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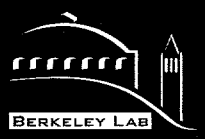

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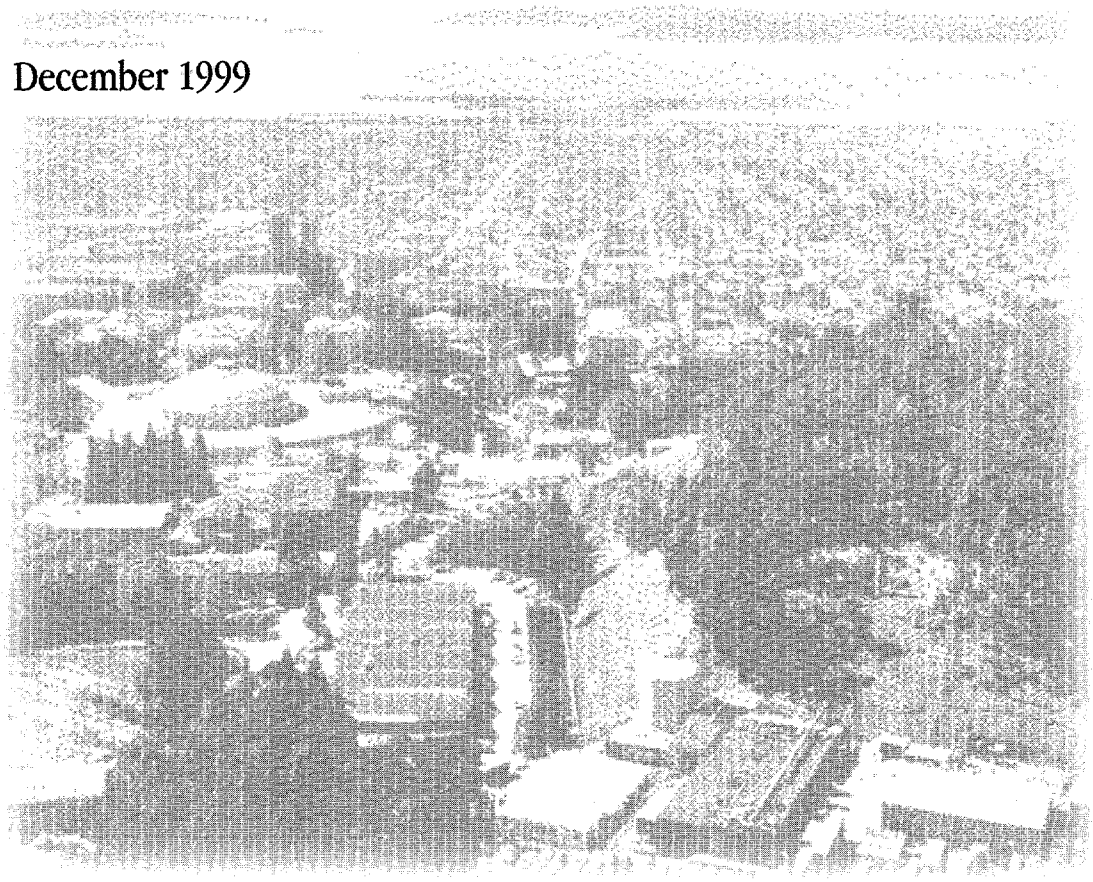
December 1999



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Comparison of Organochlorine Chemical Body Burdens of Female Breast Cancer Cases with Cancer Free Women in Rio Grande do Sul, Brazil - Pilot Study

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ABSTRACT

Background: Accepted breast cancer risk factors, such as reproductive and family history, cannot account for the majority of breast cancer cases. Exposure to certain organochlorine chemicals (OCs) may be related to increased breast cancer risk. Studies investigating the link between breast cancer and OCs have yielded conflicting results. These studies have been performed predominantly in industrialized regions where body burdens of these chemicals in human tissue have steadily declined after being withdrawn from the market and better pollution controls instituted. Health effects related to OC exposure, if they exist, could best be detected in populations with higher body burdens. Since developing countries have used OCs extensively for longer periods, their populations offer a better opportunity to study potential OC health effects.

Objectives: 1. To establish a data collection and specimen transport infrastructure in Porto Alegre, Rio Grande do Sul, Brazil. 2. To collect preliminary data to examine the distribution of known and suspected breast cancer risk factors among women living in rural and urban areas in the state of Rio Grande do Sul, Brazil. 3. To measure body burden levels of certain OCs in a small clinic-based prospective sample.

Approach: The pilot consisted of two phases. For both phases, participants were recruited from the female patients ages 18 years and older of Dr. Maira Caleffi's Clínica de Mastologia (Breast Clinic) in Porto Alegre. For Phase I, 194 completed a questionnaire regarding residential, occupational, and medical histories. In Phase II, breast adipose tissue was collected from 12 women undergoing breast biopsy surgery and transported to the Hazardous Materials Laboratory at Cal-EPA where levels of over 50 organohalogen compounds were measured.

Results: Univariate analyses of the questionnaire responses found that cases and controls differed in birth country, parity, age, menopausal status, menopausal age, income, lactation history, oral contraceptive use, and alcohol use. Rural and urban women differed in age, body mass index, education, menarche age, menopausal age, and income. The chemical analysis revealed high levels of DDE and certain PCB congeners in the pilot adipose tissue samples relative to those levels found in an on-going study of California women. Measurements of mirex, dieldrin, trans-nonachlol, hexachlorobenzene, and oxychlordane levels confirmed the presence of high OCP levels among Rio Grande do Sul female population and corroborated previous reports of high OCPs levels in human milk from Porto Alegre. Low levels of dioxins and furans were measured in the Brazil adipose samples, similar to those found in background California populations.

Future Directions: Future studies will investigate the relationship between breast cancer and exposure to OCPs, PCBs, and polycyclic aromatic hydrocarbons (PAHs) as modified by genetic factors. Specific genetic factors of interest are the presence of the *GSTM1* and *GSTT1* genes and expression of *CYP1A1* and *CYP1B1* in breast cells.

INTRODUCTION

Breast cancer is the most common cancer in women in the world accounting for approximately 19% of diagnosed cancers among women (Parkin *et al.*, 1993). Accepted risk factors, including inherited germ cell mutations, menarche before age 12, menopause after age 55, radiation to chest in moderate to high doses, nulliparity or delayed childbearing, higher socioeconomic status, and postmenopausal obesity, are present in less than 50% of cases (Kelsey, 1993; Madigan *et al.*, 1996). Due to rapid industrialization in recent decades, Brazil has experienced a shift in the main causes of death from infectious to chronic diseases, placing cancer as the third most common cause of death after cardiovascular disease and accidents (Ministério da Saúde, 1998). Breast cancer is the most commonly diagnosed cancer and the most common cause of cancer death in Brazilian women (Ministério da Saúde, 1998). Over the past few years, certain organochlorine (OC) chemical compounds with carcinogenic or estrogenic/antiestrogenic activity and ability to induce metabolizing enzymes have been proposed as possible risk factors for breast cancer. Before being banned in the 1970's, polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and other organochlorine pesticides (OCPs) were widely used throughout the United States. Due to their resistance to physical and biological degradation, OCs continue as a potential health threat.

A number of studies (Wasserman *et al.*, 1976; Unger, 1984; Falck *et al.*, 1992; Mussalo-Rauhama, 1992; Wolff *et al.*, 1993; Krieger *et al.*, 1994; Dewailly, 1994; Hardell *et al.*, 1996; Hunter *et al.*, 1997; Lopez-Carillo *et al.*, 1997; van't Veer *et al.*, 1997; Schecter *et al.*, 1997; Hoyer *et al.*, 1998; Moysich *et al.*, 1998; Guttes *et al.*, 1998; Zheng *et al.*, 1999; Petralia *et al.*, 1999; Dorgan *et al.*, 1999; Helzlsouer *et al.*, 1999) have explored possible links between breast cancer and the presence of some of these chemicals in human tissues with equivocal results and no definitive answers (Safe, 1997). These studies varied in terms of sample size, selection of cases and controls, adjustment of confounders, and statistical power. In addition to differences in the design, differences in the selection of specimen matrix (adipose vs. serum/plasma) and chemicals for analysis may have contributed to the inconsistent and conflicting results (see Table 1). Adipose concentrations of the target chemicals may be considered at steady state, whereas their serum levels vary with time. Measurements in serum and adipose tissue do not always correlate (Archibeque-Engle *et al.*, 1997; Greizerstein, 1999). In recent studies by Petreas (1997a, 1997b,1998), lipid content of breast adipose specimens varied from 10 to 90 percent, making it imperative to express chemical concentrations on a lipid basis.

All reported studies have been performed in predominantly industrialized regions where body burdens of these chemicals in human tissue have steadily declined after being withdrawn from the market and better pollution controls instituted. In contrast, OCPs and PCBs have been used extensively for longer periods in developing countries like Brazil, resulting in higher observed body burdens than in the U.S. (Beretta & Dick, 1994; Jensen & Slorach, 1990). DDT is still used in Brazil for vector control programs (Jamison *et al.*, 1993; Bretas, 1999). Populations with higher chemical body burdens offer a better opportunity to observe potential exposure related health effects.

The objectives of the two-phased pilot study were:

- To establish a data collection and specimen transport infrastructure.
- To collect preliminary data to examine the distribution of known and suspected breast cancer risk factors among women living in rural and urban areas using a cross-sectional design (Phase I).
- To measure body burden levels of certain organohalogen chemicals for a small, clinic-based prospective design (Phase II).

METHODS

Study subjects and data collection

For Phase I (June 10, 1998 to September 17, 1998), 208 participants were recruited from the adult female patients aged 18 years and older of Dr. Maira Caleffi's Clínica de Mastologia (Breast Clinic), Porto Alegre, Rio Grande do Sul, Brazil. Phase I participants were asked to complete an informed consent form and a self-administered questionnaire consisting of questions regarding residential, occupational, and medical histories. Specific questionnaire items collected data on age, education, age at menarche, age at first full-term pregnancy, parity, menopausal status, oral contraceptive use, family history of cancer, smoking history, and alcohol consumption. The questionnaire was translated into Brazilian Portuguese and back translated into English to check for translation accuracy (a copy of the questionaire appears in the Appendix). In Phase II (July 8, 1998 to August 18, 1998), breast adipose specimens from 12 women undergoing breast biopsy surgery at Breast Clinic were removed and transported to Dr. Petreas' laboratory[1] (HML) for analysis. OCP levels were measured for each individual. In addition to OCPs, the specimens were analyzed for dioxins, furans, and congener-specific PCBs. To reduce the analytical costs, dioxin, furan, and PCB analyses were performed on four composite samples of the 12 individuals. The composite samples were grouped on the basis of case/control status and rural/urban residence (i.e., rural cases, urban cases, rural controls, and urban controls). A diagram summarizing the study design is shown in Figure 1. All study procedures were conducted as per a protocol approved by the University of California at Berkeley Committee for Protection of Human Subjects (see Appendix).

Adipose tissue and laboratory analysis

In women undergoing surgical breast biopsy or wide local excision (lumpectomy or tylectomy), breast adipose tissue (1-2 grams) was obtained from beyond the edges of the biopsy or excision cavity. In women undergoing mastectomy, breast adipose tissue is obtained from a site distant from the tumor in order to not interfere with pathologic analysis. The removed adipose tissue was immediately placed in chemically clean glass jars with teflon-lined screw caps, frozen to below -20 $^{\circ}$C and stored until they were transported to the HML for chemical analysis.

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OCP and PCB levels were determined in breast adipose tissue on a lipid basis. Briefly, one to two grams of adipose sample were extracted, cleaned up and fractionated for analysis by High Resolution Gas Chromatography-High Resolution Mass Spectrometry according to HML Method 880 (HML, 1998). ^{13}C-labeled analogs of the target analytes were added as internal standards to all samples for isotope dilution. Detection levels for dioxins are in the low pg/g range and for PCBs and OCPs in the low ng/g range. Our techniques permitted measurements of the target chemicals at levels well below those reported in the literature for the general population (Dewailly *et al.*, 1994; Hardell *et al.*, 1996). Lipid content was determined by the gravimetric method in an aliquot of the extract. Standard QA/QC procedures, as specified in HML Method 880, were followed. The laboratory staff was blind to the source and status of the samples.

Statistical analysis

Questionnaire forms were checked by the study coordinator for completeness and appropriateness of responses. The data were coded and entered twice ("double-entry") by two different individuals using data entry templates developed with EPIINFO 6.04 (http://www.cdc.gov) software on a PC. Validation checks were performed, and the data were exported to pipe-delimited files for analysis in SAS 6.12 (SAS Institute) and S-Plus 3.4.2 (Mathsoft, Inc.).

Sociodemographic and risk factor characteristics of the participants were compared first by case/control status and then by rural/urban status. Means and standard deviations for continuous variables and stratum-specific proportions for categorical variables were calculated for each group. The relationship of the independent variables with breast cancer risk was analyzed using univariate methods (Pearson chi-square and Student's t tests). Univariate analysis using one independent variable to predict disease status identified potential confounding factors that may require consideration in future analyses.

RESULTS AND DISCUSSION

Of the 208 women recruited for Phase I, 194 completed questionnaires (93% participation rate). Case/control status was available for 181 of the 194 participants. Tables 2 and 3 summarize some characteristics of the Phase I participants. Univariate analyses revealed that cases and controls differed in age, age at menopause, number of children, annual income, lactation, country of birth, menopausal status, oral contraceptive use, alcohol use, and current smoking status ($p \leq 0.20$).

Cases compared with controls were older, experienced menopause later (among postmenopausal women only), had more children, had a higher annual income, and lactated for more cumulative months. The greater cumulative lactation months observed among the cases was probably due to the greater number of children born to these women. Three cases and none of the controls were born in a country other than Brazil. More of the cases were postmenopausal compared with controls which may be explained by the difference in the age distributions between cases and controls, cases being older. More controls than cases reported regular alcohol use and being current smokers.

Rural and urban women differed in age, age at menarche, annual income, and education

($p \leq 0.20$). Rural participants compared with urban participants were younger, experienced menarche at a later age, had a lower annual income, and were less likely to have completed high school.

For Phase II, all 12 recruited women participated in the study (100% participation rate). During biopsy surgery, breast adipose specimens from these 12 women were removed and transported to HML for analysis. The analysis for OCPs revealed high levels of DDE, the major metabolite of DDT exposure. These measurements corroborated high levels of OCPs (relative to the world average) reported in human milk from Porto Alegre (Beretta and Dick, 1994; Jensen & Slorach, 1990) and in breast tissue from São Paulo (Wassermann *et al*, 1976). To reduce analytical costs, portions of the adipose tissue specimens were combined into 4 composite samples (rural cases, urban cases, rural controls, and urban controls) for analysis of dioxins, furans, and congener-specific PCBs. Compared to on-going studies of breast cancer and environmental exposures in California women (Petreas, 1998), the Rio Grande do Sul group had elevated levels of DDE, Mirex, HCB, and certain PCB congeners (see Tables 4 & 5). Low levels of dioxins and furans were measured in the Brazil adipose samples, similar to those found in background California populations (Petreas, 1998). Cases had higher levels than controls for all OCPs, with some (DDE, HCB) approaching statistical significance despite the limited sample size (see Table 6).

CONCLUSION

The pilot study demonstrated the soundness of the procedures developed for and the logistical feasibility of recruiting participants, obtaining, storing, and importing specimens from Brazil. If an association between organochlorine chemicals and breast cancer risk exists, such an association will more likely be detectable in a population with a broad range of exposure levels. The wide ranges of observed body burdens in the Rio Grande do Sul population offers an excellent opportunity to better assess the impact of certain OCPs and PCBs than previous studies.

Future studies will expand the scope of the pilot study to investigate the relationship between breast cancer and exposure to polycyclic aromatic hydrocarbons (PAHs), OCPs, and PCBs as modified by genetic factors. Specific genes of interest are those coding for enzymes involved in PAH activation (*CYP1A1*, *CYP1B1*) and PAH detoxification (*GSTM1*, *GSTP1*). Genetic polymorphisms affecting the activity of these enzymes have been described. In addition, OCPs and PCBs are known to induce expression of the *CYP1A1* and *CYP1B1* genes, thereby possibly enhancing PAH carcinogenesis. Both genotype and expression of these genes will be examined as possible susceptibility factors that may modify the relationship between environmental chemical exposures and disease risk.

Table 1. Analytes found to be associated with breast cancer risk are shown along with the tissue sampled and the type of lipid adjustment, if any.

Tissue	Lipid Adjustment	Analyte(s)	Reference
ADIPOSE	GRAVIMETRIC	-	Unger, 1984
	GRAVIMETRIC	β-HCH	Mussalo-Rauhama, 1992
	GRAVIMETRIC	DDE, PCB	Falck, 1992
	NONE	DDE, PCB	Dewailly, 1994
	GRAVIMETRIC	OCDD	Hardell, 1996
	NONE	-	Van't Veer, 1997
	GRAVIMETRIC	-	Zheng, 1999
SERUM	NONE	DDE	Wolf, 1993
	NONE	-	Krieger, 1994
	GRAVIMETRIC	-	Lopez-Carillo, 1997
	CHOLESTEROL + TRIGLYCERIDES	PCB	Moysich, 1998
	CHOLESTEROL + TRIGLYCERIDES	DIELDRIN	Hoyer, 1998
	CHOLESTEROL + TRIGLYCERIDES	HCB	Dorgan, 1999
PLASMA	NONE	HCB	Dewailly, 1994
	CHOLESTEROL	-	Hunter, 1997

Table 2. Characteristics of the Phase I participants – continuous variables

	Cases (n = 83)	Controls (n = 98)	Rural (n = 46)	Urban (n = 135)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	53.4 (11.0)	46.1 (10.4)	46.6 (11.2)	50.4 (11.1)
p-value	0.000		0.045	
Age at Menarche (years)	12.8 (1.5)	12.5 (1.6)	12.9 (1.7)	12.5 (1.5)
p-value	0.292		0.135	
Age at Menopause (years)	48.4 (4.4)	45.4 (6.2)	47.2 (6.1)	47.3 (5.1)
p-value	0.010		0.967	
No. Children	2.4 (2.6)	1.5 (1.7)	1.8 (1.5)	1.9 (2.2)
p-value	0.002		0.729	
Age at First Full-Term Pregnancy	25.8 (4.7)	25.5 (4.7)	24.8 (4.0)	25.9 (4.9)
p-value	0.711		0.219	
Annual Income (U.S. dollars) [a,b]	19,228 (19,662)	13,227 (10,042)	11,958 (9,949)	17,484 (16,906)
p-value	0.030		0.079	
Body Mass Index (kg/m^2)	25.4 (5.1)	25.3 (12.2)	23.8 (4.1)	25.8 (10.9)
p-value	0.929		0.228	
Lactation (months)	12.9 (16.1)	8.4 (12.6)	11.7 (17.0)	10.0 (13.6)
p-value	0.033		0.473	

a Conversion based from reals to U.S. dollars based on the rate 1.2 reals = 1 U.S. dollar.
b The only continuous variable with greater than 3% unknown responses was Annual Income which had 30.3% unknown responses.

Table 3. Characteristics of the Phase I participants – categorical variables

	Cases (n = 83)		Controls (n = 98)		Rural (n = 46)		Urban (n = 135)	
	No.	%	No.	%	No.	%	No.	%
Country of Birth								
Brazil	79	95.1	98	100	46	100	131	97.0
Other (Germany, Poland, Uruguay)	3	3.6	0	0	0	0	3	2.2
Unknown	1	1.2	0	0	0	0	1	0.7
p-value		0.093				0.571		
Education								
< High School diploma	31	37.3	32	32.6	23	50.0	40	29.6
≥ High School diploma	51	61.4	65	66.3	23	50.0	93	68.8
Unknown	1	1.2	1	1.0	0	0	2	1.4
p-value		0.607				0.024		
Marital Status								
Never Married	10	12.0	19	19.3	9	19.6	20	14.8
Ever Married	72	86.7	78	79.5	36	78.3	114	84.4
Unknown	1	1.2	1	1.0	1	2.2	1	0.7
p-value		0.257				0.571		
Parity								
0	16	19.3	25	25.1	13	28.3	28	20.7
≥ 1	67	80.7	71	72.4	33	71.7	105	77.8
Unknown	0	0	2	2.0	0	0	2	1.5
p-value		0.370				0.424		
Menopausal Status								
Premenopause	28	33.7	65	66.3	27	58.7	66	48.9
Postmenopause	55	66.3	33	33.7	19	41.3	69	51.1
p-value		0.000				0.328		
Oral Contraceptive Use								
Never used	23	27.7	12	12.2	7	15.2	28	20.7
Used ≤ 5 years	35	42.2	43	43.9	21	45.6	57	42.2
Used > 5 years	24	28.9	40	40.8	17	37.0	47	34.8
Unknown	1	1.2	3	3.1	1	2.2	3	2.2
p-value		0.106				0.935		
Alcohol Use								
Never	26	31.3	30	30.6	13	28.2	43	31.9
Only special occasions	46	55.4	43	43.9	23	50	66	48.9
Monthly, Weekly, or Daily	11	13.3	22	22.4	8	17.4	25	18.5
Unknown	0	0	3	3.1	2	4.3	1	0.7
p-value		0.196				0.936		
Mother or Sister with History of Breast Cancer								
Yes	9	10.8	14	14.3	5	10.9	18	13.3
No	49	59.1	56	57.1	30	65.2	75	55.6
Unknown	25	30.1	28	28.6	11	23.9	42	31.1
p-value		0.670				0.694		
Ever smoke ≥ 100 cigarettes in life?								
Yes	29	34.9	44	44.9	19	41.3	54	40.0
No	49	59.0	48	49.0	24	52.2	73	54.1
Unknown	5	6.0	6	6.1	3	6.5	8	5.9
p-value		0.214				0.990		
Smoke now?								
Yes	8	9.6	20	20.4	8	17.4	20	14.8
No	69	83.1	69	70.7	35	76.1	103	76.3
Unknown	6	7.2	9	9.2	3	6.5	12	8.9
p-value		0.062				0.907		

Table 4. Selected PCB congeners (ng/g fat) in breast adipose tissue for Phase II participants of the Rio Grande do Sul Pilot study and from an on-going study of California women. PCB congeners (ng/g fat) in Rio Grande do Sul tissues were measured in composite samples.

	Rio Grande do Sul (n=12)				California Breast Cancer Study (n=60)			
PCB #	Mean	SD	Min	Max	Mean	SD	Min	Max
153/132	206	307	27	561	159	97	44	55
180	282	417	26	763	139	78	55	49
138	175	260	24	475	98	68	16	40
182/187	92	142	7	256	47	31	15	21
170	136	199	15	366	60	34	21	16
196/203	96	149	6	268	36	20	12	13
194	155	236	7	427	42	23	16	11
199	105	163	5	293	29	17	9	10
156	44	67	5	122	34	28	4	16
118	38	36	11	79	27	16	6	8
206	119	191	2	340	22	19	6	11
183	21	31	3	57	18	13	6	7
99/113	5	5	2	11	18	13	5	8
177	20	30	2	55	18	14	3	8

Table 5. Selected OCPs measured (ng/g fat) in breast adipose tissue from the Rio Grande do Sul Pilot study and from an on-going study of California women.

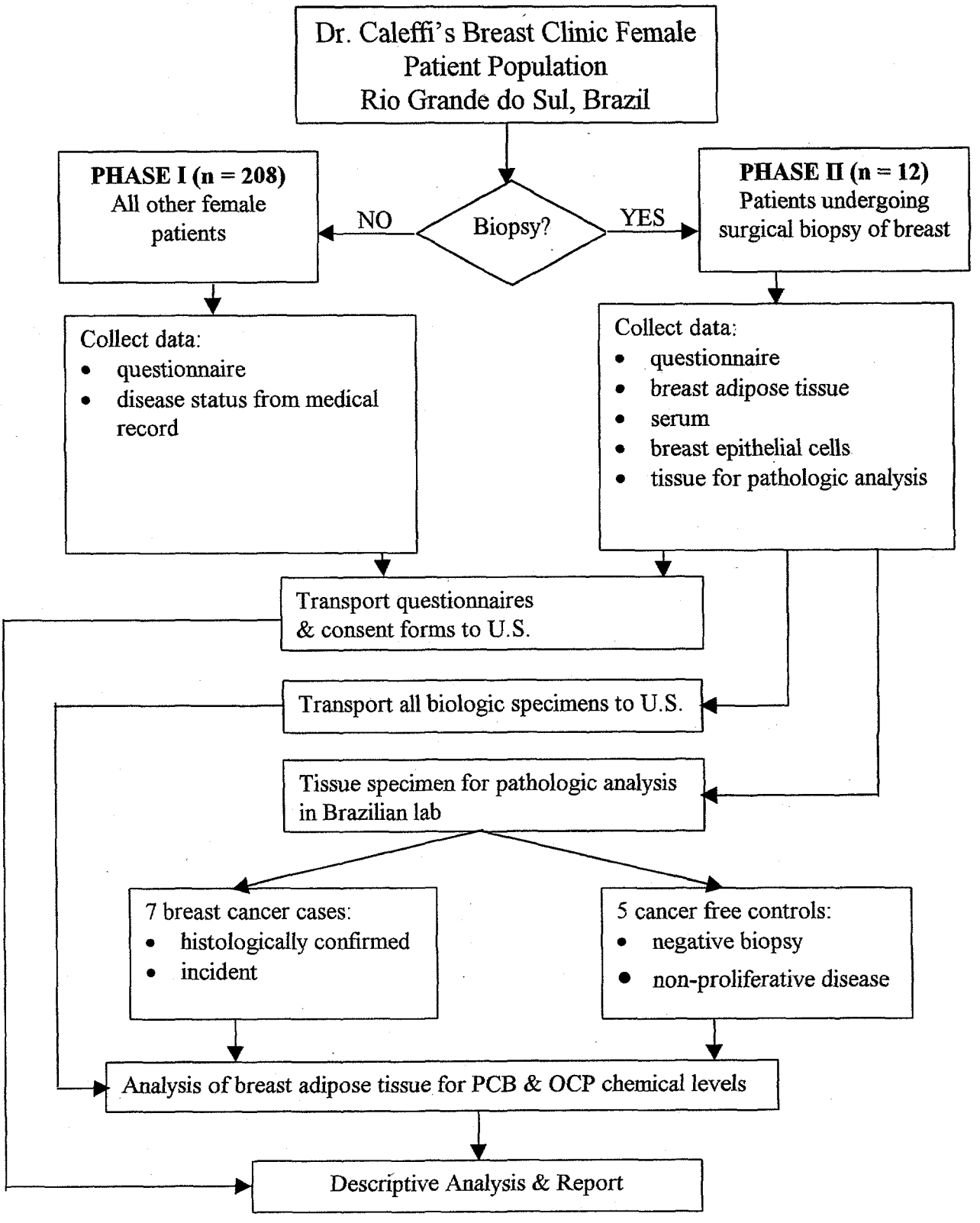
	Rio Grande do Sul (n=12)				California Breast Cancer Study (n=60)			
Chemical	Mean	SD	Min	Max	Mean	SD	Min	Max
DDE	1170	790	86	2600	745	364	120	2200
trans-nonachlor	62	58	11	210	136	148	20	690
Oxychlordane	28	16	11	67	72	57	17	340
DDT[2]	NA	NA	NA	NA	50	43	8	260
HCB	50	80	15	300	46	28	14	170
Mirex[3]	45	14	25	75	NR	NR	NR	NR
Dieldrin	27	15	10	58	34	30	8	230

Table 6. Selected OCPs measured (ng/g fat) in breast adipose tissue for Phase II participants of the Rio Grande do Sul Pilot study.

	Cases (n = 7)				Controls (n = 5)			
Chemical	Mean	SD	Min	Max	Mean	SD	Min	Max
DDE	1510	619	747	2605	667	810	86	1866
trans-nonachlor	68	72	11	210	54	31	19	85
Oxychlordane	28	21	11	67	27	10	13	39
HCB	67	103	22	298	24	5	18	29
Mirex	47	18	26	75	43	7	37	53
Dieldrin	29	16	13	58	25	12	10	44

[2] NA = Not Analyzed
[3] Mirex was only found in some California subjects and is not reported here, whereas, Mirex was found in all Brazilan subjects.

Figure 1. Overview of the study design.



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APPENDIX A

Study Protocol approved by the University of California at Berkeley Committee for Protection of Human Subjects

Title:
Comparison of Pesticide Exposure Histories of Female Breast Cancer Cases with Cancer Free Women in Rural Rio Grande do Sul, Brazil: Preliminary Study

Related Projects:
None.

Nature and Purpose:
The overall objective of this study is to test the hypothesis that a history of exposure to pesticides in independently associated with increased breast cancer risk for women living in rural areas of Rio Grande do Sul, Brazil. I will collaborate with Dr. Maira Caleffi. My part in the research is to prepare the survey instrument and train Dr. Caleffi's staff to assist in collecting and storing in a secure manner any responses received from the participants. Once data collection is completed I will return to Brazil to pick up the questionnaires for compilation and analysis. All data and analyses will be shared with Dr. Caleffi. This preliminary study will examine whether women living in rural areas differ from women living in urban areas with respect to known and suspected breast cancer risk factors. Known risk factors include early onset of menarche, late onset of menopause, late child bearing, and family history of breast cancer. Suspected risk factors include alcohol use, oral contraceptive use, and exposure to some classes of pesticides. Since there is very little data available on the distribution of breast cancer risk factors in the state of Rio Grande do Sul, we have designed this questionnaire to get a better sense of which areas of the state on which to focus our later study.

Subjects:
Phase I
Approximately, 100-200 participants will be recruited from the female patients of Dr. Maira Caleffi of Porto Alegre, Rio Grande do Sul, Brazil. Dr. Caleffi is a physician specializing in breast diseases. Only patients ages 18 years and older will be asked and allowed to participate. Members of racial or ethnic minorities may be included since all female patients over the age of 18 years will be invited to participate.
Phase II
Approximately, 12-18 participants who are scheduled to undergo breast biopsy surgery will be recruited prosepctively from the Phase I study population. To maximize the information that can be obtained from the minimal number of participants, the following 2 x 2 design was chosen: 3 rural cases, 3 rural controls, 3 urban cases, 3 urban controls.

	Case	Control	
Rural	**3**	**3**	**6**
Urban	**3**	**3**	**6**
	6	**6**	**12**

"Rural" is defined as having lived for at least the past 10 years in a munipality

where the female population is less than xx% as indicated by the 1991 Census. "Urban" is defined as having lived for at least the past 10 years in the municipality of Porto Alegre. "Case" status is defined by a malignant result of the biopsy. "Control" status is defined by a non-malignant, non-proliferative disease result of the biopsy.

Recruitment:

Phase I

Copies of the informed consent forms and questionnaires will be made available to prospective participants from Dr. Caleffi at their regularly scheduled appointments.

Phase II

For patients scheduled for breast biopsy surgery, copies of the questionnaires and Phase II informed consent form will be made available from Dr. Caleffi during the pre-operative appointment.

Screening Procedures:

Phase I

None.

Phase II

To minimize extreme values, the ages of the participants will be restricted to between 40-60 years, inclusive.

Procedures:

Phase I

Prospective participants will be asked to read the study's informed consent form and sign and sign the form if they elect to participate in the study. The informed consent form and questionnaire will be presented in Brazilian Portuguese. If they agree to participate, they will proceed to complete a questionnaire, also presented in Brazilian Portuguese, printed on paper using a pen of blue or black ink. Participants may complete the study questionnaire at Dr. Caleffi's office or they may take the questionnaire home for completion and return it to the office later. If the participant has any questions or concerns regarding the study, they may discuss them during their appointment with Dr. Caleffi or may contact the researchers at any other time. The office staff will be available to assist participants in completing the surveys, if needed. The questionnaire consists of questions regarding residential, occupational, and medical histories. With participant permission, their medical records held by my collaborator, Dr. Caleffi, may be reviewed. Of interest to the research would be diagnosis, stage of disease, etc.

Along with the informed consent and questionnaire materials, participants will be given two envelopes. One envelope will say, "Please place your questionnaire in this envelope when you have finished with the questionnaire." The other envelope will say, "Please place your signed informed consent form and detachable identification page in this envelope." The participant may elect to have another person write their answers to the questions for them. The envelopes will be picked up later by the principal investigator, Christine A. Erdmann.

If follow-up contact with the participant is needed for additional clarification of responses, they will be contacted by mail or phone by either Dr Caleffi or myself. They will be reminded of their participation in the Study of Women's Health conducted by Dr. Maira Caleffi and Christine Erdmann and will be asked if they would willing to clarify some of their questionnaire answers. If the participant is willing, the questions and responses requiring clarification will be communicated to the participant. Participants will not be asked to answer questions that were initially left blank. If the participant is not willing, the participant will not be contacted again.

Phase II

Prospective participants will be asked to read the Phase II informed consent form and sign the form if they elect to participate in the study. If they agree to participate, they will partake in the procedures of Phase I. In addition, samples of breast fat tissue and epithelial cells that would otherwise be discarded will be separated from the biopsy material. About 2 grams (2 teaspoons)of breast fat tissue will be placed in glass jars. About 1/2 gram (1/2 teaspoon) of breast epithelial cells will be placed in a plastic tube. At the time of surgery, 7 milliliters of blood will be drawn. Standard veni-puncture procedures will be followed. Serum will be separated from the whole blood and transferred to another tube. All collection containers will be labeled with an identification number only.

Immediately following the surgery, the breast fat and serum specimens will be transported by a study staff member to a locked freezer for storage. The epithelial cell specimens will be either stored in a locked storage space at room temperature or will be dissected, then stored in a locked freezer. Ideally, specimens will be stored until all Phase II specimen collection is completed (at least 12 sets of specimens). However, it may be necessary to ship the breast epithelial cells as quickly as possible since freezing may interfere with later analyses. All specimens will be transported from Porto Alegre, Brazil to Dr. Myrto Petreas, Environmental Biochemist, Hazardous Materials Laboratory, California Environmental Protection Agency, Berkeley, California either by Federal Express or hand transported by Dr. Caleffi or myself in accordance with the Centers for Disease Control and Prevention (CDC) guidelines.

Dr. Petreas will forward the breast epithelial cell specimens to Dr. Regine Goth-Goldstein, Staff Scientist, Department of Cell and Molecular Biology, Lawrence Berkeley National Laboratory, Berkeley, California.

Benefits:

There is no substantial benefit to the participant from the research. We hope that the research will benefit society by revealing the clues as to the causes of breast cancer.

Risks:

Phases I & II

There are no known risks to the participant from partaking in this study. Discomfort may be experienced while responding to personal questions. The participant will be assured that they may skip any question.

Phase II only

Temporary discomfort, bruising, or discoloration, and rarely infection may result

from drawing the blood specimens.

Confidentiality:

Phases I & II

Each questionnaire will include a detachable page on which the participant will be asked to give identifying information, including her name, birth date, biological mother's name, biological father's name, and the name of the person filling out the questionnaire if it is not the participant. This "identification page" will also include an identification number, which will correspond to the identification numbers appearing on all other pages of the questionnaire. The identification page will be detached after completion of the questionnaire and will be stored in a locked cabinet in Dr. Caleffi's office separate from locked cabinet where the completed questionnaires will be stored. This identification page will be used only in the event that participant needs to be contacted for clarification response(s) to the questionnaire. Every 4-6 months I will transport the questionnaires, informed consent forms, and identification pages in locked bags from Dr. Caleffi's office to my own office in Berkeley, California. The questionnaires and identification pages will be transported in separate bags with different locks. In Berkeley, the identification pages and informed consent pages will be stored in a locked filing cabinet separate from the questionnaires.

Any information abstracted from the participant's medical file will be recorded on a form identified only with the participant's study number. Abstractions will be performed by myself, Dr. Caleffi, or Dr. Caleffi's office staff under her supervision.

Phase II only

All biological specimen containers will be labeled with the participant's identification number only. Specimens will be transported in accordance with the Centers for Disease Control and Prevention (CDC) guidelines. Specimens will be stored in secure research laboratories until analysis. All laboratory results will be labeled with the participant's identification number only.

Informed Consent:

Phase I

An invitation to participate in the study will be extended to prospective participants via an informed consent form distributed by Dr. Caleffi's office receptionist. The prospective participant will be asked to sign the consent form if she agrees to participate. A second copy of the form will be given to the participant to keep.

Phase II

An invitation to participate in Phase II will be extended to prospective participants via an informed consent form presented by Dr. Caleffi during the patient's pre-operative appointment. The prospective participant will be asked to sign the consent form if she agrees to participate. A second copy of the form will be given to the participant to keep.

Written Materials:

Two copies of the questionnaire are attached to the original protocol.

Signatures:

See CPHS Application Coversheet.

Telephone numbers:
See CPHS Application Coversheet.

APPENDIX B

Questionaire in English

INSTRUCTIONS

- Please print.
- Please use a pen of black or blue ink.
- Place an X in the box next to the answer that fits best or write a response in the space provided.
- If the participant does not know how to respond to a specific question, please mark DK (which stands for "Don't Know").
- When the questionnaire is completed, please give it to the research nurse.

NOTE ABOUT ANSWER CHOICES:

"DK" means that the participant "does not know" the answer

"NA" means "non-applicable", that is, the question does not apply to the participant

QUESTION EXAMPLES:

Example 1. What is your date of birth?

_____/_____/_____
day month year

Example 2. Where were you born?

_____/_____/_____
a. City b. State c. Country (if not Brazil)

Example 3. What is your marital status?

[] 1 single
[] 2 married or live with companion
[] 3 widow
[] 4 separated/divorced
[] 9 DK

|_|_|_|_|_|_|

The information on this page will be treated as confidential.
It will be stored in a secure location, separate from the other pages of this questionnaire.

1. Today's date _____

2. Your complete name:

a. _____ _____ _____
first last middle

b. Are you known by other names?

[] 1 Yes
[] 2 No

c. List the other names
_____

3. What is your date of birth?

_____/_____/_____
day month year

4. Where were you born?

_____/_____/_____
a. City b. State c. Country (if not Brazil)

5. What is your biological mother's name?

_____/_____/_____
first last middle

6. What is your biological father's name?

_____/_____/_____
first last middle

7. Indicate the name of the person filling out this questionnaire.

_____/_____/_____
first last middle

|_|_|_|_|_|_|

1.9.a. What is your ethnic origin?

[] 1 Italian
[] 2 Portuguese
[] 3 German
[] 4 Polish
[] 5 African — b. Which ? _____
[] 6 Other — c. Which ? _____
[] 9 DK

1.10 In the case where you have foreign ancestors, what is your Brazilian generation?

[] 1 1st [] 2 2nd
[] 3 3rd [] 4 more than 3rd

1.11 What language do you speak the majority of time at home?

Language _____

1.12 Do you know how to read and write?

[] 1 yes
[] 2 no
[] 3 only sign name

1.13 What is the highest level of school that you attended?

[] 1 primary incomplete [] 2 primary complete
[] 3 secondary incomplete [] 4 secondary complete
[] 5 high school in complete (*) [] 6 high school complete (*)
[] 7 college incomplete [] 8 college complete
[] 9 never went to school
(*) = includes technical school and normal school

|_|_|_|_|_|_|

Part 1. PRELIMINARY INFORMATION

1.1 Today's Date _____

1.2 What is your date of birth?

_____/_____/_____
day month year

1.3 What is your height? _____ m

1.4 What is your weight? _____ kg

1.5 Where were you born?

_____/_____/_____
a. City b. State c. Country (if not Brazil)

1.6 What is your marital status?

[] 1 single
[] 2 married or live with companion
[] 3 widow
[] 4 separated/divorced
[] 9 DK

1.7 What is the date of birth of your mother?

_____/_____/_____
day month year

1.8 What is the date of birth of your father?

_____/_____/_____
day month year

Part 2. MEDICAL HISTORY

2.1 At what age did you first menstruate?

_____ years

2.2 Do you still menstruate?

[] 1 yes [] 2 no

2.3 If your response to question 2.2 above was YES, go to question 2.4?

a. At what age did you stop menstruating?

_____ years

b. Why did you stop menstruating?
[] 1 had surgery, what kind _____
[] 2 accident, explain _____
[] 3 natural causes
[] 4 other reason(s), explain _____

2.4 a. Have your ovaries been surgically removed?
[] 1 yes, one ovary [] 2 yes, two ovaries
[] 3 no [] 9 DK

b. If your response to question 2.4 above was NO, please go to question 2.5.

At what age were your ovaries surgically removed? _____ years

2.5 a. Have you every had surgery for benign breast disease?

[] 1 yes [] 2 no

b. If your response to question 2.5 above was YES, then respond to the question below:

At what are did you have surgery for benign breast disease? _____ years

2.6.a Have you ever been pregnant?
[] 1 yes
[] 2 never

|_|_|_|_|_|_|

2.7 If your response to question 2.6 was YES, then respond to the following:

a? How many times have you been pregnant? _____

b. How many children have you had (or have): _____children

2.8.a Have you every had a miscarriage?
[] 1 yes b. How many? _____
[] 2 no

2.9 At what age did you have your first child? _____ years

2.10 Did you breast feed your children?
[] 1 yes [] 2 no

2.11 If your response to question 10 above was YES, respond to the following. For how much time did you breast feed each child?
a. first child: _____ months less than one month []
b. second child: _____ months less than one month []
c. third child: _____ months less than one month []
d. fourth child: _____ months less than one month []

[] Check here if you breast fed more than 4 (four) children.

2.12 Have you ever used the pill?
[] 1 yes [] 2 no

2.13 If your response to question 2.12 above was YES, then respond to the following:

a. Have you used the pill for more than 5 (five) years?
[] 1 yes [] 2 no

b. At what age did you first start to use the pill? _____ years

c. For how long did you or have you continued to use the pill?
_____ years or _____ months

d. At what age did you stop using the pill? _____ years

|__|__|__|__|__|__|

2.14 What type of alcoholic beverage to you like most to drink?

[] 1 wine
[] 2 beer
[] 3 distilled spirits (whisky, vodka, gin, etc.)
[] 4 liquors
[] 5 others -> -> -> -> -> Which? _____
[] 6 don't drink alcoholic beverages

2.15 When do you drink alcoholic beverages?

[] 1 every day
[] 2 weekly
[] 3 only on special occasions
[] 4 never

|_|_|_|_|_|_|

2.16 How frequently do you drink the following beverages:

a. Coffee
_____ per day
_____ per week
_____ per month
[] 999 never
[] 998 DK

b. Tea
_____ per day
_____ per week
_____ per month
[] 999 never
[] 998 DK

c. Soda
_____ per day
_____ per week
_____ per month
[] 999 never
[] 998 DK

d. Beer
_____ per day
_____ per week
_____ per month
[] 999 never
[] 998 DK

e. Wine
_____ per day
_____ per week
_____ per month
[] 999 never
[] 998 DK

f. Hard liquor, vodka, gin, whisky
_____ per day
_____ per week
_____ per month
[] 999 never
[] 998 DK

g. Maté
_____ per day
_____ per week
_____ per month
[] 999 never
[] 998 DK

|_|_|_|_|_|_|

Part 3: YOUR OCCUPATIONAL HISTORY

3.1 Have you ever worked or lived for more than one month or currently live on a ranch or farm?

[] 1 Yes
[] 2 No
[] 3 DK

a. If your response was YES, please list the years:
_____
_____
_____

3.2 Have you ever worked in the any of the following occupations for more than one months? (mark all that apply)

[] 1 Gardener
[] 2 Groundskeeper
[] 3 Landscaper
[] 4 Agricultural Worker
[] 8 No
[] 9 DK

Please list the years that your worked in these occupations:
a. _____
b. _____
c. _____
d. _____

3.3 Have you ever worked for more than one month in any activity where you mixed, produced, or manipulated pesticides, insecticides, fungicides, or herbicides?

[] 1 Yes
[] 2 No
[] 3 DK

a. If your response was YES, please list the years:
_____
_____

3.4 Now, we would like to know about your work activities for the past 20 years, including different activities with the same employer. Please also include any part-time paid, full-time paid, and voluntary work.

|_|_|_|_|_|_|

1st Job

City and State of the Company (02)	Describe your job and activities (09):
What did the company do or make? (04)	
When did you work there? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

2nd Job

City and State of the Company (02)	Describe your job and activities (09):
What did the company do or make? (04)	
When did you work there? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

3rd Job

City and State of the Company (02)	Describe your job and activities (09):
What did the company do or make? (04)	
When did you work there? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

4th Job

City and State of the Company (02)	Describe your job and activities (09):
What did the company do or make? (04)	
When did you work there? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

[] Mark here if you have had more than 4 jobs.

Part 4: YOUR HUSBAND'S OCCUPATIONAL

[] Mark here if you have never been married and skip to Part 5.

4.1 Has your husband ever worked or lived for more than one month or currently live on a ranch or farm?

[] 1 Yes
[] 2 No
[] 3 DK

a. If your response was YES, please list the years:
_____
_____
_____

4.2 Has you husband ever worked for more than one month in any of the following activities? (mark all that apply).

a. Please, list the dates (years)

[] 1 Gardener a. _____
[] 2 Groundskeeper b. _____
[] 3 Landscaper c. _____
[] 4 Farmer d. _____
[] 8 No
[] 9 DK

4.3 Has your husband ever worked for more than one month in any activity where you mixed, produced, or manipulated pesticides, insecticides, fungicides, or herbicides?

[]1 Yes
[]2 No
[]3 DK

a. If your response was YES, please list the years:
_____
_____

4.4 Now, we would like to know about your husband's work activities for the past 20 years, including different activities with the same employer. Please also include any part-time paid, full-time paid, and voluntary work.

|_|_|_|_|_|_|

1st Job

City and State of the Company (02)	Describe his job and activities: (09)
What did the company do or make? (04)	
When did he work there ? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

2° Job

City and State of the Company (02)	Describe his job and activities: (09)
What did the company do or make? (04)	
When did he work there ? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

|_|_|_|_|_|_|

3° Job

City and State of the Company (02)	Describe his job and activities: (09)
What did the company do or make? (04)	
When did he work there ? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

4° Job

City and State of the Company (02)	Describe his job and activities: (09)
What did the company do or make? (04)	
When did he work there ? (05-07) [] 1 Full-time [] 2 Part-time from_____/____ to _____/____ month year month year	
Did your co-workers smoke in your work area? (08) [] 1 Yes [] 2 No [] 9 DK	

[] Mark here if your husband had more than 4 jobs.

Part 5: INCOME

5.1 What is your monthly income? (include salary, pension, and retirement income)

_____ reals or _____ minimum salaries

5.2 What is your family income, approximately? (include all members of your household)

_____ reals or _____ minimum salaries

5.3 The family income listed in question 5.2 includes how many people? _____

Part 6: YOUR RESIDENTIAL HISTORY

6.1 Please list *all* of the places where you have lived for one month or more since birth until today. If you have had more than one address at the same time, list them both. We are interested in the municipalities where you have lived and the dates that you lived there.

1st Residence

	Date arrived	Date left	How long did you live here?
_____ City / State	____/____ month year	____/____ month year	_____ years
_____ Zip Code			_____ months

2ª Residence

	Date arrived	Date left	How long did you live here?
_____ City / State	____/____ month year	____/____ month year	_____ years
_____ Zip Code			_____ months

3ª Residence

	Date arrived	Date left	How long did you live here?
_____ City / State	____/____ month year	____/____ month year	_____ years
_____ Zip Code			_____ months

|_|_|_|_|_|_|

4ª Residence

	Date arrived	Date left	How long did you live here?
_____ City / State	_____/____ month year	_____/____ month year	_____ years
_____ Zip Code			_____ months

6.2 Have you had other residences?

[] 1 Yes
[] 2 No
[] 9 DK

|_|_|_|_|_|_|

Part 7: MEMBERS OF YOUR FAMILY

We would like to ask you about your biological family. The questions below refer to members of your family with whom you are biologically related. This does not include relatives through adoption or marriage, such as sisters-in-law and brothers-in-law.

7.1 Your mother

Name (01)	Birth year (a)	If deceased, year of death (b)	Did this person ever have cancer or a tumor? (c)	Did this person ever have breast cancer? (d)
	_____ year	_____ year	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

7.2 Your father

Name (02)	Birth year (a)	If deceased, year of death (b)	Did this person ever have cancer or a tumor? (c)	Did this person ever have prostate cancer (d)
	_____ year	_____ year	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Your sisters or half-sisters

Name (03)	Birth year (a)	If deceased, year of death (b)	Did this person ever have cancer or a tumor? (c)	Did this person ever have breast cancer? (d)
[] 1 Sister [] 2 Half-sister	_____ year	_____ year	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Name (04)	Birth year (a)	If deceased, year of death (b)	Did this person ever have cancer or a tumor? (c)	Did this person ever have breast cancer? (d)
[] 1 Sister [] 2 Half-sister	_____ year	_____ year	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Name (05)	Birth year (a)	If deceased, year of death (b)	Did this person ever have cancer or a tumor? (c)	Did this person ever have breast cancer? (d)
[] 1 Sister [] 2 Half-sister	_____ year	_____ year	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Name (06)	Birth year (a)	If deceased, year of death (b)	Did this person ever have cancer or a tumor? (c)	Did this person ever have breast cancer? (d)
[] 1 Sister [] 2 Half-sister	_____ year	_____ year	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

[] Mark here if you have more sisters or half-sisters.

Maternal Grandmother (mother of your mother)

Name (20)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
	[] 2 Deceased _____ age at death	[] 2 No	[] 2 No
		[] 9 DK	[] 9 DK

Maternal Grandfather (father of your mother)

Name (21)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have prostate cancer
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
	[] 2 Deceased_____ age at death	[] 2 No	[] 2 No
		[] 9 DK	[] 9 DK

Maternal Aunts (sisters or half-sisters of your mother)

Name (22)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
[] 1 Aunt	[] 2 Deceased _____ age at death	[] 2 No	[] 2 No
[] 2 Half-aunt		[] 9 DK	[] 9 DK

Name (23)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
[] 1 Aunt	[] 2 Deceased _____ age at death	[] 2 No	[] 2 No
[] 2 Half-aunt		[] 9 DK	[] 9 DK

Name (24)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
[] 1 Aunt	[] 2 Deceased _____ age at death	[] 2 No	[] 2 No
[] 2 Half-aunt		[] 9 DK	[] 9 DK

Name (25)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
[] 1 Aunt	[] 2 Deceased _____ age at death	[] 2 No	[] 2 No
[] 2 Half-aunt		[] 9 DK	[] 9 DK

Name (26)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
[] 1 Aunt	[] 2 Deceased _____ age at death	[] 2 No	[] 2 No
[] 2 Half-aunt		[] 9 DK	[] 9 DK

[] Mark here if you have more maternal aunts.

Paternal grandmother (mother of your father)

Name (40)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
	[] 2 Deceased_____ age at death	[] 2 No	[] 2 No
		[] 9 DK	[] 9 DK

Paternal grandfather (father of your father)

Name (41)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have prostate cancer?
	[] 1 Alive _____ age in years	[] 1 Yes	[] 1 Yes
	[] 2 Deceased_____ age at death	[] 2 No	[] 2 No
		[] 9 DK	[] 9 DK

Paternal Aunts (sisters or half-sisters of your father)

Name (42)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
[] 1 Aunt [] 2 Half-aunt	[] 1 Alive _____ age in years [] 2 Deceased_____ age at death	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Name (43)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
[] 1 Aunt [] 2 Half-aunt	[] 1 Alive _____ age in years [] 2 Deceased_____ age at death	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Name (44)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
[] 1 Aunt [] 2 Half-aunt	[] 1 Alive _____ age in years [] 2 Deceased_____ age at death	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Name (45)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
[] 1 Aunt [] 2 Half-aunt	[] 1 Alive _____ age in years [] 2 Deceased_____ age at death	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

Name (46)	Vital status and age	Did this person ever have cancer or a tumor?	Did this person ever have breast cancer?
[] 1 Aunt [] 2 Half-aunt	[] 1 Alive _____ age in years [] 2 Deceased_____ age at death	[] 1 Yes [] 2 No [] 9 DK	[] 1 Yes [] 2 No [] 9 DK

[] Mark here is you have more paternal aunts or half-aunts (sisters or half-sisters of your father).

|_|_|_|_|_|_|

Part 8. SMOKING HISTORY

8.1 Has your husband ever smoked at least 100 cigarettes in his life?

[] 1 Yes
[] 2 No
[] 9 DK

8.2 At what age did he start smoking?

_____ years.
[] 999 DK

8.3 Does he still smoke?

[] 1 Yes
[] 2 No
[] 9 DK

8.3[a]. When did he quit smoking?

_____/_____
month year
[] 99/9999 DK

8.4 Has he smoked in the past three (3) months?

[] 1 Yes
[] 2 No
[] 9 DK

8.4[a]. Approximately, how many cigarettes per data does he smoke during this time

_____ cigarettes per day
[] 999 DK

8.5 In the last three months, has anyone besides you or your husband regularly (one time or more per week) smoked cigarettes, cigars, or pipes in your house?

[] 1 Yes
[] 2 No
[] 9 DK

8.6 Have you ever smoked at least 100 cigarettes in your life?

[] 1 Yes
[] 2 No
[] 9 DK

|_|_|_|_|_|_|

8.7 How old were you when you first began to smoke?

_____ years.
[] 999 DK

8.8 Do you still smoke?

[] 1 Yes
[] 2 No
[] 9 DK

8.8[a]. When did you quit smoking?

_____/_____
month year
[] 99/9999 DK

8.9 Have you smoked in the past three (3) months?

[] 1 Yes
[] 2 No
[] 9 DK

8.9[a]. Approximately, how many cigarettes have you smoked during this time?

_____ cigarettes per day
[] 999 DK

Part 9. OTHER COMMENTS

Here we finish our questions. Thank you for your time to complete this questionnaire. If you have any comments or would like to provide any other information, you may use this sheet. If necessary, please add additional sheets.