

Collaborative on Health and the Environment (CHE)
Environmental Contaminants and Fertility/Early Pregnancy Compromise

New Science Reports

Working Group Call # 7 Mon. June 27, 2005 9:00-10:00 am.Pacific (40 callers)

Speakers:

Dr Michael Skinner, Director and Professor, Center for Reproductive Biology, Washington State University - *Transgenerational epigenetic alterations in spermatogenesis in successive generations of male rat offspring following transient exposure of pregnant females to a fungicide (vinclozolin) or pesticide (methoxychlor): How a pregnant mother's environmental toxin exposure can effect her great grandchildren's disease status.*

Dr Shanna Swan, Professor, Dept of OB-GYN, University of Rochester School of Medicine and Dentistry: *Prenatal phthalate exposure at environmental levels can adversely affect human male reproductive development.*

Followed by **Q&A and discussion.**

Facilitator: Alison Carlson

Moderator: **Dr Pete Myers**

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ALISON CARLSON: A few brief notes:

- A number of you have been involved in giving very valued comments on the Vallombrosa Consensus Statement on Fertility and the Environment that is arising out of the small workshop on this topic that CHE co-hosted a few months ago with Women's Health @ Stanford. For those of you who are wondering what's happening on that document, it's just now undergoing a second rewrite, which we hope to conclude by July 10th, after which we'll be handling copy editing, circulating for sign-on, etc. And rather than releasing it in the sleepy summer months, we're holding it for release in the fall, and are going to be encouraging widespread dissemination and use of this statement. You'll be hearing more about it when the downloadable PDF version is available. Accompanying the consensus statement will be a lay backgrounder on the issues and the science, something we hope will be useful for those of you with environmental health education, policy and advocacy programs. Both of these documents will be in downloadable PDF form, and we're going to encourage borrowing, stealing, and wholesale use of it on your Web sites, and in your libraries, meetings, conferences and talks, etc.
- A reminder: You should have all by now seen the first periodic call for member announcements via our listserv and the results of that listed in my latest e-newsletter over the weekend. So, don't hesitate to email me or Mary Wade at any time with news of your upcoming presentations, publications, research studies, conferences, and that sort of thing.
- In preparation for today's call Pete Myers set up a hidden url with resources, including PDFs of the study publications and PowerPoint slides for Dr. Swan's presentation. You should have gotten that url in the call reminder, but you can also just go to www.ourstolenfuture.org. On the front page there is a temporary link in the center: "CHEfertility resources"...right beneath the top of the page.
- A reminder to those of you who will speak today: please explain acronyms, and keep your remarks geared so that our lay participants can grasp the science...with lay translations when necessary.

Dr. Pete Myers is CEO of Environmental Health Sciences - known to most of you as the originator of our very appreciated daily digest of environmental health media, science and organizational reports; and as the co-author of *Our Stolen Future*, with Theo Colborn, who is on the call today. Pete will give us a couple of brief capsules of especially notable new science reports that have come out over the last weeks in addition to those being featured today, and then go ahead and introduce our speakers.

PETE MYERS: This has been a truly remarkable month in the history of research on contamination and reproductive health. There has been almost a non-stop series of relevant publications appearing in the scientific literature and then picked up by the press and broadcast through main-stream media around the world. I've never witnessed anything like this last month...certainly not within the last decade. We have two of the main contributors of that pulse of new information, Dr. Michael Skinner and Dr. Shanna Swan, on the call today, and we will be listening to their presentations and have a chance to ask questions in a moment.

I'd like briefly to point out two of the several other papers that have come out over the most recent time period. And you can find links to summaries of these papers at the bottom of the page of www.ourstolenfuture.org that has the CHEfertility resources on it. Ana Soto and her colleagues, led by Munoz de Toro, published a remarkable paper in *Endocrinology*, now in press, looking at the consequences of *in utero* bisphenol A exposure at 25 nanograms per kilogram. That dose was delivered via an implanted osmotic pump, and it's certainly lower than the level that anyone else has reported for *in vivo* impacts with bisphenol A.. They found very striking changes in mammary gland development at time of puberty following that *in utero* exposure. The second paper in some ways parallels what Dr. Swann will be talking about in a moment. This is a study published in *Human Reproduction* by a Japanese team of human epidemiologists and medical researchers who were stimulated to do this research by research that Pat Hunt, Terry Hassold and their team had published about two years ago, again looking at low-level bisphenol A exposure, in that case in mice, and finding impacts on meiotic aneuploidy....very large increases in the frequency of meiotic aneuploidy in that mouse model. This Japanese team, led by Sugiura Ogasawara and his colleagues, tracked pregnant women with a history of recurrent miscarriage, through the end of their current pregnancies and compared it to controls and found a significant elevation in bisphenol A levels in the women with a history of recurrent miscarriage, and they also found evidence of meiotic aneuploidy in aborted fetuses. There are many more details; that was a preliminary study with a relatively small sample size that needs replication, but given that it was based on predictions coming out of Pat Hunt's work, I think it is really noteworthy. And hopefully someone on this call will follow up with a second round of studies.

With those two reports behind us, let me move to the next section of the talk, introducing **Dr. Michael Skinner**. Dr. Skinner is a professor of molecular biosciences at Washington State University at Pullman, and since 1996 he has been the Director of the Center for Reproductive Biology there. In early June he published a truly groundbreaking piece of research on the effects of exposure, during the developmental period of sex determination in mice, on subsequent generations via epigenetic mechanisms. Mike, you can explain this in much greater depth and authority than I, so please, take about 10 minutes to walk us through what you did.

MICHAEL SKINNER: I'm happy to participate today, and look forward to any questions you might have. I'm a professor of molecular biosciences and have been studying reproductive biology for a number of years. I study things on a molecular and cellular level in terms of how the testes and ovaries function. We were doing a series of experiments at the time of sex determination in embryos and sort of stumbled across this phenomenon that truly has some significant impacts in terms of how environmental factors might affect health and disease states, and not just reproduction. How I start this explanation, to allow the lay people to develop an understanding, is: if your grandmother was exposed during mid-gestation to an environmental toxin at appropriate levels, you might have a disease state, even though you never received a

dose, and you're going to potentially pass this on to your great-grandchildren. This is what's called a trans-generational effect, where an environmental toxin, or something, can cause an alteration that then permanently generates a genetic trait that goes to all subsequent generations. That's the initial way to think about this.

What we did for the experiment is we exposed pregnant female rats to an environmental endocrine disruptor. We used two different "model" endocrine disruptors: one is vinclozolin, which is an anti-androgenic substance, used as a fungicide in the wine industry and other agriculture. The other is methoxychlor, which is one of the primary pesticides used to replace DDT, and it's an estrogenic and anti-estrogenic substance. We wanted to study the initial phases of gonadal development, ovary or testes development, and so we exposed the pregnant female just transiently for a short period of time during the time of the initial stages of sex determination, when a testes or an ovary develops. Then we stopped the treatment.

We didn't see major effects on the embryos right away, but what we found was that in the adult offspring, the first generation, the adult males had abnormal spermatogenesis. There was an increase in sperm cell death; a decrease in sperm numbers and sperm motility; and so overall there was lower fertility in these first generation animals. This wasn't overly surprising; there have been a number of reports previously showing that if you expose pregnant females to appropriate toxins you might get effects in the first generation.

But what was done then in the lab was that the F1 generation was bred to make 2nd generation animals; so a treated male and treated female from different litters were bred to make 2nd generation animals. Surprisingly, males of that generation also had the same phenotype; that is, reduced fertility, reduced sperm numbers and so forth. And then when those 2nd generation animals were bred together to form a 3rd generation, they also had the same phenotype. We went out four generations - and over 90% of males in every single generation had this low fertility phenotype, of decreased sperm numbers and sperm motility and increased sperm cell death in the testis.

Now, there is no way for a common genetic mechanism to cause this level of frequency of the phenomenon at greater than 90%...there is no way a normal mutation event involving DNA sequencing can cause that frequency...mutations are usually at 1% or less of the animals. And because it was trans-generational, this needed to be what's called an "epigenetic" phenomena, where the DNA apparently gets chemically modified, and that modification is permanent going forward; and the only way that can occur is during the germ line...the sperm or the egg of the animal, because the germ line is actually what transfers the genetics to the next generation. What we've stumbled across is an epigenetic, trans-generational phenomenon, that can actually cause a disease state.

The publication that came out in June was primarily dealing with male reproduction. However, we're just now submitting a publication with the same phenomenon which shows that if you take these animals and age them just a little more, then we start seeing a whole host of disease states, everything from cancer to prostate disease, to kidney disease...a number of disease states, suggesting this phenomenon can be actually inducing a large variety of disease states, not just a male reproductive defect. So this epigenetic, trans-generational phenomenon is new phenomenon identified, one we're quite interested in...it provides a new avenue to understand genetics and the basis of disease.

There are a couple of major impacts of these findings. First, the potential hazards of an environmental toxin that can cause a permanent reprogramming of a genetic trait for all subsequent generations increases the potential hazards of those toxins. What we need to do now is evaluate trans-generational effects of these toxins, not just the effects on the individual exposed and his/her offspring. That needs to be incorporated into our thinking on and analysis of environmental toxins.

Secondly, this opens up a fairly significant avenue for understanding how disease develops, or disease etiology. Genetic mutations are clearly part of the disease phenomenon, but if epigenetic changes—not through DNA sequence changes—can cause a variety of diseases, this opens up whole new areas, such as: 1) we could have new diagnostic markers for diseases...diagnosed before the disease develops whether you're going to get it, and then with that information, there could be 2) a whole series of therapeutic treatments to potentially prevent the onset of the disease. This would be a whole area of medicine that we didn't appreciate before, which may significantly enhance therapeutic approaches to very common diseases in our society. The 3rd impact is for general biology. Evolutionary biology has been thought to be driven primarily through DNA sequence modifications that will lead to adaptations for natural selection. If indeed an environmental impact can actually influence a permanent genetic trait in a sub-population, then you could have an environmental factor driving evolutionary biology, and that could be a factor we didn't previously appreciate. And so it makes us re-evaluate the basic biology of evolution by having a new factor...In fact, there are several things in evolution that we couldn't really explain, that this could explain, as well.

One caveat I'd like to end with is that the levels of toxins we used are higher than anticipated in the environment, so we can't really claim anything about the toxicology of these compounds. The critical thing is that this phenomenon that is very new has been identified, and now we can go back and do the toxicology and find what is the lowest level that can induce this phenomenon, and is it close to the levels of environmental exposures. So we can't really discuss the toxicology of the compounds; the main point is this new phenomenon that has been described.

MYERS: Thanks very much, Mike. Truly phenomenal, and I'm sure there will be many questions. But what I'd like to do now is turn to Shanna so that we can have questions after both presentations are complete. **Dr. Shanna Swan** is Professor of Reproductive Epidemiology at the University of Rochester School of Medicine and Dentistry. She has adjunct appointments as Professor in the Departments of Environmental Medicine and Community and Preventive Medicine. And she is the new Director of the Center for Reproductive Epidemiology, that has just been established at U Rochester, that will be focusing on questions of reproduction and fertility. Dr. Swan's research over the last several years on endocrine disruption has opened up a lot of new questions about the affect of exposures at everyday levels. And this most recent publication which she will summarize has done that quite dramatically.

SHANNA SWAN: I'm pleased to have the chance to tell you about our new study. There are a couple of slides on the website and I'll just mention them as I go through. What did we do: our study examines the relationship between fetal exposure, *in utero* exposure, to a class of chemicals to which most of us are regularly exposed--- phthalates---and male genital development, including an unfamiliar end point, anogenital distance. First some definitions: phthalates – and there are now metabolites of seven of these that are routinely measured by the Centers for Disease Control—phthalates are chemicals that are used widely in consumer and industrial products, including cosmetics, fragrances, cleaning materials, medical tubing, a variety of plastics, and are measurable in many of our exposures in the home in drinking water, house dust and so on.

So, what is anogenital distance? This is a new marker for human studies (almost). You can think of anogenital distance as the length of the perineum. It's usually about twice as long in males as in females, certainly in rodents, and now we think in humans as well, based on our study and one other study. What did we do in our study? It builds on finding from three different areas of research that I'll briefly summarize. First, it's been known for quite a while that development of the male genitals and anogenital distance (AGD) in particular, is under the control of the males hormones, the androgens. And it's been known that in rodents, these levels are higher in males than females, and as a result AGD is longer in males than females. AGD seems to be the measurement that is the most, or certainly very sensitive, to prenatal androgen exposure. We hypothesized that by measuring AGD we could sensitively detect changes in genital development which resulted from prenatal exposure to androgens or anti-androgens. Secondly,

the chemistry has progressed to the point that we can now measure low levels of metabolites of phthalates in human urine and the CDC, using state of the art techniques, has been doing that and has shown that phthalate metabolites can be found at very low levels, parts per billion or higher, in most of the US population. Thirdly, a large body of work around the world has shown that some of these phthalates are indeed anti-androgens and can reduce testosterone during the critical window of sex development, and so the male pups that are born after this exposure to an anti-androgen have a shorter, or more female-like, AGD. They also show a cluster of features that include, for example, impaired testicular descent, smaller genitals, and together these alterations have been called the “phthalate syndrome” in rodents.

Recognizing the interrelationship among these lines of research led us to hypothesize that perhaps prenatal phthalate exposure in humans could produce the human counterpart of the phthalate syndrome, and that’s what our study was designed to address. We had a sample of women from our previous study, the Study for Future Families, and had stored samples of urine that the women provided during pregnancy. We could measure phthalate metabolites in these urines and relate these to development of babies born to these women. Pediatricians in our study examined these babies using a standardized exam and obtained genital measurements in the first two years of life. Then we calculated the AGD we would expect, on average, for a boy of that age and weight. It’s like going to the pediatrician’s office and saying, “is my baby on target in terms of growth?” We have a much smaller sample, so is not really normative data, but we can say within our population of 134 boys, on average a boy of, say, 12 months and a certain weight, has a certain expected AGD. Then we can look at a particular boy and ask, “Is he shorter than expected? Average? Longer than expected?” If the boy’s AGD by this measure was below the 25% percentile, we called him “short AGD.” On the slide you’ll see “AGI” -- that’s AGD divided by weight, just a statistical trick--but it’s the same thing...we’re talking about AGD.

What did we find? First, we did indeed find phthalates detectable in most prenatal samples...slide 5 gives you levels of four phthalate metabolites. You can see that many were high and that there was a wide range. Secondly we found, as predicted by rodent studies, that for several of these metabolites concentrations were higher in the mother’s urine when the boy was classified as having a “short” AGD compared to an “intermediate” or a “long.” In slide 6, I show I show the amount of phthalate metabolites for the groups, short – medium – long.

Let’s look at one of these, MBP, mono-butyl phthalate, one of those associated in rodents with AGD. If the concentration of MBP in the mother’s urine was high--and by “high” I mean above the 75th percentile for our subjects (and there were 85 of these who had urine measured)---then the boy was actually 10 times more likely to have an AGD shorter than expected (slide 7.) This result is statistically significant, as it is for each of the four metabolites that we found associated with AGD (slide 8.) Then, we noticed that these phthalates were correlated, that they tended to travel together, and that most women were exposed to many of them. So we hypothesized that we might see an even stronger association when the mother was exposed to several of these at high levels. To look at this we examined the “summary phthalates score” which measures joint exposure to all four of these phthalates (slide 9); this score was highly significantly related to the proportion of boys with short AGD. In fact, (slide 10) there were 10 boys who were exposed to most of these phthalates at high levels—they had the highest scores-- and all but one of them had short AGDs. On the other extreme, of the boys who had none of them, or almost none of them, higher than the very lowest levels, the lowest scores - only one had a short AGD. This is very statistically significant: Odds ratios were huge, but this study doesn’t have large numbers of subjects and needs to be replicated. These results are shown graphically in slide 11, and I can answer questions on that.

None of the boys we examined had frank malformation or disease. 87% of these boys had both of their testicles which were normally descended; however the amount of testicular descent varied with AGD. Among short AGD boys, 21% had incomplete testicular descent and smaller scrotum, compared to 8% of the other boys. Also AGD was significantly correlated with the volume and size of the penis; that is, the shorter the AGD, the smaller the penile volume. So, the pattern of genital changes we saw is consistent

with the phthalate syndrome which had previously been seen only in rodents. The levels of phthalates at which we saw these changes were not particularly high; in fact, the levels in the short AGD boys were comparable to those seen in 1/4 of the female population based on CDC analysis of a national sample.

Why is this important? What does it matter, why do we care about AGD? In rodents the phthalate syndrome has adverse consequences that include low sperm count--- and which Russ Hauser (whom I think is on the call) and his group has reported this in adult men in association with phthalates exposure--- also infertility in rodents and some testicular tumors. Our findings are also consistent with a syndrome that has been defined in humans: testicular dysgenesis syndrome. So we may have identified the first biomarkers for a measure of testicular dysgenesis syndrome. We need to do this study in larger samples and to replicate this study, of course. In rodents these changes are permanent, but in humans we need future studies to determine whether these boys will be permanently affected.

MYERS: Thank you, Shanna. (opens the floor to questions)

LINDA GIUDICE: Thanks Mike and Shanna. I have a question of ignorance about what's going on in California. I missed an NPR report that someone told me about, regarding the body burden assessments in selected populations in CA, that was aired in the last 3 days or so. Can anybody tell me more about that?

MICHAEL LERNER: Are you referring to the report on the News Hour with Jim Lehrer? It covered a number of studies; one of them was a project undertaken by Commonweal with individual Californians to test their body burden... People who would be willing to talk about it. I didn't hear the call myself... I can get you information on that (if you are interested in their phthalate levels...).

MYERS: I have the link to the News Hour show, so you can read the transcript.

PAUL ENGLISH: (CA Dept of Health.) Shanna, did you find any relationship between prenatal exposure to phthalates and growth retardation or gestational age at birth; and my second question is: I've seen some rat studies on impacts of PBDE exposure and male infertility and I was wondering if a similar study with PBDE prenatal exposure might be something to look into.

SWAN: We haven't looked at growth retardation, but my educated guess on looking at the birth weights and so on of these babies... they didn't look unusual. It's possible that in a larger sample we could see that. As far as PBDEs, I have to admit that I don't know the mechanism of action of PBDEs... are they anti-androgenic? (Laura from State Health Dept. says that they are thyroid disruptors.) Right, so... I don't know of any mechanism other than anti-androgen action that would lead to the syndrome we're reporting. But of course on their own, PBDEs would be extremely interesting to look at, since they are a common exposure now.

LERNER: Question for Michael Skinner, and again this is ignorant... what do we understand (at this time) about the mechanisms of trans-generational epigenetic alterations?

SKINNER: This process is called methylation of DNA, so methyl groups get stuck on DNA, and most of the genes in the genome get reset after fertilization, every time. So, when there's fertilization, in the early embryo, all the genes get de-methylated, then re-methylated before implantation of the embryo. It turns out there's a small sub-set of genes called "imprinted genes," and those genes have a certain pattern of methylation. Through a mechanism that we do not know today, those patterns get transferred to the next generation. And apparently those patterns can influence the activities of those genes and down-stream genes as well. We actually sat on the data for about four years because we couldn't explain it; but about two years ago there was a phenomenon described where the germ line (the migrating, primordial germ cells) during embryogenesis, right before sex determination, gets completely demethylated. So, in the gonad, at the time of sex determination the germ line is de-methylated; and then upon sex determination

there is a re-methylation of the genes. Evidently what we did was interfere with that re-methylation and altered the imprinting pattern of a whole series of potentially new, or previously unknown, imprinted genes. Then that is what gets transferred to the next generation. That's our current thoughts on the mechanism, that we're affecting permanently the methylation pattern of the germ line.

TED SCHEITLER: I'm curious if this is the first time that an alteration of methylation patterns in the germ line has been demonstrated, versus somatic cells?

SKINNER; Right, somatic cell reprogramming, of course, is not permanent, because that gets reset in each generation in the early embryo. It's only the germ line that can actually transfer genetic information to the next generation. So a trans-generational phenomenon has to involve the germ line to be activated. That germ line methylation pattern in the new embryo can affect a whole host of down-stream somatic cell and different tissue methylations, so that's how these various disease states (potentially) develop later on, because that germ line is carrying some kind of programming process that then goes to all the tissues later in development. But this is the first time that an environmental toxin has been shown to cause a trans-generational affect, and the mechanism appears to be epigenetic.

CARLSON: For Dr. Skinner. It sounds like you have your hands full looking at other end points aside from the male repro, but will you be, or have you started, looking at lower doses?

SKINNER: Instead of lower doses, what we're interested in up front is the actual compound itself. It turns out that methoxychlor (the pesticide, used to replace DDT, that we used) has three activities ---- it is an estrogenic compound, an anti-estrogenic compound, and it's also anti-androgenic. It's a mixed bag, and that's one of the reasons we used it---we wanted a general endocrine disruptor. Currently we're in the process of using a "pure" anti-androgen called flutamide, which preliminary studies suggest does cause this trans-generational affect, so we know an anti-androgenic substance can do this. We do not know yet whether an estrogenic substance can do this, and that's something we're in the process of testing. Once that's done, we will go back and actually do some dosing. But we wanted to find out what the specific compound, or class of compound, was that was causing the effect.

EDITH EDDY (Compton Foundation): For both Michael and Shanna: I'm interested in who is funding this research.

SWAN: The first phase, the Study for Future Families, was funded by NIEHS, and the phthalates analysis was funded by EPA.

SKINNER: My initial studies were funded by the EPA Star program, and then subsequently it's been picked up by NIEHS.

MARY LOU BALLWEG (Endometriosis Association): Do you have any plans for looking at the females? As I'm sure you know, methoxychlor has been tied to the development of endometriosis in some EPA work with Audrey Cummings.

SKINNER: Yes, we have been looking at the females. Initially in all analysis of the developing gonad, we didn't see any dramatic effects upon the female. The only female phenotype, as we mention in the paper, was we saw about a 10-20% pre-eclampsia type condition where the pregnant mothers...actually some of them died or developed severe pre-eclampsic-like conditions. We're now analyzing whether pre-eclampsia may be induced by the environmental (exposure.) However, if we age the animals longer, so that they are about 6-8 months old (which is still a young rat, but a little older), then we start to see disease states coming up in the female, particularly kidney disease and some tumors. So we do think the females will be affected; we just need to look at the right disease states. And we don't know about endometriosis (of course the rodent is not the best model for this) but that would be something interesting to look at.

CARLSON: For Shanna. Some folks may know that phthalates are not very persistent, a short half-life in our bodies. If we're extrapolating here, do we need to worry about phthalates exposures, and what do you say to consumers about phthalates exposure concerns?

SWAN: That's a hard question; I don't have a lot of good answers. As far as the timing of the exposure, there's some limited evidence suggesting that levels are pretty stable, so, they're related, probably, to product use...we don't know for sure, but people tend to use the same products every day...and that would also be true for what's in the water, etc. But as to what to tell people, that to me is the most important next question. I'd like to be able to answer the question if a pregnant woman calls me and asks, "what are the three things I can do to make the greatest impact on my exposures?" We don't have an answer for her at this point. There are some resources; there's a Web site, www.nottoopretty.org, that people can look at for alternative cosmetic choices, but in our data we're not convinced that the majority of phthalates exposure comes from cosmetics, based on preliminary data. There's also the fact that they're hidden...in pesticides, in drinking water....so really, at this point aside from maybe changing your cosmetic use and not microwaving in plastic....I don't have a lot of advice. That bothers me and I hope we can get some work going to answer that question.

MYERS: Shanna, immediately following the publication of your paper there were comments from industry that were critical of some of your statistics. Would you care to comment?

SWAN: Well of course industry will attack us because they are feeling attacked. That's fine; they're doing their job, I'm doing my job. I attempted to answer those criticisms and, Pete, did you put that up on the website, too?

MYERS: Yes, the document is there. I just thought that, as there had been questions prior to the call about this point, I wanted to be sure people had a chance to talk with you about it.

SWAN: I think the major criticism was this question of, were we just hunting through the data until we found something significant? In fact, this study was not based on looking at lots of phthalates and lots of endpoints to see what would come up significant, because it was driven by the rodent data; in fact, this was a hypothesis-driven study. So, the question of what was the true statistical significance, which is a statistical issue, I personally don't think is relevant in this case. But, this may be too technical...more than people want to hear...Pete, maybe you could give the website where people could read about it, then email me or call me. I don't mind talking about it, but it will not be something that everybody is interested in.

CARLSON: Shanna, at this point is it for others to replicate the study, using more subjects, or do you have a plan to look at it again using a larger number of subjects?

SWAN: I'm in the process of designing a multi-center pregnancy cohort study to replicate this and to do it better and address ahead of time some of the criticisms that have been raised. I hope other people will do it as well. Then also to look at the question of exposure assessment, in other words where phthalates are coming from and what are our primary routes of exposure? Those are the things I'm thinking of doing next.

SCHETTLER: I just want to emphasize, and listen to any additional comments about, the third point that Michael Skinner made, about the implications of this for evolutionary biology. Because if you look back in the history of understanding of evolution, Lamarckian ideas of environmental influences being heritable were sort of cast aside in the later part of the 19th Century. It's sort of striking how some ideas resurface when we begin to understand some biological mechanisms that might make this not such a far out idea, and we begin to think about how we might change entire germ lines by environmental interventions where

the heritable consequences are to be realized by many generations to come. I think it's really quite remarkable to think about this and ought to give us all cause for reflection.

SKINNER: I agree. A lot of what Lamarck proposed *was* incorrect. But there are some subtleties in terms of what's called "neo-Lamarckianism." This is through reprogramming of the germ line, which is a very defined time in embryonic development. So both for evolution and for the human population and disease, the critical exposure is for pregnant women in mid-gestation, for any species. It is sort of a sub-set of what Lamarck proposed, but the concept he put forward is correct in the sense that an environmental toxin could permanently alter the germ line of the population and could have some fairly significant effects on disease and evolution.

Just a comment: we now have a couple of major evolutionary biologists who are now interested in doing some things on sexual selection and testing whether that is indeed the case.

MYERS: Mike, you mentioned that you have a couple of follow up publications in process, dealing with disease states in the later life of the exposed mice. Where do those publication plans now stand?

SKINNER: *Science* is reviewing one, on the multiple other diseases that are trans-generational, so we'll hopefully hear in a few weeks on whether they are going to thoroughly get into it, then it might be out this fall. And that paper basically suggests that several major diseases that we see in the human population like cancer and aging diseases like prostate disease, breast disease are coming up in these animals at fairly high percentages.

MYERS: When you say 'fairly high percentages...'

SKINNER: Over 85% of all offspring get some disease state. Approximately 30% of any population gets the disease state like prostate disease for the male, and about 20% of animals get tumors, mostly breast. So this does have some fairly significant impacts on what we would consider some of our major disease states. Epigenetics may be a significantly more important factor than we previously appreciated.

LERNER: Mike, what you say to industry skeptics who say, "These are rat studies; there's no reason to assume the same would be true in humans" ...since that's a knee-jerk response...I'm curious how you would respond.

SKINNER: For about the last four decades we've demonstrated that pretty much the basic biological process for any species is similar. If you look at mammals, the physiology of the liver, of the stomach, lots of different tissues, are very similar among species. That's why we can take fruit flies and study a developmental process that turns out to be correct in the human. The majority of scientific information available suggests a higher degree of evolutionary conservation than differences. Species are just not completely different from each other, so that is not a real scientifically sound argument. Now, the specifics of whether a given disease state comes up could be argued, but what we do know is that epigenetics occurs in almost all mammalian species, that there is a programming of the germ line, and all of them have imprinted genes, so that developmental process is consistent. I would say that would suggest that the likelihood that there would be similarities would be much stronger than that there would be differences. But of course the next phase after we define this more in animal models would be to do, as Shanna did, to evaluate them in humans. One of the ways we're going to do that is, we've identified a set of about 50 genes with altered DNA methylation that are trans-generational and we potentially now can use those genes as diagnostics to see if the disease state gets transferred and comes up. If we can translate that to the human population then we may not have to do the toxicology studies but may be able to look for altered methylation patterns to determine whether your lineage has been exposed and you're susceptible to a disease state. That's our future plans for the human.

SWAN: Do you know whether this altered methylation occurs as a result of DES exposure?

SKINNER: That would be of course an estrogenic affect, and we're analyzing right now whether estrogenic compounds can do this. There are estrogen and androgen receptors in the gonad at the time this occurs, so it is potentially possible, but we don't know yet.

SWAN: Because we have available, and being followed to some extent, the second generation DES cohort that could be studied epidemiologically to look for trans-generational affects.

SKINNER: Sure, once we have diagnostic markers, that would be an ideal population to study.

MYERS: There are both epidemiological and animal data on second generation effects of DES, so it would be a logical place to look.

HEATHER SARANTIS: (The Breast Cancer Fund) Shanna, I work on the Campaign for Safe Cosmetics and, in regard to your comment that you're not convinced the majority of phthalate exposure come from cosmetics... Are there any good reports or reliable sources on where the majority of phthalate exposure does come from?

SWAN: I'll tell you why I said that, and then the answer to the second question is "No, I don't think we have good guesses." We asked women when they gave their postnatal samples, which we also have, what cosmetics, personal care products, etc. they used in the 24 hours before they gave their samples. Russ Hauser's group also did this. We looked at the levels in the urine in conjunction with their reported use, and we know how long ago they used them and some other details about the products. For MEP, the metabolite of diethylphthalate (and this is not published, by the way but we will be writing it up for publication), we did see associations.....not huge, but...the more women reported using make up and bath products, the higher the levels of monoethylphthalate. But this didn't hold true for the other phthalates...and in fact MEP is the only one of the phthalates for which we found associations that has not been reproductively toxic in rodents to date. So there's something else there, I think, and that it's not driven by cosmetic use.

MYERS: I would add that even though Shanna correctly says we don't know the majority of the sources of the exposures, that doesn't mean the exposures from cosmetics is not significant.

SWAN: Correct.

MYERS: Could Ted comment...he gave an excellent paper in Denmark, in January about pathways of phthalate exposures.

SCHETTLER: I would briefly comment. I think one of the hidden and poorly quantified sources of dibutylphthalate exposure could be pharmaceuticals and nutritional supplements. In follow up to the paper I gave in Denmark, I've done further investigation and found that there are literally hundreds and hundreds of pharmaceuticals that are patented and permitted to have dibutylphthalate in the coating of the tablets, as common as antihistamines, cold tablets, over-the-counter laxatives and a whole variety of pharmaceuticals. I think this could be an important source of dibutylphthalate. As Shanna mentioned, many insecticides and insect repellents have either dimethylphthalate or dibutylphthalate in the carrier, which is not reported on the label of the insecticide. These could be an important source of transdermal exposure for some people.

SWANN: Also ingestion, because it's in the drinking water, perhaps via pesticides...I don't know.

RUSSELL HAUSER: To add to what Shanna said about the use of personal care products, we have a paper under review now of our study in which we found that adult men that use cologne or after shave, there were higher levels of MEP in the urine. It was really as Shanna said that the personal care products are contributing primarily to dimethylphthalate exposure rather than to dibutylphthalate. To second what Ted said about dibutylphthalate in some medications: we just had a case report of one individual who was taking Asacol, which is used to treat ulcerative colitis and had extremely high levels of monobutylphthalate in his urine.

NANCY BRADSHAW (Toronto Environmental Health Clinic): Does anybody know of any good literature around the issues of safe microwaving and how much phthalate might be transferred through microwaving the softer plastics?

SWAN: I personally don't yet have that information...I would love to hear the answer, though.

HAUSER: I don't have that either. I think it's a different type of plastic that's used in plastics that are microwaved...I'm not aware of whether they do or don't contain phthalates.

BRADSHAW: It's my understanding that it's the softer plastics... if you put, say, a yogurt container in the microwave there would be leaching of phthalates. I haven't heard anything about those labeled "microwave safe," the Tupperware-type containers, etc. We just tell our clients to use glass whenever possible. But I didn't know if there was much that has been published in this area.

MYERS: There hasn't been a lot. Consumer Reports did an analysis several years ago, but it's an area that would benefit from some serious research...

So, sincere thanks...especially speakers Drs. Shanna Swan and Michael Skinner for presenting their extraordinary research.

CARLSON: Note that next teleconference will be late September/Early October.