

10068 - Environmental contamination and thyroid function; the New York State Angler Cohort Study. Bloom, MS, Weiner, JM, Vena, JE, Beehler, GP. Department of Social & Preventive Medicine, University at Buffalo, State University of New York.

This preliminary study investigated associations between selected organochlorine environmental contaminants and thyroid function in a sample of Western New York sportsmen (n=66) selected from the New York State Angler Cohort Study. Blood samples were analyzed for contaminants based on literature cited evidence of thyroid disruption. These included hexachlorobenzene (HCB) and polychlorinated biphenyl congeners IUPAC #'s 19, 28, 47, 118, 153, 169, 180, 183, and 187. Serum triglycerides, cholesterol, high and low density lipoproteins, body mass index, age, and cigarette smoking were considered for each participant. Potential associations between environmental contaminants and serum thyroxine (T₄), controlling for potential confounders, were examined using multivariable linear regression models. The models reported consisted of all variates being entered (the "full" model) and forward stepwise selection of variates using the criteria of maximum partial correlation at each step. Several procedures were considered to address contaminant data below the limit of detection. Sample power was determined for the current sample as well as that required for a more definitive analysis of the current results. The linear weighted average of all covariates in the full model resulted in R²=0.38. A stepwise regression selection procedure identified HCB (β =-0.113) and age (β =0.007) as predictors of serum T₄ (R²=0.08) in the "selected" model. Power analysis suggested that although current power to detect the observed HCB effect was 53%, a doubling of sample size (n=122) would provide 80% power. An inverse association between serum HCB and serum T₄ was identified at the α =0.15 significance level. It is possible that there are contaminants related to HCB and to T₄ that are confounding the relation seen in the stepwise selection. That possibility is suggested by

the difference in R^2 between full and selected models. In addition, an approximate doubling of the current sample size should permit the elucidation of a statistically significant inverse association between serum HCB and serum T_4

10148 - Timing of DDT Exposure and Breast Cancer Before Age 50

Barbara A. Cohn (Public Health Institute), Mary S. Wolff (Mount Sinai School of Medicine), Piera M. Cirillo, Robert I. Sholtz, Roberta Christianson, Barbara J. van den Berg (Public Health Institute), Pentti K. Siiteri, (Emeritus, University of California San Francisco School of Medicine)

The insecticide dichlorodiphenyltrichloroethane (DDT) was introduced in 1945 and is still used for malaria control where needed. The United States banned DDT in 1972, as have other countries, because DDT disrupts wildlife reproduction. DDT is no longer detectable in the majority of human samples in the United States, although a common DDT metabolite, dichlorodiphenyldichloroethene (DDE), is still found in most human serum specimens.

Previous breast cancer studies have measured DDE as a surrogate of lifetime DDT exposure, usually in specimens obtained decades after DDT had been banned. This design may have obscured true past exposure due to variation in exposure intervals and elimination rates; these prior studies have been largely negative. Previous studies also did not consider timing of exposure.

We tested the hypothesis that DDT is a stronger breast cancer risk factor for women who are exposed during childhood and adolescence, when undifferentiated cells are most vulnerable. We measured DDT and DDE in blood samples drawn during active DDT use.

We measured serum levels of DDT and DDE during the early postpartum in 131 age-matched case-control pairs in a prospective, nested case-control study in the Child Health and Development Studies, a pregnancy cohort based on the Kaiser-Permanente Health Plan, near Oakland, California. Women entered the study while pregnant, between 1959-1967, before DDT was banned. The average time between serum sampling and breast cancer (before age 50), was 16 years. DDT and DDE were detected in all subjects. Median DDT was 11.64 ng/ml and median DDE was 46.31 ng/ml. We estimated DDT associations with breast cancer in conditional logistic regression models adjusted for age at first pregnancy, age at menarche, parity, race, DDE, total cholesterol and total triglycerides. We found that risk of breast cancer increased with increasing concentrations of serum DDT for women exposed in childhood or adolescence. The odds ratio (OR) for the second versus the first tertile of DDT was 3.9, 95% Confidence Interval (95% CI)= 1.4, 10.9. The OR increased to 10.4, 95% CI= 2.5, 43.2 for the third tertile of DDT exposure versus the first. This trend over quartiles was significant ($p < 0.001$). The DDT association with breast cancer was significantly stronger for women exposed to DDT before age 15 than for women exposed after age 15 ($p < 0.02$). Similar results were obtained with lipid-adjusted DDT and DDE.

Our findings are consistent with prior studies of radiation effects on breast cancer (for atomic bomb and for therapeutic radiation) where increased risk of breast cancer was reported primarily for women exposed in childhood or adolescence. Our study provides further support for the hypothesis that environmental risk factors have maximum effects on early breast cancer when exposure occurs prior to reproductive maturation.

Urinary phthalate monoesters at general population exposure levels are associated with altered semen quality.

Susan M. Duty, MSN¹, Manori J. Silva, Ph.D.², Dana B. Barr, Ph.D.², John W. Brock, Ph.D.², Louise Ryan, Ph.D.^{3,4}, Zuying Chen, M.D.⁵, Robert F. Herrick, Sc.D.¹, David Christiani, M.D., M.P.H.^{1,6} and Russ Hauser M.D., Sc.D.¹

Currently scientific and public concern exists about several commonly used chemicals, such as phthalates, which are hormonally active and associated with reproductive toxicity in animals. The general population is exposed to phthalates through household and personal care products, as well as through diet and medical treatments. In the NHANES III and 1999 surveys, four phthalate metabolites were present in more than 75 percent of U.S. subjects sampled. The present study explored whether general population levels of phthalates were associated with altered semen quality. Male partners of a sub-fertile couple who presented to the MGH Andrology lab for semen analysis were recruited. Semen parameters were dichotomized based on WHO (1999) reference values for sperm concentration (less than 20 million/ml) and motility (less than 50 percent motile), and Tygerberg Strict criteria for morphology (less than 4 percent normal). The comparison group was men with all three semen parameters above the reference values. In urine, eight phthalate metabolites were measured with high performance liquid chromatography and tandem mass spectrometry. Specific gravity adjusted phthalate levels were dichotomized using the median into high and low categories. High mono-butyl phthalate (MBP) levels were associated with sperm motility and concentration below reference values with odds ratio (95 percent confidence interval) of 2.37 (1.13 to 5.00) and 2.41 (0.80-7.23), respectively. Suggestive evidence of an association between high mono-benzyl phthalate (MBzP) levels and below reference value sperm concentration and between high mono-methyl phthalate (MMP) and poor sperm morphology were found. MBP, MBzP and MMP were associated with altered semen quality.

10290 - Breastfeeding and serum p,p'-DDT levels among Mexican women of childbearing age. A pilot study.

Luisa Torres-Sánchez,¹ Lizbeth Lopez-Carrillo,¹ Jacqueline Moline,² Karen Ireland,² Mary S Wolff.²

¹National Institute of Public Health, Morelos, Mexico. ²Division of Environmental Health Science, Mount Sinai School of Medicine, N.Y., U.S.A.

For almost 50 years, millions of Mexicans have been directly and/or indirectly exposed to p,p'-DDT. The potential related health outcomes of this exposure are of international concern.

Objective: To determine the effect of breast-feeding on serum levels of p,p'-DDE (1,1-Dichloro 2,2'-bis(p-chlorophenyl)ethylene) and p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) in women of childbearing age who were residents of the state of Morelos, Mexico.

Materials and Methods: In March 1999, we examined a sample of 24 women, aged 21 to 36 years, who came with their children to the outpatient facility of a county Health Center in the state of Morelos.

Results: The geometric mean for p,p'-DDE was 21.8 ng/ml \pm 2.58 (GSD) and 2.9 ng/ml \pm 2.84 for p,p'-DDT. For each month of breast-feeding we observed similar significant decreases for both p,p'-DDE (β =-0.0403 per log concentration unit, p=0.001) and p,p'-DDT (β =-0.0309, p=0.03) serum levels, adjusted by mothers' age and number of children. The adjusted half-life estimate for p,p'-DDE serum levels was 17 months.

Conclusions: Breast-feeding leads to rapid removal of p,p'-DDT from the body (< 2 yr half-life) compared to non-lactational elimination rates (>5 yr half-life). In this population, serum p,p'-DDE levels were similar to those found 20 years ago in the United States.

10291 - Preterm birth in relation to maternal organochlorine serum levels.

Laura Torres-Arreola,^{1,2} Gertrud Berkowitz,³ Luisa Torres-Sanchez,² Malaquíás López-Cervantes,⁴ Mariano Cebrian-García,⁵ Marisela Uribe,⁵ Lizbeth Lopez-Carrillo.²

¹Epidemiology and Health Services Research Unit, National Medical Center "Century XXI". Mexican Institute of Social Security, Mexico. ²National Institute of Public Health, Morelos, Mexico. ³Division of environmental Health Sciences, Mount Sinai School of Medicine, N.Y., U.S.A. ⁴Coordination of the National Institutes of Health, Mexico City, Mexico. ⁵Center for Research and Advanced Studies of the National Polytechnic Institute, Mexico City, Mexico.

Considerable attention has been focused on a possible link between the persistent organochlorine insecticide p,p'-DDT and the risk of breast cancer. Relatively little, however, is known about its potentially adverse reproductive effects. A recent US study of serum, obtained from a cohort several decades ago, reported that the risk of preterm birth increased steadily with increasing concentrations of p,p'-DDE, the most persistent of the p,p'-DDT metabolites. Although p,p'-DDT was banned in many industrialized countries during the 1970s, it is still being utilized in many malaria-endemic regions around the world.

We performed a case-cohort study of the associations of serum levels of p,p'-DDE and two other persistent organochlorine pesticides, β -HCH and HCB, in relation to preterm birth in 233 pregnant women in Mexico City. The mothers were recruited at three large maternity hospitals in Mexico City during 1995. Serum levels were obtained shortly after delivery. A non-significant increased risk of preterm birth in relation to serum p,p'-DDE levels was observed. There was also a suggestion of an increased risk of preterm birth among women in the highest tertile of β -HCH (adjusted odds ratio 1.85, 95%CI=0.94-3.66, p value for test of trend p=0.08) compared with the lowest tertile. No association was found between HCB serum levels and preterm births.

These findings suggest that p,p'-DDT and other organochlorine pesticides may pose a risk to preterm birth in countries that continue to use such insecticides for malaria control.

Xenoestrogenic response of serum from PCB-exposed women

Rasmussen TH, Nielsen F, Nielsen, JB, Grandjean P.

Environmental Medicine, Institute of Public Health, University of Southern Denmark, DK-5000 Odense C, Denmark.

PCBs are mainly deposited in the lipid compartments of the body and occur in the lipid fraction of human serum. These substances easily pass the placenta and are excreted in human milk. Prenatal and early postnatal exposure to PCBs or other endocrine disruptors may induce irreversible damage during the sensitive period of organogenesis and sexual differentiation. Thus, women in the reproductive age group is of special concern. We analyzed the cumulative xenoestrogenic activity in 200 serum samples from healthy pregnant women (third trimester) from the Faeroe Islands, a population group with considerable dietary exposure to PCBs from consumption of whale blubber. Furthermore, we analyzed serum from healthy pregnant women (third trimester) from Denmark as a reference group. Since PCBs and PCB metabolites may display different types of action, such as estrogenic, anti-estrogenic or anti-androgenic effects, we used an assay specifically designed to measure the integrated and functional xenoestrogenic response from complex mixtures. We used solid-phase extraction of the serum samples with a newly developed polymeric sorbent that exhibits both hydrophilic and lipophilic retention characteristics, thus allowing extraction of a wide polarity range of xenoestrogens and environmental toxicants. In order to separate xenoestrogens with polarities close to endogenous hormones, we apply a HPLC method using a gradient that covers the mid-polar area. The lipophilic fraction, which include the most dominant parent PCB congeners and hydroxylated PCB metabolites in biological extracts, are then tested in the E-screen bioassay, a sensitive and reliable screening system for functional estrogenicity in biological samples. Serum from the Faroese women had a higher estrogenic response than the reference group. The causative compounds have not yet been identified, but it is conceivable that a number of substances, such as PCBs and PCB metabolites, each present at low ineffective concentrations may act together to produce significant effects.

Abstract # 10505

Evaluation of urinary phthalate levels in a pilot study of Vietnamese commercial fishermen and their spouses from counties surrounding Matagorda Bay, Texas

Grace Tee, Anne Sweeney, David Busbee, Robert Taylor, Elaine Symanski
(University of Texas School of Public Health, College of Veterinary Medicine, Texas A&M University)

Recent evidence from the third National Health and Nutrition Examination Survey (NHANES III) and the 2000 Report on Exposure to Environmental Chemicals from the CDC highlights greater than expected urinary levels of phthalate esters in the general US population. Phthalates are used in a myriad of consumer products and human exposure is ubiquitous. While phthalates are known to have low acute toxicity in humans, there is increasing concern they may cause deleterious reproductive effects in humans by acting as endocrine disrupters. Animal studies have shown teratogenic effects in male offspring and toxicity in germ cells in rodent models. However, longitudinal assessments of phthalate exposure and studies investigating adverse reproductive effects in humans have not been conducted to date. As a result, we have undertaken a pilot study using commercial Vietnamese fishermen and their spouses from 2 counties (Calhoun and Matagorda) surrounding Matagorda Bay, Texas to describe urinary distribution of phthalates among participants, evaluate the relationship between consumption of seafood obtained from Matagorda Bay and phthalate levels and identify determinants of body burden. Several industrial complexes are located in the counties bordering Matagorda Bay including one of the world's largest polyvinyl chloride manufacturers. Regular discharge into coastal waters of Matagorda Bay has resulted in heavy environmental contamination and may be an important source of human exposure among regular consumers of marine wildlife in this ecosystem. Thus, Vietnamese commercial fishermen and their families whose diets are presumed to contain a large proportion of seafood may be a highly exposed population. A complete list of all licensed commercial fishermen in the State of Texas from September 2000 to August 2001 was obtained and used to select 20 Vietnamese fishermen by simple random sampling. Individuals were eligible for participation if they are between 18 and 50 years of age, hold a Texas commercial fishing license and are of Vietnamese ethnicity. Personal interviews are used to collect demographic, lifestyle (including use of cosmetic products and perfumes), reproductive history, occupational and seafood consumption information (type of seafood, source, frequency, duration, preparation, and cooking,). Participants are also asked to provide a urine sample 3 hours after consuming a meal containing seafood from Matagorda Bay. Total concentration of phthalate metabolites will be determined using the same protocol developed by Blount and colleagues from the CDC. Data collection is ongoing until June 2002. Descriptive statistics and stratified analysis will be used to describe the distribution of urinary phthalate levels among participants by age, sex, education, type of seafood most frequently consumed, duration of consumption, cooking and preparation method.

Abstract # 10796

We assessed potential health effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) concentration in serum on thyroid function in US Air Force veterans involved in Operation Ranch Hand, the unit responsible for the aerial spraying of herbicides, including TCDD-contaminated Agent Orange, during the Vietnam War from 1962 to 1971. Other Air Force veterans who were not involved with spraying herbicides were included as Comparisons. We analyzed thyroxine (total T4), thyroid stimulating hormone (TSH), triiodothyronin percent uptake (T3% uptake), the free thyroxine index (FTI), and thyroid diseases against serum TCDD levels. Data were available for 1,009 Ranch Hand and 1,429 Comparison veterans compliant to any of five examinations in 1982, 1985, 1987, 1992, and 1997. Each veteran was assigned to one of four exposure categories based on serum TCDD levels, named Comparison, Ranch Hand Background, Ranch Hand Low Elevated, and Ranch Hand High Elevated. Cross-sectional analyses found statistically significantly increased TSH means at the 1985 and 1987 examinations in the High category and a significant increasing trend across the three Ranch Hand TCDD categories in 1982, 1985, 1987 and 1992. A repeated-measures analysis found significantly increased TSH means in the Low and High TCDD categories. We found no significant relation between the occurrence of thyroid disease and TCDD category. These findings suggest that TCDD affects thyroid hormone metabolism and function in Ranch Hand veterans. Further follow-up will be necessary to understand the relation, if any, between thyroid disease and TCDD levels.

Abstract # 10820

Exposure to DDT and Metabolites in Relation to Menstrual Cycle Length among Laotian Immigrants.

Gayle Windham, Patrick Mitchell, Myrto Petreas, Diana Lee, Bill Lasley. California Department of Health Services, California EPA, University of California, Davis.

This study was initiated because of concern about the effects of endocrine disrupting chemicals (EDCs) on reproductive capacity in wildlife and lab animals, but little data available in humans. It was designed to examine effects of potential EDC's on ovarian function, as measured by the frequency of menstrual cycle characteristics, in Laotian immigrants who may have higher exposure from their home country as well as from sport fish consumption. Working with local Lao communities, we hired and trained Lao field workers to recruit women of reproductive age (18-39) who were born in Southeast Asia and consumed fish regularly. Participants were asked to complete two interviews, provide a blood sample, and collect first morning urine samples daily during three menstrual cycles. The urine samples were assayed for metabolites of estrogen and progesterone, from which several menstrual cycle parameters were calculated. These methods were similar to those of a larger study we conducted among a general population, which can serve as a comparison. The serum was assayed for organochlorine compounds including pesticides, PCB's, and PBDE's. Fifty women completed the study by collecting urine during 148 complete cycles, with information on an additional 39 cycles with partial urine collection. The mean cycle length in complete cycles was 30.4 days (standard deviation (SD) 7.0). Focusing on DDT, it's metabolite DDE, and their ratio, we found all women had detectable levels with a mean of 1.7 ppb (SD 3.5) and 20.3 ppb (SD 22.5) respectively, indicating the possibility of more recent exposure than found in typical U.S. populations. The covariates related to mean exposure level included age, parity, breastfeeding, and time in the United States, some of which were inter-related. Examining quartiles of exposure, cycle length was decreased with increasing exposure in preliminary analyses. Using methods that account for repeated measures, at the highest quartile of DDE exposure mean cycle length was decreased by 3.5 days (95% confidence interval (CI) -7.6, 0.58) and at the highest quartile of DDT exposure by 4.4 days (CI -8.2, -0.58), compared to the lowest quartile. Adjusting for total lipid level attenuated the decrements only slightly. Adjusting for other demographic variables attenuated the decrements to 2.0- 2.5 days, with fairly wide confidence intervals. We will explore these findings further as age was also associated with length of time in the U.S., which may be on the pathway to exposure level and thus over-controlling. In summary, our data suggest a possible association of DDE/DDT exposure with changes in menstrual function, but based on small numbers and potentially confounded by other variables. If exposure is associated with shorter cycles, this could reflect a hormonal pathway. We will examine additional endpoints and EDC's as well.

10833 - Hormonal carcinogenesis: prostate cancer and environmental xenoestrogens

Gomes J MSc PhD^{1,2}

¹Department of Community Health & Epidemiology, University of Saskatchewan, ²Centre for Agricultural Medicine

Environmental xenoestrogens are ubiquitous and exposure during critical phases of life (fetal development, adolescent and andropause/menopause periods) is suspected to adversely effect reproductive health in humans. Wildlife research has confirmed suspicions that environmental xenoestrogenic chemicals are detrimental to normal male genital development in utero. Male sexual differentiation, which is dependent on androgens, during the organogenesis period, could be disturbed by increased exposures to exogenous and endogenous estrogens and unbalanced androgen/estrogen ratios, both, during and post uterine growth. Prostate gland is androgen dependent, and excessive exposure to estrogens and alterations in androgen/estrogen ratios, during the critical phases of life, could adversely effect development and functioning of this gland. Adverse effects on the prostate gland from low and sustained exposure may only manifest and attain clinical significance during andropause years. This study, therefore, proposes to develop and test a polygenic model to understand hormonal carcinogenesis of the prostate gland.

We hypothesize that functionally relevant genes, involved in exogenous and endogenous hormone metabolism, may act together and also interact with other hormonally related risk factors to modify prostate disease (benign prostatic hyperplasia (BPH) and prostate cancer) risk. A combination of such genes each with minor variations in expressed activity could provide a degree of separation of risk, which in several years could manifest in clinically significant conditions – BPH and/or prostate cancer. Exogenous and endogenous exposures to estrogens and antiandrogens during andropause could increase hydroxylated metabolites, which may cause DNA damage and genetic alterations leading to prostate carcinogenesis. However, similar exposures during critical phases of life could stimulate cell proliferation of estrogen dependent target cells and reprogramme cellular differentiation and gene expression leading to carcinogenesis through epigenetic mechanisms. Our polygenic model examines genetic and epigenetic factors and interplay between them in the development of BPH and hormonal carcinogenesis of the prostate.

To test the model we have designed a case-control study and recruited age matched incident cases (diagnosed with prostate cancer), controls (diagnosed with BPH) and references (men from any prostate related problems). Exposure to xenoestrogenic chemicals is examined by computing job exposure matrix (JEM) for lifetime exposures for all the research subjects. Period and frequency of exposure during different periods in life is also examined. Blood and tissue samples are analyzed to assess DNA damage and examine the profile of prostate specific genes and enzymes. Prostate specific genes, for our model, include androgen receptor gene (AR), steroid 5alpha-reductase type II gene (SRD5A2), estrogen responsive gene (pS2), prostate specific antigen gene (PSA), 3beta hydroxysteriod dehydrogenase gene (HSD3B2), epidermal growth factor gene (EGF), transforming growth factor gene (TGF-B) and kertinocyte growth factor gene (KGF).

We hope to indicate that hormonal carcinogenesis is most likely a result of interplay between genetic and epigenetic factors. The level of exogenous exposure to xenoestrogenic chemicals during the critical phases of life may play an important role in prostate disease development. Specific exposures during specific periods in life may predict the nature of prostate disease in andropause years.

10902 - Residential indoor air and dust measures for pesticides, alkylphenols, phthalates, and other endocrine disruptors

Ruthann Rudel, Chris Swartz, Julia G. Brody; Silent Spring Institute, Newton,
David Camann, Alice Yau, Michelle Zuniga; Southwest Research Institute, San Antonio,
TX

John D. Spengler; Harvard School of Public Health, Boston, MA

In order to characterize exposures to chemicals of interest for research on breast cancer and other hormonally mediated health outcomes, residential air and dust samples were analyzed for up to 100 target compounds that 1) have been identified as animal mammary carcinogens or hormonally active chemicals and 2) are used in commercial or consumer products or building materials. Selected phthalates, pesticides, parabens, PAHs, PCBs, and PBDEs were extracted and analyzed by GC/MS-SIM. Phenolic compounds including nonylphenol, octylphenol, bisphenol A, and the methoxychlor metabolite HPTE were extracted, derivatized, and analyzed by GC/MS-SIM. In data from the first 30 of 120 homes sampled on Cape Cod, MA, 44 of 68 target compounds were detected in at least one air sample and 68 of 93 were detected in at least one dust sample. Chemicals present at highest concentrations in these air samples include: diethyl phthalate (DEP) (range 128-2,113 ng/m³), dibutyl phthalate (DBP) (56-851 ng/m³), ortho-phenyl phenol (oPP) (43-958 ng/m³), and nonylphenol (NP) (48-416 ng/m³). Other compounds frequently detected in air samples include di(2-ethylhexyl) phthalate (DEHP), nonyl- and octylphenol mono and di ethoxylates, 4-tert-butyl phenol, PCB congener BZ 52 and certain pesticides. Most abundant compounds in dust include: DEHP (range 125-1082 microgram/g) as well as butyl benzyl phthalate and several other phthalates. Other compounds commonly detected in dust samples include NP, OP and their ethoxylates, bisphenol A, benzo-a-pyrene, and several pesticides. Pesticides detected in at least 50% of the households (air or dust) include: oPP, permethrin, piperonyl butoxide, carbaryl, methoxychlor, heptachlor, DDT, propoxur, pentachlorophenol, chlorpyrifos, chlordane, and diazinon. It is of interest that the most abundant phthalates in indoor air (DEP, DBP) are also most abundant in human urine samples collected by CDC for a reference population of US adults. While indoor air concentrations of phthalates are too low to account for a substantial portion of daily intake, it is possible that indoor air levels are proxies for more substantial exposures occurring during product use. In this ongoing study, air and dust samples from 120 homes have been analyzed, and urine samples have been assayed for phthalate and pesticide metabolites. Self-reported data on product use in the home has been collected from participants, and estrogenic activity in air samples is being evaluated by E-SCREEN bioassay. These sampling and analytical methods identify hormonally active chemicals and animal mammary carcinogens that are widespread in indoor environments, making them priorities for future research and regulatory evaluation. They also provide new exposure assessment tools for the study of hormonally mediated health outcomes.

Abstract # 11068

Epidemiologic Evaluation of Perfluorooctyl Compounds as Endocrine Disruptors in Males: Analytical Methods and Study Design. J. H. Raymer^a, M. Gardner^a, Y. Hu^a, T. Wilcosky^a, and D. Walmer^b. ^aRTI International, Research Triangle Park, North Carolina. ^bDuke University Medical Center, Durham, North Carolina.

The potential for endocrine and reproductive ramifications in human males as a result of exposures to environmental concentrations of perfluorinated chemicals, including perfluorooctylsulfonate (PFOS) and perfluorooctanoate (PFOA) is the focus of this epidemiologic study. Products containing these chemicals have been largely withdrawn from the market by a major manufacturer amid concerns of persistence, toxicity, and widespread population exposures to these chemicals. This study is focused on the assessment of semen quality and endocrine status of a potentially susceptible sub-population, i.e., men of couples who present at a fertility clinic. A case-control design in that the study population will include a high prevalence of men experiencing reproductive problems (“cases”) as well as men with a normal fertility status (“controls”). By using a case-control design (the approach of choice for investigating rare outcomes), the study will be able to efficiently detect any important exposure-related reproductive problems. If exposure levels are associated with male reproductive problems, the study participants are expected to represent a range of exposure to PFOA and PFOS, which will be measured in samples of blood and semen; concentrations in these biological media will reflect the multi-route exposures to these chemicals experienced by virtually all people in our society. The samples (500µL of plasma or semen) are made basic using sodium carbonate, tetrabutylammonium hydroxide is added and the sample is extracted into methyl-*t*-butyl ether (MTBE). The MTBE is evaporated and the extract is reconstituted into methanol prior to analysis using liquid chromatography/mass spectrometry/mass spectrometry. Semen quality is assessed using both routine measures and a test designed to more accurately and reproducibly assess normal, motile, and fertile sperm. Measurements of Follicle Stimulating Hormone, Luteinizing Hormone, Prolactin, Estradiol and free and total Testosterone reflect the hormonal status of the males and will provide evidence of perturbed endocrine function. If the exposure effect is limited to a sensitive subset of the general population, our study is more likely to detect an association compared to studies that sample on PFOA/PFOS exposure status in an occupational setting. In this presentation, the developed analytical method and the study design will be presented.

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