Collaborative on Health and the Environment (CHE)
Environmental Contaminants and Fertility/Early Pregnancy Compromise Working Group
Implantation/Miscarriage and Environmental Chemicals
Teleconference # 8 Thursday, Dec 8, 2005 9:00-10:00 am Pacific (41 callers)

Facilitator:
Alison Carlson

Moderator:
Dr Ted Schettler

New Science Updates:
Dr John Peterson Myers
Venners, SA, S Korrick, X Xu, C Chen, W Guang, A Huang, L Altshul, M Perry, L Fu, and X Wang. *Preconception Serum DDT and Pregnancy Loss: A Prospective Study Using a Biomarker of Pregnancy*. (2005) American Journal of Epidemiology.

Mackenzie, CA, A Lockridge and M Keith. *Declining Sex Ratio in a First Nation Community*. (2005) Environmental Health Perspectives 113: 1295-1298.

Speakers: (see introductions below)
Dr Warren Foster – Overview of Environmental Contaminants and Spontaneous Miscarriage
Dr Hugh Taylor – Presentation of recent research demonstrating effects in mice of a commonly used endocrine disrupting pesticide, methoxychlor, on the expression of a gene required for reproductive tract development and function: permanent developmental suppression of Hoxa10 expression and diminishing uterine desidual cell response necessary to support embryo implantation. Fei, X, H Chung, and HS Taylor. *Methoxychlor Disrupts Uterine Hoxa10 Gene Expression.* Endocrinology 146:3445–3451.

Followed by Q&A and discussion.

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ALISON CARLSON: (WELCOME & "HOUSEKEEPING" NOTES) Welcome to our 8th quarterly teleconference. Today we are featuring a couple of brief science updates by Dr Pete Myers, and we'll follow that with an overview by Dr Warren Foster on what the science tells us about environmental contaminants and implantation problems and pregnancy loss, or miscarriage. Then we have a presentation by Dr Hugh Taylor of his recent study on a common pesticide and its effects on the expression of a gene important to reproductive tract development and function.

We have many new CHE Fertility members, some of whom are on a CHE teleconference for the first time, so I'd like to remind new members who haven't yet had a chance to send their email introductions to me for distribution over our listserv to please do so.

An addition to this call's guest list since I circulated the participant list last week is a science and environment writer, Michelle Nijuis, who is observing as a part of her research for an article on CHE for Orion magazine. We've okayed Michelle's observing today on the condition that, in the event she is interested in covering any of the content of the call in her article, she obtain permission. If anyone wishes to register in advance that their participation in, or remarks on, this call are off the record, please say so now.

CHE Fertility is very eager for our members, guests, friends and colleagues to forward the announcement of the Vallombrosa Workshop proceedings far and wide, and to share the downloadable PDFs as you see fit. Certainly post or link to them from your web pages. You can find them at the CHE Fertility page at www.healthandenvironment.org/working_groups/fertility – and don't hesitate to ask me to send you the announce, or hard copy printed booklet of *Challenged Conceptions*, the lay monograph we commissioned as a companion piece to the Vallombrosa scientific consensus statement on environmental contaminants and human fertility compromise. CHE Fertility is happy to ship multiple copies of the booklet for meetings and conferences where it would be relevant, especially if you can help with or cover shipping costs.

A reminder for all of you who speak today, please explain acronyms – and keep your remarks geared so that lay participants can grasp the science…so, provide lay translations and take-aways where necessary.

Finally, introductions:

Dr John Peterson Myers, PhD is founder and CEO of Environmental Health Sciences, which publishes www.EnvironmentalHealthNews.org and www.OurStolenFuture.org. And he is a co-author along with Theo Colborn of the 1996 book *Our Stolen Future.*

Dr Warren Foster, PhD, is Professor and Director of IVF and the Reproductive Biology Division in the Dept of OB-GYN at McMaster University. Prior to joining McMaster's faculty, he served as Assoc Director of Women's Health and Director of Research at Cedars-Mt Sinai Med Center in Los Angeles, and as the head of Reproductive Toxicology at Health Canada. Dr Foster is a steering committee member and participant in the American Society of Repro Medicine's Environment Special Interest Group-in-Formation – and his research interests are broadly focused on investigation of the impacts of env toxicant exposures on human health, including into: The relationship between developmental exposures and thyroid and immune function in children; The relative potency of toxicants on aromatase expression and activity; Toxicant induced modulation of tissue remodeling enzyme expression in estrogen sensitive target tissues; And dioxins and dioxin-like chemicals in endometriosis.

Dr Hugh Taylor, MD is a board certified specialist in OB-GYN and in reproductive endocrinology. He is Director of Research in Reproductive Endocrinology as well as Associate Director of the Division of REI at Yale. His postdoctoral training included a fellowship in molecular biology, and his current clinical research centers on implantation, endometriosis and menopause. His basic science research focuses on the regulation of developmental gene expression by sex steroids – and he is best known for his work on the genetic regulation of implantation and estrogen regulation of uterine development.

Dr Ted Schettler, Chair of CHE's Science Work Group and Science Director of the Science and Environmental Health Network, has kindly agreed to moderate this call, so I am turning the conducting of the speakers and ensuing discussion over to Ted…

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TED SCHETTLER: Good morning. We'll start, as we often do, with a brief update by Pete Myers on some important research results.

PETE MYERS: With these calls being only quarterly, it's hard to choose, because there's so much research coming out now. I'd like to draw your attention to two papers. There are links to these and other resources for this call posted online. **[If you are a CHE Partner, you can go to: www.healthandenvironment.org, log in with your CHE Partner user name and password, then click on working groups/fertility and early pregnancy compromise]** If you go to [the url during the call was] www.ourstolenfuture.org/CHEfertility.html, you'll find the links.

First paper: Venners et al published in the *American Journal of Epidemiology* earlier this fall, a fascinating study on early pregnancy loss in Chinese women exposed to DDT. They measured chorionic gonadotropin levels to detect conception and early pregnancy loss in 372 clinical pregnancies. In that study group there were 128 early pregnancy losses and 36 spontaneous miscarriages after clinical detection of pregnancy. They then grouped the subjects into tertiles of DDT serum concentration, and they found a significant monotonic relationship between DDT levels and likelihood of early pregnancy loss, with the odds ratios comparing the lowest exposed group to the highest exposed group of over 2; and the lower confidence interval on that was 1.26. This study is important for a number of reasons, not the least of which is that it's important data for the ongoing debate on the costs and benefits of DDT use for malaria control efforts.

The second paper: published by Mackenzie, et al in *Environmental Health Perspectives,* also earlier this fall. Many of you will know that there have been several papers noting a small but significant decrease in population sex ratio at the country level…Canada, the US, European countries…with fewer boys being born than predicted by the normal sex ratio of roughly 0.50 to 0.51 boys out of 100. A small number of papers have also noted large decreases in relatively small populations; for example after the Seveso accident in 1976, and also a group of people studied around a Russian pesticide manufacturing plant. In both cases there were very large deviations from the expected sex ratio, and the deviations took place in the offspring of exposed fathers, not the exposed mothers.

The large-scale changes among countries have been interpreted by some as being a small but significant population-wide decline, from a factor---whatever it might be---that could be affecting the population at large, significantly but slightly shifting the probability of fathering a boy. The other interpretation is that the shift is a result of a growing number of hot-spots, like the Seveso and Russian examples, that pull down the probability for the population as a whole.

Mackenzie's study deals with a population of First-Nation Canadians who, in an industrial complex in southwest Ontario, a place called Sarnia, had dropped in sex ratio in the last decade from about 0.51 in 1992 to about 0.3 in 2004. Prior to 1992 the sex ratio was relatively stable. They also looked at the ratio for another community of the same First-Nation, the Cree, living nearby but away from the industrial plant, which was stable. The media, who have been following this, just reported that Canadian whites living near Sarnia also declined in sex ratio, but not as much as the First-Nation people. I think this study suggests that it might be very interesting for reproductive health epidemiologists to begin looking systematically at "fenceline" communities for deviations from expected sex ratios. It could be very important step that could probably be taken relatively simply.

TED SCHETTLER: Next is Warren Foster, who has prepared a 10-minute overview of the science on environmental contaminants and implantation and pregnancy loss-related issues.

WARREN FOSTER **[To view Dr Foster's Powerpoint slides, please log in at www.healthandenvironment.org; go to Working Groups, then Fertility and Early Pregnancy]**: I'm going to speak on spontaneous abortion(SA) and give an overview of the science linking environmental contaminant exposure with SA. I've not done one of these before, so I've got slides here to help guide me. I will make them available to Alison for the CHE Web site, if she would like to post them. I'm going to start with my objectives for today, which is to give you some definitions, discuss the highlights of the literature (in 10 minutes we obviously cannot go into detail), and to describe a "weight of evidence" approach I've taken in analyzing this data. I've also been working on a review paper that I will also provide to Alison for the Web site.

Definitions (so everyone's on the same page): Spontaneous abortion is classically defined as the loss of the products of conception prior to viability of the fetus at 20 weeks of gestation, or below 500 grams. There's a condition that we also deal with in the fertility clinic, individuals who are "habitual aborters:" These are patients who serially miscarry three or more times. You'll see this come up in the literature as well.

When I look at the data set, we're getting studies coming from epidemiology, exposures and biomarker studies, animal study literature, in vitro studies. In order to make sense of these different types of data and the abundance of data, we follow a "weight of evidence" approach. Are there changes in the prevalence of the disease or adverse outcomes of interest? What does the epidemiologic literature, on balance, tell us about potential associations? Is the literature consistent in what it's finding? Does the exposure precede the adverse outcome we're interested in, or does it occur roughly at or shortly after the adverse event started to change? And what does the experimental evidence tell us: are we seeing in our animal models changes that we think are consistent with what's being seen in the human population? Then we look at biological plausibility; in this case we're looking at things like dose-response; and is there a logical mechanism that can actually explain the association that we're looking at? In other words, if we think it's being mediated by an estrogen receptor-regulated pathway, does the compound that we've identified as the "bad actor" actually act through this receptor-mediated pathway, or not? The last issue is probably the most difficult to look at: the issue of reversibility. So, if we take people out of the contaminated region, or if there's remediation to sites, do we see improvement in their condition, or changes in the adverse outcomes? Those are the types of things that we look for when examining the literature.

Just some background information: On risk factors for spontaneous abortion are things like maternal smoking, advanced maternal age, and previous history of fetal death. What causes a spontaneous abortion: primarily what we think about and see most often are things like chromosomal abnormalities, and they often occur in the first trimester and result in the loss of the pregnancy. Other things like chemotherapy and radiation, as well as anesthetic gases, have also been linked. There's growing interest and concern that chemicals that are present in our environment and in our food may be linked with these adverse outcomes as well.

One thing I've tried to look for is trend data. I've performed an electronic search of the literature using PubMed, MedLine, ToxNet and others, to find articles relating to spontaneous abortion and incidence prevalence and frequency; and to date I haven't been able to find compelling evidence that there's a change. It doesn't mean there isn't one; the issue is more that it's not being written about, or the data's not being collected. This paucity of data represents a major gap in this area, and hopefully one we can see addressed in the future.

Epidemiological data: There have been numerous reports that have failed to find associations with pesticide exposures and organochlorine exposures - but there have been others that have shown a link, and are quite interesting. I'm going to highlight a couple: One is a study by Aschengrau and Monson that showed that paternal exposure to the pesticide 2-4-D was linked with an increased risk of spontaneous abortion. The interesting thing is that this was paternal exposure - which links nicely with a study conducted by Savitz et al as well as one by Arbuckle et al - that demonstrated there was an increased risk of spontaneous abortion in couples who worked on a farm or had exposure to thiocarbamates, carbaryl, and unclassified pesticides. What was interesting was that the study showed the critical window of exposure was the three months prior to conception, including the calendar month of conception. This seems to be something that's emerging as a consistent theme, that exposure can have an effect on male semen quality: We see drops in sperm count, we see changes in sperm chromatin structure assay, that would suggest there's direct effects there, and seems to be following through also with adverse outcomes in spontaneous abortion. Other studies have shown that wives of workers exposed to organochlorine pesticides have an increased risk of spontaneous abortion and stillbirth (e.g. Rupa et al, 1991). In India as

well, circulating levels of DDT were found to be higher in women with spontaneous abortion and stillbirth, which is consistent with data coming out of China more recently.

It's not just pesticides - although they are found to be a major concern and supported more recently by work by Bell et al published in 2001, which found that maternal pesticide exposure that occurred during the third to eighth week was a critical window of exposure for gestation, and had the greatest effect on spontaneous abortion…Other compounds that are potentially worrisome are things like drinking waters disinfectants, like the trihalomethanes, chloroform and so forth…These were associated with increased risk of spontaneous abortion as well in work by Waller et al., published in 1999. What's interesting is when we look at these and the acceptable level of drinking water disinfection by-product exposures in places like Winnipeg, in certain months of the year they far exceed the allowable level of exposure. This has created an opportunity to look at communities and see whether there is a relationship there. However, that has not been followed through, to my knowledge, yet. The issue I want to highlight from these studies is paternal exposure three months prior to and including conception, as well as maternal exposure during the third to eighth weeks of exposure are critical windows, and in future epidemiological studies these are windows that we would certainly want to look at.

There are also weaknesses in the data. I summarize these as: In the majority of studies there is no direct measurement of exposure. In many cases the exposure level is estimated retrospectively by asking subjects to recall their pesticide use prior to and during pregnancy, or "how close did you actually live to a chemical plant, or a site where there was an accident, or a waste incinerator." Other problems with the literature review are such things as inadequate methodology; failure to control for confounders; and in some of them there are problems with small sample sizes as well. Regardless, I think on balance there is enough evidence from the epi data to say that there's something going on here that we really need to be focusing on, and that the link is potentially credible.

Experimental evidence is a little more interesting. My group has previously shown that the effects of hexachlorabenzene, when we treated Cynomolgus monkeys with it, we found there was a change in the circulating levels of progesterone during the luteal phase. This is particularly important because it's progesterone that's providing support to maintain the endometrium for implantation of the conceptus, and if we've altered that and reduced it below a threshold, the probability is pretty good that the pregnancy will not be able to be maintained. Indeed there is one study done by John Jarrell looking at women in Turkey who had exposure to HCB, and they were found to have a higher risk of spontaneous abortion. The "big nasties" that everyone's familiar with - TCDD, dibenzofurans, PCBs - have also been associated with abortion in rhesus monkeys and several studies in rodents as well, where there have been problems with implantation failures or spontaneous re-absorptions.

Anne Greenlee, I think she's on the line, had a nice study in 2004 that demonstrated that several pesticides can attenuate embryo growth as well as increase programmed cell death, or apoptosis, in embryo cells. This is a really nice study; if you haven't had a chance to look at it, I suggest you do. She's also conducted another study looking at in vitro experiments with o,p'-DDT and shown again here that there is retarded development at the morula stage as well as increased apoptosis in the blastomeres. These are nice experimental studies from in vitro that support that with compounds people are being exposed to, there could be adverse effects. She's using levels that people are being exposed to in the general population - so these studies provide nice evidence that, if these levels are being achieved at the uterus and within the conceptus, that there could indeed be adverse effects. There's been a similar study from Japan, looking at bisphenol A, and showing much the same outcomes, although they didn't look at apoptosis.

I think on the basis of the evidence from the animal data as well as the epidemiological data, there is biological plausibility that these things could be having adverse effects. Emerging evidence from in vitro

fertilization demonstrate that slight media changes can alter gene expression and could play a role in implantation failure, which also provides support.

In summary, taken together, epidemiological, animal, and in vitro evidence - with biological plausibility – provide moderate support that environmental contaminants, and pesticides in particular, can induce spontaneous abortion. I would recommend that further epidemiological studies include direct measurement of exposure. I would support further animal studies on implantation and embryo development. Also studies that look more closely at the maternal/ fetal placental interaction and identify relevant mechanisms of action that are relevant. And the emerging epigenetic area is particularly exciting.

TED SCHETTLER: Thank you very much, Warren. Hugh, can you take about 10 minutes to present a summary of your interesting study of pesticides and gene expression?

HUGH TAYLOR: I want to talk to you about a particular pesticide, methoxychlor, which is a known endocrine disruptor, which we've studied both in human cells and in a mouse model. This group well knows that many endocrine disruptors are estrogen-like molecules, that I think disproportionately affect the reproductive tract. We've been studying for many years now the genes that regulate the development of the reproductive tract and the differentiation of the endometrium that in the adult allows for fertility and implantation of the embryo. We initially reported an effect of DES on the genes that we studied; so that's how we got interested in the endocrine disruptor field.

Let me give you a bit of background, to put this in perspective, on the development of the reproductive tract and in particular the HOX genes that we study, and their role in reproductive tract development. The female reproductive tract develops from a uniform paramesonephric duct, and it eventually differentiates into fallopian tubes, uterus, and upper vagina. It turns out that HOX genes help to give differential identity to different portions of the developing female reproductive tract. These HOX genes are highly evolutionarily conserved genes that are actually involved in patterning of the entire embryonic body axis; so there are HOX genes at the anterior end of the embryo that drive that toward development of a head, or brain, and those at the posterior or caudal end drive development of a tail in an animal or the lumbar-sacral region in a human. It turns out these same genes that are very important for giving identity to the developing body axis also give developmental identity to the reproductive tract. So a particular HOX gene expressed in one portion of the developing tract will cause it to become a fallopian tube, and so forth.

One in particular, HOXA10, the subject of this study, drives development of the uterus. Several years ago we reported that DES changes the expression pattern of these HOX genes - particularly the ones that drive the development of the reproductive tract. These HOX genes are very sensitive to estrogen. After DES exposure, the pattern of expression of these genes is altered such that the genes tend to be expressed lower in the reproductive tract. So we think that maybe what's happened with, for example, vaginal adenosis that is found in women who were exposed to DES (in the womb,) is that glandular tissues that are normally found higher in the reproductive tract have been driven to lower positions in the tract. This occurs because the genes that govern their development are expressed in the wrong position as a result of the DES exposure. Similarly other effects on DES-exposed women could be explained on this basis, that the developmental genes that give identity to the reproductive tract are shifted and expressed in the wrong place.

After we found this mechanism of DES action in the genes that encode development of the reproductive tract, we started to screen for some of the environmental and industrial endocrine disruptors that are known to act like estrogens, to see if any of those had a similar effect, a similar mechanism of action on the female reproductive tract. The report I'll talk to you about today is on methoxychlor, an organochloride pesticide originally developed to replace DDT, and which is known to be a xenoestrogen, to have estrogen-like effects, and to be an estrogen-like endocrine disruptor. We looked at the effect of

giving this pesticide to mice early on, just after birth when *their* reproductive tracts are just developing. A newborn mouse reproductive tract is similar to a 20-week gestation human reproductive tract. The female reproductive tract develops later in the mouse than the human. We administered methoxychlor to these mice, and saw an effect very similar to that of DES: expression of the gene that drives development of the uterus, HOXA10, was much lower in the mice exposed to methoxychlor. And then, weeks after methoxychlor exposure, expression of this gene was still much lower. So methoxychlor locked in some sort of permanent repression of this gene that would carry on into adulthood. In mice, when this gene is knocked out, we get zero implantation. In mice, when this gene is knocked out, we get zero implantation. Embryos can't implant; even a wild-type, normal embryo, can't implant in a uterus of a HOXA10 knock-out mouse. So, after exposure to methoxychlor we're seeing decreased levels of this gene that we believe may very well be a mechanism of reduced fertility, and perhaps some subtle developmental defects of the uterus.

We also looked at this in human cells in culture. In human cells we found that high doses of methoxychlor actually increased this gene, but when given in combination with an estrogen (estradiol) the methoxychlor seemed to block the estrogen receptor binding to the regulatory regions of the HOXA10 gene and block its expression. So methoxychlor alone worked as a weak agonist and had a partial estrogen effect, but when given at high doses with estrogen, blocked the effect of estradiol. So it looked like, both in human cells and in mice, this pesticide, methoxychlor, could block this gene, potentially leading to problems with uterine development, potentially leading to problems with implantation and pregnancy. We have found that humans with certain implantation defects - fairly common diseases - often have very low levels of HOXA 10 expression. So that the permanent changes we saw in the mouse after exposure to methoxychlor correlate with some of the conditions we see in humans – when expression of this gene is lower, implantation rates are lower. Methoxychlor could potentially impact implantaion and fertility by altering expression of this gene.

It's hard to do these studies in humans, because methoxychlor is not stored, it's metabolized. So if it's *in utero* exposure we're concerned about, when the reproductive tract is developing, it's not something we can measure later on when someone presents with recurrent miscarriage or failed implantation. But it very well may be that disrupted expression of this gene is a marker of exposure. It seems to be a common mechanism, at least of DES and of methoxychlor action. This gene is one of the common targets of these estrogen-like endocrine disruptors that effect the reproductive tract. We may have found a common mechanism of action. I'd like to further test humans for exposure to these agents, if I can identify a population that has a single exposure. However, as you know, most humans have so many exposures that it's hard to isolate a single compound. But this seems pretty convincing, that at least this compound and DES work in a similar way to alter the expression of key genes that are necessary for development of the reproductive tract and are essential for implantation later on. Proper expression of these HOX genes is necessary for fertility. So, we believe that this effect of methoxychlor is probably playing a key role in this process. The doses we gave, though, are higher than people would typically experience in every day life, but similar certainly to what someone could potentially get from an occupational exposure. For example, someone working on a farm with exposure to methoxychlor. We think this is relevant to certain segments of the human population.

TED SCHETTLER: Thank you Hugh, for that interesting summary. We're going to open the floor for questions and comments, now. Let me get this started by asking Anne Greenlee, from Oregon Health Sciences University, who has been doing some of this research…do you have reflections on what you've heard?

ANNE GREENLEE: I've very much enjoyed morning and the very nice presentations. I have a question for Hugh Taylor and his study on methoxychlor. Building on what Mike Skinner found on the epigenetic observations with regard to vinclozolin and male fertility effects. I'm wondering if you've exposed these

mice in utero, and have you seen reduced implantation rates, and whether or not that carries on in future generations, suggesting that there may be some sort of epigenetic regulation, not necessarily a gene change, but a change in the way HOXA10 is expressed?

HUGH TAYLOR: Absolutely. We started to explore that and have shown that HOXA10 is methylated in certain medical conditions, and we're looking right now to see if exposure to DES or methoxychlor results in similar or other changes in methylation, or other epigenetic changes that would regulate this gene. We have not looked for multi-generation effects, but based on Skinner's work, absolutely, we're going to do that. Again, the effect on expression of this gene is there long after the exposure is gone, so there must be an epigenetic-type mechanism at play that keeps this gene repressed throughout the life of the mouse.

TED SCHETTLER: I think I also heard Gayle Windham come on. Gayle is a reproductive epidemiologist with California Dept of Health Sservice, who has also done some work with environmental contaminants and miscarriage. Gayle, do you have thoughts or comments?

GAYLE WINDHAM: Maybe a couple of comments. I also enjoyed the talks. On the specific topic, we looked at hormone levels in women exposed to DDT, DDE and also found lower progesterone, which ties in with some of Warren's work. However, we didn't see it with hexachlorabenzene, but they were more exposed to a few things….

WARREN FOSTER: Yes, in the Turkish couples and our animal studies are much higher levels, so…

GAYLE WINDHAM: It was interesting the comments about the trend data…I think it's the nature of this topic. Miscarriage hasn't been subject to screening programs; I think because it's difficult to diagnose. People are becoming more aware of it…When we do studies now we can find higher rates, but I think that's because our technology allows us to detect it earlier and earlier. Because it's very dependent on when you detect pregnancy…Most losses occur early and there's kind of a continuum of loss. It is a difficult thing to monitor on a population basis, which does make it harder to make these links.

TED SCHETTLER: Thanks, Gayle. Now I'd like to open up the discussion more broadly.

PAT HUNT: I'd just like to reinforce what Gayle just said. Because miscarriage seems to be so common in our species and sort of hot-wired into our species, I think the bias in reporting is something we all have to be concerned about. I always have concerns when the data are not stratified by age, because we know there's an increase in pregnancy losses - both chromosomally normal and abnormal with age. So, a study that would get some baseline data, be able stratify by age - *and* karyotype would be really important. For these implantation failures, you would expect to see a dramatic increase in loss of chromosomally normal pregnancies. So just karyotyping miscarriages could go a long way in giving us mechanistic data.

RHODA NUSSBAUM: In listening to the conversation, I hear a lot about the dearth of data that's available, and I wonder if people are having difficulty finding funding for these studies, or finding actual resistance to being able to do their work.

PAT HUNT: There were several studies in the early '70s, one in New York and one in Hawaii, that were 10 year studies of miscarriages, first trimester losses, and they were karyotyping all the losses, and looking for racial differences, and looking for rates of chromosomal abnormalities - and these gave us some of the first data we have on chromosomal abnormalities. Those studies are hideously expensive to do, and the argument for trying to do them again has been hard to make. But in view of the environmental concerns, we could certainly design some new studies that would get favorable attention, I think.

GAYLE WINDHAM: I think in the past they were harder to fund, (the attitude was) this is a natural loss. I think it's gotten better. One problem with the karyotype studies is that, of course, the woman has to save a tissue specimen, which is difficult to do. You tend to get them from later pregnancies. So there are a lot of methodologic issues. We (and others) have done several studies where people collect their urine - Warren mentioned one of the studies looking at HCG - but again that's very labor-intensive for participants, so you tend to get a select group, potentially.

MARY LOU BALLWEG: I'm with the Endometriosis Association. * We have a great deal of epidemiological data on miscarriage in women with endometriosis. We have been looking for someone who has a good background in miscarriage to help us analyze that data.* I'd like to just throw that out there…we have not found the right person to help us look at that data in a really scientific way.

WARREN FOSTER: Hugh, you looked at methoxychlor, and you mentioned the levels you used were higher than what someone might usually encounter; however they might be reflective of someone in an occupational setting. I'm wondering if you've thought about looking at methoxychlor in combination, say, with an o,p'-DDT or bisphenol A to see whether or not you can get the same type of changes you documented with methoxychlor but at lower dose levels. In other words, are you getting any additivity?

HUGH TAYLOR: We haven't looked at that yet. We've been screening a lot of other compounds, including BPA, which has not quite the same effect, but does affect HOX gene expression. We've got a manuscript submitted on that right now. We're at the point right now where we're screening to see which have any effect at high dose levels on HOX gene expression. We've got several to do. We're inclined to think BPA does. But our next step will be to try combinations at lower doses, which probably more closely mimics human exposure.

WARREN FOSTER: Another question that comes at it from a totally different direction is, have you looked at any potential therapeutic interventions, or things like Resveratrol or folic acid that might antagonize or mitigate against these effects.

HUGH TAYLOR: We have not yet, no. We're still identifying and characterizing them. But that's a long range plan.

WARREN FOSTER: That makes sense.

SARAH JANSSEN (University of California, San Francisco): A couple of questions for Dr Taylor. One is, is the HOXA10 gene directly regulated by estrogen? In other words, is there an estrogen response element in the motor region, or is this a downstream effect?

HUGH TAYLOR: There is an estrogen response element; we've published that. We've shown it is actually differentially through that element regulated by DES, even if in an unusual way. But yes, it is directly regulated through <inaudible>.

SARAH JANSSEN: A follow up question: Estrogen is also very important for male reproduction. Estrogen receptors are expressed at very high levels in the head of the epididymis. So I'm wondering if the HOXA10 knock-out male mice are infertile.

HUGH TAYLOR: They're not infertile; but they do have some defects, but not enough to impair fertility.

SARAH JANSSEN: The defects are lower sperm count? Or….

HUGH TAYLOR: Just anatomic defects; the shortening of the epididymis, and assorted defects, but not enough to impair fertility. But the females are absolutely infertile; you get zero implantation. Again, the embryos are fine; you can remove them and put them in a wild-type pseudo-pregnant mass and they'll develop normally. The knock-out animals will ovulate and produce embryos that can be transferred will do fine. But if you put a wild-type embryo into the uterus, they do not implant. It's a clear implantation-uterine defect.

ALISON CARLSON: I would like to ask Hugh, from a fertility patient perspective, typically in speaking to our doctors about repeat losses or a loss, we hear the message that it's really usually an embryonic problem, not an implantation problem. I just wonder what you have to say about that, because the messages that patients get are that, you know, they are looking for problems in the embryo…

HUGH TAYLOR: Yes. In infertility practices often we are seeing older women, and as Pat Hunt pointed out there is certainly an increase in aneuploidy which is a function of age. In a younger patient I would be more vigilant in looking for other causes of pregnancy loss, including exposure to these agents

TED SCHETTLER: I recall a paper recently that described abnormal expression of HOXA10 in women with endometriosis, which ties into what Mary Lou Ballweg was commenting about. Do you have a comment on that?

HUGH TAYLOR: Absolutely. We found that many things can influence the expression of this gene. And certainly patients with endometriosis have many genes that are mis-expressed or have altered expression in their endometrium, and we think leads to lower rates of implantation. In women with endometriosis what we've found is that they have some expression of this gene. In women without endometriosis it is normally up-regulated in the window of implantation, right around the time when the embryo normally reaches the uterus in the early-to-mid luteal phase. In women with endometriosis, on average, this does not occur; the expression is much more constant.

We've also reproduced this in collaboration with Asgi Fazleabas. We presented it at the last SGI meeting and we have a manuscript ready to submit. We looked at experimental endometriosis in a baboon model. We were able to show that by creating endometriosis in the baboon, the eutopic endometrial expression of HOXA10 drops after approximately a year of endometriosis exposure. So, clearly the endometriosis is causing a change in the endometrium, and causing a change in one of the key genes that we know is absolutely necessary for implantation to occur. So we think that endometriosis does have an impact on implantation; certainly there are several reports in the clinical literature that would suggest that, including a summary, a large meta-analysis, done by a group at UPenn that suggests that in IVF, women with endometriosis have lower implantation rates. We think it is in part a direct effect on endometrial gene expression, genes that are important for implantation, such as HOXA10.

LAURA FLETCHER: I'm an emergency room physician who recently began working in a hospital in the Central Valley, California. It's an interesting juxtaposition of the scientific data we're discussing and what I'm actually seeing in the emergency room in terms of women who are having miscarriages - many young women. It is an area of high pesticide use. I wonder if there are any epidemiological studies that are going to collect this data in areas of high pesticide use, such as the Central Valley.

TED SCHETTLER: Warren, do you have comments on any on-going studies addressing areas high pesticide use?

WARREN FOSTER: I don't know of any. I know of the farm hand study conducted in Canada, and the work Gayle Windham has done, but I don't know of any ongoing in California at this point in time.

GAYLE WINDHAM: I don't know of particular studies on miscarriage. We have an environmental public health tracking system, and certainly we are looking at that area in relation to other reproductive outcomes; but because it's a tracking system, it's based on outcomes that are monitored, so we don't have miscarriage in there.

DIANA LEE: In reference to the previous question, Dr Brenda Eskenazi at UC Berkeley is conducting a study on pesticide use in the Central Valley, known as CHAMACOS, and other staff in our office may have additional information also.

ALISON CARLSON: Diana, sorry I couldn't hear you. You're saying that the CHAMACOS study *is* looking at pregnancy loss?

DIANA LEE: I'm not sure…I know they're looking at pregnancy outcomes.

GAYLE WINDHAM: But pregnancy loss isn't included, I don't think.

TED SCHETTLER: They are tracking development of the children, including neurodevelopment, but I don't know of any tracking of pregnancy loss in that population.

ALISON CARLSON: And what about fertility, I'm assuming nothing on the upstream end?

TED SCHETTLER: No, I think not. They're enrolling women who come into the clinic for prenatal care, and entering them into the various studies.

LAURA FLETCHER: I'd just say that of all the hospitals I've ever worked in, I'm seeing more spontaneous abortions in the Central Valley hospital than I ever have, on the basis of regular shifts.

TED SCHETTLER: You know, it raised for me…and I'm sorry Warren had to get off the phone…when he went through his criteria for drawing conclusions about exposures and outcomes, it sounded a lot like the Bradford-Hill criteria, with some added information having to do with laboratory animal work. When all was said and done, he had reviewed the literature and concluded that all the criteria had been fulfilled, that there was a weight of evidence that suggested a link between pesticide exposure and spontaneous abortion. So, at what point does this turn into a public policy conversation where we say causality according to the Hill criteria has been met, and what do we do about this?

ALISON CARLSON: That's a great point, and a question I've come to a lot. And often I've run into the problem of trend data. To hear that a ER physician in the trenches is seeing earlier and more spontaneous abortion in practice is very interesting to me; but it seems like in the jump to the policy level, there's always questions about trend data, and that's where I find it very frustrating.

HUGH TAYLOR: This is where some of the mechanistic, or gene expression data may be helpful. If we know that some of these agents have a common mechanism of action, and we can show that these genes are being affected in this population then it's more than just trend data, we'd have a strong causal link to the mechanism of action. * I'd love to take a look at endometrial biopsies from women in these areas of high exposure. We haven't done that, but if someone could help me do that I'd love to look at that. *

ALISON CARLSON: Hugh, you said if someone could help you get biopsies in the problem areas?

HUGH TAYLOR: Yes, then we could see if some of the changes in gene expression that we're seeing are showing up in these women. We can create it in an animal model, and we can show in disease states that these genes are essential for implantation. But if we could show that these same effects are showing up in

these women, although not complete proof of cause and effect, is certainly very suggestive that the trend is related to the same sort of biological phenomenon that we've described in experimental scenarios.

MARY LOU BALLWEG: Hugh, I think you probably could get those tissue biopsies from Linda Birnbaum and the cluster study in West Virginia, where Agent Orange was produced during the Vietnam War, and they are diagnosing endometriosis cases in incredible numbers and studying it in depth. Linda Birnbaum is the EPA officer in charge of it. I can't imagine that they couldn't get those tissue samples for you.

TED SCHETTLER: We are coming to the close of this call, so if anyone has a last comment?

ALISON CARLSON: Just many thanks to presenters. I will send notice of the audio file and transcript, along with Dr Foster's slides and related items, when they are posted on the CHE Fertility Web page. And I will announce our next teleconference early in 2006. Thanks to all who participated today, and happy holidays.