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TITLE: Total xenoestrogen body burden in relation to mammographic density,  
a marker of breast cancer risk

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| 14. ABSTRACT<br>Humans are also exposed to a large number of environmental chemicals which have estrogenic activity ("xenoestrogens") and therefore may raise breast cancer risk. This study is evaluating the association of total xenoestrogen burden with mammographic breast density, which is a strong intermediate marker of breast cancer risk. All study procedures and manuals of operation for this study have been finalized, and IRB approval obtained. Subject recruitment is currently ongoing. 108 subjects in total have been recruited as of November 14, 2008. Recruitment will continue until 200 subjects are recruited. Data analysis will ensue upon completion of subject recruitment and the analysis of xenoestrogen levels in blood samples and the measurement of breast density on the mammograms. Since no analyses have been conducted, no scientific knowledge has been produced yet.                                      |                  |                          |                                             |                                                           |                                              |
| 15. SUBJECT TERMS<br>Breast cancer, xenoestrogens, environmental pollutants, mammography, breast density                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                  |                          |                                             |                                                           |                                              |
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## TABLE OF CONTENTS

|                                    |   |
|------------------------------------|---|
| INTRODUCTION .....                 | 4 |
| BODY .....                         | 4 |
| KEY RESEARCH ACCOMPLISHMENTS ..... | 6 |
| REPORTABLE OUTCOMES.....           | 7 |
| CONCLUSION.....                    | 7 |
| REFERENCES .....                   | 7 |
| APPENDICES .....                   | 7 |
| SUPPORTING DATA.....               | 7 |
| APPENDIX: Study Questionnaire      |   |

## INTRODUCTION

Breast cancer risk increases with higher endogenous estrogen levels and with use of pharmaceutical estrogens. Humans are also exposed to a large number of environmental chemicals which have estrogenic activity (“xenoestrogens”). Previous studies have focused on the relation between single xenoestrogen chemicals and breast cancer risk, with little evidence to support an association. The recent development of an assay to measure the sum estrogenic activity of xenoestrogens in biological samples presents a novel opportunity to evaluate total xenoestrogen exposure in relation to breast cancer risk. This study will evaluate the association of total xenoestrogen burden with mammographic breast density, which is a strong intermediate marker of breast cancer risk. To accomplish this aim, 200 healthy postmenopausal women receiving their regularly scheduled screening mammogram at a clinic in Madison, Wisconsin, will provide a blood sample and complete a questionnaire regarding established breast cancer risk factors and potential sources of xenoestrogen exposure, including diet, occupation, and lifestyle factors. The blood samples will be analyzed for total serum xenoestrogen burden, and breast density will be measured from participants’ mammograms as continuous percent density. Statistical analyses will be used to identify important predictors of total xenoestrogen burden and to measure the association between total xenoestrogen burden and breast density. This study will describe current xenoestrogen exposure levels, assess their relation to breast density, and provide direction to future studies of the potential health effects of these ubiquitous compounds.

## BODY

The approved Statement of Work for this grant includes:

### Task 1. Finalize procedures, Months 1-6:

#### a. Finalize manual of operations for blood collection, processing, and temporary storage

Progress report: All study procedures and manuals of operation for this study have been finalized.

#### b. Obtain IRB/Human Subjects and HIPAA regulatory approvals

Progress report: Final human subjects protection approval for this study was obtained February 18, 2008 from the University of Wisconsin Institutional Review Board.

#### c. Pilot test questionnaire

Progress report: The study questionnaire was piloted and finalized in February 2008 (see Appendix).

#### d. Pilot E-Screen bioassay on 5-10 anonymous samples

Progress report: IRB approval is pending for approval of the receipt of anonymous blood samples from UW Hospital Clinics for piloting of the E-Screen bioassay.

**e. Pilot quantitative density measurement on 5-10 anonymous mammograms at Group Health (Seattle)**

Progress report: Anonymous mammograms were recently (November 2008) delivered to collaborators at Group Health. Piloting of the density measurement process will occur in December 2008.

**f. Finalize Microsoft Access database to track participant recruitment, questionnaires, blood samples, mammograms, and mammogram reports**

Progress report: A Microsoft Access database was finalized in June 2008 to track participant recruitment, questionnaires, blood samples, mammograms, and mammogram reports.

**Task 2. Recruit participants, Months 7-12:**

**a. Recruitment of 200 women obtaining screening mammograms at the UW Health West-Madison Clinic**

Progress report: In June 2008 we began recruitment of subjects for the study. 59 subjects had been recruited and completed participation by September 14, 2008 (the one year anniversary of the grant start date). Subject recruitment during this initial period was accruing more slowly than we anticipated. To improve recruitment, we started recruiting at an additional study site on September 15, 2008. 108 subjects in total have been recruited as of November 14, 2008. Recruitment will continue until 200 subjects are recruited.

**b. Obtain signed permission for release of radiology report corresponding to the screening mammogram from participants**

Progress report: Signed permission has been obtained for all 108 subjects recruited. Permission will continue to be obtained from newly recruited subjects.

**c. Collect blood sample and questionnaire from participants at the Clinic**

Progress report: Blood samples and questionnaires have been obtained for all 108 subjects recruited. Collection of blood samples and questionnaires will continue as subjects are recruited.

**d. Implement questionnaire data entry and quality control measures**

Progress report: Data entry from the questionnaires for 99 subjects has been completed. Data entry will continue as subjects are recruited.

**e. Submit annual progress report to the DOD**

Progress report: This constitutes the annual progress report.

**Task 3. Analyze blood samples and mammograms, Months 13-18:**

**a. Transport blood samples from Office of Clinical Trials to the Wisconsin State Laboratory of Hygiene (both located in Madison, WI)**

Progress report: Blood samples for the first 49 subjects have been transported to the WI State Laboratory of Hygiene. Transport of samples will continue as subjects are recruited.

**b. Perform E-Screen blood sample analysis for total xenoestrogen burden**

Progress report: No analyses of the blood samples have been conducted. These will be conducted upon completion of subject recruitment.

**c. Deliver mammogram copies from UW-Madison to Group Health (Seattle) for quantitative measurement**

Progress report: No study mammograms have been delivered to Group Health. These will be delivered after completion of the pilot measurements, and continue as new subjects are recruited.

**d. Interpretation of mammograms for quantitative density measurements**

Progress report: No analyses of the mammograms have been conducted. These will be conducted upon completion of subject recruitment.

**Task 4. Data analysis and communication of results, Months 19-24:**

- a. Conduct statistical analysis of potential sources of xenoestrogen exposure**
- b. Conduct statistical analysis of relation between total xenoestrogen burden and mammographic density**
- c. Prepare manuscripts and final report to the DOD**

Progress report: No statistical analyses have been conducted yet. No publications have been prepared. These tasks will be conducted upon completion of Tasks 1-3.

**KEY RESEARCH ACCOMPLISHMENTS**

- All study procedures finalized

- IRB approval obtained
- Recruitment of 59 subjects during first year of grant

## **REPORTABLE OUTCOMES**

- Poster presentation of the study design at the 2008 DOD Era of Hope Conference.<sup>1</sup>
- Funding has been obtained from the Susan Komen Foundation for an ancillary study of sex hormones and breast density. The Komen Foundation is providing funds to analyze sex hormone levels in the blood samples obtained in this study. The relation between sex hormone levels and mammographic breast density will be assessed.

## **CONCLUSION**

All study procedures and manuals of operation for this study have been finalized. Final human subjects protection approval for this study was obtained February 18, 2008 from the University of Wisconsin Institutional Review Board. In June 2008 we began recruitment of subjects for the study. Subject recruitment is ongoing. 108 subjects in total have been recruited as of November 14, 2008. Recruitment will continue until 200 subjects are recruited. Data analysis will ensue upon completion of subject recruitment and the assessment of blood xenoestrogen levels and mammographic breast density.

Since no analyses have been conducted, no scientific knowledge has been produced yet.

## **REFERENCES**

1. Sprague BL, Trentham-Dietz A, Sisney GA, Hemming J, Buist DSM. Total xenoestrogen body burden in relation to mammographic breast density, a marker of breast cancer risk. Presented at “Era of Hope: the Department of Breast Cancer Research Program Meeting”, June 25-28, 2008; Baltimore, MD.

## **APPENDICES**

1. Study questionnaire (attached)

## **SUPPORTING DATA**

None

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# Total Xenoestrogen Body Burden in Relation to Mammographic Density, a Marker of Breast Cancer Risk



Study Survey

This study looks at the connection between breast density, as seen from a mammogram, and the levels of environmental pollutants measured in the blood. Your assistance is very important to the success of the study. Your answers in this survey will give us information that may help us to better understand what factors in the environment are related to breast density. Your participation in this study is completely voluntary.

Please try to answer every question. However, you are free to skip any question if it makes you uncomfortable. Please take the time to read each question carefully, and check the box that best represents your response.

This first section includes some general questions.

- February 7, 2008  
Version 5

**6. What is the highest degree or year of school you have completed?**

- ☐ None
- ☐ Grades 1-7 (some grade school)
- ☐ Grade 8 (completion of grade school)
- ☐ Grades 9-11 (some high school)
- ☐ Grade 12 (high school diploma, GED, or any high school equivalent)
- ☐ 1-3 years college (junior college)
- ☐ 4 years college (college degree)
- ☐ Advanced degree (M.A., Ph.D., M.D., J.D., etc.)

**7. What is your current marital status?**

- ☐ Married
- ☐ Living with a partner
- ☐ Divorced or separated
- ☐ Widowed
- ☐ Single (never married)

|                                                      |
|------------------------------------------------------|
| <b>SECTION 2: REPRODUCTIVE AND MENSTRUAL HISTORY</b> |
|------------------------------------------------------|

These questions deal with your reproductive and menstrual history.

**8. How old were you when you had your first menstrual period?**

\_\_\_\_\_ years old

**9. Are you still having periods?**

- ☐ Yes
- ☐ No ➡

**9a. If No, how old were you when your menstrual periods stopped?**

\_\_\_\_\_ years of age

**9b. Why did your menstrual periods stop?**

- ☐ Natural menopause
- ☐ Use of birth control pills or female hormones
- ☐ Hysterectomy (removal of the uterus)
- ☐ Removal of one or both ovaries
- ☐ Other: *Please describe-* \_\_\_\_\_

**10. Have you ever given birth? (Include all pregnancies that lasted at least 6 months, live births, still births, or cesarean sections. Do not include miscarriages and abortions.)**

☐ No

☐ Yes ➡ **10a. If Yes, how old were you when you first gave birth?**

\_\_\_\_\_ years old

**10b. How many times have you given birth?**

\_\_\_\_\_

**10c. Did you breast feed any of your children?**

☐ No

☐ Yes ➡ **10d. If Yes, how long in total did you breast feed all of your children?**

\_\_\_\_\_ weeks \_\_\_\_\_ months \_\_\_\_\_ years

|                               |
|-------------------------------|
| <b>SECTION 3: MEDICATIONS</b> |
|-------------------------------|

Below are questions regarding medications you have taken.

**11. Have you ever taken birth control pills?**

☐ No

☐ Yes ➡ **11a. If Yes, how long in total have you taken birth control pills?**

\_\_\_\_\_ weeks \_\_\_\_\_ years

**11b. How old were you when you last took birth control pills?**

\_\_\_\_\_ Years of age

**12. Do you have diabetes (high blood sugar)?**

☐ No

☐ Yes ➡ **12a. If Yes, how old were you when you were diagnosed?**

\_\_\_\_\_ Years of age

**12b. If Yes, how do you treat your diabetes (check all that apply)?**

- ☐ Special diet
- ☐ Medications by mouth
- ☐ Insulin injections
- ☐ Other: *Please specify*- \_\_\_\_\_

|                                 |
|---------------------------------|
| <b>SECTION 4: BREAST HEALTH</b> |
|---------------------------------|

This section deals with your breast health history.

**13. Before today, how many mammograms have you had in the past five years?**

- ☐ 0
- ☐ 1
- ☐ 2
- ☐ 3
- ☐ 4
- ☐ 5
- ☐ 6 or more

**14. Before today, when was your last mammogram?**

\_\_\_\_\_ (month) \_\_\_\_ \_\_\_\_ \_\_\_\_ (year)

☐ I have never had a mammogram before today

**15. Has a physician ever removed tissue from your breast or done a biopsy (this does not include removing fluid from a cyst using a needle)?**

☐ No

☐ Yes ➡

**15a. If Yes, how many times has this occurred?**

\_\_\_\_\_ times

**15b. Which breast(s) were involved?**

- ☐ Both breasts
- ☐ Left only
- ☐ Right only

**16. Have you ever been diagnosed with breast cancer?**

- ☐ No
- ☐ Yes

**17. Have you ever had any of the following breast procedures? (Check all that apply)**

- |                                                         |                                                                 |
|---------------------------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/> Cyst aspiration                | <input type="checkbox"/> Breast reconstruction                  |
| <input type="checkbox"/> Lumpectomy (for breast cancer) | <input type="checkbox"/> Breast reduction                       |
| <input type="checkbox"/> Mastectomy                     | <input type="checkbox"/> Breast implants                        |
| <input type="checkbox"/> Radiation therapy              | <input type="checkbox"/> I have not had any of these procedures |

**18. Have any of your blood relatives been diagnosed with breast cancer?**

- ☐ No
- ☐ Yes ➡ **18a. If Yes, has your mother ever been diagnosed with breast cancer?**
- ☐ Don't know

- ☐ Yes
- ☐ No
- ☐ Don't know

**18b. Have any of your sisters ever been diagnosed with breast cancer?**

- ☐ Yes ➡ How many sisters have been diagnosed? \_\_\_\_
- ☐ No
- ☐ Don't know
- ☐ I don't have any sisters

**18c. Have any of your daughters ever been diagnosed with breast cancer?**

- ☐ Yes ➡ How many daughters have been diagnosed? \_\_\_\_
- ☐ No
- ☐ Don't know
- ☐ I don't have any daughters

## SECTION 5: PESTICIDES

These questions deal with potential sources of exposure to pesticides on farms or around your house. Pesticides are not the same as fertilizers. Pesticides are used to kill unwanted pests, and include insecticides which kill insects, herbicides which kill weeds, and fungicides which kill molds.

**19. Are pesticides used to control pests (ants, for example) inside your house?**

☐ No

☐ Yes ➡ **19a. If Yes, how often?** \_\_\_\_\_ times per year

**20. Do you personally apply pesticides to control pests inside your house?**

☐ No

☐ Yes ➡ **20a. If Yes, how often?** \_\_\_\_\_ times per year

**21. Are pesticides used to control pests (rodents, for example) in your yard or gardens?**

☐ No

☐ Yes ➡ **21a. If Yes, how often?** \_\_\_\_\_ times per year

**22. Do you personally apply pesticides to control pests in your yard or gardens?**

☐ No

☐ Yes ➡ **22a. If Yes, how often?** \_\_\_\_\_ times per year

**23. Do you use insect repellants on your skin, hair, or clothing?**

☐ No

☐ Yes ➡ **23a. If Yes, how often?** \_\_\_\_\_ times per year

**24. Have you ever lived on a farm?**

☐ No

☐ Yes ➡ **24a. If Yes, for how many years in total have you lived on a farm?**

\_\_\_\_\_ years

**24b. If Yes, were pesticides used to control pests (weeds, insects, molds) on this farm?**

☐ No

☐ Yes ➡

**24c. If Yes, did you personally apply pesticides to control pests?**

☐ No

☐ Yes

**25. Have you ever worked on a farm?**

☐ No

☐ Yes ➡

**25a. If Yes, for how many years in total have you worked on a farm?**

\_\_\_\_\_ years

**25b. Were pesticides used to control pests (weeds, insects, molds) on this farm?**

☐ No

☐ Yes ➡

**25c. If Yes, did you personally apply pesticides to control pests?**

☐ No

☐ Yes

## **SECTION 6: DIET AND LIFESTYLE**

This section deals with your diet, lifestyle, and current health status.

**26. Do you take multivitamins, such as One-A-Day, Theragran, or Centrum-type multivitamins?**

☐ No

☐ Yes ➡

**26a. If Yes, how often? \_\_\_\_\_ times per week**

**27. Do you take mineral supplements, such as selenium, zinc, or manganese?**

☐ No

☐ Yes ➡

**27a. If Yes, how often? \_\_\_\_\_ times per week**

**27b. If Yes, which minerals? \_\_\_\_\_**

**28. In the past year, have you taken fish oil supplements?**

☐ No

☐ Yes ➡ **28a. If Yes, how often?** \_\_\_\_\_ times per month

**29. Do you drink bottled water?**

☐ No

☐ Yes ➡ **29a. If Yes, how often?** \_\_\_\_\_ times per week

**30. On average, how often do you eat a serving of meat, including beef, chicken, lamb, or pork?**

☐ Never or less than once per month

☐ 1-3 servings per month

☐ 1 serving per week

☐ 2-4 servings per week

☐ 5-6 servings per week

☐ 1 serving per day

☐ 2-3 servings per day

☐ 4 or more servings per day

**31. On average, how often do you drink one 8 oz serving of cow's milk, including on cereal?**

☐ Never or less than once per month

☐ 1-3 servings per month

☐ 1 serving per week

☐ 2-4 servings per week

☐ 5-6 servings per week

☐ 1 serving per day

☐ 2-3 servings per day

☐ 4 or more servings per day

**32. What type of cow's milk do you usually drink?**

☐ I do not usually drink any cow's milk

☐ Whole milk

☐ 2%

☐ 1%

☐ Skim or nonfat

☐ Milk directly from a farm

**33. Do you usually drink organic cow's milk?**

- ☐ No, I do not usually drink organic cow's milk
- ☐ Yes, I usually drink organic cow's milk

**34. On average, how often do you eat a serving of soy products (tofu, soy milk, etc.)?**

- ☐ Never or less than once per month
- ☐ 1-3 servings per month
- ☐ 1 serving per week
- ☐ 2-4 servings per week
- ☐ 5-6 servings per week
- ☐ 1 serving per day
- ☐ 2-3 servings per day
- ☐ 4 or more servings per day

**35. On average, how often do you eat a serving of store-bought fish?**

- ☐ Never or less than once per month
- ☐ 1-3 servings per month
- ☐ 1 serving per week
- ☐ 2-4 servings per week
- ☐ 5-6 servings per week
- ☐ 1 serving per day
- ☐ 2-3 servings per day
- ☐ 4 or more servings per day

**36. In the past year, did you ever eat sport-caught fish, that is, caught by you or given to you? (This usually does not include fish that you buy at a store or restaurant.)**

☐ No

☐ Yes ➡ **36a. If Yes, How often did you eat sport-caught fish?**

- ☐ Less than once per month
- ☐ 1-3 servings per month
- ☐ 1 serving per week
- ☐ 2-4 servings per week
- ☐ 5-6 servings per week
- ☐ 1 serving per day
- ☐ 2 or more servings per day

**36b. Was any of this sport-caught fish from the Great Lakes? (Including Lakes Michigan, Huron, Erie, Superior and Ontario plus mouths of rivers feeding into the lakes. Please also include Green Bay and other parts of the lakes that have separate names.)**

- ☐ No  
☐ Yes

**36c. In the past year, how often did you eat lake trout and salmon (Chinook or Coho) that was sport-caught from the Great Lakes?**

- ☐ Less than once per month  
☐ 1-3 servings per month  
☐ 1 serving per week  
☐ 2-4 servings per week  
☐ 5-6 servings per week  
☐ 1 serving per day  
☐ 2 or more servings per day

**37. Do you microwave food or beverages in plastic containers?**

- ☐ No  
☐ Yes ➡ **37a. If Yes, how often?** \_\_\_\_\_ times per week  
\_\_\_\_\_ times per month

**38. In the winter, how many hours on average do you spend outdoors each week?**

\_\_\_\_\_ hours per week

**39. In the summer, how many hours on average do you spend outdoors each week?**

\_\_\_\_\_ hours per week

**40. In the summer, when you are outside during the day, how often do you use sunscreen?**

- ☐ Always  
☐ Usually  
☐ Sometimes  
☐ Rarely  
☐ Never

**41. Do you use underarm deodorants or antiperspirants?**

☐ No

☐ Yes ➡ **41a. If Yes, how often?** \_\_\_\_\_ times per day  
\_\_\_\_\_ times per week

**41b. If Yes, how often do you use underarm deodorants or antiperspirants within 1 hour after underarm shaving?**

\_\_\_\_\_ times per week

**41c. Please list which products you use as underarm deodorants or antiperspirants:**

\_\_\_\_\_

**42. How many hours on average do you spend each week doing physically vigorous activities that cause large increases in heart rate or breathing? For example: lifting or carrying heavy objects (such as small children or bags), heavy gardening/field work, climbing stairs, participating in sports activities, jogging, fast swimming, or cycling uphill.**

\_\_\_\_\_ hours per week

**43. How many hours on average do you spend each week doing physically moderate activities that cause small increases in heart rate or breathing? For example: fast walking, cycling without going uphill, cleaning windows, mopping, vacuum-cleaning, moderate gardening, light gym, leisurely swimming.**

\_\_\_\_\_ hours per week

**44. How often, on average, do you drink one 12 oz bottle, glass, or can of beer?**

- ☐ Never or less than once per month
- ☐ 1-3 servings per month
- ☐ 1 serving per week
- ☐ 2-4 servings per week
- ☐ 5-6 servings per week
- ☐ 1 serving per day
- ☐ 2 or more servings per day

**45. How often, on average, do you drink a 5 oz glass of wine?**

- ☐ Never or less than once per month
- ☐ 1-3 servings per month
- ☐ 1 serving per week
- ☐ 2-4 servings per week
- ☐ 5-6 servings per week
- ☐ 1 serving per day
- ☐ 2 or more servings per day

**46. How often, on average, do you have a drink containing a shot of liquor?**

- ☐ Never or less than once per month
- ☐ 1-3 servings per month
- ☐ 1 serving per week
- ☐ 2-4 servings per week
- ☐ 5-6 servings per week
- ☐ 1 serving per day
- ☐ 2 or more servings per day

**47. What is your current height? \_\_\_\_\_ feet \_\_\_\_\_ inches**

**48. What is your current weight? \_\_\_\_\_ pounds**

**49. What was your weight 1 year ago? \_\_\_\_\_ pounds**

**50. What was your weight at age 18? \_\_\_\_\_ pounds**

**51. Have you smoked at least 100 cigarettes in your entire life?**

☐ No

☐ Yes ➡ **51a. If Yes, how old were you when you started smoking cigarettes?**

\_\_\_\_\_ years

**51b. Do you currently smoke cigarettes?**

☐ No ➡

☐ Yes **51b. If No, how old were you when you stopped smoking?**

\_\_\_\_\_ years

**51c. On average, over the entire time you smoked, how many cigarettes or packs of cigarettes did you smoke a day?**

\_\_\_\_\_ cigarettes \_\_\_\_\_ packs

**52. Do you currently live with someone (for example, husband) that smokes cigarettes in your home?**

☐ No

☐ Yes ➡ **52a. If Yes, how many cigarettes or packs of cigarettes do they smoke per day?**

\_\_\_\_\_ cigarettes \_\_\_\_\_ packs

**53. In general, would you say your health now is:** (please check one)

☐ Excellent

☐ Very Good

☐ Good

☐ Fair

☐ Poor

**54. Please enter your zip code and county of residence:**

Zip code \_ \_ \_ \_ \_

County \_\_\_\_\_

**55. We want to learn more about the environment in different communities. Please provide your full street address to help us do this. We can then link to databases in the state which provide information on air pollution and other environmental factors.**

Street Address: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**56. How long have you lived at this address?** \_\_\_\_\_ years

