Supporting Statement A

Longitudinal Investigation of Fertility and the Environment - NICHD

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JUSTIFICATION

A. 1. Circumstances Making the Collection of Information Necessary`

This is a request submitted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), the National Institutes of Health that the Office of Management and Budget (OMB) approve under the *Paperwork Reduction Act of 1995*, clearance for the NICHD to continue an epidemiologic study entitled "Longitudinal Investigation of Fertility and the Environment". The original information collection request was approved (OMB Clearance 0925-0543) following publication in the Federal Register on January 9, 2004, page 1589 and December 2, 2004, page 70153. The 60-Day FRN for the current application was published in the Federal Register on January 16, 2008, Vol.73, No. 11, p.2925. This research will be done by the Division of Epidemiology, Statistics and Prevention Research consistent with the NICHD's mission to conduct basic, clinical and epidemiological research focusing on factors and processes associated with human reproduction and development, thereby, ensuring the birth of healthy infants capable of reaching full adult potential unimpaired by disabilities. The authority for the NICHD to conduct research studies is contained in The Public Health Service Act, which outlines the research and information dissemination mission of the NICHD in the area of child health [42 USC 285g].

A. 2. Purpose and Use of Information Collection

The data collected as a part of this study will be analyzed to answer growing concerns about the effect of persistent environmental chemicals, in the context of lifestyle and a couple's previous

reproductive history, on human reproduction and development. Findings from wildlife populations suggest that persistent environmental agents are adversely affecting reproduction and development (Thomas & Colborn, 1992), particularly so-called endocrine disrupting compounds (EDCs). These compounds are alleged to have estrogenic, anti-estrogenic, androgenic, or anti-androgenic activity and can interfere with the synthesis, secretion, transport, binding, action, or elimination of the body's natural hormones that are required for homeostasis, reproduction, development, or behavior (Crisp et al., 1998). EDCs are reported to be capable of enhancing (agonist) or inhibiting (antagonist) hormone activity. A recent summary has identified exogenous substances that may affect sex hormone function in humans: 1) estrogenic effects of high potency (e.g., diethylstilbestrol), medium potency (e.g., dietary phytoestrogens), and low potency (e.g., bisphenol A, octylphenol and nonylphenol pesticides); 2) anti-androgenic effects (e.g., pesticides) and 3) other effects (e.g., dioxin-like PCBs) (Joffe, 2003).

Recent findings suggest reductions in male fecundity, and disruption in mammalian oocyte maturation and follicle physiology associated with exposure to organochlorine chemicals and polychlorinated biphenyls (Faroon et al., 2001; Dallinga et al., 2002; Rozati et al., 2002; Hauser et al., 2002; Pocar et al., 2003). Many human studies focusing on PCB exposure and reproduction and development have relied upon proxy measures of exposure (e.g., fish consumption) or retrospective collection of exposure and outcome data. Virtually no prospectively collected longitudinal data exist regarding the effects of persistent environmental chemicals on human reproduction and development in the context of lifestyle factors that are also suspected of exerting deleterious effects on human reproduction and development. The absence of convincing data is in sharp contrast to public belief that environmental chemicals adversely

impact human health (Sharpe and Irvine 2004; SIRC 2004). Further concern about EDCs has resulted in a congressionally mandated response by the U.S. Environmental Protection Agency (EPA) to form the Endocrine Disruptor Screening and Testing Advisory Committee (Brown 2003). The Agency for Toxic Substances and Disease Registry (ATSDR) is charged with the preparation of toxicologic profiles of environmental chemicals at geographic sites of concern for human health impact.

The NICHD investigators in conjunction with principal investigators (PIs) at two competitively awarded research institutions are leading data collection and/or analyses. One of those two sites has completed enrollment, leaving only one site at which data collection is still ongoing. A data coordinating center is overseeing the collection of de-identified information from the research sites and the two governmental laboratories quantifying exposures and male fecundity, i.e., National Center for Environmental Health and the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

This research originally proposed to recruit 960 couples who are interested in becoming pregnant and willing to participate in a longitudinal study. Given the uncertainty associated with the percentage of U.S. couples planning a pregnancy at any point in time, our design assumptions were conservative resulting in a higher estimated cohort size. To date, fewer than expected couples were enrolled during the first three years of the project (n=350), predominantly due to the fact that more couples were ineligible for participation than had been originally estimated. Our statistical methodology and underlying assumptions have been revised in the context of new information and empirical evidence generated from the LIFE Study as described throughout this

document. The current revised study plan is to enroll a total of 500 couples (i.e., 150 additional couples for this OMB request), a sample size that will not compromise the main study objectives, given that additional exposure data has relaxed some of our earlier assumptions. Prime reasons for needing an OMB extension include: 1) our Texas site was required to temporarily stop recruitment during Hurricane Rita and in the following year given extensive flooding in the region and 2) one of our original three study sites was closed for budgetary reasons leaving two remaining sites for available recruitment. One of the two remaining sites has completed recruitment, given that they depleted their sampling framework. Data are being collected using a combination of telephone interview for purposes of initial screening, in-person questionnaire for baseline information, longitudinal collection of entries in a daily fertility journal or monthly pregnancy journal, and electronic fertility monitors. The longitudinal capture of information is necessary given the timed, highly interrelated aspects of human reproduction and development. The data collection schedule is illustrated in Attachment 1.a. The study's primary environmental exposures include: organochlorine pesticides; polychlorinated biphenyls; polybrominated diphenyl ethers; metals; perfluorinated compounds; cotinine; and phytoestrogens. A listing of study compounds by biologic media is presented in Attachment 5.a. These agents are largely persistent in the environment raising the likelihood of ubiquitous exposures for the American public.

Nurses are instructing couples in the proper use of commercially available home fertility monitors to aid couples in becoming pregnant and home pregnancy tests for the earliest recognition of pregnancy. Both kits are being provided as a part of the research protocol free of charge to participants and both are being used according to the manufacturer's instructions. Of

added note is the acceptability of the kits by study participants and women can easily incorporate use of the kits to aid them in becoming pregnant. Four types of biospecimens are being collected as illustrated in Attachment 1.a. The nurses are collecting blood and urine specimens (see Attachments 1.n. and 1.o.) from men and women and forward them to the laboratories at the Centers for Disease Control and Prevention for toxicological analysis. Two semen samples from male partners are being collected for use as a global measure of male fecundity as measured primarily by sperm concentration, morphology and 24-hour motility and to quantify contaminants in semen (see Attachment 1.k.). Two saliva samples are being collected from women (see Attachments 1.i. and 1.j.) to measure cortisol levels as a marker of stress so that the relation between environmental factors, stress and human reproduction can be assessed. Couples are being asked to complete short daily diaries on lifestyle factors suspected of adversely affecting the probability of conception or ability to carry a pregnancy to term (see Attachments 1.f. and 1.g.). These data will allow us to identify lifestyle factors (amenable to public health intervention) that adversely affect reproductive and developmental outcomes and to adjust for potential confounders when analyzing environmental chemicals. Additional questions on menstruation and sexual intercourse are being asked on the female journal so that time-to-pregnancy is accurately measured and to help women know when to use fertility monitors (see Attachment 1.l) and pregnancy test kits (see Attachment 1.m.). Women becoming pregnant are being asked to complete a short journal each month about their health status and behaviors while pregnant (see Attachment 1.h.) to aid in the analysis of our 4^{th} and 5^{th} hypotheses regarding length of gestation and baby's birth size. These data are critical for separating the effect, if any, of environmental agents from lifestyle factors known to adversely affect fetal growth and development.

We expect to write a number of scientific papers for a diverse audience, especially key papers responsive to the five principal null hypotheses that there is no association between environmental chemicals in the context of other lifestyle factors and: 1) time-to-pregnancy; 2) infertility; 3) pregnancy loss; 4) length of pregnancy or gestation; and 5) infant's birth size. Data sharing is consistent with NIH policy http://grants.nih.gov/grants/guide/notice-files/NOT-OD-03-032.html. These data will help to answer remaining questions regarding the association between EDCs and human reproduction and development as previously discussed.

A. 3. Use of Information Technology and Burden Reduction

Research nurses are using programmed laptops to administer the baseline interview and to record participants' responses. All data are being uploaded via a web-based data management system that allows monitoring of data collection and processing and extraction for analytic purposes. The computer-assisted interviews have reduced burden and loss by tailoring the questionnaire to the participants' responses thereby avoiding unnecessary questions. The need for double data entry has also been eliminated, which will reduce errors and generate a more valid dataset. Furthermore, the web-based data management system has facilitated data sharing at the conclusion of the study, as required by NIH policy.

All study participants have had the choice of providing their daily and monthly journal entries either by mail-in card or by online web-based form provided they have access to an Internet-connected computer. Online forms were specially designed for respondents use to make data

entry simple and with a minimum of technical limitations. The study allows respondents to switch between the two modes of journal entry at their convenience. Internet access in the target population was estimated at 30% on the basis of surveys conducted by the Pew Internet & American Life Project in September 2000 and August 2003 [www.pewinternet.org]. This estimate took into account such factors as income level, educational level, age, race or ethnicity, urban or suburban versus rural location, and interest in parenting. Respondents with Internet access also have had the convenience of viewing via the study public web site information about online journal entry and a summary of their journal data for the past week, similar to the mail-in cards.

Digital fertility monitors are helping couples optimize their chance of conception and at the same time record daily information on women's fecundity as two reproductive hormones are tracked by the monitor. The monitors minimize burden by electronically recording test results, which can be uploaded by research nurses when they visit the home to provide additional test supplies. Digital home pregnancy test kits are helping women recognize pregnancy as early as possible. Retail sales of pregnancy test kits commenced in 1976 with approval of the Medical Device Amendment of the Food, Drug, and Cosmetic Act and amount to 19 million tests (Pal, 2003; Lipsitz, 2000). We are using a digital pregnancy test kit hat displays "pregnant", "not pregnant", or "error" to avoid user misinterpretation; it has been documented to be one of the most sensitive and reliable pregnancy tests currently available on the market (Cole et al., 2004).

A. 4. Efforts to Identify Duplication and Use of Similar Information

There is no duplication of information, since there are no prospective pregnancy studies relating environmental exposures to reproductive and developmental end points in the context of longitudinally collected lifestyle factors. Online searches for government-funded research revealed no such studies, nor did our many personal discussions with other government agencies or academic and private research institutions. This includes electronic searches of extramurally funded grants and intramural research projects supported by the National Institutes of Health and other research entities.

A. 5. Impact on Small Businesses or Other Small Entities

No small businesses will be recruited to participate in this study. Per the Small Business Administration and 13 CFR, we have contracted with a small business, The EMMES Corporation, to act as the data-coordinating center for the study.

A. 6. Consequences of Collecting the Information Less Frequently

For our study to be scientifically valid, we need data collection at critical windows reflecting the highly interrelated and timed nature of human reproduction and development. Exposures occurring outside critical windows may not exert an adverse effect underscoring the need for collection of time-varying data (Wilson 1965; Selevan et al., 2000). We are focusing on persistent environmental agents collected at baseline necessitating only *one* blood specimen. Three urine samples will be collected mainly to look at changes in phytoestrogens and cotinine that are expected to be more variable than persistent compounds. Two semen samples are

needed to globally assess male fecundity (WHO Manual, 1997); two saliva samples are needed for the detection of changes in stress cortisol levels.

Longitudinal capture of exposure information on other study covariates while couples are attempting pregnancy is needed to assess timing of exposures in relation to conception and gestation. Support for this approach comes from recognition that periconceptional exposures tremendously impact (un)successful human development such as the role of folic acid in reducing neural tube defects or other recently identified periconceptional exposures (Wald et al., 1991; Chapin et al., 2004). Retrospective recall of such exposures including chemicals is reported to be inaccurate (Anwar 1993; McCauley 1998).

A. 7. Special Circumstances Relating to the Guidelines of 5 CFR 1320.5

Study participants are being asked to report more than on a quarterly basis. To determine eligibility, selected individuals are being asked to take part in a five to ten minute screening instrument (Attachments 1.b. and 1.c.). Once enrolled, each partner completes a baseline interview (Attachments 1.d. and 1.e.) that takes approximately 20-25 minutes depending upon response patterns. The daily time to pregnancy journals (Attachments 1.f. and 1.g) and the monthly pregnancy journal (Attachment 1.h.) require approximately, two minutes a day and five minutes a month, respectively. While this may seem ambitious, the literature strongly supports that women/couples will complete daily diary information (and often collect daily urines for hormonal assays) including sensitive information and withdrawal, if any, from the study tends to occur in month one (Buck et al., 2004). We anticipate that 60% of couples will conceive within

three months, though it may be sooner given the use of fertility monitors to help identify the fertile window. Assuming that pregnancy lasts nine months, most couples will be followed for one year. The world's literature relating to retention in prospective pregnancy studies with preconception enrollment is summarized in Attachment 5.b.

Questionnaire data and biospecimens collected at baseline or before couples begin to attempt pregnancy are critical for quantifying exposures before the study outcomes so that a temporal relation can be established. The baseline interview serves another purpose in that the nurse also uses that time to teach the couple how to use home fertility monitors and pregnancy test kits. The collection of daily information on journals while the couple is attempting pregnancy is essential for determining whether the woman's menstrual cycle was at risk for pregnancy, which is the basis for assessing time to pregnancy as well as infertility (defined as the inability of couples to conceive with 12 months of trying). To estimate the incidence of infertility in this cohort, we allow couples up to 12 months at which time many will seek medical care. In addition, these data permit the longitudinal collection of information on lifestyle factors that may exert an acute or chronic effect on human reproduction and development. Another reason for the collection of daily information while couples are attempting pregnancy is so we can formally evaluate the timing of exposures in relation to the menstrual cycle (i.e., proliferative or secretory phase) and ovulation. Collection of monthly (ideally it should be more frequent) journal data from pregnant women is our attempt to identify exposures or events that adversely affect fetal growth and development so that we can control for important confounders when evaluating environmental agents. Every effort is being expended to ensure the collection of internally valid and, to the extent possible, externally valid data. Given that this is an etiologically oriented

study, interval validity is a critical aspect. Our ultimate success in recruiting couples from a defined population will impact the generalizability of our findings, i.e., external validity.

A. 8. Comments in Response to the Federal Register Notice and Efforts to Consult Outside Agency

Under the provisions of Section 3506(c)(2)(A) of the Paperwork Reduction Act of 1995, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), the National Institutes of Health submitted to the Office of Management and Budget (OMB) a request to review and approve the continuation of information collection for the current study. This official notice requesting a continuation of the project was published in the Federal Register on January 16, 2008 (Vol. 73, No. 11, page 2925) and allowed 60-days for public comment. In addition, the 30-day notice was published in the Federal Register on March 21, 2008 (Vol. 73, No. 56, page 15162) allowing 30 days for public comment.

Only one public comment, from a concerned citizen, was received during the 60-day comment period. She indicated that she felt that the government was already "…bankrupt due to the war…" and that the study should be stopped due to budget concerns.

We will consult with study participants on a monthly basis largely through the research nurses who are checking in with couples to collect data or to provide additional test supplies. Any issues can readily be brought to the nurse's attention for communication to the Project Officer. Furthermore, participants are able to contact us by telephone or e-mail, regardless of reason. We

are using these modes of communication to monitor participant burden and related issues during the active phase of data collection.

A. 9. Explanation of Any Payment of Gift to Respondents

Each respondent receives a total of $75 cash remuneration for provision of biological specimens during two home visits, including one blood specimen ($25), two urine specimens ($5 per specimen), two saliva specimens ($20 per specimen for women only), and two semen specimens ($20 per specimen for men only). These amounts are well within incentives used in other nationally representative studies (e.g., NHANES III) and individually less than the $50 amount permitted by U.S. law for each blood withdrawal for transfusion, scientific or research purposes [Title 24, Chapter 1, Section 30]. Several small non-cash incentives also are provided to participants as shown in the data collection schedule in Attachment 1.a.

A review of the world's literature on the use of incentives in prospective pregnancy studies reflected a range of cash incentives from $10 every two months for daily urine collection and storage to $500 for completion of an intensive protocol (see Attachment 5.b. for details). Fertility monitors and pregnancy test kits are an essential component of the research plan and are *not* being used as incentives. We are sensitive to the ethical principle of distributive justice (Beauchamp and Childress 1994) given our interest in the inclusion of minority and socio-economically disadvantaged couples in our study.

A. 10. Assurance of Confidentiality Provided to Respondents

Neither the NICHD nor the Data Coordinating Center (DCC) has any identifying information about study participants. Each contracted research site is responsible for assuring study participants' privacy and the confidentiality of their data. Personally identifying information is kept in either a secured project share drive or in a locked filing cabinet located in a locked office or other restricted access room. Only research staff with signed confidentiality agreements have access to the personally identifying information. None of these data are being shared with outside parties.

Upon enrollment, participants are assigned a site-preferred participant ID (SPID) for use in the DCC's centralized data system. The SPID is comprised of a site identifier and a unique code randomly generated by the data system. The only personally identifying information that is included in the centralized data system includes ethnicity and date of birth. Participants' data are transmitted to the DCC from the research sites and laboratories using a password-protected online distributed data entry system that uses Secure Socket Layer (SSL) encryption. All electronic data are stored behind a firewall and are available only to individuals involved in the project.

The Longitudinal Investigation of Fertility and the Environment protocol incorporates standard operating procedures meant to ensure participants' privacy and the confidentiality of their data. Telephone calls to conduct eligibility screening interviews are being made from private offices at each research site using landline telephones. Baseline interviews and biospecimens collection are being done in the privacy of participants' homes. Mail-in daily and monthly journals

completed by participants include only the SPID and numerical answers, which are meaningless to someone who does not have access to the corresponding questions. As an alternative to the mail-in method, participants are able to complete their journals online. Individuals who choose this option can log in from the privacy of their home using a username and password provided by the enrolling research site. Journal data, identified only by the SPID, are transmitted securely to the Data Coordinating Center. Online journal forms provide additional security by limiting access to previously entered data to only the current week and by automatically logging off the computer when data are submitted. Participants are instructed how to ensure their privacy while entering data online, including proper use of the secure data entry system. Only participants with computer systems meeting minimum standards for data encryption and transmission are permitted to use online data entry.

Each biological specimen is labeled with a bar code. No identifying information accompanies shipped specimens other than the SPID. Blood and urine specimens are shipped by the sites to the CDC's National Center for Environmental Health laboratory for toxicology analyses. Residual samples are stored frozen. Semen specimens are collected by male participants and shipped directly to the National Institute for Occupational Safety and Health (NIOSH) laboratory for andrology analyses then to CDC's toxicology laboratory for analysis of seminal fluid. Saliva samples collected by female participants are shipped for analysis of salivary cortisol. All specimens are tracked via the DCC.

Institutional Review Board (IRB) approval for the conduct of this study has been obtained from Texas A & M, RTI International, The EMMES Corporation, and the *Eunice Kennedy Shriver*

National Institute of Child Health & Human Development (see Attachment 3). A Certificate of Confidentiality has been approved for this study under the auspices of the NICHD informs participants that: 1) researchers will resist demands for information, other than from the respondents themselves, which would identify the respondents but that the Certificate cannot be used to resist demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects; 2) researchers might voluntarily disclose respondent identity if it is necessary to protect the respondent or others from serious harm, such as in situations of child abuse, reportable communicable diseases, possible threat to self or others, or high levels of toxic chemicals in the environment (e.g., as suggested by lead levels in blood); and 3) persons from the local Institutional Review Boards and the Federal Office for Human Research Protections may review their information, though confidentially [http://grants.nih.gov/grants/policy/coc/appl_intramural.htm].

In accordance with the Privacy Act of 1974, the study brochure indicates that the NICHD is conducting this study as a part of its mission to sponsor research focusing on factors impacting human reproduction and development (42 USC 285g). The informed consent documents approved by the individual research sites outline how we plan to protect individuals' privacy and the confidentiality of their data. Further, the consent documents state that participation in the study is entirely voluntary.

A. 11. Justification for Sensitive Questions

We are asking no third party questions. Two questions that are being asked may be sensitive for some people: 1) frequency of sexual intercourse to quantify the length of time couples are attempting to become pregnant and 2) frequency of ejaculations for the assessment of male fecundity. Valid time-to-pregnancy requires counting only menstrual cycles at risk for pregnancy or those where intercourse occurs five days before or on the day of ovulation (Dunson et al., 1988; Wilcox et al., 1995). This information is critical for validity of time-to-pregnancy and for couples seeking medical treatment if so desired.

All data inclusive of potentially sensitive information are stored free of personal identifiers according to the IRB requirements at each research site and as fully described in Section A. 10. All study participants are given an informed consent (see Attachment 2.c.), including full disclosure of potential risks and benefits and other essential elements such as the voluntary nature of research, privacy and confidentiality statements. Further, all consents are consistent with the language requirements of the Certificate of Confidentiality.

A. 12. Estimates of Hour Burden Including Annualized Hourly Costs

We will be enrolling a total of 500 couples into the study (i.e., 150 additional couples). The estimated number of response sets per respondent varies by gender, with women asked to complete six sets and men three sets. This difference is attributable to menses and to pregnancy occurring only in women. A single estimate of burden cannot be estimated, as length of participation will vary based on gender and when and if a couple conceives (see Table A.12-1a for the detailed calculations).

Specifically, the time interval for participation will range from nine months (for couples becoming pregnant in the first month of trying) to 21 months for couples requiring a full 12 months to become pregnant plus nine months of pregnancy. The annual burden per male respondent ranges from 2.10 to 5.48 hours per year, depending on when and if pregnancy is achieved. This translates to a total annual burden of 1,050 to 2,740 hours among the 500 male partners that will be recruited. For women, the annual burden per participant ranges from 3.28 to 9.90 hours, which corresponds to a total annual burden of 1,640 to 4,950 hours for the 500 female participants that will be recruited. Please note that 350 out of the total 500 couples have already been recruited, so the additional burden hours that are being requested with this collection extension are actually 70% lower than what is stated. These figures are conservative estimates of participant burden (i.e., overestimates) in that the literature suggests that 60% of couples are expected to become pregnant within one to three months, with 80% becoming pregnant by six months. Further, the literature suggests that most study participants who drop out of prospective pregnancy studies with preconceptional enrollment, drop out within the first few months of participation (see Table A.12-1b).

Table A.12-1a. Estimates of Hour Burden by Gender of Respondents and Type of Response

Gender of Respondent and type of Response	Number of Respondents*	Frequency of Response	Average Time per Response (hours)	Annual Hour Burden**
Female Partner of Couple				
Screening instrument	500	Once	0.17	85
Baseline questionnaire	500	Once	0.42	210
Time to pregnancy and daily early pregnancy journals	500	30 entries/month	0.03	15
Fertility monitors	500	11 tests/month	0.12	60
Pregnancy testing	500	Two tests/month	0.07	35
Biospecimens collection	500	Blood: once Urine: three times Saliva: twice	Blood: 0.13 Urine: 0.05 Saliva: 0.10	65 25 50
Pregnancy journal	500	One entry/month	0.08	40
Total	500	-	-	15-210
Male Partner of Couple				
Screening instrument	500	Once	0.17	85
Baseline questionnaire	500	Once	0.42	210
Time to pregnancy journals	500	30 entries/month	0.03	15
Biospecimens collection	500	Blood: once Urine: twice Semen: twice	Blood: 0.13 Urine: 0.05 Semen: 0.33	65 25 165
Total	500	-	-	15-210

Note: Duration of study participation is expected to range from one month to 21 months depending on when and if a couple conceives (i.e., twelve months of trying to conceive plus nine months of pregnancy). Majority of couples are expected to conceive in months 1-3 per past literature based on convenience sampling.

* We expect to enroll 500 respondents of which approximately 40 are expected to drop out following the baseline visit and an additional 60 thereafter; however, burden estimates are not adjusted for these withdrawals.

** By design, burden is overestimated in that it assumes complete participation, which is unlikely. In addition, time to pregnancy will vary across couples with the majority of couples becoming pregnant in first few months. Hence, time to pregnancy coupled with use of fertility monitors and pregnancy kits are greatly overestimated.

Table A.12-1b. Expected Distribution of Pregnancies and Drop Outs by Month of Participation

	Baseline*	Months											
		One	Two	Three	Four	Five	Six	Seven	Eight	Nine	Ten	Eleven	Twelve
Dropout this month	40	10	10	5	5	12.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Cumulative dropouts	40	50	60	65	70	82.5	85	87.5	90	92.5	95.0	97.5	100
Number remaining in the study	460	450	440	435	430	417.5	415	412.5	410	407.5	405	402.5	400
Pregnant**	0	100	180	228	260	280	292	300	304	308	312	316	320
Not pregnant	460	360	320	272	240	220	208	200	196	192	186	184	180

* Month of baseline interview. Dropouts reflect attrition after completing baseline questionnaires but before submitting any monthly diary data.

** Based on population-based studies reviewed in Buck et al., 2004

Table A.12-2. Annualized Costs to Respondents

Participant	Number of Respondents	Frequency of Response	Hourly Wage Rate	Respondent Cost
Male partner	500	3	$10	$15,000
Female Partner	500	6	$10	$30,000
			TOTAL	$45,000

A. 13. Estimate of Other Total Annual Cost Burden to Respondents or Recordkeepers

There are no additional costs to respondents or recordkeepers stemming from the collection of information.

A. 14. Annualized Cost to the Federal Government

FY07 contract awards for participating sites are:	
The EMMES Corporation	337,879
RTI International	56,734
Texas A&M University System Health Science Center	3,107,616
Subtotal	3,502,229
Interagency agreement costs:	
CDC's National Center for Environmental Health laboratory & NIOSH's Reproductive Health Assessment Section	308,963
Total	3,811,192

The NICHD Project Officer Dr. Germaine Buck Louis, has committed 30% effort to the study. Additional contributions have included 50% effort from Dr. Courtney Lynch (who has recently left the NICHD for academe), 15% effort from Dr. Aiyi Liu (biostatistics) and 5% effort from Dr. Enrique Schisterman (biomarkers & methodology). The estimated cost of Federal employees working on this project, including salary and fringe, is $131,973.72. Additional cost for a bi-annual site visit at the remaining site is $1,000 annually.

A. 15. Explanation for Program Changes or Adjustments

This is a request for an information collection extension for an ongoing project (OMB Clearance 0925-0543). This extension is needed given the initial delays in obtaining all human subjects approvals in 2005. Also, recruitment was temporarily halted in our participating Texas site following Hurricane Rita and extensive flooding in 2005. Based upon past recruitment rates, the remaining couples needed for recruitment goals can be recruited within the next 12-18 months.

A. 16. Plans for Tabulation and Publication and Project Time Schedule

Several papers will be published from this as developed from the analytic plan supporting the five hypotheses. With univariate analysis, the association of each exposure with the outcome will be analyzed to evaluate if that exposure is statistically associated with the outcome, and to decide which exposures will be further considered in the multivariate

analyses. Potential confounders also will be considered in the model (e.g., change of the **$**-coefficient estimated when confounders are entered into the model). The multivariate model will evaluate simultaneously the exposures associated with the outcome, while adjusting for potential confounder effects to search for the "best" group of exposures that provide maximum prediction of the outcome.

Hypothesis One

The discrete time Cox's proportional hazard regression model will be used to analyze environmental exposures, relevant covariates such as past reproductive history and time-to-pregnancy (TTP) outcomes. We anticipate fitting a discrete time multiple events Cox's proportional hazard model that considers the joint distribution of multiple TTP outcomes contributed by reproductive history. Another approach will be to put the history TTP outcomes as independent variables into the model; results from this approach need to be interpreted with caution, using conditional expectation arguments. Of particular interest is the probability of being pregnant during a certain menstrual cycle. These probabilities will be estimated from fitting the discrete time Cox's proportional hazard regression model, along with 95% confidence intervals (Cox and Oakes 1984). The probability of delayed conception (>6 months) can also be estimated from the fitting of the model. Meaningful interpretation of the results relies on correctly defined cycle 1 of a woman underscoring the importance of the baseline interview.

Other alternative methods can also be used to analyze the number of cycles required for conception, and the probability of conception per-cycle. One useful method is to employ

the Beta-Geometric distribution to model fecundability (Weinberg and Gladen, 1986). Effects of exposures and other covariates will be analyzed by modeling the mean structure as a function of exposures and other covariates. Estimates of the effects can be obtained using expectation-maximization (EM) algorithm.

Hypothesis Two

We propose two methods to analyze infertility and estimate the incidence rate and to assess the exposures' effects on infertility. First, let T be the number of cycles a woman takes to getting pregnant (i.e., T is the discrete time to pregnancy). Then, the rate of incident infertility is simply the probability of $\{T>12\}$, the survival function of the time to pregnancy at 12 months. This probability and its association with exposures and confounders can then be evaluated from fitting the discrete time Cox's proportional hazard regression model, or the Beta-Geometric model, for TTP, as discussed above. Another method would be to fit a logistic regression model with infertility outcomes (0=pregnant before 12 months and 1= being infertile) as the dependent variable and exposures and confounders as independent variables. Fitting this model also will provide estimates of the infertility rate and the odds ratio of each exposure as a measure of effect on infertility. Technically, if the couples were followed indefinitely, the discrete time Cox's proportional hazard regression model approach, or the Beta-Geometric model, would be more appropriate since it captures the survival (time to pregnancy) nature of the outcome. We will evaluate the fit of both methods.

Hypothesis Three

Pregnancy loss, as a dichotomous outcome, may occur more than once during the 12-month conception period. Furthermore, a woman may also report in her pregnancy history previous pregnancy losses. These situations result in multiple dependent binary outcomes. We propose using the generalized estimating equation (GEE) model with the logistic regression model as the marginal model to analyze pregnancy loss outcomes. In the model, exposures and confounders will be entered as independent variables and pregnancy loss as dependent variables. We will fit separately the model assuming the intercept to be a random effect or an unknown constant to be estimated. We will explore several working correlation structure, the independent, the compound symmetry, and the autoregressive structures in particular. Estimates of the **$**-coefficient or odds ratio will be obtained along with their robust standard errors. The AIC criterion will be used to evaluate model fitting with each working correlation structures. We also will treat previous pregnancy loss (or other previous adverse pregnancy outcomes) as independent variables in the model, to assess what these previous pregnancy outcomes may provide in predicting the current pregnancy losses. We hypothesize that women with previous pregnancy outcomes have increased risk of pregnancy loss as observed in the study period.

Hypotheses Four and Five

Gestation will be analyzed both as a continuous variable (days from conception to birth date) and as a dichotomous variable (short gestation versus normal). When treated as a continuous variable, linear models will be used with gestation as a dependent variable and exposures, confounders and other factors as independent variable. Data may be log-

transformed to stabilize variance and to achieve better normal approximation. When input as a dichotomous variable, logistic regression models will be employed for analysis. When previous gestation as reported in a woman's pregnancy history is considered, the GEE version of these models will be subsequently used to account for dependence among gestation outcomes. Intercept parameter and working correlation structures will be dealt with as described above for other outcomes. Alternatively, previous gestation will be entered as dependent variables to assess their predictability of the current gestation. Birth weight and other measures of birth size also will be analyzed using the above models (linear and logistic regression models).

Lastly, our analytic plan includes model diagnostics, detection of outliers and influential observations and missing data. Model fit will include examining the residuals of the model. If a peculiar residual pattern occurs, we will reexamine the model by using strategies such as adding a quadratic term into the model, adding interaction terms, trying non-linear models, as may be suggested appropriate by the residuals. Outliers and inferential observations will also be examined by comparing the fitting of two models, one with and the other without the suspected observation or outlier. Significant change in the model fitting by removing the observation signals abnormality and will be reported to the Data Coordinating Center for quality assurance and to the study investigators and Steering Committee for decision-making. Missing values are anticipated and introduce uncertainty to the estimates, p-values and confidence intervals of the statistical analysis. To evaluate such uncertainty, we will use the multiple imputation technique (Rubin, 1987, Schafer 1997). We will assume missing at random, unless otherwise informed. For

each item that has missing values, several imputations (three to five according to Schafer, 1997, Chapter 4) will be carried out, each generating a "complete" set of observations. This then yields a set of p-values, estimates and confidence intervals for the parameter of interest. These p-values, estimates or confidence intervals will then be combined to create a single p-value/estimate/confidence interval for the parameter of interest (Schafer 1997).

Table A.16-1. Project Time Schedule

Activity	Time Schedule (months)
Letters to eligible individuals	1-2
Recruit couples	1-18
Follow couples attempting pregnancy	1-18
Follow couples during pregnancy	1-18
Analyses (time-to-pregnancy)	19-24
Analyses (pregnancy loss, gestation, birth weight)	19-24
Publications	24-36

Note: Assumes allowing couples up to 12 months to conceive and 9 months for average length of pregnancy.

A. 17. Reason(s) Display of OMB Expiration Date is Inappropriate

The OMB expiration date is displayed in the upper-right hand corner of all study forms near the control number. [New number and expiration data will replace the existing number and date on all instruments and other study documents upon receipt from OMB.] The statements referring to the Privacy Act and the Paperwork Reduction Act also appear on all letters and brochures seen by study participants.

A. 18. Exceptions to Certification for Paperwork Reduction Act Submissions

There are no exceptions to the Certification requirements.

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