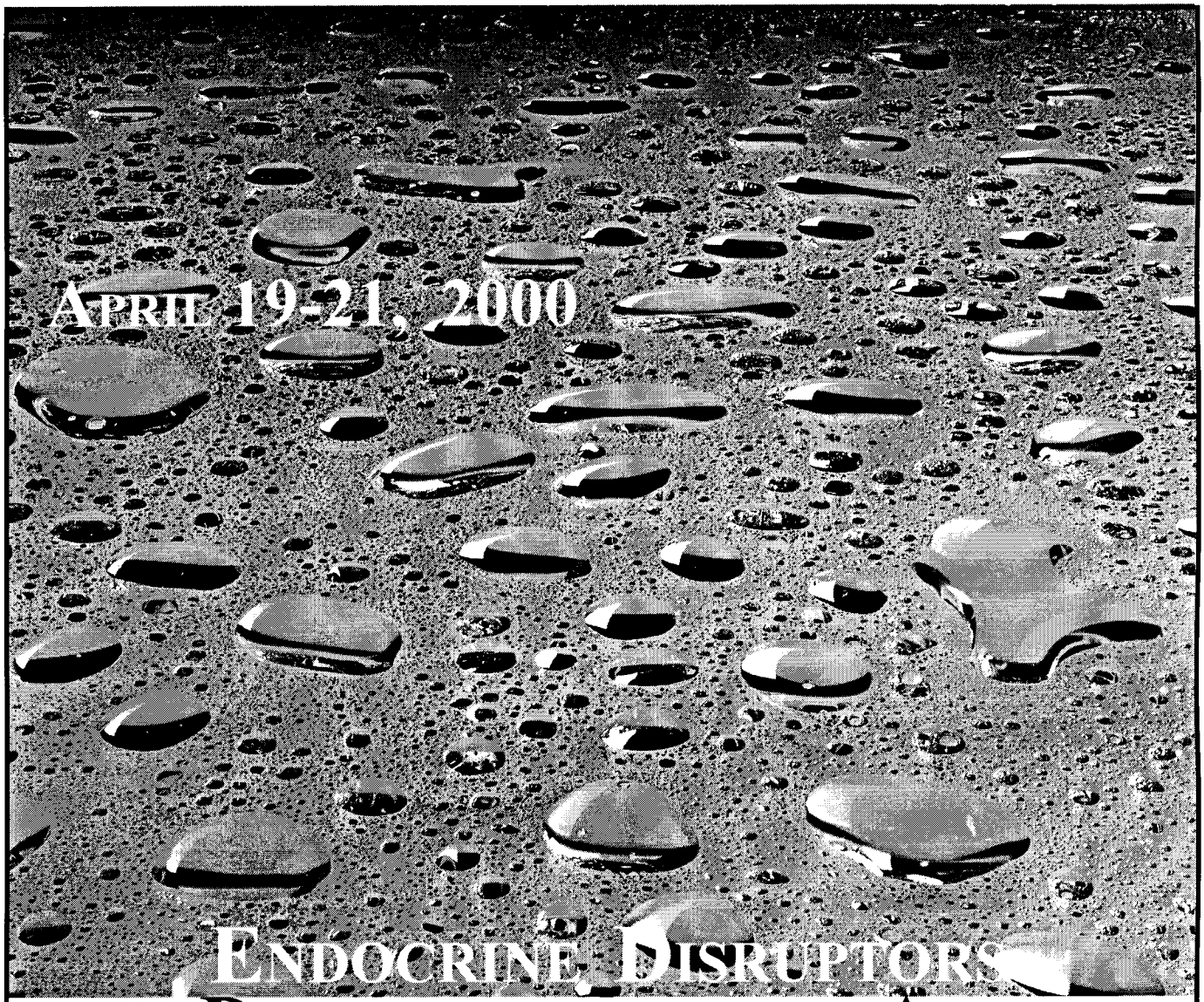

Endocrine Disruptors & Pharmaceutically Active Chemicals in Drinking Water

The University of Iowa
Center for Health Effects of
Environmental Contamination

Sponsors:
American Water Works Association
Research Foundation
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Preface

The ***Endocrine Disruptors and Pharmaceutically Active Compounds in Drinking Water*** workshop was held in Chicago, Illinois on April 19-21, 2000. The workshop was co-sponsored by the American Water Works Association Research Foundation, Water Environment Research Foundation, WateReuse Foundation, and The University of Iowa Center for Health Effects of Environmental Contamination (CHEEC). CHEEC served as organizer and host of the workshop.

The workshop provided an overview of research and scientific findings to date, and addressed questions and concerns that may have an impact on the drinking water and waste water communities. Plenary sessions included in-depth reviews of identification and occurrence of compounds, industry research and concerns, ecological and human health effects, regulatory activities, and international activities and perspectives of interest to water industry professionals. International in scope, the workshop attracted experts from municipal utilities, industry, academia, and government policy makers to discuss this emerging environmental health issue. The workshop was a first of its kind effort organized specifically for the drinking and waste water industry.

Organizers would like to thank Gary Benjamin (Des Moines Water Works), George Breuer Ph.D. (UI Hygienic Laboratory), George Hallberg Ph.D. (The Cadmus Group, Inc.), Katherine Kramer (Western Center for Environmental Decision Making), Dana Kolpin (United States Geological Survey), Joseph LeBeau (Dow Chemical Corporation), L.D. McMullen (Des Moines Water Works), John North (Cedar Rapids Water Department), Gene Parkin Ph.D. (Director CHEEC and workshop co-chair), Peter Thorne Ph.D. (UI Department of Occupational and Environmental Health), and Richard Valentine Ph.D. (UI Department of Civil and Environmental Engineering) for planning and participating in the workshop. Additional thanks go to Jarka Popovicova (AWWARF Project Manager) and Jami Montgomery (WERF Project Manager) for their thoughtful guidance with conference preparations; and to Beverly Prostine (UI Center for Conferences and Institutes) for the exceptional job and composure she demonstrated as conference coordinator. Appreciation is extended to Sarah Greenley, Jiji Kantamneni, Mark LeRette, Mary Skopec, and Samantha Van Nyhuis for their contributions ensuring the success of the workshop. Finally, to Lakshmi Kantamneni and Casey Novy for the time and care spent in the production of these proceedings.

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Public Health Issues: Endocrine disruptors and pharmaceuticals in drinking water and wastewater

Dr. Henry Anderson, Wisconsin Department of Health & Social Services

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I would like to present the public health issues of endocrine disruptors and share information on research and the pro-public health approach used in Wisconsin. One thing that has to be done first is a public health assessment. Where do endocrine disruptors fall on the scale of all health issues? On the research community and regulatory sides, people are taking a close look at endocrine disruptors. From a public health standpoint, endocrine disruption is very interesting because it can occur at very low levels; levels below the traditional lowest exposure level producing an effect, which many of the regulations looking at cancer endpoints use. In humans, very tiny concentrations of certain chemicals control the endocrine system - much smaller amounts compared to other chemicals that we are exposed to.

In broader terms, thousands of chemicals are manufactured; the top 2,000 chemicals are each manufactured at a rate of one million pounds yearly. We want to keep many of these chemicals out of food, water, and air. So the first key issue is determining what might reasonably reach water. We want to insure safe water and food supplies. Secondly, as a physician in public health I am often asked, "Well, you can find something in the water or in the air, but what does it mean?" There are potentially many contaminants in water and air, but what are the key ones to worry about? Do we understand the human toxicology? For many of these compounds, that is where we have fallen short. All the information has not been gathered to understand the actions of the chemicals, where they go, and what they do. Third is the laboratory issue, which revolves around what is being detected. We are trying to find out what is there, in which water supplies. Also, can various chemicals be detected during the water treatment process? So sensitive laboratory methods must be developed. Additionally, will the methods be available commercially or only at research laboratories?

From the human side, understanding how an exposure is integrated into the body is critical. There are many different sources of exposure. Even though we can say there is an exposure,

we want to know if the pathway has been completed and get a sense of what is going on in the human body. That process is first developed in a laboratory test. You then have to try to understand the toxicology, how it might be metabolized, and then how it can be measured in the body. Right now we can use biomarkers to assess exposure, as opposed to looking at effects from very low levels. For the endocrine system, we can begin to look at making an association between an exposure and an effect. A key factor through all of this is addressing remediation and prevention alternatives.

Another public health issue is whether there is sufficient capacity to respond to emerging concerns. The water treatment industry also thinks about capacity issues - the press covers something and the public calls you wanting to know what you're doing about it. In public health, I get lots of those calls. We all need to be proactive in responding to public concerns. One thing we do in public health is recognition of water associated disease. Not only is there exposure monitoring, but also there are disease surveillance systems available to track diseases and answer "how good are we?". The ultimate key is disease prevention. We want to know if existing treatment systems for drinking water and wastewater are effective. Identifying and protecting high-risk individuals needs to be considered. Other considerations are the need to strengthen old partnerships and establish new ones; bringing public health, environmental enforcement, utilities and advocates together to address this issue.

It is important to remember that the public health system was designed and built to protect humans from infectious diseases. Despite our best efforts, there are still about 99 million cases of gastrointestinal infection that occur every year in the U.S. About one-third are related to waterborne infections - drinking water and recreational water use. Roughly half of the identified waterborne outbreaks are due to recreational swimming. The good news is most of these types of infection are cured without treatment, unless there is special risk factor in the individual. There are roughly one million individuals with HIV/AIDS in the population, there are more than 40,000 transplant recipients, and there are chemotherapy patients as well. All of these individuals are at greatest risk to infectious agents. These individuals serve as sentinels for infectious disease in the community, unfortunately.

With respect to microbial contamination, all exposed individuals generally have the same risk of developing a disease. A major concern is high-risk populations who are more likely to develop severe life threatening illness, or develop chronic infections. Individuals who are immuno-compromised are at risk of developing some of these diseases, and curing them often proves unsuccessful. A good example is cryptosporidiosis. In 1996, Milwaukee experienced a huge outbreak of cryptosporidiosis, which resulted from ineffective treatment of the city's drinking water. A review of hospital information in Milwaukee showed that pre-outbreak, there was a lot of gastrointestinal disease appearing at emergency rooms. But during the outbreak period there were considerably more visits, with two to three times as many individuals in the medical care system. The estimates of 400,000 cases are based on telephone surveys, not based on estimates on those who sought medical care. One question we asked is, "was this outbreak going on before at a smaller rate?" The data during the outbreak do show an association between turbidity in the water and the disease. As turbidity units went up during the outbreak so did the number of individuals appearing at hospitals. If you look at data during other periods, similar associations are seen in relation to turbidity.

From a public health side, the capacity of public health to monitor and look at this all the time is not possible. What happened in Milwaukee involved an unusual set of circumstances that initiated an investigation, which found an association between the drinking water and the disease. It was something going on that previously had been lost in the noise of all the other infectious disease appearing in emergency rooms.

What are the evolving public health concerns? First, assessing the adequacy of current water treatment methods for infectious agents. Many treatments were put in place at the beginning of the 20th century, when awareness of various agents was quite different than they are today. As public health is moving toward non-infectious water contaminants, the question now is how well does water treatment deal with non-infectious agents? On the public health side, how adequate is disease and exposure surveillance? On the endocrine disruptor side, a major question is "what should we be looking for?" What are the diseases we should be tracking? That is very difficult to come up with, particularly when comparing it to the infectious disease side. There is much stronger laboratory capacity to target and measure infectious agents in humans, and then be able to detect that agent in the water. For non-infectious agents, that is not the case.

What are needs and opportunities with respect to endocrine disruptors? We need to establish some type of epidemiologic surveillance system. We have a pretty strong system for infectious disease. The public health community across the country is trying to improve the ability to identify and measure chemicals in water. There is movement on the endocrine side to characterize potential exposures. Combinations of water, food, air and soil all have to be considered. Laboratory development and QA/QC is a very critical issue particularly when comparative studies, information and consistency across water supplies are considered. Biomarkers are important too. Even though a chemical may be in the water, determining if the pathway in an individual has been adequately completed so that individuals are actually having their tissue exposed at what levels continues to be an unknown.

What do we need to do? EPA's direction so far has been to consider mixtures and to consider aggregate exposures. And finally, one strategy might be to look for chemicals that have common modes of action. An example of a possible endocrine disruptor that has been closely studied is PCB. With PCBs you can have exposures from multiple sources; in the Great Lakes the consumption of sport fish is an important exposure.

A survey was done looking at people eating Great Lakes sport fish and their exposure to PCBs. A large percentage of people from the Great Lakes basin eat sport fish on an annual basis. From state to state there is variability - in some states the fish are coming from the Great Lakes, in other states the fish are from much smaller lakes. If you eat the fish, it is likely PCB will bioaccumulate in your tissue. Even if you don't eat fish, your tissue will still have some PCBs. Males have twice the body burden of PCBs as females. The background rate for non-sport fish eaters is low for PCBs. But in long time male sport fisherman levels are still almost 5-10 times higher than a comparison population that don't eat sport fish. That is a group exposed by one source to a level of considerable concern, which could perhaps cause effects. So one of the best predictors of PCB values in tissue is the number years an individual has been eating sport fish. While we can argue the PCB levels of concern and the toxicology, the reality is if people aren't getting the word it doesn't matter where we set that level. Those states with the

largest public awareness programs tend to have the highest informed citizens on sport fish advisories. If you put something in a fishing license booklet, it goes to the person that buys the license, which tends to be a male. However, that information probably doesn't get passed along to those who are going to eat the fish. Obviously, if this is the case, public health is not doing a good job. Water utility professionals have to consider this - are you getting the word out? You have to reach the high-risk target populations.

The issue of mixtures is also important, and the number of multiple detections needs to be addressed. For example, 35% of private well water samples in Wisconsin had one chemical in them. But 11% of the water samples had more than 5 chemicals. Addressing what that means is important. Do they have a common mode of action for these multiple detections? Should they be added together for exposure assessment levels? How do we approach this, when in fact regulation and advice is given for a single chemical? What are the primary prevention issues? For groundwater, prevention includes addressing wellhead protection, land use, well construction and functioning septic systems. For surface water, prevention practices include wastewater discharge, the impact of agriculture and non-point sources and storm water control. Keep in mind when it comes to pharmaceuticals, more than half of all the antibiotics used in the country are used in an agricultural setting. Most are not used to cure animals that are ill, but to promote growth. Other secondary prevention measures include disinfection, how we treat water, what works, and what are the best indicators of potential problems. We don't want to overreact and take action that is unnecessary.

In closing, I would like to stress the global view. As we get into the issue of sustainable water use and environments, the key to remember is best captured in a quote by Rene Dubos, who said "Improved health owes less to advances in medical science than to changes in external environment, and to a favorable trend in the standard of living. We are healthier than our ancestors not because of what happens when we become ill but because we do not become ill: and we do not become ill not because of specific protective therapy but because we live in a healthier environment". Public utilities are a key component of that. When you think of teamwork, don't wait for the state infectious disease person or environmental health person to come calling because they think you might have a problem. As a utility operator, you should work with them in advance. Learn what their system is and how it works. Your job is to keep after them and ask "is disease occurring?" and give advance warning before a serious problem happens. The proactive approach to working together will serve everyone well.

Impacts of endocrine disruptors on wildlife

Louis Guillette, Ph.D., University of Florida

Louis Guillette is a Professor of Zoology and Distinguished Alumni Professor at the University of Florida. He is internationally recognized for his research examining the role of endocrine-disrupting contaminants during embryonic development in various wildlife species. His current work examines the effects of embryonic exposure to modern use and "old" pesticides as well as the role of ecosystem resilience and evolution in ecotoxicological theory. Dr. Guillette served on the National Academy of Sciences Committee on Hormonally Active Agents in the Environment.

He is an award winning teacher and researcher, having received among other awards, the highest faculty honor - Teacher/Scholar of the Year - at the University of Florida.

My task is to provide an overview of endocrine disruption and wildlife. Beginning in the 1950's and 1960's looking at Rachel Carson's work and many others at the time, wildlife has been used as environmental sentinels. The crazy part of the endocrine disruption story has been a classic catch-22: if problems in wildlife are found that does not tell anything about humans, yet when pharmaceutical agents are tested with animals it does tell something about humans. This is true of endocrine disruption.

A lot of the early work was done on birds that fed in wetland systems. Many of those species have recovered, but there are still problems around the world. In the early 1990's sub-lethal responses in wildlife started to be addressed. Not cancer endpoints - if wildlife get cancer, they are dead before it is discovered. The only place cancer is seen in wildlife is in harvesting tons of fish and looking at thousands of individuals. For the average species, cancer is not going to be seen. There are other endpoints; that is really important to recognize. It is not that wildlife do not get cancer, it is just that they start to get sick and they get eaten. Species are not out there for a wildlife biologist to come along and catch.

The other important thing to consider when addressing wildlife and endocrine disruption issues is that an awful lot of screening is done based upon a molecular model. This is the idea that hormones are produced by an endocrine source, then enter the blood, then enter cells or interact with receptors that are on the membrane or in the nucleus, and they do some action in the cell. The focus on endocrine disruption has been on environmental estrogens and environmental anti-androgens. These are steroidal components in which the receptors are nuclear based. They are transcription factors and are associated with turning on or shutting off gene action. It is very important to recognize that there are other ways to have endocrine disruption. Endocrine disruption is not just the ability to turn on or shut off a receptor or to interact with a receptor. Enzymes that synthesize hormones can also turn on and shut off; enzymes that degrade hormones can also be turned on or shut off. All of these are forms of endocrine alteration. This is how circulating hormones in our own bodies are controlled, by modifying how much hormone is produced, how much is stored in the body and how much is degraded at the liver. All of those components are important in endocrine disruption.

We started our work in 1985 and 1986 in Florida, not because we were studying endocrine disruption and not because I was trained as a toxicologist. I am actually trained as a reproductive endocrinologist and spent most of my life studying the evolution of the endocrine system, understanding the evolution of maternal fetal communication, and how the developing embryo actually communicates with the mother during pregnancy by establishing and maintaining pregnancy. I began studying reptiles when the state of Florida asked me to study the American alligator, so they could use the species as a renewable resource. People still like alligator skin shoes, they still like to eat alligator meat at $30 a pound in New York and Chicago. Research began with the study of populations by going out on warm summer nights and counting the number of animals to get an idea of their size, which is measured by the distance between the eyes. Alligator populations are growing on many lakes, but human population in Florida is growing b y

700 - 1,000 people a day, too. In many areas where people are complaining about too many alligators, their lawn happens to be last year's nesting site. Swimming pools have been built where the alligators used to swim.

On many lakes, these animals are not at carrying capacity; there are reproductive problems. The positive control for the study is a high exposure lake called Lake Apopka, which is near Orlando. This lake is compared to Lake Woodruff National Wildlife Refuge, which is the best lake we can find as far as the general health of the wildlife, not just for alligators. It is a wildlife refuge that has never had modern agriculture around it, has never had a modern municipality dump raw sewage nor processed sewage into this area. It does have some aquatic weed control, so it is not pristine, but it is the best we could find. Comparatively, Lake Apopka has been used as a sewage treatment plant for some time with raw sewage coming from the city of Wintergarden, and it has had extensive agricultural activity all around the lake. In 1980 it had a pesticide spill. It is, if you will, the worst case scenario.

Later, I will show data on Lake Okoboji and other lakes, because one of the major criticisms of my work has been "why are you surprised?" Lake Apopka is near a Superfund site; it is highly contaminated. So I am not surprised wildlife are having problems. Lake Apopka is being used as a positive control; 90% of the animals in Lake Apopka have had some measurable abnormality. That compares to less than 5% on Lake Woodruff. These lakes are being used as two extremes, as in a classic toxicology program where there are positive controls and negative controls. The question all of us are concerned with is not whether water supplies associated with high impact areas have problems, it is whether the general water supply and general agricultural activity constitutes an environmental risk, be it to the public or to the ecosystem.

One research interest is looking at young animals. It takes 12-15 years for an alligator to reach sexual maturity; females reproduce every other year, laying 35-50 eggs. The average hatching rate on most lakes is about 50% - one-half the embryos die before they are ever born. This number is 40% below what it should be. The best lakes have 90% hatchability, which leaves questions about hatching. Research looked at a number of features of developing embryos. It is known that circulating levels of hormones in developing embryos and in a neonate are critical for future reproductive health. This is not just in alligators; it applies to humans as well. The circulating levels of hormones in the first couple of months of life in baby boys sets up the number of Sertoli cells in the testes and sets up future sperm counts. So circulating levels of hormones are very important in early embryonic development.

Two principal steroids for almost all species are testosterone and estradiol 17 Beta. The enzyme aromatase is critically important in turning on aromatase activity in herbicides as a mechanism of endocrine alteration. It is not the fact that men have testosterone and women have estradiol and never the twain shall meet. The fact is there is a relative amount of aromatase that gives you the difference and that men and women have both of these hormones. It is a ratio and how much is being produced at a given time that are critically important during development.

Our initial study was published in 1994; it has been replicated every year since. Nine-month-old animals, either hatchlings or yearling males have similar levels of plasma estradiol. Females from the contaminated lake, Lake Apopka, had almost half as much estradiol, and there are elevated levels of estrogen. Does

that actually mean anything? A typical normal ovary has a single nucleus and a single oocyte in one follicle. Every follicle contains the cells that support the growing oocyte; there should be only one oocyte and one nucleus per follicle. Every animal studied on Lake Apopka had a condition called polyovular follicle, which is associated with having multiple oocytes per follicle, sometimes multiple nuclei per oocyte. This is an abnormal condition; it is a condition that has been described in DES daughters and in mammals exposed to estrogen during embryonic development. It is a condition that occurs at a low frequency in many vertebrate females.

Animals in Lake Woodruff have a low frequency of this condition, but every female on Lake Apopka has this condition. It is not possible to follow these individuals, because it takes 12-15 years for them to reach sexual maturity. This condition is associated with a very high incidence of infertility. The eggs can be fertilized but many of them die early in the gastrula phase, so there are adverse reproductive outcomes associated with this condition. These are conditions that are associated with estrogen exposure during development. Looking at androgen, in this case plasma testosterone, females appear to be similar at this stage, but there is a dramatic difference in males. Males from the contaminated lakes have a fourth to a fifth of the circulating androgen than animals from the reference lake.

Anyone who is a physician or endocrinologist knows that hormone levels change. Many people believe that steroids can be used as biomarkers, but it is important to design the experiment appropriately. For these studies, samples are taken within an hour of one another. All the field data on the lakes is taken within two days of each other. Are the problems in these animals something they grow out of it, or is this going to be something which is persistent, something which is actually going to last for a lifetime? As it is difficult raise animals for 12 years, the solution is to go out into the population and ask the question there. So the studies go and catch the teenagers; these animals are nocturnal so we go out on warm summer nights. Animals are collected and blood, tissue biopsies, DNA and mRNA samples are taken. Juvenile females are usually 5-8 years old. These animals have growth rings in their bones just like trees, they are ectotherms. They go through seasonal changes in temperature and growth rates and so it is possible, until about age 10, to know exactly how old the animal is. In females, testosterone levels seems to have no difference. However, the females have elevated levels of dihydrotestosterone (DHT) on Lake Apopka. Estradiol levels are suppressed; in som e data sets, estradiol levels are significantly suppressed. So in the teenage or older animals there is a switch in levels. Females no longer have elevated levels of estrogens, it is now suppressed, and they have elevated levels of androgens. If you look at total androgens there is, in fact, a significant increase, as it is with DHT.

If you go in and look at the ovary, you see a different story. There no longer are polyovular follicles. Instead, there is a dramatic reduction in stage C and D, which are the larger follicles. So a huge number of ovarian follicles are wiped by aptosis or by cell atresia. While the larger class follicles are knocked down, there are still similar numbers of smaller ones, which probably means that these animals are not infertile but their fertility has been altered as well as their reproductive potential. In males, this abnormality in testosterone persists, so there is a dramatic change in circulating levels of testosterone. DHT is altered as well, and some males have elevated levels of estrogens. So there are feminized males. As an endocrinologist, I know that different tissues

are responsive to hormones. Was it possible to look at a morphological feature that is androgen dependent (in this case testosterone was suppressed) and is it a lifetime reduction? Is there a marker that is dependent upon androgen and could it be determined whether it was an alteration? Finally, instead of doing huge amounts of surgeries, we wanted to be able to go out and sample the population and get lots of numbers relatively easily. The answer to many of these questions was to look at phallus size.

In alligators, phallic development is very similar in many ways to mammals. The structure to begin with is indeterminate. If the developing embryo sees androgens, then it develops a penis, which is an intermittent organ that has vascular tissue. It is structurally a little bit different than the phallus, but the endocrine structures and the endocrine response is similar. One difference is that (unlike the human condition or a lot of mammals) if this structure sees estrogen, then aptosis happens - some cell death and some reduction in size. So, there is shrinkage in female exposure to estrogens and an increase in morphological development of the exposure to androgens.

Data that include Lake Apopka, another bad lake called Lake Griffin, and Lake Okoboji, which is more in the intermediate water quality range, show: 1) these reproductive effects are not just a Lake Apopka problem, 2) Lake Apopka is not the worst case scenario, and 3) Lake Woodruff isn't so good that no other lake can measure up. Reductions in phallus size are occurring with the average reduction about 20%. That does not mean that these animals are precluded from reproducing, it means that there is something going on with testosterone and DHT. It is an indicator. If there is an alteration, it is probably likely that other structures that are androgen dependent, including possibly behaviors that are endocrine dependent, have also been altered. But there are no data at this point to support that.

There is also interesting research on the garfish, a large long-lived species. There are intriguing problems and differences in the research findings, many of which support the alligator work. Other work is looking at mosquito fish. Mosquito fish are everywhere; they have a gonopodium, which is a highly modified fin used to transport the sperm packet to the female. It is androgen dependent; it grows under the control of testosterone and it appears to be possibly DHT-sensitive. Last year a report from Australia showed that male mosquito fish downstream from a sewage treatment plant had a reduced gonopodium size. We are in the middle of a project and have three different sample times during the year with literally hundreds of animals. The results show a significant reduction of gonopodium size in mosquito fish from Lake Apopka. This will contrast to the data that shows females growing gonopodium in other populations.

As a final note to the alligator story, my research has also looked at Lake Okochobi. There is a debate about the Everglades and Everglades restoration; the water source for the Everglades restoration is going to be Lake Okochobi. This is a lake that has been highly impacted from cattle and dairy farms on the north, the west is not quite so impacted, and the south end has huge amounts of sugar cane production. It has been hypothesized that the largest atrazine deposition in the United States is not in the Midwest, but on the south end of Lake Okochobi. Three areas of the lake are part of the study. The north end, where the Kasumi River comes out and drains through the marsh. Another area out in an emergent marsh, where animals are caught as they come through, mostly fish and alligators. The third area is near Belle Glade, where there is sugar

cane backflow. On the north end and the south end there are significantly reduced androgen levels. The study also employs an in-the-field two-hour stress test on alligat ors, which looks at the response of capture stress. There are dramatic reductions in testosterone in the two populations that are least affected, but there is not much of a response in the south at all. That brings up an interesting question of whether, in fact, the alternations in testosterone are just due to stress. That is, if animals are swimming in a toxic soup, it might not be a lot of fun and might be causing the stress.

Looking at plasma corticosterone, which is the stress steroid in these animals, and other aspects of stress, you find there is no difference among these sites. These animals are not perceiving that they are swimming in a toxic soup. There are other mechanisms being stressed. What could those other mechanisms be? One mechanism could be hepatic degradation of the hormone is being altered. That is one way of recognizing, or at least regulating, hormones. Research has been able to look at hepatic androgen metabolism and it quite clearly shows there have been alterations in the way the liver functions. Male and female livers function differently as far as recognizing hormones. Females degrade androgens at a much higher rate than males. There is sexual dimorphism here, at least in a number of the enzymes such as oxidoreduction hydroxylation. This is not unique to alligators as fish, birds, humans, and various mammals have it as well. Findings in Lake Apopka show an alteration, but interestingly enough it is not that the males have become more female-like, it is that the females have become more male-like. This fits with the elevated levels of circulating androgens. Females also have enlarged clitori.

There are multiple mechanisms to get endocrine disruption. The hypothesis we have been working on is that it is a maternal contribution problem; there is something being picked up in the diet and transported to the embryos. Studies looking at the nest environment have gone out and grabbed animals and surgically removed eggs before they reach a nest. The eggs that are incubated under pristine conditions develop the same problems as eggs hatched in the wild. So it is not the nest environment that these eggs are seeing in the first few days of life, but something is going on about in mom's uterus or what mom is actually transferring to the embryo. We have also looked at contaminant data that includes metals, organochlorines and PCBs. There are a few differences, but for the most part, metals by themselves do not seem to point to something that is critical. In contrast, organochlorines demonstrate fairly dramatic differences. There are still huge amounts of DDT and its breakdown products in the environment. Many of us assume that this an old chemical. For someone like me who works throughout the tropics, DDT is not an old chemical. This chemical is still being used throughout the world. In fact, the best statistics suggest that in 1995, which is the last data I can find, more DDT was used than in any previous year. DDT comes to us in Florida from across the Gulf; it is used in Mexico. So there is new DDT; there are also DDD and DDE, which are metabolites. In Lake Apopka, animals have lots of serum contaminants.

One hypothesis for these abnormalities is that they are organochlorine effects, or at least pesticide metabolite-based. There is a mechanism to test that. Alligator sex determination is temperature dependent, but it is also hormonally dependent. If eggs are incubated at 33 degrees, they result in 100% males; at 30 degrees it is 100% females. But if eggs are incubated at 33 degrees and a topical application of estrogen is applied to the surface of the egg, it is picked up by the chorioallantois membrane and delivered to the

embryo. The result is sex reversal to female. Only estrogens work, so this also can be used to test chemicals that might be estrogenic. One of the data sets looks at different dosages applied to the outside of the eggshell. The data show that between 2- 40% of a chemical makes it across the eggshell into the embryo. From one part per trillion to ten parts per million were tested for many of these chemicals. We did not try one part per trillion for the contaminant, as we assumed that was too low. Well, we were wrong. It ends up that everything from a hundred parts per trillion to ten parts per million are ecologically relevant. I know that sounds high but that is what is being measured in some of the alligator eggs in these lakes; at these levels there is sex reversal. In this system there is a nice dose response curve with estradiol, which is what is expected. The intriguing part is there is not a dose response curve with these other compounds. Some compounds are acting more like a mixed function agonist, similar to how tamoxifen and reloxifin function. It works depending upon the background circulating levels of hormone, and also the receptor depends upon whether there is a greatest response or a classic dose response curve. Sometimes the shape can be an inverted 'U'. It shows the highest dose does not always give the greatest response. That has been a very disturbing issue for many people trying to do risk assessment in toxicology.

What happens to animals that do not get sex reversed but get exposed? In males the testes make more testosterone than the ovaries, but what happens if males are exposed to estradiol or dicophol, a commonly used miticide in the citrus industry? Other exposures or mixtures might be DDD and transnonachlor. Transnonachlor, DDD, or dicophol exposure by itself, while not enough to sex-reverse the animals, in low concentrations actually altered the testes so that production of testosterone is more like an ovary than it a testes. The biochemistry has been altered. Pathologically though, it still looks like a testes.

With respect to pesticides, there will be those who say there is no need to worry about endocrine disruption. I beg to differ. I do not think there is a debate anymore about whether there are chemicals in the environment that cause endocrine disruption. I think the debate is whether in fact the effects being measured are adverse and whether they are acceptable to the public. Another question being asked involves farming. On many farms, antibiotics and pharmaceutical grade steroids are being used as growth factors. The story coming out of the water works in England is that there are many of these substances in surface water, things like nonaphenol, octophenol, and ethinyl estradiol, the major component of birth control pills. There is also natural estradiol 17 beta. When it comes into the sewage treatment plants in England it is not active; it has been glucuronated, sulfated, etc. The body has inactivated it and made it water-soluble so it can get rid of it. But in sewage treatment, the bacteria cuts off the added groups and it becomes an active hormone again. It is released out of sewage treatment plants as an activated steroid.

The question we asked was "It is known that farms are using huge amounts of these compounds, can we measure an ecological response from their use?" The initial study did not address whether there were natural steroids that happened to be in the feces or whether it was chemical grade pharmaceuticals. Most laboratories in the U.S. can't measure these compounds at a reasonable level. The laboratories are still dealing with part per million concentrations, which are inappropriate for most biological systems measurements, so the study was a collaborative effort with colleagues in Europe. Water samples from this study are currently being analyzed. The study is also utilizing

wild fathead minnows that live in these rivers; most of this work was initiated in rivers in Nebraska. The study shows that animals will have a significant reduction in circulating levels of testosterone, testosterone synthesis from the testes, and a significant reduction in testicular mass at a contaminated site (immediately below a feed lot) , or even at an intermediate site that has a feedlot and agricultural input compared to a reference 'clean' site. Similar things are occurring in females. So, it is possible to measure an actual alteration in the endocrine system and the reproductive system of these fish.

Other research is studying fish downstream from pulp mills. In this case, the worry is not dioxin, but what happens when a tree is processed, and a large amount of waste material is dumped back into a river. The river for this research is the worst case scenario; the Fenholloway River in Florida looks like a beautiful river except it is pitch black. Female mosquito fish in this river have started to develop gonopodia; there are androgens in this water. In collaboration with Earl Gray at the Human Effects Research lab at Research Triangle Park for EPA and Gary Ankley up at Duluth-EPA laboratory, we have been able to show there are components in this water that are not only androgenic, but they bind to the human androgen receptor and turn on gene action. We are now trying to identifying the chemicals. These results seem to be paralleling work that colleagues in Canada are doing. I point this out because it does not seem to be just one river or one site phenomenon where these females with the development of the gonopodia occur. This phenomenon is only dependent on androgens and is occurring in many places.

The wildlife story is telling us an interesting thing. That is that many of the cases of endocrine disruption or alteration occurs in developing embryos. Embryos require hormonal signals; blocking those signals or adding signals to the system alters the developing embryo. I don't care whether it is alligators or human beings; these embryos have windows of opportunity where signals are required to differentiate cells. It is not just whether there is the right kind of cell, but also whether the right constituents of expression are present, for example, receptors, enzymes, hormones and proteins.

The other thing that is critically important is understanding the system. There have to be appropriate endpoints; understanding normal is essential before you can go out and study abnormal. That includes how studies in laboratories are done with highly inbred species. The fact is we study single compounds in laboratory conditions and then try and go out in the field and make predictive comments about public health and ecosystem health. It does not work that way. It is frustrating for someone like me who can take off my developmental endocrinology hat and put on my evolutionary biology hat and say "Come on folks, wake up". Understanding relevant mixtures and ecologically relevant mixtures is crucial. Understanding relevant endpoints in the study species is crucial too. No matter how much estrogen is given to a pregnant woman, it will not sex reverse her baby boy into a girl. It is not going to happen. It can be done in alligators, but it is not working in humans. However, testicular function can be altered, p enis development can be altered, liver function that is critically important can be altered too.

It is very important for all of us to be a bit more creative and get beyond what has been the traditional way of thinking. By tradition, I mean we only study the chemicals we have the machinery to study, we only study what we are interested in. We have to understand if in fact we are going to study water, we can't just continue to go out and study chemicals in

water. This actually has to be true interdisciplinary work. Water professionals have to work with ecologists, biologists and molecular biologists. "Interdisciplinary" does not mean organic chemists talking to physical chemists. It means chemists talking to biologists talking to ecologists talking to molecular biologists talking to public health physicians. This is the only way to address the long-term ramifications of not just water pollution, but also air pollution, etc. I think it comes down to actually understanding what the normal system is doing. Why do I think this is important? Because I don't think pesticides and endocrine disruption is the only problem in the environment. I think it is critically important. Lots of words are put into my mouth all the time whether it be in the press or elsewhere. I stand here as a scientist, one that actually works in the environment trying to understand what is going on.

Hurricane Floyd came through this past fall and just missed Florida. I mention this because if a hurricane like this hits Florida, whole ecosystems are wiped out. Animal populations are destroyed or knocked down. Why do animals have the reproductive potential they have? So they can respond to ecological disasters. They may not express that potential on an everyday basis. As long as things stay stable, or as long as they are sitting in a cage and get the amount of food needed, they do just fine. But, the ecosystem as a whole has to be understood. In your field, water is not just what comes out of a sewage treatment plant, but it is what is in the aquifers, it is what is in the rivers, it is storm water runoff, it is all of those issues tied together. But it is tied together with the biology and the ecology of these systems.

I want to finish by saying that I am not a toxicologist, I'm a biologist. I think it is important for us to understand the balanced equation. We understand that the use of chemicals in our environment is critical; we understand that none of us want to go back to the Middle Ages and have outbreaks of encephalitis or cholera. We also have to start balancing the equation. We have to make sure that the benefits are balanced by the costs and the costs are not just the money to buy the product. Whether the public and whether we decide that certain risks or certain abnormalities or certain detriments are acceptable; that is a completely different debate. It is not a debate about whether it is happening or not. It is happening. We just have to decide to what degree we want to let it continue to happen.

Human embryogenesis and birth defects

Dr. Kathleen Sulik, University of North Carolina-Chapel Hill

Kathleen Sulik is Professor of Cell Biology and Anatomy, University of North Carolina at Chapel Hill with a joint appointment in Ophthalmology. She is a member of Skipper Bowles Center for Alcohol Research, and the University of North Carolina Birth Defects Center. Research in her laboratory is directed toward achieving a better understanding of the mechanisms and pathogenesis associated with a variety of environmentally induced or genetically based birth defects. A past-President of the Teratology Society, Dr. Sulik has been described as the person most responsible for the effective teaching of embryology to clinicians and medical geneticists and teratologists in the United States. She has a Ph.D. in anatomy from the University of Tennessee.

Dr. Sulik's presentation relied heavily on a slide presentation using images from scanning electron microscopy. The rich texture of her presentation cannot be captured by merely transcribing the audio. A web based tutorial of embryogenesis in which she and her colleagues prepared can be found at:

http://www.med.unc.edu/embryo_images/

The environmental endocrine hypothesis: premature verses precautionary science
Sheldon Krimsky, Tufts University

Sheldon Krimsky is Professor of Urban & Environmental Policy at Tufts University in Medford, Massachusetts. His research has focused on the linkages between science/technology, ethics/values and public policy. His current book is titled 'Hormonal Chaos: The Scientific and Social Origins of the Environmental Endocrine Hypothesis'. Dr. Krimsky was chairperson of the Committee on Scientific Freedom and Responsibility for the American Association for the Advancement of Science for 1988-1992. Currently he serves on the Board of Directors for the Council for Responsible Genetics and as a Fellow of the Hastings Center on Bioethics. Dr. Krimsky has been elected Fellow of the American Association for the Advancement of Science for "seminal scholarship exploring the normative dimensions and moral implications of science in its social context.

I spent four or five years studying the origins and development of the endocrine disruptor hypothesis, and I want to share with you some of what I have learned. Firstly, politics and science are symbiotic. Politics creates space for which science is done - public health science is done this way. Science nurtures the political entities that go out and fight for more science or the interpretation of science. Recently I was teaching a group of medical students about science based policy. I invited a person who had conducted research in the 1970s on the effect of phthalates on primates. It was very provocative data; the primates were given infusions or transfussions similar to those that people would be given using PVC tubing. The PVC tubing leaches phthalates. The investigator found some important adverse consequences, but for the most part, nobody cared. Now the politics have heated up; the persistent organic pollution convention began to heat up, and of course people are now looking at his research. That's an example of this very important symbiosis between politics and science.

Another example is the fact that both the Safe Drinking Water Act of 1996 and the Food Quality Protection Act of 1996 had provisions dealing with endocrine disruptors. The provisions were put in, perhaps, largely because of breast cancer activists working on Long Island. They had politicked for understanding the research into the possibilities that the rise of breast cancers on Long Island were related to synthetic organic chemicals in the environment. The political actions of these individuals, working with then Republican Senator Al Damato, resulted in getting endocrine disruptor testing programs into legislation. This provides another example of the interactive role of politics and science.

During the 1990s a small group of scientists began to raise concerns that an undetermined number of synthetic and natural chemicals

could interfere with hormonal systems in animals. They proposed a generalized explanation that postulated a link among a wide diversity of developmental and adult onset abnormalities. I have referred to this as the environmental endocrine hypothesis, because most of the chemicals under consideration are xenobiotics - chemicals used in agriculture or in industry - and the effects to which their exposure has been associated are linked through the endocrine system. Others have referred to this as the endocrine disruptor hypothesis to emphasize these chemicals can create disturbances in the development of the organism by mimicking or interfering with the messages of the body's natural hormones. Still others have referred to it as a theory of endocrine disrupting chemicals. While its precise status within science has not been discussed, this hasn't stopped some individuals from calling the hypothesis false or disconfirmed, refuted, tentative, or confirmed. Much of the debate has focused on whether this or that effect can be explained within the rubric of endocrine disrupting chemicals. The debate has not been looked at through the epistemological status of the claims. By this I mean, are we speaking about an explanation, an observation, a mechanism of action, a model, a theory, or some type of hypothesis - causal or associational. Without some recognition about what the claim means in science, it is difficult to understand what the role of evidence is with respect to the claim. Evidence has a different relationship to a theory, a model, an explanation, an observation, a hypotheses or a causal law. I am going to discus the nature of the debate, some of the politics of the debate of endocrine disrupting chemicals, and issues of contested knowledge in the context of the type of scientific product we have in the so-called environmental endocrine hypothesis.

One of the earliest generalized statements of the hypotheses came from a meeting organized by Theo Colburn in 1991- the first Wingspread Meeting. Twenty-one scientists adopted a statement, which said "A large number of man-made chemicals that have been released into the environment as well as a few natural ones have the potential to disrupt the endocrine system of animals, including humans." They then listed some of the types of chemicals. On the surface this statement is nothing more than an observation; it certainly is not concrete enough to develop a testable hypothesis - one that derives from the statement itself. This observation that certain chemicals have the potential to interfere with chemical messages in an organism is hardly news. Scientist have known about reproductive toxins since 1950, when it was discovered that if DDT was administered to newly hatched roosters, the secondary sex characteristics of those animals were effected. They became estrogenized. In the late 1960s and early 1970s it was d etermined that DDE - a metabolite of DDT- accumulated in the fatty tissues of the adult female bald eagles. DDE impaired the calcium release necessary for normal eggshell formation, resulting in thin shells and reproductive failures. We have known the pesticide DBCP (dibromochloropropane) was the likely cause of male sterility among workers who handled it in the 1960s and 1970s. In 1975, kepone was shown to lower the sperm count of workers and that it can activate very specific estrogen associated genes in the oviduct of chickens that natural hormones activate. If we go back to Silent Spring, which has many prophetic statements, Rachel Carson speculated that foreign chemicals can interfere with the body's ability to eliminate estrogen. An over-estrogenized body, she believed, would result in cancer.

In some sense we have already had evidence in the past about endocrine disruptors. The Wingspread statement says "..a large number of man-made chemicals". Does this become

an important scientific thesis because of its scope of application? I believe this is partially true. If there is a theory lurking behind this statement, its field of application and its universe of discourse are fairly broad. Its scope includes many different species, and potentially many different chemicals. The discovery of new effects that are postulated to occur among many different species seems too broad to be labeled a mere hypothesis, which is often relegated to cause-effect relationships of much narrower scope. Such are the examples as H. pylori as a cause of duodenal ulcers, or PCBs affecting the reproduction of salmon. Not only did the Wingspread scientists emphasize a large number of chemicals, but they also cited a large number of species that are covered by their findings. They stated in 1991 "the impacts include thyroid dysfunction in birds and fish; decreased fertility in birds" , and on and on. It is a very impressive list of effects in wildlife, including mammals.

In addition to the diversity of species effects, the range of effects are also quite varied. They become even more varied when new findings for the past nine years since the first Wingspread meeting are added in. So there is a general statement that a class of chemicals has the potential to disrupt the endocrine systems of animals, comprising a broad group of species resulting in a broad range of effects. I would state that these propositions are still not specific enough to be a hypothesis; it needs to be in the form that has testable consequences. Nor does it have the form of a theory. One can impose a hypothesis on it, but it doesn't lend itself specifically to a clear hypothesis, or a theory, which is a set of propositions that are very closely integrated and from which one is able to derive testable hypotheses. Theories are falsifiable. That is, one can imagine negative evidence. What would give this thesis a more precise focus?

Three elements would give these statements a more precise focus. Firstly, an independent means of identifying the chemicals, which might be a mechanism or several mechanism of action. The second would be a relationship between the mechanisms and the chemicals. The third element is the effects. The 1991 Wingspread meeting provided additional focus to the thesis by citing four general points that refer specifically to fetal effects. These points emphasize the contextual role of chemicals and human health and development. These include time and place of exposure, and that the cause and effect may be separated in time. The chemicals form some type of imprint in the organism in the developmental stages and the effects occur in some later stage of the organism's maturation. The chemicals of concern may have entirely different effects on the embryo, fetus or perinatal organism than on adults. That begins to focus attention a specific type of effect; the effects are most often manifested in the offspring, not in the exposed parent. The timing of exposure in the developing organism is crucial in determining its character and future potential. Although critical exposure occurs during embryonic development; obvious manifestations may not occur until maturity. These propositions help to focus the endocrine disruptor thesis. It refers to a class of chemicals that may not harm the adult but may affect its offspring if the exposure occurs during certain windows of development. Furthermore, these effects may not be observed until the organism reaches maturity. Thus far, there is nothing in the thesis about a mechanism or what distinguishes the type of chemical that can induce such effects. From the scientific literature there is no single mechanism that defines the pathways of endocrine disruptor effects. Several have been postulated.

For example, xenobiotics can attach to a hormone receptor and either promote or suppress the expression of the receptors

androgenic hormone. The chemical pathway to promotion or suppression of an androgenic hormone contains many more steps than a foreign chemical attaching to a hormone receptor. In other words, it's much more complicated than the often-simplified versions that are found in lock and key models. It has to enter the cells and it has to bind to a hormone receptor. Elements upstream of the gene regulated by the hormone come into play and there could be interferences or rogue messages at any point of the multistage process of hormone expression. Another mechanism involves non-receptor-mediated pathways in which hormonal messages can be scrambled. Xenobiotics can interfere with metabolizing enzymes in the cytoplasm. Thyroid diseases in animals have been identified as possible results of chemical interference during development. A third mechanism: animals have chemicals that bind with androgenic hormones to prevent them from reaching the brain; the bound hormone is rendered biologically inactive. For example, the female rodent is protected from influences of estrogen by estrogen binding proteins, which bind the free estrogen and prevent if from reaching the brain. But xenobiotic estrogens - synthetic estrogens - can bypass the protective mechanism of estrogen binding factors resulting in an over estrogenized organism.

So clearly, if there were a describable, specifiable and novel mechanism for action for toxicity, that would satisfy the conditions for a new theoretical framework for endocrine disruptors. That theoretical framework could look something like the following: A class of xenobiotic chemicals (C1 through Cn) that acts through a mechanism (MI) (it could be several mechanisms) when in the presence of the developing fetus during stage of development (S) at concentration (Ck) that does not adversely effect the adult will, with some probability (P) potentially adversely effect the development of the offspring. Moreover, mechanism (MI) is different than that which describes the acute toxicological effects of these substances.

If we had a thesis formulated like this we would be distinguishing it from more conventional toxicological hypotheses and theories. This new perspective on toxicology is based on the idea of chemical signaling. It states that the toxicology of some environmental pollutants may be the result of natural signals being sent by an unnatural signaling molecule. For the past decade, scientists have been trying to fill in the gaps. Let's call this formulation of the environmental endocrine hypothesis the Theory of Endocrine Disrupting Chemicals. Let's assume this formulation satisfies that theoretical perspective.

Thus far, scientists have learned that this class of chemicals cannot be defined by their structure. There are no telltale clues in the physical shape of the molecules - no phenol or hydroxyl ring that is characteristic of all estrogen mimics. The chemicals have to be tested for their unique endocrine effects. A highly developed theory might enable us to predict, or at least explain, which chemicals are most likely to produce the effects. The constructed theory about endocrine disrupting chemicals has the following components: some class of chemicals called endocrine disruptors, chemicals in this class interfere with naturally signaling mechanisms, the effect is most pronounced in the developing fetus, and the effects may not follow a monotonic dose response relationship. Higher concentrations may not show the effect, while lower concentrations might show the effect. There are already observed cases that support this thesis. But we cannot make any predictions from these propositions as long as we do not have an independent means of classifying the chemicals as endocrine

disruptors. Since we cannot make any predictions, we cannot make any false predictions. We can make guesses, but we don't have a theoretical framework from which to say the evidence is now falsifying the theory if we don't have a tightly knit theoretical framework. We can use the framework to establish guesses and focus research to determine if the guesses are plausible or whether they are consistent with the framework.

For example, we know that in certain parts of the country there is a sex ratio skew in the breeding of gulls. The male population has been reduced relative to the females. Some people think that this is happening to humans. Also there have been observations of female/female pairings in the nesting behavior of gulls. So we have an effect; can we show the effect is consistent with the propositions in this theory? First, we need a chemical agent that could be the cause of some effect. The pesticide methoxychlor has been identified as one possibility. Is there a mechanistic explanation that would tie methoxychlor to one of several outcomes? Perhaps the larger number of females in the population through some hormone mediated process, or a behavioral change in some female gulls that take on male roles, or behavioral changes in some male gulls that have been developmentally feminized and exclude themselves from breeding. Therefore if the males don't want to participate in the nesting then females take the place.

We may also find some effect that does not conform to this set of propositions. Does that refute the thesis? It might not, although if enough cases are found that are anomalous to the thesis, then the scope of the thesis would be limited. So the thesis is more than a hypothesis because of its breadth, but it is less than a theory because its propositions are not tightly connected. If we were to develop this into a tightly connected set of propositions, we could then begin deriving some hypotheses that would be tested, and which could in fact provide supporting evidence or falsifying evidence for the theory. The way the thesis has been developed thus far it is not a developed theoretical framework.

A model or framework is not falsifiable, at least in the way I have viewed the endocrine thesis thus far, in the sense that Aristotle's theory of motion is falsifiable and was refuted by Galileo. If there was an independent means of identifying endocrine disruptors by structure or short-term assays, or some mechanism of action, then we could infer testable hypotheses that would allow us to predict outcomes. For example, a chemical with a high estrogenicity potency factor - mainly it binds tightly to the estrogen receptors - would be more likely to result in developmental disorders in the offspring in pregnant animals who were exposed. That would be a hypothesis that could be derived from the theoretical perspective. Critics of the environmental endocrine hypothesis do not dispute the facts that the principal thesis is confirmed in some animal species. The major thrust of the controversy, both scientific and political, is whether the propositions that have been confirmed for some animal species also apply to some human effects. There is no consensus among scientists over a human effect that conforms to the principal thesis of the environmental endocrine hypothesis.

The postulated effects that I have identified cover quite a range. If even a small number of these effects were true (or confirmed) it would be a significant public health concern. Where we have the best animal data we don't have the best human data. Where we have the best human data we perhaps don't have the best information about the mechanism.

The sperm issues have been discussed widely. There was a string of articles on the hypothesis of sperm decline; much of this has been reported in the popular press rather skeptically. There have been numerous meta analyses on this. The scientists looking at the data cannot agree on whether or not there is a worldwide decline in sperm. There is even greater disagreement over what the cause of any decline might be. There have been a lot of interesting studies on human effects. Jacobson studied the effects on the children of mothers who consumed PCB contaminated fish. A recent study looked at the intellectual impairment in children whose mothers were exposed to biphenyls during pregnancy. This was a pretty sophisticated study that followed children until age eleven. The New York Times never connected this to endocrine disruptors; they decided this was an isolated issue. That's how the politics of this emerges. There are the lumpers and the splitters. I have to say that it's in industry's interest to be a splitter, to treat all the chemicals individually. Other interests, who see a larger picture, are the lumpers. The very fact that you have the term endocrine disruptors suggests a larger theoretical framework that these are not chemicals that are just acting outside of a larger mechanism that is common to many chemicals. That is another part of this political science dichotomy - some people would prefer to see these studies as very independent and not connected and others would prefer to see them as connected to a broad theoretical framework.

When you look at pictures drawn by four-year-old children who were exposed to agricultural pollutants in a research study and compare them to a normal four-year-old you see that those exposed cannot draw expressive figures. You have to say that something is going on with them developmentally. The pictures are quite provocative. On the other hand, these data simply don't convince many people that there is a human effect that is taking place. You will often hear that this is just another environmental scare that has been debunked by some body of evidence. The fecundity of the theoretical framework will depend on how many hypotheses can be confirmed. Lou Guillette, for example, has given very persuasive data about alligators earlier this morning.

I have created four hypotheses to present and discuss. As the mechanism of this first hypothesis becomes more fully understood, it may point to several signaling pathways that are characteristics of endocrine disrupting chemicals. It seems that Guillette is on his way to uncovering more than the phenomenological effect but also the mechanistic effect. That will certainly strengthen the theory or help build a theory.

With respect to hypothesis two, scientist have been studying the relationship between certain organochlorines (especially PCBs and DDT) and breast cancer. This hypothesis has to be tested indirectly, as we cannot easily perform experiments on humans. The data have not been consistent; the most recent comprehensive studies show no relationship between serum concentrations of DDT and PCBs and breast cancer. However, the theory from the Wingspread workshop suggests that the initiating causes of outcome are critical exposures that occur in utero. If that is the case, then the exposures for the breast cancer hypothesis must be measured during gestation. Moreover, the female neonate must be followed until she reaches maturity - sort of like the DES issue, which is a very important path to understanding endocrine disruption. Remember, when pregnant women were given DES it wasn't until their children were ages 20-25 that we began to see the effects of DES - clear cell carcinoma of the vagina. The theory suggests that the imprint of breast cancer occurs during pregnancy, perhaps

through a certain group of abnormal cells. This hypotheses could be falsified or confirmed for specific chemicals if longitudinal studies were done. It is possible that it could be another form of the theory based on a different mechanism that is triggered during the life of the adult. Leon Bradlow and Devra Davis, who postulated an alternative mechanism that doesn't depend on in utero exposure, have proposed such a mechanism. There are some studies being done to test their hypothesis but so far the evidence is not there.

The third hypothesis concerns itself with chemicals contributing to the declining sperm count. The evidence does point to certain regional declines. Studies in rodents show that a single maternal exposure to dioxin and PCBs reduces semen quality of adult offspring. It will take a very imaginative experiment to test the hypothesis for humans. The fact that there are regions in the world that do not show declines in sperm count does not refute the hypothesis. Unless we understand the theoretical framework we are looking at we won't know whether evidence supports or doesn't support it. The fact that there are some regions where the sperm count is not declining doesn't mean that in other regions where it is declining there might be an effect based on in utero exposure of chemicals. Skakkebk in Denmark has evidence that suggests that there are some early cells in the development of males that are abnormal and that may be the result of some in utero exposure. What I am trying to say is that we have seen very glib presentations that try to falsify a hypothesis or a theory without even stating what it is they are trying to falsify.

The fourth hypothesis is that in utero exposures to endocrine disruptors can result in human cognitive deficiencies and behavioral abnormalities. The data are epidemiological. As I pointed to earlier, pregnant women who ate higher amounts of PCB contaminated salmon had children with lower IQs - up to six points. They also had more behavioral problems. Studies of lead were the reason why we finally got lead out gasoline. Those studies were not a whole lot stronger than these kinds of studies. However, these studies do not demonstrate causality. But if the mechanism by which these chemicals could effect brain development are worked out they provide a much stronger confirmation of the theory and the hypothesis. EPA's testing program for endocrine disruptors is based on the idea that short term *in vitro* assays that measure hormone receptor binding and short term animal tests that measure any developmental abnormalities like frog metamorphosis assay or fish gonadal assay would be predictive of wildlife effects and human effects, if there are any.

In conclusion, science is still in its early stages of theory building with respect to endocrine disruptors. As a result, the thesis as currently postulated is less a theory than a heuristic or a framework that guides research. It suggests that if there is an abnormality that could be developmental and not genetic, one clue might be the exposure of the developing fetus to endocrine disrupting chemicals. The history of the nature/nurture debate eliminates that whole period of development. It is a false dichotomy to think that everything is either due to genes or to everything that happens after birth. There is that very important third element in the debate that is rarely discussed in the popular literature. Those who claim that one disconfirming test, like male sperm counts in a region of the world, weakens the entire edifice do not understand the nature of the scientific structure that is underlying the endocrine hypothesis with a theory of endocrine disrupting chemicals. Its explanatory power will be enhanced if its theoretical framework is developed and we can derive testable hypothesis from it. An

advanced theory requires well-defined terms, an integrated set of theoretical statements describing cause and effect relationships, and rules of correspondence linking the theoretical terms and propositions to observable and testable hypotheses.

Finally, here's a paragraph from the National Research Council's report on Hormonally Active Agents in the Environment. This was a long and detailed report, hotly contested by members of the committee. It says "Much of the division among committee members appears to stem from different views of how we come to know what we know. How we understand the natural world and how we decide among conflicting hypotheses about the natural world is the province of epistemology. Committee members seem to differ on some basic epistemological issues, which led to different interpretations and conclusions on the issues of HAAs and the environment." Basically, is says people couldn't even agree on the definitions. Until we begin making the definition more precise and creating a theoretical framework with testable hypotheses we will have the same kind of contested science that we have seen thus far.

Wednesday April 19, 2000

Session 2: Identification and Occurrence of Compounds

Challenges associated with quantification of trace concentrations of pharmaceuticals in a complex matrix

David Sedlak, University of California-Berkeley

David Sedlak is Professor of Civil and Environmental Engineering at the University of California at Berkeley. His research interests are related to the fate and transport of pollutants in the environment. Presently, he is studying the environmental chemistry of pollutant metals and polar organic compounds in surface waters. Other research interests include transport and transformation hormones in the aquatic environment, and pharmaceutically active compounds (PhACs) in the aquatic environment. Dr. Sedlak received the National Science Foundation CAREER development award in 1998. He is on the editorial board member of Environmental Technology, Project steering committee member of the Water Environment Research Foundation, and Campus coordinator, University of California Toxic Substances Research and Teaching Program Coastal Toxicology Group.

I would like to look at the engineering issues associated with pharmaceutically active compounds and endocrine disruptors. I hope this morning's introductory talks convinced you that there is some interesting and provocative science related to endocrine disruptors and pharmaceuticals in the environment. But as yet, engineering challenges and solutions have not been discussed. One reason for this workshop is that the water industry wants to be proactive about these issues. To be proactive as engineers and scientists, we have to know what to do in order to prevent these problems or to avoid the difficulties associated when trace contaminants are present. Addressing them in a timely manner is important.

There are many trace contaminants that present problems for the water industry because the industry was not proactive enough. I will discuss these compounds and try to define the problem better. The title of my talk implies that I am going to cover the challenges associated with the quantification of these compounds. However, I am not going to spend a lot of time getting into the nitty-gritty details of how to analyze the data. Instead, I want to go through the thought process of how to take thousands of different compounds and start defining a more reasonable problem - one that the water industry can have some impact on.

Let's begin by talking about what are driving the concerns related to pharmaceuticals and endocrine disrupting compounds in the environment. An example is population issues. The population in California is predicted to double. As that happens, California will have a lot of new infrastructure needs, which will be addressed by engineers. This isn't a problem unique to California. In the Southwest and in southeast Florida, populations are growing very quickly and are going to outstrip local capacity to provide water. These issues need to be looked at in terms of all aspects of environmental engineering, but probably most importantly in terms of water and the water industry.

With respect to population increases, environmental engineering programs have taught students to address increases in population by building more. That is not the kind of solution that is going to work, especially in parts of the world where there is a large population but not a lot of water. This conference is being co-sponsored by the

WateReuse Foundation. Growing populations and growing water demands will have to consider the issue of water reuse - either indirect water reuse or direct water reuse.

In thinking about how to accommodate larger populations it becomes apparent that wastewater discharge becomes the source water going into many drinking water treatment plants. This process, called water reuse, has been practiced for many centuries around the world and it should not strike us as something unusual. Indirect water reuse has been practiced for many years. Water is discharged directly into a river and it comes out kind of brown. There is some sort of natural attenuation of the contaminants in the water. The contaminants will go away as it goes down river, or dilution comes into play as more and more freshwater supplies come in to dilute the pollutants. There is also a concept of direct water reuse that it is a valuable resource. Rather than discharging this wastewater to the surface water body and letting someone else use it, utilities capture the wastewater after it has been treated, store it in a reservoir or groundwater system, and pump it back up and use it as a water supply when it is ne eded.

There are a lot of interesting engineering issues associated with both the ideas of direct water reuse and indirect water reuse. Indirect water reuse is not emphasized nor thought about too much, but it does occur. When direct water reuse projects are proposed, issues with pharmaceuticals and endocrine disruptors can and do come up. People see that it is sewage water and it is becoming drinking water somehow. An interesting issue associated with indirect water reuse involves aquatic habitats. Potable water reuse is not discussed unless this water is reused for drinking water supplies. Treated wastewater, though, is acceptable as an aquatic habitat. For example, water reuse projects can create a new wetland or augment the flow in a stream. Or wastewater from a very large population center results in discharges that create an effluent-dominated surface water - that is, the stream flow or most of the water is coming from wastewater. There are some important aquatic habitat issues, because there is an ecosy stem that is living in treated wastewater.

Parts of the Midwestern U.S. or other rainy areas in the world have the luxury of not having to think very much about water reuse because there are ample supplies of water, and when wastewater is discharged, it is quickly diluted. In other parts of the world, water reuse is, has been, and will be a reality. In California there are water reuse projects going on right now that are providing drinking water. Indirect water reuse, happening either intentionally or unintentionally, is having large impacts on aquatic habitat. The Rio Hondo spreading basin in Los Angeles is one place where treated wastewater is discharged and infiltrates into the ground. While it may or may not end up in the drinking water supply, it certainly plays a role in current water supply in the Los Angeles area and in future planning for water supply as the population grows. All around California there are projects where either drinking water or aquatic habitat is being augmented by wastewater supplies. The City of San Diego is known for i ts infamous "toilet-to-tap" project that proposed using wastewater discharged into a reservoir and turning it into drinking water. Closer to San Francisco, the Livermore Valley project takes highly treated wastewater effluent and injects it back into an aquifer. Potable reuse is a very important issue in parts of the country where the water supply is limited.

An example of aquatic habitat reuse is in South San Francisco Bay. One million people in SiliconValley have their wastewater go through a treatment plant that discharges into the South Bay. The salinity of the South Bay, above the Dumbartin Bridge, is similar to sea water. Below the bridge, the salinity is between 5 - 10 parts per thousand. Between 50 - 90% of the

freshwater in this area in the summer time is derived almost entirely from wastewater effluent. The sensitive aquatic habitat living in these marshes - endangered species and salmon fisheries - all are living in treated wastewater. Any endocrine disrupting chemicals that are there are obviously an issue. In the particular case of the South Bay, there is also a proposal to take an ephemeral stream and augment its flow with wastewater effluent to improve the salmon habitat. Obviously, there would be issues associated with pharmaceuticals or endocrine disruptors. But all over California there are cases where wastewater is important to aquatic habitat.

So, water reuse is important in parts of the world where there is a limited water supply. Water is reused for drinking water purposes or for aquatic habitat. Engineers are doing these things and are planning them for the future. Projecting where water for increasing populations is going to come from in the future is crucial, and these projects are going to consider water reuse.

There are concerns about water reuse that have large implications. For example, the National Academy of Science (NAS) issued a report in 1998 that presented an interesting and balanced assessment of water reuse. Their charge was to look at indirect water reuse. There is a controversial sentence or two in the executive summary of that report which says "Our general conclusion is that planned indirect potable reuse is a viable application of reclaimed water. However, indirect potable reuse is an option of last resort. It should only be adopted if other measures have been evaluated and rejected as technically or economically infeasible." There were two main reasons why the NAS expressed concern about water reuse: pathogens that might not be removed completely during treatment, and chemicals that were poorly defined or unknown. At that time, NAS was not sure what those chemicals might be, because all the chemicals that could be present in wastewater had not been characterized. There was reason enough to exercis e caution. In the past couple of years some papers have come out, most notably in Europe, that indicate pharmaceuticals and endocrine disrupting compounds could be present in municipal wastewater, and this would be one reason caution should be taken when practicing water reuse. Some of the projects involving water reuse that I mentioned previously (Livermore Valley project and the San Diego project) encountered issues of endocrine disruptors and pharmaceuticals during public discussions, which created an uncomfortable position for the water industry.

The objective of my talk today is to discuss this huge list of potentially problematic compounds. Think about all the different drugs manufactured by the pharmaceutical industry or all the different compounds that the toxicology community has identified as potential endocrine disruptors. I want to take that list and break it down into a smaller number of compounds. As members of the water treatment industry, you have to realize that if you are going to address these compounds you can't address the whole universe. You have to prioritize which compounds are going to be important and identify those that are most interesting to study; the next step is to quantify them. I'll try to show some of the approaches for quantifying the priority pollutants that don't lend themselves very well to measuring pharmaceuticals. If the industry tries measuring these things in the environment, laboratories may need to be re-tooled and new skills must be developed. Finally, I want to discuss predicting the environmental fate of these compounds because it will have implications for the employment of treatment technologies.

One approach for identifying pharmaceuticals involves looking at records from the pharmaceutical industry. It is very difficult to get records of what drugs are prescribed in the

U.S., they are closely guarded by the industry. We have been able to find data on the top 200 prescription drugs administered in the U.S.; we have taken that survey of drugs and broken it down by calculating the size of the dose and the number of prescriptions. We have also talked to pharmacists about how many drugs might be present and what their concentrations would be in wastewater influent. You can take the size of the dose, the active ingredient, and data on the metabolism of the drugs and then calculate out with dilution in the wastewater stream what the concentration coming into the wastewater treatment plant might be. These data are good to one significant figure. Some of the top 200 prescription drugs have the same active ingredient in several different formulations, so there are around 100 different compounds of interest. Based on our best estimates, we calculated that about 50 of them would be present coming into a treatment plant at greater than 1.0 microgram per liter. Another 10 compounds would have a concentration between 0.1 - 1.0 microgram per liter, and the final 50 at less than 0.1 microgram per liter.

In addition to drugs prescribed by doctors, there are a number of other drugs that could find their way into the water supply. For example, the over-the-counter drugs ibuprofen, aspirin and acetaminophen. There are not good records on how much of these substances might end up in wastewater, but based on estimates of other compounds, we are pretty confident that it would be greater than 1 microgram per liter for many of these compounds. There are a number of compounds that are used only in a hospital setting that are potentially problematic too, because they can be very toxic. HIV, chemotherapy and organ transplant patients take very strong drugs that can potentially end up in the water supply.

There are a very large number of endocrine disrupting compounds; I will limit my talk to the pharmaceuticals that are actual hormones. In the list of the top 200 drugs there are several hormones that are considered to be endocrine modulators. From that list there are about 10 pharmaceuticals present at about 1 nanogram per liter that are hormones. Those include things like estrogens, androgens and thyroid hormones. Many of these are very commonly used. For example, the number one prescription drug in the U.S. in 1997 was Premarin, which is used in hormone replacement therapy. Premarin is an equine estrogen. In addition, there are endogenous hormones produced within the body that come out in the urine.

The next step is to consider which compounds in widespread use will get into the wastewater, then which ones might be coming out in the wastewater treatment plant effluent. A German study by Ternes (1998) on pharmaceuticals in wastewater showed influent and effluent concentrations for a selected group of pharmaceuticals. Not all compounds behave the same way in the wastewater treatment plant. Some compounds, like propanole, are very well removed; while there may be 4 - 5 micrograms per liter coming into the treatment plant, much less than 0.2 micrograms per liter comes out. Propanole is removed either by biotransformation or by absorption onto solids that settle out in the treatment plants. Other compounds, such as carbamazepine, are barely removed at all in the treatment process. As we decide which compounds we should be most interested in monitoring, we should think about those that might be present in high concentrations, including those that are being metabolized. The Ternes data show benzafibrate come s into a treatment plant at a relatively high concentration. Leaving the plant, there is still more than 1 microgram per liter, but this compound will probably disappear in the environment over a short amount of time. Other compounds coming in at relatively low concentrations may be very recalcitrant and you may still find them in the environment.

I must caution those of you that have started looking at the literature on pharmaceuticals. There is a bias. Most of the data and recent literature come from studies in Germany, Switzerland and other European countries. There is spatial variability, especially with respect to pharmaceuticals. Doctors are different, pharmaceutical companies are different, and patent law is different in different countries, therefore prescription drug use is different. Different pharmaceuticals are prescribed and used depending on the country. Even within the U.S. there likely is some spatial variability. Clofibric acid and its precursors provide an example of this. There are a variety of compounds that are metabolized into clofibric acid. This compound got a lot of people thinking about pharmaceuticals in the environment, from Berlin and Rhine River data where clofibrate was detected. The influent concentration of clofibrate in the U.S. is less than 0.1 microgram per liter, because it is a very uncommon pharmaceutical in the U.S. Other compounds have largely replaced it. Although it is a very interesting compound with respect to its behavior, it is not a pharmaceutical of interest in the U.S. Benzafibrate has a similar story; it is not heavily used in the U.S. Other compounds have very good agreement spatially; my calculated influent concentration agrees very well with Ternes' calculated influent concentration. Other compounds are much more widely used in the U.S. For example, gemfibrozol is calculated to have about five times more use in the U.S. than in Germany. Zithromyacin is found in concentrations less than 1 microgram per liter in Switzerland, but in the U.S. there are 8 - 9 micrograms per liter. Don't be fooled by data from one country into thinking that you'll see it in the U.S.

I'd like to focus on analytical issues by addressing the question "why are pharmaceuticals being found in the environment?" Chemicals have been studied in the environment for many years. Many people have taken wastewater and extracted it and stuck it into a GC/MS to see what chemicals are there. Why, all of the sudden, are we seeing pharmaceuticals and endocrine disruptors? The priority pollutants, the VOC's, the base neutrals, the pesticides and the PCBs all have properties that make them amenable to analysis by GC/MS. That is, they either have a high vapor pressure that allows them to go into the gas phase and be analyzed by GC, or they are relatively hydrophobic, which allows them to be analyzed structurally by GC/MS. On the other hand, pharmaceuticals have a low vapor pressure, a relatively low partition quotient, and functional groups that cause them to hang up on GC columns, so they don't behave well in GC/MS systems. That is why pharmaceuticals aren't detected very much when priority pollutant anal ysis is done. However, they are there and the reason we are starting to see them now is that new technologies for measuring chemicals in the environment are taking advantage of other approaches for analyzing these chemicals, most notably LC/MS or LC/MS/MS.

Some techniques used to analyze organic compounds will have to be abandoned or modified to analyze pharmaceuticals and endocrine disruptors. Another reason a new analytical approach is necessary is these compounds occur at relatively low concentrations. For the priority pollutants, liquid extraction was used. Many of the pharmaceuticals require solid phase extraction, often times with specialized solid-phase extraction media. For cleanup, silica gel could be used for things like PCBs and pesticides. This method won't work well for pharmaceuticals because they have similar polarity to the compounds found in natural organic matter. Instead, some sort of reverse phase clean up might be tried. For detection, GC/MS is fine for some compounds. With low concentrations, we often want to go to tandem mass spectrometry GC/MS/MS or liquid chromatography with mass spectral detection.

LC/MS suffers from problems with humic substances interfering or LC/MS/MS or immuno-chemistry, which involves antibodies as detectors.

I would like to give you an example from our research of how we've gone about looking for estrogenic hormones in the environment. We would expect to find the estrogenic hormones 17-B estradiol, ethylene estradiol for birth control, equiline and dihydroequiline in wastewater. We predict somewhere between 15 - 20 micrograms per liter of 17-B estradiol and about 2 micrograms per liter of ethylene estradiol. The lowest observed effect level is the point at which studies of fish feminization can be induced in the laboratories at concentrations that low. If you realize that these effects are additive, because you are working on the same receptor, we can definitely expect some endocrine disruption or feminization of fish by exposure to wastewater effluent.

Data from a secondary effluent in an activated sludge treatment plant, a tertiary effluent plant with an activated sludge treatment plant that also has nitrification and effluent filtration, and an advanced treatment plant that employs microfiltration and reverse osmosis prior to a water reuse project show that we can detect these compounds at several nanograms per liter reliably. There is a correctional relationship to the degree of sophistication of the treatment plant and the concentrations in the effluent. The more sophisticated the treatment plant, the lower the concentrations. The compounds can be barely detected in reverse osmosis water; they are detectable but not quantifiable at those levels. So the concentrations are reproducible within a treatment plant. We find both 17-B estradiol and ethylene estradiol. We didn't analyze for the other estrogens.

What about surface waters? There is interest in the fate of hormones in surface waters. We have been studying the Santa Ana River in Orange County and the data are quite puzzling. The measurements were made in wastewater treatment plants that discharge into the river. This is a river that is almost 100% wastewater effluent. On one day in November, the concentrations of the effluent were about the same as the concentrations in the river, suggesting very little removal. On another day, there were very high concentrations near the treatment plant and lower concentrations in the river. We are going to be studying the fate of these compounds in an engineered treatment wetland, because what the data show so far is that there is probably not a lot of removal in the surface water. The Santa Ana River is shallow water that doesn't have a lot of life in it; it doesn't have a lot of attached surfaces for bacteria to grow. We are going to look at the fate of these compounds when they go out of the Santa Ana River and int o an engineered treatment wetland, to get a sense of removal there.

To summarize, there are endocrine disruptors and pharmaceuticals in wastewater. Concentrations can be predicted in wastewater influent; in many cases, they can be measured in the effluent. Now we need to start thinking about advanced wastewater treatment systems. Some data suggest that hormones can be removed in these facilities, but little is known about other pharmaceuticals. Effluent dominated surface waters and indirect water reuse will drive the need to investigate the fate of these compounds in waters. The best place to start looking is in waters with high concentrations or high fractions of wastewater effluent, because it is very difficult to detect these things when there is also dilution to consider. Groundwater recharge systems where partitioning and bacteria attachment to surfaces occurs are going to be important in metabolizing these compounds.

Finally, pharmaceuticals and endocrine disrupting compounds are not the kind of issues

that we should sit passively and wait for regulations to be handed down to the water industry. There are engineering treatment alternatives to consider as we design drinking water systems, wastewater systems and water reuse systems. The role of natural attenuation should be considered - how quickly are these compounds removed once they are discharged? Maybe that will affect decisions about surface water recharge versus infiltration versus direct recharging to an aquifer. Management alternatives are very important. For example, as water reuse is considered, we have to decide whether this is the right thing to do before we invest money in it.

Screening and testing for endocrine disruption: chemical mixture issues

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Previous speakers have touched on the importance of studying mixtures. Obviously, we are all exposed to mixtures. In fact, each individual in this room is a rather unique chemical mixture. Everything in our universe is composed of chemicals, so we are exposed to multiple chemicals. That should be reason enough for studying mixtures. In the area of endocrine disruption, there are some additional reasons. For example, the Food Quality Protection Act (FQPA) has a specification for testing cumulative toxicity that occurs from exposure to multiple chemicals that operate by the same mechanism. Another reason is that the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) recommended the screening of some specific chemical mixtures. The Science Advisory Panel reviewing that report advised EPA to delay that recommendation and go forward only when screening had been conducted for a number of individual chemicals, which resulted in some basis for fine tuning the screens and assays and interpreting the results.

There have been a number of agency activities on mixtures. In 1990, EPA finalized guidance on identifying chemicals and other substances that produce toxicity by a common mechanism of action. In addition, EPA finalized some risk assessment guidelines for chemical mixtures. I would like to give an explanation as to why I think the Science Advisory Board and Science Advisory Panel were wise in recommending pulling back on screening and testing for mixture effects. It is a very complex issue. I will then finish up by looking at published data on interactive effects of estrogenic mixtures.

There are basically two broad categories of approach for screening and testing mixture effects. The first category is the whole mixture

approach; within that are a couple of subdivisions. This approach tests the mixture itself; it is often done with specific defined mixtures, such as product formulations. A subdivision of this is to test a surrogate or a similar mixture. There is some economy of scale that can be attained by testing a surrogate mixture instead of every individual mixture. This approach was illustrated in an article on reproductive endpoints by Heindel, et al: A 1995 Article, *Fundamental and Applied Toxicology*. The second category is the component-based approach, which can be subdivided into two general categories: approaches that assume additivity, and approaches that assess interactions. A component-based approach derives toxicity data on the chemical components of the mixtures individually or in small defined combinations, two or three chemicals at a time, rather than testing a whole mixture. Some prediction about the toxicity of the entire mixture is then made.

What are the pitfalls of doing it this way? Testing the mixture itself raises several questions. What exactly is the mixture? If drinking water is tested, what is the mixture? Will the drinking water itself be tested? Will it be water from Chicago or San Francisco? Will the test involve simply feeding it to rats or mice or alligators? Will the mixtures first be analyzed to determine what the contaminants are and at what level? One of the reasons this is an important question is toxicity studies require multiple doses. If a mixture of Chicago water is being tested, how will the doses be determined if the contaminants are not taken into account and then concentrated in some way?

This is important because the toxicological effect of a mixture can be concentration-/dependent. It can also be dependent on the ratio of the constituents; those ratios can vary across different drinking water sources. The question then becomes how are regulations and decisions made concerning individual contaminants on the basis of whole mixture results? There are a lot of complexities involved in the simple approach of just taking the mixture and testing it; the whole matrix of the water might be important. Is it assumed that only contaminants are important? Or are pH, hardness, and other characteristics of the water important? Will those define the toxicity?

Testing a similar mixture raises additional questions. How is similarity defined? Is it a toxicological similarity? Is it defined as a mixture of chemicals that will produce a similar effect, or is it defined based on chemical similarity? There may be problems basing it on chemical similarity. Estrogen, estradiol, and testosterone all look similar at a molecular level; toxicologically, pharmacologically and physiologically they are different. If it is based on toxicological similarity, how is validation done for those mixtures if they are indeed similar? Again, the concentration and ratio of the constituents is important.

This kind of complexity underscores the reasons why there are so many component-based approaches to the testing of mixtures. I categorize them a bit differently, by saying that component-based approaches can fall into two broad groups. One approach is based on the mode of toxicity. Some approaches are called "mechanism of toxicity approaches", but they are not.

The mode of toxicity approach includes several sub-groups. The hazard index approach typically is used in circle-style risk assessments. For these, concentrations of chemicals are added up, then specific chemicals are split out by target organ of toxicity. This is applied to non-cancer endpoints. Another mode of toxicity approach is the toxic equivalency factor (TEF), which is

used for dioxins and dibenzofurans. TEF is based on a presumed feature of a mode of toxicity, or a mechanistic feature that is presumed to have something to do with the mode. Another approach is used for the FQPA that stipulates assessment of cumulative toxicity occurs by a common mechanism of action. The criteria the Act references is really a mode of action approach.

What is the difference between mode and mechanism? Mode refers to the broad general features of the effect of a chemical. Mechanism is the detailed sequence of events that occurs between absorption of the chemical into the body and the manifestation of its effect. Mechanism is much more detailed and requires more data to understand. These are basically mode of toxicity approaches. Previously, I mentioned EPA's draft mixture guidance for risk assessment, which discusses a number of different approaches. The EPA document states that additivity should be assumed for chemicals with a common mode of toxicity. The problem is defining additivity; I will discuss that later in my talk.

There are mechanistic approaches to mixture assessments. I am familiar with one that is ongoing in the pharmaceutical industry, which tries to avoid the development of a drug that may interact with another class of important drugs. For example, it would be a waste of money to develop a drug to treat arrythmias that interacts with a drug that lowers blood pressure, because patients often take those two types of medications together. In order to try to avoid this very early on, the pharmaceutical industry is asking the question, "What actual biochemical and physiological mechanisms lead to adverse drug interactions?" If it can be understood mechanistically, perhaps *in vitro* or short term *in vivo* assays can be developed to test individual chemicals for their ability to act through that mechanism. If they do, that raises a red flag that there may be an interaction with another class of chemicals.

There are other approaches, which I call hybrid approaches, that are very powerful. el-Masri published a review of these in Critical Reviews in Toxicology in 1997. Three approaches combining data from different types of experiments - pharmacokinetic data, mechanistic data, and assumptions about interactions - produced an overall model to predict the result of exposure to a mixture of chemicals.

What are some of the pitfalls of these component-based approaches? Additivity approaches have been criticized because they assume that when chemicals are present together their effects are going to be additive. Is that scientifically valid? Do chemicals really add together when they are present in mixtures? Is that a protective assumption? Risk assessments are interested in being not just predictive, but also protective. Is making the assumption based on the mode of action really valid? Is mode of action really predictive for the mixture effects of two or more chemicals? These questions present weak points in the data. The other approach would be to assess interactions rather than making an assumption, which can lead to some really muddy waters.

There is a great disparity in the terminology used within the field of interaction analysis. Different scientists working in different areas use different terms for similar things. In addition, methods employed in the study designs are highly disparate; there is not much consistency, which can lead to inconsistency in the data quality. Barenbom, the father of interaction analysis, has suggested the method that predominates is the "no method" approach. In this approach, it is assumed that the results achieved with any combination of chemicals are somehow self-evident, and a

rigorous study design is never applied. Another criticism is that some very large study designs are required.

Let's expand on the mode of action. Mode of action refers to the general features of a chemical's effects, not the detailed step-wise sequence of events. Should we assume that toxicologists and pharmacologists know what mode of toxicity is? I went to some toxicology textbooks to see what the major categories of mode of action were. While we think we know what we are talking about with mode of toxicity, no two textbooks do it the same way. Casarret and Doull does it two different ways, Hayes does it a different way, Sullivan and Krieger a different way, and Rand, in Fundamentals of Aquatic Toxicology, yet a different way. There is no consensus on what is meant by mode of toxicity or how to go about lumping chemicals together by mode.

The International Life Sciences Institute (ILSI) convened a panel to look at the FQPA proposals that addressed cumulative toxicity occurring by a common mechanism. Is it valid to assume that all organophosphate pesticides, for example, work by a common mechanism? The panel came up with five criteria for assessment: the chemicals had to have the same critical effect, the same molecular target, the same target tissue, the same biochemical mechanism and the same toxic intermediate. The panel primarily looked at organophosphate pesticides, which have a very specific mechanism of action. This is a dose issue too. All chemicals have a multitude of effects. Those used as drugs or as pesticides have one mechanism of action and one type of effect that occurs in the dose range far below the dose range that causes all of their other effects. That confers some specificity, so we can use those as drugs or pesticides. It is optimistic to think that this mode of action approach is going to lead to accurate predictions ab out mixture toxicity.

For example, let's say we have a mixture of ethanol and methanol where effects would be reproductive, developmental, central nervous system, hepatic, and optic nerve toxicity. Adding ethylene glycol makes it even more difficult. There is similarity in some endpoints - reproductive and developmental - with ethanol and methanol. Ethanol is the treatment of choice for methanol poisoning; it antagonizes the optic nerve toxicity and this effect is rather species-specific. But it is not possible to predict those based on animal laboratory studies. Moving from single chemical effects and adding those effects in a mixture becomes very dicey.

Where are we at this point with assessing hazards and risks? I think no single approach will be universally applied. It will require a lot of effort using different approaches. The sophisticated hybrid models are probably premature for most chemicals, because they are only as good as the data upon which they are based. It appears to be a promising way to go, but it will take a lot of effort. I have discussed why the mode of toxicity may be a red-herring. Many of these approaches require predictions or assumptions about interactions, and require some data on interactions. I believe that additivity is the best-supported assumption, especially if your goal is to be protective.

Let's address the different types of chemical interactions. Keep in mind that there are differences in additivity. If two chemicals (A and B) are added, the expected effect, if they don't interact, will be additivity. In this case, dose additivity is a doubling of these two, or A + B. There is a presumption that chemicals act as simple dilutants of one another. This TEF approach is an example of a dose additivity assumption. Another non-interaction model is called independence, which is built on probability theory. It assumes that the action of chemical A is not

influenced in any way by the action of chemical B when they are combined. Results can vary depending upon the mode of action. Both independence and additivity are used as hypotheses or models for predicting the effect of two or more chemicals in a mixture if they do not interact. Synergy and antagonism are relative to this expectation of what might happen if they don't interact. That is going to depend on your model of non-interaction. Synergy encompasses lots of things, like potentiation and super-additivity. It can be defined as an effect greater than expected based on some model of no interaction, and antagonism can be defined as an effect less than expected.

Low dose concerns are another reason mixture assessments are important in the study of endocrine disruption. Low dose may imply that there may be no threshold; low doses may interact producing a non-monotonic dose response curve. Using this idea, what would be a no-interaction hypothesis for two chemicals having dose response curves that might be non-monotonic? An interesting risk assessment approach would be one that determines the effective dose 10% (ED10) of each chemical when the chemicals are combined. The first question is which ED10? Which model of non-interaction is best for these chemicals? Can dose additivity be assumed? That assumption requires that chemicals act as simple dilutions of one another and their dose response curves would be parallel.

The complexity of the endocrine system has been described in the literature as being homeostatic, adaptive, feedback-controlled, and optima-based. In other words, adverse effects occur with too little hormone or with too much hormone. The endocrine system integrates inputs from many different sensory systems. Hormonal effects have been described as transient, that timing of exposure could be important, that effects can be compensatory, that hormones keep an organism on an even keel. The endocrine system can be multivariable because it is feedback- controlled and integrated, or it can be deficiency-induced. As John Ashby pointed out in 1997, there are a lot of problems reproducing data that may be partially due to this complex system.

Despite this complexity, there have been a number of reports of estrogenic synergy. Before starting a research project, my colleagues and I looked for reports of estrogenic synergy; we found a number of reports that persistent chlorinated organics and steroidal estrogens synergize. I will talk about two of these studies because they are the best in terms of study design.

EPA believes it is best to use specific mixture data; the second best data is from a similar mixture. If neither is used, a component-based approach is suggested, where data on interactions are investigated. For the component-based approach, assessment and evaluation of data quality that has reported possible interactions is used, instead of using the default assumption of additivity. EPA does not give a detailed guidance on how to do that. We reviewed several hundred papers in the pharmacological literature and pulled out dozens of methods of analysis for mixtures. We identified five key points from the methods. 1) Know the dose response curves of the individual chemicals. 2) Define an appropriate no interaction hypothesis when evaluating data. Is it dose additivity, is it independence, or is it some other model? 3) Assess combinations of the components at more than one ratio. Effects can be dependent upon the ratio of constituents and the level of constituents; for mixtures it is not only the dose that makes the poison, it is also the dose ratio of the constituents that makes the poison). 4) Apply an appropriate statistical analysis.

Apply that statistical analysis to your expected effect according to your non-interaction hypothesis as opposed to your observed effects to find out if there is really a departure from no interaction. 5) Assess interactions at relevant levels of biological organization. There are examples in the literature where synergy at a receptor results in antagonism in the whole organism and vice versa.

Bergeron et al. published a report on estrogenic synergy in Environmental Health Perspectives in 1999, which said there does need to be concern about very low levels of chemicals in the environment, because these low levels can synergize. If endogenous hormones work through a synergistic mechanism and a very weak estrogen (such as estrone) can synergize with a stronger estrogen (such as estradiol), that may lay the foundation for assuming that relatively less potent xenobiotics might also synergize with a natural hormone system. These experiments were done in a yeast construct; this is a transcriptional activation system construct with a human estrogen receptor in yeast. Activation of that human estrogen receptor by some estrogen will cause transcription of the gene for B-galactosidase, which is an enzyme that can be measured in the growth medium. Increased activation of the estrogen receptor in this system results in increased enzyme activity, measured by Miller units.

The Bergeron experiment is well designed. The study looked at the dose response curves of estrone doses of 1, 10, and 100 nanomolar, with no estradiol. Similarly they looked at 17 B-estradiol doses at .1, .5, 1, and 10 nanomolar, with no other compounds. Finally, they looked at combinations of doses of estradiol and 17 B-estradiol. The paper reported observed values and then, in parentheses, the expected additive values. What I was curious about was what no-interaction hypothesis did they use? It was not stated in the paper. Bergeron used a model that assumes an effect addition or response addition - it assumes a linearity of the dose response curves by simply numerically adding the effects. Remember, it is adding or combining dose response curves, not just individual points on that curve.

We went back and recalculated all the expected additive values, because only a few were reported. We put them on a percent basis and found that there were synergistic combinations, though it was not mentioned in the paper. There were also antagonistic combinations. The data also show that at higher doses of estradiol, the dose response curve is plateauing - perhaps due to a saturation of the receptor. It was curious that there was an antagonistic point that was not mentioned in the report. If there is plateauing of the dose response curve, that implies that there is not a linear dose response curve. Giving the researchers the benefit of the doubt for not reporting this would require that you think the dose response curve might be plateauing and you would get antagonism because the system could not go any higher.

I want to try to explain dose additivity and response additivity. As an example, think of an interactive experiment with a single chemical. There are two doses in this experiment - dose 1 and dose 2. There are also two effects- effect 1 and effect 2. From this, predictions are made about the dose response curve. Perhaps there is not quite enough data for a curve; in many toxicological studies only three doses are done. In this example, no dose and two doses characterize the response curve. Combining D1 plus D2 gives D3. Combining dose 2 plus dose 2 yields dose 4 along the line. But actually doing an experiment with this chemical at dose 3 or dose 4 levels may lead to a different result - it may show a dose response curve that is not linear. That might lead to the erroneous conclusion that the

chemical synergizes with itself at these higher doses, as it no longer looks linear.

This example demonstrates the importance of knowing the dose response curve of individual agents. Our interaction studies are in their third year and we're just beginning to nail down the details of the dose response curves. The assumption of linearity can give a very different result as opposed to looking at the whole shape - especially when addressing a seasonal assay for animals that have reproductive peaks. It does matter how it is added.

With this in mind, let's go back to the Bergeron study. I mentioned giving them the benefit of the doubt for not reporting the high dose effects; a plateau would have to be assumed. We looked at the same data points but in a bit different way. Bergeron's study looked at the dose response curves for estradiol in the presence of no estrone and then increasing concentrations of estrone. Another way to look at the same data is a dose response curve for estrone plus increasing concentrations of estradiol. That curve does not show evidence for linearity; looking at the data this way discounts the appropriateness of applying a linear model.

We recalculated the results based on an appropriate model of non-interaction and found four synergistic combinations in the data. In the lower dose region of the dose response curves, there is antagonism that is almost of the same magnitude as the synergistic effects. There is antagonism at the low doses. I pointed out researchers have to be very careful when looking at the literature for interaction analysis studies. These issues are not amenable to very simple approaches. Ultimately, more data are needed before some of these assumptions can be used in lieu of default assumptions.

In addition, interaction effects can be different at different biological levels of organization. It is possible this interest in synergy has to do with something other than biology. It is possible that in the drive to produce exciting results, it is forgotten that synergy is useful in pharmacology and pesticide toxicology, because finding dose combinations of chemicals that increase efficacy without increasing the dose has value. That is an active area of research in antibiotics and chemotherapy. Synergistic combinations are hard to find. If they were really that easy to find, there probably would not be as many problems with cancer, antibiotic resistance, or insect resistance to pesticides. The idea that synergy is lurking around every corner might be more about the exciting results than it is about the biology.

Occurrence and fate of selected anti-inflammatory agents in surface waters.

Thomas Poiger, Hans-Rudolf Buser, and Markus D. Müller
Swiss Federal Research Station for Fruit-Growing, Viticulture and Horticulture

Thomas Poiger is a research scientist at the Swiss Federal Research Station for Fruit-Growing, Horticulture and Viticulture in Wädenswil, Switzerland. His main research interests are the occurrence and environmental fate (degradation, transport, sources) of modern and persistent pesticides in the environment; and characterization of pesticides of natural origin (botanicals/green chemicals). Poiger studied chemistry at the Swiss Federal Institute for Science and Technology (ETH), Zurich, Switzerland, completed his Ph.D. studies on the behavior of fluorescent whitening agents in wastewater treatment at the Swiss Federal Institute for

Environmental Science and Technology (EAWAG), Dübendorf, Switzerland in 1994, and completed post-doctoral work at the US Environmental Protection Agency Lab in Athens, GA, researching and analyzing dyes in wastewater.

Residues of numerous man-made chemicals are frequently identified in surface waters, e.g. industrial chemicals, household chemicals, pesticides etc. Among these compounds are also numerous pharmaceuticals of various classes, such as hormones, antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), beta blockers etc (for a review see e.g. CG Daughton and TA Ternes, *Environmental Health Perspectives* 107 (1999) 907-938 and references therein). Their wide-spread occurrence indicates input to surface waters via human consumption, rather than via production wastewater. As many of these chemicals pass through sewage treatment largely unaffected, their concentrations can reach rather high concentrations, particularly in small streams.

Prior to registration, pharmaceutical compounds are extensively tested on pharmacological and toxicological grounds. Consequently, a vast amount of information is available concerning uptake, metabolism, excretion, mode of action, side-effects and human toxicity. However, very little is known about the environmental behavior and fate of pharmaceutical compounds and about their effects on aquatic ecosystems.

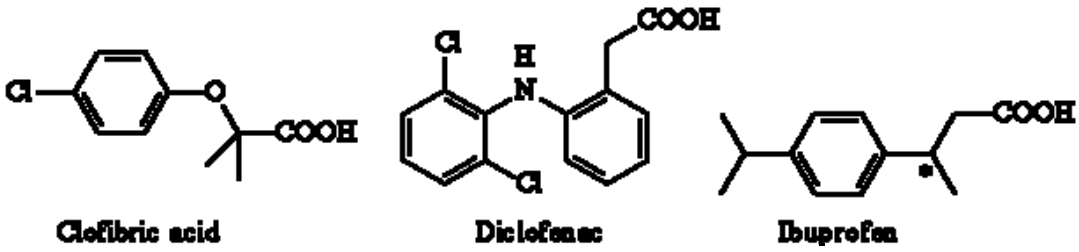

Figure 1: Structures of the pharmaceutical compounds studied

Our research group mainly focuses on research of pesticide occurrence and fate in the environment. Among other tasks, we are currently involved in a monitoring program for pesticides in selected Swiss lakes. Within this project, monthly vertical concentration profiles of various neutral and acidic pesticides are collected and the annual inputs of these pesticides are calculated with mathematical modeling. To distinguish between agricultural and non-agricultural sources of these pesticides, we frequently analyze samples from the tributaries of the lakes and from wastewater treatment plant (WWTP) in- and effluents. Besides the pesticides, we consistently detected other man-made chemicals, such as ibuprofen and diclofenac (non-steroidal anti-inflammatory agents), and clofibric acid (a blood lipid regulator) in these samples, which appeared in the same extracts as the targeted phenoxyalkanoic acid herbicides. The relative concentrations of these three compounds changed considerably from WWTP influent to effluent and from lake influent to effluent, pointing to a different environmental behavior of these

compounds (Table 1). In the following, the results of the investigations of the behavior and fate of the three acidic pharmaceuticals in the catchment area of Lake Greifensee is summarized compound by compound. Lake Greifensee was selected for this study, because of the high population density in its catchment area and, consequently, the high mass loading of anthropogenic compounds to this lake.

Table 1: Concentrations of clofibric acid, diclofenac, and ibuprofen detected in samples from the catchment area of Lake Greifensee (ng/L).

Compound	WWTP		Lake	
	influent	effluent	influent	effluent
Clofibric acid	= 100	= 100	2 - 11	2 - 9
Diclofenac	470 - 1920	310 - 930	11 - 270	< 1 - 12
Ibuprofen	990 - 3300	2 - 81	< 0.2 - 2	2 - 8

Clofibric acid

Clofibric acid (CA), the active metabolite of the drug clofibrate, is formed in the human body by cleavage of the ethyl ester group. CA is structurally related (isomeric) to the herbicide mecoprop (2-[4-chloro-2-methylphenoxy]propionic acid). This compound was one of the first compounds reported in sewage effluent. It was also detected in water from the north sea in concentrations similar to those of mecoprop. In that study, however, because of similar mass spectrometric properties, CA was suspected to be an isomeric form of mecoprop contained in the herbicide formulation.

In the WWTPs around Lake Greifensee CA was detected at concentrations around 0.1 g/L. In incubation experiments with activated sludge, no degradation of CA was observed. Also, no degradation was observed in incubation experiments with fortified lake water over a period of 38 d. This apparent persistence may explain why CA could even be detected in the north sea at concentrations similar to those of the herbicide mecoprop.

(HR Buser, MD Müller, N Theobald, *Environ Sci Technol* 32 (1998) 188-192)

Diclofenac

Diclofenac (DICL) is a popular drug in human medical care as an analgesic, antiarthritic and antirheumatic compound belonging to the group of NSAIDs. DICL was observed in wastewater in- and effluents at concentrations of 0.3-1.9 g/L, corresponding to a daily mass loading of 2.5 mg/person. Comparison of influent and effluent concentrations suggested a moderate removal of DICL (5-50 %). However, water samples were not from exactly corresponding water packages. Laboratory experiments with activated sludge indicated no degradation of DICL.

Concentrations of DICL in the tributary of Lake Greifensee ranged from 11-370 ng/L, which corresponded well with the predicted DICL concentration in the lake (60 ng/L), calculated using the average mass loading and average flushing. In the lake effluents, much lower DICL concentrations (<1-12 ng/L) were observed, pointing to an efficient removal (>90 %) of this compound in the lake.

When DICL was incubated in lake water, rapid removal occurred when exposed to direct sunlight (no DICL detected after 4 d), whereas no removal was observed in the dark. Because DICL was also degraded in sterilized water, we concluded that photolysis was the predominant degradation process. Photolysis experiments under more controlled conditions (quartz vials, short sampling intervals, Figure 3) confirmed that DICL is degraded rapidly with a first-order reaction rate of = 2.5 d^{-1} (summer, noon, 47N latitude). The first photoproduct in the degradation of DICL is the de-hydrochlorination product carbazole-1-acetic acid, which degrades at an even faster rate. Depending on the reaction conditions (nitrogen-saturated solution, traces of organic solvent), further products can be observed. In air-saturated solutions and thus under conditions similar to those in a lake, carbazole-1-acetic acids reacts further to products which were not amenable to our GC/MS procedure.

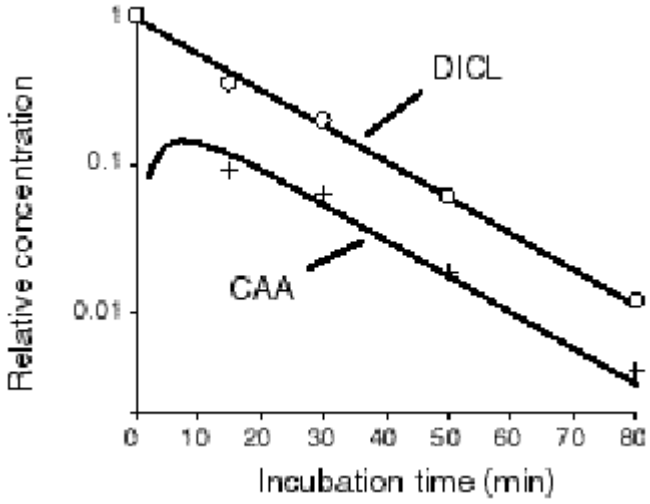

Figure 3: Photolysis of diclofenac and formation and degradation of carbazole-1-acetic acid

Vertical concentration profiles of DICL were measured in February, July, and December 1998 (Figure 4). In February, a homogeneous distribution of DICL (11-13 ng/L) was observed, consistent with the temperature profile which indicated that the lake was vertically mixed during this time. In July the lake was stratified. DICL concentrations were lower (1-9 ng/L), particularly near the lake surface. In December, after the lake overturn, DICL was again homogeneously distributed and concentrations were higher (8-10 ng/L) in July. The observed seasonal and spatial distribution of DICL in the lake was consistent with a photolytic degradation in the lake.

To verify this hypothesis, a lake model was constructed that included input, mixing, flushing, and photolysis. The photolysis rate constants were extrapolated to the situation of lake Greifensee taking into account the seasonal variation of the sunlight intensity, the attenuation of light by overcast skies, the light reflection at the lake surface, and the attenuation of light in the water body by dissolved organic matter and by particles. The modeled DICL concentrations corresponded well with the measured ones, indicating that photolysis is the predominant removal process for DICL in the lake (Figure 4).

(HR Buser, T Poiger, MD Müller, *Environ Sci Technol* 32 (1998) 3449-3456)

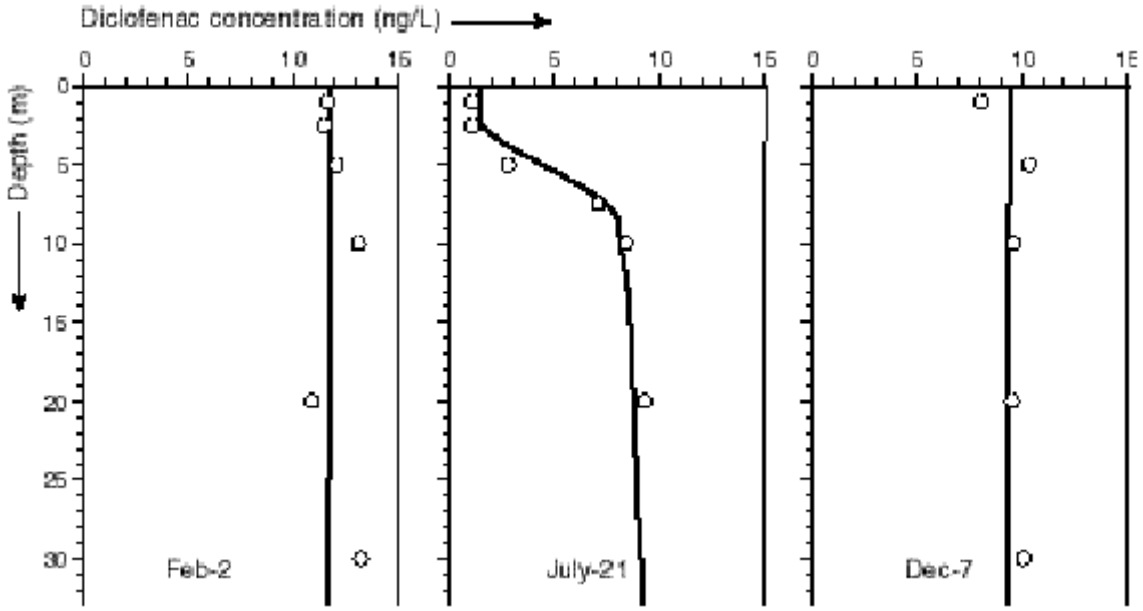

Figure 4: Vertical concentration profiles of diclofenac in Lake Greifensee (circles, measured values; lines, calculated using lake model).

Ibuprofen

Ibuprofen (IB) is a non-steroidal anti-inflammatory (NSAID), analgesic and antipyretic drug, widely used in the treatment of rheumatic disorders, pain and fever. It is an important non-prescription drug and has a relatively high therapeutic dose (600-1200 mg/d). It is excreted to a significant degree as the parent compound or in the form of metabolites. The desired pharmacological effects reside almost exclusively in the *S* enantiomer, yet the racemic compound is used as the drug. It has been shown that in humans and other mammals the inactive (*R*)-(-)-IB undergoes extensive (unidirectional) chiral inversion to yield the active (*S*)-(+) compound. The principal metabolites of IB are hydroxy-IB, carboxy-IB and carboxy-hydratropic acid (carboxy-HA), all of which are chiral.

IB was detected in the WWTP influents around Lake Greifensee at concentrations of 1-3.3 g/L and an enantiomer ratio ER =5.5-8 (S/R). The concentrations of the metabolites were even higher, indicating that the IB detected was extensively metabolized prior to its discharge. However, in the effluents of the same WWTPs, no metabolites could be detected and concentrations of IB were much lower (2-81 ng/L) and exhibited smaller ERs of 0.9-2, pointing to an efficient biodegradation of these compounds. IB and its metabolites were also extensively degraded in a laboratory activated sludge experiment (Figure 5). The laboratory incubation experiment showed that for complete degradation of IB, a residence of the wastewater in the activated sludge system in excess of 6 h is required. As this condition is by far not met by all currently operating WWTPs, it is not surprising, that very high IB concentrations in WWTP effluents are reported for some German plants.

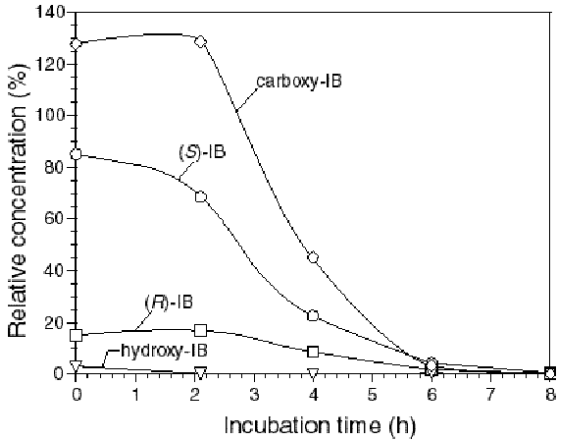

Figure 5: Degradation of ibuprofen and its metabolites in activated sludge

In the effluent of Lake Greifensee, IB was detected at low concentrations of 2-8 ng/L. IB incubated in lake was degraded with a half-life of $\tau = 20$ (Figure 6). Degradation was enantioselective and the degree of enantioselectivity depended on whether the water was exposed

to sunlight or not. The fact that no degradation occurred under sterile conditions indicated that degradation in lake water is biological.

(HR Buser, T Poiger, MD Müller, *Environ Sci Technol* 33 (1999) 2529-2535)

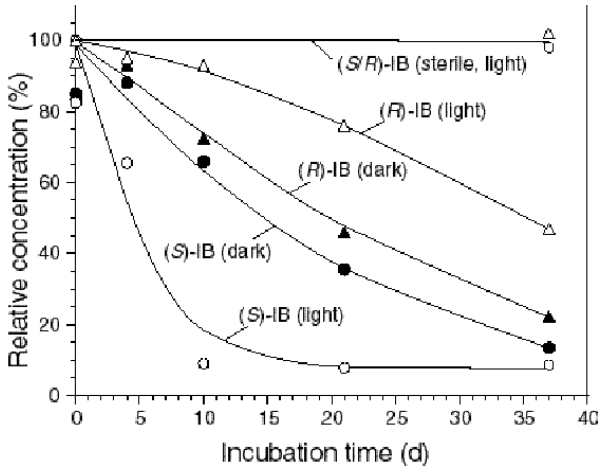

Figure 6: Degradation of ibuprofen in fortified lake water in the dark and exposed to daylight

Regional mass balances of CA, DICL, and IB

From the field data, as well as the degradation rates determined in the laboratory, the following tentative regional mass balances for the three pharmaceuticals in the catchment area of Lake Greifensee can be derived (Figure 7). Clofibric acid was shown to be rather persistent. No degradation was observed in laboratory experiments with activated sludge and fortified lake water. Although CA is discharged in much smaller amounts than the other two compounds, its concentrations are similar in lake effluent. In fact, CA is the only compound of the three that could be detected in water from the north sea.

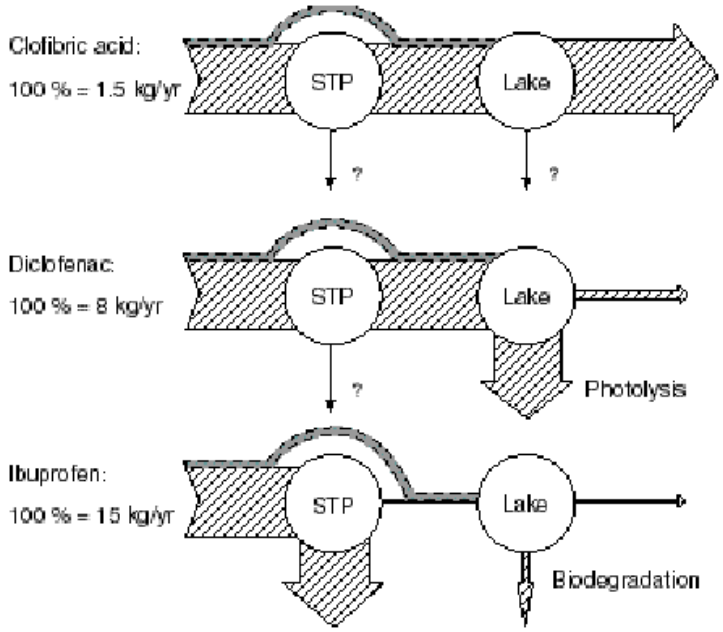

Figure 7: Regional mass balances of clofibric acid, diclofenac, and ibuprofen in the catchment area of Lake Greifensee.

DICL passes sewage treatment largely unaffected, as shown in the incubation experiment with activated sludge. In the lake, however, DICL is photodegraded efficiently, so that >90 % are removed from the lake.

The amount of IB (and its metabolites) that is discharged with raw wastewater in much greater amounts than the that of the other two compounds. However, IB is efficiently biodegraded during sewage treatment. A small fraction of wastewater, however, is discharged directly to the lake (overflow during storms) and is expected to contribute significantly to the overall discharge of IB to the lake. IB is also biodegraded in the lake, so that IB concentrations in the lake remain small at all times. No IB metabolites were detected in lake water.

Pharmaceuticals, hormones, and other wastewater related compounds in U.S. streams

Herb Buxton, United States Geological Survey

Herb Buxton currently is the Coordinator of the USGS Toxic Substances Hydrology Program. The goal of the program is to provide unbiased scientific information on the behavior of toxic substances in the Nation's hydrologic environments. The Toxics Program conducts: (1) intensive field investigations of representative cases of subsurface contamination at local releases; and (2) watershed- and regional-scale investigations of contamination affecting aquatic ecosystems from nonpoint and distributed point sources. He received a B.S. in Geology from Rensselaer Polytechnic Institute, and M.S. in Geology through a Sea Grant Fellowship at the State

University of New York at Fredonia. After working as a Research Associate with the University of South Carolina Hydrogeology Program, Herb started a 22-year career with the U.S. Geological Survey. He has been the Hydrologic Simulation Specialist of the New York District, and the Assistant Chief for Hydrologic Systems Investigation and Research of the New Jersey District.

(Editors note: The following is an abstract of Mr. Buxton's talk. The Toxics program webpage is http://toxics.usgs.gov/index.html)

In 1999, the U.S. Geological Survey (USGS) Toxic Substances Hydrology Program implemented a reconnaissance of stream waters across the United States to determine the occurrence of human and veterinary pharmaceuticals (37 prescription and non-prescription drugs, including 18 antibiotics); sex and steroidal hormones; and other wastewater-related compounds (antioxidants, plasticizers, and other industrial compounds of which at least 18 are suspected endocrine disrupting compounds). The objectives are: (1) to develop analytical methods to detect and quantify these compounds in ground and surface waters at nanogram-per-liter levels, and (2) to provide baseline data that determine if these compounds are entering the environment and that define their occurrence in streams considered susceptible to contamination.

Specific and sensitive analytical methods are being developed at USGS research laboratories across the Nation. The methods employ: (1) high performance liquid chromatography/mass spectrometry using positive ion electrospray, (2) selected ion monitoring gas chromatography/ mass spectrometry, and (3) radioimmunoassay and immunoassay tests (for screening purposes).

One hundred stream sites, sampled in 1999, were selected based on expected susceptibility to contamination by human, animal, and industrial wastes. The streams represent watersheds that fall into four general categories: intense urban activities, intense livestock production, mixed land use, and undeveloped controls. The watersheds range from less than 1 to 1,125,300 square miles; sampling points are located in 24 states. In 2000, additional streams are being sampled.

Compounds detected at submicrogram-per-liter levels include: antibiotics of the sulfonamide, tetracycline, and macrolide classes; selected alkylphenols and alkylphenol ethoxylates (detergent metabolites), acetaminophen (analgesic); acetophenone (fragrance); BHT (antioxidant); bisphenol A (used in polymer manufacturing); caffeine (stimulant); codeine (narcotic analgesic); cotinine (a nicotine metabolite); diltiazem (coronary vasodilator); 17beta-estradiol (hormone); and triclosan (antimicrobial disinfectant).

Thursday April 20, 2000

Session 3: View from Industry - Research and Concerns

The European water industry's view of endocrine disruptors and pharmaceuticals

Michel Gibert, Vivendi Water, Paris

Michel Gibert is the leader of the Prospective and Development Team (Research Development and Management) at Vivendi Water. In this capacity, he oversees regulations, trends and emerging issue in the water quality industry. Trained as a chemical engineer with experience in the chemical industry, he joined Compagnie Generale des Eaux in France in 1982 to manage the Central Laboratory and analytical R&D for 15 years. This position brought him the experience on practical quality issues in drinking water supply, wastewater treatment and related environmental problems. With the reorganization of the company at the worldwide level with new partners as Vivendi Water, he joined the R&D management.

I am pleased to see that water industries all over the world are studying the possible impacts of chemicals with respect to endocrine disruption. We are far from having all the answers, but it is very important that we start to think about the issue. It is a very important concern; all compartments of the water business are involved. A lot of research has shown there may be an impact on aquatic life from endocrine disrupting substances in wastewater. With respect to drinking water research, we can't say they are clear problems with adverse human health effects, but this is definitely an area that should be addressed. We have seen that some chemicals are easily removed by water treatment, which is good news. However, many of these chemicals are probably in the sludge, and so they are still in the environment. The problem of sludge treatment and recycling or disposal is very important. Currently, a couple of European research programs deal with the fate of endocrine disruptors in sludge.

Where wastewater is reused for irrigation, there are indications we might have problems with endocrine disruptors (by a complex mechanism) through the food chain. So, in the water industry, we are encountering the problem of endocrine disruptors and pharmaceuticals. We understand why it is difficult to have answers and that it is a complicated issue, made up of a large number of highly specialized scientific domains. In the past, scientists from the different sectors have not had the opportunity to work together; it is difficult to work in an area without having an understanding of what others in the field are doing.

Finding a solution involves a very complex process. First, we have to deal with chemical fates and environmental analysis; we have to know the biochemistry. Then we look at how the chemical affects the cell. Even if the cell is affected, it doesn't say anything about what that means for the individual. This is a problem of reproductive capacity. There are many other factors, especially when it comes to human beings, which make it difficult to understand what an endocrine disruptor will do. Even if individuals are affected, we still have to determine what is happening at the population level. At a recent workshop on endocrine disruptors, feminization of males was reported in fish populations. Is that the limiting factor? Not necessarily. If we see feminization of males by vitellogenin induction, on the other side would we have superfemales? A couple of recent studies

show that we don't have superfemales. Of course, we have to deal with ecology; do we really get the practical answers we need at this level for humans?

What is the water industry doing with respect to research and development? Most of the time, we are looking at treatment processes, management tools, quality surveys and resource management. "Quality surveys" refers to analytical methods that you have confidence in and that are inexpensive as well. Let's talk about fundamental research being done by the water industry. The goal is to gain the public's confidence in minimizing impacts on health and on the environment through regulation. Regulation is not a goal itself; regulation is an expression of public demands. We need the public to understand risk assessment; this is not always easy to explain to the public, especially when newspapers and the media can mislead them. Public acceptance of regulations and confidence in measures taken to meet regulations are also important. A key to creating acceptance by the public is early communication on sensitive issues. The public is more or less aware of what the industry is doing; we should make an effort to communicate to the public about what we don't know, and when we may expect to have answers.

Applying regulations is a reflection of public demand. What can the industry do about regulations? The target depends upon the regulation, for example, it could be biodata. We may see some effects, but we are not able to identify them for some chemicals, as the list of chemicals that may be endocrine disruptors is endless. Even so, we may have regulations that are driven by biodata. This is quite difficult for the water industry, because you have to understand what chemicals are involved and then develop a solution to solve the problem. In the meantime, regulation of chemicals is moving forward. But which chemicals, and at what levels? Depending on whether it is brominated flame-retardants, phthalates or estradiol, the technical answer would be totally different. Depending on a specific chemical, are you talking about micrograms or nanograms per liter? The technical answer could be totally different.

Some European countries have started to ban chemicals because of endocrine disruption effects, which is good news for the water industry but not for the chemical industry. We know that some chemicals may remain in the environment. For example, in Europe we still find DDT in soil sediment although it has been banned for many years. It is very difficult to start research and development until we have the answers as to what will be regulated and how. Even if we have that, it may not be enough. The target will depend on the actual situation. It is clear that if we have a large number of similar problems, we may try to develop technologies because there will be a market for them. For example, if there is an estradiol problem in many places, it will be valuable to develop technologies that will solve the problem. However, it could be that we have locally specific problems, in which case we will have to find a local approach to solve those problems.

What are the similarities and differences between endocrine disruptors and pharmaceuticals from the industry's viewpoint? With endocrine disruptors, we start with the effects and then try to work backwards to find out which chemicals are involved and what the sources are. The problem with that approach is we can start to do something but we don't know what the target is. With respect to pharmaceuticals, we start off knowing what the chemicals are, then we go about finding their pathways and effects in the environment.

There are also some similarities between endocrine disruptors and pharmaceuticals.

Antibiotics and pathogens are found in the water environment, so the water industry may have a problem with antibiotic resistance. Until now, the concern has been about pathogens that are already antibiotic resistant. In instances where these pathogens occur, we might ask water utilities to be more stringent about their removal. Another similarity between endocrine disruptors and pharmaceuticals involves public perception. We have to understand the public's perception about "forced medication". People can generally deal with pesticides and other toxic compounds that are designed "to kill". However, pharmaceuticals and medicines are made to cure people. The public feels that when these compounds come through the drinking water it is, in essence, forced medication.

How does regulation work in the water industry? At the research level, you first discover the problem or issue and then begin to study the causes and risks. Finally, you are able to assess the situation. Regulation starts with temporary rules, based on what is known about causes and risks. With the final assessment, you create final regulations and means for enforcement. The water industry can't do anything until they know what the regulation looks like, so that technological innovations can be made. It is important that the regulation strikes a balance between the risk and the economics, or what can be done for which price. The industry also needs a lot of time to implement the technology.

When you change something or create a new process, you try to design it so it lasts for 10-20 years. The public is aware of the problem from the beginning; they demand regulations and enforcement of those regulations. The problem with starting too late is you may delay application of regulations as well as raise public concerns. The problem with starting too early is you may have research and development results that are based on speculative data. It could also be possible that new data may not support previous data. What can be done in the meantime? We must push forward to address important issues; we should try to develop less expensive analytical tools for large surveys. Currently, we have nice equipment and clever technologies, but they are very expensive. We need tools to identify responsible substances in real world situations. We need to know what chemicals are involved in biotests; this will not be easy. We should stimulate the study of actual environmental situations and practical risk assessments. We must be able to understand the magnitude of the problem in order to know how critical the situation is. Finally, communication with the public is key.

At Vivendi Water, we sponsor research on TIE (toxicity identification and evaluation), in cooperation with the Swiss Institute on the Environment. We believe in utilizing technologies that identify chemicals that have positive response from biotests. We also support workshops to help scientists, regulators, water industry professionals and chemical industry professionals with their needs. We also participate in the COMPREHEND European project, which looks at endocrine disruptor wastewater impacts in Europe; this effort involves developing a comprehensive map of problem areas. We sponsor a French study on aquatic antibiotic resistant pathogens, and Vivendi Water is collaborating with the University of Technology in Sydney on water reuse for irrigation. Future programs we hope to get involved in include investigating the fate of chemicals in wastewater treatment processes and identifying the presence of endocrine disruptors in drinking water.

The public will judge all of us - scientists, regulators, and industries - on our capacity to manage issues together in as quick a manner

as possible. The public wants us to solve problems quickly. If we don't do our job, the precautionary principle will do it for us. Some people say if you don't have the answer, I have a black box that will tell me what to do. It may sometimes lead to a decision that protects neither people nor the environment.

Forest products industry environmental research addressing effluent effects issues
Robert Fisher, National Council for Air & Stream Improvement, Research Triangle Park, North Carolina

Chlorinated compounds and endocrine disruption
Terry Quill, Beveridge & Diamond P.C., Washington D.C.

Terry Quill is Of Counsel in Beveridge & Diamond, P.C.'s Washington, D.C. office where he provides legal counseling and litigation services concerning a variety of environmental and regulatory issues. Much of his practice focuses on legal and technical matters associated with potential human and environmental exposures to toxic substances. Mr. Quill has extensive experience in the regulatory and political processes concerning dioxin and in assessing the toxicity and environmental fate of dioxins, PCBs and other chemical substances. Mr. Quill is a graduate of the University of Michigan Law School and holds M.S. degrees in biology (Wayne State University) and toxicology (University of Michigan). Prior to his legal career, Mr. Quill held research positions specializing in molecular and developmental genetics, immunochemistry and tumor immunology.

I have worked quite a bit with the chemical industry on the endocrine disruption issue, so I can share with you the general industry views and positions on a number of issues in this area. There is not a lot of agreement on some of these issues even within the chemical industry. My presentation deals mainly with the research issue; my comments will focus on research and needed research. It is important to start with the question "what are endocrine disruptors?" Endocrine disruption is a maze. We don't know where we will end up; in fact, we don't know whether we will get out of the maze. We have to take a step back and view the maze from the top to determine where we want to go. Part of this involves trying to determine exactly what we need to research.

The hypothesis that needs to be tested is whether low level chemical exposures cause adverse endocrine-related effects on humans or wildlife. That is what our research should revolve around to a large extent. Some people will say that we have the evidence that there are endocrine-related adverse effects in wildlife, and we're certain it is happening in humans. They will say that we don't need to research that anymore. This position is of great concern to me. If we are not willing to go forward and test this hypothesis, we have given up on the scientific process and the scientific method. Instead, what we will have is a situation where we rely upon observational science. That is fine for developing hypotheses, but not for testing them. We need to back up and think about good science. We need to think about conducting our research programs in an effective manner. My purpose isn't to show the weaknesses in current science in order to disprove the hypothesis. Rather, we need to go forward with good science to prove or disprove the hypothesis.

There is a lot of misunderstanding about industry's position. Some portions of the public think industry usually delays and does not put money into research. This impression has the

industry very concerned. If you talk to people individually, many would say that there are hypotheses out there that need to be tested. Let's get the information we need to come to some conclusion and determine what type of actions, if any, should be taken. While industry has already done a lot of research, there is a lot more that needs to be done. For example, do chlorinated compounds pose a unique endocrine-related risk? Much of the emphasis is on chlorinated compounds.

It is important that we address the question "what are endocrine disruptors?", as it is difficult to design research programs, and assess the results of those programs, if we don't know what we are looking at. Is simply binding to a hormone receptor endocrine disruption? Is that where we should be focusing our research? Is that really going to tell us anything? Should we be looking for non-adverse effects when we do research? We need to think about why we are doing research. Close to the end of the maze is risk assessment. What kinds of evidence are we going to generate that will help us do a risk assessment? It is not going to be information concerning non-adverse effects. How do we account for normal fluctuation in hormone levels? This goes to the adverse effect versus non-adverse effect issue. This is not a simple process. There are ten to twelve issues on the endocrine system that have to be considered, including fluctuation of hormone levels and feedback loops. Is a slight increase in circulating estrogen an adverse effect in a woman? What if it is within normal monthly fluctuations of estrogen? What if it is within the normal range of women in the environment?

These are questions that we haven't really addressed yet, but we have to start thinking about. What is normal? Are naturally occurring substances in the diet endocrine disruptors? Everyday we are exposed to compounds in our diets that have estrogenic effects. An example is isoflavones, which commonly occur in many vegetables. One would think that potency plus the level of exposure should be driving the issue. Why is low level exposure to chemicals important? What does it tell us about what might be happening with chemicals? This is something that really hasn't been addressed yet.

The second question on endocrine disruptors is "does it act through an endocrine-related mechanism?" One of the main examples of endocrine disruption in wildlife is DDT and eggshell thinning. The question is does eggshell thinning occur through an endocrine-related mechanism? There have been a lot of theories put forth, none of which are endocrine-related. We have to be sure, when making observations in the environment, to ask ourselves if an endocrine-related mechanism is involved. Why does this matter? Some would say who cares? If you see eggshell thinning and you can show some kind of connection to DDT or some other chemical, just ban it. End of story. To some extent, that is true. Why does it matter? It matters because with endocrine disruption we are dealing with a different kind of methodology. Some people are proposing we throw away our traditional toxicological methods, our dose-response methodology, because endocrine disruptors are somehow special.

For example, some people claim that if you see a slight fluctuation in hormone levels, there is endocrine disruption occurring. They say that because you have a background, which is always normal, anything that adds or subtracts from the background is adverse. That position is based on thinking about endocrine disruptors as a special science. Even if we assume that is correct, the very least we must do when looking at a chemical is determine whether it acts through an endocrine mechanism. Many times these are things that people ignore.

We are still trying to define "endocrine disruptor". EDSTAC (Endocrine Disruptor Screening and Testing Advisory Committee)

was a process that came out of the Food Quality Protection Act. Congress told EPA it must put together a screening and testing program for pesticides and chemicals that might have a cumulative effect. EPA put together an advisory group that looked at the issues in more depth than anyone has before. In the end, they still couldn't agree on a definition for endocrine disruption. It is interesting, but also problematic with respect to how we are going to design research programs if we can't even agree on the basic definition underlying the research program.

EPA put together an endocrine disruption screening program policy statement. They didn't even use the words endocrine disruption in the document, except in the title. The American Academy of Science abandoned the term altogether and used the term "hormonally active agents". Industry's view is that it should be defined as "substances that cause adverse effects through endocrine- mediated mechanisms." We are using this definition and moving forward to identify potential research projects.

Do low level chemical exposures cause adverse endocrine-related effects in humans or in wildlife? My purpose is not to tell you there is no evidence, and therefore the hypothesis is incorrect. My purpose is to say that it has not been determined yet and we have a lot of research to do. First of all, there is no evidence of adverse human effects at environmental levels. Right now this is a fact, based on all the data that have been presented. EPA's interim report and the National Academy of Science report say that, at this point in time, no conclusive evidence has been brought forward to show that low level exposures to chemicals have an adverse effect through an endocrine-related mechanism.

We do know that high dose effects have been reported; DES is the most prominent. How relevant are high dose effects? I calculated a dose equivalent for DES. The amount of estrogen equivalents given to a DES mother over a three-month period equaled the amount of normal hormone production in a woman over 100-150 years. That is a huge exposure. I would've been surprised if we hadn't seen an effect somewhere. We did see an effect and it was devastating.

Is that proof that a very low-level exposure somewhere else is causing an adverse effect in humans? The science certainly isn't there yet to support that. Birth control pills are designed to be endocrine disruptors; when you look at it from a reproductive standpoint and measure the ability of a female to reproduce, it is an adverse effect. It is also a therapeutic effect because that is the effect that is intended. Birth control pills are given at high doses. Claims of adverse effects on human populations from endocrine disrupting chemicals are at best equivocal. We've heard a lot of reports of breast cancer risks and low sperm counts. If you look deeper into the issues you will find that studies do not support any kind of correlation between an exposure and adverse effects for DDT, PCB's and other bioaccumulative chemicals.

If you see normal sperm counts in one population and lower sperm counts in another population, does that mean there is an effect? A recent book addressed decreasing sperm counts, which could potentially lead to population problems. They compared a population in one area (20-30 years ago) to another population in another area some time later. You can't compare one geographical location to another geographical location to show a decreasing trend. If one were to take sperm counts in New York and compare them to sperm counts in California, they would be different, and there are a lot of reasons for that. You really have to know the populations you are dealing with. It goes back to the scientific method; we need to be very careful about what the observations really mean.

What is the relevance of high exposure to endocrine-active substances in the normal diet? This has to do with isoflavones. Why is that or is that not endocrine disruption? EPA's interim report and the American Academy of Science report stated there was no evidence of an adverse effect at low levels in humans. There have also been a lot of European bodies that have made similar statements.

Let's turn from humans to reports of low dose effects in limited animal experiments. The U-shaped dose response curve suggests that at higher doses you hit a no-effect level. If you test below that dose, you see an effect again. That is not what would usually be assumed in toxicology. If you find the no observable adverse effect level (NOAEL), you can be sure there is not going to be an effect below that. You can then regulate based on that NOAEL. The low dose endocrine theory throws the NOAEL concept up in the air. Maybe we haven't been regulating chemicals correctly at all. Maybe there are some lower doses, below the NOAEL, where the response is higher. This is an issue that we have to do more research on.

Anybody can report an observation. But is an observation repeatable? Can other labs repeat that process? That is part of the scientific process. In one case where there is increased prostate weight, we still must determine what the implication is for humans. The press played it out as though that was the reason we see higher rates of prostate cancer in humans. There is no evidence for that; there is very little reason to believe that, but that is the way it is played out in the press.

Is there an effect in wildlife? We haven't correlated chemical exposures to effect. For certain species, they have found places with high hatchability that have higher chemical concentrations, and places with lower hatchability that have lower chemical concentrations. That makes one wonder whether it is due to exposure to chemicals. Early on, the effects seen in alligator egg hatchability were said to be due to exposure to dicophol. The data don't support that. If there is something going on in the lake, we need to figure out the cause; we can't just assume it is due to chemical exposure. This is where the scientific method comes in. We have an observation, but a lot more research needs to be done to determine why we are seeing what we are seeing.

Are observed effects endocrine-related? Again, with DDT and eggshell thinning, what is the cause of the effect and is it through an endocrine-related mechanism? Those questions are typically not asked. In other words, it is possible that we have effects out in the environment. The question is "are these effects truly related to low dose exposures when we are looking at bioaccumulative substances?" Let's take DDT for example. Why was it so devastating to eagles? Because DDT biomagnifies in the food chain. The top of the food chain is the bald eagle; they were getting huge doses of DDT.

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Session 4: Potential Needs for Treatment

Water utility perspective on endocrine disruptors

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My talk will focus on issues and philosophies that a water utility manager needs to think about when addressing endocrine disruptors. Regardless of the size of the town, there are certain issues that water utilities will face when dealing with endocrine disruptors.

An interesting aspect of being a utility manager is that over the years, pharmaceutical use, the ability to create new compounds and the ability to identify compounds have all increased. Should we be surprised that we find endocrine disruptors and other chemicals in our source waters? Probably not. Is it alarming to find those contaminants? We might be surprised, because we wouldn't expect to find them. The history of our industry over the last twenty to thirty years has been that when we look for things, we find them. Do we know what the data mean? Do we know what it means to utilities? Do we know what the health impacts are? Do we know how to incorporate our findings of what we know into our risk assessments? These are the harder questions that the utility manager wrestles with. It is what a manager does about what they don't know that drives a decision. That is not to say research is not needed. Research is needed in a number of areas. Often times, utilities are forced to take action and make decisions.

Regardless of the size of the utility, they all are faced with addressing these kinds of issues. While a very small system may employ only one person - the operator also takes care of the roads and the streets - larger utilities have departments and staff to address these areas. Public relations and public perception are very important to utilities; utilities respond to their customers. I was recently asked to give a rundown on current trends in the water utility industry to a group of people in the business of developing consumer products. At the end of my presentation someone asked, "What about endocrine disruptors?" It was on their minds and they knew it was on the customers' mind. The customer buys consumer products; our customers are thinking about this.

Utilities cannot control where customers get information about drinking water. While utilities can present information to the public, there are other sources of information as well. If utilities do not communicate effectively with their customers, those customers will go elsewhere for information. The public may develop

attitudes or perceptions that may or may not reflect reality. The other interesting thing is you can't make people play by your rules. Frustration occurs because we each have different ideas on how things should happen, and sometimes they are not followed. Customers may develop attitudes that are not justified; nevertheless, their perception is their reality. Utility managers, who are a highly educated group, must respond to that. In my service area there are customers who have a fundamentally different way of thinking. Different values and different attitudes can result in different conclusions. They want different things. As a utility manager, I cannot ignore that, I must respond to that in some way. Not only on endocrine disruptor issues, but on other issues as well. So put yourself in a utility manager's position. If you had to make some of the decisions in terms of budget and how to respond, what would you do? Given that reality, what should be done?

Consumers make choices in terms of willingness to pay for new technologies through rate increases. They also make choices to go to alternative water sources. They make most of these choices based on their perceptions. Utility companies need to respond in some way to that, keeping in mind that a non-response is a response. If you do nothing, that is a choice on how to react to customer perceptions. Other forces will fill the void and motivate customers. The fact that there may be endocrine disruptors in source water leads to some questions. Does that mean customers are exposed to those substances in tap water? What are the risks? How would that affect our actions as utility managers?

It is difficult to make decisions regarding water reuse. Source water selection and source water management are very difficult. Engineering and construction of facilities to provide protective barriers, production and transmission (getting water from the treatment plant to the tap), and managing the distribution system through pipes are all jobs of a utility manager.

There is a U.S. regulatory structure that sets maximum contaminant level goals (MCLGs), which are non-enforceable goals based on health effects. Health effects assessments are done on contaminants and MCLGs are set with a margin of safety. The MCLGs are deemed to be protective with that margin of safety. The enforceable standard, the maximum contaminant level (MCL), is set considering feasibility. A lot of people ask, "How come you can't reach the health goal? Why do you have to consider feasibility and cost?" This has to be approached with an eye for reality and practicality; the enforceable standard needs to be achievable. Our enforceable standards force those utilities that are lagging behind the leaders to shift their treatment practices. Throwing a water utility manager in jail does not make the water cleaner.

A question to consider for possible endocrine disrupting compounds is "have the potential effects in setting MCLGs for those compounds been accounted for in currently regulated contaminants?" Many of the regulated compounds are synthetic organic compounds, including pesticides. Their MCLGs are based on a cancer effect; those MCLGs are zero. The fact that there may be another effect may not be relevant. Water provided to the tap must meet the MCLs. The regulatory mechanism is in place to account for endocrine disruption or any other health effect if there are data on what those effects are. However, additional health research is necessary to feed into that process. From a regulatory compliance point of view, there is a mechanism in place to account for effects as drinking water standards are set, if health effects data are available for use in the system. In many peoples' minds, endocrine disruptors are another specific chemical. There is arsenic, benzene, tetrachloroethylene, and endocrine disruptors; some customers actually

think that way. This is an educational process, and one of the challenges is educating people who don't want to be educated. There is a regulatory mechanism on the drinking water side to consider endocrine disruption based on health data. Utilities wonder if this changes the picture. Does it change current standards? If the MCLG is already zero, it won't go any lower. If changes are required in other areas of utility operation, additional financial resources will also be required. The way utilities relate to customers will also be affected. This process happens everyday in many utilities as we respond to current concerns and issues that are on the customer's mind.

Let's consider some other areas. All stakeholders involved in a decision, whether at the utility level or the national level, participate in the process, and all stakeholders think differently. They have different attitudes and different points of view. Utility managers work with other managers who in turn wrestle with how to apply technical information in order to make real-world decisions. All of these interactions are between people that do not all think the same way. In addition, customers see the world differently. They come in with a base of information on any given topic. This information can be viewed in the following ways: facts - things that they know are true; speculation - what they believe is true; and fantasy - what they know is not true. Sometimes what we think is true is actually fantasy, but we don't know it. When trying to make real world decisions relating to endocrine disruptors, there are different views. We lump facts into the truth category and fantasy into the false category. In be tween things may be possible, probable and improbable. Is it probable that water treatment processes provide an adequate barrier to all contaminants that may have these effects? Where would you put that statement? We have many different points of view; participants need to decide where to put that statement. Utility managers have a customer base with diversified views. Through process and education with stakeholders, different epistemology and different ways of knowing, utility managers have to sort out these views. Do they wait for something to be 100% certain before making a decision?

Recently, I provided technical advice to a group of ethicists that were studying the ethics of a particular water treatment process. In the course of conversation I asked them, "Regardless of science, should a water utility respond to what the customer wants?" A third of them said yes without hesitation, a number of them didn't necessarily agree with that and the rest were unsure. This is an issue a utility manager faces on a daily basis. I am not saying science is not important. But the better we can apply the science, communicate to our customers and help utility managers who are in the position to make better decisions, the more effective our research will be applied.

The number of drinking water regulations has increased in recent years; utility people have been feeling the pinch. There are over 90 contaminants regulated. I expect to see modest increases in the number of contaminants in the next few years under the provisions of the Safe Drinking Water Act. There are a number of new regulations coming out that utilities will face. The Stage One rules states that by December 2001, large water systems (serving more than 10,000 people) must be in compliance with a new rule for disinfection by-products. That rule is structured very uniquely and very deliberately. There is a new MCL for total trihalomethanes of 80 micrograms per liter and a MCL for the sum of five haloacetic acids at 60 micrograms per liter. Those limits were set mainly to control those particular groups of contaminants, but also to control other by-products that can be identified or by-products that we don't know about yet. In addition to MCLs, there is a treatment technique requirement for surface water systems using conventional treatment,

which is targeted to removing precursors to disinfection by-products. If we remove the precursors, humic substances, and other chemical precursors that react with disinfectants to form by-products, fewer by-products will be formed. The strategy is to remove the precursors; we are attempting to control both known and unknown by-products by doing that. This is a regulatory mechanism for unknown disinfection by-products that are trying to be controlled. These rules are currently being implemented.

The long-term agenda is driven by the Drinking Water Contaminant Candidate List and the National Primary Drinking Water Regulations and Revisions. The Drinking Water Contaminant Candidate List substances could potentially require regulation. There will be some new contaminants there. We will go through some health effect assessments; if data are available this will include endocrine effects as well cancer and non-cancer effects. EPA will be making determinations off that list as to whether contaminants need to be regulated. The other long-term driver is the National Primary Drinking Water Regulations and Revisions. The Safe Drinking Water Act requires that EPA revisit drinking water rules every six years. So if there is a reason to revisit a rule based on new health data, there is a regulatory mechanism in place for that to happen if the data are available, which highlights the importance of health effects data.

I have heard endocrine disruptors characterized as being like a killer asteroid - it is out there but maybe it won't hit. A recent story struck me regarding the similarities it has to the endocrine issue. A grenade was found at an airport in a bag that was sent through a metal detector. The owner of the bag did not speak any English. He had no way of communicating with the police so he started to run; he panicked. Ultimately it turned out that the grenade was not real but was an exact replica of one. When relating to endocrine disruptors, I wonder have we found a bomb but not the knowledge and understanding to communicate whether or not it is a real risk and where it fits in the spectrum of risks that we face everyday? Do we need to worry about it or not? The kind of research that gets us there helps the water utility and the water utility manager make good decisions.

Current and emerging drinking water treatment technologies

Kenneth Carlson, Colorado State University

Kenneth Carlson has over 14 years experience designing and researching drinking water treatment systems. Dr. Carlson's area of expertise has been physical and chemical treatment of drinking water working as a utility engineer (Fort Collins Water Utility), as a consultant (CH2M HILL) and now as an assistant professor of Civil Engineering at Colorado State University. He received his Ph.D. in environmental engineering from the University of Colorado - Boulder and a BS in chemical engineering from the University of Wisconsin - Madison.

My purpose is to give a general overview of drinking water treatment. Assuming that endocrine disruptors are present in raw water influent to treatment plants, I hope to give an idea of what typical treatment processes will do to reduce or eliminate those compounds. We will look at it in two ways. The first is the manner in which existing plants could treat or are treating endocrine disruptors, the second is how we might design plants in the future to accomplish the removal of endocrine disruptors.

In general, a water supplier's objective is to provide safe, aesthetically pleasing water at an affordable price. The customer, assuming that

the water supplier assures the water is safe, drives this. The industry believes they could treat water to almost any standard; if the public wants pure water, they could come close to that, but it would be at a great cost. This is the battle utility managers face, that is, balancing treatment with costs. While we can provide any level of treatment, it is not always obvious how much that will cost. This balance is different for different water consumers. Industrial consumers of water versus household consumers of water are willing to pay different amounts for safe water. For example, a household with three infants is willing to pay more than an industrial user who wants safe water, but does not want to overspend. What are treatment processes typically designed to accomplish? Primarily, it is pathogen removal; this is the main goal for utility managers and operators. There are some site-specific situations where certain contaminants are more important, but in general, the focus is on removal of particles and pathogens. The industry has two approaches for reducing the risk of pathogens. First is the physical removal of the pathogens, the second is disinfection. The top priority is reducing the risks of pathogen contamination, which can be accomplished by physical removal and also by disinfection. Another objective is the removal of natural organic matter (NOM); this is a more recent objective driven by regulations. The reason is disinfection by-products. Another objective is the removal of compounds that contribute to objectionable taste, odors and color. Some say this is the number one concern of customers, as customers assume you will take care of the other problems. The aesthetic appearance of water can be more important than anything else, because people believe that if it doesn't look good, there must be something wrong with it. This issue can drive the treatment strategy for a utility. Another goal is the removal of trace concentrations of individual contaminants, which can also be very important. For example, if a utility is exceeding the MCL for arsenic, then that obviously goes to the top of the list. In general, our treatment strategies are not developed to address trace contaminants.

Granular media filtration for physical removal, preceded by chemical coagulation are the steps used in most treatment plants today. Aluminum or iron-based salts are usually added. Several mixing steps are added and there is an opportunity for larger particles that have coagulated to settle. They don't rely on the settling process as a complete barrier; some type of filtration is next, usually a simple filter of sand underneath coal. These often are large concrete basins. This technology was developed in the earlier part of the 20th century. While many refinements have been made, it has been essentially the same since then. Greater than eight percent of existing plants rely on this for particle removal. We rely on charge neutralization of natural colloids either through the formation of an aluminum or iron solid or directly with a charged positive metal hydroxide polymer. We are interested in building a particle that will either settle or filter. The effective pore size of a granular media filter is much greater t han the particles that are removed. In other words, they are not being removed through a sieving effect. The chemistry is very important in a conventional process like this. The unstable particles, which are naturally negatively charged, are not sticky. Chemicals are added to make them sticky. They stick to each other and settle or stick to the filter.

With respect to emerging technology, membranes are the future. There are various applications of membranes. If your objective is particle removal, low-pressure membranes involve either microfiltration or ultrafiltration. The virus removal credit that you get with ultrafiltration is higher than that with microfiltration. In reality, very few surface

water utilities have the luxury of focusing on just particle removal, there are always other treatment objectives. If particle removal is the only objective, a low-pressure membrane would replace concrete basins and could potentially replace the coagulation chemicals. You still may have to add pH and buffering-type chemicals. Now that the processes have been improved, they can be operated at lower pressures. They are now cost competitive with conventional treatments. The driving force is pressure; there is either a vacuum inside, a pressure outside or a pressure inside forcing the water out. There is an absolute pore size, which rejects anything greater than that process. The entire process is done without any chemicals. Electrostatic rejection can also be important. Rejection of particles smaller than the pore size may also occur.

Particle removal may or may not result in a reduction of endocrine disruptors. Endocrine disrupting compounds are not large; they are about 0.1 to .03 microns. If we use ultrafiltration, endocrine disruptors will not be removed. If we are using a conventional process where we add a salt, form a hydroxide surface, the secondary impact of particle reduction with coagulation also removes dissolved organic matter. The primary objective is to kill bacteria, viruses, and protozoa (like cryptosporidium), which are easily rejected by membranes and disinfected by the emerging disinfectants. When we started using disinfection processes we did not take into account things like taste and odor control or organic contaminant oxidation. We often do iron and manganese oxidation up front. Taste and odor-control are aesthetic issues that are not health-related. Often we know what by-products are at issue. They are by-products of algae, which are present at nanogram per liter concentrations. That is similar to what we ar e looking at with endocrine disruptors. We have developed processes to deal with this issue.

In terms of disinfectants whose impact we need to better understand, chlorine is the most commonly used. Ozone has been, until recently, the most emergent disinfectant. Chlorine dioxide is used regionally; some regions use it more than others. Chloramines are used mostly as residuals in the distribution system. While the tap water might have a chloramine residual in it, it wasn't the disinfectant that was applied to get the original kill to meet regulatory disinfection requirements. The emerging disinfectant is UV, which we know a lot more about now than we did several years ago. We know that it kills crypto and that it is cheaper than ozone, which is what the industry has been looking for. UV is very promising with respect to those two issues.

We don't use the hydroxyl radical as a disinfectant as it is too short-lived; it is too reactive to develop a residual concentration. However, if we are really trying to target endocrine disruptors, we might have to consider it, as it is used to oxidize target organic compounds. It is the strongest oxidant that can be applied. To summarize disinfection processes, they result in oxidation in most cases. The question is to what extent? That is certainly an area for additional research. The industry was moving towards ozone because it appeared that it effectively killed crypto. However, this does not now appear to be the case. The effects of UV are not known; it may be possible to use UV to destroy or oxidize compounds, but research needs to be done in that area first.

Why do we care about the removal of natural organic matter (NOM)? It is only important when we apply free chlorine as the disinfectant; if a treatment system doesn't use chorine, then it is not relevant. Many people use hypochlorus acid. Free chlorine hydrolyzes to hypochlorus acid. If there is bromide in the water, the three react in various

combinations with NOM, and the disinfection byproducts (DBPs) trihalomethanes (THMs) and haloacetic acids (HAAs) are formed. A mass balance on the chlorinated species shows that this is less than 50%. The strategy for minimizing DBPs is twofold. The first goal is to minimize the disinfectant that is applied initially. If the chlorine applied is minimized we could minimize disinfection. We care about NOM, which is quantified as total organic carbon (TOC) concentration. We now have regulations that help us do that.

The most common approach for minimizing NOM or TOC is what we are already doing. We have a process for particle removal, in which we add aluminum or iron salts and form aluminum-hydroxide solids and ferric-hydroxide solids. One mechanism for NOM removal is through adsorption to the solid surface. This is an effective method for about 20% - 60% of NOM. Not all of the NOM adsorbs to the solids that we provide. It is not a lack of sorption sites; the fractions are not sorbable. The more hydrophobic natural organic molecules can be precipitated. If we are interested in the removal of endocrine disruptors through a standard coagulation process, this is the most likely mechanism. Adsorption to a metal solid that is provided would work best.

Membrane filtration is a rapidly emerging technology for particle removal and pathogen control. Low-pressure membrane processes such as microfiltration and ultrafiltration will not remove organic matter. In order to do that, smaller pore sizes are necessary along with higher-pressure membrane processes. Nanofiltration or reverse osmosis (hypofiltration) will remove a lot of NOM and hardness (calcium and magnesium), and RO removes TDS salts and low molecular weight synthetic organic compounds. The difference in costs between nanofiltration and RO, and microfiltration and ultrafiltration is substantial. Unless there is a driving force to implement higher-pressure membrane processes it will probably not happen; people will rely on standard coagulation. It is possible to combine coagulation and a low-pressure microfiltration process and get NOM removal. It isn't an all or nothing situation. If chemicals are used, NOM removal can be achieved using membranes as the ultimate particle removal mechanism. Another NOM removal technique is using granular activated carbon (GAC), which is an adsorptive packed bed with activated carbon. It is quite expensive. When NOM is oxidized, it is more biodegradable and allows the granular media filter to act as a biofilter, which can enhance NOM removal. Both of these would be very effective in removing endocrine disrupting compounds.

Algae by-products, such as methylisobornial, can cause aesthetic problems if found in nanogram per liter concentrations. Utilities are used to dealing with these types of concentrations. How can we eliminate trace organic compounds? Some utilities add powdered activated carbon (PAC), which involves an absorption process that works in conjunction with coagulation. Standard coagulation typically doesn't remove these compounds. PAC can be effective, but often times the doses are too high to make it cost-effective. A more common method is oxidation. Chlorine dioxide has been used to address taste and odor concerns. People that have real taste and odor problems use ozone. Using ozonation is not always driven by disinfection requirements; often times, the ozone dose and the process developed is driven by taste and odor. It is in excess of what is needed for disinfection.

For iron manganese we add a pre-oxidant before adding the coagulants. We can put in chlorine dioxide, ozone, chlorine or potassium permanganate to oxidize these reduced compounds to something that we can remove

with solid-liquid separation. Hydrogen sulfide can cause odor problems. However, it is usually oxidized to sulfate so it doesn't smell. The point is, if I am already doing this I might get some other benefits that I don't know about in terms of endocrine disruptor removal. If we understood it, we could modify those processes slightly and get additional benefits; we could optimize an existing process for two or three objectives. Removal of trace contaminants and ion exchange deals with membrane filtration. There must be a serious problem to be willing to pay the cost to start removing ions with membrane filtration; it is not that common.

How does this relate to the removal of endocrine disruptors? There isn't a lot of U.S. data; most of the work in this area has been in Europe. Pesticides and herbicides are a problem that we have dealt with for a while in the U.S. If something is an endocrine disruptor, we now know how to deal with it, not always in the most cost-effective manner, but we can remove it. Some people have tried to control atrazine with PAC; there is variability in the removal rates. If we promote the development of the hydroxyl radical, we often get better removal. However, promoting the development of the hydroxyl radical is counter to disinfection. Nanofiltration is effective, but expensive. Surfactants are moderately biodegradable in the raw water of a treatment plant if they have one or two hours detention time. In a situation where we could get real biodegradation, like a filter, we might be talking minutes of contact time. Ozone oxidation is very effective here.

We don't have a lot of data on removal of pharmaceutically active compounds with conventional coagulation. Nanofiltration and RO appear to be effective. A lot of the data comes from reuse applications. In water reuse, it is more important that the highest level of treatment is provided so nanofiltration and R/O are part of the picture. We have some data from those studies. We would expect GAC absorption to be effective. Oxidation with ozone alone does not seem to be effective for all compounds, but we know it will be improved if we promote the formation of the hydroxyl radical.

Data recently published by a German group (Zweiner and Frimmel) looked at the pharmaceutically active compounds clofibric acid, ibuprofen and diclofenac. They also looked at ozone and enhanced the oxidation by adding peroxide, which enhances generation of the hydroxyl radical. The dose was 1 milligram per liter, which is a little low. The dose is driven by water quality. If there is a lot of dissolved organic matter, ozone will react with that, and not with the compound that you are after. It is important to overcome a background demand that is based on alkalinity and dissolved organic matter. With ozone alone, 1 milligram per liter ranged from 8% - 97% removal. With the addition of peroxide at a stoichiometric dose (ozone still at 1 milligram per liter) increased the removal to 12%. This number is still rather low. Researchers found they had to go to a relatively high dose of 3.7 milligrams per liter to get 93-99% removal. They added peroxide at a stoichiometric dose; it is not easy for an ozone pla nt to double their dose and add peroxide in order to remove all the necessary compounds.

As for conclusions: 1) Membrane processes are most effective for removing endocrine disruptors, although they are rather expensive; 2) Oxidation processes, which exist in virtually every water utility in the U.S., appear to be effective in oxidizing endocrine disruptors, particularly ozone. We might need to go to higher doses or enhance hydroxyl radical formation by going to a higher pH or adding peroxide; 3) The existing treatment processes may be removing some endocrine

disruptors that may be coming into the plant. We don't necessarily know what process is at work, but it involves meeting the objectives of particle removal, disinfection and preoxidation. We preoxidize to oxidize reduced metals to precipitated oxidized metals. Taste and odor removal processes would also be assumed to remove endocrine disruptors; and 4) The removal rates vary significantly from water to water.

Research needs: 1) We need a lot more data on the effectiveness of typical coagulation processes with and without PAC, with and without adding an absorbent. Most of the data are with ozone, which is the strongest disinfectant; 2) Ozone does not oxidize all compounds instantly to 99.9%. The effectiveness of weaker oxidants, such as chlorine dioxide, and chlorine impacts aren't fully known. We need to understand what their impact would be; 3) The most important aspect of oxidation is to understand the degradation pathways. "Removal" just means that the compound being looked at isn't there anymore. Metabolites of the oxidation process could also be of concern. Before we begin oxidizing compounds, it is important to understand what the compound is being oxidized to; 4) People use other processes such as biofiltration that might provide additional information that may make this process more beneficial. For example, a utility may be trying to decide whether to allow the filter to operate biologically. In other words, de ciding whether or not to provide chlorine or another oxidant to keep the biological activity out. It is done for various reasons, such as HPC control and aesthetics. This might be indicating that if we operate it biologically, there will be additional benefits. Bank filtration, which is frequently done in Europe, is becoming something that we are talking about more in this country. Maybe that provides an additional barrier to these sorts of compounds; and 5) We are developing additional nanofiltration RO removal data through the reuse applications. We need to continue doing that.

Overview of UK research and treatment technologies

Arnold Bates, Bristol Water plc, Bristol, United Kingdom

Arnold Bates is Quality Director at Bristol Water plc, which is based in Bristol in the southwest of England. Bristol Water has been a private English water supply company since 1846. He is responsible for water quality assurance including emergency planning, risk management, information technology and research and development.Dr. Bates is involved in several national activities with Water UK (the water industry's trade association) and manages toxicology research carried out for UK Water Industry Research Limited, the UK water industry's collaborative research arm. He has a Ph.D. in peptide chemistry fromthe University of Liverpool. Prior to recieving his Ph.D., he was a bench chemist with a major chemical manufacturer.

Water reuse is usually not thought to be a concern in the wetter parts of the world. However, in many wet parts of the world, water reuse has been practiced for many years. It surprises many people, but in the United Kingdom (UK), because of the density of the population, the per capita rainfall is low, so the pressure on water resources is high.

Drinking water in the UK is supplied by private companies in England and Wales, and by public or government utilities in Scotland and Northern Ireland. Many water companies were privatized in 1989. Bristol Water has been a private water company since 1846. The UK water industry is very heavily regulated;

there are about 14 different regulators with an interest in the industry in Wales and England.

Our charging scheme is set on a five-year cycle; the Office of Water Services (OFWAT) sets charging. Capital and revenue spent is capped, hence it is charged to customers. Profits are not capped, so we do the best we can with the amount we can charge and the output we deliver in terms of capital and revenue expenditure. Our performance is measured quite closely. In addition to the charging regulator, another regulator looks after drinking water quality. There are also effluent standards for companies on the effluent treatment side; they are regulated by environmental agencies in England and Wales. Our financial outputs are monitored closely by the regulator, as is customer service.

Water UK is the trade association for all the water utilities. This arrangement is good because it includes the private and public sectors in Scotland and Northern Ireland, creating good cross-fertilization of ideas. Each water company in the UK has its own research interests. Clearly, there are specific situations that each must handle. To get some uniform research on "one-voice" issues, Water UK commissions its work from UK Water Industry Research (UKWIR), which is managed by people within the industry. UKWIR also manages the Engineering & Physical Sciences Research Council (EPSRC). This is government funding for research, water infrastructure and treatment areas. There is further funding which is managed by the industry's captive research organizations.

What are the UK water industry's priorities? First and foremost is wholesome drinking water and public health. Second is customer confidence. There is no point in having a good product if the customers don't believe it is a good product. Environmental protection leading to sustainable development is also a key area of interest. Because we are so heavily regulated, we believe that sound regulation based on scientifically derived standards is the way forward.

To meet regulatory requirements, we need to understand the processes and hence provide cost effective treatments. That leads to elements of fundamental research. We must remember that water is the universal solvent; outside the laboratory it is never pure. This is something that customers don't necessarily appreciate. Most natural constituents are relatively benign at the concentrations found in water. The biggest health risk comes from pathogens. There is also the potential risk of contamination through anthropogenic contaminants. Until recently, cancerous endpoints have been the main concern; this concern has broadened into the reproductive area.

The basis for many of the drinking water standards is the World Health Organization (WHO). There is a lot of learning from others in terms of standard setting. In the UK, the directive is translated into UK law through water quality regulations. These mirror, to a degree, what is present in the directive. They specify the parametric standards. Sampling frequencies, monitoring requirements and analytical control requirements are all set through this mechanism.

The methods of drinking water analysis always have to be performance tested and proven. As a matter of interest, we recently purchased a new Inductively Coupled Mass Spectrometer (ICPMS). It took over a year to get the instrument's performance tested to meet the standards set by the drinking water inspector. We must be able to demonstrate that we can monitor within 10% of the required standard. The water quality regulations cover the reporting these results. We have had to make our water quality

register available to the public for the past 10 years. While the average customer doesn't have that degree of interest, the register and annual report are made available to the public.

Other speakers have addressed the principles of drinking water management. Watershed management is an important part of that process. We must locate our abstraction point appropriately. Contaminants taken out of drinking water are frequently put back into the watershed, but usually downstream. By law, utilities have to use appropriate treatment technology depending upon the raw water source. The drinking water inspector specifies the degree and types of treatment. If it is groundwater, treatment will be simple disinfection. If it is a lowland river source, treatment will include multiple barriers of disinfection. The multiple barrier principle is quite complicated. These barriers are not total barriers; usually a series of three barriers are employed, each of which takes out a proportion of contaminants. Finished water will have a reduced level of contamination. Depending on what the barriers consist of, there will be different degrees of treatment or removal for different components.

The quality regulator audits the risk assessments used for defining the barriers that are in place, the specifications, and the operation and performance of a treatment plant. Each company is subject to several audits annually. Some are surprise audits. The audits follow the same line of attack as a financial audit; they ask for the complete audit trail and the specified requirements for a particular analysis.

During treatment, a limited amount of chemicals are introduced. While other speakers have mentioned coagulants, flocculent and oxidants, no one has yet addressed corrosion and disinfectants. We need to understand what effect additives have during treatment and their possible by-products. Contaminants from the raw water source or that occur as a result of treatment come out from the water treatment works and go to the customers tap.

Our drinking water inspector knows that raw water contains particles; these particles may include cryptosporidium. Due to the inability to prosecute water companies for these types of contaminants, the UK water industry now faces new regulations on treatment for cryptosporidium. It is a criminal offense in the UK to supply water with more than 1 crypto oocyst in 10 liters of water. Unfortunately the method of analysis that has to be used is not tuned for giardia. Those of us who are currently monitoring water for giardia have to repeat the analysis at a great expense. If we move to the statutory requirements, we don't get the giardia information.

To get rid of these beasties, we are installing membrane microfiltration followed by chlorination. What remains after microfiltration is a waste product. These are small groundwater sites, usually remote from river sources. We have a waste to dispose for the first time on those plants. There are additional challenges. Reverse osmosis is also a possibility. A disadvantage of reverse osmosis is that although it is very effective as a water treatment, the water needs to be remineralized afterwards. Virtually everything has been taken out of it.

An "advanced water treatment" plant schematic would have these processes: raw water intake gets immediate ozone, which helps the coagulation process, particularly by oxidizing manganese. Then ferric, aluminum sulfate, or polyaluminum sulfate is added, and flocculent with an organic coagulant. Sedimentation is the next stage, followed by rapid gravity filtration, which offers a degree of biological treatment as well. The next stage

is ozone, after that comes granular carbon absorption, followed by pH stabilization and final chlorination. If necessary, super-chlorination is done and then the water is discharged into the network.

There may also be site-specific treatments. For example, Bristol Water operates a facility that uses hydrogen peroxide. The principal reason for this is to put in ozone and granular activated carbon; hydrogen peroxide was added for pesticide control. The added value resulted in taste and odor control.

Phosphoric acid is added for stabilization. Ozone seemed like a good idea, until bromate came along. The new directive's standard (for the next 5 years) is 25 micrograms of bromate per liter of water, after which the standard drops to 10 micrograms per liter. The UK drinking water inspector decided that we will skip the 25 and go straight to 10. So we have to achieve 10 micrograms per liter maximum of bromate in the water, which can be done by pH control. The alternative is reverse osmosis. That will require a large capital investment. Nitrate removal is also a potential issue on river sources. Nitrate removal is contentious, if we go to ion exchange it is very costly. We don't fluoridate nor soften the water. At the moment we are not trying to create designer water.

What are the research issues? Currently, we use quite a few chemicals in the treatment of water, and we need to be certain that those chemicals are not adding to the burden of endocrine disruptors. Our response for endocrine research has been to look at treatment chemicals as potential sources of contamination; this has turned towards endocrine disruptors. The conclusion was that the standards and specifications for the inorganic treatment chemicals are adequate to ensure that any contamination is insignificant. With respect to organic chemicals, specifically polyelectrolytes, researchers concluded there was no need to do further work. That recommendation still stands, as control of polyelectrolytes by the inspector is very much on a dose basis. We are not able to analyze for the components of polyelectrolytes at the appropriate level. By dose control and control of the specification, we can meet all the international standards.

The effectiveness of the water treatment process is being researched. We looked at the literature and carried out some lab trials, and concluded that alkylphenols react extensively with both chlorine and ozone. Experimental coagulation had a limited effect. There probably is some scope for optimizing coagulation; activated carbon is a very effective absorbent, particularly in the presence of ozone. Our researchers found that steroid hormones react extensively with both chlorine and ozone. We are confident that there isn't an issue with these compounds under these treatment processes. We have not found any new information to suggest that the steroid hormones are not knocked back well with the treatments that we are applying based on a reuse principle. We are satisfied with the inorganics, we believe that the oxidants that we are using are effective in breaking down these compounds, and carbon is a very good absorber.

We are confident that we don't have these compounds coming through in the treated water. The next issue is dealing with customer confidence, which is a difficult area. Since the hypothesized link between substances in the environment and disorders in wildlife receives such wide public and academic media attention, it clearly undermines customer confidence. The thirst for information has not been well satisfied by any of us. That applies to the industry, the academics, and the regulators. In the absence of hard evidence the media present their own answers.

How can we prove drinking water quality? The first thing is demonstrate the quality through the development and application of exceedingly robust analytical methodology. All statutory analysis in the UK has to meet very stringent performance requirements; it has to be demonstrably fit for purpose. Some of the published work actually uses analytical methods that are fit for purpose. It is important to remember that endocrine compounds are designed to be biologically active at very low concentrations. Therefore, analysis has to be meaningful at that low level to enable the rest of the research to produce meaningful results. The UK industry's response to this was to stress the importance of the regulator and the regulatee working together.

In 1997, work was commissioned to develop a sensitive analytical method for steroid concentrations in sewage effluence and treated drinking waters. The method was developed, performance tested, and demonstrated performance at the sub-nanogram per liter level for 17-B-estradiol and ethinyl estradiol. Once the method was perfected, we applied it to several drinking water samples from a variety of source waters. In the worst cases of source water samples analyzed, none of the free steroids were found at these very low levels of detection. Further work in the UK has supported these findings.

Yesterday, we heard a little bit about direct water reuse. One of our facilities is faced with that situation and the customer's response to it. They've had to do a great deal of work to prove the water quality is satisfactory to both regulators and customers. Customer confidence can now be built as this important milestone of demonstrating these compounds are not being found in the water.

The general conclusion in much of the endocrine disruptor research was that any confirmed reproductive changes in men were almost certainly due to multiple factors. There is a vertical child health study in Bristol, which is following children for the first 15 years of life. There was a report that suggested that boys with hypospadias were more likely to be born from mothers who were vegetarians, the suggestion was that phytoestrogens could be a source of estrogenic activity. We believe there is no evidence for a major role in drinking water in creating public health problems. An article from the London Times stated that "Of course, we're not talking here about what comes out of the tap - complete with chlorine, fluoride, human body fluids and contraceptive oestrogen. For wholesomeness, beauty and sheer bloody chic, bottled water is the thing." That type of statement from a newspaper creates a real a real public relations challenge. It makes super headlines but the true story is very difficult to get across.

As an industry, we are responsible for the entire water cycle, including sustainable development and consideration of environmental impacts. Bristol Water is the largest discharger of treated domestic and industrial effluent to the environment in the UK. We must not forget that widespread sewage treatment represents the greatest achievement in public health improvement over the past century. If it weren't for improvements in sewage treatments systems, water treatment systems worldwide would not be as effective as they are. Sewage treatment is key to environmental process and sustainable development.

Let's take a brief look at sewage treatment, starting with crude sewage. Crude sewage could be domestic sewage or it could also contain industrial waste. Regardless of what it is, the treatment process begins with a rough screening process, then some sort of settlement either with or without coagulant aid, followed by a form of biological filtration. It could be trickling filters, activated sludge or

deep-shaft. Afterwards, there is some sort of secondary settlement to create an effluent. These days, a tertiary treatment may be applied, which could be specific to a particular component of the waste. The important thing is these processes produce sludge. However successful we are at taking any particular component out of the effluent stream, it will end up in the sludge. If you don't get rid of the sludge correctly, it comes back to create further issues. On the wastewater side, we believe research activities should focus on estrogenic substances in the environment. The industry needs to work with others who are conducting research on these substances and specifically consider their fate during sewage treatment. To guide our future research, we commissioned a workgroup to recommend research activities for steroids. The environment agencies have detected these compounds in sewage effluence.

The point was made earlier that without sound standards, we aren't going to get sound regulation. Regulation covers routine monitoring; it is a normal feature of water supply or sewage discharge. There are few places in the world where steroid hormones are currently controlled; the first elements of control are being put into place in the UK. The European Union recently published its strategy for endocrine disruptors. The industry in the UK is encouraging the philosophy that any new standard should be scientifically based, and UK regulators have adopted the same approach. Our own studies of the fate and behavior of steroid estrogens in rivers is addressing these science issues.

A review has been published by an environment agency on pharmaceuticals in the environment; there is a governmental inter-departmental group on endocrine disruptors that recently published its final report. Very recently the environment agency published its strategy for endocrine disrupting substances in the environment. They looked at steroids, alkylphenols, pesticides, and compounds like dioxins, PCBs and furans. The report suggests that the main pathway for steroids are sewage treatment discharges. The proposed action is to develop environmental standards and targets, identify high-risk areas, and make improvements to sewage treatment processes. The interesting aspect is the time scale. Their proposal for the release of steroids from sewage treatment works is to develop an environmental quality target for total steroids by the end of 2000 (they've got nine months to go), refine the work on identifying rivers which are likely to have high steroid concentrations, target river monitoring at these sites through surveys (2001-2003), and set up collaborative projects on high risk areas to investigate ecological relevance of endocrine disruption (2000-2004). They also plan to find options for reducing inputs, including research and wastewater treatment, and take cost-benefit assessments. The last step will occur just in time for when we are renegotiating with the regulator in 2005 for our charges. If the environment agency is keen on setting standards for sewage discharges beginning in 2005, there could be massive costs. We do know in the case of direct reuse that the biggest issue is that the environment agency has set standards for discharging from the sewage works into the holding reservoir. The reservoir is intermediate between the sewage works and the water works. Those standards are particularly stringent. The regulators are getting their act together. If it happens in Europe it will soon happen in the United States.

The keys to relevant standards are behavioral knowledge and suitable analytical techniques. In order to achieve cost-effective treatment, we need new and robust analytical technology. Currently analytical technology is not particularly good for the conjugated compounds. It is not good in a dirty sample;

when looking at sewage and sludges, it is very difficult to get reasonable results. There is a lot of work in progress, though. Recent studies have indicated potential methods, but the performance still needs to be ascertained. There are crucial gaps in knowledge that need to be filled before the fate and behavior of these compounds can be established adequately.

Our response has been to keep abreast of work in other areas, and to work with regulators, industry and academia. We have co-funded work with regulators on environmental issues. We have reviewed fate and behavior. We carried out, along with the drinking water inspector and the environmental agencies, some inter-laboratory performance testing of the analytical method for steroids. Because it was a research method on the frontiers of equipment capability, it does not translate easily into other laboratories. If we are going to achieve a wide range of cost-effective samples, we need cost-effective analytical methods that translate easily.

We have been looking at developing a highly sensitive method for steroid conjugates; that work is in progress. To understand the mechanism of steroid removal we need to better analyze at low-levels. Although we have achieved it, we realized it is more complex than we anticipated. It relies on LC/MS/MS.

The long-term objective is to use a methodology to follow compounds through sewage treatment. We also want to identify the processes taking place within the treatment works in order to optimize treatment. We recently looked at stability studies on selected conjugate compounds using radio labeled estrone sulfate, estradiol, and glucuronide. We intended to look at adsorption studies on radio labeled estradiol, ethinyl estradiol, and estriol and we decided to start some work of quantitative structure activity relationships. This has not been discussed much at this workshop, but there was a comment suggesting this may be a difficult area to work in. But if we can develop it in the endocrine area and pesticides, we can start to migrate the knowledge into the pharmaceutical area.

In terms of fundamental research, the first priority is analysis. The second priority is analysis, and the third is also analysis. Without the fundamental analysis we don't make progress. After that, we need to understand fate and behavior, and we are pinning some hope on structure activity relationships. We need to understand the process and we must have some sort of holistic risk assessment.

Some results from the later work showed that ethinyl estradiol binds rapidly to sludge flocculent and remains stable afterwards, provided the floc doesn't break down. It appears that estradiol disappears rapidly (in about 10 minutes) from sludge to form estrone. Estriol disappears to form hydroxyestrone. The question that still hasn't been answered is whether ketones reduce back to alcohols before discharge. The majority of estrone sulfate is removed within 12 hours; only a limited formation of free estrone occurred. We don't know where it went but it was somewhat of an unexpected finding. We now have to consider pathways and metabolites. The synthesis of estradiol glucuronide, the tritiated form, is proving to be extremely challenging. The manufacturer can make it but cannot recover it. The synthesis of the estriol glucuronide has been abandoned. It is so mobile that the glucuronide falls off immediately. This provides an indication that these compounds are not stable in the sewage treatment environment. Our intention is to continue this work using 17-glucuronide, which we know is available as a radio labeled compound.

Finally, we need to set the risks in perspective. There was some degree of rift between the UK and the United States over genetically modified foods. As a water industry, we don't want endocrines to be the next GM foods.

Endocrine disruptors in wastewater

Matthew Davis, Brown & Caldwell, Seattle, Washington

Matthew Davis received his Master's degree from Cornell University in hydraulics and hydrology and has spent the last four years as a water resource engineer with Brown and Caldwell, a leading environmental engineering and consulting firm with offices throughout the United States. Mr. Davis is based in Seattle and has helped clients address issues relating to endocrine disruptors; he actively participates in efforts to keep the wastewater community abreast of new developments in the field. He is co-author of a 1999 paper titled 'Endocrine Disruptors In Wastewater: Is There Cause For Concern?' (Article available at: http://www.brownandcaldwell.com/tech/717.pdf

I work for an environmental engineering firm that does wastewater work; I'd like to share my perspective as a practicing wastewater engineer and talk about how the endocrine disruptor issue is developing. I first came into contact with endocrine disruptors in wastewater while working on a wastewater comprehensive plan for the wastewater partnership in Olympia, Washington. The plan had three primary objectives; the first was to provide just-in-time capacity. As the area is going to double in population by the year 2020, they will need new wastewater capacity and new conveyance capacity. Instead of building facilities that won't be fully utilized for the next 20 years, we proposed building smaller modular facilities that we can bring on in a "just-in-time approach" so we can make full use of our economic investment. This approach also has benefits for equitably distributing development costs.

The second objective was to begin to treat wastewater as a resource. While Olympia is not a particularly arid area, we are still interested in beneficial reuses. The third objective was to decrease marine discharge. The treatment plant discharges into the southern most extent of Puget Sound, which does not have particularly good circulation. The idea was to go to more upland recharge. We developed a framework for new capacity which involves siting small satellite treatment plants near areas that have opportunities to reuse reclaimed wastewater, when there isn't enough demand to recharge it to groundwater. An environmental impact study was part of this report, as well as a public review and comment period. Citizens voiced some concerns about endocrine disruptors. The main comment was that "the study does not address the potentially significant harm done to the receiving environment by endocrine disrupting, estrogen-like chemicals. Such an assessment should be conducted." That was when I became invo lved with endocrine disruptors. I did a literature review to see what kind of information was out there. This is a tough concern to respond to because we don't know a lot about it; we did our best to explain the information that was available.

This issue has the potential to affect the wastewater industry by changing the types of treatment technologies. It has already had an effect at the planning level, and it has the potential to change the way the industry is regulated. In addition, beneficial uses for wastewater reuse are being discussed. For example, there is a lot of interest in using reclaimed wastewater for stream flow augmentation. In the Seattle area, there is a

real desire to keep stream flows going in the summertime for salmon runs. We are also considering using reclaimed wastewater for supplying fish hatcheries. Are these kinds of uses appropriate?

The endocrine disruptors in wastewater issue first came to light in the mid-1980's, when some anglers who were fishing in sewage treatment lagoons in the U.K. reeled in fish that had both male and female sexual characteristics. Researchers confirmed the presence of the inter-sexed fish, which is pretty uncommon, so it was a surprising discovery. This event is often cited as the initiating observation that led to the line of research that has developed on sexual differentiation in fish.

There are some lines of evidence that wastewater does have endocrine disrupting, particularly estrogenic, properties. Researchers have used vitellogenin as a sensitive biomarker for exposure to estrogens. Vitellogenin is normally produced by female fish as a precursor to egg-yolk formation; it is formed by the liver under action of estrogen. While male fish do not normally produce vitellogenin at any appreciable levels, they will begin to produce vitellogenin if they are exposed to estrogen in the lab, and they can produce it in large quantities relative to their background concentrations. Researchers placed male fish in cages in wastewater treatment effluent streams. After a certain amount of time, they sampled for blood vitellogenin levels. They found highly elevated vitellogenin levels; results vary depending on what type of species used. Typically, male rainbow trout have been used. A study done by Sumpter et al. placed male rainbow trout in a wastewater treatment effluent stream for a week. In t he control group, vitellogenin production was 0.1 micrograms per milliliter; after a week, vitellogenin levels had increased more than three-fold in the effluent stream fish. This is a sensitive biomarker.

Another recent study reported widespread sexual disruption of a wild fish (roach) in U.K. rivers. Roach were collected upstream and downstream from wastewater treatment plants and checked for gender. Researchers found significantly higher inter-sexed rates downstream from wastewater treatment plants. In a laboratory control group, the inter-sexed rate was 4%. Downstream from two wastewater treatment plants on two different rivers, they found 100% of the male roach fish had both male and female sexual characteristics. Researchers also looked at vitellogenin and found increased levels in fish downstream from wastewater treatment plants. The conclusion was that wastewater treatment plants are responsible for this occurrence. This is a specific example where wastewater treatment plants do seem to be disrupting the environment.

Municipal wastewater is composed of a variety of substances. Domestic wastewater comes from residences and commercial facilities, institutional and recreational uses, industrial flows from bottling companies, pulp-paper mills, etc. There is also an unwanted infiltration inflow component in many sanitary flows, typically storm water that gets in through cross-connections between storm water systems and sanitary systems. There could also be groundwater leaking in through cracks in pipe joints and other defects. The domestic and industrial sources could potentially contribute a host of endocrine disrupting compounds. Certainly, wastewater receives alkylphenol and polyethoxylates, which eventually results in their degradation products. Wastewater could receive heavy metals, pesticides, bisphenol A, persistent organo-halogens, phytoestrogens, endogenous estrogens and pharmaceutical estrogens. As

there are a lot of compounds, how can we begin to narrow the list down?

If we plot wastewater concentration and estrogenicity, we find that as wastewater concentration and estrogenicity increases, the potential for disruption also increases. Compounds with low wastewater concentration and estrogenicity include phytoestrogens, heavy metals, persistent organohalogens and pesticides. These compounds can occur in wastewater if, for example, someone decides they don't like their weed killer anymore and dumps it down the drain, which will result in a slug of pesticides. We see universal vitellogenin induction in many caged fish studies. It is rather unlikely that these compounds are the major contributors to the estrogenicity.

Nonylphenol is a weakly estrogenic compound that can occur at relatively high concentrations (in the microgram per liter range) in wastewater treatment plants. Estradiol is very potent estrogenically; it occurs at relatively low concentrations of about 10 nanograms per liter. Ethinyl estradiol is a potent estrogenic compound that occurs at low concentrations. In our quest to find what is causing the estrogenicity of wastewater, we must look at the compounds that are frequently detected. Typical wastewater treatment effluent concentrations of estradiol, estrone, ethinyl estradiol, nonylphenol, and 4-tert-octylphenols are important to record. Estradiol occurs at a range of 1.8 to 48 nanograms per liter; a typical value is about 10 nanograms per liter. Estrone is generally a bit higher, with a concentration of about 15 nanograms per liter. Ethinyl estradiol occurs at low concentrations, around 1 nanogram per liter. Nonylphenol is present at much higher concentrations of around 3000 nanograms per liter, or 3 mic rograms per liter. This concentration can vary depending on the sources contributing to the wastewater stream. If pulp and paper mills are contributing effluent, that number may be a bit higher. 4-tert-octylphenols has low concentrations in wastewater of around 50 nanograms per liter.

In order to determine which compounds may be causing the estrogenicity of wastewater, it is useful to compare typical wastewater concentrations with concentrations you would need to produce increased vitellogenin production in fish. It takes about 50 nanograms per liter concentration to induce elevated levels of vitellogenin production in male fish. While you can increase vitellogenin production at much lower levels, this is certainly a level where there is significant production. For estrone, it takes about 100 nanograms per liter. For ethinyl estradiol, some studies have shown that one-tenth of a nanogram per liter can induce significant vitellogenin production. Orders of magnitude higher than 35,000 nanograms per liter and 10,000 nanograms per liter for 4-tert-octyphenol elicit the same reaction for male rainbow trout. The higher the concentration of a particular contaminant in wastewater, divided by the concentration required to significantly increase vitellogenin production, the greater the ratio of what is present in the wastewater to what it takes to cause a reaction in fish. Ethinyl estradiol of 1 nanogram per liter is 10 times higher than what has been shown in the lab to cause this reaction in male fish. Estradiol, estrone and nonylphenol are in the range of 8%-20%. Any of these compounds could be contributing to the large estrogenicity of wastewater.

In addition, these compounds don't occur by themselves in wastewater, so there are concerns about possible additive or synergistic effects. We also need to consider bioaccumulation of possible degradation products, including compounds like nonylphenol. Researchers have found bioconcentration factors in wild fish ranging

from 10 to 480. There are some conflicting reports out on mussels. One study found a BCF of 10 and another found a BCF of 3400. Some studies have shown that the half-life is pretty short; for mussels it is about 0.3 days and for fathead minnows about 1.2-1.4 days.

What compounds are traditionally targeted for removal by wastewater treatment plants? Certainly suspended solids, biodegradable organics, carbohydrates and fats are typically quantified in terms of biological and chemical oxygen demand. Pathogens, and more recently, compounds like nutrients, priority pollutants, refractory organics and heavy metals have also been targeted.

Removal rates of various types of estrogens were measured in several different treatment plants in Brazil, Germany, Ontario and a combination of 27 plants in Japan. Ethinyl estradiol is not reduced significantly in the German and the Ontario wastewater treatment plants. This supports research by Ternes on the transformation of ethinyl estradiol in the activated sludge process in which he found hardly any conversion of ethinyl estradiol at all.

While the literature contains reports of influent concentrations of many substances, including estrone and estradiol, it does not include their conjugates. This needs to be taken into account when looking at the estrogen coming into a treatment plant. Removal of estrone in an Ontario treatment plant was around the 70% level, which was higher than that of the Brazil treatment plant. For estradiol there is almost complete removal in the Brazilian treatment plant. The Japanese treatment plants had a little over 50% removal. Alkylphenols, 4-tert-octylphenol and nonylphenol were recorded across various treatment processes in a treatment plant, which includes removal after primary treatment, tertiary treatment and final treatment. In general these removal rates are greater than 90%.

In conclusion, endocrine disruptors definitely have the potential to change the way we do wastewater treatment. We know that many compounds may have estrogenic properties; suspected compounds include natural estrogens, pharmaceutical estrogens and nonylphenol. Source control for these compounds will be difficult; it is very difficult to convince people to stop excreting natural estrogens. It is also difficult to convince people to stop taking their oral contraceptives. Some European countries have taken the step of banning alkylphenolic compounds, but they are still in widespread use in the United States. Another conclusion is that natural and pharmaceutical estrogens are potent at very low concentrations, in the nanogram per liter range. Ethinyl estradiol may not be significantly removed by our current wastewater treatment processes.

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Session 5: Human Health and Ecological Health Issues

Very low doses of bisphenol A and other estrogenic chemicals alters development in mice

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My objective is not to teach you the molecular biology of the developing reproductive tract, although I am going to go into a little bit of that. People have been talking about low dose issues, pretty much presenting this as a phenomenon that has essentially no data associated with it and has yet to be proven as to whether it exists or not. What I want to do is present information that we have and that other people have generated with regard to why we see things occurring at very low dose and why the endocrine system is so dose sensitive over extremely narrow ranges of dose. These are concepts that a lot of people may not have encountered depending on the particular field. Even biologists don't often have a lot of experience with developmental biology - particularly development endocrinology, which is a sub-specialty. What I will focus on is low dose issues from the perspective of a physiologist. I'll give you a sense of what I mean by that when I cover physiological relevance of dosing, and I'll get into som e new terminology.

I'm going to start by talking about bisphenol A, which is a chemical that has generated a lot of debate. It was synthesized by Dodds, along with a whole series of chemicals. This was back in the heyday of estrogen and androgen synthesis, when steroids were beginning to be synthesized for drug use. Bisphenol A was a chemical synthesized as an estrogenic drug in the 1930s; it was published to be fully estrogenic in the journal *Nature* in 1936. A few years later, Dodds synthesized diethylstilbestrol (DES), which dropped off the radar screen for a couple of years. Then along came polymer chemists who said "hey, this bisphenol is a really neat product, you can polymerize this and make all kinds of things out of it." A couple of decades after this chemical estrogen was synthesized it was used to make baby bottles and other plastic products. I don't think you see that in the product literature. But that's the way it was. I didn't know much about bisphenol A as a plastic, I just knew it was an estrogen. It wasn't un til after I made the terrible mistake of doing an experiment with it, and the experiment showed something that the plastics industry didn't like, that I realized I had stepped on a big elephant's toe.

What does bisphenol A actually get used for today? Four corporations in the United States make almost two billion pounds of it; there are a lot of people who have a very big interest in this multi-billion dollar product. It's one of the 50 top chemicals in production. Some uses: the resin lining in most cans is made from bisphenol A. It's used to make polycarbonate products such as baby bottles. It's polymerized as a dental sealant. It's used as a hardening agent, it's the major flame retardant called tetra bromo bisphenol A. It's used as a dispersant, it has a zillion different uses. One of the things now being found is that there are exposure data on the release of bisphenol A. When I first started researching this, I found that articles basically said plastic products are essentially non-degradable. Then I started talking to polymer chemists, and they indicated these molecules came right apart, they are very unstable. In fact, we presented at the 'Estrogen and Environment' meeting in conjunction with U.S. (USG) Geological Survey Lab in Columbia, Missouri, who did the high resolution mass spec for us, and we did the bioassay. If you take a polycarbonate rat cage that people put frogs and fish in and do other research in (a cage that has been around for awhile), you can measure up to 100 micrograms per liter of bisphenol A just floating around in the water. That is a biologically active amount - that will sex reverse a frog in there. I don't house my animals in those cages - I use polypropylene cages that have little known phenol in them, but not bisphenol A. Again, billions of pounds of this are synthesized every year, and when you throw products containing bisphenol A in the landfill, they will degrade and there will be bisphenol A leaching out of them. That's a very important thing for water quality people to consider. The reason there is not much information up to now is that the assays for this chemical have been very complicated. CDC researchers, Japanese researchers and the USGS have really come a long w ay in making it possible and much less expensive to measure bisphenol A. When I first started this, it cost a couple thousand dollars a sample.

Regarding the concept of environmentally relevant dose, that is where we have exposure information, where there is monitoring going on. We can come up with environmental relevant amounts of human exposure, which will vary from environment to environment. The best data on this were presented in Japan in December 1999 by Chisato Mori's group at the University of Kyoto. Their research found 0.2 - 2.0 parts per billion bisphenol A in the umbilical cord of the human fetus. That is the best data you can get right now. That's what's getting to the baby. There are other reports looking at the amount coming out or being monitored from dental sealant. There are also other measurements that are leaching from various polycarbonate products. Others have shown amounts coming out of baby bottles. We're beginning to see more data on how much humans are being exposed to through various products.

The second issue - the physiologically relevant amount - is how we actually got into looking at bisphenol A. We look at it in terms of bio-equivalence relative to the natural hormone estradiol. How much estradiol does it take to stimulate the uterus or the prostate or other organs? Then give us a dose of bisphenol A and you get a relative binding coefficient in terms of how much binds to the receptor, and then what kind of biological readout you get. The interesting thing for bisphenol A is the dose range for the physiologically relevant dose and environmental relevant dose overlap. That's obviously not going to happen for many chemicals.

The focus of endocrine disruption is disruption of development of a baby. The fetus is very sensitive to hormonal disturbance. Hormones coordinate the development of all organs in the body - the differentiation of all tissue. Howard Bern (a very famous endocrinologist) coined the

term 'the fragile fetus'. You are never going to get at the damage being done by a chemical to a fetus by studying the adult. The most classic example of that is thalidomide; another example is DES. There were no effects on the mothers, but there were devastating effects on the babies. There is also a strange twist to these chemicals. As people get older, they may actually have some therapeutic value. For example, it may be valuable for people to take estrogenic chemicals as they age - for bone loss, cardiovascular disease, Alzheimer's, etc. However, if you are pregnant or in your reproductive years, these things are bad. Exposing babies to artificial chemicals is not good.

There are periods in differentiation in the human during which hormones are controlling development - this is at the end of the embryonic period and at the beginning of organogenesis, when the reproductive tract and the brain and other organs are differentiating. We know from the DES literature that children of women who took DES after this timeframe don't have identifiable damage. However, if DES was taken during this time there was severe damage, even at very low doses. So, the idea that only massive doses of DES cause problems is not accurate. It really has to do with timing of exposure as dose. Why is this? Two hormones, testosterone and estradiol, are critical for differentiation of cells - what they do is bind to receptor sites associated with genes, they activate some genes. The cells go off in one differentiation direction; testosterone activates genes in another cell and they stay permanently on and other genes stay permanently off. One thing we have learned is this process is NOT a "girls have thi s" and "boys have that" kind of thing. But it is an exquisitely dose sensitive process. We see differences on the order of 5/100 of a trillionth of gram per milliliter (ml) of blood of estradiol leading to differences in the course of differentiation of reproductive tract tissues. The sensitivity of this differentiation process to dose is mind boggling. We know this because of technical advances in the ability to work in the lab to identify substances in the very low dose range. Twenty years ago we couldn't know that because we didn't have the assays that could detect those levels.

Now I will tell you a little bit about my research on the male reproductive system/ specifically the prostate organ. Our research on the CF1 mouse pretty much explains what happens in the human. During the CF1's early life the prostate grows and then in the adult phase the prostate stops growing; it stays exactly the same size. Throughout the entire period of adulthood there is a very consistent prostate size. In many of our experiments, the control animals have exactly the same size prostate - the range of values is very similar. There is tremendous consistency and reliability in the experimental model system, this is a very important issue. When these guys hit the beginning of middle age, the prostate goes back into a ballistic growth mode. It has nothing to do with body weight - prostate size and body weight are totally independent of each other. The big question with benign prostatic hyperplasia and cancer is, what is doing this? Sixty-five percent of men at the a ge of retirement have this happening to them. It's one of the largest medical care costs in the United States. We don't have an answer. Forty thousand men die per year of prostate cancer; it is a very serious problem. We are trying to develop animal models to study it. I hope to tell you something that may be relevant as to why prostates show abnormal growth; I think we have some answer to that. We did an experiment - we gave pregnant mice 2 parts per billion bisphenol A, that is 2 nanograms per gram body weight, for the 7 days in the end of pregnancy that matched up with the period of human fetal sexual differentiation of the reproductive organs. So just around the time that many of the sexual organs are differentiating we fed pregnant females bisphenol A once a day at a very low dose. In a 1997 paper in *Environmental Health*

Perspectives we go into great detail about why we selected these doses, based on the biology of bisphenol A. It's a very long series of mathematical presentations, which I don't w ant to go into now. There was a very good reason to choose the very low dose and expect it to be biologically active.

Here are some results from our study. Relative to control animals, prostate weight for adult males was higher in treated animals. Treated animals got seven days of bisphenol A during development and never were exposed to it again, and there is an increase in prostate size by about 30%. The whole glandular area is enlarged. Interestingly, the epididymis and seminal vesicle are smaller. At first that was very confusing. Then we took a look at the embryology and saw that the urogenital sinus is totally different embryonic tissue. This tissue differentiates under a totally different set of hormonal control mechanism than the tissue of the Wolfian duct, seminal vesicle, vas deferens and epididymis differentiate from. It is a totally different control mechanism. If you give a chemical, you have two different embryonic tissues responding to that chemical in very different ways: inhibitory effects in one area, stimulatory effects in the other area. We now know what is happening in the Wolfian duct derivatives (such as the seminal vesicle) is that even at very low doses of estrogen we down-regulated production of 5-alpha reductase - a critical amplifying enzyme that takes testosterone and converts to DHT. This is sensitive to extremely low doses; any dose inhibits it. You don't get any stimulating and then inhibiting -not in these tissues - all you get is inhibition at any dose. This is a more potent hormone and you can't produce enough of it if you fetal estrogenize these animals, because there is a deficit in this enzyme, so essentially you end up with a smaller organ. That is the basis of the small tissues in the epididymis and the seminal vesicles. An enzyme is being down regulated.

In the prostate, something entirely different is happening. When you give very low doses of estrogens, it stimulates and increases the number of glands that form in the zone of the prostate. More glands are induced and more branching of the glands themselves is occurring and they get bigger. We were able to see this happening by collecting prostates on the first day of prostate development in the embryo; after twenty-four hours we could already see that result. If you add a little more fetal estrogen, there are more glands, more branches which all lead to more volume - it's just becomes a bigger organ. We now know some of the molecular mechanisms of this. It has been shown that 1/10 of one trillionth of a gram of free estradiol per milliliter of blood in a mouse fetus is enough to stimulate and increase an androgen receptor in the fetal prostate. That is a phenomenally low dose - we did this by putting a capsule of estradiol in the mother and then very slightly tweaking up estradiol levels in the fetus. This can be done because the assays have been developed to measure hormones at very low levels in rodent fetuses. This has since been replicated by Dr. Gupta at the University of Pittsburgh who has done exactly the same experiment with DES and bisphenol A showing that 1/10 of one trillionth of a gram of DES and 50 parts per trillion of bisphenol A stimulate androgen receptor increase.

Testosterone drives development of prostatic cells as a function of how many androgen receptors it has to bind to and what kind of output it can create. What you are doing with estrogen is jacking up androgen responsiveness in the cell. Dr. Gupta has also published a paper in *Proceedings of Experimental Biology and Medicine* and another paper in *Neurological Sciences*. What she reports is that second protein epidermal growth factor is stimulated. It synergizes by binding to receptors on its own cell such that if you take ten units of normal output of testosterone and ten units of output of cell growth of VGF, when you put them

together you get synergism. That is, you get about seventy units of output. When you have these two together you have synergistic output. Anybody who has ever studied endocrinology knows that one of the central features of endocrine responsiveness is amplification. You have amplifying systems in target cells, so an extremely small signal can lead to a very large output. Think of this as a stereo with the gain turned all the way up. That's the way evolution has created your cells. It is able to take very small amounts of endocrine signals and have a very large output. We are working out the molecular details of the amplification and how very small quantities of estrogenic chemicals lead to molecular changes in the cell that lead to this cell putting out signals that then drive the epithelial glands to grow at a much greater rate. As a result of the EGF and androgen receptor up regulation, you have a much greater output of growth factors that are driving gland development and gland growth in the prostate. Once this happens in the embryo, the genes that are acted on are permanently up-regulated in their activity and that event lasts throughout the life of the individual. It is an irreversible event; we don't have the technology to go in and down-regulate genes that have been permanently up regulated during fetal life. Now comes the real killer with regard to the issue of dose: if you take 1000 t imes higher dose of DES, high doses of bisphenol A or high doses of estradiol you get exactly the opposite set of events. You inhibit all of the systems that are up regulated at the lower dose. They are simply shut off at a higher dose. They just stop. In fact, in a male exposed to 100 micrograms per kilogram of DES, essentially there is no prostate. You have a rudimentary prostate, practically undetectable levels of androgen receptors and virtually no responsiveness. Exactly the opposite outcome: high doses of hormones shut down endocrine response systems, low doses stimulate them. There is an immense body of literature on this. It's like the first week of lecture in a class on the endocrine system - you talk about receptor down-regulation and inhibitory feedback.

Over the years, I have been studying sources of natural variation in hormones and how that impacts our life history. In the rodent, for example, you have a natural source of individual difference in fetal hormones, which is called the "intrauterine position phenomenon". I published a series of papers in *Science* on identifying this back in the late 1970s and early 1980s. What we find is that if a female fetus is between male fetuses, she gets supplemental testosterone. The fetuses will transport hormones to each other. If a female is next to other females, they get supplemental estrogen. But the differences are phenomenally small. We're talking about less then 1/10 of a trillionth of a gram of estradiol that distinguishes this female from this female. How could that possibly matter? How could so little hormone possibly translate into anything? Well, one thing it does is it translates into discriminating whether animals respond to bisphenol A or not. This was an astonishment to us. We took pregnant females and fed them a 2 microgram per kilogram dose of bisphenol A during the last one-third of pregnancy when the reproductive system is differentiating. This dose is well within the realm of what human babies would be exposed to by drinking out of baby bottle made of polycarbonate. So, this is an environmental relevant dose. We fed these pregnant females, caesarian delivered them, categorized what position the babies were in and then watched their growth to determine when the females went into puberty. We found something very interesting. In terms of body growth, the bisphenol A animals were heavier. Everyone knows, if you want to make livestock bigger put a little estrogen in them; estrogen is a growth-promoting hormone. The fact that estrogenized animals were a little bigger was no big surprise. What was interesting was that in terms of body growth, the females that had the lowest endogenous levels of estrogen showed no growth effect of maternal bisphenol A treatment.

If they were intermediate in their levels of f etal estrogen, they had a slight effect, but if they had the highest levels of background levels of estrogen, they had the really big effect on their body growth. They were 22% bigger at puberty than the untreated females. If you look at the timing of puberty, if you are between two males and you have the lowest background levels of estrogen, it had no effect on the timing of puberty. With intermediate levels of estrogen puberty was slightly accelerated by bisphenol A, with the highest levels of fetal estrogen you have the biggest effect. Does this have any relevance to humans?

In a woman's first pregnancy, she runs higher levels of estrogen than in subsequent pregnancies. With twins, she'll have higher levels of estrogen than with single births. Black women have higher levels of estrogen than white women, and Asian women have different levels of hormones than white women. There are also age-related changes in the production of sex hormones by women. There are all kinds of natural sources of variation. It suggests that these natural sources of variation are going to set the stage for differential responsiveness to environmental chemicals. We think that the ability of individuals to respond to bisphenol A or other xenoestrogens depends on their background levels of hormones. This raises a very interesting issue with regard to threshold. We know that the natural levels of estrogen in fetuses between females are above threshold already with the endogenous level, there's enough estrogen in there to be affecting development. In some fetuses this external event drives them over that. Some animals have already exceeded that with their endogenous level of estrogen. The total level of estrogenic activity isn't just the added external dose, it's the internal dose plus the external dose. Anybody into risk assessment and into threshold models of risk assessment has to take this into account. These data were published in *Nature*.

Why is bisphenol A so potent? We did a MCF-7 cell assay where we put in human breast cancer cells and plated them into the cells and then add doses of estradiol. You begin to see cell proliferation at below 1/10 of a trillionth or picogram per ml. The cells show maximal proliferation; when you get up here to 10 micrograms per ml, the cells die. It is lethal. With regard to estradiol, the MCF-7 cells with a mutation grow with no ligand - no estrogen present. Adding the ligand doesn't matter. But they die in response to estradiol or bisphenol A at exactly the same dose whether they have estrogen receptors or not. So acute toxicity is not a receptor-mediated event. That is, these are lipids, you are just melting down the cells and killing the cells, it has nothing to do with functioning of the endocrine system. The dose that causes death is not predictive of the dose of different chemicals that are hormonally active. Bisphenol A in this assay is 100,000 times less potent than estradiol in terms of human breast cancer cells. If you were to take this dose and extrapolate ten fold safety factors (used in animal to human variability), use a LOAEL (lowest observed adverse effect level) rather than a NOAEL (no adverse effect level), you would extrapolate down to this threshold and say this is the dose that is absolutely safe for estradiol, and you would be whoppingly off. But if you do that for bisphenol A it wouldn't look like you would be whoppingly off. Bisphenol A does not look like a powerful chemical. But how are we feeding it to females so it is getting it into babies in this range and are we seeing effects?

We were very curious about that. Can you extrapolate from very high doses and predict the way hormonal systems operate in the very low dose range? It turns out this is a problem. The chemical industry had estimated the NOAEL for bisphenol A based on not finding a no effect dose but coming up with a LOAEL, 50 mg/kg per day. They put a thousand fold safety factor on it and said 50 micrograms/kg was the

acceptable daily intake, or reference dose. That's a starting point. Now we're giving 2 micrograms/kg per day. We're 25,000 below the low effect dose. We are seeing effects at that level. So we don't know what the no effect dose is. How is this possible?

A long time ago, a Dow chemist came to me and said "we are looking at bisphenol A in adults and it's cleared through their system very rapidly.therefore none of your research makes any sense." I said, "Come on. You're telling me that you're doing an adult study and telling me about the kinetics of this stuff in a fetus?" He said, "Actually no, I shouldn't be doing that." I said, "Why aren't you doing a fetal study?" He said, "The lawyers for the plastics industry won't approve the study." So my lab did it. We fed pregnant females radioactive bisphenol A for 5 days at 20 micrograms/kg body weight and spiked it with tritiated bisphenol A, then on the 17th day of pregnancy we gave one feed and collected blood over 24 hours. We saw bioaccumulation - about a 10-fold bioaccumulation due to pre-feeding. In an adult non-pregnant female we get exactly what everyone else gets with this chemical - no bioaccumulation, very rapid clearance and a half life of about 90 minutes. This stuff leaves the adult body very fast. We don't know where this stuff is hanging up in the pregnant female; we're still searching around the fetal organs. But we get bioaccumulation in pregnancy; we do not see that in adults.

We were looking at plasma proteins, which increase dramatically during fetal life and sequester most of the circulating estrogen, blocking it from entering cells. Bisphenol A had a very low binding affinity for these barrier proteins; where you have 0.2% free of estradiol, you have about 10% free of bisphenol A. So these things bypass blood binding proteins. A couple years ago, a new estrogen receptor was located in the prostate - ER beta. It has almost an order of magnitude higher affinity for bisphenol A than ER alpha does. So suddenly there is a unique prostate estrogen receptor that likes to bind bisphenol A.

So you have a very interesting set of events. You have bioaccumulation in females accounting for a ten-fold increase in bioactivity relative to what we expected based upon the human breast cancer cell typical E screen. Then, due to bypassing plasma-binding proteins, you have another 20-fold increase in potency. Together that gives you about a 200-fold increase in potency. The data on ER beta is another 8-fold, so now we are up to about a 2,000-fold increase, and the synergistic activity of increasing androgen receptors, increasing EGF, and that's at least another 10-fold increase in bioactivity. That gets us to about a 20,000-fold increase in bioactivity increase in the fetus relative to initial predictions. None of this is predicted by the *in vitro* assay with human breast cancer cells. None of it would have been predicted by studying the ways the chemicals act in adults. The only way you know is to study it at the very low physiological dose range in the fragile fetus during development.

My conclusion is that testing high doses of endocrine disrupting chemicals may be useful for assessing toxic effects, but it has not (in any assays) predicted receptor mediated response that are inhibited at high doses. We have similar kinds of data for DDT and for methoxychlor. We are finding effects below published acceptable daily intake levels for these chemicals. What we are recommending is to rethink the idea of high dose testing; a linear extrapolation by these constant factors is not going to be predictive of low dose endocrine effects. Recognize that the research focus should be on development; on low doses on babies. The issue is not adult bodies, but babies bodies. We need a totally different kind of endpoint. I am not saying don't study cancer because that is a major part of what I do, but we need to look at other endpoints. You're not

going to see the kinds of endpoints we are seeing with EGF gene regulation and androgen receptor up-regulation, that is, the extra gland branching, on conventional histology. The glands don't look abnormal, there's just a lot more of them. The way we do it is we take these sections and feed them into a computer and reconstruct the entire prostate in 3 dimensions, then do morphometry on it, and we see the entire structure of the prostate is different. If you look at a two-dimensional histological section, it looks pretty good, you don't see what's happening. You have to move to a different level. The other issue that needs to be brought out is these are chemicals for which there is published evidence of endocrine disruption. People like Steve Safe are going to tell you "DDT is going down in the environment, PCBs are going down in the environment, therefore there is no problem with endocrine disruptors." But we're just developing assays for many of the chemicals that are on the list. Until recently, nobody was monitoring bisphenol A or other plastic components, or many other chemicals in the environment. To say that two banned chemicals are going down and that is therefore reflective of our exposure to the wider issue with regard to endocrine disruptors is really misleading.

A book called *Toxic Ignorance* by the Environmental Defense Fund reports that for 75% of the top volume chemicals in commerce with respect to exposure (that is more than one million pounds per year) essentially there are no data. They are declared safe because of the absence of information. I am one of the authors of the National Academy of Science report *Hormonally Active Agents in the Environment*. The executive summary says "you can not take the absence of evidence as evidence for the absence of harm." There is a huge difference - because we know nothing doesn't mean we are safe. There is consensus about this on the NAS panel. That isn't to say we're sure there is harm, but we should be careful to discriminate what we know and what we don't know.

Evidence of endocrine disruptors impact on human health

Stephen Safe, Texas A&M University

Stephen Safe is a Distinguished Professor of Veterinary Physiology & Pharmacology at Texas A&M University. He currently serves as the Director, Center for Environmental and Rural Health at Texas A&M. Research in Dr. Safe's laboratory is focused on environmental chemistry, toxicology, biochemistry and mechanisms of action of polychlorinated biphenyls (PCBs), dibenzo-p- dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds. He is a Senior Scientist, Institute of Occupational and Environmental Medicine, Texas A&M University, and Adjunct Professor, University of Guelph. He holds a M.Sc. from Queen's University and a Ph.D. from Oxford University. Dr. Safe served on the National Academy of Sciences Committee on Hormonally Active Agents in the Environment.

My talk will focus on the impact of endocrine disruptors on human health, why there has been some concern, what the recent data show, and where we are going from here. I will balance as much of this as I can but that may be difficult. I will also try to give you the reasoning supporting my opinions on these issues.

Firstly, the endocrine disruptor problem or concern emerged during the early 1990s - particularly with the work of Theo Colburn, in which she synthesized information from the environment. This information suggested that developmental problems in some regions in the environment are due to contaminants that disrupt the endocrine system of animals.

Supporting that were data showing that there were correlations and perhaps causations for problems not only for environmental effects, but also in laboratory animals. The laboratory animal data for endocrine disrupting chemicals, particularly those that go through the estrogen receptor and the Ah or dioxin receptor, is really strong. The strength of these data comes from the standpoint that many chemicals can disrupt the endocrine system, particularly during early exposure periods, early post-natal or in utero periods. This is coupled with the fact that diethylstilbestrol (DES), a potent estrogen given at fairly high doses, caused a lot of the same effects seen with other estrogen and dioxin-like chemicals during these early exposure periods. DES caused problems not only in laboratory animals but also in humans. The environmental data, the laboratory animal data and the DES data are clearly sufficient to cause people to be concerned about estrogen-like chemicals in the environment. The question is - is there a problem with humans? Are exposures high enough?

I want to go into the problems with humans. The endocrine disruptor hypothesis states that there are chemicals out there that a) are endocrine-like, primarily estrogens, but dioxin-like compounds as well, and b) are at environmental levels that are causing effects. I think we have to be concerned about that; what I want to look at are some of the human data.

The first piece of data that really synthesized and brought all this together was a paper published in 1992 in the British Medical Journal by a Danish group that analyzed sperm counts from fertility clinics worldwide from 1940 to 1990. Their analysis showed about a 50% decrease in the number of sperm - going from about 113 million per milliliter (ml) to 60 million per ml. Now 60 million sounds like a lot and it is, but the real point of their discovery was the 50% decline. They realized there were problems in this.

Sharp and Skakkebaek (in Lancet) hypothesized that there was a worldwide decrease in male reproductive capacity that could be related to exposure to estrogen-like chemicals. They based this hypothesis on the animal data, the DES data, the implications from environmental studies, the sperm count data from that one study and some data on hypospadias, which are defects in males exposed in utero to estrogens. They did not confine their hypothesis to environmental or industrial derived compounds, but included flavones, soy products, and dioxin- like chemicals, which act a little like estrogens during early exposure in utero. That's some background. What that did quite rightly was spur a lot of research. What I want to deal with is the research on sperm counts, which is very interesting.

What happened was the data from these various studies was re-analyzed. Some thought the data was not analyzed properly, others thought it was analyzed wonderfully. Then there was analysis of the reanalysis. The proof in terms of what the data is worth is to look at the new data. Sperm count studies were carried out in many laboratories in many countries. For the most part, these were studies of men who presented themselves at clinics for one problem or another, so this group of people is not representative of a random study of men. There are not many random studies of normal people; these are all people who are self-selected volunteers. The results of these studies were initially quite confusing. Here are two examples.

In some studies, sperm counts over a time period of 15-25 years were down in these clinics; in other studies they were up. That was confusing. It obviously indicated that there could have been a difference between location one and location two. A really good example is a study published in the New England Journal of Medicine, from a group in Paris, clearly showing that sperm counts were down. A second study from Toulouse, France, on a

smaller number of patients, used the same approach. They suspected that in Toulouse, like in Paris, the sperm counts would be down, but they were not. Right away that tells you something. In Paris they are down and in Toulouse they are up, but the overall trend hadn't changed over a 15-25 year period. This kind of variation was found in a number of locations. The study that really got people thinking was one by Harry Fisch and his colleagues at the Columbia-Presbyterian Medical Center. They looked at vasectomy patients in three locations - New York, Minnesota, and California - a nd found that for the period between 1970-1994, there was no change in sperm counts, volume, or motility. The interesting thing was they did find a monster difference with respect to location. Sperm counts were high in New York - about 120-130 million per mil. In Minnesota sperm counts were about 100 million per mil and had not changed. In California they were pitifully low at around 73-74 million per mil. So what they had was huge demographic variability. Since then, researchers in several places around the world have looked at different locations within large countries and within small countries, and have found significant differences in sperm counts depending on location. This has been found in the United States, Canada, Denmark and France.

The Canadian study is interesting because they looked at sperm counts in 11 clinics across the country, and depending on how you put the data together, there was either a small decline or no decline. Over the longest time period there was no decline. While 5 of the centers showed an increase, the other 6 centers showed a decrease. Between centers there were up to three fold differences in sperm counts. Across Canada you went from 120 million in one center to 40 million in another center. The main thing is the differences between centers in Canada were much greater than the decrease reported in the 1992 paper in the British Medical Journal. In another study, David Handelsman at the University of Sydney looked at 5 different groups of volunteers over a period of 3-4 years. These were self-selected people for different sperm count studies. The results within these 5 groups over a short time period shows their sperm count varied from about 60 million to 140 million. He concluded that this highlights the 'invalidity of extrapolating sperm counts of self-selected volunteers to the general male community'. We don't know enough about sperm counts. In clinics, sperm counts are up and down; the biggest variability is not the temporal decrease but the regional differences. Why are there regional differences? I don't know. Should something be done about it? Yes, and a number of groups are studying this. So this should be interesting. Has it been related to any chemicals? Not as far as I know.

Another issue related to decreased male reproductive capacity is fertility. Two studies are important and may tell us more in the future. Firstly, there is a paper by Wilcox published in the New England Journal of Medicine in 1995 that compared offspring of DES treated women with offspring of a control group that received a placebo. They only looked at men and measures of fertility. Specifically, they found no difference between the unexposed versus the exposed with respect to the proportion of partners pregnant at the start of the cycle, and how long it took them to get pregnant. They concluded that there was no effect of DES on fertility. This doesn't mean that DES doesn't do anything, because it does. But it did not effect fertility. A Finnish study published in late 1999 looked at reproductive effects of in utero exposure to estrogens. In Finland, DES was not used, but estrogen alone or estrogen plus progesterone was used for treatment of problems in women during pregnancy. Women did not get trace levels of estrogen, but rather pharmacological doses of estrogens during pregnancy. The study looked at the offspring of these women. There were minimal effects, but

overall effects on males and females from mothers exposed to pharmacologic doses of estrogens during pregnancy showed no effects on fertility. That is important.

This is estrogen exposure of humans during the critical exposure period where at least animal models and environmental models show marked effects. With DES we get marked effects. This is a hallmark of the endocrine disruptor hypothesis, a foundation. The Finnish study shows that it might not just be estrogens that are causing the problem, but perhaps some unusual properties of DES combined with estrogenic activity. We don't seem to have the marked effects of DES in the offspring of women exposed to estrogens alone. That is important. I don't know all the details of the study, but I can't think the effects are marked or they would have been reported and obvious like DES. It may be a good group because it may distinguish DES for whatever reason from estradiol.

Two other things are worth noting in terms of male reproductive capacity. The initial hypothesis indicated possible increases in hypospadias and cryptorchidism, which are problems in male offspring and infants. There were some indications that these problems were increasing. A recent study by Paulozzi published in Environmental Health Perspectives (1999) indicated there were some increases prior to 1985 in both hypospadias and cryptorchidism. However, since 1985, there were no increases - one actually decreased. The important thing is that in different countries the rates or incidence of hypospadias were very different. Like sperm counts, there are demographic differences. The so-called increase for both of these defects appears not to be real.

The other thing I would like to mention on male reproduction is a study by Ekbom published in Lancet in 1996 that reported a big difference in testicular cancer rates between Denmark and Finland. There's a three-fold difference in incidence. When DDE was first discovered to be an antiandrogen, it was hypothesized that it might be responsible for or associated with testicular cancer. What Ekbom showed is that DDE levels have gone down about 90-95% between 1965-1995 in all Scandinavian countries. There are no differences in breast milk levels of DDE between the countries. This would not explain it. The levels of exposure in each country are similar and have been decreasing, and would not account for the differences. Is a chemical responsible for testicular cancer? Why is the incidence going up? I do not think we know those answers. Could it be chemicals? It could be but we don't know what those chemicals are. This is an issue the Scandinavians are addressing. Of all the effects of male reproductive problems, testicular cancer is the one of most concern. What is needed are new hypotheses that include diet, occupation, genetics, and chemical exposures in order to tease out the factors that may be responsible for the increase in testicular cancer almost worldwide, and the big differences between countries.

Briefly, a second issue is breast cancer in women. I have NIH-funded research looking at estrogen action and the molecular biology of estrogen action. I'm also looking at dioxin as an antiestrogen. Dioxin interacts with the Ah receptor; many laboratories have shown cross talk between the Ah receptor and the estrogen receptor signaling pathways. There is an antiestrogenic effect. In addition, in some places like Seveso, Italy, where there has been high exposure to dioxin-like compounds, there's actually been a decrease in breast cancer.

In 1993, a couple papers came out. One said that DDE levels were higher in breast cancer patients than in controls, another paper showed that PCB levels were higher in breast cancer patients than in controls. That paper hypothesized that these chemicals may be associated with the development of breast

cancer. I took exception to that because in high worker exposures to PCBs, there is no relationship to breast cancer. PCBs in animal models and in mixtures we are most exposed to in fact protect against mammary cancer in rodents. The link between DDE and breast cancer is just not there; papers show it both enhancing and protecting. The hypothesis was not based on good biological data. Subsequent studies from around the world have not shown that DDE or PCB is higher in breast cancer patients versus controls. Why they were in the early studies I don't know. The same analytical laboratories participated in many of the later studies and it doesn't appear to be holding up. I don't think it's biologically plausible. A lot of recent studies are teasing out individual PCB congeners and individual compounds. Some studies are showing higher levels of individual compounds in breast cancer patients versus controls. If you look at it carefully and compare between studies, you see that in some studies the levels are higher, in other studies the levels are lower than controls. Do PCBs or pesticides play a role or not? My bias is to say probably not, but obviously more work needs to be done. They may play a role in association with genetic polymorphisms of some genes. A lot of people are working on this and maybe something will come out of it. But the evidence doesn't support the early hypothesis.

The other thing to be aware of is that we are exposed to trace levels of environmental estrogens, in terms of intake. In terms of potency, it depends on how potency is classified. Just because some environmental estrogens bind to the estrogen receptor and turn on a reporter gene does not mean they are all the same. You heard Dr. vom Saal that bisphenol A seems to be a little different. I agree with that. That is consistent with the way the estrogen receptor works - when a compound binds it changes the shape of the estrogen receptor. In a specific cell context or tissue context, one estrogen can be different from another estrogen. Tamoxifen, which is used to treat breast cancer, blocks estrogen action in the breast, but in the uterus it acts like an estrogen. Not surprisingly, a lot of the xenoestrogens act like that, even though they bind to the estrogen receptor, they are different. We don't know enough about them; we need to know more. I could globally say that basically they are usually weakly estrogenic, with few exceptions. If we look at our diet, we see that we have a huge dietary load of all sorts of estrogenic compounds. This is increasing almost monthly with new chemicals in foods, particularly phytochemicals are estrogenic or antiestrogenic. There is a big load of estrogens, not only in our diet but natural estrogen levels in the blood of individuals are quite high. The only other thing measured in blood in terms of foreign estrogen or industrial estrogens are the organochlorines. These are relatively low compared to natural estrogens in a soy fed infant. How you equate that I don't know. I think to make a real scientific evaluation of exposure to endocrine active chemicals, industrial chemicals and natural chemicals must be included. We have got to find out how much we are absorbing. People will argue quite rightly that the organochlorines that we take in are not rapidly metabolized and are persistent, even though levels are low. That has to be taken into account. But we now know that natural estrogens can also be detected in serum in relatively high levels. Although they are turned over quickly, the levels we take in are large and we take them in at one or two meals per day, so the levels can remain high. We don't understand what that means. More work needs to be done in that area to really evaluate natural estrogens and antiestrogens and other endocrine active chemicals in the diet verses trace levels of some other chemicals. In addition, some of the chemicals of concern - the phenolics that are estrogenic and in some studies show some real adverse effects - we have to find out how much is in our serum. Are they of concern? Will in utero exposure occur? A lot of work needs to be done on that.

To summarize, results of most recent studies suggest that sperm counts, hypospadias, cryptorchidism and fertility are highly variable in regions and it is unknown whether they have gone down or not. With hypospadias and cryptorchidism it doesn't look like there has been a recent increase. In addition, the correlation of DDE and PCBs being high in breast cancer patients versus controls does not seem to be the case in most recent studies. If we want to look at endocrine effects we need to take into account all endocrine active chemicals - both synthetic and natural.

U.S. Food and drug administration's statutory framework and the evaluation of pharmaceuticals for potential environmental impact

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I'd like to start out by giving you a little bit of an overview of the Food and Drug Administration (FDA). The FDA Center for Drug Evaluation and Research (CDER) regulates human drugs. The Center for Biologics Evaluation and Research regulates blood products, vaccines and biotech products. Other centers within FDA include the Center for Veterinary Medicine, the Center for Food Safety and Nutrition, the Center for Devices and Radiological Health and the National Center for Toxicological Research. The FDA has inspectors throughout the U.S. that conduct fieldwork, including inspections of manufacturing facilities and food imports. You can see that FDA is a very diverse agency. FDA's main focus is regulation of products in interstate commerce rather than being a science-based agency; we do research, but it is not our main focus. CDER employs almost 1600 people from various disciplines, including medical doctors, toxicologists, pharmacologists, epidemiologists, chemists and statisticians. When a pharmaceutical company develops a new drug, that company conducts a series of studies according to our requirements and submits the results to us. We peer review the studies to determine if they meet standards, regulations and statutory requirements before the drug can be marketed in the U.S.

CDER deals with a lot of laws, regulations and statutes. There are two regulations related to endocrine disruptors, these being the Federal Food, Drug & Cosmetic Act (FFDCA) and the National Environmental Policy Act (NEPA). FFDCA is a congressional statute that guides FDA in its operation. NEPA is a very general and broad statute that applies to all federal agencies. FFDCA requires FDA to approve a drug if there are no grounds for denying approval. One reason for denying approval in humans is a lack of substantial evidence that the drug will have the effect it claims to have. For example, a drug may claim to lower blood pressure, but the data do not support that claim. Another reason to deny

approval is that there is insufficient information to show that the drug is safe for use under the conditions included in the labeling.

There are eight reasons to deny approval for a new chemical coming on the market; the same number applies to generic equivalents. The statutes are concerned with safe and effective use of a product in humans, none relate to environmental issues. Utilizing these statutes, the mission of CDER is to ensure that safe and effective drugs are available to the American public. The effective part of the analysis is that drugs are tested in humans in limited quantities to show that drugs do what they claim to do.

Previous speakers have raised the question of whether animal models predict impacts on humans. In reality, FDA frequently uses animal information to approve drugs because they don't conduct tests in humans for carcinogenicity. The government certainly does conduct epidemiology studies to see if exposure to certain substances may be associated with risks for cancer.

Drug companies must submit extensive information to CDER regarding the safety of a drug; the studies they conduct are usually non-clinical. Pharmacokinetic studies are done *in vitro, in vivo* and in humans. Other studies investigate absorption after a single dose, absorption after repeated doses, cumulative excretion, excretion into bile, organ distribution, plasma protein binding, studies in pregnant or nursing animals, metabolism *in vitro* and *in vivo*, possible metabolic pathways, induction and inhibition of drug metabolizing enzymes, and drug-drug interactions. Drug-drug interactions are very important in geriatric populations, as elderly people often take handfuls of drugs to combat arthritis, high blood pressure, high cholesterol and other health problems. Are these drugs interacting in the body to cause any effects? Single-dose and repeat-dose toxicity studies, as well as genotoxicity, carcinogenicity and reproductive toxicity studies are all performed. The reproductive toxicity studies look at everything from fertility and early embryonic development to implantation studies, effects on embryo-fetal development, and effects on pre-natal and post-natal development including maternal function, and local tolerance studies.

An international agreement exists between the U.S., the European Union and Japan on the types of studies for supporting drug product applications, including information on safety issues for direct use by humans and indirect consumption through incidental exposure. These are conservative tests that are multi-dose and multi-species; the conservative view is always taken when making a judgement about use in humans.

NEPA requires federal agencies to assess the environmental impacts of their "major" actions. It allows an agency to say "yes" or "no" to something happening. This is the same Act that requires the government to evaluate the environmental impacts of constructing a building or a highway. Under NEPA, FDA evaluates the environmental impacts of drug products; approving a drug is considered a major federal action. NEPA is a decision-making tool that is intended to help officials make decisions based on understanding potential environmental consequences in order to take actions that protect, restore and enhance the environment. The Act does not require an agency to take the most environmentally beneficial course of action, it just requires the agency to evaluate it. With respect to the introduction of a new chemical, often the most environmentally beneficial action is to not approve the chemical. However, public health is not served by not approving new pharmaceuticals.

FDA is required to operate within the statutory limits of the FFDCA and NEPA regulations. However, if FFDCA and NEPA conflict, NEPA gives way; this was decided in the courts. FDA evaluates environmental impacts, but must follow its mission statement, which is to ensure safe and available drugs for the American public. While this dynamic is quite different from the drinking water realities, many of the issues are the same. We deal with consumer advocate groups, industry and Congress. You might expect any given group is generally on the same side of the issues, but they change. For example, sometimes consumer advocate groups say we regulate too little, sometimes they say we regulate too much. Industry tends to say that we regulate too much. Congress changes from day to day.

FDA's decisions are generally challenged, either by Congress or through the courts. For example, dietary supplements and tobacco have been in the news recently. Dietary supplements are not regulated as drugs, as Congress passed an act stating that they are considered food, not drugs. With respect to tobacco, FDA asserted they had the right to regulate tobacco products. However, the Supreme Court ruled that FDA does not have that authority. While FDA works within the statutory framework, what we ultimately can or can't do is frequently decided by outside forces.

The Council on Environmental Quality issued regulations for implementing NEPA. There are three documents prepared under the NEPA process: categorical exclusions, environmental assessments (EA) and environmental impact statements (EIS). The goal is to help federal agencies make the most informed environmental decisions by making us aware of the potential impacts. The overall purpose is to reduce paperwork and reduce delay, not to be a barrier to decision making. The process promotes sculpting and tiering to try to get to the answer faster. A categorical exclusion is a decision made by the agency on a category of actions that normally do not have any environmental impact. There is usually no EA or EIS prepared if there is a categorical exclusion. However, there are always exceptions. If no environmental impacts are expected, FDA is not going to require any environmental documentation. The exception is the extraordinary circumstances provision within NEPA regulations.

FDA requires an EA for any specific action that is categorically excluded if information indicates the action may significantly affect the quality of the environment. NEPA regulations provide a list of extraordinary circumstance provisions that should be considered. If FDA's approval of a drug would cause a violation of an environmental law, we would not be able to categorically exclude that action. An example would be approving something that might affect a national historic site. While that is more for the construction of buildings and highways, it is also part of our consideration. Within FDA regulations, several categorical exclusions are specified. For example, if knowledge or data exists to support the position that a chemical is expected to adversely affect the environment, an EA would be requested.

Another categorical exclusion under our regulations is investigation and approval of new drug applications. New products are usually produced in low volumes and very few people will be taking them; these have always been and will continue to be categorically excluded. Abbreviated new drug applications, for generic drugs or duplicate drugs, also fall under the categorical exclusion. These occur when the approval will not increase the use of the drug or when the concentration of drug expected to enter the aquatic environment is 1 part per billion (ppb) or less. Next on FDA's environmental impact assessment chain is the

EA. This is a concise document that provides information to determine whether we go further and prepare an EIS, or whether we write a finding of no significant impact (FONSI). This basically ends the evaluation for that particular action.

Actions normally requiring an EA include situations when concentrations expected in the environment are over 1 ppb, or when wild plants or animals are involved. This occurs under the extraordinary circumstance provision, as these products are often low volume products, but a lot of trees may be cut down in the process. EAs are not required if they are using plantation or cultivated materials, but if wild plants or animals are involved, an EA must be prepared. Cutting down trees or harvesting the rainforest causes much more direct environmental impacts than other types of actions.

There are no FDA actions that routinely require the preparation of an EIS. The only EIS we have prepared related to human drugs was in 1978 with chlorofluorocarbons (CFCs). The topics covered in this workshop remind me of the CFC issues and battles being fought about whether or not CFCs affect the ozone layer. FDA prepared the EIS on CFCs in collaboration with a number of federal agencies, based on information from the National Academy of Science and the National Science Foundation. Under the EIS, the government said that all products would be considered adulterated, which basically means they can't be marketed. There was a phaseout period on products containing CFCs, except if they were medically necessary. There are some drugs that have CFCs in them. We are supposed to go through a phaseout period under the Montreal Protocol, which calls for the elimination of CFCs. It is not an easy task to remove CFCs from drug products and replace them with something else. A number of asthma drugs and inhalers contain CFCs; there is drug development work going on for replacements or substitutes. Obviously, it is not a simple issue.

A couple of years ago, FDA developed a plan on how to withdraw products from the market that use CFCs, once alternatives were available. Products would not be taken off the market if there were no alternatives or only one alternative. After publishing this plan, we received about 10,000 comments from consumers asking us to not take away asthma drugs; we also had to deal with Congress on that. The public's perception was that we were going to take drugs away, and the resulting reaction was huge. Another interesting example was an EIS that was prepared by the U.S. Forestry Service, because the drug being approved was made from trees that were being harvested from a national forest. Generally, it is very rare that an EIS is filed; normally an EA is done and that is the end of the story.

Let's re-visit the 1 ppb in the aquatic environment clause. In April 1995, the President announced the Reinventing Government Initiatives. Part of this involved increasing the number of categorical exclusions from the EA and EAS requirements. FDA was told to go back and review what we were doing. When we started doing environmental assessment work, we didn't know what to look for or what to expect. After years of gathering data, we realized we were getting a lot of information that we didn't need, and we were not getting some information that we did need. In 1997, FDA published a final rule revising the regulations. The categorical exclusion of 1 ppb in the aquatic environment was added as part of that rule. The retrospective review of the data supported this conclusion.

The acute toxicity studies used EPA and OECD methods. On review, we found no data under 1 ppb in those studies. We didn't

statistically analyze the data because it was in many different formats. You have no observed effects, EC50's, and greater thans because of limits of solubility. There weren't definitive numbers in every case. Over 90% of data we reviewed had values above 1 part per million (ppm); about 10% were between 1 ppb and 1 ppm. So at the time, we didn't have any data below 1 ppb. Since that time, we did find one compound that was in the low parts per trillion, which wasn't a surprise because it was an anti-parasitic.

When looking at the historical data on doses between 1 ppb and 1 ppm, everyone thought cancer drugs would be at the lowest levels. We were surprised to find that one third were a mix of compounds, one third were antibiotics, and one third were anti-depressants and other central nervous system drugs. The ones that showed effects at the lowest levels tended to be anti-depressants. A lot of the central nervous system drugs have special characteristics that allow them to get into cells quicker. They cross the blood-brain barrier so maybe that was the rate-limiting step in this type of process.

There is a lot of talk about aquatic versus terrestrial pharmaceuticals. There are some pharmaceuticals that might end up in the terrestrial environment, but we set our regulations so that the aquatic environments are more sensitive. We assume a drug will get into the aquatic environment, so we steer testing in that direction because we think we will get more conservative results. The data have demonstrated that aquatic organisms are more sensitive than terrestrial organisms by 1-2 orders of magnitude. Many times, if the drug is getting into the environment through the wastewater treatment process, it adsorbs to the sludge. It can actually adsorb quite tightly, so it might be less available. Drugs tend to be very soluble. That is why we steer testing to the aquatic environment and estimate the expected environmental concentrations in the aquatic environment. An estimate of the concentration of a drug being released into the environment is calculated by dividing the amount of water going through the wastewater treatment facilities by the estimated U.S. sales figure for the drug. This estimate assumes there is no removal of the drug during the wastewater treatment process, so all the product will be going into the environment.

Toxicity data we review includes fate and effects information from the EAs. We also gather data on physical/chemical characterization including water solubility, dissociation constant, octanol water partition coefficient, vapor pressure, and absorption and desorption properties. We collect depletion mechanism information such as photolysis, hydrolysis, and biodegradation from EPA and OECD standard tests.

Again, we use a tiered approach and steer testing towards aquatic organisms because we feel we will get the more conservative number out of that. First, we decide where the drug is going to go; normally it is aquatic. Is there a rapid removal mechanism? If it hydrolyzes very quickly, and isn't expected to enter the environment, there is no need to do toxicity-type organism testing. If the drug is expected to bioaccumulate, you might have to go to chronic studies. In general, this is not a concern for human drugs, as they are mostly very soluble and not lipophilic. If a drug bioaccumulates, it wouldn't be approved for human use, as humans are the first biological filter in this process. However, there are always exceptions. For example, pesticides used to control lice may have bioaccumulation potential but the benefits outweigh the risks. There is a chart outlining the environmental assessment guide on the FDA webpage if you'd like to see more details.

I want to briefly talk about the National Center for Toxicological Research (NCTR), which is part of FDA. NCTR's mission is to conduct scientific research to aid future regulatory needs and actions. This involves fundamental and applied research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by FDA. This research is aimed at understanding critical biological events in the expression of toxicity, and developing methods to improve assessment of human exposure, susceptibility and risk. Quite a bit of work is done at NCTR on endocrine issues, many of which are focused on issues under the Food Quality Protection Act, or how FDA evaluates toxicity for human drugs. NCTR does a lot of work to support FDA in making the best decisions in approving safe and effective drugs. They also have a component doing basic research in the endocrine area, including working on the low dose hypothesis and developing screening methods. A lot of that information is expected to come out fairly soon.

I want to bring up the point that not all pharmaceuticals are endocrine disruptors, and not all endocrine disruptors are pharmaceuticals. We have analyzed drugs using standard toxicological approaches from the environmental side because that is what is available. Our main mission is to approve drugs. We follow the lead of agencies like EPA with respect to environmental work because that is where the expertise lies. We use the tiering scheme because it is based on work done by EPA. There may be evidence that suggests this may no longer be the right model, and FDA is looking into this. We are concerned about endocrine disruption from the human standpoint, so we are developing models and information to address the issue mainly from the human impact side.

European research on endocrine disruptors in the aquatic environment

Thomas Hutchinson, Astra-Zeneca, Brixham, United Kingdom

Tom Hutchinson is an ecotoxicologist with Astra-Zeneca in the United Kingdom. He has over 12 years laboratory and field experience in the ecological risk assessment of chemical products and process effluents. He has extensive experience in several areas of ecotoxicology, especially aquatic hazard assessment, endocrine disruption, genetic toxicology, immunotoxicology and fish disease. In addition to his laboratory based research, he has also conducted field work in Bermuda, Germany, the Netherlands and the North Sea. Dr. Hutchinson is an active member within several international ecotoxicology working groups, including European Chemical Industry Council -Endocrine Modulators Study Group (CEFIC-EMSG), Chemical Manufacturers Association (CMA), and the Organisation for Economic Cooperation and Development (OECD).

I was asked to talk about the broader aquatic environment. Activities very relevant to wastewater are going on in Europe, where this issue has developed exponentially over the last 10 years. I'm going to try and fill in a few more pieces of the puzzle, and raise a few issues that are with us today or may be coming up in the near future. Astra-Zeneca was formed last year by the amalgamation of Swedish and UK-based pharmaceutical companies. I work at the corporate environmental health laboratory for Astra-Zeneca. We also do work for government agencies and industry; that collaborative work is what I am going to present.

There are many definitions of "endocrine disruptor". The definition industry subscribed to at an international meeting in the UK in

December 1996, along with the Organisation for Economic Cooperation and Development (OECD), the European Commission, and the US Agency for International Development (USAID), focuses on "adverse effects in intact organisms or their offspring subsequent to changes in endocrine function". The subtext around this definition is to look at effects on whole animals and to consider reproduction and developmental effects in critical life stages, as well as behavioral effects. An example of a behavioral effect might be the ability of laboratory or wildlife animals to breed. All of these concepts are in the definition. This is a definition that industry and others can work with for the implementation of new tests at the international and regional levels in dealing with this important question.

Most of the work is based on *in vivo* approaches. Earlier in the workshop, someone said that we have always worked from the end to the beginning for research on ecological risk assessment, particularly for pesticides. This was done using multigenerational studies. Now we have been asked to think more about critical life stages and about trying to predict from windows of *in vivo* exposure possible long-term effects. That has been supported more in considering wastewater contributions to endocrine disruption in the aquatic environment by the use of *in vitro* or test tube techniques.

I'd like to talk briefly about what is happening in Europe with freshwater fish populations. We cannot think of protection of the aquatic environment purely in terms of one or two species; we have to think about the complete food chain. That is enshrined in effluent testing, in terms of whole effluent testing or wet testing, using algae, fish and invertebrates. For pharmaceuticals and other chemicals, a tiered approach is used. There is some interesting data in Europe where impacts of endocrine disruptors led to the extinction of certain invertebrate populations; that is a critical case study that has moved this debate forward, particularly in the eyes of the environmental groups.

I want to talk about some of the work going on in our laboratory on complex mixtures and suspected endocrine disruptors. I'm the chairman of the OECD fish expert group that is studying endocrine disruptors. This group is trying to get international harmonization for fish testing methods for a range of substances that are relevant not only to products, but also regionally to effluent discharges. I'd like to give you an update on what is happening with test methods.

We do a lot of work in marine risk assessment and marine science at our lab, which is 300 miles west of London in a coastal location close to Plymouth. The highest frequencies of intersex in a particular species of carp are found in the industrialized areas of Northern England. The biggest city on the River Aire is Leeds, with a population of 8 million people. The textile industry is a very strong part of Leeds' heritage, and a lot of the inputs to the river are related to that industry. The River Trente is another major river with a very high frequency of intersex fish. Smaller rivers leading into the north of London also show a high frequency of intersex fish. Even some small rivers such as the Arun, which drains agricultural areas through the south of England, have up to 82% of the fish intersexed.

Many sites were looked at for these studies. Having a control site in the environment is not always a good idea because you can never control everything. The reference site used was in the Irish Republic, where they found an intersex frequency of 4%. It was very surprising to find so many fish populations in Britain that had very high frequency of intersex fish. The impacts on the fish populations are still being studied. This is complicated by the fact that there are re-stocking programs in the fish populations, so it is difficult to determine if the fish are breeding and if the population is viable

if left on its own. Maybe in the future they will step back from the management and input of new fish stocks and do some case studies to see if the population is viable.

Another complicating factor is that for many fish species, the actual trigger for the sex of the animal is not known. We know that genes and chromosomes control sex determination in humans and in mammals. In fish it is probably genetic, but may be overridden by the environment. It is not known whether intersex fish are males that have been feminized or if they are females that have gotten some masculination. There are still some basic fishery questions that need to be analyzed. The weight of evidence is that this an abnormal situation and we need to find the causes.

In the intermediate future, we will see tests with biochemical endpoints being used in monitoring effluent discharges from both municipal and industry sources. There are already programs in Europe where pharmaceutical effluents have been monitored using fish assays looking for endocrine specific endpoints. For an adult female fish in normal breeding season (when day-length and water temperature increases) an environmental signal occurs through the neuroendocrine axis which stimulates the ovary to produce estrogen or estradiol. This is shipped via the blood to the liver. In the normal course of things, the female's vitellogenin (VTG) is shipped via the blood to the ovary.

When research on fish began in the 1980s and 1990s, we saw intersex fish in the London area very close to factories that make contraceptive pills. These factories were discharging into surface waters. Researchers found that male fish caged in these areas were producing high levels of VTG; this is something that clearly shouldn't have been the case. We now use assays as detective tools to understand where there are spatial and temporal trends in terms of estrogen in the environment. There is no other explanation as to what causes induction of VTG in male fish or in very young. The estrogen hypothesis is certainly the obvious one to study. To an extent, the more we look the more we see. In Sweden, Germany, Belgium, and the Netherlands, we are seeing induction of VTG in fish caged downstream of sewage effluent plants as well as in laboratory studies.

The issue is not limited to invertebrates. There are three key examples where chemicals of concern for humans were initiated by observations in wildlife. A classic example of field biology raising a flag was the 1950s UK work on birds of prey affected by organochlorine pesticides. Another example is where marine snails have been affected by the biocide tributylin. The female mollusks grow a penis or other male genitalia that block the egg canal, making it impossible for the animals to breed. There was a complete collapse of snail populations in many areas of coastal Europe (and elsewhere) in the 1970s and 1980s. Several years ago, a ban was imposed on boats less than 25 meters in length prohibiting use of tributylin on hulls. That improved the situation dramatically; there is now an agreed phase out (supported by industry) to stop the use of tributylin entirely. Work in Germany in the last 3 - 4 years has shown that the mechanism by which these snails are masculanized is probably due to one of the key enzymes that produce steroid hormones. This enzyme is also very important in human metabolism; it is a major target enzyme for helping to treat breast cancer in women. Hermaphroditic marine shrimp have been found near sewage outfalls in Scotland. This is a very unusual phenomenon that has not been explained. Evidence in other parts of the world shows unusual patterns of sex ratio in wildlife. If you are interested in an overview of what has been happening with wildlife, a recently published article reviews the weight of evidence for impacts of endocrine disruptors, covering fish, invertebrates and the whole situation in Europe from seals to polar bears, etc.

(Editors note Citation: Vos JG, Dybing E, Greim HA, Ladefoged O, Lambr D, Tarazona JV, Brandt I, and Vethaak AD. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Critical Reviews in Toxicology 30(1): 71-133, 2000.)

In the months and years ahead, regulators likely will be issuing lists of substances called "endocrine disruptors" rather than "estrogens". The compounds initially on the scene were ones that interact with the estrogen receptor or the androgen receptor. Examples are bisphenol A, diethylstilbesterol, the contraceptive pill, ethinyl estradiol and, more recently, nonylphenol, all of which are able to bind to the estrogen receptor. Other studies show some pesticides (like DDT) or fungicides (like vinclozolin) can bind to the androgen receptor and produce adverse effects in laboratory animals. Other drugs, like the biocide TBT, don't affect hormone receptors, but they affect the enzyme systems that lead to the production of endogenous hormones. This is a very important part of the scientific landscape where we will see more research and more tests being developed.

I would like to do a plug for the pharmaceutical industry. In trying to understand the risk of endocrine disruptors to wildlife and to understand contaminant effects, we must have a good grasp of the basic processes affected by well-understood compounds. We have mentioned aromatase, an enzyme that controls the synthesis of natural estrogen from natural testosterone. A very important breast cancer drug called fadrozole (produced by Novartis) is also used to understand how to control the production of coho salmon for aquaculture. There is a large database of the effects of fadrozole in fish. Novartis has donated fadrozole to environmental scientists in the chemical industry to develop new tests that we hope will be part of the international battery of testing.

Astra-Zeneca has donated anti-estrogens, which work by blocking the estrogen receptor. Tamoxifen is a partial blocker. A new compound that doesn't yet have a name, ZM189154, is a pure estrogen receptor antagonist. We are putting this through assays with fish and invertebrates so we have an understanding of the baseline before we move on to environmental contaminants. Some drugs work via endocrine mechanisms; that is how they do their jobs. Not all drugs are endocrine disruptors and not all endocrine disruptors are drugs. There is an important scientific cross-talk in our understanding of these two issues at the moment.

I'd like to review work going back to 1994 on potential endocrine disruptors. A UK group published a key paper suggesting that steroidal estrogens, including synthetic estrogens and alkylphenols, were probably the causes of feminization of fish in UK rivers. To be frank, our group in Brixham didn't feel that was the complete story. We felt this was jumping to synthetic substances before the question of natural substances had been addressed. We know that many microorganisms can produce very powerful biologically active compounds, an example is penicillin. It was important to look at natural hormones along side synthetic ones, so we started a project in 1995 to do just that. You've heard about the caged studies with VTG induction in trout. A key paper is Clair Desbrow's work, in collaboration with UK groups, suggesting that levels of natural and synthetic steroids, estrones, estradiol and ethinyl estradiol, were at high enough concentrations in UK effluent to explain the feminization responses. More recent wo rk has verified UK observations that there are natural and synthetic steroids in domestic sewage effluence. In Germany, they have reported the compound mestranol, which is closely related to ethinyl estradiol, in sewage effluent discharges. That has not yet been reported in the UK. More data are coming from the Netherlands showing the

same basic pattern - the more you look the more you see, and steroids are there.

I want to talk about some of the broad scientific principles for the aquatic environment, and I will link this back to whole effluent testing of municipal effluents. When you take concentrations in the water for the effects across various species and endpoints for nonylphenol using algae, invertebrates and fish, the effects on invertebrates are most sensitive. Test tube assays for many compounds show results at very low concentrations that are not very predictive of a whole animal response. There are cases, due to metabolism and the way the chemical presents itself to the whole animal, where test tube assays can underestimate the potential risks to animals and to the environment. It is for these reasons that the international scientific community is not putting any effort into test tube or *in vitro* screening assays at this point in time. The current priorities for OECD are to get whole animal bioassays with rats, fish, shrimp, or frogs.

The River Aire, in the north of England, has a range of nonylphenol concentrations in surface waters. It is quite plausible that nonylphenol alone could be causing the induction of VTG in caged fish; this is another key piece of the puzzle. The actual levels (due to voluntary agreements from industry working with the water utility) show that the nonylphenol levels have gone down. There is still the question of what is in the sediments. Caged fish from surface water are now not showing feminization, but we still have the intersex populations.

Coming back to natural hormones and the work that has been done in our laboratory, we have been interested to see what effect concentrations of natural hormones have on fish in our laboratory, where we use VTG assays on male fathead minnows. A biochemical change like VTG alone is not a signal of an adverse effect, but it is a flag that shows there has been an exposure to estrogens. What does it mean? Even after 21 days of exposure to these levels of estradiol or estrone, there were significant inductions of VTG and significant increases in the relative weight of the testes.

An important fact that we have learned from other life-cycle studies with steroids in fish, is that when we get a 100 or a 1000-fold increase of VTG in blood, it becomes almost like syrup. There is so much glycoprotein in the blood of these fish that it is very difficult to bleed them. In male fish there is nowhere for the egg-yolk protein to go. The kidneys are trying to clear it and are failing as a result. So the fish change color and become stunted; they become very sick. There is a toxic effect associated with high levels of VTG of the order of 100 to 1000-fold above baseline. These are from a limited number of studies; more work needs to be done. The key point is that VTG is responding to environmentally relevant levels of these compounds; the testes of exposed fish are smaller. We began this work about 4 or 5 years ago, when we did not have the analytical techniques to apply to wastewater to look at parts per trillion (ppt) concentrations of free and conjugated steroids. We used the bioassay approach. Work published by Grace Panter used VTG to study estradiol exposure, in which a control of freshwater was used then adult male fish were exposed to estradiol for three weeks at 320 ppt. This was done before anybody had reported the actual levels in effluents. We thought it was ppt levels. At this level there was a marked induction of VTG. We also exposed the fish to equimolar concentrations of the glucorinide conjugates. We did not see *in vivo* any induction of VTG in these fish.

In addition to aquarium systems in our laboratory, we also have simulated wastewater treatment plants systems, where we can look at the impacts of biodegradation on metabolites. We can then test those metabolites for their environmental effects. This simulated

wastewater plant has both influent and effluent. We spiked the influent with the same concentration of estradiol glucorinide, that influent was not estrogenic to the fish. The effluent however, was estrogenic, suggesting that wastewater systems were actually deconjugating the normal metabolites from humans back into the free form. When you look at clinical microbiology, this is very plausible. It is known for an enterohepatic recirculation of hormone for women on the contraceptive pill. If women are taking antibiotics, this suppresses the gut microflora. That means there has not been free steroid released in the gut, absorbed back over the intestinal wall, and back into circulation to maintain the therapeutic dose. The medical literature supports this. The deconjugation in wastewater plants is part of the issue that we have to deal with. We are working right now with support from the water and chemical industries to develop the analytical techniques and go back and repeat these studies in a more extended form with the support of specific analyses of key steroids.

We began mixture work after a paper was published by Arnold et al. (1996), which said that 1+1=1000 *in vitro* in a test tube assay. Clearly mixtures are absolutely essential to the understanding of this issue in the real world. We began a program co-funded with the UK environment agency using the fish assay as one of our tools for looking at responses of animals and fish to chemicals exposed by a natural route. We used juvenile rainbow trout. We are very interested with effects on trout and salmon that could be migrating through estuaries where there are discharges from both factories and municipal plants.

We used the same principle of VTG. We asked if nonylphenol and estradiol together have an interactive effect, and if so, what would that effect be? The binary mixture of estradiol and nonylphenol was found to be additive, not synergistic, *in vivo.* We have basically shown that for the given concentrations of estrogen, there is an interactive affect. The affect *in vivo* appears to be additive at environmentally relevant concentrations. There are not really any surprises with that; what is surprising is that we see antagonistic effects with nonylphenol and methoxychlor, and estradiol and methoxychlor. I have not yet seen any evidence of synergism in fish exposed to endocrine disruptors. Obviously, there is a lot more work to be done. This year we began a study on binary mixtures of estrone, estradiol and ethinyl estradiol and how those steroids interact. The question is "how much is permissible in the aquatic environment to protect aquatic life?"

We have discussed short-term data generated over 21 days. The fish expert group of OECD met in Tokyo in March 2000. The Sumitomo Corporation published some data where they used all these compounds as reference compounds (para-nonylphenol, bisphenol A,17b-estradiol17a-ethynyl estradiol, diethylstilbestrol, methyltestosterone) to help develop screening tests for endocrine disruption. They show the standard short-term lethality tests. They then exposed another species of fish to these substances for up to 56 days and looked at changes in development and reproduction, and recorded the concentrations where they saw statistically significant effects. The point is that within the whole family of endocrine disruptors that are under debate, some of the ratio of short-term or long-term are within the standard way of thinking for toxic effects of chemicals. These ratios are scientifically very important. The idea that you could take the value for ethinyl estradiol, the contraceptive pill ingredient, and divide it by 100, and have the safe effect for fish is clearly not there. We have to do a lot more work. For these classes of substances, steroids and androgens for example, we need to look again at the way we predict chronic effects from short-term data sets.

We are also looking at effects on invertebrates as a key part of our safety assessment for chemicals. An unpublished dataset took the hormone receptor that controls molting and development in insects and crustaceans and used a test tube assay that looked for how chemicals will interact with the receptor. The mammalian estrogens (like estradiol) and their analogs (like DES) do not interact with these insect receptors. However, there is very weak activity from compounds like bisphenol A, diethylphthalate and lindane. We have no structural activity relationships as yet to understand this; we are doing a lot more work to try and understand what could be the cause of this pattern.

To conclude, in the UK and other European countries, there is a high level concern over pharmaceuticals and endocrine disruptors in the environment. The two are distinct but overlapping in the eyes of regulators and the public. In terms of fish populations, is it the case that the more we look, the more we see? There is a lot more work going on to find out what the baseline and the extent of intersex is in European fish populations. The idea of 100% of intersex fish is not considered to be normal. The bottom line is we need to get a much closer link between laboratory studies and field studies. We need continued and increased collaboration between government, academia, the water industry, and the water utilities. My colleagues have all been feeding into this work. The co-funding came from several companies: Astra-Zeneca, Avecia, ICI, The European Chemical Industry, and the UK government.

Friday April 21, 2000

Session 6: Regulatory Activities and Risk Perception/Communication

Risk perception, communication, and regulatory reality

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I'm going to try to give you a little perspective on how the endocrine disruptor issue is playing across America to the public at large - construction workers, soccer moms, computer scientists, and the broad population of the country.

In the U.S., citizens lay claim to a large role in decisions, both risk assessment and risk management. We have to pay attention in our discussions to what citizens are thinking and where they are going on these topics. Actually, an analysis can be done because we know quite a bit about how citizens make decisions based on risk. Scientists always want what is referred to as "good science". Congress uses that term a lot, as do people at EPA and at other agencies. The public's view of risk is much more complex. There are two examples to underscore this point in the U.S. The nuclear power industry is one topic where there is not a huge "risk," because there are not a whole of deaths. Yet it is a complete non-starter for the public. Therefore, we don't have a vibrant nuclear power industry. Another topic is Yucca Mountain - the site in Nevada chosen as the repository for nuclear waste. The people in Nevada say that it will never open. Both of these illustrate the differences we deal with in this country regarding risk and the tremendous influence citizens have on the decisions.

As Dr. Paul Slovik said, "Public perceptions of risk have been found to determine the priorities and legislative agendas of regulatory bodies such as the EPA, much to the distress of agency technical experts. The experts argue that other hazards deserve a higher priority". I am not going to question whether the public is right or wrong. At this point, we realize that is the way it is and we can live with it.

There are some localized hot spots when it comes to endocrine disruptors. The people in Las Vegas know this, they are getting the calls and questions. I also contend, however, that it is really not a big issue yet for a majority of the public. How many constituent calls have you

received in your role as scientist, regulator, or utility official in the last year on endocrine disrupting substances? Most people haven't received more than 1-5 calls, if any. There are not many calls from the media either. There has been a collective yawn from the public about endocrine disruptors. There are a number of reasons for that. There was one article in The Washington Post in the last two years, and one article in The New York Times in the last year relating to endocrine disruptors. That translated into one local article in The Rocky Mountain News and one call to my local health department water official about endocrine disruptors. There is a lot of good information being generated, but it is not getting out in the popular press and it is not being discussed colleague to colleague.

I got a lot of responses when I talked to my friends and colleagues. They are mostly high tech workers or environmental people, and are more educated than the general population. Some of their questions were along the lines of "is that the problem with frogs?" A pregnant woman asked me "should I be worried about that because there are so many things that I have to be worried about when I am pregnant?" Based on some testing and some anecdotal information, there does not appear to be a lot of concern out there. The question is "will there be a concern in the future?" I think the answer to that is both no and yes.

The public is overwhelmed with and pretty tired of environmental disasters. This has been borne out in the polling. Everyday we hear that we are going to be extinct in 10, 20, or 100 years because of "X", and yet we see the population numbers increasing dramatically. We can't make that fit in our minds. We think that the public thinks that the environment is getting safer. The public is still very committed to the environmental movement. In fact, in the latest Gallup poll (which came out before Earth Day) the goals of the environmental movement were supported by 83% of the American public; 43% were strong supporters. We are concerned about it, we are aware of it, we think it is good, but we also think it is getting better. In 1990, only 14% of the public thought a great deal of progress had been made in dealing with environmental issues. A week ago, 26% of the American public felt that a great deal of progress had been made in environmental issues. So we think things are getting better. In 1992, 68% of Americans thought that the government was doing too little to protect the environment, whereas today it is 58%. People are calling for less governmental interaction. We are very supportive of the goals. In terms of the diversity of our society, it is amazing that we have 83% of people agreeing on anything. People feel that problems are being solved.

The science of endocrine disruptors is very complex. The number of chemicals, the effects, and what the information means are difficult to understand. The public knows what "chemicals" means, but most don't know what "endocrine" means. So what does that mean? Do we call them endocrine disrupting chemicals? Pharmaceutical chemicals in water? Hormonally active agents? The public will probably not embrace those terms. I disagree with using terms like toxic eunuchs, hormonal chaos, drugged waters, toxic culture, Pandora's poison, infertile Americans. All of these are missing the point. It won't be any of you that will turn the next phrase to describe this. It will probably be someone in the media. It will change the way Americans view this topic dramatically. Silent Spring really captures what Americans feel - that title brings an image to mind.

Endocrine disrupting chemicals have great potential to cause a lot of concern to the public. They are a classic example of how the public deals with risk that makes them greatly feared. Dr. Willett Kempton wrote a book called Environmental Values in American Culture in which he said that people in the U.S. take the

facts they hear from media and friends and put them together into a mental model. That mental model actually helps them make decisions, gives them the base of their values, and it helps them solve problems. The public did this very effectively with Silent Spring. They heard some facts about thinning eggshells. They heard some other facts about dead birds. They formed from that a mental model of the food chain. They put themselves in that food chain and they became concerned and aware of this. This is by no means a scientific process by the public. However, you should be aware that the facts and information will not just float in their heads separate from one another. The information will come together, and a conclusion will be formed and applied within their own lives.

Using this mental model, I think there is a great chance the public will start hearing about endocrine disruptors and will put that information into a mental model. 50% of alligator eggs in contaminated lakes are not hatching. Female mosquito fish are growing odd male parts. Female gulls are nesting together. Those are the kinds of facts that are going to really grab the public and have them say, "There is a crisis in natural reproductive systems. Maybe there is a crisis in human reproductive systems." Let's talk briefly about the public's approach to risk. This research was done back in the 1980s. To the public, risk is not the chance of injury, damage, or loss; it is a much more complex process. The public has invented the concept to help them understand and cope with dangers and uncertainties of life. Therefore, you don't see a lot of bodies on the high-risk issues that the public deals with.

Paul Slovik wrote an article thirteen years ago in Science, in which he described several factors that we can apply to endocrine disrupting chemicals. The first is the unknown. Unknown risks include things like pesticides, satellite crashes, nuclear weapons, and water fluoridation. Known risks are things such as bicycles, bridges, auto racing, and handguns. We can count deaths from known risks, but it is a little harder to count them with unknown risks. On the other hand, the public fears the unknown risks much more; they rank them higher in terms of their risk. Endocrine disruptors fall in the unknown category. The public also has a factor they use called "dread". People dread risks from nerve gas accidents, large dams and uranium mining more than they dread risks from power motors, caffeine, aspirin or other things they are familiar with. Controllability is very key. If a person feels like they have control, for example driving a car or using fireworks, they rank the risks low. If there is no control, like in nuclear war or commercial aviation, they give it a higher risk. There are many other factors we have to understand that are part of the equation the public uses. There is also the question of the likelihood of an effect on future generations. The public feels this is a very critical concern, and it factors in a very strong way in their risk calculations. Obviously, radioactive waste, DDT and other pesticides have this fear. Endocrine disruptors will also score quite high in this column.

Some of the chemicals we have been talking about over the past few days have different types of risk. Unknown risk has to do with things that aren't observable, such as unknown risks to the exposed, delayed effects, new risks and risks unknown to science. Those are usually above the line. Below this line are observable (known) risks to those exposed, immediate effects, old risks and risks known to science. Bridges, bicycles, and general aviation are below the line; DDT and others are above the line. Using Slovik's model, things like pesticides, DDT, PCBs and DES fall into the area where there is the greatest unknown and therefore the greatest fear by the public. The public wants more regulation on these same areas. We also assess costs for all these things. Cost of a year of life saved by various interventions. Toxic control is extremely expensive. Endocrine

disruptors are the classic case of chemicals that the public will call for increased regulation. They will also demand a lot of costs to meet the public s needs for feeling safe with these issues.

There is a lot of regulation already in place for many of these chemicals, so regulation may not require a major change. The public will ask for a different type of regulation for pharmaceuticals; that will be a big change. They will also ask to see some refinement on how various public water systems deal with these chemicals. There is one thing missing in all this, and that is "the event". Usually you have some facts that you've heard about. Then there is an event that is a catalyst for bringing these together in your own mind and forming this mental model. Endocrine disruptors won't have an event like the Exxon Valdez or Three Mile Island. But what they might have is an event similar to what is occurring with Parkinson's disease.

Parkinson's disease has been a well-known disease for many years. There has been funding for Parkinson's disease for research to find a cure, but not an overwhelming support for that funding. They had just been moving along at a basic level. Recently Michael J. Fox came out and said he had Parkinson's disease and quit his job as an actor to devote full time to raise funds for Parkinson's. I predict that in three to five years, we will have the funding he is requesting, which is 10 times what is currently being provided. His hope, and that of many people in the field, is that we will have a cure in five to ten years. I think this could very well happen because of Michael J. Fox's efforts. I don't know whether there will be an endocrine disruptor person. It is obviously not as direct. It might be someone who learns about this and makes it his or her cause. If that happens, that is when it will start rolling in.

Endocrine disruptors have all the classic characteristics to cause great fear in the public and great public outcry. I think this outcry will lead to greater public awareness for more information. Whether it is right or wrong, the public will use what they know to develop a mental model to make decisions. There will be cries for more regulation. There will be cries for more research dollars, but the public isn't very patient once they get an idea. They won't be willing to wait long periods of time for this.

The real concern is going to be with public utilities. There is going to be a cry from the public to "make drinking water safe", which could translate into big infrastructure costs. At the same time they are crying for this, they are going to start buying bottled water. So the public will take the income they should be giving to public utilities to increase infrastructure and quality, and spend it on bottled water. They will get used to spending an amount on bottled water and protest increasing rates by utility companies. It is already happening in Las Vegas. I was told that after a USGS study came out two years ago on feminized carp in Lake Mead, 70% of Las Vegas is using bottled water. They are spending a lot of money on research and a lot of money to communicate with the public about what it means.

What I'm saying is "You ain't seen nothing yet." When this starts rolling, there could be huge costs; we should all be prepared for that. My way of being prepared is to change some of the terminology that has been used. Many people at this workshop have been wise in saying that we should tell the public what is going on. The public wants communication with the public. They want their concerns and fears to be heard. They want to start a dialogue and mutually solve the problem. Sharing information is what the public wants.

U.S. policies and perspectives on endocrine disrupting chemicals

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When I first studied endocrinology, the last thing I expected to be doing is talking about science policy before a group of professionals, most of whom are engineers. I shouldn't be surprised that endocrinology has become so important in my professional life. Today I am going to discuss policy. I'll start with covering the history of the endocrine disruptor issue. Second, I will discuss science policy questions and how science is affecting what's going on currently. Finally, I will talk about what is ahead. These are some of the things we have been doing at Virginia Commonwealth University (VCU) at the Center for Environmental Studies, where urban studies and planning, chemistry, biology, ecology and regulation management are all combined.

By "environmental policy" I do not mean science policy. I mean how policy is used in a specific sense to articulate what actions will be taken based on the values and beliefs that we hold. The FDA has a course of action; that is the generic term for "policy". In the particular case of dealing with the environment, that policy is set because of values and beliefs that we have. It doesn't matter how we come to these beliefs, whether they are perceptual or conceptual. Science policies are set to govern the scientific enterprise in the U.S. This has to do with funding of private and public research and encouraging various groups to participate.

Science that influences environmental policy relies on two things. First, history is very important because institutions and individuals are loath to go back on what they once said. Institutions more so; they don't like to admit that they have made a mistake. Congress really hates to admit that it made a mistake. The White House is no less excited about the prospect of admitting error, regards of who is occupying it. Second, environmental policy relies upon, but does not depend on, scientific information. In this particular case you will recognize the phrase that "science is useful but not necessary" for making policy. It is necessary but not sufficient.

Science policy is articulated in several different instruments in the U.S., mostly through federal law. It is also implemented through executive orders. Two examples are the environmental justice executive order and the one issued more recently on children's health. There are also court decisions. An earlier talk described how FDA could and could not take action. In one sense, that is a policy instrument or vehicle. Finally, there are international agreements that are approved by the Senate. This gets back to the same two bodies that have to do with elected officials. It

is important to understand these factors in regard to endocrine disruptors because "who said what" and "who did what and when" provides a context of where we are going next. One of the better examples is the 1972 Clean Water Act, which very specifically states not only the objectives of the Act, but that it is the national policy. This is one way in which national policy is articulated in the U.S.

The history of endocrine disruptors goes back to 1992, when Theo Colborn convened a group of scientists (who had previously not been colleagues) at the Racine, Wisconsin conference center at Wingspread. They came up with a consensus document, a section of which reads "we are certain of the following: A large number of man-made chemicals that have been released into the environment have the potential to disrupt the endocrine system of animals, including humans." From 1992 to 1996, the discussion on endocrine disruptors came out of the scientific community. The media picked up these discussions. There were some hearings in Congress, briefings on the Hill, and a few symposiums were held. There wasn't too much in the news about it during that time. During that time the issue grew largely as a scientific one.

In 1996, two pieces of legislation were passed that altered the situation significantly; these were the Safe Drinking Water Act and the Food Quality Protection Act. Both of these Acts are important policy vehicles. They included the language "develop a screening program, using appropriate, validated test systems and other scientifically relevant information, to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the administrator may designate." The important thing is that the legislation had no opposition; it was virtually unanimously passed.

One of the reasons this legislation was successful was because it was supported and sponsored by conservative Senator Alfonse D'Amato of New York. His constituents were acting in a very constituent-like fashion. They encouraged their senator to take action regarding their health; a breast cancer coalition pushed him locally and got national attention. We saw the melding of two usually non-agreeable groups on the political spectrum getting this legislation passed. That is a huge activity in terms of setting federal policy. When you see bipartisan agreement, when you see normally disagreeable groups or constituents coming together to hold hands and go before their colleagues and friends and agree on legislation it is quite powerful. When that happened in 1996, EPA convened the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC).

There are more than just two pieces of legislation that formed the basis for regulating chemicals that might or might not be endocrine disruptors. There are at least 12 federal laws that regulate chemicals, and an additional group of federal laws and executive orders also exists. Each one has its own policy statement, goals, and implementing language that either allows, permits or requires a federal agency to undertake some sort of activity. Under each one of these, there is the opportunity to control endocrine disruptors. As EPA began moving forward in the mid-1990s on endocrine disruptors, they faced not only the possibility or the probability, but the certainty that whatever resulted from the discussion on endocrine disruptors would affect all of EPA, the Fish and Wildlife Service, USDA, the Forest Service, FDA, and other federal agencies. This was going to be very comprehensive and it was going to be far-reaching.

The framework we now have for endocrine disruptor policy in the year 2000 was set by a

few key events put in motion in 1996. The actors and players at that time knew that it would affect federal legislation. The industry knew, the environmental organizations knew, the health advocacy organizations knew, and citizens and the government knew that this would have far-reaching effects. The framework in 2000 includes the scientific literature that has been discussed at this workshop, congressional hearings and legislation, scientific evidence and existing policy. All of this information provides the backdrop for what is happening now and what will happen in the future.

I will focus on the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) and the National Research Council (NRC) report that you have heard so much about. I will also give you some information on what happened with one professional society. The reason I will focus on one professional society is that the issue began with the discussion of human health. We have heard a lot in the news about breast cancer, sperm counts, and sperm quality and how it might affect human health. However, the environmental movement began (as a movement) in the late 1960s, not with concerns over human health, but on what happens with wildlife. In the endocrine disruptor movement, Theo Colborn's book brought scientists together whose greatest certainty was in wildlife effects; the issue rapidly moved into the area of human health. We lost the wildlife component along the way very quickly, except for the Society for Environmental Toxicology and Chemistry (SETAC). Over the years, SETAC has conducted a series of workshops and published articles on the effects of these chemicals on wildlife.

The NRC Committee set the scientific backdrop. While the NRC does seek consensus among parties, their intention is not to make policy recommendations. The NRC gathered the scientific information and explored the validity of the endocrine disruptor hypothesis. Does it have any scientific evidence? Do we find that there are adverse reproductive and developmental effects? How certain are we about that? How good is the science? The report doesn't say what should be regulated, and it doesn't say whether the policies and federal laws are adequate or inadequate. In addition to saying what the scientific information does and does not show, where the data are adequate or inadequate, it also recommends how to fill in some of those blanks. What new information should we have? It talked about gaps in the data and the fact that we do not know whether or not exposure levels we are discussing have been documented as causing anything in human populations. We have some data on wildlife populations, but we really don't have much information on humans, because we haven't looked.

EDSTAC was interested in a very different sort of question. EDSTAC was charged with coming up with a series of screens and tests. It was intended to be a consensus driven process whereby experts from a variety of constituencies gathered together to identify the most effective and usable methods in the laboratory in order to identify endocrine disruptors. EDSTAC came up with a series of recommendations on which bioassays to use and which ones not to use. Some of EDSTAC's major conclusions are helpful. One recommendation was that screening should include adverse effects to wildlife ecosystems as well as to humans. The charge was to examine the effects on more than just one type of hormone, so we see that estrogen, androgen and thyroid hormone systems are included. Reference was made to the fact that there are other hormone systems out there. There are tests, but they need to be verified, and work

needs to progress. These were some of the comments made by EDSTAC.

EDSTAC stopped short of extending their review beyond the vertebrates, other than a handful of paragraphs. A little over a year ago, SETAC convened a workshop and extended the scope of the discussion to the vast majority of life on earth. We recognize that 95% of animals on this earth do not have a spine or any sorts of bones; these are the invertebrates. We used them in assays, and have seen examples where female snails have male reproductive parts growing. SETAC put together a collection that summarizes the endocrinology of invertebrates and what we know about testing and screening protocols. Those of you that have been in this business for awhile recognize that some of these assays have been around for a long time. The mice and shrimp bioassay is not new at all; the invertebrate assays have been around for a long time. The report on invertebrates and endocrine disruptors extended the observations into the predictive mode. If you start looking for more consequences of the activities we have carried out, you will probably find them. Will you find them to be severe? Will you find them here or there? That is not known. EDSTAC and NRC worked on the vertebrates; SETAC worked on invertebrates.

EDSTAC made its recommendations and EPA conducted a task force, which is now ended and will be transformed into a federal advisory committee. The reason for this is that a component of the NGO community pressured EPA into restructuring the task force into a formal advisory committee, which makes it open to the public and announces it in the Federal Register. So the environmental organizations, the health advocacy organizations and the individual companies that had all been sitting together working on EDSTAC were joined by another player at the table. The original players are still in the game, including the partners who helped form the legislation that created EDSTAC and the partners that formed everything that has happened since then. Now there are more partners and new dynamics, which tells you that this has a longer life. From this, we can predict that there will be more research going on.

The number of federally funded studies on endocrine disruptors is quite high. Agencies such as NIEHS, DOD, NCI, DOI, EPA, NOAA, NSF, CDC, NIH, DOE, ATSDR, USDA, FDA and the Smithsonian all fund some sort of research in the area. There is also funding by private industries and a substantial effort by the Chemical Manufacturers Association as an association or by individual companies. This topic has transformed federal research in this area in basic endocrinology, applied endocrinology and environmental endocrinology. One group of people who could not be happier is the endocrinologists, because that funding had dried up; they are finally receiving some new funding. It is fostering research in areas like the relationship between the hormone that controls molting in insects and the hormone that controls reproductive function in women.

As we go forward, we are going to see more federal agencies working in partnerships, with private interests, business, and academia continuing to play an active role. We have seen an increase in the number of organizations involved. Expect to see more and expect them to be local. Expect them to get more deeply involved and more up to speed on the nature of the science, where it goes, and how it can be used to manage their particular issue. We have also seen an unprecedented partnership. I have spoken with a number of people in Washington who said never before have they seen the chemical industry walk hand in hand with the

environmental community, up to Capitol Hill and into the White House to say, "Do this." The partnership between the World Wildlife Fund, the Natural Resources Defense Council, Physicians for Social Responsibility, and The Chemical Manufacturers Association resulted in more than a doubling of the funding that EPA spent on developing screens and tests for endocrine disrupting chemicals. It is what EDSTAC recommended. It tells you something about the nature of the issue.

Here are several conclusions. Science is affecting federal policy in a very large and substantial way. The basic science, the science of receptors and the growth of alligator reproductive parts, is being discussed in Congress at a level that it has never been before. You have already identified areas where research is absolutely necessary in order to carry out the regulatory requirements of federal and state law. That will continue. Some of those research areas will involve new ways of looking at this issue, new biological assays, and looking at new categories of animals and animal systems.

One area that will be new is that we will be looking small. When the invertebrate endocrine disruptor workshop was convened in Amsterdam in December of 1998, we all had a copy of *Science* magazine. It had a nematode on the cover. A roundworm was the first animal to have its genome completely sequenced. The second one was the Drosophila fruit fly. That is why I predict we should look small. These two animals whose genome is known first and better than any animal on earth, provide some of the keys that we are looking for. One key I hope someone is looking for is where we can find an animal that gives us a more integrated and predictive response. We have to look small. My prediction about chemicals is that some of our chemicals are going to go away. Whether they are banned by regulation or whether they simply go out of use, it doesn't matter. Others will stay. A third category will be those applications and uses that simply change because we find ways to make them a little safer. There will also be new chemicals that fit a new set of criteria. They will be neither biologically active nor accumulative nor persistent.

Finally, we need to learn more and learn it a lot quicker. We have all been scanning the literature, but no matter how much we look there is always something missing. The latest issue of *Science News* had an article about more waters testing positive for drugs. It documents cases in which pharmaceuticals have been found not only in surface waters but also in groundwater, and made their way into drinking water. Another article from *Chemosphere* discussed how testicular cancer is associated with occupational exposure to PVC plastic manufacturing. Several journals now regularly contain relevant articles either on assays for endocrine disruptors, epidemiology, whole-animal toxicology, new consequences, and new results. New information is coming out all the time. All of these things are going to change the way in which we do business. They are going to change it in large and fundamental ways. One of the reasons is because it affects human health. The other reason is because it effects the world around us.

Endocrine disrupting chemicals - activities within the European commission

Peter Part, European Commission Environment Institute, Ispra, Italy

Peter Part has been the Head of Unit Environmental Impact at the European Commission Joint Research Centre-Environment Institute, Ispra, Italy since June 1997. Prior to this he was a Lecturer (assistant professor) in Environmental Toxicology, Uppsala University, Sweden from 1986-1997. His areas of research are in comparative physiology and aquatic toxicology. He currently is Editor-in-Chief of Aquatic Toxicology. Part holds a Ph.D. in zoophysiology, Uppsala University, Sweden and a Docent in Environmental Toxicology, Uppsala University, Sweden, 1990. He has served as a visiting scientist and professor at Lab Jean Maetz, Villefranche/Mer, France, University of Nijmegen, Holland, University of Helsinki, Finland, McMaster University, Hamilton, Canada, and University of Miami, USA.

My talk will discuss current activities within the European Commission on endocrine disruptors. Let's start with a bit of background. The European Union (EU) consists of fifteen member countries, so there are many different nationalities and habits that have to be coordinated. The EU was formed in 1959 and had only seven members at that time. The European Commission is the acting body of the EU. The Commission consists of a number of Directorate Generals dealing with different aspects of everyday life. Those dealing with endocrine issues include the Directorate General of Environment, the Directorate General of Research, and the Directorate General Joint Research Centre, where I work. The Joint Research Centre is the only research body within the Commission. It employs about 2000 people; 60% of these people have research backgrounds. Our main mission is to provide scientific support to the other Commission directories and organizations.

The Commission prepares information for the European Parliament, which makes the decisions. In the case of endocrine disruptors, the deciding body is the Council of Ministers of the Environment, which consists of the ministers from the fifteen member countries. This is the link between the Commission and the Parliament to the member countries. The decisions that are made within the Council are anchored to the independent member countries.

A meeting was held in 1996 in Weybridge, England that involved the US EPA, the European Commission and SETAC. While that meeting created a scientific framework in the area of endocrine disruptors, the real work in the field started with the initiative of the European Parliament in 1997. One of the parliamentarians posed a simple question about endocrine disruptors, and the Parliament began their own investigation into the area. They identified a number of priorities - research on the reproductive system, epidemiological studies, test systems and biomarkers, and lists of priority chemicals. This resulted in the resolution accepted by the European Parliament in 1998. The resolution called for the Commission to take specific action concerning endocrine disruptors including developing a legislative framework to reinforce research efforts and making the information available to the public. So the Commission had a mandate, which was the responsibility of the Directorate General of the Environment. After a long p eriod of preparation and consultation, a strategy was adopted in December 1999. This strategy outlined work priorities for the Commission, the EU and the member countries. Four key elements were identified: 1) the need for further research; 2) the need for international coordination and cooperation; 3) the need for communication to the public; and 4) the need for policy action.

An important step was defining "endocrine disruptor". While the International Program of Chemical Safety (IPCS), the European Commission and the US EPA agreed upon a definition, there is also an alternative definition for "potential endocrine disruptor". From a current regulatory activity standpoint, there are no accepted endocrine disruptors present or identified. The reason for this is we don't have agreed upon test methods to show that a substance is an endocrine disruptor. We are waiting for the development of test methods, so we cannot yet point out those chemicals that are endocrine disruptors. In order to have something to work with, there was an alternative definition of "potential endocrine disruptor". This definition is basically the same as the definition of "endocrine disruptor".

The strategy is divided into three actions: short-term, medium-term and long-term. The short-term action puts priority on the development of agreed upon testing methods. This is done together with the Organisation for Economic Cooperation and Development (OECD). The next short-term priority, which is during the first two years, is to identify endocrine disrupting substances, estimate the exposure to humans and wildlife and identify critical users. The third priority is to communicate the current findings to the public as well as the associated risk communication in the field.

The goal of medium-term action (2-5 years) is to see results from research move towards policy, and to strengthen the research program. Other goals include facilitating synergies across research actions and across national programs, and identifying substitutes and considering voluntary initiatives to find replacements for chemicals. Finally, goals for long-term action (over 5 years) are to amend existing legislative instruments for chemicals, consumer health, and environmental protection to cover endocrine disruptor effects. This is the basic strategy of the Commission, on which different Commission Directorates have to work. It is also the guiding document for my work on endocrine disruptors with the Joint Research Centre. To further strengthen the European initiative, a program on endocrine disruptors was recently adopted at a meeting of the environmental ministers. The program connects the Commission activities to activities and responsibilities within the independent member countries.

The Council of Ministers of the Environment, recognizing the importance of this issue, welcomed the community strategy on endocrine disruptors. They called on the member states and the Commission to implement the strategy; it is a clear mandate that member states be involved in the process. They stress that the precautionary principle must be applied in order to respond quickly and efficiently when necessary. Furthermore, the Council calls upon the Commission, in close consultation with stakeholders, to strengthen and speed up efforts to establish a dynamic list of priority substances. It is essential that the Commission and the member states ensure the development of agreed upon toxicological and ecotoxicological test methods. They also stress the importance of international collaboration so that efforts are not duplicated. To conclude, the strategies of the Commission and the Council have two priorities: to develop agreed upon testing methods and to use these methods to establish a list of priority chemicals in consultation with stakeholders.

There is a strong recommendation for more research in this field on the EU worldwide web site. EU research is directed through the Framework Program, which is organized in different topics, every topic has a number of key actions. We are currently in the 5th Framework Program. The problem for

endocrine disruptors is that the Framework Program was written three years ago, when endocrine disruptors did not have the prominence that they now have. While there is no specific key action addressing endocrine disruptors, three different key actions involve endocrine disruptors: food nutrition and health, environment and health, and sustainable management and water quality. The 5th Framework Program will continue for another 2-3 years, during which time there will be new calls and new possibilities for research.

There will be specific possibilities for US scientists to work with colleagues in Europe within a specific treaty, the EU/US Science and Technology Cooperation Agreement. This agreement identifies four different research priority areas where collaboration should be established. One of the priority research areas is endocrine disruptors. Last year at a meeting in Ispra, Italy, scientists from the US and Europe agreed on priorities for this research. The priority areas for collaboration where added value existed for US and European scientists to work together were in human epidemiology, wildlife ecotoxicology, screening and testing methodology, and integrated risk assessment animal toxicology. This activity is now implemented and is progressing at different speeds in the US and in Europe. In the US, EPA and NIEHS have already made up a framework of how this could be achieved. In Europe, the research has to be within the 5th Framework Program. As I noted, endocrine disruptors were not identified directly as a key action in this framework. So it has been a bit difficult, from an administrative point of view, to get this agreement to work. Recent developments look promising on getting this to work on the European side.

There are three different activities on endocrine disruptors in the Joint Research Centre. The European Chemicals Bureau is involved with risk assessments of chemicals and is the centralized organization in Europe for registration of chemicals. There is also the European Center for Validation of Alternative Methods. Finally, there is an environment institute that directly works on endocrine disruptors; that is the one I am involved with. Our work focuses on identifying biomarker endpoints for endocrine disruptors. We work on both human and wildlife health - mostly fish and invertebrates.

We are also involved in a lot of international activities. One of these is the Global Endocrine Disruptor Research Inventory (GEDRI), which was an initiative originally started by the US EPA. It transferred to Ispra, Italy under the IPCS. GEDRI is a directory of all the research going on involving endocrine disruptors; it is accessible on the Internet through the EPA and the EU websites. The directory is active and new projects are being added continuously. This is a useful tool for scientists and administrators to get an overview of the different research activities in the field of endocrine disruptors. A second international activity is the Global Assessment Document on Endocrine Disruptors, which was prepared under IPCS. This document, which involves scientists in Europe, Canada, the U.S. and other parts of the world, is supposed to be finished by the end of the year. It will be a global assessment document on the problem of endocrine disruptors.

There are many other activities going on Europe. Europe has many independent countries; it is not always easy to coordinate the activities of the individual countries within some of the organizations and structure I have talked about.

Scientific assessment of endocrine disrupting substances in canada

Mark Servos, National Water Research Institute, Environment Canada

Mark Servos is Chief of the Priority Substances Exposure Project at the National Water Research Institute, Environment Canada, where he leads a team of scientists investigating the fate, bioavailability and effects of environmental contaminants in the environment. His current research interests are on exposure and effects of endocrine disrupting substances in aquatic environments, particularly agricultural runoff and municipal effluents. Prior to joining Environment Canada, Dr. Servos worked with the Great Lakes Laboratory for Fisheries and Aquatic Sciences, Department of Fisheries and Oceans. He studied the fate and effects of contaminants in the Great Lakes as well as pulp and paper effluents across Canada. He is a past president of the International Association of Great Lakes Research and is currently vice-president of the Society of Environmental Toxicology and Chemistry. Dr. Servos is the co-chair of the Environment Canada and 5-Natural Resource Departments Working Group on Endocrine Disrupting S ubstances in Canada.

I am the co-chair of a research consortium in Canada, which is comprised of the five Canadian natural resource departments - environment, agriculture, fisheries, natural resources and human health. There are three things I want to cover: 1) how the endocrine disruptor issue has developed in Canada; 2) what data are being generated on sewage outfalls in Canada; and 3) a workshop held in Canada to deal with risk assessment, risk management questions, and establishing a national agenda on these issues.

There is growing public concern and awareness of the issue in Canada. There's been identification of potential subtle effects of endocrine disruptors and an enormous explosion in the amount of science, with results pouring out in the literature. There is a lot of international development and activities that influence Canadian policy. Canada is a small country in terms of population. The international activities influence Canada because we have to harmonize with the US, the Organization for Economic Cooperation and Development (OECD) and other countries. The development on the international testing and screening programs are going to have a big impact on Canadian policy. One thing that recently happened is the Royal assent of the new Canadian Environmental Protection Act (CEPA).

Language was included in the CEPA committee process making it mandatory for the Canadian government to conduct research on endocrine disrupting substances. It also defined an endocrine disrupting substance as "a hormone disrupting substance." It is a pretty comprehensive definition - "a substance having the ability to disrupt the synthesis, secretion, transport, binding, action or elimination of hormones in an organism, or its progeny, that are responsible for the maintenance of homeostasis, reproduction, development or behavior of an organism". One interesting thing is that there is no comment on "adverse" in the definition. The CEPA definition relates only to research, not in terms of action or how the rest of the Act is applied.

In Canada, each department has their own activity on endocrine disruptors. There are ongoing research programs in Environment Canada and in Fisheries, where research on endocrine disruption has been going on for a long time, particularly in the Great Lakes and in the Arctic. There are a variety of working groups, and there have been a number of workshops that focus research activities. There

are departmental endocrine research strategies and activities. All have been contributing to international activities. There's an inventory of endocrine disrupting substances and research that Canadian agencies have contributed. I want to point out that when you look at endocrine disrupting research in Canada compared to the US, the distribution is very different. There is much stronger and larger contingent of people working on environmental issues in Canada in the human and ecology research and less on the chemistry.

Canada has a new program called the Toxic Substances Research Initiative, in which endocrine disruption is a major theme. It is a $10 million per year program - $2.5 million will go into endocrine disrupting research for the next three years. The other thing Canada has done is to recognize that the departments could not work independently, because endocrine disruption is a complex issue. A working group was created to coordinate the research and activities of all the units in Canada.

The issue has evolved rapidly in Canada over the last 5 years - very similar to how it evolved in the US and in Europe. The issue was preliminarily looked at as a science issue; there wasn't a big public outcry at the beginning. It has evolved rapidly due to public pressure to translate all of the science into decisions and take action. There is a recognition within the government that we have to be proactive so that we reduce the uncertainty and make decisions based on sound science. In addition, the precautionary principle is in the forward position with the CEPA; all decisions must take the precautionary principle into consideration. The two issues have gotten tangled together.

When the endocrine issue began, we were sitting up north asking "what's going on, we've been studying this stuff quite awhile in the Great Lakes?" We have seen a whole variety of impacts going back into the 1970s in the Great Lakes - effects on birds and the effects of PCBs. Much of this work was predominantly on historical contamination of persistent organic pollutants (POPs). There has been action already taken on those kinds of chemicals. The other group of chemicals that raised concern in Canada were the non-persistent bioaccumulating toxic substances, things coming out of pulp and paper mills. If you compare white fish testes during spawning near pulp and paper mills to those that are not near the mills, there is a dramatic decrease of the gonadic size of fish near the mills. Thirty percent of the pulp mills in Canada are reporting reduced gonad size in fish (from data in the Environmental Effects Monitoring Program). Other information is starting to trickle out on unanticipated and subtle effects on development and other processes regulated by endocrine systems.

Researchers studying Atlantic salmon were correlating population returns to a pesticide called Matacil; they found a two year delay in reduced populations returning if the watershed was sprayed with Matacil for spruce budworm control. Matacil contains nonylphenol, so they looked at whether the nonylphenol had an impact on Atlantic salmon returns. The study exposed fish in static tanks to two 24 hour periods of 20 ug/L (or parts per billion - ppb) nonylphenol before they underwent smoltification, and then let them grow in salt water for 60 days. Thirty percent of the fish died and the rest had stunted growth, which likely means they don't migrate back to spawn. Nonylphenols, which are very common contaminants in sewage, and other estrogenic compounds produce estrogen-mediated effects. Until recently in Canada, they were common in pulp and paper mill effluent. There's very short exposure in estuary areas where most of the sewage treatment plants are located; this exposure could cause these type of responses.

The other thing we are concerned about in Canada is the type of responses we have seen

reported in the UK. Susan Jobling published work comparing testes and gonads in normal fish to testes and gonads in fish exposed to sewage treatment plant effluent- those exposed to the treatment plant effluents have intersex organs. There is quite a degree of change to the intersex depending on the exposure. We were interested in finding out whether we are having some of these types of responses in the Canadian environment. There is nothing unique about Canadian sewage, because we see vitellogenin (VTG) induction at sewage treatment plants as well. Some of the data measuring VTG in caged rainbow upstream and downstream clearly show VTG induction. A control site 5 kilometers downstream of another site shows the effect is either diluted or dissipated very quickly. Toxicity Identification Evaluation (TIE) research using a comparison of standard chemicals coming out of sewage show responses corresponding with estradiol, est rone and ethinyl estradiol. There is also a response with nonylphenol and other compounds. This is the same response UK researchers saw, so Canadian sewage is not unique. This has been repeated at 4-5 different sites, and we get the same result at each.

If you measure hormones in Canadian raw sewage effluent, primary effluent and final effluent you find estrone, estradiol and ethinyl estradiol, which degrade quite rapidly (but not completely) in the sewage treatment plants. Sometimes the measurements don't degrade, which might be due to conjugated steroids that we are unable to measure. We are only measuring the free material in the raw and final sewage. We don't know what is happening during the treatment process; it's not possible to mass-balance this right now.

A national survey for estrogenic compounds in sewage treatment has been completed in 30 sites, where we have tried to characterize the watersheds and treatment plants. Some preliminary data shows that 17B-estradiol and estrone are present in primary effluent in measurable concentrations at almost every plant. These substances are treated very efficiently in almost every plant. However, plants with short sludge retention times show a little different result; the concentrations of estrone for final effluent are actually higher compared to the raw effluent.

I want to talk about the risk assessment just completed on alkyphenols, which are another type of compound commonly found in sewage effluent that people have been very concerned about (editor's note: the document is available at www.ec.gc.ca/cceb1). The sewage component of that risk assessment illustrates the complexity of looking at the risk of these kinds of compounds to the environment. Nonylphenol polyethoxylates come into sewage plants and are broken down into carboxylic or ethoxylates and eventually into nonylphenol and carbon dioxide. Nonylphenol ethoxylates (NP9E0) also comes into the sewage treatment plant. You can see there is lots of NP9E0 for a primary treated sewage treatment plant; there is lots of nonylphenol, but very few of the carboxylics. As more and more treatment occurs, the nonylphenol and NP9E0 disappears; the carboxylics are created because they are much more water soluble and they are not going down to the sludge.

We've completed a large survey of these compounds in Canada. If you do a cumulative rank percent of the compounds, you have to deal with many types of compounds in mixture; you have to consider the exposure. There's also a lot of data and literature on nonylphenol; from these data you can look at the LC50s and the chronic and acute ratios and plot the cumulative rank percent. It turns out for this compound that about 4:1 for chronic things - relevant to the fat head minnow chronic test and acute test. If you move that line over, it corresponds quite nicely with one of the most sensitive species, which is the Mysid growth chronic test. In Europe they

used an algal test and they are using a value of 0.33 for nonylphenol for their risk assessment for the level of concern. In Canada we picked a value of 1.0 for nonylphenol. A lot of other compounds are in the sewage too, not just nonylphenol. There is quite a bit of good literature on the acute/chronic toxicity of nonylphenol, so we took a TEQ (Toxic Equivalents) approach and applied it that way. We then looked at the exposure data and expressed it as TEQ . The first set of data uses nonylphenol; we then add data points by combining the ethoxylates, and then combine nonylphenol, the ethoxylates and carboxylics. The carboxylics are relatively non-toxic so there's not much change between the second and third one. But remember, well-treated sewage has high levels of carboxylics in the final effluents.

What about taking endocrine disruption into account? The first problem you have is selecting the endpoint. How is the level of concern determined? Intersex occurs at concentrations less than 100 ppb, smoltification at less than that, VTG induction occurs around 10 ppb. For nonylphenol, you could probably select a level of 1.0 ppb - your chronic test are very similar to endocrine values as the endpoint value.

What about other compounds you have to take into account if you have VTG induction reported in trout? Jobling also reported that nonylphenol, ethoxylates and carboxylics had very similar responses in terms of VTG induction. So the potency of 0.6 of nonylphenol induction of VTG is quite different from the chronic toxicity. Remember that the carboxylics had very low toxicity relative to nonylphenol. That changes the result when you plot it out in terms of TEQ. We have made huge assumptions when it comes to mixtures. The scenario of nonylphenol mixed with ethoxylates and carboxylics show one half the sites across the country now have a concern - before there were only two treatment plants sites. There is a lot of controversy on how to calculate that relative potency. In our own assays, nonylphenol carboxylic doesn't bind to the estrogen receptor. But in the trout cell line, they are almost the same. You can't always extrapolate from the receptors to the trout cell lines to whole organisms. How you apply this in a risk assessment is very complex thing; you have to work with that data you have. But that data is very incomplete.

There's huge uncertainty associated with doing risk assessment of endocrine compounds in municipal effluent. There is difficulty in selecting the appropriate endpoints, there is complexity in the chemistry in the fate in the particular system, there is complexity for determining the relative potencies, determining point estimates from dose responses is complex, and assessment of the mixtures is complex. Obviously there are lots of assumptions in doing it this way.

What are the next steps in Canada? We need to assess if knowledge and current approaches are adequate to address this group of chemicals and our concerns. How do we make decisions in the face of this enormous uncertainty? Can we reduce uncertainty in our scientific assessments? What are the implications of all the international activities? In order to address these questions, Canada held a workshop in February 2000 titled Establishing a National Agenda for the Scientific Assessment of Endocrine Disrupting Substances. The workshop included people from many departments, industry, academia, and NGOs. Seventy-two scientists gathered to discuss and evaluate the activities and research and develop a national agenda on how to progress. The objectives were to review the current situation in Canada, review the adequacy of the risk assessment and management framework, identify potential implications for international activities and Canadian policy, review and identify the sources of uncertainty in the scientific ass

essments, and identify the major knowledge gaps and research needs - particularly specific to Canada and the Canadian environment and culture.

The preliminary conclusions were that there are concerns that low level and/or multigenerational effects are occurring in humans or the environment which are currently going undetected in Canada. The major areas of concern are municipal effluents, pulp and paper effluents, intensive agriculture (pesticides and pesticide runoff, animal wastes), textile mill effluents, mining effluents, POPs in the arctic (including aboriginal diets), and historically contaminated sites, particularly in the Great Lakes. Endocrine disrupting substance issues need to be broadened to draw attention back on subtle effects on reproduction and development rather than a single mode of action (endocrine function). There are many people looking at receptors, but the feeling of Canadian scientists is there is strength in doing fieldwork and maintaining focus - the real issue is trying to detect those subtle impacts on reproduction and development.

The current Canadian approach to risk assessment is appropriate for endocrine disrupting substances (e.g., Priority Substances List Program) but requires continuous improvement. Adequate data are rarely available for even priority substances like nonylphenol, and there is a lot of data for nonylphenol compared to other substances. Endocrine disrupting substances can be addressed within current Canadian legislation, regulation and management frameworks. The screening and testing of new and existing substances (e.g., Domestic Substances List) needs to be harmonized with the OECD and the US. It is critical that Canada contributes and participates in the international efforts to standardize this testing. Otherwise, Canada will just have to live with what others produce. If Canada has a special perspective, we want to have some influence on that process now and not have to worry about it five years from now. Canada agrees with the tiered screening and testing approach that considers both hazard and exposure, a nd believes that should be incorporated into Canadian programs once it has been validated and accepted internationally. The validation is an important component. We envision something similar to the areas Tom Hutchinson discussed in an earlier talk in terms of a tiered approach in future testing. The research needs to continue to reduce the uncertainty associated with scientific assessments of these types of substances and effects. It was noted that the *in vitro* tests and screens included Structure Activity Relationships (SAR), which are currently not developed well enough to predict effects at higher levels of organization. It is at the higher levels of organization that the functional endpoints we are concerned about exist.

Research in Canada should continue to focus on our strengths in field studies, identifying impacts, and defining cause and effect relationships. There is a long history of Canadian science trying to understand impacts in the real world. There is a strong move to discourage replication of international efforts on screening and testing because there is limited amount of money to put into that. Canada can contribute to the international effort by calibrating some of the tests in the real world as opposed to trying to develop new ones that are specific to the Canadian environment.

The list of needs and priorities is quite long. We need to establish the connection between lab tests and actual world - are tests predictive of real world effects? Are there things happening in real populations that are not predicted? We need to link screening/testing methods to the ecological relevance, especially with respect to sensitive life stages of various organisms. We need better knowledge of exposure and dispersal of endocrine disrupting substances in the

environment, and better monitoring. We need better ecological monitoring as well and monitoring in human populations, not just chemical monitoring alone. A framework needs to be developed for risk assessment and risk management of endocrine disruptors, especially for mixtures and complex effluents. We need to improve the understanding of the role and importance of naturally occurring hormonally active substances, especially with regard to testing. We need to address the low dose and threshold effects issues. These are all things that almost every single talk has mentioned. We need to define "adverse effect" for risk managers, as they must be able to decide when to take action. Improving basic knowledge about early development of endocrine and reproductive organ systems from fertilization onwards in vertebrates is needed. We need to have better understanding of the basic biology of humans and basic biology of organisms. We need to improve the basic knowledge on the role of hormones in the development of nervous, reproductive and immune systems in human and relevant species. The effects of timing of exposure and windows of sensitivity for a variety of sentinel species needs to be improved.

In the future, we must continue to consider potential subtle effects on reproduction and development in scientific assessments and screens in various programs within the government. We need to support research to address critical knowledge gaps. We need to keep in mind that endocrine disruption is a mode of action, not an endpoint of concern. Collaboration and consultation with stakeholders on this issue must continue. We need to keep industry, NGOs and public advisory groups informed. International as well as local activities will continue to influence public perception and policy on this issue.

A scientist who joined us at the workshop brought her young daughter along. It was pointed out that it is her daughter who, twenty years from now, will decide whether or not we were successful and followed through - she will decide whether anything useful comes of these meetings. Remember that actual concerns are for subtle effects on the development and reproduction of people and the environment. For those of you who would like more information, proceedings of the Canadian workshop are at www.cciw.ca or www.ec.gc.ca>. A special issue of the *Water Quality Research Journal of Canada* will have summaries of the Canadian perspective, too.