Collaborative on Health and the Environment (CHE)

Environmental Contaminants and Fertility/Pregnancy Compromise
Discussion Group Call # 3 June 23, 2004 9:00 am. PDT (20 callers)

ALISON CARLSON: Welcome to the third teleconference of this discussion group. Today we have two distinguished presenters speaking about their research – but first we'll do a roll-call, followed by two brief updates – and then after the speakers – we'll have time for some open discussion and "Members On Topic" announces - and a run through of Pete Myers' multilayer online information service and what is called an RSS feed that many of you may find extremely useful. For this run through, you will if possible want to be in front of a computer with your browser open to www.environmentalhealthnews.org.

NEW PARTICIPANT INTRODUCTIONS (SINCE LAST CALL):
Barbara Davis, VMD, PhD, Dipl. A.C.V.P.
Acting Chief, Laboratory of Women's Health, NIEHS, NIH, DHHS

Michael Diamond, MD
Professor of OB/Gyn, Wayne State Univ. School of Medicine

Eleni Sotos
Program Coordinator, CHE

Ellen Stein, MD, MPH
Medical Director for Maternal and Child Health, City and County of San Francisco

Julie Wirth, PhD
Michigan State University and Dept of Community of Community Health

(Other new discussion participants not making this call: Noah Chalfin, CETOS; Sophia Kolehmainen, Cedar Tree Foundation; Vina Lee LeBlanc, B.S.N, A.R.N.P., Seminole County Health Dept, FL; Susan Duty, Harvard School of Public Health and Asst Prof of Nursing, Simmons College)

UPDATES:

~ **LINDA GIUDICE** - on the environment and fertility/repro health conference proposal. Our interdisciplinary proposal to Stanford's Institute for the Environment, submitted in April, made the final round but was not chosen for funding this year. We were encouraged to resubmit for 2005, but have decided to move forward under different auspices, but still at Stanford. Linda, Michael Lerner, Edith Eddy and Alison Carlson met by phone to plan basis for a conference to be held in Northern California, hopefully at Stanford, on fertility and environmental chemicals. We're thinking along the lines of a think tank retreat over first day or two with a cross section of individuals from invited organizations [to be followed by a one-day public forum]. We're looking for a carefully chosen set of speakers on reproductive health syndromes and trends to identify what we know about hard evidence, and what is the soft evidence. It will be important for the patient groups to have a critical voice at this forum.

BARBARA DAVIS: What are the funding sources you have or are looking for?

LINDA GIUDICE: We haven't yet identified funding for sure but will contact a variety of organizations and would like to put in an application to NIH much as we've done for the Gordon Research Conferences. If anyone has suggestions, I'd be happy to hear them.

ALISON CARLSON: Thank you to Linda. Ted Schettler is the Science Director at Science and Environmental Health Network, Chair of CHE's Science Work Group, a physician in Boston and co-author of Generations At Risk: Reproductive Health and the Environment. Ted has offered to do a brief new science update, and will speak about a report recently out from the Proceedings of the National Academy of Sciences…

~ **TED SCHETTLER** - I'll briefly describe a couple of experiments that show impact of air pollutants on DNA mutations in germ line cells. These studies were prompted by earlier observations that herring gulls in the Great Lakes region near steel mills had DNA mutations at higher rates than birds in other areas. It wasn't clear what the cause was – it could have been diet or some other factor. So, in these recent studies, researchers put mice in cages in the steel mill region for ten weeks, and a control group of mice in a rural area. They brought the two groups of mice back to the lab after 10 weeks. After another six weeks, the mice bred. The researchers then detected DNA mutations in the germ line of the offspring of the mice exposed to the steel mill pollutants – and did not see those mutations in the controls. The mutations were seen in areas of the DNA that do not code for protein, called "expanded simple DNA repeat sections." But those sections can affect gene expression. And they are unstable, so in small study groups you can really see if an exposure is increasing mutation rates.

A second experiment followed: The same model was repeated but this time the researchers used Hepa [high efficiency particulate air] filters to filter the air for the mice near the steel mill, and the DNA mutation impact in the exposed animals was not seen. They concluded that the small particles in the air pollution near the steel mill were responsible for the mutations seen. Their hypothesis was that it was polycylic aromatic hydrocarbons (PAHs) attached to the small particles that were likely responsible, since PAHs are known to be mutagenic.

The implications of these studies for humans is not certain. But there is a correlation between these kinds of mutations and radiation exposure. And there are dose-response curves seen for mutations in the coding regions of DNA. Thus it is plausible there could be implications for humans. There have been studies that have shown sperm alterations in humans that are consistent with these results in mice. One was in Environmental Health Perspectives in 2000, reporting that men living in areas of the Czech Republic with high coal burning pollution, which has high PAHs, exhibited sperm morphology, motility and chromatin patterns.

These rodent studies are significant in that they show intergenerational, heritable sperm impacts – mutations passed on to offspring. It isn't known whether it will go further than that.

JULIE WIRTH: Were the exposed group both males and females and were they mated with unexposed mice?

TED SCHETTLER: Yes, both males and females, and there was cross-breeding – but the statistically significant impacts were seen only in the paternal lines.

MARY LOU BALLWEG: This study is reassuring to physicians and others who have advised using Hepa filters for a long time…They are effective particle filters.

ALISON CARLSON: Thanks Ted. Dr Barbara Davis is Acting Chief at NIH's Laboratory for Women's Health. She is going to tell us about her research on phthalates and reproductive health, and her upcoming presentation at the Ovarian Workshop in Vancouver titled, "Lessons Learned When Environmental Chemicals Disrupt Ovarian Function." Dr Davis' web bio includes interesting notes on her work in the area of PCOS (polycystic ovarian syndrome) and environmental factors, and on a fibroid growth study. I read that your research "seeks to determine how environmental toxins and stresses influence health and

disease in women over a lifespan…with the goal of reducing the burden of environmentally related diseases in women, by integrating genetics, endocrinology, immunology, pathology, epidemiology..." I saw a list of foci ranging from breast cancer to ovarian cancer to ovarian dysfunction to pregnancy/parturition disorders. It looks like you have quite a job on your hands…

~ BARBARA DAVIS – Thanks for the invitation to speak today. This group's interest gives me hope for the future. I've had a great opportunity to build a laboratory of women's health, and try to understand the impacts of the environment. We think it is critical to do work in the laboratory in order to understand women's health. I've focused most of my research on the ovary because it has an effect not just on reproductive health but also the general health of women. We want to understand the cellular/molecular level mechanisms of action of environmental factors. What we've learned is that the ovary is susceptible to damage from an array of environmental chemicals. And each chemical's effect relates to specific cell populations in the ovary, with distinct mechanisms.

For my Ovarian Workshop talk, I convey that chemicals disrupt signaling systems that hormones work through, and use two examples. First is the phthalates, the most abundant synthetic chemical in our environment. We and others have shown that one phthalate, DEHP and it's metabolite MEHP - we knew from work in males that it is a reproductive toxicant- specifically a testicular toxicant - in rodents. This helped us define questions to pursue in the female system.

We showed that this phthalate affects the ovary – specifically cell populations called the granulosa cells, which are responsible in the ovary for hormone synthesis, mostly estrogen. This phthalate works through signaling activating receptors called PPARs, peroxisome proliferator activated receptors. When cells are exposed to the chemical, it activates PPARs that in turn suppress aromatase – the rate limiting enzyme that converts testosterone to estradiol. That means estradiol gets suppressed. In the animals we study, that means adult female rats fail to ovulate (anovulation).

There is some evidence in humans that women facing high occupational exposures to this phthalate may exhibit similar effects of decreased estrogen and anovulation. So this could be a way that human fertility is affected by phthalate exposures.

We've also done global gene or microarray studies to try to identify the cell signaling pathways PPARS were activating – aside from aromatase. We saw changes in gene expression of the Ah receptor, which is a receptor that responds to polycyclic hyrocarbons and dioxin. This might suggest some interaction between phthalates and these other chemicals. Another altered gene was Fatty Acid Binding protein. This protein is changed both in cell cultures and in animals at low doses previously said to have no effect. The lesson learned: hazard identification at specific dose ranges are only as good as our biomarkers of effect. We need to find sensitive biomarkers to be able to bridge animal models to relevant levels in humans. We'll pursue this over the next years, and my research colleague is presenting these low dose exposures at the Society for the Study of Reproduction. We are happy when reproductive groups want us to come talk about toxicology.

I can contrast effects of phthalate type chemicals with ethylene glycol monomethyl ether, EGME, an organic solvent used widely in industry – which we are concerned about in occupational settings, especially in the semiconductor industry. Legal cases you've probably been hearing about revolve mostly around these glycol ethers. Rodent models show that they are male and female reproductive toxicants. Again, a lot of work has been done in the male, which helped us develop questions about female systems and mechanisms of action. We found something we didn't predict: exposed rats show enlarged corpora lutea and continue to secrete progesterone when they shouldn't as long as the exposure is happening. We tested this by cell culture as well, and think this is an unusual finding. Metabolites on human and rat luteal cells produced the same effect: excess progesterone.

Others have found that these chemicals enhance effects of steroid receptor pathways, so it has profound implications for fertility, repro effects, and cancer. A number of groups are looking at this novel type of endocrine disruption.

So lessons learned: we have very different effects by different chemicals and we must know more about molecular mechanisms in order to be able to apply what we are learning in public health arenas.

SHANNA SWAN: I'm excited to hear about your work on glycol ether mechanisms. I'd like to speak with you about our studies on glycol ethers in semiconductor industry because we saw effects in women, but knew nothing about mechanisms. It would be interesting to put these together. About phthalates, we are doing a study involving urine measurements of phthalates in pregnant women and then postnatally. Also then in their offspring. We could talk about that. We have information about their pregnancy progression, and about their children's sexual development.

LINDA GIUDICE: I'm interested that you say phthalates are working through PPARs because we are working with granulosa cells in humans and looking at PPAR gamma agonists. These are endogenous and insulin sensitized. We found the same aromatase inhibition and decrease in estradiol. In light of our interest in treatment for PCOS, it's encouraging that we've found this common mechanism. Hopefully we can talk at some point about collaboration potential?

PETE MYERS: Can you say more about exposure levels in your studies on glycol ethers and phthalates?

BARBARA DAVIS: In our initial phthalate studies, it was one gram per kilogram of body weight. So that is high compared to what humans show exposure to according to the CDC. That's 100 fold higher. My point was that if it's that high [….unable to hear Dr. Davis' response over next minute] …We see gene induction as low as we've looked to date. So we're at 1 micromolar in vitro – and we can see the changes in Fatty Acid Binding Protein. We don't yet know what the significant pathways are.

ALISON CARLSON: Apologies for cutting in here, but we have to move to our other speakers, so perhaps we can take more questions for Barbara later, or we can handle questions via email.

Dr Michael Diamond is a professor of OB/GYN and director of the Division of Reproductive Endocrinology and Infertility at Wayne State School of Medicine. He has been involved in the largest study to treat infertility in women with PCOS and is renowned for his expertise in gynecological surgery and adhesion prevention. His collaborator on the study [on organochlorines and male infertility] he is speaking of today is also on the call. Julie Wirth is an assistant professor at Michigan State University, and also works with the State of Michigan Department of Community Health as an environmental epidemiologist.

~ **MICHAEL DIAMOND** - Thank you for this opportunity to share with you all the work we are doing. We're in the middle of our study, so there are no results to share at this time. But it's a collaborative effort between Wayne State, Michigan State, University of Michigan, and the Michigan Department of Community Health.

This work began in a number of ways. It grew in large measure from the work of Nigel Paneth at Michigan State, a group that includes Julie Wirth, looking at environmental influences over several decades. Julie is the principal investigator of our grant – and we have offshoots of work from that grant as well that are not funded but we are pursuing them all the same. Our grant is NIEHS and specifically looks at organochlorine effects on male fertility. Collaborators have different strengths. The epidemiological experience from the MSU group looking at environmental toxicants; experience with the infertility patient population we see here at Wayne State. As a practitioner, I've seen patients over a long time and had the opportunity to collect semen specimens as a part of routine evaluations of patients.

What were doing in a case-controlled study is looking at relationships in presenting patients between organochlorine exposure and fertility. Patients are not provided information about the specific purposes of the study – but do know that we are interested in environmental influences. We use a 50 page questionnaire for male partners that pertains to infertility/fertility history, economic status, demographics and factors we know contribute to male factor problems. We get information on hunting and fishing experience, occupational exposures, and lifestyle issues.

We tie this to the work of the State of Michigan on waterways and specific regions within the state, specific types of fish…There is good information about specific toxicants in fish caught in different areas, including the Great Lakes, rivers and inland areas. So we are now combining our questionnaire with individual fertility information, including repro hormone tests (LH, FSH, testosterone/estrogen, inhibin B), serum organochlorine levels (over 100 samples), DNA samples analysed for polymorphisms. And heavy metal levels. In partnership with the CDC we'll also look at phthalate levels in some of these men.

One of our collaborators at Wayne State, Steve Krawetz, has been doing a lot of work with microarrays in sperm, isolating sperm RNA as a means of diagnosing male infertility and looking at possible effects of environmental toxins. There is particular importance to a part of our study in light of our paper in *Nature* last month. That paper showed that in addition to DNA from the father, RNA from the father is transferred to the egg during fertilization. This brings up questions about what role they have in embryo development, implantation, miscarriage and early infancy.

I'll stop here and see if Julie has anything to add?

JULIE WIRTH: I can't add much, but in terms of the RNA that Steve has isolated, this provides for the first time a mechanism for how an environmental effect in the father can be transmitted to offspring.

SHANNA SWAN: This is exciting. We are doing a study of fertile men, sperm counts, and pesticides in current use. We have found associations. We hope to get funding to look at infertile populations on the same questions. What is your participation rate? How many men agree to go through the lengthy questionnaire and agree to give samples? This information might help us plan our next studies…

MICHAEL DIAMOND: Participation is one of the challenges. Women often come to see us alone, without their husbands with them. But currently with new couples coming to our clinic, we are getting close to 50%. This is a guesstimate.

BARBARA DAVIS: This *is* exciting. I haven't seen your *Nature* paper. Are these RNAs that get transcribed? What is the function of these RNAs?

MICHAEL DIAMOND: There were six RNAs identified in that paper. They are unique to sperm. They are not in the oocyte. Hamster oocytes. This is why we know they are coming from the sperm. They're shown to be important in early development. There are others identified that are going to be in future papers, so I am not at liberty to discuss them yet, but your questions should be answered then.

BARBARA DAVIS: It's very interesting and we'll want to look for your papers. Julie, can you say what is most significant about this. It's always been thought that DNA damage was the route of male transmission. Now you say it's RNA transmission too…

JULIE WIRTH. That's right. There can be paternal gene silencing in the dad that is transmitted to the fetus. This could lead to differential expression as an adult.

BARBARA DAVIS: This has profound implications. Have you identified types of syndromes, say infertility syndromes, that would be …

JULIE WIRTH: We are just beginning. It has been only a couple of years since we knew that sperm even has RNA.

ALISON CARLSON: Thank you very much to Michael and Julie. To all three speakers. I am really impressed by the range of possibilities and potentials the work described today suggests. If there are more questions for the speakers, I can put anyone interested in touch with them. We can do this via email or through the CHEfertility listserv.

For lack of time, we'll need to skip what should come next: the "Members On Topic" announces – but we can share those via CHEfertility, so please use this listserv for announces about papers, presentations, studies, comments, questions etc.

We'll move ahead to Pete Myers' demonstration of his online environmental health news search service and RSS feed. Dr Myers is the founder and CEO of Environmental Health Sciences. He's co-author of the groundbreaking book, *Our Stolen Future*. And it is he who manages that website as well as www.environmentalhealthnews.org and CHE's science pages at www.protectingourhealth.org.

~ **PETE MYERS** - For those at computers, the url is www.environmentalhealthnews.org. Some background with these websites is that we want to make it easier for reporters and others to write intelligently about environmental health and new findings. There is "bait and reward" for reporters, people doing research and health affected constituencies. You can see the three columns: on the left is news; the center column contains synopses of new science and background materials; on the right are organizational reports. [Pete then ran us through how one conducts multilayered searches by starting on the Archives page using the left side menu options. These are hierarchical searches where you can use the layers on the left to refine your search as desired, refreshing at any time to switch your lens.]

One of the novel features of this website: No matter where you are on the website, under the masthead it says "syndication" which explains the RSS news feed. You can see two examples of RSS feeds on that page. [We clicked on Our Planet's, which opens up a United Nation's webpage that tailored the feed as per their own specifications and displayed it]. Half a million people a month come to this page…You see the top three news items, and then you can click on "Other Stories," where you can see their choice to display the top 15 stories on a second page. They've chosen environmental health globally in broad terms…

BARBARA DAVIS: This is fabulous.
ALISON CARLSON: So, Our Planet set up this RSS feed according to layers of information that they chose?

PETE MYERS: Exactly. Our Planet is one example of what it can look like. When someone hits on their site, their site asks me for their tailored feed – they ask us for the answers to their pre-set query. We send it back and they format it the way they've prefigured. And it's constantly refreshed.

ELIZABETH SWORD: Another example is at our website, tailored for children's environmental health at www.checnet.org.

NANCY HEMENWAY: And we at INCIID have installed it also with an aggregator on our webpages that will launch soon.

PETE MYERS: Going back to Archives at www.environmentalhealthnews.org, you can form any search you want…Let's do infertility. You can simply type in the word infertility in the text box – on the left. Or

under Reproductive Disorders, you'll also see the subcategory of infertility. IF you click on that you come up with results. If you scroll all the way to the bottom of the page, you'll see the orange RSS feed link. [We then clicked on that and saw how one can define, grab and format an RSS feed…]

ALISON CARLSON: That was a fabulous and fast run through. Because I speak to so many different constituencies with varying interests in the topic, I can say that this is an incredible service – especially for our NGOs looking to understand what it is they need to be learning and paying attention to, and secondly, how they can be talking to their constituencies about it. This service will go along way in many ways and for many people, and for the patient groups developing web pages on the environment and fertility topic.

PETE MYERS: One additional thing is that we are now stepping up our synopses of new science, so this site and service should have additional value going forward from that perspective.

BARBARA DAVIS/SHANNA SWAN: How far back do you go on Environmental Health News?

PETE MYERS: News stories go back about a year. Citations go back about 5 years.

ALISON CARLSON: Respecting our commitment to keep these calls to one hour, I need to bring this call to a close. Could we agree on a date and time for our **next quarterly call** in September? [Tentative agreement on **September 27, 2004 at 9:00am CA time and noon EST**. **barring any complications that come to light over the summer.** Please hold that time on your calendars.]

All participants please feel free to contact me for other participants contact information so you can keep your dialogues going.

Thank you.