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a marker of breast cancer risk.

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14. ABSTRACT Humans are 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 are finalized, and IRB approval obtained. Subject recruitment is complete. Breast density measurements on all participants have been completed. Analysis of xenoestrogen levels in the blood samples is currently ongoing. Data analysis will ensue upon completion of the analysis of xenoestrogen levels in blood samples. Since no statistical analyses have been conducted, no scientific knowledge has been produced yet.				
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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: Anonymous blood samples were obtained from UW Hospital Clinics and used for piloting of the E-Screen bioassay. Use of these blood samples revealed quality control issues that have just recently (October 2009) been resolved.

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

Progress report: Anonymous mammograms were delivered in November 2008 to collaborators at Group Health and used for piloting of the density measurement process.

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 March of 2009 we completed recruitment of 200 subjects for the study. Recruitment took longer than anticipated due to delays in obtaining IRB approval and slow subject accrual during the recruitment period. The addition of a second study site in September 2008 enhanced the rate of subject accrual and allowed us to reach our goal.

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 recruited subjects.

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

Progress report: Blood samples and questionnaires have been obtained for all recruited subjects.

d. Implement questionnaire data entry and quality control measures

Progress report: Data entry from all questionnaires has been completed, with double-data-entry on a sample of questionnaires for quality control.

e. Submit annual progress report to the DOD

Progress report: An annual progress report was submitted in 2008. This constitutes the second 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 recruited subjects have been transported to the WI State Laboratory of Hygiene.

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

Progress report: Assay validation and quality control delayed the start of these blood analyses. However, analyses of the blood samples are now ready to begin (October 2009).

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

Progress report: All study mammograms have been delivered to Group Health.

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

Progress report: Quantitative density assessment has been completed on all study mammograms.

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 the serum xenoestrogen analyses.

KEY RESEARCH ACCOMPLISHMENTS

- All study procedures finalized
- IRB approval obtained
- Recruitment completed
- Questionnaire data entered
- Mammographic breast density assessment completed

REPORTABLE OUTCOMES

- Poster presentation of the study design at the 2008 DOD Era of Hope Conference.¹
- 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. Recruitment of 200 subjects was completed in March 2009. All questionnaire data has been entered and all quantitative breast density assessment has been completed. The analysis of xenoestrogen levels in the blood samples is set to begin in October 2009. Statistical data analysis of the study aims will ensue upon completion of the assessment of blood xenoestrogen levels. 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

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)

SECTION 2: REPRODUCTIVE AND MENSTRUAL HISTORY
--

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

SECTION 3: MEDICATIONS

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-* _____

SECTION 4: BREAST HEALTH

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)

- | | |
|---|---|
| ☐ Cyst aspiration | ☐ Breast reconstruction |
| ☐ Lumpectomy (for breast cancer) | ☐ Breast reduction |
| ☐ Mastectomy | ☐ Breast implants |
| ☐ Radiation therapy | ☐ 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 ➡ **51b. If No, how old were you when you stopped smoking?**
- ☐ Yes

_____ 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

