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# Biomarkers for Assessing Human Female Reproductive Health, An Interdisciplinary Approach

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Identification of environmental hazards to reproductive health and characterization of the adverse outcomes necessitate a multidisciplinary approach. Epidemiologic studies are required for the identification of adverse health effects in human populations and then to confirm that specific exposures are responsible. Clinical studies are required to develop assays for reproductive biomarkers and to validate these assays prior to their application in the field. Assays for field use must be formatted and streamlined for large-scale applications and, whenever possible, computer algorithms should be developed to interpret biomarker data. Appropriate animal models must be identified, biomarker assays validated for that model, and animal experiments conducted to identify the mode of action and target organ of a putative reproductive toxicant. Finally, in vitro studies at the level of the cell and cell organelle are essential for mechanisms of toxicity to be clearly identified and understood. In this article we describe the interdisciplinary approach that we have developed for study of the effects of environmental agents on female reproductive functions. This effort requires specific skills of toxicologists, epidemiologists, physicians, biochemists, and physiologists. — *Environ Health Perspect* 106(Suppl 4):955–960 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/955-960lasley/abstract.html

Key words: interdisciplinary studies, reproductive hazards, dioxin, biomarkers, early pregnancy

## Introduction

The identification of environmental agents that have adverse effects on reproductive health is particularly challenging. As much as 37% of all unexplained infertility in the United States is thought to be caused by environmental factors (*1*), but few human reproductive toxicants have been clearly identified and the mechanisms of chemical toxicity to reproductive tract cells are not well defined. Unlike other aspects of human health, disturbances of reproduction are likely to go unrecognized or unreported. For example, only limited assessments of reproductive health are possible in over 50% of women in the United States because of elective or age-related infertility. Infertility may be periodic and it may be a relative effect rather than an absolute condition. Couples who succeed in conception after many years of attempting pregnancy or have a smaller family than they desire are afflicted with infertility, even though they may not recognize the condition. Many men and women do not think of infertility as a disease and may not seek medical advice if reproductive failure is not accompanied by any physical symptoms. The incidence of reproductive disease in lower socioeconomic groups may be disproportionately underreported because of the limited access that these individuals have to elective medical care. Whether or not there is any immediate discomfort or long-term concern associated with reproductive disease, for women, in particular, the sequellae can be devastating, and may involve irreversible effects on the skeletal, cardiovascular, and central nervous systems. Thus, infertility can be regarded as a silent and well-tolerated disease that is underreported and sometimes misdiagnosed. Contemporary methods for diagnosing infertility rely heavily on advanced, clinic-based technology and do not lend themselves to application in population-based studies. Surveillance of human populations for the incidence of reproductive disease requires the development of practical and sensitive biomarkers that can be used by epidemiologists in place of the labor-intensive and costly methods that are used for clinical evaluations.

Even when environmentally induced reproductive disease has been documented in a human population and one or more toxicants is suspected of being responsible, it is still a challenging task to identify the cellular target(s) of toxicity and the mechanism(s) of toxic action. This information cannot be obtained by experimentation with human subjects. Many aspects of reproductive biology are so specific to primate species that only a nonhuman primate model may be suitable as a surrogate for humans in experiments that identify the target of a reproductive toxicant. Nonhuman primates are very expensive and their supply is limited. Nevertheless, results from whole-animal studies are usually required to select and design *in vitro* experiments that use more widely available cells from humans or other animals.

The basic requirements for comprehensive evaluation of an environmental hazard to human female reproduction are similar, regardless of the source of toxicity. In this chapter, we outline the steps and processes that we have developed to approach such problems. This effort involves an interdisciplinary team that includes epidemiologists, clinical investigators, biochemists, toxicologists, and comparative physiologists. This article is not intended as a comprehensive review of contemporary methodology for population-based studies of female reproductive health. For this information, the reader is referred to specific state-of-the-art reviews (*2,3*).

## Overview

Efforts to characterize the adverse reproductive health effects of an environmental exposure can be divided into three areas which we conceptualize as cycles of activities (Figure 1). These activities encompass human field studies, whole-animal toxicology studies, and *in vitro* mechanistic studies. One or all of these activities may be appropriate to accomplish the objective, depending on specific circumstances. For
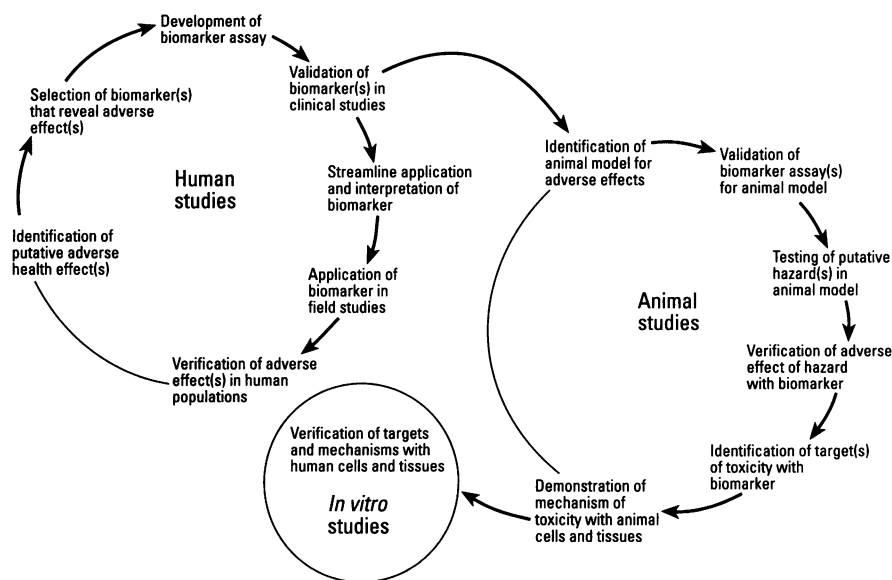
**Figure 1.** A schematic of the overall concept to illustrate the areas of activities for the interdisciplinary approach to the identification and characterization of an environmental hazard. The activities start and end with the first cycle, human studies, where an adverse health effect is perceived and documented.

example, the information which might be gained from one cycle of activity (e.g., the target of toxicity or the mechanism of action) may already be known.

The first cycle of activity, which we will refer to as human studies (Figure 1), is composed of a logical series of research steps that are driven by the suspicion or recognition of an adverse reproductive outcome in an exposed human population. The identification of the population at risk and the possible sources of exposure may be activities of the epidemiologist members of the interdisciplinary research team, or this information may be developed by others. The activities that follow involve a collaborative effort by epidemiologists, endocrinologists, and clinicians to develop and validate a biomarker or set of biomarkers which can be used to study populations at risk and to verify an increased incidence of adverse reproductive outcomes in exposed individuals. In general, these investigations provide biologic data that not only verify a self-reported or clinically reported outcome (e.g., an increased incidence of infertility, menstrual dysfunction, or spontaneous abortion), but also provide an indication of the cause of the disorder (e.g., anovulation).

A second and related series of activities requiring animal experimentation may be needed to confirm a target of toxicity that is indicated by the results of the field study, and in some cases to verify that the putative environmental hazard causes the types of reproductive disorders that were observed

in the human populations. We will refer to these activities as animal studies (Figure 1) and they involve the efforts of reproductive physiologists, toxicologists, and endocrinologists. For these experiments, an appropriate animal model is selected, and for this model, comparable biomarkers to those being used in the human field studies are developed and validated. Ideally, the animal model is exposed to environmentally relevant concentrations of a toxicant to which humans are exposed and the biomarkers of effect are analyzed to determine whether the outcome of the human exposure can be replicated in the model. If the toxicant has not been identified, the animal studies may utilize model compounds to identify target(s) of toxicity at the organ-system level and to correlate these findings with the results of the biomarker data.

The third area of *in vitro* studies (Figure 1) follows from the whole-animal studies when specific organ targets of toxicity are identified. Experimental studies utilizing cells and tissues from humans, nonhuman primates, or other animal models can be designed to investigate the mechanism of toxic action at the biochemical and molecular levels. Such experiments are carried out by biochemists, toxicologists, and molecular biologists. The results of these studies allow predictions to be made about the toxicity to reproductive tract cells of related classes of chemical compounds. Information obtained at each step in these three cycles of investigation is

relevant to the overall objective of confirming that a putative toxicant is responsible for the adverse effects that are revealed by the reproductive biomarkers. The interdisciplinary research that is portrayed in the three cycles of activity requires close and continuing interaction between the investigators. Although many of the activities in one area are dependent on information obtained in other areas, the overall organization of the program allows for simultaneous progress at all levels of the investigation.

## Human Studies

The stimulus for concerted research activity in human reproductive toxicology is the identification of exposed individuals with impaired reproductive functions. In the case of females, clinically apparent reproductive diseases include sterility, infertility (delay to conception), spontaneous abortion, and menstrual dysfunction. The instruments that are used to identify these adverse reproductive outcomes include menstrual calendars and daily diaries (4,5) as well as measurements of urinary biomarkers (6,7). These biomarkers are metabolites of reproductive hormones. Under field conditions, multiple biomarkers may be needed for detection of complex reproductive outcomes such as pregnancy loss (6,8).

Clinical expertise is essential for understanding the reproductive diseases that are suspected to have an environmental cause, and for selecting the endocrine parameters that can be used to identify these diseases. In general, female reproductive function is described not by absolute levels of hormones, but rather by the patterns of hormone fluctuation throughout the menstrual cycle and during pregnancy. It is important to understand the relationships between the patterns of circulating hormones and the patterns of hormone metabolite excretion in urine. During the development of assays for urinary hormone metabolites, consideration must be given to the practicality of assay systems if they are to be cost-effective in large-scale, population-based studies. Efficiency and economy are as important as reliability and accuracy in situations where tens of thousands of samples must be analyzed. Enzyme-based, microtiterplate-formatted assays provide one method of reducing the costs of both labor and materials. Such assays have been developed in our laboratory for the urinary metabolites of estradiol ($E_2$) and progesterone (Po) (9), luteinizing hormone (LH) (10), follicle-stimulating hormone (FSH) (11), and human chorionic gonadotropin (hCG) (12).

The process of validating assays for biomarkers of adverse reproductive outcomes includes laboratory exercises, as well as observational studies of human subjects in either a clinic or field setting. Initial laboratory studies involve the addition of pure standards to biologic fluids (usually urine samples) and characterization of the assay in terms of sensitivity, specificity, reliability, and repeatability (11). Ideally, assays for reproductive biomarkers are validated using biologic samples collected in population-based studies, but such samples are frequently not available, and as mentioned above, multiple sequential samples rather than single samples are usually required to assess reproductive functions. Therefore, most validations of reproductive biomarkers are carried out using samples from clinic patients with the reproductive disorder that will be investigated in subsequent field studies.

Biologic samples that are obtained in field studies must be easily collected, handled, and stored; this consideration is an important aspect of biomarker assay development and validation. Self-collected urine samples have been used in most studies that have investigated the adverse effects of environmental hazards on reproduction. Although this approach has been shown to be feasible in a field setting, new methods are currently being developed for collecting urine on filter paper to create a dry sample for easier storage and shipping (13). Because urinary hormone metabolites are not commonly measured by clinicians during evaluation of abnormal reproductive function, there is relatively little information in the literature on the urinary hormone profiles that are characteristic of various reproductive disorders. Therefore, clinical studies in normal women that compare hormone concentrations in blood and the corresponding concentrations of urinary hormone metabolites are a necessary first step in the validation process. Such comparative studies have been published by our laboratory on the profiles of $E_2$ and Po (9), LH (10), and FSH (8,11).

After laboratory and clinical/field validations, biomarker assays should be pilot tested in a small field study to demonstrate the adverse effect of a specific exposure. In the pilot studies the biomarkers should be compared to gold standards such as physical examination, serum hormones, and clinical assessments, and they should be characterized in terms of ability to detect the adverse effect (sensitivity) and ability to discriminate between abnormal and normal subjects

(specificity). This phase of assay development should also be used to streamline and simplify the biomarker assay and the analysis of assay results. If possible, computer algorithms for interpretation of biomarker data should be developed so that large data sets can be managed with a minimum of manipulation. An example of this kind of simplification of methods is the algorithm for detecting abnormal ovarian function using a single biomarker assay for Po metabolites (14). The algorithm was shown to have a sensitivity of 75% and a specificity of 89.5% for identifying anovulatory cycles in comparison with a serum Po "gold standard." The method was applied in a population-based study of 403 healthy women recruited from a health care plan, and 4.9% of these women experienced anovulatory episodes (7).

Some endocrine patterns, such as those associated with early pregnancy loss [also known as early fetal loss (EFL)], are so complex that multiple biomarkers must be employed and individual assessments of cycle-specific data are required to identify adverse outcomes. We initially believed that EFL could be identified as an outcome in population-based studies if only hCG was measured. However, clinical studies in which circulating levels of hCG were measured on a daily basis suggested that false negative results would be obtained if only hCG is measured, as some cases of EFL may not produce detectable levels of hCG in urine (15). False positive detection of EFL was demonstrated in population-based field studies as a result of the presence of hCG in the urine of nonpregnant women (6). In an effort to minimize both false positive and false negative results, we investigated a number of ovarian hormones and how their secretion patterns were changed in response to circulating hCG. Changes in some of these hormones such as relaxin (16) and ovarian androgens (17) could be measured in blood but were not suitable as urinary biomarkers. We had previously developed urinary assays for the metabolites of $E_2$ and Po (9), and demonstrated that the production of these steroids is accelerated by hCG (18). Together with hCG, these biomarkers were measured in a large prospective study of EFL involving analysis of approximately 70,000 daily urine samples collected in more than 2500 menstrual cycles from 448 women employed in the semiconductor industry (6). When hCG was present in an ovulatory cycle and during the time when implantation could take place, the cycle classified as having EFL. Surprisingly, only

21 of the 38 cycles confirmed to be positive for heterodimeric hCG met these criteria (6). Altogether, five separate assays for four different reproductive biomarkers were required in order to fully interpret the urinary hormone data.

Application of recently developed assays for pituitary gonadotropins is likely to reduce the number of biomarkers needed to detect EFL. The assay that we previously developed for urinary Po metabolites (9) can be used to recognize the luteal phase of the cycle, and identification of the midcycle surge of FSH can be used to determine the approximate day of ovulation (8), and thus an implantation window in which hCG excretion is likely to be a result of pregnancy (6). An algorithm has been developed for detection of pregnancy using only the urinary FSH biomarker. The FSH algorithm identified conceptive cycles with a sensitivity of 88.9% and a specificity of 89.3%, compared to the serum hCG gold standard, and all six cases of EFL that were evaluated were detected by the FSH algorithm (8).

To determine whether toxicity to the mother or conceptus is responsible for EFL, we have studied the biologic and biochemical characteristics of hCG as biomarkers of embryonic well-being. The primary tool in these investigations is a luminescence LH/hCG bioassay based on the clonal human fetal kidney cell line 293 which is permanently transfected with human LH/hCG receptor cDNA and a luciferase reporter gene driven by a cAMP-dependent promoter (19). First, we developed a radioreceptor assay utilizing the membranes from this transfected cell line which express recombinant human LH/hCG receptors (19). Next, we used the bioassay, the radioreceptor assay, and two different immunoassays for hCG to characterize circulating hCG in the periimplantation period of normal and failing pregnancies (20). We found that a steep rise of bioactive hCG is a consistent feature of the periimplantation period of normal pregnancies. On the other hand, most failing pregnancies produced hCG with lower biological activity than the hCG of normal pregnancies (20). The results of culture studies with human trophoblast cells suggest that the lower bioactive/immunoreactive (B:I) hCG ratios observed in failing pregnancies are related to reduced or impaired trophoblast differentiation (21). Because the B:I hCG ratio appears to identify pregnancies with normal implantation, it may be possible in future epidemiologic studies to evaluate separately this subset of normal pregnancies

for evidence of abortion resulting from subsequent environmental exposures.

## Animal Studies

Animal studies are an essential element in the process of reproductive biomarker development, because all human studies are, by ethical necessity, observational rather than experimental. Therefore, all investigations that aim to identify the toxicant, its target of toxicity, and its mechanism of action must be carried out using animal models. Such activities are particularly appropriate after the identification of toxicants with putative effects on human reproduction. In the case of reproduction, the choice of models is limited because of species specificity in most of the reproductive processes. Unique primate characteristics involving gamete transport, implantation, hormonal rescue of the corpus luteum, and spontaneous sloughing of the endometrium (menstruation) necessitate the use of a nonhuman primate model for these investigations, and most studies have been carried out with the laboratory macaque. Limitation of animal numbers and the expense of animal care and resources dictate that such studies be thoroughly justified and carefully planned to provide the necessary information with the smallest number of animals possible.

Regardless of the similarity between the physiology of the primate model and the human, whenever possible, the same biomarkers that are used for human surveillance should be developed for the primate and validated in that species using the same approach as in the human studies. Initially, we established serum assays for macaque $E_2$ and Po and for their urinary metabolites (22). A urinary assay for the metabolites of FSH (11) also has been validated for assessing macaque pituitary function (23). Previously, there was no reliable assay for monkey chorionic gonadotropin (mCG) and this assay also has been developed (24). These assay systems have enabled us to design studies in which the biomarkers that are used to monitor human reproductive functions in epidemiologic studies are also employed to assess the results of experimental manipulations in the monkey model.

We have used the monkey model for investigating primary ovarian failure as a result of follicle depletion (25,26). Such a model is essential as an adjunct to ablation experiments or experiments with toxicants in which ovarian function is compromised. This model has permitted us to characterize the escape of pituitary gonadotropins as a consequence of release from ovarian negative

feedback in the monkey, and to compare that phenomenon with perimenopausal events in the human. The data from the monkeys 22 years of age and older reveal oligomenorrhea, low ovarian steroids, and elevated FSH excretion (26), as predicted from our characterization of perimenopausal women (27). These data validate the approach that we use to determine central versus ovarian targets of toxicity.

Our ongoing studies of dioxin toxicity in the laboratory macaque are an example of how animal studies use the principles and technologies of the human biomarker development and can lead to the identification of targets of toxicity and mechanisms of action. Macaques which had been treated with 2,3,7,8-tetrachlorodibenzo-$p$-dioxin (TCDD) were evaluated for ovarian function more than a year after treatment (28). Animals that received higher doses of TCDD exhibited oligomenorrhea, low ovarian steroid levels, and elevated levels of FSH excretion. In contrast, the animals that received lower doses of TCDD had normal menstrual calendars and normal steroid profiles with low-normal FSH excretion (28). These data suggest that the ovary is a target for TCDD toxicity, an outcome that was also observed when TCDD was given to macaques during early pregnancy.

Prior to initiation of the whole-animal studies of the effects of toxicants on pregnancy, we developed methods for detection of early pregnancy using endocrine assays for mCG (24) and ultrasound evaluation (29), both of which are capable of detecting pregnancy by gestation day (GD) 12 (29). To characterize the biomarkers associated with EFL in the macaque, luteectomy (surgical removal of the active corpus luteum) was performed to eliminate ovarian support of the pregnancy, or methotrexate was instilled into the uterine cavity via ultrasound guidance to simulate early demise of the conceptus (30). The results of these studies not only confirmed the choice of animal model by comparison to the urinary profiles in human spontaneous abortions, but also provided critical information for demonstrating that biomarkers could be used to identify effects of toxicants on specific target organs.

The in vivo experiments that included TCDD treatment of pregnant animals followed basic pharmacodynamic and pharmacokinetics studies in nonpregnant animals and confirmed the adverse effects of TCDD on early pregnancy in the primate model. The experiments also demonstrated how well the biomarkers detected

EFL and, by comparison to the biomarker profiles following surgical- and chemical-induced EFL, they revealed the targets of dioxin toxicity. Females were administered single doses of 1, 2, and 4 µg/kg TCDD ($n = 4$ per dose group) by nasogastric intubation on gestational day (GD) 12 of pregnancy. Hormones were monitored in serum ($E_2$, Po, mCG) and their metabolites were measured in urine to assess ovarian and placental endocrine status (31). Early pregnancy loss occurred between GDs 22 and 32 in 10 of the 12 animals treated with TCDD. None of the seven control animals treated with vehicle had a pregnancy loss. The primary endocrine alteration associated with embryo loss was lowered steroid levels (Po and $E_2$) indicative of a direct effect of TCDD on ovarian function. Although the urinary metabolites of $E_2$ and Po did not have the same profile as the serum biomarkers, evaluation of the urinary biomarkers led to similar conclusions regarding the time and site of toxic action (31). As these urinary metabolites are the same biomarkers that are measured in population-based studies, this demonstration of their application in the nonhuman primate model will enable future comparisons to be made between human biomarker data and laboratory animal experiments in which the putative human reproductive toxicant is studied. In addition, the results of animal studies demonstrating organ targets of toxicity shift the focus of investigations to in vitro systems in which mechanistic studies can be performed.

## In Vitro Studies

Following establishment of dose- and time–response relationships for toxicants in vivo and identification of the target organ(s) and/or cell(s), in vitro studies of the mechanisms of toxicity at the biochemical and molecular levels become meaningful (Figure 1). The design of these studies is guided by results obtained in the whole-animal experiments, and in particular the tissues and cell types that were identified as targets of the toxicant. Some in vitro studies can be carried out with tissues or cells from the primate model (32); in other studies, experiments with human cells and tissues are appropriate (33). When appropriate, the mechanistic studies should focus on effects of the toxicant on hormone production and/or hormone action in reproductive tract cells. A continued focus on the production and action of the reproductive hormones ensures continuity and relevance between all areas of the integrated research

program. Specifically, the population-based human studies in which these hormones serve as biomarkers are linked to the animal studies in which the fluctuations in these hormones are shown to cause disease. These activities in turn are linked to the *in vitro* studies, in which the mechanisms of toxic action are demonstrated with hormone-producing and/or hormone-responsive cells. Once the *in vitro* studies using animal tissues have demonstrated the adverse effects of the toxicant, then the same types of human tissues can be studied in experiments of similar design. On the basis of the knowledge gained from these human *in vitro* experiments, strong inference can be made that the putative toxicant was responsible for the reproductive disorders observed in the exposed human populations.

The identification of ovarian cells as a primary target of toxicity in whole-animal studies of TCDD toxicity (*31*) led to investigations of ovarian cells after *in vitro* exposure to TCDD (*34,35*). These studies with human luteinized granulosa cells demonstrated an effect of TCDD on the production of $E_2$ (*34*) as well as Po (*35*). Such experiments were useful in documenting that the adverse effects of *in vivo* exposures can be detected and studied *in vitro*. The *in vivo* exposures with *in vitro* follow-up also allow us to demonstrate that TCDD was not acting indirectly or through activation prior to targeting ovarian cells. Following the demonstration of a direct effect of TCDD on ovarian cells, a series of *in vitro* studies was designed, focusing first on time course and dose-response characteristics (*35*). We were able to use human and monkey cells simultaneously and demonstrate a negative effect of TCDD on steroid production in both cell types (*32,34,35*). In this specific example, the results of the *in vitro* studies demonstrated that TCDD acts through the Ah receptor in both human and monkey granulosa cells (*32,35*).

Related research with rodent models provided mechanistic insights that were applied directly to the design of experiments with TCDD and primate reproductive tract cells. The results of initial experiments with guinea pig and monkey adipose tissues demonstrated that one mechanism of TCDD toxicity involved phosphorylation cascades that modulate AP-1 and other transcription factors (*36*). Subsequent studies with rats have shown that these effects can be modulated by the actions of sex steroids (*37*). Our experiments with human granulosa cells suggest that in
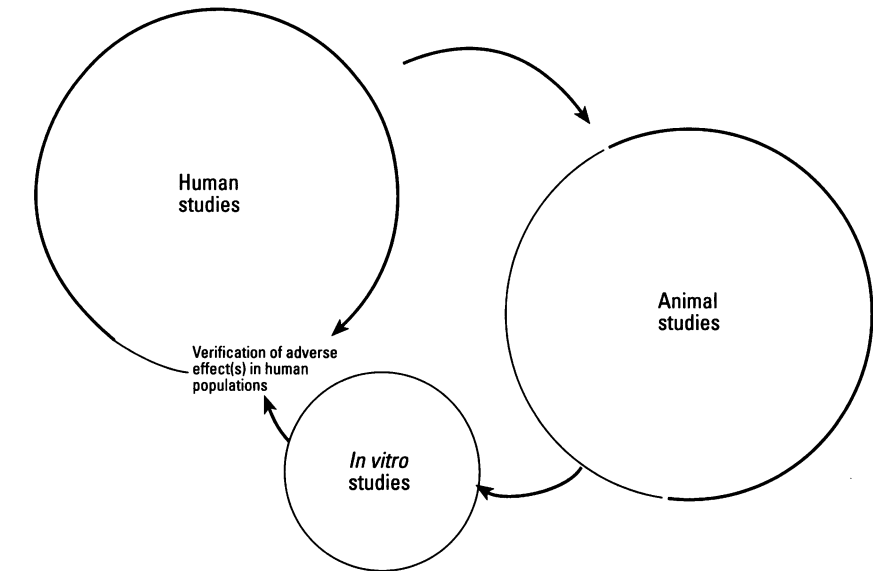


**Figure 2.** A composite of the individual activities from all three cycles are shown as they occur in a single integrated study. Although the sequence of progress may vary with specific circumstances and individual activities may be added or deleted, the overall objective of the plan is to develop methods and knowledge that enable an epidemiologic study to be conducted to verify or deny that an adverse reproductive health effect has resulted from exposure to a specific environmental hazard.

reproductive tract cells, as in adipose cells, one mechanism of TCDD toxicity involves interference with cytosolic phosphorylation cascades (*34*).

## Integration

In the context of an integrated research program (Figure 2), this study design enhances the prospect of identifying and verifying hazards to reproductive health that affect human populations. Depending on the technology and information that is already available, many of the steps and even some phases of the study design would not be necessary. The key element is the focus on reproductive hormones. The logic for using the metabolites of these hormones as reproductive biomarkers is derived from clinical experience in diagnosing infertility and is supported by animal experiments in which perturbations of these hormones result from toxicant exposures. Finally, the circle is closed by demonstration at the cellular and molecular level of the mechanisms by which toxicants affect hormone production and hormone action.

## Summary and Conclusion

An integrated research program is described that is designed to investigate the adverse effects of environmental hazards on female reproductive health. Although this program has been designed to investigate hazards to reproduction, the same general

approach could be used to study any adverse health effect or environmental factor. The logical progression of steps ensures that appropriate tools for future health surveillance will be available, increases the likelihood that toxicants will be identified, and provides an efficient and effective approach to investigate the mechanisms of toxicity. The program requires collaboration between investigators with diverse backgrounds and skills as well as continuous transfer of information and technology between them. Such an approach is appropriate for multidisciplinary centers such as the Superfund Basic Research Centers.

**REFERENCES AND NOTES**

1. Government Accounting Office. Reproductive and Developmental Toxicants. Publ No PEMD-92-3. Washington:Government Printing Office, 1991.
2. Stein ZA, Hatch MC, eds. Reproductive problems in the workplace. In: Occupational Medicine: State of the Art Reviews. Vol 1, No 3. Philadelphia, PA:Hanley & Belfus, 1986;361–539.
3. Gold EB, Lasley BL, Schenker MB. Reproductive Hazards. Occupational Medicine: State of the Art Reviews. Vol 9, No 3. Philadelphia, PA:Hanley & Belfus, 1986;363–372.
4. Gold EB, Eskenazi B, Hammond SK, Lasley BL, Samuels SJ, Rasor MO,

Hines CJ, Overstreet JW, Schenker MB. Prospectively assessed menstrual cycle characteristics in female wafer-fabrication and nonfabrication semiconducter employees. Am J Ind Med 28:799–815 (1995).

5. Gold EB, Eskenazi B, Lasley BL, Samuels SJ, Rasor MO, Overstreet JW, Schenker MB. Epidemiologic methods for prospective assessment of menstrual cycle characteristics in female semiconductor workers. Am J Ind Med 28:783–797 (1995).

6. Lasley BL, Lohstroh P, Kuo A, Gold EB, Eskanazi B, Samuels SJ, Overstreet JW, Lasley BL. Laboratory methods for evaluating early pregnancy loss in an industry-based population. Am J Ind Med 28:771–781 (1995).

7. Waller K, Swan SH, Windham GC, Fenster L, Epstein D, Elkin E, Schaefer C, Lasley BL. Use of urine biomarkers to evaluate menstrual function in healthy women. Am J Epidemiol (in press).

8. Qiu Q, Kuo A, Todd HE, Dias JA, Gould JE, Overstreet JW, Lasley BL. Enzyme immunoassay method for the β subunit of urinary follicle stimulating hormone (FSH) and its application for measurement of total urinary FSH. Fertil Steril 69:278–285 (1997).

9. Munro CJ, Stabenfeldt GH, Cragun JR, Addiago LA, Overstreet JW, Lasley BL. Relation of serum estradiol and progesterone concentrations to urinary excretion profiles of their major urinary metabolites as measured by enzyme-immunoassay. Clin Chem 37:839–844 (1991).

10. Clough K, Cole FX, Seaver SS, Danti A, Kuo AY, Lasley BL. Enzyme immunoassay for total α gonadotropin subunits in human urine samples. Fertil Steril 52:1241–1245 (1992).

11. Qiu Q, Overstreet JW, Todd HE, Nakajima ST, Stewart DR, Lasley BL. Total urinary follicle stimulating hormone as a biomarker of early pregnancy and periimplantation spontaneous abortion. Environ Health Perspect 105:862–866 (1997).

12. Taylor CA, Overstreet JW, Boyers SP, Canfield RE, O'Connor JF, Lasley BL. Prospective assessment of early fetal loss using an immunoenzymometric screening assay for detection of urinary human chronic gonadotropin. Fertil Steril 57:1222–1224 (1992).

13. Shideler SE, Munro CJ, Johl HK, Taylor HW, Lasley BL. Urine and fecal samples collected on filter paper for ovarian hormone evaluations. Am J Primatol 37:305–315 (1995).

14. Kassam A, Overstreet JW, Snow-Harter C, DeSouza MJ, Gold EB, Lasley BL. Identification of anovulation and transient luteal function using a simple urinary pregnanediol-3-glucuronide ratio algorithm. Environ Health Perspect 104:408–413 (1996).

15. Stewart DR, Overstreet JW, Celnicker AC, Hess DL, Cragun JR, Boyers SP, Lasley BL. The relationship between hCG andrelaxin in normal pregnancies versus periimplantation spontaneous abortions. Clin Endocrinol 38:379–385 (1993).

16. Stewart DR, Nakajima ST, Overstreet JW, Boyers SP, Lasley BL. Relaxin as a biomarker for human pregnancy detection. In: Progress in Relaxin Research (MacLennan AH, Tregear GW, Bryant-Greenwood GB, eds). Singapore:World Scientific Publishing, 1995;214–224.

17. Castracane VD, Stewart DR, Overstreet JW, Gimple T, Lasley BL. Maternal serum androgens in human pregnancy: early increases within cycle of conception. Human Reprod 13(2):460–464 (1998).

18. Lasley BL, Gold EB, Nakajima ST, Stewart DR, Overstreet JW. Classification of adverse reproductive effects can be improved by measurements of multiple biomarkers for ovarian toxicity and early fetal loss. J Toxicol Environ Health 40:423–433 (1993).

19. Ho H-H, O'Connor JF, Overstreet JW, Lasley BL. Characterization of hCG peptide variants with a radio-receptor assay using recombinant human LH/CG receptors. EPBM (in press).

20. Ho H-H, O'Connor JF, Tieu J, Overstreet JW, Lasley BL. Characterization of hCG in normal and failing pregnancies. EPBM (in press).

21. Ho H-H, Douglas GC, Qiu QF, Thirkill TL, Stewart DR, Overstreet JW, Lasley BL. The relationship between trophoblast differentiation and the production of bioactive hCG. EPBM (in press).

22. Shideler SE, Shackleton CHL, Moran FM, Stauffer P, Lohstroh PN, Lasley BL. Enzyme immunoassays for ovarian steroid metabolites in the urine of Macaca fascicularis. J Med Primatol 22:301–312 (1993).

23. Todd HE, Shideler SE, Laughlin LS, Overstreet JW, Pohl CR, Byrd W, Lasley BL. Unpublished data.

24. Munro CJ, Laughlin LS, VonSchalscha T, Baldwin DM, Lasley BL. An enzyme immunoassay for serum and urinary Levonorgestrel in human and non-human primates. Contraception 54:43–53 (1996).

25. Roberts JA, Gilardi K, Lasley BL, Rapp PR. Reproductive senescence predicts cognitive decline in the aged female monkey. Nature 8:2047–2051 (1997).

26. Gilardi K, Roberts JA, Shideler SE, Valuerde CR, Lasley BL. Characterization of the onset of menopause in the rhesus macaque. Biol Reprod 57:335–340 (1997).

27. Shideler SE, De Vane GW, Kalra PS, Benirschke K, Lasley BL. Ovarian-pituitary hormone interactions in the perimenopause. Maturitas 11:331–339 (1989).

28. Morán FM, Scott MA, Tarara R, Hendrickx AG, Overstreet JW, Lasley BL. Unpublished.

29. Tarantal AF, Laughlin LS, Dieter J, Overstreet JW, Tieu J, Hendrickx AG, Lasley BL. Pregnancy detection by ultrasound and chorionic gonadotropin during the peri-implantation period in the macaques (Macaca fascicularis). EPBM (in press).

30. Stewart DR, Stouffer R, Overstreet JW, Hendrickx AG, Lasley BL. Measurement of periimplantation relaxin concentrations in the macaques using a homologous assay. Endocrinology 132:936–938 (1993).

31. Hendrickx AG, Peterson PE, Otianga-Owiti GE, Tarantal AF, Dieter JA, Lasley BL, Overstreet JW. Endocrine and morphological biomarkers of early pregnancy loss in macaques. In: Proceedings of the 11th Primate Symposium, May 1997, Münster/New York:Waxmann-Verlag, 1997.

32. Morán FM, Enan E, Stewart DR, VandeVoort CA, Overstreet JW, Lasley BL. 2,3,7,8-Tetrachlorodibenzo-p-dioxin effects on pregnant monkey luteal cell function in vitro. In: Proceeding of the 29th Annual Meeting of the Society for the Study of Reproduction, August 1996, London, Ontario. 1997;180.

33. Stewart DR, VandeVoort CA. Simulation of human luteal endocrine function with granulosa lutein cell culture. J Clin Endocrinol Metab 82:3078–3083 (1997).

34. Enan E, Moran F, VandeVoort CA, Stewart D, Lasley BL. Mechanism of toxic action of 2,3,7,8-tetrachlorodibenzo p-dioxin (TCDD) in cultured human luteinizing granulosa cells. Reprod Toxicol 10:497–508 (1996).

35. Enan E, Lasley BL, Stewart DR, Overstreet JW, VandeVoort CA. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) modulates function of human luteinizing granulosa cells via cAMP signaling and early reduction of glucose transporting activity. Reprod Toxicol 10:191–198 (1996).

36. Enan E, Overstreet JW, Matsumura F, VandeVoort CA, Lasley BL. Gender differences in the mechanism of dioxin toxicity in rodents and in nonhuman primates. Reprod Toxicol 10:401–411 (1996).

37. Enan E, El-Sabeawy F, Moran F, Overstreet JW, Lasley BL. Interruption of estradiol signal transduction by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) through disruption of the protein phosphorylation pathway in adipose tissues from immature and mature female rats. Biochem Pharmacol 55:1077–1090 (1998).