

To: MaryFran Sowers, Principal Investigator
Repository Organization

From: MaryFran Sowers, Repository User Applicant

Re: Letter of Intent for Use of Repository Resources

Introduction and Goal. We propose to conduct an investigation of the role of variation in intermediate metabolites of the estrogen pathway in relation to selected health outcomes important for the health of women. The prevailing dogma is that the differential expression in estrogen metabolism imparts differences in estrogen-related adverse outcomes. The concept is based on data that indicated that different metabolites have different, even opposite, effects on estrogen-sensitive end organs. Variation in circulating estrogen metabolites may be due, in part, to the variability in the induction of cytochrome P450 enzymes that are responsible for the oxidative metabolism of estrogens. The goal of this application is to examine whether cultural/lifestyle practices such as smoking and diet are potential sources of “endocrine disruption” of the ovarian axis, as measured by hydroxylation products of estrone, and to relate this endocrine disruption to measures of the menopausal transition, cardiovascular status and bone status. While the existing questionnaire data provides a great deal of information regarding lifestyle and general exposures, it is unrealistic to expect that self-reporting can accurately identify all exposures. It is also unlikely that identifying specific compounds leading to the induction of selected cytochrome P450 enzymes would be sufficiently comprehensive as to be informative regarding biological consequences. We propose, therefore, to accurately measure the arylhydrocarbon receptor (AhR)-ligand load to provide an estimate of the amount of P450-induction can be attributed to this well-defined signal-transduction pathway.

Study Design. Two study designs are proposed for this application. First, data from the SWAN baseline (year 00 or 01) plus new data (estrone metabolites and AhR-ligand load) will be related to intermediate health outcomes, specifically oxidized LDL cholesterol, bone mineral density, age at menopause as assessed in year 05. Then, the 2-OH and 16-OH estrone metabolites will be measured in urine samples collected throughout one menstrual cycle in each of the five annual collections from the SWAN Daily Hormone Study (60 women randomly sampled within each of the 5 race/ethnic groups).

Specific products proposed for measurement using the Repository serum/urine resources:

1. The **2-OH and 16-OH urinary metabolites of estrone** will be measured with the EstrametTM 2/16 urinary ELISA kit (Immuna Care Corp., Bethlehem, PA).
 - requires 0.5ml of urine
 - prefer single thaw/refreeze—must know thaw/refreeze history
 - would like to have samples from all participants at either Baseline or Year 01 to be able to link with outcomes observed at Year 05
2. The total circulating **AhR-ligand load** will be determined by measuring the ability of serum samples to induce an AhR-dependent expression of a reporter gene using a functional assay for TCDD-like compounds. A mouse hepatoma cell line will be used which has been transfected with a wild-type dioxin response element-luciferase reporter plasmid (DRE- luc). This transfected cell line has been transferred to Dr. Lasley's laboratory from the laboratory

of Dr. Michael Denison for collaborative purposes. Relative light units for the standards, internal controls and unknowns will be processed using an in-house ELISA program.

- requires 0.5ml urine
 - prefer single thaw/refreeze—must know thaw/refreeze history
 - would like to have samples from all participants at either Baseline or Year 01 to be able to link with outcomes observed at Year 05
3. Since one of the outcomes proposed is the most atherogenic lipid, we propose to characterize **oxidized LDL cholesterol**. It has been suggested that estradiol or its intermediate metabolites has a role in the degree of oxidation of LDL cholesterol.
- requires 0.5 ml of plasma
 - prefer no previous thaw/refreeze—must know thaw/refreeze history as well as fasted state of participant and any particulars about sample handling
 - would like to have samples from all participants in Year 05

Laboratory Resources and Environment.

- The **2-OH and 16-OH urinary metabolites of estrone** will be measured in the CLASS Laboratory of Dr. Dan McConnell at the University of Michigan. This Laboratory has served as the endocrine laboratory for the SWAN Study and has as its direction and focus the development and implementation of assays related to the hypothalamic/pituitary/ovarian axis and the intermediate metabolic pathways associated with that axis.
- The total circulating **AhR-ligand load** assay will be conducted in the laboratory of Dr. Bill Lasley at the University of California at Davis. This Laboratory has collaborated with Dr. Mike Denison for this particular cell line but has also been involved in active characterization of ligand load associated with the estrogen receptor and the LH receptor.
- The **oxidized LDL cholesterol** will be assayed in the MRL laboratories under the direction of Dr. Evan Stein. MRL has characterized markers of lipids, coagulation, and bone turnover for the SWAN Study. The Laboratory has international recognition for its contribution to studies of cardiovascular disease.
- NIH CV's are attached for the investigators from these laboratories.

Variables required from the SWAN Data base.

- Characteristics of the population: site, age, ethnicity, body size, “healthy” status
- Outcome measures of interest: bone mineral density (BMD) including indices of QA, lipid profile including HDL, LDL, triglycerides and indices of QA, and the measures of menopause transition including an index of menstrual status a 00 or 01 and again at 05, age at menopause and hot flash history. Annual or biennial data for BMD and lipids are preferred.
- Covariates of the intermediate metabolism: estradiol and FSH concentrations at baseline or Year 01 as well as for the 300 women from the blood draw at the time they began their monthly urinary collection. If sEstrone exists, this would be helpful.
- Estrone concentrations measured in the 300 women selected for studies of the monthly urinary collection
- Aromatic Hydrocarbon related exposures: Smoking, active and passive, nutrient estimates of flavonoids, phytosterols, citrus fruit consumption frequency, cruciferous vegetable frequency consumption, total fat intake consumption, total protein consumption, total alcohol consumption and total energy intake.

Sample Size Justification for Limited Resources.

We estimate that we will be able to detect an effect size of less than .25 between past and never smokers with respect to the mean 2OH-E1 AND 16OH-E1 measures at baseline with the projected 2700 samples. For the association of 2-OH-E1 and 16-OH-E1 and oxidized cholesterol, we project a per-quartile sample size at the last visit of 333 women. This sample size will permit us to detect a between-group difference in mean oxidized cholesterol of 0.26 standard deviations, a small effect size. We also will be able to detect a difference in the annual rate of change in bone density of 0.0039 g/cm²/year for the first versus the fourth 2OH-E1 AND 16OH-E1 quartiles. Regarding the association between 2-OH-E1 and 16-OH-E1 and age at menopause, we project an 80%+ power to detect a relative hazard of 1.3 or greater for the fourth versus the first 2OH-E1 and 16OH-E1 quartile, for a median time to final menstrual period in the reference group (first 2OH-E1 and 16OH-E1 quartile) of 6-24 months. To determine whether 2OH-E1 and 16OH-E1 values “track” over time, departures from “tracking” can be detected by comparing the within-woman slopes over time across groups, e.g., the first baseline 2OH-E1 and 16OH-E1 quartile versus the fourth baseline 2OH-E1 and 16OH-E1 quartile. For such a comparison, (N=75 per quartile group or cycle data across time from 300 women), we will be able to detect a difference between groups of the magnitude of 0.56 times σ_G , which is a medium effect size.

Projected investigators on the application:

Members of the Repository Organization plus a designated SWAN Core Study Investigator

Fiscal Resources.

The fiscal resources are not currently in place to undertake these activities. We would propose to submit an application as a response to the request for application (RFA) “OH-01-001 Endocrine Disruptors: Epidemiologic Approaches” to investigate the relationships between exposure to endocrine disruptors and adverse health effects. This application would be evaluated in February, 2001 with availability of funding July, 2001. This is a single-time solicitation; therefore, if we were unsuccessful in securing the funding, we would notify the Repository Organization at the time we received our score and would withdraw our request for use of materials (had such a request been accepted by the Repository Organization).

Assurances.

We assure the Repository Organization of the following:

- We will include a member of the SWAN Core Study as a co-investigator to our research effort to facilitate appropriate use of the rich SWAN data base and appropriate interpretation of findings given the sampling, population, and characteristics of the SWAN Core Study.
- We understand that any commitment of resources by the Repository Organization for this application is limited to this application and that the commitment would be valid for the time period specified in the commitment letter.
- We will sign and execute by both intent and practice the contents of the agreement that has been delineated by the “Letter of Understanding from Applicant Investigator to the Repository”.
- We will submit the Letter of Understanding from Applicant Investigator to the Repository” as well as a Letter of Commitment from the Repository Organization when applications are submitted that require the use of Repository specimens and data.