winter and summer, respectively. Although this removal efficiency might seem sufficient, 30 bacteria per milliliter translates to more than 10 trillion (10^{13}) tetracycline-resistant bacteria released each day from this treatment facility into our waterways.

We also investigated the efficacy of anaerobic digestion at the Western Lake Superior Sanitary District (thermophilic process) and the Empire Wastewater Treatment Facility (conventional process), which are located in Duluth and Farmington, respectively. Both of these plants have also earned awards for operational excellence. We again detected about 100,000 (10^5) tetracycline-resistant bacteria per milliliter in the waste stream entering the anaerobic digestors at each of these treatment facilities. However, we were unable to detect any tetracycline-resistant bacteria in the waste stream leaving the anaerobic digestors at these two treatment facilities, in part because the research method we used is unable to detect levels of tetracycline-resistant bacteria below 1,000 (10^3) per milliliter of sludge solids. However, this suggests that both anaerobic digestion processes were able to inactivate at least 99% of antibiotic-resistant bacteria. We are currently attempting to develop an alternative technique to measure the efficiencies by which these anaerobic digestors inactivate antibiotic-resistant bacteria.

The second goal of our research was to characterize the antibiotic-resistant bacteria in sewage. From the three treatment facilities, we isolated and identified 173 bacterial strains that were resistant to tetracycline. All of these bacterial strains were pathogenic (disease-causing—e.g., *Shigella* or *Klebsiella* spp.), possibly pathogenic (e.g., *Escherichia coli*), or non-pathogenic but related to pathogens (e.g., *Citrobacter* spp.). In more than 50% of these bacteria, we also detected at least one gene encoding for tetracycline resistance.

Based on these initial data, we then studied 14 different tetracycline-resistant bacterial strains in more detail. All 14 of these strains contained an integron and were resistant to at least three different antibiotics (we tested resistance to amoxicillin, ampicillin, chlortetracycline, enrofloxacin, erythromycin, sulfamethoxazole, trimethoprim, and tylosin). We also tested these bacteria for lateral gene transfer. Although this work is still ongoing, many of these bacterial strains are capable of

exchanging with other bacteria a gene encoding for tetracycline resistance.

Following our work on tetracycline-resistant bacteria, we isolated an additional 65 different bacteria that were resistant to ciprofloxacin. Ciprofloxacin is a relatively new antibiotic and there is not much known about bacterial resistance to it. Once again, we found that all of these bacterial strains were pathogenic, possibly pathogenic, or related to pathogens. We then focused our efforts on 11 of these strains, all of which were resistant to at least four different antibiotics. About half of these strains contained an integron or a gene encoding for resistance to tetracycline. Although this work is also ongoing, our analysis revealed that several of these strains were capable of laterally exchanging genes encoding for resistance to ciprofloxacin.

Conclusion and Policy Recommendations

Our research has demonstrated that extremely high numbers of antibiotic-resistant bacteria are released from municipal wastewater treatment plants, even when disinfection is performed. Our original hypothesis was that disinfection would adequately inactivate antibiotic-resistant bacteria in treated municipal wastewater, and that an outcome of our work would be to encourage the implementation of year-round disinfection. Instead, we learned that although a 99% inactivation looks encouraging, 1% of a very large number (10^{15}, or 1 quadrillion) still represents a very large number (10^{13}, or 10 trillion) of antibiotic-resistant bacteria that are released from the Metropolitan Wastewater Treatment Facility each day.

The bacteria that we studied were all pathogens or related to pathogens and all were resistant to multiple antibiotics. A substantial fraction of these bacteria (greater than 50%) harbored genes encoding for tetracycline resistance. These bacteria frequently harbored integrons (genes that allow bacteria to accumulate multiple genes for antibiotic resistance) and some of them were capable of transferring their resistance to other bacteria. The frequency of lateral gene transfer of ciprofloxacin resistance, which occurred in more than 40% of the strains we studied, is particularly worrisome because this trait is typically very rare (less than 1%) among clinical strains of ciprofloxacin-resistant *E. coli*. Simply put, the bacteria that we detected in municipal wastewater are some of the most resistant bacteria ever studied. There is a substantial need, therefore, to prevent these organisms from reaching the environment.

At first glance, the most obvious solution to the problem of antibiotic-resistant bacteria in treated municipal wastewater would be to require more stringent disinfection. The majority of municipal wastewater is disinfected using chlorine, which poses a security risk (chlorine gas is very dangerous) and generates disinfection by-products that are known or suspected carcinogens. Although we recommend a policy shift to include year-round wastewater disinfection, we do not recommend that more stringent disinfection regulations be imposed because of these unwanted consequences.

Instead, we recommend that wastewater effluents be passed through a sand filter prior to disinfection. Sand filters can physically remove antibiotic-resistant bacteria from treated wastewater, but without the use of potentially dangerous chemicals. At the present time, sand filters are rarely used in wastewater treatment, but they are commonly used at drinking water treatment facilities, so the technology is well-developed and well-understood. Additional research is needed, however, to optimize the removal/inactivation of antibiotic-resistant bacteria by our proposed combination of sand filtration and effluent disinfection.

Although our research on the fate of antibiotic-resistant bacteria in anaerobic digestors was inconclusive due to the limitations of our research method, we suspect that our ongoing research will demonstrate that thermophilic anaerobic digestors achieve substantially better inactivation efficiencies than conventional technologies. This ongoing research is particularly pertinent because of a recent shift in policy that emphasizes the application of treated wastewater solids to land rather than putting these residues into landfills—that is, the "environmental friendly" practice of applying wastewater solids to land may have unexpected and undesirable consequences in terms of the proliferation of antibiotic-resistant bacteria.

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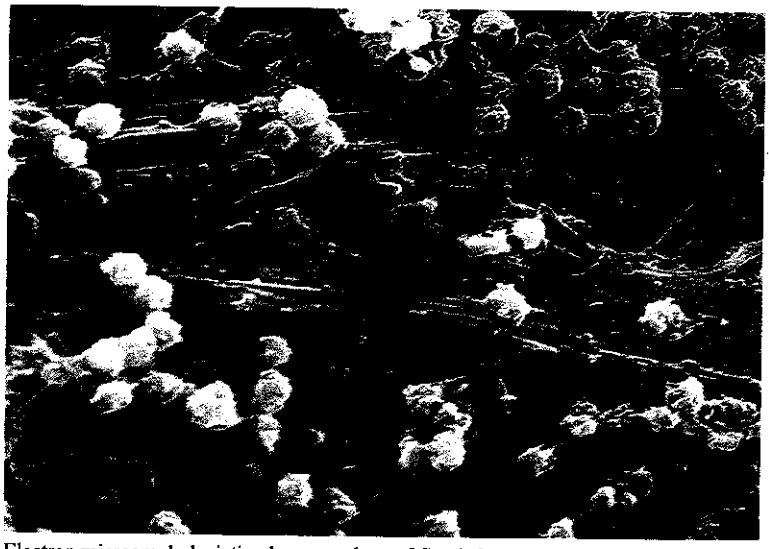
This study was supported by a grant from CURA's Faculty Interactive Research Program. The program was created to encourage University faculty to carry out research projects that involve significant issues of public policy for the state and that include interaction with community groups, agencies, or organizations in Minnesota. These grants are available to regular faculty members at the University of Minnesota and are awarded annually on a competitive basis. Additional support was provided by grants from the Undergraduate Research Opportunity Program (UROP) at the University of Minnesota.

A Microscopic Arms Race:
The Battle Against Antibiotic Resistance

LAURA STERNICK '08

In 1928, bacteriologist Alexander Fleming noticed that a culture of staphylococcus bacteria had been contaminated by mold. This rather mundane event stirred Fleming's curiosity when he noticed that the mold was dissolving the bacteria it touched. Fleming cultured the mold and found that it produced a substance that could kill several disease-causing bacteria (1), including pneumococci, streptococci, meningococci, and gonococci (2). He named the substance–the first antibiotic–penicillin, after the *Penicillium* mold that produced it (3). It would be the first of many antibiotics to revolutionize the field of medicine. After the discovery, Howard Florey and Ernst Chain studied penicillin's properties and developed the first antibiotic drugs. Penicillin was in mass production by the 1940s (1). Fleming, Florey, and Chain shared the Nobel Prize in Medicine in 1945 "for the discovery of penicillin and its curative effect in various infectious diseases" (4). The development of antibiotics is widely considered to be one of the greatest public health achievements of the past century. The use of antibiotics has led to a massive improvement in the treatment of infectious bacterial diseases and has made invasive surgical procedures much safer to perform. However, the efficacy of antibiotics has encouraged overuse. Doctors would prescribe them to treat minor infections, for which antibiotics are unnecessary. Moreover, livestock breeders give antibiotics to their animals in order to protect them from disease. The overuse of antibiotics has led to the evolution of resistant strains of bacteria. Strains now exist that elude even the strongest antibacterial agents. The World Health Organization estimates that two million people in the United States are infected with antibiotic-resistant bacteria, resulting in the deaths of 14,000 each year (5). Once an indispensable weapon in the fight against disease, antibiotics have ushered in a new public health threat.



Electron micrograph depicting large numbers of *Staphylococcus aureus* bacteria, found on the luminal surface of an indwelling catheter. The sticky looking substance is composed of polysaccharides, and is known as "biofilm." This biofilm has been found to protect the bacteria from attacks by antimicrobial agents such as antibiotics. Image magnified 2363x.

Image Courtesy of CDC/Rodney M. Donlan, Ph.D.; Janice Carr

A recent article in *Scientific American* describes a report from Colorado State University about the presence of DNA that promotes antibiotic resistance in drinking and non-drinking water. Amy Pruden, the primary author of the study, chose to look for the genes that create antibiotic resistance, rather than for antibiotics themselves since this approach provided more accurate information about the amount of antibiotic resistance in these water sources. DNA samples were obtained from bacteria found in bodies of water in northern Colorado. The researchers looked for genes that conferred resistance to tetracycline and sulfonamide to bacterial hosts. According to the report, bodies of water in close proximity to urban areas or farming regions had levels of drug-resistance genes that were hundreds to thousands of times higher than those of isolated bodies of water. However, antibiotic resistance genes were found in every water source tested (5).

The study links the increase in antibiotic resistance to the overuse of drugs in humans and animals. According to *Scientific American*, "up to 95 percent of antibiotics are excreted unaltered, seeping into the environment and possibly encouraging antibiotic resistance there" (5). Microbes that are able to survive the dose of antibiotics that they encounter will be selected for survival.

The presence of antibiotic resistance genes in drinking water is particularly troubling. Water filtration systems are designed to remove bacteria and toxic minerals, rather than DNA, which can pass through undetected. Pruden and her team are currently working to develop water treatment methods using ultraviolet light, peroxides, or both to clean up contaminated water (5). Others are working to prevent antibiotics from reaching water sources in the first place. A movement to end the use of antibiotics in livestock is gaining momentum. In Europe, a January 2006 law made it illegal to give antibiotics to animals for nontherapeutic purposes. The antibiotic avoparcin, a relative of vancomycin, has been

outlawed since its overuse was correlated significantly with an increase in vancomycin resistance in microbes in the human intestine (6).

But antibiotic resistance threatens more than our water supplies. Hospitals have become breeding grounds for antibiotic-resistant bacteria. These strains are associated with increased lengths of stay, higher costs, and mortality (7). According to data from the Centers for Disease Control and Prevention (CDC), the proportion of infections that are resistant to antibiotics has increased (7). One such strain is known as methicillin-resistant *Staphylococcus aureus* (MRSA), which has become more common in recent years. In 1974, MRSA infections represented 2% of the total number of staphylococcus infections; in 1995 it was 22%; in 2004 it was about 63% (8). Ironically, it was *S. aureus* that was killed by penicillin in Fleming's original culture (3).

The most common sources of MRSA in hospital settings are patients who are infected with MRSA but who do not display symptoms. It is transmitted to others most commonly by healthcare workers who come into contact many patients and families each day. Individuals most susceptible to MRSA infection are those with severe disease, particularly those with compromised immune systems. Groups with the highest infection rates include: individuals recovering from surgery, individuals who have internal medical devices, such as urinary catheters, and hospitalized patients. (7).

In terms of pathology, MRSA produces different symptoms in patients compared to methicillin-susceptible *S. aureus* (MSSA) strains. Individuals with MRSA infections are more likely to develop symptomatic infections. Higher fatality rates are also associated with MRSA infections, compared to MSSA infections. A number of studies offer explanations for these observations. One suggested that the delayed delivery of vancomycin, which is considered the "last-hope treatment" for MRSA (6) might be responsible. The same study also mentioned the possibility that vancomycin has decreased in efficacy against the strains of MRSA in question (7). Another study hypothesized that the particular bacteremia associated with the strains of MRSA involved in the study was particularly lethal (7).

Interestingly, genetic analyses suggest that only a small proportion of the MRSA strains found in hospitals contained mutations that promote peculiar aggressiveness or persistence, which could allow them to transmit to other individuals easier. It was also found that most MRSA strains were genetically unique compared with strains prevalent in hospitals. This suggests that the MRSA strains may have been the product of MSSA strains that acquired genes for antibiotic resistance (7).

The CDC has taken steps to monitor and reduce the presence of MRSA in healthcare settings. It conducts extensive surveillance of MRSA transmission rates and has published guidelines for hospitals on how to deal with MRSA infections. CDC also conducts epidemiologic and laboratory research on MRSA in hope of finding ways to actively treat infections (9).

Some believe that the hospitals and public health organizations in the U.S. are not doing enough to prevent MRSA transmission. Experiments with new containment measures in European hospitals have succeeded. In Denmark and the Netherlands, hospitals isolate all incoming high-risk patients until lab results confirm that they are not infected with MRSA. Currently, less than 1% of staphylococcus infections in Dutch hospitals are MRSA, compared to 64% of staphylococcus infections in the U.S. (6). According to *Scientific American*, American hospitals have been hesitant to follow the European example, citing the high costs of such intense screening. However, considering that MRSA infections increase costs of care, it might be economically viable to implement an extensive screening process (6).

Physicians and researchers are engaged in an arms race with bacteria, and bacterial evolution has begun to outstrip human scientific progress. At the moment, the best defense against antibiotic-resistant bacteria is prevention of transmission. Public health agencies around the world are working to find more aggressive treatments, but unless a means of stalling evolution is found, the battle with bacteria is far from over.

CDC conducts MRSA surveillance, prevention, epidemiologic and laboratory research, and outbreak and laboratory support. For more information, see http://www.cdc.gov/ncidod/dhqp/ar_mrsa_CDCactions.html

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NewsTarget.com printable article

Originally published October 10 2006

Prescription drugs found in soil sludge used for lawns and gardens

by Jerome Douglas

(NewsTarget) According to research by Chad Kinney, assistant professor of chemistry and biochemistry at Eastern Washington University, fertilizer made from sewage sludge may be adding pharmaceuticals, flame retardants and other chemicals to the land.

Kinney's research showed that no less than nine different biosolid products were produced by municipal wastewater treatment plants in seven different states -- Washington, Arizona, Wisconsin, Kansas, Colorado, Texas and Iowa. These biosolid products were analyzed for 87 different organic wastewater contaminants, which represents a cross section of medicinal, industrial and household compounds.

These compounds are able to enter wastewater treatment plants and may be discharged without being completely metabolized or degraded -- causing them to show up in the sludge that is then processed into certain garden and yard fertilizers. In fact, 55 of the contaminants were detected in at least one biosolid product sold as lawn and garden enhancements, and 25 compounds were found in every single one of the samples.

Kinney went on to say that "No matter what biosolid we looked at, there were some of these compounds in it." His research was published in online edition of the journal Environmental Science and Technology. Kinney, who is a postdoctoral fellow at the United States Geological Survey (USGS), has the support of the USGS's Toxic Substance Hydrology Program as well, who supports his research.

Government regulators and health officials say there is no immediate risk to public health; however, the study's authors called for more research on the long-term impact on the environment. Thomas Burke -- a professor of public health policy at Johns Hopkins University in Baltimore -- believes that Kinney's research is a sobering reminder for the Environmental Protection Agency, which has promoted biosolids for decades because they contain the same nutrients found in fertilizers.

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Executive Summary

Chemicals of Concern

Iowa's First Field Research Area for Emerging Contaminants

Douglas Schnoebelen
Dana Kolpin
Larry Barber
Edward Furlong
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April 2006

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The Iowa Policy Project Executive Summary

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Chemicals of Concern

Iowa's First Field Research Area for Emerging Contaminants

By Douglas Schnoebelen, Dana Kolpin, Larry Barber, Edward Furlong, Michael Meyer and Mary Skopec

Emerging Contaminants: Chemicals of Concern

As Americans, we use a wide variety of chemicals in our homes and our jobs, whether we work at factories, on farms, or in offices. Recent research has shown compounds not previously considered contaminants are present in the environment.[1] These include human and veterinary prescription drugs, diagnostic agents, hormones, cosmetics, dyes, preservatives, detergents, and numerous other organic compounds. There are increasing concerns about the potential environmental effects that may occur from such "emerging contaminants" (ECs). ECs are defined as:

> Any synthetic or naturally occurring chemical or any microorganism that is not commonly monitored in the environment, but has the potential to enter the environment and can cause suspected adverse ecological and/or human health effects. In some cases, release of emerging chemical or microbial contaminates to the environment has likely occurred for a long time, but may not have been recognized until new detection methods were developed. In other cases, synthesis of new chemicals or changes in use and disposal of existing chemicals can create new sources of emerging contaminates.[2]

Most ECs are not routinely monitored. Indeed, water-quality monitoring in the United States is largely driven by regulations of the Clean Water Act and Safe Drinking Water Act. Over the last three decades, much of the water-quality monitoring work has focused almost exclusively on the conventional "priority pollutants," however this is only one piece of the larger environmental puzzle.[3] Recently ECs have begun to be examined in limited studies using newly developed laboratory analytical methods and techniques allowing detection at much lower levels. Furthermore, the possibility that environmental contaminants may be complex mixtures that can interact synergistically or antagonistically has increased the need to understand ECs.

In order to minimize ecologic effects from ECs, it is essential to understand how a contaminant moves and is altered in the environment. Investigations of processes influencing transport (e.g. sorption, dispersion, degradation, etc.) require a systematic evaluation of a variety of hydrologic, landscape and anthropogenic factors. The purpose of this paper is to provide a short synopsis of ECs as potential contaminants of concern and to highlight an 8-km reach of Fourmile Creek in central Iowa as an ideal research site to investigate the transport, fate and effects from an urban source of ECs.

Possible Effects of ECs: Endocrine Disruption and Antibiotic Resistance

The potential toxicological behavior from the environmental occurrence of ECs and mixtures of ECs are largely unknown. In particular, the effects of ECs on aquatic organisms are difficult to measure because concentrations of these compounds are generally low (nanogram per liter range) and, over the life of the organism produce no acutely toxic effects. However, detrimental effects to organisms from ECs may be subtle and go unnoticed until some cumulative threshold is reached. In recent years, the presence and effects of endocrine disrupting compounds (EDCs) in the environment has become an important issue.[4] The endocrine system is the "key control system" of most organisms.

The presence of low concentrations of some chemicals in the environment (e.g. natural and synthetic hormones, alklyphenols, pesticides, solvents and pharmaceuticals) could affect or damage the function of the endocrine system.[5] For example, nonylphenol (a detergent degradation product found in laundry and dish detergents), and AHTN (a polycyclic musk found in perfumes, laundry products, air fresheners and cosmetics) have been shown to disrupt reproduction and growth in fish by affecting endocrine systems.[6] A variety of ECs have been shown to bioaccumulate in fish tissue.[7] Data from laboratory experiments suggest that EDCs in the aquatic environment may impact the reproductive health of fish populations.[8] Linking EDCs to observed changes in fish populations, however, remains an open challenge.[9] As the ecological risk assessment of EDCs is in its infancy stage, less is known about potential effects to other aquatic species, yet early research suggests effects to aquatic organisms are possible.[10]

Antibiotics are an important class of pharmaceuticals and their prevalence in the last 60 years has brought dramatic and often even "miraculous" progress in fighting bacterial infections in humans and animals. In livestock farming, sub-therapeutic doses of antibiotics are often used to promote more rapid animal growth.[11] Despite their widespread use, antibiotics have only recently received attention as environmental contaminants. However, the increase of resistant bacterial strains and the spread of bacterial resistance have become a worldwide concern.[12] Concerns also exist for antibiotic use and increasing antibiotic resistance in livestock confined feeding operations.[13] Many antibiotics are only partially metabolized after administration to humans or animals.[14] Concentrations of select antibiotics in animal manure have been reported at milligrams per liter levels (they are typically reported at parts per billion levels).[15]

Antibiotics can reach streams and ground water via a variety of mechanisms and the potential for the aquatic environment to promote or maintain antibiotic resistance is largely unknown. Some chemicals, such as triclosan (an antimicrobial disinfectant found in many liquid soaps, dishwasher powders and plastics), are suspected of increasing the antibiotic resistance of bacteria in the environment,[16] reducing algae diversity in streams,[17] and affecting natural ecosystem functions such as soil microbial activity.[18] In addition, research has shown effects of mixtures of antibiotics to aquatic organisms.[19]

Evolution of Fourmile Creek as a Research Site for ECs

Following a national stream reconnaissance study,[20] water samples were collected in 2001, upstream and downstream of select towns and cities in Iowa during low-, normal- and high-flow conditions to determine the contribution of urban centers to concentrations of ECs in streams under varying

flow conditions.[21] This study found the number of ECs detected decreased as streamflow increased from low- (51 ECs detected) to normal- (28) to high-flow (24) conditions. Fourmile Creek near Ankeny, Iowa, was initially sampled for ECs during this study and results showed a strong gradient in EC detections during low-flow conditions between samples collected upstream of Ankeny (three ECs detected) compared to samples collected downstream (31 EC detected).

The initial EC results from Fourmile Creek,[22] led to including this stream as part of collaborative research between the U.S. Geological Survey (USGS) and the U.S. Environmental Protection Agency to better understand the fate of ECs following their discharge from wastewater treatment plants (WWTPs).[23] This research involved collecting four samples at each of 10 WWTPs across the nation: upstream of the WWTP, at the WWTP where effluent was being discharged into the stream, at a location in close proximity downstream of the WWTP, and at a location farther downstream from the WWTP. All samples were measured for 110 different ECs. Between 28 and 50 ECs were found in treated wastewater effluent being discharged to streams.[24] The similarity in chemical concentrations between WWTP effluent and proximal downstream sampling points clearly shows the contribution of WWTPs to EC concentrations in streams. Additional knowledge gained from Fourmile Creek during this study included:

1. the ECs detected in Fourmile Creek during the previous study[25] were primarily derived from the Ankeny WWTP (see Figure 1),
2. there are significant reductions of the number of ECs detected and total EC concentrations through the 8.4 km study reach (Figure 1),
3. ECs vary in their type of transport (conservative versus nonconservative) through the study reach (see Table 1),
4. at low-flow conditions, greater than 90 percent of the streamflow is derived from WWTP discharge.[26]

Figure 1. Compounds and Concentrations of Emerging Contaminants at Various Locations of Fourmile Creek

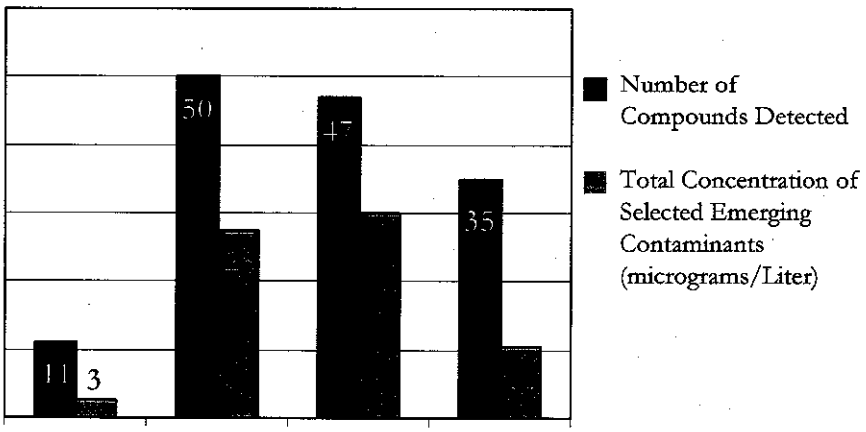

Table 1. Selected Compounds Detected, Primary Use, Reporting Level, and Concentration from Samples Collected at Various Locations of Fourmile Creek

Compound	Primary Use	Reporting Level (µg/L)	Upstream of WWTP	WWTP Effluent	8.4 km Downstream of WWTP
			(concentration in (µg/L)		
Cimetidine	Antacid	0.012	undetected	0.123	0.107
Dehydronifedipine	Antianginal	0.015	undetected	0.202	0.018
Diltiazem	Antihypertensive	0.016	undetected	0.053	0.029
Diphenhydramine	Antihistamine	0.015	undetected	0.218	undetected
Sulfamethozole	Antibiotic	0.064	undetected	0.589	0.321
Tonalide (AHTN)	Fragrance, musk	0.500	undetected	2.300	0.700
Trimethoprim	Antibiotic	0.013	undetected	0.353	0.093

In 2003, the USGS EC Project[27] was searching for a real-world setting to investigate the complex in-stream processes (e.g. dilution, sorption, degradation, dispersion, etc.) that can affect ECs following their discharge from a WWTP and determining if such input is having an effect on the aquatic ecosystem. Such research requires the integration of multi-disciplinary efforts at a carefully selected field site. Knowledge gained from previous research[28] and other unique aspects of Fourmile Creek led to its selection as a field setting to help answer these important research questions. Critical aspects of Fourmile Creek include the following:

1. A single source WWTP effluent-dominated stream. This allows for the examination of EC concentrations as water moves downstream without complications from additional inputs.
2. Data documented the input of a wide variety of ECs from WWTP discharge. Previous research found between 3 and 10 ECs present upstream of the WWTP and between 30 and 50 downstream.[30]
3. Small basin size (less than 160 km^2 size). This facilitates an increased understanding of the transport and fate of environmental contaminants.
4. Relatively simple flow system. Little to no ground-water or surface-water inputs to streamflow exist in Fourmile Creek during normal flow conditions. Thus, any changes in EC concentrations observed downstream can be attributed to in-stream processes.
5. Data documented that ECs vary in their type of transport. Undefined processes are taking place within the stream that affect EC concentrations.
6. The WWTP uses a treatment technology (conventional activated-sludge) typical of many towns and cities across the United States. Thus, the source is representative of many similar sources in the United States.
7. The hydrogeologic setting (low-gradient stream, glaciated deposits, rowcrop agriculture) is typical of the Midwest.
8. A low-head dam exists approximately 2 km upstream of the WWTP outfall. The dam provides a physical barrier to fish migration. Thus, comparisons in fish community structure and fish health assessment can be made to more accurately determine potential effects from the input of ECs by the WWTP. Research has found a range of abnormalities in fish populations (vitellogenin induction in males and juvenile females, development of oocytes in testes, etc.) downstream of WWTPs.[31]

9. A major change is anticipated to the primary source of ECs in the system. Around 2010, the WWTP is scheduled to close. This closure provides a unique opportunity to examine how a stream and aquatic biota react to the removal of the primary source of ECs and allows a novel "before" and "after" assessment not been previously available in EC research.

Future Work

Future work on ECs will involve not only the occurrence of these compounds, but also their fate, transport and possible effects in the environment. Several large-scale studies in the United States by the USGS Toxic Substances Hydrology Program have already documented the occurrence of ECs in the environment.[32] These studies have shown that a wide variety of ECs are commonly detected in streams, streambed sediment, and ground water as complex mixtures of compounds. Other studies have documented the occurrence of ECs globally.[33] Many of these same EC compounds have been detected in a study of Iowa's streams.[34] Indeed, the data on ECs collected at Fourmile Creek are consistent with similar national studies. However, the effects of long-term, low-level exposure to these mixtures of emerging contaminants on aquatic life and humans are currently unknown. Research on the effects of ECs in the environment is only in the beginning stages.

The field research site established at Fourmile Creek will continue to build a framework for better understanding of the transport, fate, and effects of ECs in the environment. One goal of the field research site at Fourmile Creek is to move beyond documenting the occurrence of these compounds to examine what happens to these compounds once they enter the environment and their potential effects to aquatic ecosystems.

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PULSUS GROUP INC
THE CANADIAN JOURNAL OF INFECTIOUS DISEASES & MEDICAL MICROBIOLOGY
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July/August 2001, Volume 12, Number 4

The case against land application of sewage sludge pathogens

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There is currently a public debate about whether health, agricultural and environmental authorities should continue to allow sewage sludge to be spread on farmland. Some of the concern in the debate is about the pathogen content of sewage sludge. This concern was heightened by the tragedy at Walkerton where *Eschericha coli* 0157:H7 and other pathogens contaminated the drinking water supply of this Ontario town. The Canadian public were reminded how vulnerable they can be to disease when agricultural practices ajoin population centres without adequate health and environmental controls.

But pathogens are not the only contaminants of concern in sewage sludge. Sewage sludge also contains potentially harmful levels of toxic metals and environmentally persistent chemicals such as polychlorinated biphenyls and dioxins (1).

In the past, farm application was often not the favoured method of disposal. Sludge was incinerated or landfilled, and only a small percentage was applied to farmland. With air quality concerns increasing and landfill capacity at a premium, municipalities have moved to farm disposal of these waste sludges, especially in Ontario, where almost all wastewater sludges are applied on farms.

Have the risks to human health been adequately evaluated? Should government promote the transfer of these wastes to the countryside? Is it reasonable to ask rural residents to live next to sites where human excrement mixed with industrial waste is stockpiled and spread on the land?

Background

Flush toilets were invented almost 200 years ago, yet we as a society are still trying to manage the health and environmental impact of our own waste. The whole idea of 'public health' developed in response to the contagion created by allowing urban streets to flow with septic wastes. Eventually, the crises in pests, disease and plagues led to the creation of an infrastructure of sewers to remove these wastes and confine them away from the public in a sewage treatment plant.

Over the past 20 years, with the public desire to protect surface waters from sewage contamination, sewage treatment plants (accurately renamed wastewater treatment plants) are designed to separate the water in the sewer system from the solids and contaminants, and return the water fraction the lake, river or ocean in a relatively purified form. Sewage sludge, sometimes called 'biosolids', is the solid fraction of this waste. Wastewater treatments plants are not designed to treat the the sludge effectively; they are primarily designed to clean the water fraction.

What 'treatment' is required?

The industries that promote the placement of sewage on rural and agricultural land like to speak of 'treated' sewage, 'treated' sludge or 'biosolids'. However, there is little in provincial statutes that sets out what 'treatment' may be required before sewage is placed on land. Requirements for

sewage treatment vary from one wastewater facility to the next and may or may not be detailed in a Certificate of Approval or permit from the provincial Ministry of the Environment.
In Ontario, septage, which is untreated sewage waste pumped out of septic tanks and abbatoir waste, is also allowed to be spread or sprayed on rural land. No treatment is required.
Often, the Ontario government adopts the American requirement for 'treatment' that the sludge should contain less that two million fecal coliforms colony forming units/g. However, not all sewage treatment plants achieve this level. The Ashbridges Bay Treatment Plant in Toronto, Ontario processes sludge for about 1.7 million people. There are not enough digesters to allow for the recommended minimum 15 to 30 days of digestion of sludge in anaerobic tanks. Therefore, Toronto sewage sludge may receive as little as eight to 10 days of digestion (personal communication, Interim Compliance and Monitoring Committee, City of Toronto, Toronto, Ontario). The Ontario Ministry of the Environment has facilitated the spreading of this sludge with elevated pathogens by allowing the City of Toronto to average the test results according to a specific formula, so rural residents have no assurance that specific standards of disinfection have been met by sludge delivered to farms.
The fecal coliform levels in the City of Toronto sewage sludge have often exceeded even the above permissive standards. In 1999, levels of fecal coliform exceeded two million colony forming units/g, even when the city was allowed to average the test results over several weeks.
Toronto sewage has been trucked to a facility in Halton, Ontario where it was stored in an open pit the size of a football field. Neighbours of this facility have protested because foul odours have stopped them from being able to leave the house or open the windows. Residents near the facility have become seriously ill with enteric viruses, which they are concerned may be the result of their exposure to the sludge (personal communication, Laura Eagles).

Do wastewater treatment plants 'treat' sludge?

Scientists at the University of Ottawa Faculty of Medicine prepared a research report for the City of Ottawa on whether the Ottawa Pickard Centre sewage treatment plant was effective in reducing pathogens in Ottawa sewage sludge (2). Their report concluded that "it is clear that the pathogen content of biosolids from the Pickard Centre may be relatively high.". The report also concluded that there was no reduction on giardia cyst levels, only a 49.9% decrease in cryptosporidium oocyst densities and only slightly reduced levels of somatic coliphages, suggesting a similarly small reduction in enteric virus loading in the land applied sewage (3).
A more detailed report on the health risks from pathogens associated with land application of sewage sludge can be found in "Hazards from pathogenic microorganisms on land-disposed sewage sludge" (4), which outlines the range of pathogens found in sludge and their longevity in the soil.
A study of sewage workers at the Toronto Ashbridges Bay Treatment Plant showed increased incidence of disease com-pared with a similar group of workers not exposed to biosolids (5). The United States National Institute for Occupational Safety and Health has posted a health alert for those who work with biosolids (6). *The Journal of Agromedicine* also published a report linking odours with health risks (7). The research has implications for those workers, farmers and rural residences who are exposed to sewage sludge.

RISKS FROM SEWAGE SLUDGE

Venues for pathogen transfer: Land application of sewage sludge can lead to the transport of pathogens through bioaerosols downwind of sludge storage or spreading sites, through contamination of ground water, drinking water wells, stockponds and surface waters, or through food contamination from eating food grown in sludge spread land. Pathogens can be transported to

humans who walk through sludge spread fields. Wild animals, farm animals, birds, rodents and pets may become infected by or transmit sludge pathogens. There is no requirement to post signs that sludge has been spread or stored on fields in Canada. Therefore, children, family members and others may access and traverse sludge spread sites unknowingly.

Bioaerosol risk: A recent study from the University of Arizona (8) shows that the risk of windborne pathogens is significant within a 10 km radius of a sludge spread field. In Canada, the relatively cooler and wetter climate may make this risk even higher.

Risk to water: Sewage sludge contains a high concentration of wastes; indeed, a 100-acre spread of sewage sludge represents the annual fecal output of approximately 1400 people plus the industrial waste component. To compare sludge with manure, a field of similar size would support 100 cattle.

Some provinces have 'guidelines' or other criteria that are intended to address the environmental and health issues related to the land application of sewage sludge. However, these 'guidelines' are not always enforceable, and most are not legally binding. Ontario has one of the most detailed documents on the land application of sewage sludge and other organic waste. However, the *Guidelines for the Use of Biosolids and Other Wastes on Agricultural Land* (9) are only a 'guidelines'. They are not statutes or regulations. In Ontario, even after the Walkerton tragedy, reductions were allowed in the the separation distances between sludge spreading sites and drinking wells, lakes, rivers and surface waters. Ottawa and Toronto are currently allowed to spread sludge as close as 15 m to a drilled drinking water well and as close as 15 m to surface water despite provincial law that stipulates greater, more protective distances (10).

Risk from food: The Ontario Ministry of the Environment and the Ministry of Agriculture recommend that farmers observe a waiting period between sludge spreading and the harvesting of some crops, and recommend that farmers restrict farm animals from fields recently spread with sludge. These recommendations are not enforceable under current legislation. Therefore, sludge spread fields can be used to grow small fruits such as strawberries or pasture livestock, and thus expose the consumer to contaminated produce and meats.

Animal transfer: Farm animals and pets such as cats and dogs can be expected to continue to travel through fields, even after the fields have been spread with sludge, and return to their owner's homes, spreading sewage sludge to the family home. Birds may also visit sludge spread fields, and spread viruses, bacteria and parasites in their subsequent droppings.

Issues of concern

Lack of research: The Environmental Protection Agency in the United States, the Ontario government and the Water Environment Association of Ontario have all acknowledged that pathogen risks from land application of sewage sludge have not been adequately evaluated. In both Canada and the United States, policies concerning land application are under review for this reason. In the United States, the National Academy of Science is conducting a risk assessment on the land application of sewage sludge. In Ontario, just a few months after Walkerton crisis, the Ontario Ministry of the Environment announced a review of the regulations concerning the land application of sewage sludge, septage and all biosolids.

Lawsuits in the United States: It is thought that several deaths in the United States have been attributed to the land application of sewage sludge, and lawsuits have been brought (11). One of these cases, concerning the death of Shayne Connor, who lived adjacent to a sewage sludge

spreading site, is currently making its way through the New Hampshire courts. Other lawsuits involving livestock deaths have also been filed in Georgia.

Regrowth: A study conducted in Australia in 1997 looked at pathogen regrowth in soils that had been spread with sewage biosolids (12). Researchers found that during hot, dry months, there were reduced levels of pathogens (fecal coliforms and salmonellae) in stored and spread sewage sludge, but that the concentrations of pathogens rose during the wet cool months that followed. In some instances, the pathogen regrowth was to levels higher than found in the initial tests.

No informed consent: Farmers and rural residents are told that the sludge is treated for pathogen reduction. There is no requirement to explain the health risks to farmers or those who live near sludge spreading or storage sites.
Occupational health and safety issues related to sewage sludge are starting to be addressed with the recent Health Alert from the National Institute for Health and Safety. Documents like the Safety Alert issued by the Spokane Regional Health District in the State of Washington are increasingly common in the United States and Great Britain. (13). However, action on public health education on sludge lags behind the occupational health and safety requirements for sludge handlers. The public needs to be informed of the pathogenic risk posed by sludge transporatation, storage and spreading.

No storage: When rainy weather makes sludge spreading particularly risky for runoff and ground water contamination, most municipalities do not have adequate disposal alternatives for sewage sludge. The City of Toronto sludge has been evicted from the Halton storage facility, and the City of Toronto hauler is now storing sewage sludge in open piles in Ontario farmfields. The Ontario Ministry of the Environment has allowed the Toronto sewage sludge to be stored in huge open piles on farmland in Northumberland County near Warkworth (14).

CONCLUSIONS

Open field storage of sludge and sludge spreading near wells and surface water, increase the risk that sewage sludge pathogens will be transported to workers, farmers and neighbours, and increases the environmental risks of this wastedisposal practice. It seems unreasonable to offer the farm community 'free fertilizer' and promote its use when the public health risks associated with the land application of sludge have not been addressed. The case against land application does not stop with the issues posed by pathogens; other heath risks are associated with the elevation of heavy metals in the soils and foods, the release of mercury into the atmosphere from sludge spreading and the presence of priority pollutants in the land-applied sludge.
We seem to have forgotten the public health lessons of the 1800s and the underlying reason for sewage treatment plants themselves. The pathogen levels in sewage sludge are high and can even increase over time once the sludge is stored or applied to land. Land application allows sludge pathogens to be transported by weather events, and facilitates the spread of diseases to animals and humans. It is unreasonable to expect rural residents to tolerate exposure to these diseases in land, air and water. The Canadian Infectious Disease Society should be applauded for its call for a moratorium on sludge spreading until the risks to public health and the integrity of food are thoroughly evaluated.

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Lea Brooks, a spokeswoman for the state Department of Public Health, said recycled water is treated but can contain bacteria and viruses that cause diarrhea and other illnesses.

Otay water district officials investigate water foul-up

Stores may reopen if samples test clean

By Anne Krueger
UNION-TRIBUNE STAFF WRITER

August 23, 2007

CHULA VISTA Otay Water District officials spent the day yesterday trying to determine how a Chula Vista business park had treated sewage flowing from its water taps for two years, and how to ensure it doesn't happen anywhere else.

"We thought we had a good process," said Mark Watton, Otay's general manager. "Obviously, there's a gap."

The 17 stores at Fenton Business Center in Eastlake were ordered Friday not to drink or wash their hands with tap water after tests from a private lab showed they were getting recycled water, or treated sewage, instead of drinkable water.

Otay officials are investigating, but it appears a mis-marked pipe was the cause.

Two food-related businesses " the Candy Bouquet, which creates candy baskets, and Dream Dinners, a meal-preparation store "" were shut down by the county.

If recent water samples are clean, the state Department of Public Health is expected to declare the water safe today, allowing the stores to reopen.

Otay serves about 190,000 people in a 125-square-mile district ranging from El Cajon to eastern Chula Vista.

Watton said the district's approximately 500 meters for recycled water will be inspected.

All pipes carrying recycled water are in eastern Chula Vista along thoroughfares, on public property or in business areas, not in residential sections.

Recycled water flows through a separate system of distribution pipes, colored purple to distinguish them from pipes carrying drinking water.

Watton said when the business park was built in 2002, a purple plastic sleeve placed over the copper recycled-water pipe somehow slipped below ground when the pipe was installed. Whoever hooked the pipe to the water meter didn't realize the pipe carried recycled water.

The district still hasn't determined how its inspectors or supervisors for the contractor on

the project could have overlooked the error, he said.
Some business owners are concerned a former Otay inspector who pleaded guilty to a bribery charge could be at fault.
In 2005, Otay inspector William Cooper pleaded guilty to taking $5,000 in exchange for overlooking deficiencies in underground water and sewer lines on two Chula Vista residential developments.
Watton confirmed Cooper was one of the inspectors on the Fenton Business Park project. He said he has not determined whether that is related to the bad connection.
When the business park opened in July 2005, Otay blended recycled water with drinkable water. In May, Otay reached a deal to buy recycled water from the city of San Diego, and began flowing 100 percent recycled water through its purple pipes.
When the district first received a complaint about the business park's water July 27, Otay employees tested the water at the main line on the street. The district doesn't usually test water at the tap in response to a complaint, Watton said.
The main line carried drinkable water, so the tests showed nothing wrong. Watton said Otay employees thought the water might be stagnant because the park was new and many of the offices unoccupied.
The property manager sent the water to a private lab, and the results that came back Friday showed the water contained coliform bacteria, which indicates contamination. Recycled water is safe for full body contact, though it's meant for irrigation and manufacturing, not consumption.
Lea Brooks, a spokeswoman for the state Department of Public Health, said recycled water is treated but can contain bacteria and viruses that cause diarrhea and other illnesses.

Jane Ballard, who owns a sign company in the business park, said she's told her 10 employees to see their doctors. Watton said the district would pay for the exams and compensate businesses for their losses.

"We just want to know that we're all OK," Ballard said. "Everybody is just kind of a little tense waiting."

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THE NEWS&OBSERVER
newsobserver.com

Published: Jul 25, 2007 12:30 AM
Modified: Jul 25, 2007 05:44 AM

MORE PHOTOS

Cary has 'weird' water mix-up

TOBY COLEMAN, Staff Writer

CARY - The bitter water pouring from Vinay Jain's tap was a water conservation measure gone foul.

For nearly five months, the Jain family home in Cary has been connected to the town's reclaimed-water system. That is the treated wastewater the town considers clean enough for suburban lawns but not good enough to drink.

"I had a suspicion because when we moved, I said, 'The water tastes weird,' " said Jain, a 37-year-old information technology worker. "My wife said, 'You always complain.' "

Jain lived with the funny-tasting water until Monday. Then, suddenly, the water in the house went off. Somebody had shut off the neighborhood's irrigation pipe.

Cary water workers discovered the goof after Jain's neighbors, who had water inside, realized that their sprinklers weren't spraying. Cary officials then started scrambling around town to see whether any of the other 500 homes with irrigation systems served by reclaimed water had reversed water connections.

By Tuesday afternoon, Public Works head Mike Bajorek seemed confident that the Jains were the only ones in Cary with treated wastewater coming out of their taps and drinking water coming out of their sprinklers.

"We believe that this is a unique situation," he said.

Just to be sure, though, Cary workers were completing a house-by-house check Tuesday.

Cary is the only town in the Triangle that pipes reclaimed water into residential neighborhoods to help conserve drinking water. Residents connected to the system do not have to obey outdoor watering restrictions, and they pay less for the water they use to quench their lawns. But they're never, ever supposed to wash with the stuff, much less drink it.

Cary officials say the risk from reclaimed water is low. By one estimate, you would have to drink 12 gallons of reclaimed water in a single sitting to get an infectious dose of coliform bacteria. But it is serious: State regulations ban water systems from distributing reclaimed water for consumption.

The Jains have been unnerved by the revelation. Jain and his wife, Priyanka, wonder whether their children's dinnertime claims of bellyaches were legitimate reactions to low-quality water and not the childlike ploys to avoid eating unwanted food, as they originally suspected.

"In a place like Cary, it never even occurred to me that this might even be a possibility," Vinay Jain said Tuesday as Cary workers walked through his house testing taps. "This gives the impression of a Third World country. At least in India, we knew the water was bad, and we boiled it."

The town is putting the Jains up in a hotel while the problem is repaired. The Jains said they will be able to move back in Thursday, once the town has completed follow-up testing and inspections.

It might take longer to figure out how the foul-up occurred. Currently, water workers think somebody switched the Jains' black drinking-water main and their purple reclaimed-water main between their water main installation and their final home inspection.

But why would somebody do that? And why didn't anybody catch the mistake earlier?

"That," Bajorek said, "is what we're trying to determine."

From: http://www.livescience.com/health/061023_antibiotic_spread.html

DNA Found in Drinking Water Could Aid Germs

By Charles Q. Choi, Special to LiveScience

posted: 23 October 2006 08:28 am ET

DNA that helps make germs resistant to medicines may increasingly be appearing as a pollutant in the water.

This DNA was found "even in treated drinking water," researcher Amy Pruden, an environmental engineer at Colorado State University in Fort Collins, told *LiveScience*.

The spread of this DNA could exacerbate the already growing problem of drug resistance among potentially infectious microbes. Diseases once considered eradicated, such as tuberculosis, are making alarming comebacks. Currently, more than two million Americans are infected each year by resistant germs, and 14,000 die as a result, the World Health Organization reports.

"I personally have known people with antibiotic-resistant infections, and they can be very scary," Pruden said.

Resistant microbes

While antibiotics kill off many germs that have no resistance against them, they also prompt the spread of microbes that are resistant. The over-prescription or other improper use of these drugs helps these resistant infections emerge, but experts also note that up to 95 percent of antibiotics are excreted by humans and animals unaltered, seeping into the environment and encouraging antibiotic resistance there.

Pruden's new research did not focus on the presence of antibiotics in the environment. Instead, she looked for the presence of genes that help confer drug resistance to the germs in the first place. Bacterial genes are encoded as DNA, and microbes often swap genes with each other. In principle, antibiotic-resistance genes could persist and spread long after the drugs they target have dissipated.

"The spread of antibiotic-resistance genes in the environment is undesirable, just

as is that of any other pollutant, such as PCBs or mercury," Pruden said.

Pruden and her colleagues focused on genes conferring resistance against two antibiotics, tetracycline and sulfonamide, which are linked to urban and farm activity. They investigated a range of northern Colorado waters, from relatively pristine river sediments to water from dairy lagoons to irrigation ditches. They also looked at water from drinking-water treatment plants and effluents from a wastewater recycling plant.

Everywhere

The levels of antibiotic-resistance genes were hundreds to thousands of times higher in waters directly impacted by urban or farm activity than in relatively pristine waters. Still, the researchers discovered the presence of antibiotic-resistance genes in all the waters they investigated.

"Wastewater treatment systems are not designed to treat antibiotic-resistance genes. The treated effluent is usually chlorinated, but even though this inactivates bacteria, it does not destroy DNA," Pruden explained. The DNA they found likely is inside dead or living cells, although it is possible it is floating in the water outside cells.

The researchers will further investigate what other antibiotic-resistance genes are present in the environment, such as ones against vancomycin, often considered the most powerful antibiotic of last resort. They will also explore ways to modify wastewater treatment plants to help them destroy DNA.

Pruden and her colleagues reported their findings online this month via the journal *Environmental Science & Technology*.

- Top 10 Mysterious Diseases
- Study Details Chemicals in Tap Water
- Bottled Water Sales Soar as Tap Water Safety Questioned
- Rise of Deadly Superbugs should 'Raise Red Flags' Everywhere
- All About DNA