PESTICIDE USE AND SELF-REPORTED UTERINE LEIOMYOMATA AMONG FARM WOMEN: AN ANALYSIS OF THE AGRICULTURAL HEALTH STUDY WITH ASSESSMENT OF OUTCOME MISCLASSIFICATION

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ABSTRACT

SHARON L. MYERS: Pesticide Use and Self-Reported Uterine Leiomyomata among Farm Women: An Analysis of the Agricultural Health Study with Assessment of Outcome Misclassification
(Under the direction of Dr. Donna Baird and Dr. Andrew Olshan)

Uterine leiomoyomata (fibroids), benign tumors that develop in the majority of women, are the leading indication for hysterectomy in the United States. Although it is well-established that ovarian hormones are involved in fibroid pathogenesis, few studies have examined the role of endocrine disrupting chemicals. This study investigated the relationship of pesticide use and self-reported fibroids among 16,526 women, aged 18-59, in the Agricultural Health Study (AHS). The impact of outcome misclassification from use of self-report was assessed by incorporating estimates of self-report validity.

Validity was estimated using self-report of clinical diagnosis and ultrasound findings from Right From The Start (RFTS) (*n*=2,046) and the Uterine Fibroid Study (UFS) (*n*=869). Log-binomial regression was used to estimate sensitivity and specificity and examine differences by various factors. Overall sensitivity was ≤0.50 in both studies. Sensitivity was higher in blacks than whites (RFTS: 0.34 vs. 0.23; UFS: 0.58 vs. 0.32) and increased with age. Parous white women had higher sensitivity than nulliparae. Specificity was 0.98 in RFTS and 0.86 in UFS. Ethnic differences were modest in UFS (Specificity Ratio, black vs. white: 0.90; 95% confidence interval [CI]: 0.81, 0.99). Parity was inversely associated with specificity among UFS black women (Specificity Ratio: 0.84; 95% CI: 0.73, 0.97).

The association between pesticide use and fibroid diagnosis in the AHS was estimated with odds ratios (OR) and 95% CI, adjusting for age and state (Iowa/North Carolina). Ever use of agricultural pesticides was associated with fibroids, with users of ≥3 pesticides having the highest odds compared to never users (OR: 1.31, 95% CI; 1.12, 1.53). Use of any of 10 possible hormonally active pesticides was associated with fibroids when compared with never use of any pesticide (OR: 1.28; 95% CI: 1.12, 1.45). When pesticides were grouped by chemical class, organophosphate users had slightly higher odds than users of other agricultural pesticides (OR: 1.17, 95% CI: 1.05, 1.31).

These results suggest a possible association between agricultural pesticide exposure and uterine fibroids that warrants further investigation. Allowing for the imperfect outcome measurement which was demonstrated in the self-report validity analysis resulted in estimates that were further away from the null.

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LIST OF ABBREVIATIONS

AHS	Agricultural Health Study
BMI	Body mass index
BWHS	Black Women's Health Study
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
EDC	Endocrine disrupting chemical
EPA	Environmental Protection Agency
ER-α; ER-β	Estrogen receptor α; estrogen receptor β
GnRH	Gonadotropin-releasing hormone
NHS	Nurses' Health Study
NIEHS	National Institute of Environmental Health Sciences
OC	Oral contraceptives
PCB	Polychlorinated biphenyl
RFTS	Right from the Start
SERM	Selective estrogen receptor modulator
Se; SeR	Sensitivity; Sensitivity Ratio
Sp; SpR	Specificity; Specificity Ratio
TCDD	2,3,7,8-tetrachlrodibenzo-*p*-dioxin
UFS	Uterine Fibroid Study
UL	Uterine leiomyoma

CHAPTER 1: INTRODUCTION AND AIMS

Uterine leiomyomata (fibroids) are benign neoplasms of uterine smooth muscle tissue that develop in the majority of women (1). Fibroids can cause menstrual abnormalities, pelvic pain, and pregnancy complications (2), and account for approximately 32% of all hysterectomies in the United States (3). Established risk factors include African-American race, increased premenopausal age, and earlier age at menarche (4).

The development and progression of uterine fibroids are known to be highly hormone dependent, but there is an incomplete understanding of the precise mechanisms or etiology of the disease. Earlier investigations of oral contraceptive use (as a source of exogenous hormones) have yielded equivocal results, primarily due to the possibility of reverse causality and detection bias (4). Exposure to endocrine-disrupting chemicals (EDCs)—which can either mimic ovarian hormones or exert agonistic effects on hormone receptors—may play a part in the development and growth of these tumors. Despite evidence from animal models and *in vitro* experiments to suggest that EDCs (including some pesticides) may affect fibroid pathogenesis (5-9), few epidemiologic studies have investigated these exposures as possible risk factors. To my knowledge, only one study of 25 women has examined pesticides and found higher concentrations of DDT in leiomyomatous tissue compared to surrounding normal myometrium (10).

One of the methodological challenges in observational studies of uterine fibroids is in the correct identification of women with and without fibroids. In particular, studies that use

self-reported fibroid diagnosis to identify cases and controls could result in identification of risk factors that are associated with disease symptoms or reproductive health care utilization rather than the disease itself. Furthermore, since many women with fibroids remain asymptomatic, it is likely that a sizeable proportion who report no previous fibroid diagnosis actually have undetected fibroids (2, 11).

The purpose of this dissertation research was to examine whether pesticide use is associated with prevalence of uterine fibroids among farm women in the Agricultural Health Study (AHS), a cohort of licensed pesticide applicators and their spouses in North Carolina and Iowa. Women who personally mix or apply pesticides for agricultural applications have the potential for higher exposures to more toxic restricted-use pesticides. I hypothesized that women who have used pesticides will be more likely to report having uterine fibroids, and that associations will be stronger among women who have used possible endocrine-disrupting pesticides in a farming application compared to those who have only used commercially-available pesticides in residential applications.

Because the Agricultural Health Study relies on self-reported diagnosis, I implemented methods to correct for outcome misclassification by incorporating measures of self-report accuracy in the logistic regression models (12). In order to obtain reasonable assumptions regarding self-report sensitivity and specificity, I used data from Right from the Start (RFTS) and the Uterine Fibroid Study (UFS). Both of these studies collected self-reported fibroid diagnosis from participants and performed ultrasound screening to identify women with fibroids. In addition to informing this research, I sought to provide data that could be useful to others in assessing the impact of misclassification when using self-report to classify fibroid status.

Aim 1 was therefore to evaluate the validity of self-reported fibroid diagnosis and examine the possible determinants of reporting quality in women from RFTS and UFS. RFTS is an ongoing community-based prospective study of early pregnancy, conducted since 2000, which performed early first trimester ultrasound examinations. The UFS was a cross-sectional study conducted in 1996-1999 to estimate uterine fibroid prevalence among randomly-selected members of an urban health plan.

Aim 2 was to examine the association between pesticide use and self-reported uterine fibroids among female farmers and farmers' wives in the AHS. I performed sensitivity analyses to assess the impact of outcome misclassification in addressing the following specific questions:

- Do women who report using pesticides have increased odds of uterine fibroids, compared to never users?
- Is there a difference in the magnitude of the effect depending on whether women report only residential pesticide use, or use pesticides in agricultural applications?
- Is there an association between use of select pesticides that have been identified as possible endocrine disruptors and uterine fibroid prevalence?

CHAPTER 2: REVIEW OF THE LITERATURE

2.1 EPIDEMIOLOGY OF UTERINE FIBROIDS

Uterine leiomyomata (uterine fibroids) are benign, hormone-dependent tumors of uterine smooth muscle origin. Although estimates vary somewhat, fibroids occur in 70 to 80% of women in the United States by the time they reach menopause (1). However, only 20 to 50% of women with tumors become symptomatic (2, 11). Despite their benign nature, uterine fibroids can cause significant co-morbidities such as heavy menstrual bleeding, anemia, pelvic or abdominal pain, and pregnancy complications (13-18). These factors make uterine fibroids an important public health problem.

The etiology of fibroids is not well-understood, although it is well-accepted that steroid hormones play a part in tumor development. Epidemiologic studies, therefore, have generally focused on risk factors that might influence a woman's circulating hormone levels. These include age, race, smoking, alcohol use, oral contraceptive use, and various reproductive and menstrual cycle characteristics. Of these, age, race, and certain reproductive/menstrual factors show the most consistent associations with uterine fibroids. The risk of fibroids increases with age during the reproductive years, with an estimated cumulative incidence of over 70% by age 50 (1, 19-22). They typically regress after menopause (23), and it is estimated that postmenopausal women have a 70 to 90% reduced risk of fibroid diagnosis relative to premenopausal women (24-26).

The incidence of uterine fibroids is 2 to 3 times higher in African-American women compared to white women (1, 20, 27). A higher prevalence among black women is evident in all age groups (1, 20, 28), and does not seem to be explained by racial differences in health care access and utilization or other established risk factors (20, 29, 30). Moreover, black women tend to have a younger age at diagnosis and larger and more numerous tumors than do white women (30, 31). The biological basis for these racial differences remains unclear, however, and genetic susceptibility studies are limited and inconsistent (32).

Reproductive risk factors that have been well-investigated in relation to fibroids are age at menarche, parity, and timing of pregnancies. Most studies have found an inverse association between age at menarche and uterine fibroids, leading some researchers to hypothesize that increased lifetime exposure to circulating sex hormones from earlier menses onset might play a role (33-37). Studies have consistently reported that parous women are 20 to 40% less likely to be diagnosed with fibroids compared to nulliparous women (25, 27, 34, 35, 37, 38). Some, but not all, have further shown an inverse relationship between uterine fibroids and number of births (25, 34, 38). The association with parity persisted even after accounting for the possible confounding effects of infertility (35, 37) and breastfeeding history (37) in two large prospective cohorts. Two other studies that have examined lactation, which suppresses ovarian hormones, have not supported an association between breastfeeding and fibroids (24, 34). Evidence of a protective effect of pregnancy may be limited to viable pregnancies only, since there has been no association found between spontaneous or induced abortions and uterine fibroids; these results are difficult to interpret however, because of possibilities of selection bias in the study designs (37-39).

There is some evidence to suggest that the timing of pregnancy may also be important to some degree in explaining its apparent protective effect. A large prospective cohort study of nurses (the Nurses' Health Study, NHS) which measured incident ultrasound- or hysterectomy-confirmed fibroid diagnosis, found a lower risk among women who first gave birth at ≥25 years of age compared to those whose first birth occurred at ≤24 years of age; in both groups, risk of diagnosis increased with increasing years since last birth (35). A prospective study of black women (the Black Women's Health Study, BWHS) also reported an inverse association between age at first birth and incident fibroids confirmed by ultrasound or hysterectomy, and a positive association with number of years since last birth (37). The Uterine Fibroid Study (UFS), a cross-sectional ultrasound screening study of premenopausal women, found that childbearing in the mid-reproductive years (age 25-29) was the most protective for fibroid development, supporting the theory that smaller/early fibroid tumors might be eliminated with apoptosis during postpartum uterine remodeling (40). More recently, a prospective study of pregnant women which compared early first trimester to postpartum ultrasound results found that 36% of tumors were no longer detectable at the postpartum ultrasound and 79% of the remainder had decreased in size (41).

The relationship between oral contraceptive (OC) use and fibroids is unresolved. The prevalence/incidence of uterine fibroids among women who have ever used OCs has been reported to be reduced (25, 26, 33, 34), similar (24, 35, 37), or increased (42) compared to never users. One case-control study found a steady decrease in the risk of surgically-confirmed fibroids with increasing years of OC use (25). In the NHS, no clear pattern emerged with years of use among current OC users or years since last use, and only women who reported first using OCs between 13-16 years of age had higher risk of fibroids

confirmed by ultrasound or hysterectomy compared to never users (35). The BWHS reported similar results (37). These inconsistencies could be due to differences in study design and population or to differences in the estrogen and progesterone content of oral contraceptive formulations. Spurious associations could also arise if the use of oral contraceptives is related to the degree of fibroid symptoms.

Most investigations have found a positive association between the highest body mass index (BMI) category at enrollment and uterine fibroids, but no evidence for an increasing trend among successively higher BMI categories (25, 33, 34, 43-45). BMI was not associated with fibroids in two case-control studies (24, 36) and among whites in the UFS (46). Two studies have examined other weight measures among premenopausal women. The NHS, whose population was overwhelmingly white, found positive associations with weight gain since age 18 and current waist-to-hip ratio, but little evidence of an association with BMI at age 18 or childhood/adolescent body size (43, 44). The BWHS reported increased incidence rates for ultrasound- or hysterectomy-confirmed fibroids at all BMI categories above the referent of <20.0 kg/m^2, but stronger associations among parous women (who also had elevated risks with increasing weight gain since age 18); waist circumference and waist-to-hip ratio were not associated with leiomyoma risk (45). Obesity may be involved in the progression of uterine fibroids through effects on endogenous hormone levels, although the exact mechanisms and their relative importance are unknown. Conversion of androgen to estrone by excess adipose tissue and decreases in sex-hormone binding globulin may induce a relatively hyper-estrogenic state not normally present in postmenopausal women, while decreased metabolism of estradiol into more inactive

metabolites could be a factor in increasing the bioavailability of estradiol in premenopausal women (47-50).

Other factors that are associated with obesity—such as hyperinsulinemia or hypertension—could also play a role, although few studies have examined these aspects. In the BWHS, the incidence of ultrasound- or hysterectomy-confirmed fibroids was lower in black women reporting a diabetes diagnosis compared to those without diabetes (51). The UFS found a similar association with ultrasound-detected prevalent fibroids among blacks, as well as a reduction in fibroid prevalence comparing the highest to lowest tertiles of insulin levels among black women with large fibroids (52). A case-control study found no association with a history of diabetes, but a positive association among women taking diabetes medication; these findings were based on small numbers, however (53). Fibroids (confirmed by ultrasound or surgery) were positively associated with a history of hypertension in two clinic-based case control studies (53, 54) as well as with blood pressure (treated as a continuous variable) in NHS participants (55).

Behavioral factors that have been investigated include smoking, alcohol use, and physical activity. Of these, smoking has been studied most extensively, albeit with inconsistent results. Current, but not past, smoking has been inversely associated with uterine fibroids in several studies, even after controlling for BMI in three of those studies (24, 25, 34, 56, 57). No association was found, however, in the large prospective cohorts of the NHS (43) and the BWHS (58), and a small case-control study found no association with smoking duration (33). Smoking has been hypothesized to reduce the risk of fibroids through anti-estrogenic effects, but studies of smokers and non-smokers have shown lower endogenous estrogen levels only in post-menopausal women (49, 59-61). Alcohol

consumption has been shown to increase estradiol levels in some (62, 63), but not all (64-67), studies. A positive association between current alcohol intake and uterine fibroids was reported in the BWHS (58) and the NHS (20). Physical activity has been reported to be protective for breast and endometrial cancers (68), but its association with uterine fibroids has not been well-studied. A comparison of athletes versus non-athletes found lower prevalence of fibroids in the former group, however, these results are difficult to interpret due to the possible confounding effects of diet and weight (56). The UFS also noted an inverse association among both African-American and white women for the highest category of physical activity versus the lowest after adjusting for possible confounders (46).

2.1.1 Methodological Challenges

The principal methodological issue in uterine fibroid research is that these benign tumors arise in a large percentage of reproductive-age women without coming to clinical attention because they do not cause any symptoms. It is estimated that only 20 to 50% of women with one or more fibroid tumors will experience symptoms (13). This poses certain methodological challenges for epidemiologic investigations of the prevalence of this condition (reviewed by Schwartz and Baird (69-72)). Obviously, any study design that uses imperfect disease measurement methods will suffer from some degree of misclassification bias. With regard to uterine fibroids, the misclassification can be extensive because the "non-diseased" group will most likely include a large proportion of women with subclinical fibroids. In the UFS for example, 51% of premenopausal black or white women who reported no previous diagnosis actually had fibroid tumors detected at study ultrasound, while 15% of women who claimed they had a previous fibroid diagnosis did not show evidence of fibroids on ultrasound.

Histologic evidence is considered the gold standard for uterine fibroid ascertainment (72), but studies utilizing surgically-confirmed cases can suffer from selection bias to the extent that determinants of a woman's decision to undergo surgery are related to risk factors of interest. Historically, the standard treatment for fibroids has been hysterectomy, but more conservative surgeries (such as myomectomy and uterine artery embolization) and medical treatments are increasingly available (73). A woman's chosen treatment mainly depends on the severity of symptoms, the size and location of tumors, and the desire to keep her uterus for reasons both related and unrelated to childbearing. Consequently, previous studies among women undergoing hysterectomy could show spurious associations with factors (e.g., parity) that are instead related to the treatment choice (e.g., women who have their desired number of children are more likely to choose hysterectomy). Similarly, findings from studies of other special populations—such as women recruited from family planning, gynecologic, or infertility clinics—are difficult to interpret, as the likelihood of incidental detection increases among women who seek medical care for symptoms from other gynecologic conditions. This may result in mistakenly identifying factors as related to uterine fibroid prevalence when they are actually related to those other conditions.

These challenges have impacted the design and interpretation of previous studies. As with other conditions with a long preclinical phase, little is known about the onset and progression of these tumors because the temporality of risk factors with regard to disease onset cannot be established. A good example is the UFS, which minimized the misclassification and selection biases described above by systematically screening a randomly selected sample of women, but is limited in the ability to relate certain risk factors to fibroid onset due to its cross-sectional design. On the other hand, two well-known

longitudinal designs—the NHS and BWHS—have been better able to correlate the assessment of risk factors with disease (i.e. first fibroid diagnosis) through regular follow-up of their cohorts. However, detection bias cannot be ruled out in these studies because they relied on self-reported clinical diagnosis instead of systematic screening. Although both studies found high positive predictive value of self-report in a sample of cases verified through medical records, it is likely that there is substantial case under-ascertainment and that factors related to the probability of a woman being diagnosed (e.g., access to and use of health care, other conditions, presence of symptoms) could account for some of the associations detected. An illustrative example is the BWHS validation subsample, in which 55% of cases reported being diagnosed because of fibroid-related symptoms; the remaining 45% were diagnosed incidentally, either during a routine pelvic examination (32%) or while receiving care for another condition (13%) (21). Nonetheless, these studies provide an important contribution to the relatively limited research in uterine fibroid epidemiology. Data that could be used to examine and possibly adjust for outcome misclassification—such as the sensitivity and specificity estimates from this dissertation research—would be extremely helpful, especially in the analysis of the large prospective cohort studies that are ongoing.

2.2 Role of Hormones in Uterine Fibroid Pathogenesis

Although little is known about the etiology of uterine fibroids, it is widely accepted that ovarian hormones are involved in their pathogenesis. The strongest evidence for this is the observation that fibroids occur in women in their reproductive years and typically regress after menopause (25, 74). Clinical trials showing reduction in uterine volume and fibroid regression after treatment with GnRH agonists—which create a hypoestrogenic and

hypoprogestagenic state—also provide support for the role of estrogen and progesterone activity on fibroid growth (75-77). The use of selective estrogen receptor modulators (SERMs) has had mixed results in humans, underscoring the complexity of the endocrine system and the disparate activity of endocrine-modulating compounds in different target tissues. Tamoxifen for example, which is used as an antiestrogen in breast cancer treatment, has partial agonist effects in the uterine endometrium; some case reports of women being treated for breast cancer have suggested that it may result in growth of existing uterine fibroids (78-80). Trials of the antiprogesterone mifepristone have shown promise in inducing fibroid regression (81, 82), and other selective progesterone receptor modulators are being explored.

In addition to the clinical evidence, *in vitro* and *in vivo* studies have clearly indicated the role of both estrogen and progesterone signaling in the growth of uterine fibroids (reviewed by Marsh et al. (83)). Despite the similarity in serum estradiol and progesterone levels among women with and without uterine fibroids (84), important differences between leiomyomatous and myometrial tissue have been demonstrated. Compared to normal myometrium, uterine leiomyoma (UL) cells have elevated levels of both estrogen receptors (ER-α and ER-β) (85-87) and increased proliferation and transcriptional response to estrogen stimulation (85, 88, 89). In addition, elevated expression of the enzyme aromatase, which converts androgens to estrogen, was found in fibroid smooth muscle cells, signifying the potential *de novo* production of estrogen by leiomyoma tissue (90-92).

Uterine fibroids also have increased concentrations of progesterone receptors A and B compared with normal myometrium (93, 94), and have the highest mitotic count during the luteal phase of the menstrual cycle, when progesterone production is at its peak (95). As

with estrogen, progesterone induces proliferation and up-regulates growth factors, proteins that can have inhibitory and/or stimulatory effects on cell replication (96, 97). Certain growth factors, which may stimulate fibroid growth by increasing extracellular matrix, have also been found to be over-expressed in uterine fibroids (reviewed by Maruo et al. (98)). The relative importance of and relationships between ovarian hormones, growth factors, and binding proteins in uterine fibroid development remain unclear. The fact that different tumors in the same woman can have different growth rates argues against a simple model of hormonal regulation. However, the increased sensitivity to estrogen raises the possibility that the growth of these tumors may be influenced by exposures to environmental estrogens (i.e., xenoestrogens).

2.3 ENDOCRINE-DISRUPTING ACTIVITY OF PESTICIDES

The potential for pesticides to act as endocrine-disruptors was first suggested in the early 1950s, when DDT and some of its analogs were found to have estrogenic activity in animal models (99, 100). Endocrine disruption refers to the ability of a chemical to either mimic or block the action of endogenous hormones by 1) binding directly with steroid receptors; 2) inhibiting steroid synthesis; or 3) modulating hypothalamic-pituitary feedback loops (101). With regard to pesticides, the focus of most toxicologic research to date has been on their estrogenic potential (especially organochlorine insecticides), although some have been shown to have effects on progesterone, androgen, and other hormones (101). This section will focus on the evidence for estrogenic activity and female reproductive effects.

The hallmark of estrogen action was presented by Hertz as its proliferative effect on the female genital tract (102). This definition has long been considered the standard for testing the estrogenicity of compounds *in vitro*, and is the basis for the E-Screen assay which

measures proliferation in human breast cancer MCF7 cells (103). The most widely used test of estrogenicity *in vivo* is the rodent uterotrophic assay, which measures changes in uterine weight after administration of the suspect compound (104). Other assays and combinations of testing strategies have been developed, especially in response to the initiation of the U.S. Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (information available on-line at http://www.epa.gov/endo/pubs/assayvalidation/index.htm).

Examples of estrogenic pesticides are DDT and some of its analogues, kepone (chlordecone), and methoxychlor and its metabolite HPTE (101). DDT, kepone, and methoxychlor have been ranked as "Category 1: Evidence for endocrine disruption in living organisms" by the European Union (105) and as "known" endocrine disruptors based on the weight of evidence by the Illinois Environmental Protection Agency (106). These compounds have demonstrated estrogenic activity *in vitro* and have been linked to adverse effects on fertility, early pregnancy, and reproductive tract development in murine models. One of the most well-studied pesticides is DDT and its analogues, which can be estrogenic, anti-estrogenic, or anti-androgenic. DDT and some of its breakdown products increase cell proliferation in estrogen-responsive cell lines (103, 107), show ERα and/or ERβ agonist activity (108, 109), induce human ER-mediated transcriptional activation *in vitro* (110), and increase estradiol secretion in porcine granulosa and theca cells (111-113). DDT, *o,p'*-DDT, or *p,p'*-DDT also exhibited some degree of estrogenicity by causing an increase in uterine weight and early vaginal opening in murine models (114, 115). Neonatal exposure to the organochlorine pesticide kepone in rats leads to persistent vaginal estrus and anovulation (116). Precocious puberty, persistent vaginal estrus, and problems in initiating and maintaining pregnancy were observed in mice and rats administered methoxychlor neonatally

or during the peri-implantation period (117-120). In addition to DDT and methoxychlor, the pesticides lindane, endosulfan, toxaphene, and dieldrin were shown to increase cell proliferation in the E-Screen assay (121).

A recent review by Mendola et al. (122) has described some of the existing epidemiologic findings (and limitations) with regard to environmental contaminants and female reproductive health. With the exception of one study of about 700 female greenhouse workers in Italy (123), studies published in the last ten years have found some association between agricultural or horticultural pesticide exposure and impaired fertility and fecundability such as increased time-to-pregnancy and risk of spontaneous abortion (reviewed in Mendola et al. (122)). Younger age at menarche has been associated with increased serum DDT levels in a cross-sectional analysis of 466 female Chinese textile workers (124), but not related to serum DDE and mirex in a cross sectional analysis of 138 Native American girls living near a U.S. Superfund site (125). Women who reported ever having mixed or applied pesticides had longer menstrual cycle lengths, increased risk of missed periods, and older age at menopause compared to never users in the relatively large Agricultural Health Study cohort (126, 127). A small (n = 60) study of serum DDT levels among young Chinese women enrolled at the time of their premarital health examination found no effect on menstrual cycle length or duration (128); the cross-sectional analysis of Chinese textile workers (n = 466) reported an increased risk of short cycle length (124). Inconsistencies in results in this limited number of epidemiologic studies are probably due to several factors, not the least of which is the inability to construct an adequate exposure measurement that incorporates the magnitude, duration, and timing of these exposures relative to the outcome of interest. Exposure measurement approaches varied and could

account for differences in study results. Some used serum pesticide metabolite measures, which are generally more reliable than self-reports, but even these studies could be difficult to interpret if blood samples are taken after the outcome of interest occurs or if there is inadequate adjustment for factors that might affect body burden (e.g., BMI). Recall bias may have also played a role when outcomes were assessed retrospectively. Finally, studies of special populations (e.g. greenhouse workers) that are exposed to high concentrations are difficult to compare because these groups may be exposed to varying mixtures of chemicals (measured and unmeasured) that could either lead to a spurious association with a correlated exposure of interest or could dilute its association.

2.4 ENDOCRINE-DISRUPTING CHEMICALS AND UTERINE FIBROIDS

The existing research on the relationship between EDCs and uterine fibroids is limited. Results from *in vivo*/*in vitro* studies lend support to the hypothesis that exogenous estrogens might affect fibroid growth, but the epidemiologic findings are few and inconsistent. It has been suggested that the increased prevalence of uterine fibroids in Baltic gray seals during the 1970s was related to highly elevated levels of DDT and PCBs in Baltic biota; a more recent study using a lifetime exposure index indicated that PCB concentrations, rather than DDT, better explained the variation in fibroid prevalence over time (129). Female mice exposed prenatally to diethylstilbestrol (DES) displayed reproductive tract abnormalities, including uterine fibroids, in adulthood (7, 130). Administration of environmentally relevant doses of bisphenol A resulted in increased fibroid incidence in neonatally exposed mice compared to controls (8).

The most compelling animal evidence for a possible relation between xenoestrogens and fibroids comes from the Eker rat model. The Eker rat spontaneously develops uterine

fibroids with high frequency that share many of the phenotypic characteristics of human fibroids. The ELT-3 cell line developed from these animal models has been shown to be estrogen receptor-positive and responsive to estrogen in culture (131-133). Using this model, Hodges et al. (5) confirmed that DES acts as a potent estrogen agonist in the uterine myometrium of intact animals, that ELT-3 cells proliferated in a dose-dependent manner in response to DES, and that DES was able to upregulate the expression of the progesterone receptor (an estrogen-responsive gene). The same group also showed that the phytoestrogens coumestrol, genistein, and naringenin as well as five organochlorine pesticides—methoxychlor (and its metabolite HPTE), kepone, endosulfan, toxaphene, and dieldrin—had estrogenic activity either by inducing a transcriptional response or cell proliferation *in vitro* (134). More recently, researchers at the National Institute of Environmental Health Sciences (NIEHS) found that exposure to fenvalerate, a pyrethroid insecticide, increased the rate of growth *in vitro* of human uterine leiomyomata as well as Eker rat leiomyomatous and smooth muscle cells (9).

Very few human studies of EDCs and uterine fibroids have been conducted to date. Significantly higher concentrations of DDT and its metabolites were found in leiomyomatous tissue compared to surrounding normal myometrium in a sample of 25 women with fibroids (10). The Uterine Fibroid Study (n = 1,323) reported an odds ratio of 2.4 (95% confidence interval: 1.1, 5.4) for uterine fibroids comparing white women who reported prenatal DES exposure to those who did not (135). However, a collaborative follow-up study of almost 2,700 DES-exposed and unexposed women did not find an association with self-reported fibroid surgery that was confirmed via medical records (age-adjusted Incidence Rate Ratio: 0.9; 95% CI: 0.6, 1.5) (136). The Seveso Women's Health Study, a prospective study of 956

women living in the vicinity of a chemical plant explosion in 1976, found a statistically significant inverse association between serum levels of 2,3,7,8-tetrachlrodibenzo-*p*-dioxin (TCDD) at the time of the explosion and hazard of self-reported fibroids confirmed by medical records (137), in line with data that TCDD acts as an antiestrogen in the rat uterine myometrium (138). Jackson et al. investigated possible endocrine-disrupting heavy metals in a cross-sectional analysis of uterine fibroids and endometriosis among 1,425 premenopausal women in the National Health and Nutrition Examination Survey, 1999-2002. Although women with previous uterine fibroid diagnosis had significantly higher mean blood lead and mercury levels than those without fibroids, there were no differences in the adjusted odds of fibroids across tertiles of exposure (139).

CHAPTER 3: METHODS

This dissertation research had two primary objectives: Aim 1 described the accuracy of self-reported uterine fibroid diagnoses and Aim 2 examined the relationship between pesticide use and uterine fibroid prevalence among farm women.

I used data from the Right from the Start and the Uterine Fibroid Study to examine the validity of self-reported fibroid status by comparing it to results from study ultrasound examinations. Together, these two studies comprised 2,119 white and 796 black premenopausal women aged 18-49. Sensitivity (the proportion of women reporting a diagnosis among those with ultrasound-detected fibroids) and specificity (the proportion of women reporting no diagnosis among those with no evidence of fibroids at ultrasound) were estimated using log-binomial regression. Sensitivity ratios and specificity ratios, and their 95% confidence intervals (CI) were examined to assess whether validity of self-report is associated with characteristics such as age, race, or parity.

I examined whether pesticide use is associated with self-reported fibroids among 16,526 white female private pesticide applicators and wives of private pesticide applicators, aged 21-59, enrolled in the Agricultural Health Study. My focus was on pesticides identified as hormonally active based on review of the toxicological literature. I also examined pesticide use patterns to assess whether the strength of the association with fibroid diagnosis differed according to type and number of pesticides used. In order to account for bias resulting from outcome misclassification, I used an outcome correction method (12) in the

logistic regression models, incorporating self-report sensitivity and specificity estimates obtained from Aim 1.

3.1 DATA SOURCES

3.1.1 Aim 1: Right from the Start and the Uterine Fibroid Study

Right from the Start (RFTS) is an ongoing prospective study of early pregnancy (28, 140). The study has been conducted since 2000, and utilizes community-based recruitment procedures to identify women who are pregnant or planning a pregnancy. A short telephone interview was used to screen women for eligibility and obtained informed consent. Women who elected to participate in the study were required to review and sign an informed consent form and the HIPAA authorization for Use of Protected Health Information. Starting in 2004, an abbreviated enrollment questionnaire collected demographic data and information on pregnancy status and fibroid diagnosis; more detailed information on medical/reproductive history, health behaviors, and current pregnancy was collected in a telephone interview during the first trimester. Ultrasound was also performed on study participants during the first trimester of pregnancy.

The Uterine Fibroid Study (UFS) was a cross-sectional study of approximately 1,500 randomly-selected female members of a Washington DC-area health plan (1). Enrollment and initial ultrasound screening occurred in 1996-99. Women were contacted by telephone to confirm eligibility and give informed consent to participate in the study. Postmenopausal women were interviewed about prior diagnoses of fibroids, and those with surgically-induced menopause were asked for permission to review their medical records. Premenopausal women were screened for fibroids with pelvic ultrasound. Data on medical history, demographics, and lifestyle were collected by self-administered questionnaires. Information

on reproductive and gynecologic history (including previous fibroid diagnoses) was obtained through a telephone interview.

3.1.2 Aim 2: Agricultural Health Study

The Agricultural Health Study (AHS) is a longitudinal cohort of approximately 60,000 licensed pesticide applicators and their spouses (~32,000) in North Carolina and Iowa. Individuals applying for a restricted-use pesticide license in Iowa and North Carolina were recruited at state licensing agencies between 1993 and 1997 (Phase I) (141). Those who agreed to participate in the study completed an Enrollment Questionnaire and were asked to identify their spouses for enrollment in the study. Married male private applicators were given two questionnaires to be completed by their female spouse. Women (applicators or spouses) who completed the Phase I questionnaires were eligible for follow-up telephone interviews between 1999 and 2003 (Phase II). Data on fibroid diagnoses, farm-related exposures, ever use of up to 50 pesticides, demographic variables, reproductive history, and general health and medical history were obtained from self-administered questionnaires and telephone interviews.

3.1.3 Required Approvals

The UFS was approved by the Institutional Review Boards (IRB) at the National Institute of Environmental Health Sciences (NIEHS) and George Washington University. Approval for RFTS was granted by the IRBs at the University of North Carolina, University of Tennessee, and the University of Texas. The AHS was approved by the IRBs at NIEHS, the National Cancer Institute, and their contractors which carried out the study. This dissertation research was reviewed by the University of North Carolina Public Health-Nursing IRB, which determined that this study was exempt from the requirements of the US

Department of Health and Human Services regulations, and therefore does not require IRB approval (Study #08-1907).

3.2 ANALYTIC APPROACH: STUDY AIM 1

The objective of Aim 1 was to evaluate the validity of self-reported fibroid diagnosis and examine the possible determinants of accurate reporting in black and white women aged 18 to 49 years.

3.2.1 Study Population

The study population included records from two study data sets: RFTS and the UFS. For this analysis, the population was restricted to non-Hispanic white and African-American women because the proportion of women of Hispanic ethnicity or other race was small. Further restrictions specific to each parent study data set are described below.

Although RFTS began in 2000, my analysis population was restricted to African-American and white (non-Hispanic) women who were recruited starting in 2004 (RFTS-2 and RFTS-3), when a question about previous uterine fibroid diagnosis was added to the enrollment questionnaire. RFTS-2/3 participants are women in very early pregnancy or those planning to become pregnant living near study sites in North Carolina and Tennessee (28, 140). Participants were recruited from prenatal care clinics and the general community using a variety of methods such as direct mail and promotional materials. To be eligible for RFTS-2/3, women had to be at least 18 years of age, speak English or Spanish, plan to remain in the study area for the next 18 months, not have used assisted reproductive technology, and enrolled prior to 9 completed weeks' gestation (if already pregnant). Women who were planning to become pregnant were followed for up to six months and enrolled if they became pregnant. Beginning in 2007 (RFTS-3), the follow-up period for women trying to conceive

was shortened to three months. Women were allowed to re-enroll in the study if they became pregnant again, but I only included data from the first enrollment. Between 2004 and 2008, a total of 2,411 women were enrolled and 2,341 (97%) had both ultrasound and self-report information. Women whose self-reported race/ethnicity was non-Hispanic white (n = 1,756) or non-Hispanic black (n = 290) were included in this analysis (Figure 3.1).

UFS participants were female members of a prepaid urban health plan with approximately 50% black membership and a broad socioeconomic base (1). A random sample of 2,384 women was sent an advance letter describing the study and then contacted by telephone to confirm eligibility and obtain informed consent. To be eligible for the study, women had to be 35-49 years old, members of the health plan's Washington, DC site, and able to complete the data collection in English. Approximately 88% of the original sample was contacted and screened for eligibility. Sixteen percent of the 2,102 screened women were ineligible for the study, mainly because they were no longer receiving care at the study site. A total of 1,430 out of 1,786 eligible women (80%) participated; study participation rates were similar among black and white women (1). In addition to the race/ethnicity criterion defined above, the analysis population was further limited to women who were premenopausal at the time of their baseline interview, had an ultrasound performed, and whose ultrasound results were definitive (i.e. women who had classifications of "diffuse heterogeneity" or indeterminate results are excluded). The UFS population for analysis consists of 363 Caucasian and 506 African-American women (Figure 3.2).

Figure 3.1 Right from the Start (RFTS) participants and final analysis population

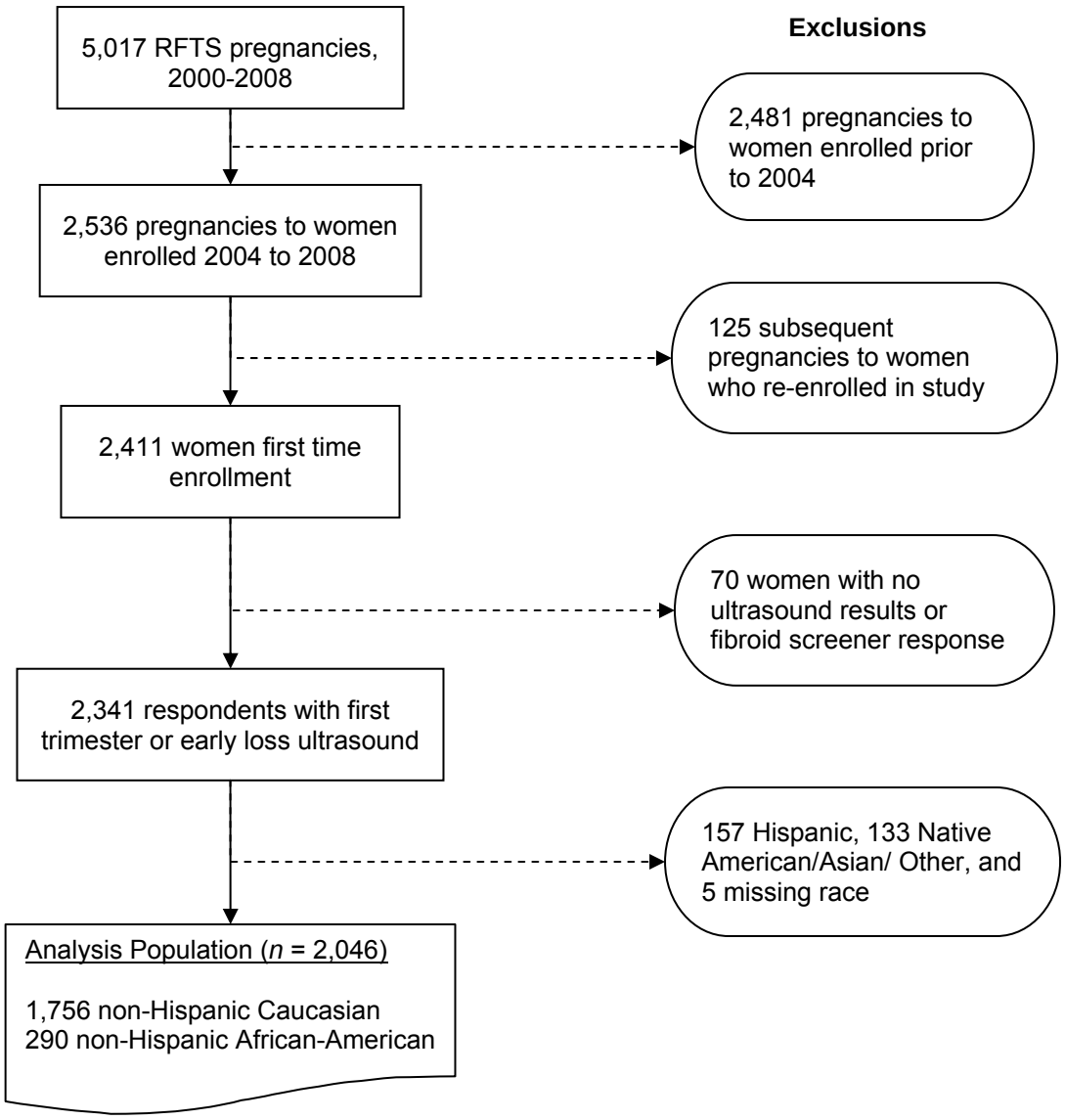
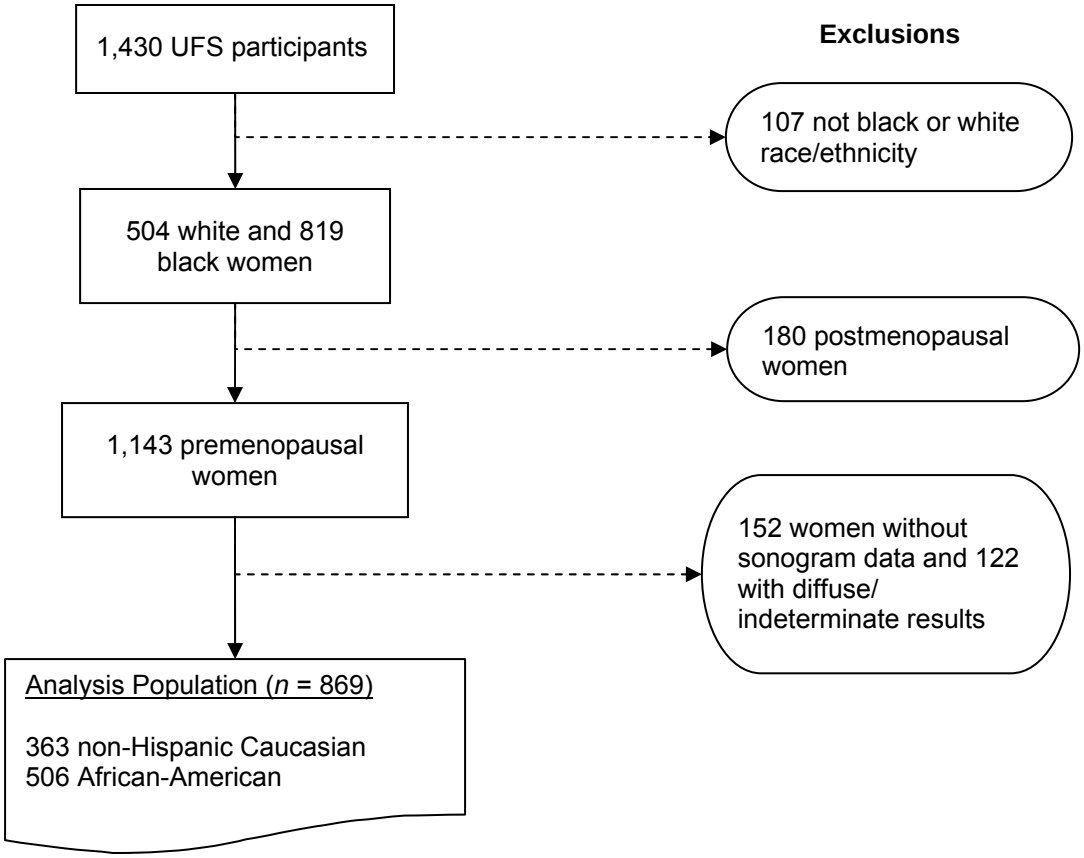


Figure 3.2 Uterine Fibroid Study (UFS) participants and final analysis population



3.2.2 Uterine Fibroid Measurements

Self-report

Women who screened as eligible in the RFTS-2/3 immediately completed an enrollment interview in which they were asked "Have you ever been told by a medical provider that you have uterine fibroids?" Previous fibroid diagnosis in the UFS was ascertained in a telephone interview prior to the study ultrasound and clinic visit. Women were asked "Have you ever been told by a doctor or other health professional that you have uterine fibroid tumors or a leiomyoma, a benign tumor of the uterus or womb?" In addition, the UFS collected an extensive history including age at first diagnosis, diagnostic and follow-

up examinations, and treatment. Women were categorized as having a previous diagnosis if they answered "yes" to the initial question and did not have any answers in the next few questions to indicate that it had been a misdiagnosis.

Ultrasound

In both the UFS and RFTS, fibroid identification at ultrasound examination was based on Muram criteria (142), modified to include masses of ≥0.5 cm in diameter. RFTS-2/3 participants underwent an endovaginal ultrasound as early as 6 and no later than 13 weeks of gestation. Previous analysis of RFTS data showed no difference in fibroid prevalence by gestational age at ultrasound (28), so detection is unlikely to be influenced by the timing of the ultrasound within this narrow window. Examinations were performed by sonographers certified by the American Registry of Diagnostic Medical Sonographers (ARDMS). Sonographers received additional study training for consistency in identifying and measuring uterine fibroids, and instructions not to discuss the results of the examination with study participants (28). Measurements included fibroid number, location, and size; each tumor was examined three times during the ultrasound to reduce the chance of misidentifying focal contractions as fibroids (28). Digital images of the ultrasound results were sent to study investigators for review.

Premenopausal participants in the UFS underwent both transabdominal and transvaginal ultrasound examinations at a clinic visit within three months of study entry. Ultrasound was performed by ARDMS-certified sonographers who recorded measurements of the size (length, width, anteroposterior diameter) of each tumor, the size and location of the two largest tumors > 2 cm in diameter, and the size of the three largest submucosal tumors (1). Results were reviewed by a radiologist trained in ultrasonography. Women who

had had a recent pelvic ultrasound at the clinic (n = 170) were not examined again, but were classified as positive or negative for fibroids based on the radiology records from the recent examination. Recorded measurements included the number, location, and size of the fibroid tumors.

Outcome Definition

Using the measurements described above, I created a dichotomous variable (SR_CORR) to indicate whether the self-reported diagnosis matched results from the ultrasound or information about prior fibroid surgeries (SR_CORR=1 if self-report and ultrasound results agreed; 0 if they did not agree). Study ultrasound can be negative for women who report a previous fibroid diagnosis and who elected to have their fibroids removed. Data on previous surgery to remove fibroids were available in both the UFS (from questionnaire and medical records) and RFTS (from the questionnaire). For purposes of this analysis, women who had previous fibroid surgery were treated as having fibroids, even if the study ultrasound did not show evidence of fibroids.

The main "outcomes" of interest are sensitivity and specificity, which were estimated by restricting the analysis to different groups as follows:

- Sensitivity (Se), defined as proportion of women correctly reporting a fibroid diagnosis and limiting analysis to women with fibroids on ultrasound or previous fibroid surgery (D+).

$$Se = Pr (SR\_CORR=1 \mid D+)$$

- Specificity (Sp), defined as proportion of women correctly reporting no fibroid diagnosis and limiting analysis to women with no fibroids on ultrasound and no previous fibroid surgery (D-).

$$Sp = Pr (SR\_CORR=1 \mid D-)$$

Although I originally proposed to also estimate overall agreement, I found this measure to be somewhat uninformative and excluded it from the analysis. For example, overall agreement was relatively good even if sensitivity was very poor (if specificity was very high), and tended to obscure the fact that many true fibroid cases were unreported.

3.2.3 Possible Predictors of Reporting Accuracy

I explored the variables described below as possible predictors of sensitivity and specificity. As there is no previous literature on this subject, I chose covariates that are either associated with uterine fibroids or may be associated with reproductive health care utilization or the likelihood that a woman might have been screened for fibroids prior to study entry.

Age: Women were asked their current age and date of birth at the start of each study. Women in RFTS ranged from 18 to 45 years old, with a mean age of 30 for white and 27 for black women. In order to have a reasonable number of women in each category, I categorized age as 18-29, 30-34, and 35-45 for RFTS. UFS participants were between 35 and 49 (by design), and both black and white women were about 41 years old on average. For the UFS, age was categorized into 5-year groups (35-39, 40-44, 45-49).

Race/Ethnicity: Race and ethnicity were self-reported by respondents in both studies. In RFTS, race and ethnicity were asked separately while in the UFS, race and Hispanic ethnicity were combined into one question (e.g., white/not Hispanic, white/Hispanic). Clinic records were used in the UFS to complete race information if missing. In both studies,

women could select more that one race category (e.g., black and white). For purposes of this analysis, race is defined as African-American if respondents selected "Black" either alone or in combination with another category, and as white if they identified themselves as "White." Respondents who identified themselves as Hispanic are not included in this analysis.

Education: Among other factors, socioeconomic status is associated with access to and use of health care services (143). I used education as a proxy measure of socioeconomic status in this analysis. Self-reported years of completed schooling is a reliable measure and a meaningful indicator of SES for adults (144). RFTS asked for years of schooling completed and was categorized as follows: <16, "High school/some college;" 16, "4 years of college;" and >16, "Post-baccalaureate." UFS participants reported their highest level of education in the self-administered mail questionnaire by selecting from among a list of eight choices, which were collapsed into the categories above. The "high school/some college" category includes a small percentage of women with less than a high school education: 19 white and 21 black RFTS women, and 11 black UFS women.

BMI (kg/m^2) was categorized following standard ranges found in the literature: $\leq$24.99, 25.00-29.99, and $\geq$30.00 kg/m^2. In the RFTS, BMI was calculated from height and weight measurements recorded at the first trimester ultrasound visit. I used self-reported height and weight "around the time you got pregnant" for six records that were missing this information. In the UFS, height and weight were measured at the clinic visit.

Parity: The First Trimester RFTS interview asked participants "How many times in total have you been pregnant, counting this pregnancy?" and then collected a detailed history of past pregnancies, including pregnancy outcome. Similarly, the UFS telephone interview included the question "Have you ever been pregnant?" Women who responded "yes" were

then asked about the outcome of each pregnancy. For each pregnancy, respondents selected from the following options (options differ slightly for RFTS and UFS): live birth, stillbirth, miscarriage, elective/induced abortion, ectopic/tubal, molar pregnancy, and other. If the respondent reported a live birth or stillbirth, gestational age was estimated based on her response to whether the baby was "born early, late, or on time" and "how many weeks (early/late)." Using these data, parity was defined as a dichotomous variable (nulliparous/parous) based on whether the woman reported any births ≥20 weeks completed gestation.

Previous miscarriage (yes/no) was classified as "yes" based on self-report of miscarriage, confirmed using the reported gestational age (if < 20 weeks completed gestation). In RFTS, women were asked if any of their pregnancies ended in a miscarriage. Women reporting a miscarriage were then asked "How far along in the pregnancy were you when the pregnancy ended?" The UFS pregnancy history section was similar, but women reporting a miscarriage were asked "How many weeks did this pregnancy last, counting from the last normal menstrual period before this pregnancy?"

Fibroid size: Uterine fibroid size was examined with respect to sensitivity only. Size of fibroid tumors may be related to the incidence and severity of symptoms such as abnormal bleeding, abdominal bloating, and pelvic discomfort (13, 145), and may therefore be related to the likelihood of a woman seeking care. Fibroid size was categorized as <2.00, 2.00-3.99, and ≥4.00 cm based on the largest measured diameter for the fibroid tumor(s) detected. A total of 16 (1%) RFTS and 23 (3%) UFS participants reported having previous surgery to remove fibroids. I assumed that tumor size in these women would have to be clinically significant to require treatment and therefore coded their fibroid size as ≥4.00 cm.

Factors influencing presence/absence of fibroids at ultrasound

For a subset of women who reported a previous fibroid diagnosis, I explored additional factors in relation to whether or not they had fibroids detected at the time of the study ultrasound. Although there are very few investigations of fibroid growth over time, there is some evidence that each tumor might have its own intrinsic growth rate, and that some tumors can spontaneously shrink (31). Pregnancy may also influence the regression of fibroids, possibly through postpartum uterine remodeling (40, 41). I used self-reported age at first fibroid diagnosis, date of study ultrasound, and the pregnancy history described above to construct the following variables: time interval between previous diagnosis and study ultrasound (categorized as ≤2, >2 to 6, >6 to 12, and >12 years); any pregnancy (yes/no); and any term birth (live or still, >37 weeks' gestation) between previous diagnosis and study ultrasound (yes/no).

3.2.4 Data Analysis

All analyses were limited to non-Hispanic white and African-American premenopausal women because of the small number of other race/ethnicities. I analyzed RFTS and UFS data separately because of differences in the age distribution and other characteristics of the study populations. Within each study, both overall and race-specific results were presented because most epidemiologic studies analyze these groups separately, and race-specific estimates would be of greatest utility for assessing outcome misclassification in other studies.

First, I examined the univariate distribution of all variables included in this analysis, including those used for constructing variables of interest. The distribution of observations (including percent missing) was assessed for categorical variables using one-way frequency

tables and for continuous variables using descriptive statistics. I assessed digit preference for self-reported age at diagnosis in the UFS by examining a histogram of the frequency distribution of the terminal digit in the reported age. The overall distribution did not vary significantly from what would be expected under the assumption of equal proportions (χ^2 test *P* value = 0.63). RFTS asked women for the date of previous diagnosis rather than age.

Next, for each study data set, I examined the joint distributions between sensitivity and specificity (the outcomes) and each of the possible predictors, stratified by race. Differences in the race-specific distributions among some of the predictors deemed it necessary to collapse some categorical variables in order to have adequate numbers for the regression analysis. In both RFTS and the UFS, for example, few black women had a graduate degree and few white women had only a high school diploma, making it necessary to collapse the categories at either end of the education variable. Similarly, there was a small percentage of black women in the "underweight" category (BMI <20.00 kg/m^2), so it was collapsed with the 20.00-24.99 kg/m^2 category.

Log-binomial regression was used to estimate sensitivity and specificity and their 95% confidence intervals. Prevalence ratios from the log-binomial model are interpreted here as the sensitivity or specificity in one group compared to that in a referent group. Estimation of sensitivity and specificity ratios was accomplished by limiting the analysis population to certain subsets as defined below and regressing SR_CORR (correct self-report: Yes=1, No=0) on each of the potential predictors separately.

Outcome	Analysis subset	Estimate interpretation
Sensitivity	Fibroids on ultrasound or prior fibroid surgery	Proportion of women who self-report a previous fibroid diagnosis among those with ultrasound-confirmed fibroids or prior surgery
Specificity	No fibroids at study ultrasound and no prior fibroid surgery	Proportion of women who self-report no previous diagnosis among those with no evidence of fibroids at ultrasound and no previous fibroid surgery

Linearity of dose-response trends for categorical predictors (including age) was examined using common referent coding and by treating categorical variables as ordinal parameters in the models. Age was categorized for purposes of displaying results of the association between age and sensitivity/specificity, but included as a continuous variable for purposes of adjustment. The linearity of the associations of sensitivity/specificity and age as a continuous variable was assessed by adding a quadratic term to the model and retaining it if the Wald *P* value was <0.05.

Age and race were evaluated as potential effect measure modifiers. These variables were selected because increasing age up to menopause and African-American race are well-established risk factors for uterine fibroids, and because it seemed plausible that differences in other (measured and unmeasured) covariates across groups might influence the association with self-report validity. Effect measure modification was assessed visually by examining stratum-specific prevalence ratios separately for race and age, and by computing a Mantel-Haenszel χ^2 test for homogeneity with a P <0.10 significance level.

Final multivariable models included covariates that were associated (P <0.10) in univariable models with sensitivity or specificity in either RFTS or the UFS. Sensitivity ratios were adjusted for parity and age as a continuous variable (with a quadratic term in the

UFS analysis to accommodate non-linearity). Specificity ratios were adjusted for parity in analysis of UFS data, but I did not adjust for any covariates in the RFTS specificity analysis due to the small number of women who reported a previous diagnosis but did not have ultrasound-detected fibroids. There were some instances in which the log-binomial model did not converge, especially in the RFTS specificity analysis. In these instances, prevalence ratios and 95% confidence intervals were estimated by Poisson regression using robust error variances (146).

Analysis of factors influencing presence/absence of fibroids at ultrasound

Based upon the results of the specificity analysis, further investigation was made among the subset of UFS women reporting a previous diagnosis in order to explore possible reasons for the lower self-report specificity compared to RFTS women. Only the UFS black women were examined (n = 221) because there were few UFS white women reporting a previous diagnosis who did not have ultrasound-detected fibroids. Factors since diagnosis (described in Section 3.2.3) were examined to determine if they were associated with whether or not a woman who reported a diagnosis still had fibroids detected at ultrasound. I excluded 14 records in which the self-reported age at diagnosis was deemed inaccurate based on review of other variables in the data set. For the pregnancy analysis, 14 women were excluded because it could not be determined whether the pregnancy occurred before or after the diagnosis.

Two-way crosstabulations by each of the factors of interest provided information on the percentage of women who had fibroids at ultrasound among those reporting a previous diagnosis. Logistic regression was used to test for statistically significant effects, controlling for age at interview.

3.3 ANALYTIC APPROACH: STUDY AIM 2

The objective of Aim 2 was to examine the association between pesticide exposure and self-reported uterine fibroid prevalence among women in the Agricultural Health Study (AHS), with a focus on pesticide use patterns and use of possible hormonally active pesticides. Results from the validity analysis of self-reported fibroid diagnosis (Aim 1) was used to assess and correct for outcome misclassification in these analyses.

3.3.1 Study Population

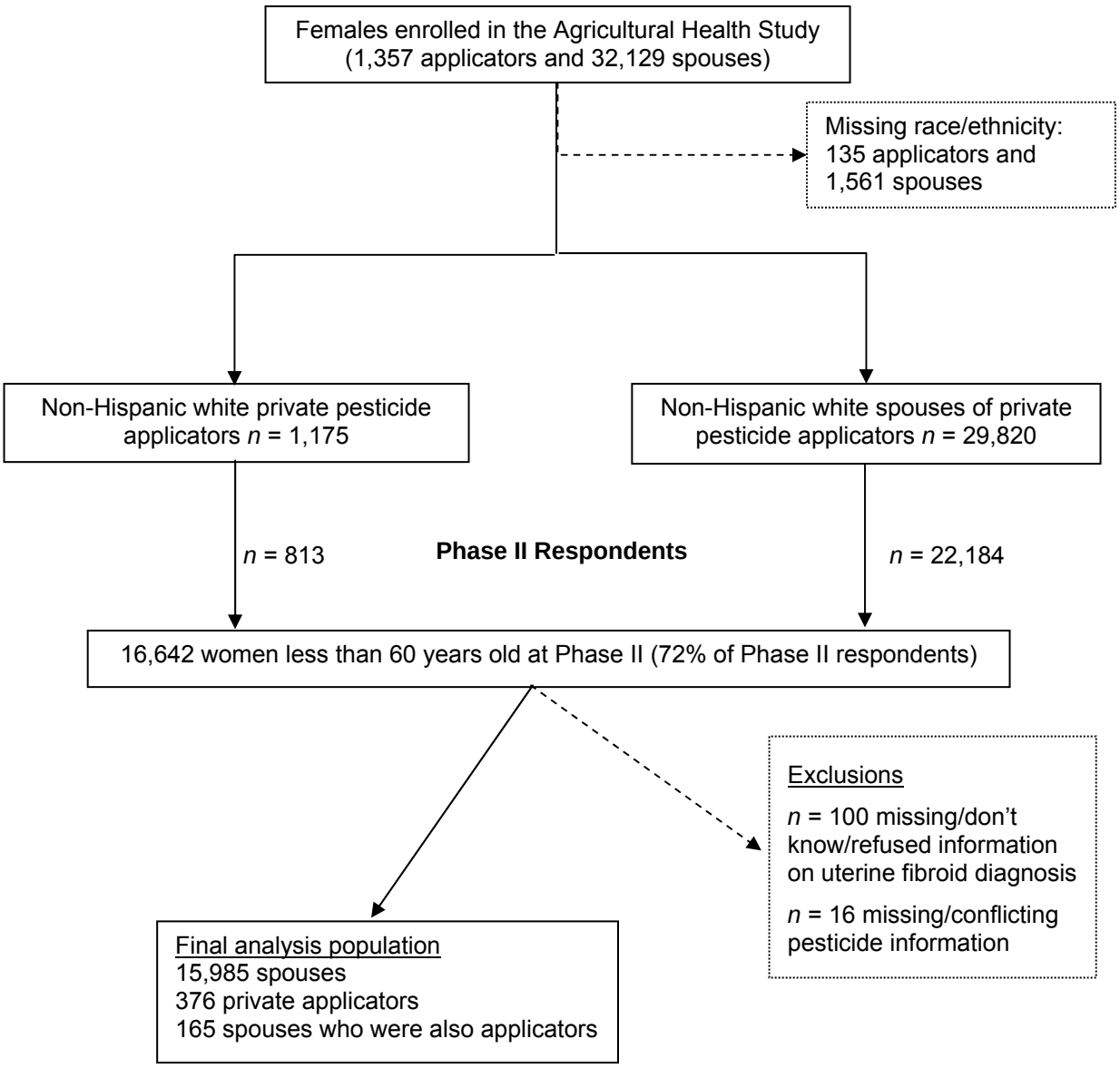
The majority of female participants in the AHS were wives of private pesticide applicators; about 4% of female participants were themselves private pesticide applicators. Approximately 82% of eligible pesticide applicators enrolled in Phase I of the study between 1993 and 1997 (147). Once enrolled, male private pesticide applicators (mostly farmers) were given a Spouse Questionnaire and Female and Family Health Questionnaire to take home to their wives. A telephone interview was conducted to collect data from non-respondents to the take-home questionnaires. Altogether, 75% of wives completed the self-administered Spouse Questionnaire (19% of whom completed by telephone). Follow-up telephone interviews (Phase II) were conducted approximately five years later, between 1999 and 2003. Approximately 69% of female private applicators (n = 921) and 74% of wives (n = 23,682) completed the follow-up interview. Compared to Phase II respondents, nonrespondents were slightly younger, had less education, resided in North Carolina, and more likely to report their ethnicity as non-white. The nonresponse rate for female applicators was lower than for spouses. Nonrespondents were also more likely to have reported never personally mixing or applying pesticides in the Enrollment Questionnaire (Table 3.1).

The study population for Aim 2 consists of 16,526 white women who completed the Enrollment or Spouse Questionnaires in Phase I and the Phase II Health Module and were 21 to 59 years old in the Phase II follow-up (Figure 3.3).

Table 3.1 Differences in demographic and other characteristics for female applicators and wives of pesticide applicators, by Phase II respondent status

Characteristic	Phase II respondent (*n* = 24,603)		Phase II nonrespondent (*n* = 8,883)	
	No.	%	No.	%
Type				
Applicator	921	3.7	436	4.9
Spouse	23,682	96.3	8,447	95.1
State				
Iowa	17,242	70.1	4,902	55.2
North Carolina	7,361	29.9	3,981	44.8
Education				
Less than high school	1,053	4.9	603	7.9
High school grad/GED	8,519	39.5	3,214	41.9
Vocational school/ some college	6,569	30.5	2,260	29.5
College degree or higher	5,377	24.9	1,583	20.6
Other	41	0.2	13	0.2
Missing	3,044		1,210	
Race				
White	23,602	98.5	8,240	97.4
Black	247	1.0	143	1.7
Native American / Asian / Other	125	0.5	78	0.9
Missing	629		422	
Hispanic ethnicity				
No	23,337	99.1	8,192	99.1
Yes	206	0.9	75	0.9
Missing	1,060		616	
Age at enrollment				
< 30	1,275	5.2	762	8.6
30 – 39	5,907	24.0	2,375	26.7
40 – 49	7,178	29.2	2,337	26.3
50 – 59	6,103	24.8	1,850	20.8
60 – 69	3,434	14.0	1,112	12.5
70 and over	705	2.9	445	5.0
Missing	1		2	
Mean (SD)	47.2 (11.7)		46.2 (13.0)	
Lifetime use of pesticides				
Never	9,674	40.3	4,150	48.9
Ever	14,336	59.7	4,333	51.1
Missing	593		400	

Figure 3.3 Agricultural Health Study participants and final analysis population



3.3.2 Outcome Definition

The outcome of interest is self-reported physician diagnosis of uterine fibroids, defined as a dichotomous variable. The Phase II questionnaires were completed via computer-assisted telephone interview (CATI), and included a Female Health Module which collected information about current reproductive health as well as past diagnoses of various

medical conditions. Women were asked "Has a doctor or other health professional ever told you that you had uterine fibroids?" If the woman responded yes, she was then asked "How old were you when the doctor first told you that you had (this /uterine fibroids)?"

3.3.3 Exposure Assessment

Exposure metrics used in this analysis are from the Phase I questionnaires. Women were asked about ever use of 50 specific pesticides: 18 herbicides, 22 insecticides, 4 fumigants, and 6 fungicides. (A list of these pesticides appears in Appendix A, Table A.1.)

Use of hormonally active pesticides: One of the specific questions to be addressed in this dissertation research was whether use of possible hormonally active pesticides was associated with self-report of fibroid diagnosis. Farr (148) conducted a review of the toxicological literature related to AHS pesticides and their endocrine disrupting potential in 2003. Based on these results and updated evidence from more recent publications (summarized in Appendix A), 17 of the pesticides had some evidence of endocrine disruption, ovotoxicity, or estrus cycle effects. For the purposes of this dissertation, I focused only on those pesticides that showed evidence of effects that may be relevant to uterine fibroid pathogenesis, such as estrogenic or progesteronic activity. Because the ovary is the primary source of these hormones in the uterus, chemicals that disrupt the estrus cycle or exert ovarian or uterine effects were also possible candidates. This narrowed the list to 10 pesticides, for which evidence is summarized in Table A.3: the organochlorines DDT, chlordane, lindane, dieldrin, and toxaphene; mancozeb; atrazine; alachlor; carbon tetrachloride; and permethrin/pyrethroids. A dichotomous variable (ever use of hormonally active pesticides) was set to 1 if the woman reported ever use of any of these pesticides and 0 if she did not use any pesticide at all.

Pesticide use patterns: An additional question was whether women who used any pesticides were more likely to report uterine fibroids than those who did not, and whether there was an increasing trend of fibroid diagnosis with type and number of pesticides used. In the Spouse Questionnaire, women were asked if they personally treated their home, lawn, or pets for pests. A categorical variable was created to capture wives' pesticide use patterns; residential use questions were not included in the Applicator Questionnaire. The referent category consists of women who never used or applied pesticides in their lifetime. "Residential only" refers to women who did not indicate use of any specific pesticide on the list of 50, but reported that they personally treat their own home for "flies, fleas, cockroaches, ants, or insects other than termites," their "lawn for pests (e.g., weeds, insects, or fungus)," or use home fumigants/flea bombs to control fleas. "Common agricultural pesticides" includes women who specified that they used one or more of the most frequently reported pesticides (149): glyphosate, carbaryl, malathion, 2,4-D, and diazinon. Women who specified use of pesticides other than the five most common were further differentiated by number used: "1-2 other agricultural" or "≥3 other agricultural." An "Other" category was added to capture women who reported mixing/applying pesticides but selected "something else" (i.e., other specify) when asked about specific pesticides used. Responses to additional questions about frequency and duration of pesticide application were varied, and one-third of these women also reported residential use. There were no consistent patterns in responses to these questions to indicate whether these women used pesticides in residential or agricultural applications.

In addition to the main exposures of interest, more general exposure variables were also examined. These included ever/never use variables for pesticide groupings by chemical

class (organochlorines, organophosphates, carbamates, and triazines) as well as for individual pesticides. Years lived or worked on a farm and whether the respondent grew up on a farm were also assessed in relation to fibroid diagnosis.

3.3.4 Covariates

Potential confounders were identified using a Directed Acyclic Graph and based on established and possible risk factors for uterine fibroids (Figure 3.4). Among these possible confounders, only age, age at menarche, and parity are established risk factors and were considered in this analysis. Data were collected via self-administered questionnaires or telephone interviews in Phases I and II.

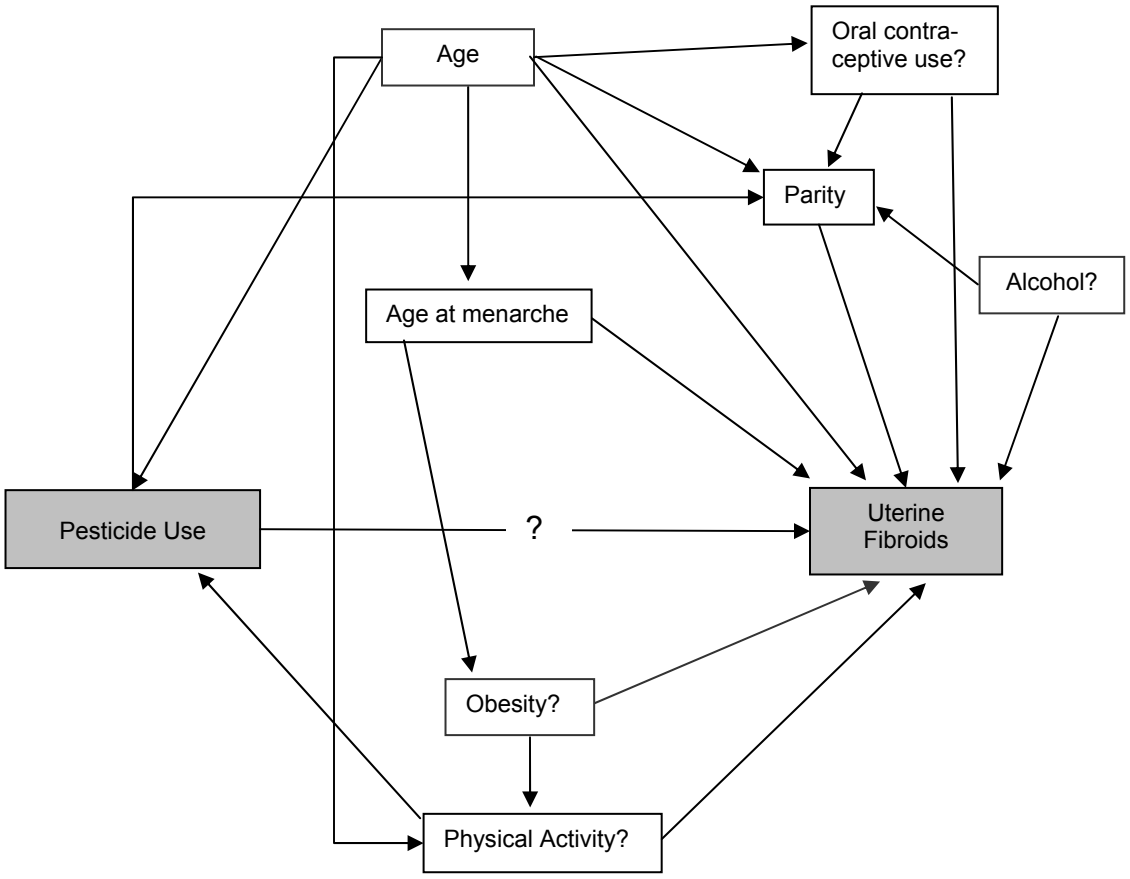
Age at interview in years was created using the date of birth reported by the respondent and the date of the Phase II interview. Birth date was first asked at enrollment and verified/corrected during the Phase II interview.

Timing of births was created using the pregnancy history reported in the Phase I Female and Family Health Questionnaire and updated in the Phase II Female Health Module. Maternal age (in years) at the birth of each child was calculated using the woman's date of birth and the date of birth of each child. A categorical variable (none, all births <24, one or more births ≥24 years of age) was created based on previous reports that births to women after their mid-twenties might partially account for the apparent protective effect of pregnancy (40).

Age at menarche was reported in the Phase I Female and Family Health Questionnaire. Respondents could select from five age categories: less than 12, 12, 13, 14, and 15 or older.

State of residence is an assigned variable based on the respondent's state (Iowa/North Carolina) at enrollment. Other analyses of Agricultural Health Study data have found a difference in personal and farm characteristics and pesticide use profiles among applicators and spouses in these two states (149, 150). Differences in the distributions of these characteristics may confound the association between pesticide exposures and fibroid risk.

Figure 3.4 Hypothetical causal diagram



In addition to these variables, data from the Female and Family Health Questionnaire and Phase II Female Health Module were used to identify women who reported having had a hysterectomy for purposes of assigning sensitivity and specificity estimates in the outcome correction model, as described below. Women were first asked "Have you gone through

menopause (the change of life) or had surgery that caused you to completely stop having menstrual periods?" Women who answered yes were then asked what type of menopause they had, and selected from a list of options. Those who indicated that they had a full or partial hysterectomy were flagged for the outcome correction method.

3.3.5 Data Analysis

Exploratory analyses were first conducted by examining the frequency distributions and descriptive statistics for each of the variables included in this analysis. Although these data have gone through extensive data editing and consistency checks, I inspected all variables for implausible or out-of-range values and where possible, used other data collected in the questionnaires to check for logic and consistency with the key variables of interest. Bivariate distributions of fibroid status with the exposures of interest and each of the covariates were also examined, as was the percentage of records with missing responses on the covariates. To get a clearer picture of the relationship between age and fibroid status, I categorized age as 21-29 (due to small numbers) and then by successive 2-year categories (e.g., 30-31, 32-33, …, 58-59), and plotted the log-odds of fibroids by age. The log-odds of fibroids tended to increase in a linear fashion for the most part, but seemed to level off (or even form a slightly inverse "U" shape) after about age 50.

"Uncorrected" regression model

Logistic regression was used to estimate the association between pesticide use and uterine fibroid prevalence. The first step was to use the uncorrected outcome, self-reported uterine fibroid diagnosis. Although effect measure modification was not a primary focus, a number of the possible endocrine disrupting pesticides were removed from the market in the late 1970s and '80s. Because the likelihood of use of discontinued pesticides is age-

dependent (e.g., younger women would not have used DDT), odds ratios and 95% confidence intervals were estimated for each age stratum (21-34, 35-39, 40-44, 45-49, 50-54, 55-59) and visually inspected for differences. I tested for statistical interaction by age of the associations between fibroids and pesticide use patterns, ever use of hormonally active pesticides, and chemical class pesticide groupings by including interaction terms for each exposure and age with a $P < 0.10$ significance level.

I evaluated the linearity assumption for categorical predictors by including disjoint indicator terms and inspecting graphs of the log-odds of fibroids plotted against the variable's categories (151). When a linear trend was seen, I modeled the variable as a single ordinal (e.g., 0, 1, 2) variable and computed a Wald *P* value for its coefficient. Based on the non-linear relationship between log odds of fibroids and age, I added a quadratic term for age in the models. The quadratic term for age was statistically significant, but resulted in very small changes in the exposure effect estimates. However, excluding the quadratic term resulted in a poorly-fit model as assessed by the Hosmer-Lemeshow goodness-of-fit test ($P < 0.0001$) (152), so it was retained.

A backward elimination approach was used to build the final multivariable logistic regression model. Age (continuous), age squared, and state of residence were forced into the models. Each of the other two covariates was dropped one at a time sequentially from the full model (starting with the covariate with the highest *P*-value in the full model and working down), and retained if it resulted in a 10% or greater change in the exposure odds ratio relative to the full model.

Outcome correction

The next step in the analysis was to run logistic regression models utilizing a method proposed by Magder and Hughes to correct for outcome misclassification (12). This method incorporates values of sensitivity and specificity into the estimation of logistic regression parameters and corresponding variances using the Expectation-Maximization (EM) algorithm to obtain maximum likelihood estimates (153). The procedure can be described as essentially performing a "…standard logistic regression considering each study subject as both diseased and not diseased with weights determined by the probability that the study subject is truly diseased given the data" (12). To paraphrase their illustrative example, suppose a woman reports that she has had a fibroid diagnosis. Given the sensitivity and specificity of the self-report and the values of that woman's covariates, the probability that she truly has fibroids is estimated as 90%. Then a standard logistic regression is performed with that woman entered twice: once as diseased with weight = 0.90 and again as non-diseased with weight = 0.10. These probabilities need to be recalculated after the logistic regression parameters are estimated because of the fact that the probabilities are partially based on the value of the parameters. This leads to new probabilities, which lead to new regression parameters. This process—estimating the probabilities and the regression parameters—is repeated until the parameter estimates converge.

The benefit of the Magder and Hughes method is that it accommodates varying sensitivity and specificity values for different subgroups of the analysis population. Based on results from the validity analysis in Aim 1, sensitivity for white women increased with age (except for the oldest age group) but specificity decreased slightly with age. The descriptive analysis of presence/absence of fibroids at ultrasound among women reporting a previous

diagnosis suggests, however, that these women may not have been wrong. Rather, tumor regression could have occurred with intervening factors such as time since diagnosis or pregnancies.

I used a SAS macro available from the authors at http://medschool.umaryland.edu/epidemiology/software.asp to perform the outcome correction. I used results from the Aim 1 analysis to inform the estimates for sensitivity and specificity of self-reported fibroids diagnosis. For the main correction model, specificity was set to 0.95 but sensitivity varied by age: 18-29, 0.15; 30-34, 0.20; 35-39, 0.35; 40-44, 0.40; 45-59, 0.30. Sensitivity was set to 0.85 for women who reported having had a hysterectomy (n = 3,022) based on the assumption that they would be better reporters of fibroid diagnosis. As above, all corrected odds ratios were adjusted for age, age squared, and state.

Additional analyses

Several secondary analyses were conducted. First, I examined associations between specific pesticides and uterine fibroid diagnosis and compared effect estimates obtained using different referent groups: 1) including never users of any pesticides as well as users of pesticides other than that of interest and 2) only users of pesticides other than that of interest (Appendix B).

Next, I evaluated the degree to which assumptions about self-report validity influence the corrected odds ratios and 95% confidence intervals (Appendix C). I used age-specific sensitivity (regardless of hysterectomy status) and specificity = 0.95 as the initial set of assumptions, and then varied sensitivity, specificity, and both. Assumptions about self-report validity among women with hysterectomy were evaluated by varying sensitivity and specificity for this subset of women only. Because the AHS population includes women up

to 59 years old, whereas the validity analysis population only includes women up to age 49, it was difficult to predict the shape of the sensitivity and specificity curves for women in older age ranges. The final sensitivity analysis was conducted to examine the influence of different assumptions regarding sensitivity in the 50-59 year age group.

CHAPTER 4: VALIDITY OF SELF-REPORTED UTERINE FIBROID STATUS

4.1 ABSTRACT

Studies using self-reported uterine fibroid status to classify cases and non-cases are subject to misclassification because many women with fibroids are undiagnosed. To assess the validity of this measure, the authors analyzed self-report of clinical diagnosis and ultrasound findings from 2,046 women, mostly <35 years of age, in Right From The Start (RFTS) and 869 women aged 35-49 in the Uterine Fibroid Study (UFS). Log-binomial regression was used to estimate sensitivity (Se) and specificity (Sp) and examine differences by ethnicity, age, education, body mass index (BMI), parity, and miscarriage history. Overall sensitivity was ≤0.50 in both studies. Sensitivity was higher in blacks than whites (RFTS: 0.34 vs. 0.23; UFS: 0.58 vs. 0.32) and increased with age. Parous white women had higher sensitivity than nulliparous whites. Specificity was 0.98 in RFTS and 0.86 in UFS. Ethnic differences were modest in the UFS (Sp Ratio, black vs. white = 0.90; 95% CI: 0.81, 0.99). Parity was inversely associated with specificity among UFS black women (Sp Ratio = 0.84; 95% CI: 0.73, 0.97). Misclassification of fibroid status can differ by factors of etiologic interest. These findings will be useful to assess bias in studies using self-reported clinical diagnosis as the outcome measure.

4.2 BACKGROUND

Uterine leiomyomata (fibroids) are benign neoplasms of uterine smooth muscle tissue that develop in the majority of reproductive-age women (1). For some, fibroids can cause

menstrual abnormalities, pelvic pain, and pregnancy complications (2) severe enough to require surgical treatment. However, many women with fibroids remain asymptomatic throughout their reproductive years. An estimated 20 to 50% of women with fibroids will experience related symptoms (11, 13), and these women will be more likely to be diagnosed.

The large proportion of women with subclinical fibroids leads to an important methodological challenge for epidemiologic studies. As with any condition with a long preclinical phase, any ascertainment method that does not attempt to identify asymptomatic women will misclassify a substantial percentage of true cases as non-cases. This misclassification can be extensive when outcome ascertainment is obtained by self-report. In one cross-sectional study with ultrasound screening, 51% of premenopausal women who reported no previous diagnosis had fibroids upon ultrasound examination (1).

Incidental detection also affects which women will be clinically diagnosed. Women who are not experiencing fibroid-related symptoms could be diagnosed during a routine pelvic exam, obstetric ultrasound, or if seeking care for other gynecologic conditions. The use of self-report could therefore result in spurious associations with factors not related to uterine fibroids, reflecting instead an underlying difference in the opportunity for diagnosis. In a large prospective cohort study that validated positive self-report among a subsample of women, 55% of cases reported being diagnosed because of fibroid-related symptoms; the remaining 45% were diagnosed incidentally, either during a routine pelvic examination (32%) or while receiving care for another condition (13%) (21).

The purpose of this analysis is to evaluate the validity of self-reported fibroid status and examine possible predictors of reporting quality. It is well-established that fibroid prevalence increases with age and that black women are at higher risk than white women at

all ages (4, 72). We therefore used data from two studies with a relatively high proportion of black participants and which, together, included women from 18-49 years old.

4.3 METHODS

4.3.1 Study Population

Data for this analysis come from two studies in which participants were systematically screened for uterine fibroids using ultrasound. Right From The Start (RFTS) is an ongoing community-based prospective study of early pregnancy conducted since 2000. Women very early in pregnancy or those planning to become pregnant were recruited from the community and clinical care sites via outreach materials and advertisements. Details of methods and study design are described elsewhere (28, 140). Eligibility criteria included: at least 18 years of age, English or Spanish speaker, and no use of assisted reproductive technology in the index pregnancy. Women who were planning a pregnancy were followed and enrolled if they became pregnant. Questionnaire data were gathered through computer-assisted telephone interview; information on basic demographics was obtained at enrollment, and questions about medical and reproductive history were asked in a first trimester interview. Weight and height were collected in the enrollment interview and at the time of early first trimester ultrasound.

In 2004, the RFTS enrollment interview was amended to include a question on previous fibroid diagnoses. Our analysis is therefore limited to women joining the study from this point onward. Although women were allowed to re-enroll in the study, we only included records from the first time they were asked about previous fibroid diagnoses. Study enrollment was required before 9 completed weeks of gestation. Between 2004 and 2008, a total of 2,411 women were enrolled, and 2,341 (97%) had both ultrasound and self-report

information. We included only women whose self-reported race/ethnicity was non-Hispanic white (n = 1,756) or non-Hispanic black (n = 290) in this analysis.

The Uterine Fibroid Study (UFS) was a cross-sectional study conducted to estimate uterine fibroid prevalence. Details have been described previously (1). To be eligible, women had to be 35-49 years old, members of the George Washington University health plan, and able to complete data collection in English. Enrollment occurred between 1996 and 1999. Approximately 88% of the original random sample was contacted by telephone and screened for eligibility. A total of 1,430 out of 1,786 eligible women (80%) participated. Information on demographic characteristics and reproductive and medical history were collected from telephone interviews and self-administered questionnaires. Height and weight were measured at the clinic visit. We excluded women whose reported ethnicity was other than non-Hispanic black or white (n = 107), postmenopausal women (n = 180), and those missing ultrasound (n = 152) or whose ultrasound results were not definitive (n = 122) to obtain our final analysis population of 363 white and 506 black women.

4.3.2 Self-Report of Uterine Leiomyomata

Both RFTS and the UFS collected information on fibroid diagnosis by telephone interview prior to conducting the study ultrasound. Women were asked if a doctor or medical care provider had ever told them that they had uterine fibroids, and responses were used to classify women's self-reported fibroid status (yes/no). The UFS interview also included a series of follow-up questions (e.g., diagnostic and follow-up examinations, treatment). Fewer than 10 women who responded "yes" to the initial question subsequently indicated that the diagnosis had been incorrect; these women were classified as having no previous fibroid diagnosis.

4.3.3 Ultrasound Detection of Uterine Leiomyomata

RFTS participants underwent an endovaginal ultrasound as early as 6 and no later than 13 weeks of gestation. Examinations were performed by sonographers certified by the American Registry of Diagnostic Medical Sonographers (ARDMS). They received additional study training for consistency in identifying, measuring, and recording uterine fibroids. Sonographers were instructed not to discuss prior knowledge of fibroid status with study participants. Measurements included fibroid number, type, and size. As described elsewhere (28), each tumor was examined three times during the ultrasound to reduce the chance of misidentifying focal contractions as fibroids, and the mean of these diameter measurements was calculated for each fibroid.

Premenopausal participants in the UFS underwent transvaginal (and, when necessary, transabdominal) ultrasound examinations within three months of study entry. Details of the ultrasound protocol are described elsewhere (1). ARDMS-certified sonographers performed the ultrasound, and findings were reviewed by a radiologist trained in ultrasonography. Recorded measurements included the number, location, and size of each tumor. Women who had had a recent pelvic ultrasound (n = 170) were not examined again, but were classified as positive or negative for fibroids based on the radiology records.

In both studies, fibroid identification was based on Muram criteria (142), modified to include masses of ≥0.5 cm in diameter. Women were classified as having uterine fibroids if the results of the ultrasound examination indicated presence of one or more fibroids. Fibroid size was categorized as <2.00, 2.00-3.99, and ≥4.00 cm based on the largest measured diameter for the tumor(s) detected. A total of 23 (3%) UFS and 16 (1%) RFTS participants reported having previous surgery to remove fibroids. For purposes of comparing self-reports

with ultrasound results, these women were classified as having fibroids, even if the study ultrasound did not show evidence of tumors. We assigned fibroid size as ≥4.00 cm for women who had fibroid surgery.

4.3.4 Statistical Analysis

The validity of self-reported uterine fibroid status as compared to the "gold standard" ultrasound examination was measured by sensitivity and specificity. Sensitivity was defined as the proportion of women who self-reported a previous fibroid diagnosis among those with ultrasound-confirmed fibroids or prior fibroid surgery. Specificity was defined as the proportion of women who self-reported no previous diagnosis among those with no evidence of fibroids at ultrasound and no previous fibroid surgery. Data for RFTS and UFS were analyzed separately due to differences in the study populations.

Log-binomial regression was used to estimate sensitivity and specificity with 95% confidence intervals (CI). Prevalence ratios obtained from the regression models are interpreted in this analysis as the sensitivity (or specificity) of self-report in one subgroup compared to that in a reference group. Sensitivity ratios and specificity ratios were used to examine differences in self-report validity according to age at interview, education, body mass index (BMI), parity, miscarriage history, and (for sensitivity only) size of the largest fibroid detected at ultrasound.

We first conducted univariate analyses to assess the association of each individual predictor with sensitivity and specificity, respectively. We tested for statistical interaction by ethnicity and age using the Mantel-Haenszel χ^2 test for homogeneity with a $P < 0.10$ significance level. Based on this *a priori* criterion, ethnicity was an effect measure modifier for the association between sensitivity and parity in RFTS ($P = 0.06$), and sensitivity and

fibroid size in UFS (P <0.05). We present both overall and ethnicity-stratified estimates below. To build the final models, we included covariates that were associated (P <0.10) with sensitivity (or specificity) in the univariate analyses. We adjusted sensitivity ratios for parity (any previous birth vs. none) and age as a continuous variable (with a quadratic term for age in the UFS analysis to accommodate non-linearity). We adjusted specificity ratios for parity in analysis of UFS data, but did not adjust for any covariates in the RFTS specificity analysis due to the small number of women who did not have ultrasound-detected fibroids but reported a previous diagnosis. Linearity of trends for categorical predictors was examined using common referent coding and by treating categorical variables as ordinal parameters in the models. Poisson regression with robust error variance was used to estimate prevalence ratios and 95% confidence intervals when log-binomial models did not converge (146). All P values are two-sided.

All analyses were carried out with the statistical software package SAS 9.1 (SAS Institute, Inc., Cary, North Carolina).

4.4 RESULTS

4.4.1 Characteristics of Analysis Population

The majority (86%) of RFTS participants was white, compared to the slightly higher percentage of black women in the UFS (Table 4.1). Overall, RFTS participants were about 10 years younger than UFS participants. On average, black women were 2.8 years younger than white women in RFTS, but there was no difference in average age between black and white women in the UFS. Black and white women differed with respect to education, BMI, and parity in both study populations. Among women with a previous pregnancy, there was no difference in miscarriage history between white and black women. The prevalence of

both self-reported and ultrasound-detected fibroids was higher among black women compared to white women in both study populations but lower in both groups in RFTS compared to UFS participants. Furthermore, a higher percentage of black than white women had fibroids ≥ 4 cm in diameter. Among those who had a previous diagnosis in the UFS, the reported age at first diagnosis was 3 years younger in black women compared to white women.

4.4.2 Validity of Self-Reported Uterine Leiomyomata

Sensitivity (Se) was low among participants of both studies (Table 4.2). Half of the UFS participants who had fibroids at study ultrasound reported a previous diagnosis (Se: 0.50; 95% CI: 0.45, 0.54). Sensitivity was even lower (Se: 0.27; 95% CI: 0.22, 0.32) in RFTS. As shown in Figures 4.1 and 4.2, black women had higher sensitivity of self-report than did white women; this was more pronounced in the UFS participants (Sensitivity Ratio [SeR] for black vs. white: 1.73; 95% CI: 1.33, 2.25). Sensitivity was associated with age at interview in both studies (Figures 4.1 and 4.2). In RFTS, overall sensitivity increased from 0.12 (95% CI: 0.07, 0.21) in 18-29 year-old women to 0.41 (95% CI: 0.31, 0.53) in 35-45 year-olds (*P* for trend < 0.005). Among UFS participants, there was increased sensitivity for black women in their 40s compared to 35-39 year-olds, but no significant differences by age for white women.

Figure 4.2 provides sensitivity ratios for additional demographic and reproductive factors. Parity was associated with higher sensitivity of self-report among white women, with the strongest association seen in white RFTS participants (SeR for parous vs. nulliparous: 2.90; 95% CI: 1.51, 5.60). Women with higher education tended to have higher sensitivity of self report, although this association was statistically significant only when

comparing UFS black women with the highest education to those with the lowest (SeR of post-baccalaureate vs. high school/some college: 1.27; 95% CI: 1.05, 1.55). Sensitivity in RFTS blacks with some graduate-level education was also elevated compared to those with less than four years of college (SeR: 1.87; 95% CI: 0.78, 4.51). Neither BMI nor miscarriage history was a predictor of self-report sensitivity in either study population.

In both studies, women with larger fibroids at the ultrasound examination had significantly higher sensitivity of self-report (Table 4.3). After adjusting for age and parity, sensitivity was three to four times as high in women with tumors ≥ 4 cm compared to those whose largest tumor was < 2 cm in diameter. This was seen among both black and white women in RFTS and white UFS participants. Among UFS black women, however, the association with fibroid size was not as strong (SeR for ≥ 4 vs. < 2 cm: 1.88; 95% CI: 1.42, 2.49).

Specificity (Sp)—the proportion of women reporting "no fibroid diagnosis" among those with no ultrasound-detected fibroids—was high in both study populations. In RFTS, overall specificity was 0.98 (95% CI: 0.97, 0.99) compared to 0.86 (95% CI: 0.82, 0.90) in the UFS (Table 4.4). Unlike the sensitivity results, there were few differences in specificity of self-report among the factors considered (Figure 4.3). In the RFTS population, specificity was almost equal between blacks and whites (0.98 and 0.97, respectively). However, specificity for black women in the UFS was lower compared to whites (Specificity Ratio [SpR]: 0.90; 95% CI: 0.81, 0.99). As shown in Figure 4.1, age at interview was inversely associated with specificity in RFTS (*P* for trend < 0.01) but not the UFS (*P* for trend = 0.15). In both study populations, parous women had lower specificity compared to nulliparae, and

this was seen among both blacks and whites (Figure 4.3). Specificity was unrelated to education, BMI, or miscarriage history.

Agreement between self-report and ultrasound fibroid status can be calculated from data included in Tables 4.2 and 4.4. Overall agreement was 88% in RFTS and 65% in the UFS (data not shown). The combination of differences in sensitivity, specificity, and prevalence of ultrasound-detected fibroids by race and age yielded the highest agreement (93%) in the youngest (ages 18-29) white women, and the lowest agreement (47%) in the oldest white women (ages 45-49). Among black women, agreement between self-report and ultrasound fibroid status was also highest in the youngest age group, and varied from 83 to 60%.

We examined factors since diagnosis—time interval, age at ultrasound, intervening pregnancy—among UFS women to investigate what might account for their lower specificity (that is, proportionally more women reporting a fibroid diagnosis when the study ultrasound was negative). Only the UFS black women were examined (n = 221) because there were few UFS white women reporting a previous diagnosis who did not have ultrasound-detected fibroids. A short time interval between prior diagnosis and study ultrasound was associated with increased concordance between self-report and ultrasound. Concordance was highest (90.4%) among women who reported a diagnosis within two years of the study ultrasound. Age at ultrasound was also important, even after controlling for years since diagnosis. Concordance was 23% higher for women 40 or older relative to that for women 35-39 years old at the time of the ultrasound examination.

4.5 DISCUSSION

To our knowledge, this is the most detailed assessment of the validity of self-reported information on fibroid status. We evaluated the sensitivity and specificity of self-report in two different study populations: Right From The Start, consisting of pregnant women, most of whom were under 35, and the Uterine Fibroid Study, which included 35-49 year-old women randomly selected from members of an urban health plan. Using study ultrasound screening results as the indicator of "true" fibroid status, sensitivity of self-report was low among both study populations: 0.27 in RFTS and 0.50 in UFS. In contrast, specificity of self-report was high (0.98) in RFTS to moderate (0.86) in UFS.

4.5.1 Factors Associated with Validity of Self-Report

Race/ethnicity. Self-report among black women was more sensitive but less specific than respective values in whites. The higher sensitivity of self-report in black women compared to white women may be related to their younger age at onset resulting in larger and more numerous tumors compared to similarly-aged whites (20, 28, 30). Better reporting among black women with fibroids could occur if these differences lead to more severe symptoms or easier detection during routine pelvic examination. In addition, increased awareness of black women as a "high-risk" group could lead to increased surveillance and a higher likelihood of detection.

Age. Age at interview was positively associated with sensitivity of self-report up to age 44, but appeared to drop in the 45-49 year age group, especially in white women. The reason for this non-monotonic relationship is unclear, but may be related to fibroid size, which our results showed to be strongly predictive of self-report sensitivity. Among UFS white women with fibroids, the proportion with tumors ≥ 4 cm in diameter was lower in the

45-49 year-olds compared to the 35-44 year-olds (0.22 vs. 0.28, respectively). In comparison, the proportion of black women with large tumors increased with age. This may reflect a real difference in the natural progression of these tumors, such that fibroids that first develop in white women in their 40's remain small enough to go undetected. In a study which tracked fibroid growth in tumors from 72 premenopausal women, older white women had a lower fibroid growth rate than their younger counterparts, while this age difference was not seen in black women (31).

In contrast with sensitivity, age was associated with a decrease in specificity in RFTS. Although not statistically significant, this inverse association was also seen in the UFS. This could reflect respondent reporting error, or resolution of previously existing tumors. In the few studies that have measured changes in fibroid size over time, tumor regression occurred in a small percentage of cases (22, 31, 154). Our finding that the proportion of apparent false-positives increased with duration between first diagnosis and ultrasound suggests that specificity may be affected by time since diagnosis as well as age at interview. Among UFS black women who reported having had a previous diagnosis within two years before the study, 90% had fibroids detected at the ultrasound examination. This was consistent with findings from two prospective cohorts with biennial follow-up, in which over 90% of positive self-reports were confirmed in a validation subsample (20, 21).

Parity. Parity was associated with higher sensitivity and lower specificity of self-report. Women who had been pregnant may have had an increased opportunity for diagnosis because of pregnancy-related ultrasound examinations. However, we did not find a further association with miscarriage history, which could have increased gynecologic surveillance and thus increased self-report. The decrease in specificity with parity might be due to a

protective effect of pregnancy in eliminating or reducing the size of fibroids. Postpartum uterine remodeling was originally put forth by Baird and Dunson (40) as a possible mechanism for the reduced risk of fibroids seen among parous women. More recently, a prospective study of pregnant women which compared early first trimester to postpartum ultrasound results found that 36% of tumors had resolved and 79% of the remainder had decreased in size (41).

4.5.2 Strengths and Limitations

The relatively large sample size in both RFTS and the UFS was an asset for this analysis. These data enabled us to examine black women (who are at higher risk for fibroids) as well as white women over a large age range. However, we were limited in our ability to assess certain factors in finer detail (e.g. education level) or to conduct some subgroup analyses due to smaller numbers. Generalizability of our findings to other groups must also be considered. Women in RFTS volunteered for the study, and most had planned pregnancies. Compared to the general population, they were more highly educated, less likely to smoke, and more likely to be married (28). They had achieved the index pregnancy without fertility treatment, so women with fertility problems (possibly due to uterine fibroids) are under-represented. UFS participants were members of a health plan and therefore had access to health care services. Self-report from women with limited access to or use of health care might show lower sensitivity than our results. The fact that we observed similar results in these two different study populations lends support to our overall findings.

An additional strength of this analysis is use of transvaginal and transabdominal ultrasound to define "true" fibroid status. Ultrasound has been shown to have high sensitivity (99%) and specificity (91%) when compared to histological results, which are

considered the gold standard (155). A potential limitation in measuring true fibroid status is that the RFTS population consisted of pregnant women whose fibroids may have grown during early pregnancy (156). Although ultrasound was performed early in the first trimester, it is possible that fibroids which were undetectable prior to pregnancy grew to a detectable size at the time of screening. Identification of any such newly-detectable cases would result in lower sensitivity for this population. Previous analyses of RFTS data showed no difference in fibroid prevalence by gestational age ultrasound (28), so detection is unlikely to be influenced by the timing of the ultrasound in the narrow time period in which examinations were conducted. Pregnancy may also have affected the validity of the ultrasound results. However, all ultrasounds were performed early in pregnancy and study sonographers were specially trained to measure each tumor three separate times to ensure that focal contractions were not mistaken for fibroid tumors.

4.5.3 Impact of Findings

Our results suggest that using self-report would result in misclassification of a large proportion of true cases, and this misclassification might differ by ethnicity, age, and parity. Previous investigations (37, 157) using self-reported fibroid diagnosis have limited analyses to women under 35 in an attempt to reduce misclassification. Our findings demonstrate that the high specificity and lower prevalence of fibroids among younger women would result in relatively fewer true cases being misclassified. However, differences in reporting quality with respect to other factors may still lead to biased estimates, and could explain some of the inconsistencies in previous findings. For example, higher sensitivity among parous women results in a higher likelihood of reporting a fibroid diagnosis. In the simplest case (i.e., assuming no confounding, other measurement errors, or selection bias), this would lead to

parity being an apparent risk factor for uterine fibroids. On the other hand, if women who report a fibroid diagnosis prior to the baseline of a prospective analysis are excluded, then cases would be differentially excluded among parous compared to nulliparous women, and parity would seem to be protective.

Sensitivity analyses performed in previous publications (44, 158) have used methods which apply the same validity estimates to the entire analysis population, not accounting for the differences in self-report validity by ethnicity, age, and parity that we detected. Our results provide more detailed estimates that could be used for a more accurate assessment of misclassification bias in existing studies. The availability of methods (12, 159, 160) that allow for varying sensitivity and specificity according to designated covariate patterns also provide an opportunity to calculate point and interval estimates that account for the differential validity of self-reported fibroid diagnosis.

In this analysis, between 35 to 90% of women with ultrasound-detected fibroids reported that they had never been diagnosed with fibroids. This finding highlights one of the critical needs in uterine fibroid epidemiology: the ability to correctly define cases and non-cases and to clarify the relationships between risk factors that may be important in the onset of fibroids and those that play a role in their growth and detection. Accurate outcome measurement, through ultrasound screening, is therefore critical to better understanding the etiology and natural history of uterine fibroids.

Table 4.1 Characteristics of Right From The Start (*n* = 2,046) and Uterine Fibroid Study (*n* = 869) analysis populations, by race/ethnicity

	Right From The Start				Uterine Fibroid Study			
	White (*n* = 1,756)		Black (*n* = 290)		White (*n* = 363)		Black (*n* = 506)	
	No.	%	No.	%	No.	%	No.	%
Age at interview								
<20	18	1.0	22	7.6	0		0	
20–24	134	7.6	80	27.6	0		0	
25–29	658	37.5	76	26.2	0		0	
30–34	629	35.8	85	29.3	0		0	
35–39	279	15.9	22	7.6	124	34.2	191	37.7
40–44	38	2.2	5	1.7	122	33.6	178	35.2
45–49	0		0		117	32.2	137	27.1
Mean (SD)	30.2 (4.5)		27.4 (5.6)		41.9 (4.3)		41.4 (4.2)	
Highest education[a]								
High school	124	7.1	74	25.5	12	3.4	104	20.9
Some college	229	13.0	95	32.8	33	9.3	228	45.8
4 years of college	665	37.9	71	24.5	54	15.3	66	13.3
Post-baccalaureate	737	42.0	50	17.2	254	72.0	100	20.1
Missing	1		0		10		8	
BMI (kg/m^2) at enrollment[b]								
<20.00	186	10.6	18	6.2	27	7.4	16	3.2
20.00–24.99	945	53.8	75	25.9	183	50.4	117	23.2
25.00–29.99	374	21.3	78	26.9	91	25.1	155	30.7
≥30.00	251	14.3	119	41.0	62	17.1	217	43.0
Missing	0		0		0		1	
Parity								
0	803	48.3	118	45.2	219	60.3	115	22.7
1	608	36.6	93	35.6	52	14.3	120	23.7
2	198	11.9	35	13.4	75	20.7	159	31.4
3 or more	54	3.2	15	5.7	17	4.7	112	22.1
Missing	93		29		0		0	
Number of miscarriages[c]								
0	669	65.4	120	62.2	147	69.7	317	69.7
1	277	27.1	57	29.5	47	22.3	112	24.6
2 or more	77	7.5	16	8.3	17	8.1	26	5.7
Missing	64		20		0		0	
Previous fibroid diagnosis (self-report)								
No	1680	95.7	255	87.9	294	81.0	271	53.6
Yes	76	4.3	35	12.1	69	19.0	235	46.4
Mean (SD) age	29.3 (5.2)		28.4 (4.0)		36.2 (5.6)		33.0 (7.2)	
Missing age at diagnosis	15		7		4		14	

table continues

Table 4.1 Characteristics of Right From The Start (*n* = 2,046) and Uterine Fibroid Study (*n* = 869) analysis populations, by race/ethnicity (cont.)

	Right From The Start				Uterine Fibroid Study			
	White (*n* = 1,756)		Black (*n* = 290)		White (*n* = 363)		Black (*n* = 506)	
	No.	%	No.	%	No.	%	No.	%
Ultrasound-detected fibroids								
No	1560	88.8	207	71.4	201	55.4	161	31.8
Yes, size of largest tumor:	196	11.2	83	28.6	162	44.6	345	68.2
<2.00 cm	96	49.0	38	45.8	62	38.3	78	22.6
2.00–3.99 cm	62	31.6	19	22.9	60	37.0	140	40.6
≥4.00 cm	38	19.4	26	31.3	40	24.7	127	36.8
Mean (SD) age of first diagnosis[d]	31.1 (5.1)		29.1 (4.7)		40.9 (5.9)		36.3 (7.5)	
Missing	15		7		4		14	

Abbreviations: SD, standard deviation.

[a] RFTS asked for years of schooling completed and was categorized as follows: ≤12, "High school;" 13-15, "Some college;" 16, "4 years of college;" and >16, "Post-baccalaureate." The "high school" category includes some women with less than a high school education: 11 black UFS women, and 19 white and 21 black RFTS women. The "postbaccalaureate" category includes 197 white and 11 black women in the UFS who reported having a graduate/professional degree.

[b] For RFTS, BMI was based on self-reported pre-pregnancy height and weight or first trimester clinic measures when missing.

[c] Among 1,087 white and 213 black RFTS women with a previous pregnancy (prior to study enrollment); 211 white and 455 black UFS women with a previous pregnancy.

[d] Among all women with fibroids previously diagnosed or newly-detected at ultrasound.

Table 4.2 Unadjusted sensitivity of self-reported uterine fibroid status among 279 Right From The Start and 507 Uterine Fibroid Study participants with fibroids detected at study ultrasound

	All Women				White Women				Black Women			
	No. Correct	Total[a]	Se	95% CI	No. Correct	Total[a]	Se	95% CI	No. Correct	Total[a]	Se	95% CI
Right From The Start												
Overall	74	279	0.27	0.22, 0.32	46	196	0.23	0.18, 0.30	28	83	0.34	0.25, 0.46
Age at interview												
18–29	11	89	0.12	0.07, 0.21	8	57	0.14	0.07, 0.27	3	32	0.09	0.03, 0.28
30–34	30	109	0.28	0.20, 0.37	15	74	0.20	0.13, 0.32	15	35	0.43	0.29, 0.63
35–45	33	81	0.41	0.31, 0.53	23	65	0.35	0.25, 0.49	10	16	0.63	0.43, 0.91
Highest education												
High school/Some college	13	65	0.20	0.12, 0.33	7	34	0.21	0.11, 0.40	6	31	0.19	0.09, 0.40
4 years of college	29	101	0.29	0.21, 0.39	19	76	0.25	0.17, 0.37	10	25	0.40	0.25, 0.65
Postbaccalaureate	32	113	0.28	0.21, 0.38	20	86	0.23	0.16, 0.34	12	27	0.44	0.29, 0.69
BMI (kg/m^2) at enrollment												
≤24.99	33	133	0.25	0.18, 0.33	24	114	0.21	0.15, 0.30	9	19	0.47	0.29, 0.76
25.00–29.99	16	70	0.23	0.15, 0.35	10	48	0.21	0.12, 0.36	6	22	0.27	0.14, 0.54
≥30.00	25	76	0.33	0.24, 0.45	12	34	0.35	0.22, 0.56	13	42	0.31	0.20, 0.49
Parity												
Nulliparous	20	131	0.15	0.10, 0.23	10	94	0.11	0.06, 0.19	10	37	0.27	0.16, 0.46
Parous	51	139	0.37	0.29, 0.46	35	97	0.36	0.28, 0.47	16	42	0.38	0.26, 0.56
Ever miscarry[b]												
No	35	113	0.31	0.24, 0.41	22	75	0.29	0.21, 0.42	13	38	0.34	0.22, 0.53
Yes	25	69	0.36	0.26, 0.50	19	52	0.37	0.26, 0.52	6	17	0.35	0.19, 0.67

table continues

Table 4.2 Unadjusted sensitivity of self-reported uterine fibroid status among 279 Right From The Start and 507 Uterine Fibroid Study participants with fibroids detected at study ultrasound (cont.)

	All Women				White Women				Black Women			
	No. Correct	Total[a]	Se	95% CI	No. Correct	Total[a]	Se	95% CI	No. Correct	Total[a]	Se	95% CI
					Uterine Fibroid Study							
Overall	253	507	0.50	0.45, 0.54	52	162	0.32	0.25, 0.40	201	345	0.58	0.53, 0.64
Age at interview												
35–39	66	149	0.44	0.37, 0.53	12	36	0.33	0.21, 0.53	54	113	0.48	0.39, 0.58
40–44	99	171	0.58	0.51, 0.66	19	48	0.40	0.28, 0.56	80	123	0.65	0.57, 0.74
45–49	88	187	0.47	0.40, 0.55	21	78	0.27	0.19, 0.39	67	109	0.61	0.53, 0.71
Highest education												
High school/Some college	136	256	0.53	0.47, 0.60	7	20	0.35	0.19, 0.64	129	236	0.55	0.49, 0.61
4 years of college	32	64	0.50	0.39, 0.64	7	22	0.32	0.17, 0.59	25	42	0.60	0.46, 0.76
Postbaccalaureate	78	175	0.45	0.38, 0.53	34	113	0.30	0.23, 0.40	44	62	0.71	0.61, 0.83
BMI (kg/m^2) at enrollment												
≤24.99	71	169	0.42	0.35, 0.50	25	89	0.28	0.20, 0.39	46	80	0.58	0.48, 0.69
25.00–29.99	83	150	0.55	0.48, 0.64	19	42	0.45	0.32, 0.63	64	108	0.59	0.51, 0.69
≥30.00	99	187	0.53	0.46, 0.61	8	31	0.26	0.14, 0.47	91	156	0.58	0.51, 0.67
Parity												
Nulliparous	73	187	0.39	0.33, 0.47	33	111	0.30	0.22, 0.40	40	76	0.53	0.43, 0.65
Parous	18	320	0.56	0.51, 0.62	19	51	0.37	0.26, 0.53	161	269	0.60	0.54, 0.66
Ever miscarry[b]												
No	154	284	0.54	0.49, 0.60	27	64	0.42	0.32, 0.56	127	220	0.58	0.52, 0.65
Yes	61	121	0.50	0.42, 0.60	6	27	0.22	0.11, 0.45	55	94	0.59	0.49, 0.69

Abbreviations: Se, sensitivity; CI: confidence interval.

[a] Subcategory numbers may not sum to total due to missing data.

[b] Among women with a previous pregnancy.

Table 4.3 Relationship between size of largest fibroid detected at study ultrasound and sensitivity of self-report in 279 Right From The Start and 507 Uterine Fibroid Study participants with fibroids detected at study ultrasound

Mean diameter of largest fibroid	White Women						Black Women					
	No. Correct	Total	Se	95% CI	SeR[a]	95% CI	No. Correct	Total	Se	95% CI	SeR[a]	95% CI
RFTS												
<2.00 cm	14	96	0.15	0.09, 0.24	1.00	Referent	5	38	0.13	0.06, 0.30	1.00	Referent
2.00–3.99 cm	12	62	0.19	0.12, 0.32	1.42	0.73, 2.79	8	19	0.42	0.25, 0.71	4.09	1.47, 11.35
≥4.00 cm	20	38	0.53	0.39, 0.71	3.08	1.76, 5.36	15	26	0.58	0.42, 0.80	4.15	1.56, 11.02
UFS												
<2.00 cm	8	62	0.13	0.07, 0.25	1.00	Referent	32	78	0.41	0.31, 0.54	1.00	Referent
2.00–3.99 cm	22	60	0.37	0.26, 0.51	2.93	1.42, 6.02	69	140	0.49	0.42, 0.58	1.19	0.87, 1.62
≥4.00 cm	22	40	0.55	0.42, 0.73	4.26	2.11, 8.60	100	127	0.79	0.72, 0.86	1.88	1.42, 2.49

Abbreviations: RFTS, Right From The Start; UFS, Uterine Fibroid Study; Se, sensitivity of self-report; SeR, sensitivity ratio; CI, confidence interval.

[a] Adjusted for age and parity.

Table 4.4 Unadjusted specificity of self-reported uterine fibroid status among 1,767 Right From The Start and 362 Uterine Fibroid Study participants with no fibroids detected at study ultrasound

	All Women				White Women				Black Women			
	No. Correct	Total[a]	Sp	95% CI	No. Correct	Total[a]	Sp	95% CI	No. Correct	Total[a]	Sp	95% CI
					Right From The Start							
Overall	1,730	1,767	0.98	0.97, 0.99	1,530	1,560	0.98	0.97, 0.99	200	207	0.97	0.94,0.99
Age at interview												
18–29	889	899	0.99	0.98, 1.00	745	753	0.99	0.98, 1.00	144	146	0.99	0.97, 1.01
30–34	590	605	0.98	0.96, 0.99	543	555	0.98	0.97, 0.99	47	50	0.94	0.88, 1.01
35–45	251	263	0.95	0.93, 0.98	242	252	0.96	0.94, 0.98	9	11	0.82	0.62, 1.08
Highest education												
High school/Some college	445	457	0.97	0.96, 0.99	312	319	0.98	0.96, 0.99	133	138	0.96	0.93, 0.99
4 years of college	620	635	0.98	0.96, 0.99	575	589	0.98	0.96, 0.99	45	46	0.98	0.94, 1.02
Postbaccalaureate	664	674	0.99	0.98, 0.99	642	651	0.99	0.98, 0.99	22	23	0.96	0.88, 1.04
BMI (kg/m^2) at enrollment												
≤24.99	1,071	1,091	0.98	0.97, 0.99	998	1,017	0.98	0.97, 0.99	73	74	0.99	0.96, 1.01
25.00–29.99	374	382	0.98	0.96, 0.99	319	326	0.98	0.96, 0.99	55	56	0.98	0.95, 1.02
≥30.00	285	294	0.97	0.95, 0.99	213	217	0.98	0.96, 1.00	72	77	0.94	0.88, 0.99
Parity												
Nulliparous	786	790	0.99	0.99, 1.00	706	709	1.00	0.99, 1.00	80	81	0.99	0.96, 1.01
Parous	833	864	0.96	0.95, 0.98	738	763	0.98	0.95, 0.98	95	101	0.94	0.90, 0.99
Ever miscarry[b]												
No	654	676	0.97	0.95, 0.98	577	594	0.97	0.96, 0.98	77	82	0.94	0.89, 0.99
Yes	346	358	0.97	0.95, 0.99	291	302	0.96	0.94, 0.98	55	56	0.98	0.95, 1.02

table continues

Table 4.4 Unadjusted specificity of self-reported uterine fibroid status among 1,767 Right From The Start and 362 Uterine Fibroid Study participants with no fibroids detected at study ultrasound (cont.)

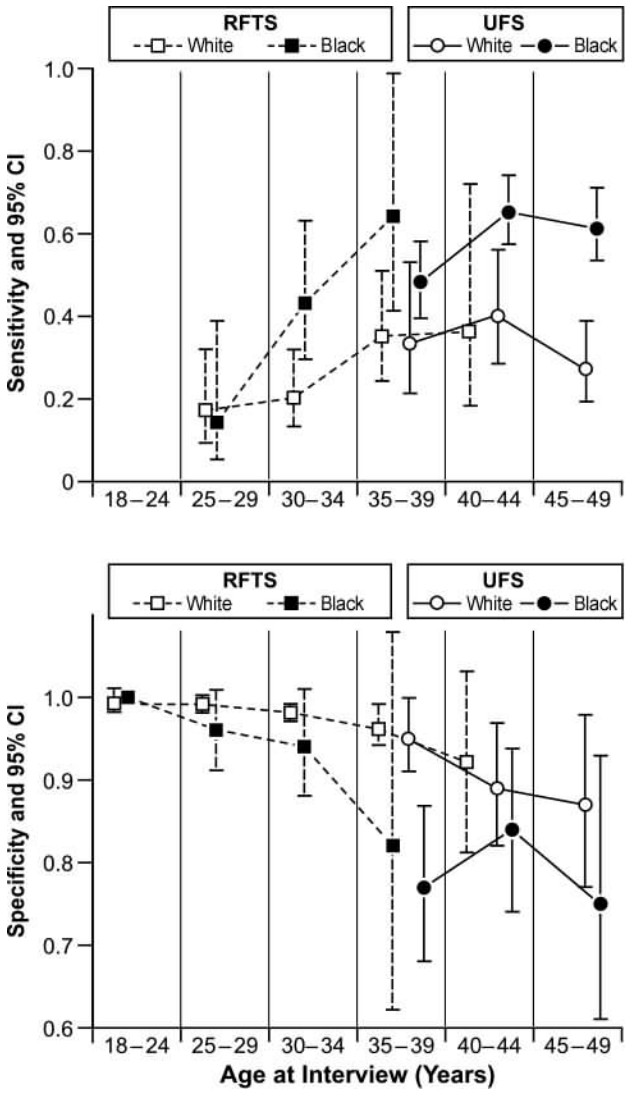
	All Women				White Women				Black Women			
	No. Correct	Total[a]	Sp	95% CI	No. Correct	Total[a]	Sp	95% CI	No. Correct	Total[a]	Sp	95% CI
					Uterine Fibroid Study							
Overall	311	362	0.86	0.82, 0.90	184	201	0.92	0.87, 0.95	127	161	0.79	0.72, 0.85
Age at interview												
35–39	144	166	0.87	0.82, 0.92	84	88	0.95	0.91, 1.00	60	78	0.77	0.68, 0.87
40–44	112	129	0.87	0.81, 0.93	66	74	0.89	0.82, 0.97	46	55	0.84	0.74, 0.94
45–49	55	67	0.82	0.73, 0.92	34	39	0.87	0.77, 0.98	21	28	0.75	0.61, 0.93
Highest education												
High school/Some college	97	121	0.80	0.73, 0.88	23	25	0.92	0.82, 1.03	74	96	0.77	0.69, 0.86
4 years of college	47	56	0.84	0.75, 0.94	26	32	0.81	0.69, 0.96	21	24	0.88	0.75, 1.02
Postbaccalaureate	162	179	0.91	0.86, 0.95	132	141	0.94	0.90, 0.98	30	38	0.79	0.67, 0.93
BMI (kg/m^2) at enrollment												
≤24.99	151	174	0.87	0.82, 0.92	110	121	0.91	0.86, 0.96	41	53	0.77	0.67, 0.89
25.00–29.99	81	96	0.84	0.77, 0.92	45	49	0.92	0.84, 1.00	36	47	0.77	0.65, 0.90
≥30.00	79	92	0.86	0.79, 0.93	29	31	0.94	0.85, 1.03	50	61	0.82	0.73, 0.92
Parity												
Nulliparous	137	147	0.93	0.89, 0.97	102	108	0.94	0.90, 0.99	35	39	0.90	0.81, 1.00
Parous	174	215	0.81	0.76, 0.86	82	93	0.88	0.82, 0.95	92	122	0.75	0.68, 0.83
Ever miscarry[b]												
No	149	180	0.83	0.77, 0.88	75	83	0.90	0.84, 0.97	74	97	0.76	0.68, 0.85
Yes	69	81	0.85	0.78, 0.93	33	37	0.89	0.80, 1.00	36	44	0.82	0.71, 0.94

Abbreviations: Sp, specificity; CI: confidence interval.

[a] Subcategory numbers may not sum to total due to missing data.

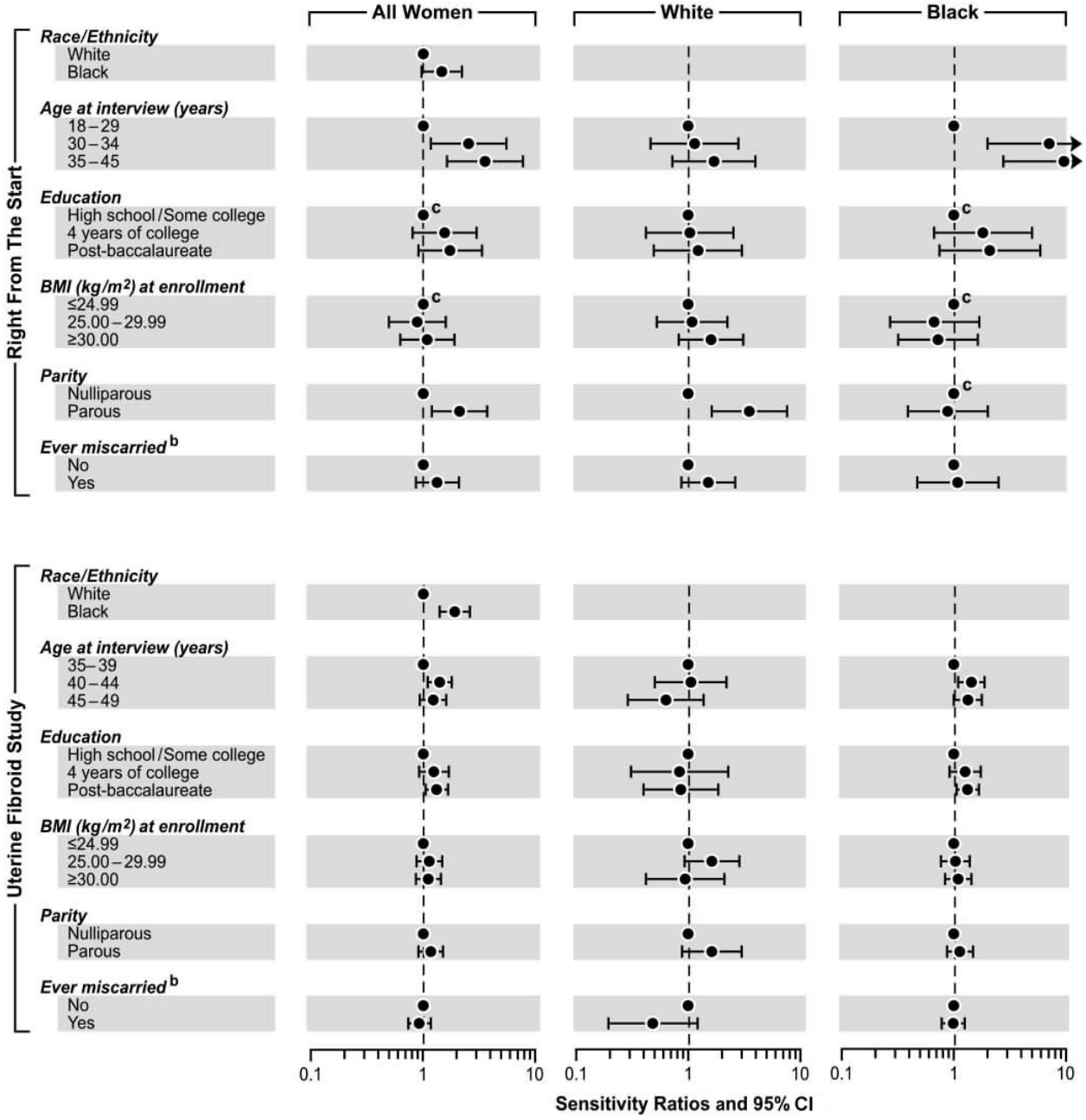
[b] Among women with a previous pregnancy.

Figure 4.1 Self-report sensitivity (upper panel) and specificity (lower panel) by race/ethnicity and age at interview for Right From The Start (n = 2,046) and Uterine Fibroid Study (n = 869) participants



Abbreviations: CI, confidence interval; RFTS, Right From The Start; UFS, Uterine Fibroid Study. Error bars indicate 95% confidence intervals. Estimates for the following RFTS age groups are excluded because there were fewer than 10 women in each race/age category: sensitivity for women aged 18-24 and black women over 40; specificity for black women aged 40-44.

Figure 4.2 Association of demographic and reproductive factors with sensitivity of self-reported uterine fibroid status among 279 Right From The Start and 507 Uterine Fibroid Study participants with fibroids detected at study ultrasound[a]

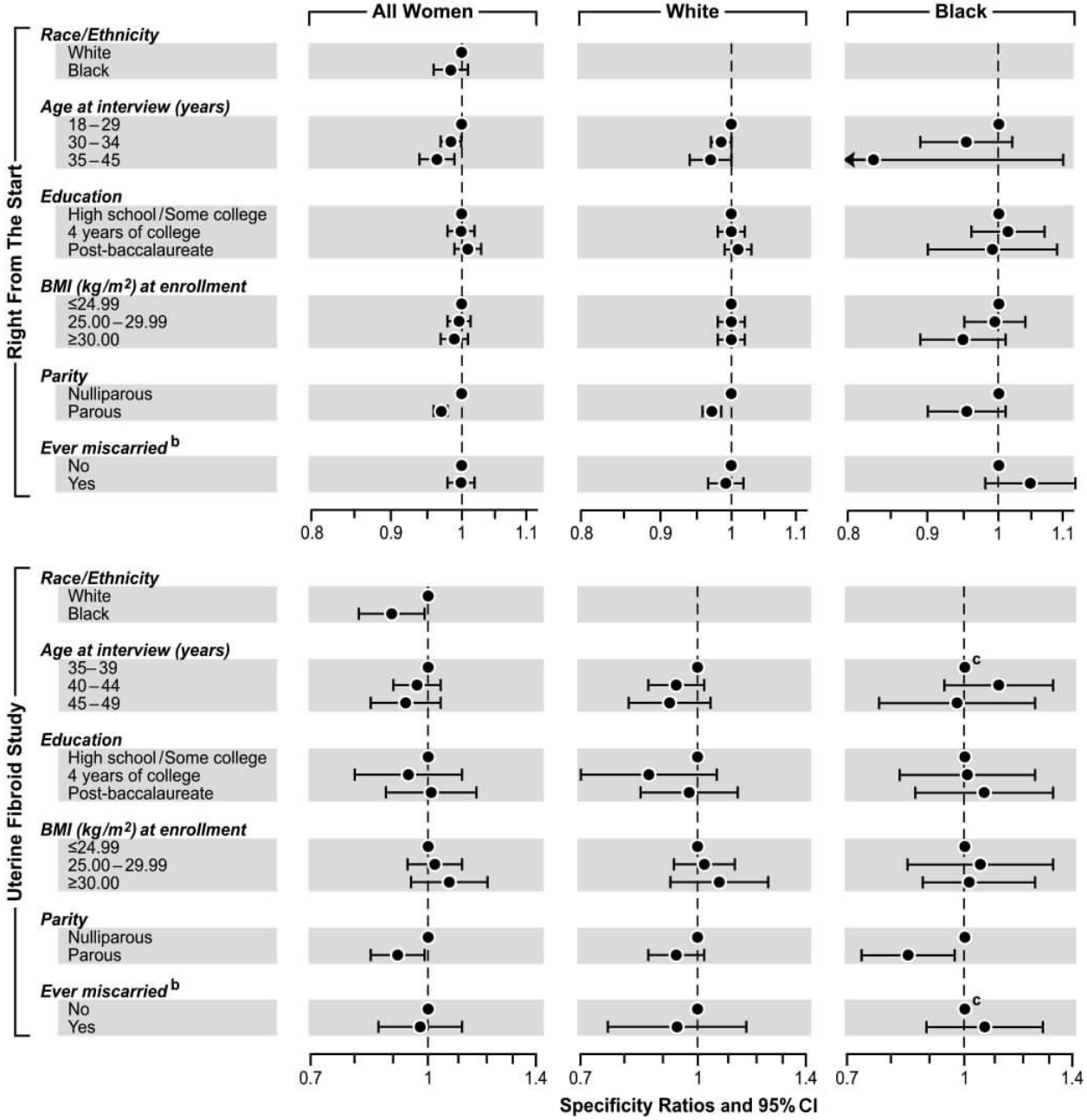


[a] Sensitivity ratios (sensitivity in a subgroup of interest compared to sensitivity in the reference group) are adjusted for age (continuous), parity, and (for unstratified estimates) race/ethnicity. A quadratic term was entered for age in the UFS multivariate analysis due to non-linearity.

[b] Among women with a previous pregnancy.

[c] Estimates obtained using Poisson regression.

Figure 4.3 Association of demographic and reproductive factors with specificity of self-reported uterine fibroid status among 1,767 Right From The Start and 362 Uterine Fibroid Study participants with no fibroids detected at study ultrasound[a]



[a] Specificity ratios are specificity of self-report in subgroup of interest compared to specificity in the reference group. UFS specificity ratios are adjusted for parity, and (for unstratified estimates) race/ethnicity. RFTS estimates are unadjusted.

[b] Among women with a previous pregnancy.

[c] Estimates obtained using Poisson regression.

CHAPTER 5: PESTICIDE USE AND UTERINE FIBROIDS AMONG WOMEN IN THE AGRICULTURAL HEALTH STUDY

5.1 ABSTRACT

Uterine fibroids are the leading indication for hysterectomy in the US. Although it is well-accepted that ovarian hormones play a role in fibroid growth, few studies have examined endocrine-disrupting chemicals as potential risk factors. Using cross-sectional data from the Agricultural Health Study, the authors investigated the association between pesticide use and fibroids among 16,526 women aged 18-59. Self-reporting of fibroids is known to be inaccurate because many women with fibroids are never diagnosed. Therefore, sensitivity and specificity estimates obtained from a separate analysis of fibroid reporting accuracy were incorporated in logistic regression models to correct for outcome misclassification. Both uncorrected (aOR) and corrected (cOR) estimates were adjusted for age and state (Iowa/North Carolina). Ever use of agricultural pesticides was associated with fibroids, with users of ≥ 3 pesticides having the highest odds compared to never users of any pesticide (aOR: 1.31, 95% CI: 1.12, 1.53; cOR: 1.44, 95% CI: 1.11, 1.87). Use of any of 10 pesticides classified *a priori* as potentially hormonally active was associated with fibroids when compared with never use of any pesticide (aOR: 1.28, 95% CI: 1.12, 1.45; cOR: 1.48, 95% CI: 1.20, 1.82), but odds were not increased when compared to users of other pesticides. When pesticides were grouped by chemical class, organophosphate users had slightly higher odds than women reporting use of other agricultural pesticides (aOR: 1.17, 95% CI: 1.05,

1.31; cOR: 1.21, 95% CI: 1.00, 1.45). These results suggest a possible association between agricultural pesticide exposure and uterine fibroids that warrants further investigation.

5.2 BACKGROUND

Uterine leiomyomata (fibroids) are benign neoplasms of uterine smooth muscle tissue that develop in the majority of women (1). Fibroids can cause menstrual abnormalities, pelvic pain, and pregnancy complications (2), and are the leading indication for hysterectomies in the United States (161), accounting for over one-third of these surgeries (3). Although a highly prevalent condition, fibroids remain clinically silent in a significant proportion of women with the tumors. There is an incomplete understanding of the etiology of uterine fibroids, but it is well-established that their growth and development is dependent on estrogen and progesterone (83, 162).

Given the relationship between hormones and fibroid pathogenesis, the possible role of exogenous hormonally active agents deserves attention. Exposure to chemicals that either mimic ovarian hormones or exert agonistic effects on hormone receptors may play a part in the growth of these tumors. Animal models and *in vitro* experiments support the role of some pesticides and persistent organic pollutants in uterine leiomyoma cell proliferation (5, 6, 9). Prenatal diethylstilbestrol and bisphenol A exposure in mice has been shown to increase reproductive tract abnormalities, including uterine fibroids (7, 8). Exposure to agricultural pesticides has been associated with menstrual cycle irregularities (127), age at menopause (126), and increased time-to-pregnancy (163) in humans, indicating that these compounds may have an effect on the female hormonal milieu. However, the possible role of pesticides in the etiology of uterine fibroids has not been explored in epidemiologic studies.

Women living on farms have increased opportunity for both direct and indirect pesticide exposure compared to the non-farming population. We therefore examined the association between pesticide use and self-reported uterine fibroid status among women enrolled in the Agricultural Health Study, focusing on hormonally active pesticides and pesticide use patterns. Because many women with fibroids remain asymptomatic and undiagnosed, the use of self-report to identify cases and non-cases is subject to misclassification. In order to account for this misclassification bias, we employed an outcome correction method (12) using self-report sensitivity and specificity estimates obtained from a separate analysis of two study populations that were screened for fibroids using ultrasound.

5.3 MATERIALS AND METHODS

5.3.1 Study Population

The Agricultural Health Study (AHS) is a longitudinal cohort of approximately 60,000 licensed commercial and private pesticide applicators and 32,000 spouses of private applicators in North Carolina and Iowa. Study details have been described previously (141), and questionnaires are available on-line (www.aghealth.org/questionnaires.html). Individuals applying for a restricted-use pesticide license were recruited at state licensing agencies between 1993 and 1997. Those who agreed to participate in the study completed an Enrollment Questionnaire and were asked to identify their spouses for enrollment in the study. Married male private applicators (mostly farmers) were given two questionnaires to be completed by their female spouse. A telephone interview was conducted to collect data from spouses that did not complete the take-home questionnaires. Seventy-five percent of identified spouses enrolled in the study by completing the Spouse Questionnaire (19% of

whom completed by telephone). The Spouse and Applicator Enrollment Questionnaires collected data on pesticide use, farming activities, general health and lifestyle factors, and demographics. All women (applicators and spouses) also received the Female and Family Health Questionnaire which gathered information about reproductive history. Institutional review boards of the National Institutes of Health and their contractors approved the study.

Women (applicators or spouses) who completed the enrollment questionnaire were re-contacted for follow-up telephone interviews approximately five years later, between 1999 and 2003. Data collected in the follow-up included a more extensive health history (including fibroid diagnosis) and updated reproductive history and pesticide use information. Approximately 69% of female private applicators (n = 921) and 74% of applicators' wives (n = 23,682) completed the follow-up interview. In this analysis, women who did not participate were about one year younger on average, had less education, and were more likely to reside in North Carolina, to report their ethnicity as non-white, and to report never personally mixing or applying pesticides in the Enrollment Questionnaire.

This analysis focused on female pesticide applicators and spouses of pesticide applicators who completed the follow-up interview. We excluded women if they did not identify themselves as non-Hispanic white (n = 1,606) or were 60 or older at the time of follow-up (n = 6,355). We further excluded 100 women missing uterine fibroid data and 16 women with missing or conflicting information on pesticide use. The final analysis subset of 16,526 women included 15,985 wives of pesticide applicators and 541 women who were licensed private pesticide applicators.

5.3.2 Exposure Assessment

Lifetime pesticide use was reported at baseline (Spouse or Applicator Enrollment questionnaires). Women were asked if they had ever personally mixed or applied pesticides in their lifetime, whether for agricultural or residential use. Those who answered yes were then asked about ever use of 50 specific pesticides. We constructed variables for ever/never use of individual pesticides, as well as pesticide groupings according to chemical structure (organochlorines, organophosphates, carbamates, and triazines). Women also reported whether they grew up on a farm ("Before age 18, did you live at least half your life on a farm?") and the number of years they had lived or worked on a farm.

Additional questions on residential pesticide use—whether or not they personally treated their own home or lawn for pests—were only asked in the Spouse Questionnaire. We constructed a variable to capture wives' pesticide use patterns based on answers to these questions and the individual pesticide questions. The referent category included women who reported never mixing or applying any pesticides. Women who reported residential use but did not report mixing or applying any of the 50 specific pesticides were categorized as "Residential only." Wives who reported use of any of the five most frequently reported agricultural pesticides—glyphosate, carbaryl, malathion, 2,4-D, and diazinon—were categorized as using "common agricultural pesticides." With the exception of diazinon, these pesticides are classified as general use pesticides by the US Environmental Protection Agency (EPA), and are generally considered to have low toxicity (149). Women who used any of the specific pesticides other than the top five were categorized based on the number used: "1-2 other agricultural" or "≥3 other agricultural." Women who reported that they

mixed or applied pesticides but did not report using any of the 50 specific pesticides that were queried were classified as using "Other pesticides."

Using the National Library of Medicine's PubMed database, we updated an earlier literature review (127) on the endocrine-disrupting potential of pesticides listed in the AHS questionnaires. This identified 10 possible hormonally active pesticides that showed effects possibly relevant to fibroid pathogenesis: the organochlorines DDT, chlordane, lindane, dieldrin, and toxaphene; mancozeb; atrazine; alachlor; carbon tetrachloride; and pyrethroids. Pesticides were selected based on *in vivo* or *in vitro* evidence either for estrogenic/progestagenic activity or follicle-stimulating hormone disruption. Because the ovary is the primary source of steroid hormones in the uterus, we also included pesticides with evidence of estrus cycle disruption or effects on the ovary or uterus in animal models. For the analysis, we created a dichotomous variable to indicate ever/never use of any of these 10 pesticides.

5.3.3 Study Outcome and Covariates

The outcome of interest was uterine fibroid diagnosis as reported by participants in the follow-up interview. Women who answered "yes" to the question "Has a doctor or other health professional ever told you that you had uterine fibroids?" were counted as cases. Possible confounders were identified based on previous literature on uterine fibroid risk factors and included age at follow-up, age at menarche, and parity (4). Age at menarche (<12, 12, 13, 14, ≥ 15) was reported in the baseline Female and Family Health (FFH) Questionnaire. Parity has been inversely associated with fibroids (reviewed by Laughlin et al. (4)), and there is some suggestion that births to women after their early-twenties might partially account for the apparent protective effect of pregnancy (40). A categorical variable

was created to capture timing of births (none, all births before age 24, one or more births ≥24 years of age) based on the reproductive history obtained in the FFH and updated in the follow-up interview. State of residence (Iowa/North Carolina) was classified at the time of enrollment. We also identified women who reported having had a hysterectomy (baseline FFH questionnaire or the follow-up interview) for purposes of assigning sensitivity and specificity estimates in the outcome correction model, as described below.

5.3.4 Statistical Analysis

Logistic regression was used to estimate the cross-sectional association of pesticide use at enrollment in relation to uterine fibroid diagnosis. Analyses were carried out with the statistical software package SAS 9.1 (SAS Institute, Inc., Cary, North Carolina). To get a better picture of the shape of the relationship between age and fibroid status, we examined the log odds of fibroids by age, categorized as 21-29 and then by successive 2-year categories (e.g., 30-31, 32-33). Based on this assessment, a quadratic term for age was added to the model and retained if the Wald *P* value was <0.05 (all *P* values are two-sided). We evaluated the linearity assumption for categorical predictors on the log scale using indicator variables with common referent coding (151). When a linear relationship was observed, we modeled the categorical predictor as a single ordinal variable and calculated a Wald *P* value for its coefficient.

The main exposures of interest in this analysis were pesticide use patterns and use of hormonally active pesticides. We also examined ever use of individual pesticides (limited to those with at least 10 exposed cases) and chemical class groupings as main exposures in separate analyses. All prevalence odds ratios (OR) and 95% confidence intervals (CI) were adjusted for age at follow-up (continuous), age squared, and state of residence. We assessed

for confounding and effect measure modification for the main exposures and the chemical class groupings, because assessment for the individual pesticides would be unwieldy. Timing of births and age at menarche were not confounders of the association between fibroid diagnosis and pesticide use as assessed by at least a 10% change relative to the age- and state-adjusted odds ratios. We tested for statistical interaction by age at follow-up by including an interaction term for each exposure and age and a P <0.10 significance level. Age at follow-up did not modify associations with any of the exposures tested.

Because fibroid status was ascertained via self-report, we used an outcome correction method in the logistic regression models to produce point estimates and confidence intervals that accounted for outcome misclassification bias. Details of this method are described elsewhere (12) and the SAS macro is available on-line (http://medschool.umaryland.edu/epidemiology/software.asp). Briefly, given known or assumed values for sensitivity and specificity of the outcome, an expectation-maximization (EM) algorithm (153) is used to obtain odds ratios and their variances. We applied sensitivity and specificity estimates from a separate analysis of self-report validity that was conducted in two study populations which had self-reported fibroid status and "gold standard" ultrasound examination results (data not published). Together, these studies included over 2,000 white premenopausal women aged 18-49. Results demonstrated that sensitivity of self-report was generally low, but increased with age with a slight decline in women aged 45-49. In contrast, specificity was generally high but had a small negative association with age. Additional investigation suggested that women who reported having had fibroids previously but did not have them at ultrasound may not have been wrong.

Tumor regression could have occurred in association with intervening factors such as time between diagnosis and study ultrasound or pregnancies.

Therefore, for our main correction model (referred to as Corrected-1), we varied sensitivity by age but set specificity to 0.95. We used the following values for sensitivity by age in the main outcome correction model: age 18-29, 0.15; 30-34, 0.20; 35-39, 0.35; 40-44, 0.40; 45-59, 0.30. We assumed that women who reported having had a hysterectomy (n = 3,022) would be better reporters of fibroid diagnosis than those who had not, and set sensitivity to 0.85 and specificity to 0.95 for this subgroup.

Because of our specific interest in hormonally active pesticides and pesticide use patterns, we performed additional sensitivity analyses to evaluate the influence of our assumptions in these models. We examined the assumption of high self-report specificity by running models in which specificity decreased with age, as observed in the earlier validity analysis (Corrected-2). We also ran models in which sensitivity and specificity varied with age as in the Corrected-1 and -2 models, but treating all women as if they had not had a hysterectomy (Corrected-3 and Corrected-4). Details of these additional analyses are provided in Tables 5.4 and 5.5.

5.4 RESULTS

5.4.1 Characteristics of Analysis Population

The majority of the study population was enrolled in Iowa, but women who resided in North Carolina at the time of enrollment had higher odds of reporting fibroids (Table 5.1). The odds of fibroids increased with age at follow-up (P for trend <0.0001) and decreased with age at menarche (P for trend <0.0001). Women who gave birth before age 24 were more likely to report being diagnosed with uterine fibroids than those who had at least one

birth at 24 or older. Slightly more than 18% of women had a hysterectomy by the time of follow-up and of these, 39% reported having had a fibroid compared to 9% of women who had not had a hysterectomy. Number of years living or working on a farm was positively associated with fibroids (*P* for trend <0.0001), but the association was no longer statistically significant after adjustment for age and state (data not shown).

5.4.2 Pesticide Use Patterns

The analysis of pesticide use patterns was limited to 15,985 wives of pesticide applicators who completed the Spouse Questionnaire that asked about residential pesticide use (Figure 5.1). After controlling for age and state, the odds of fibroid diagnosis were not significantly different when comparing women who reported only residential use to those who never mixed or applied any pesticides (adjusted odds ratio [aOR] = 0.92; 95% CI: 0.76, 1.10). There was a trend for slightly increased odds of fibroids across categories of agricultural pesticide use, with women who used three or more agricultural pesticides having the highest odds compared to never users of any pesticides (aOR = 1.31; 95% CI: 1.12, 1.53). Odds were somewhat elevated for women who reported personally mixing or applying pesticides, but did not use any of the 50 specific pesticides (aOR = 1.15; 95% CI: 0.86, 1.53). Estimates from corrected models were further away from the null than uncorrected estimates (Figure 5.1), but after correction, there was little difference in the odds of fibroid diagnosis among agricultural pesticide use categories, with a corrected odds ratio (cOR) of 1.42 (95% CI: 1.16, 1.72) for common agricultural pesticide use and 1.44 (95% CI: 1.11, 1.87) for ≥ 3 other agricultural pesticides. Sensitivity analyses that varied assumptions used to correct for outcome misclassification generally resulted in odds ratios further away from the null. The greatest difference between uncorrected and corrected estimates appeared in the Corrected-4

model, which varied both sensitivity and specificity by age and did not distinguish between women who did or did not have a hysterectomy (Table 5.4).

5.4.3 Hormonally Active Pesticides

Approximately 16% of women in this analysis reported using one or more of the 10 pesticides identified as possible hormonally active agents, estrus cycle disruptors, or utero- or ovotoxic (Table 5.2). The odds of fibroid diagnosis in these women were 1.28 times as high as women who never mixed or applied any pesticides (95% CI: 1.12, 1.45). Correcting for outcome misclassification further strengthened this association (cOR = 1.48; 95% CI: 1.20, 1.82) (Table 5.2). Results of sensitivity analyses (Table 5.5) followed similar patterns as above, with the strongest association seen in the Corrected-4 model (cOR = 4.23; 95% CI: 1.86, 9.61). However, there was no difference in the odds of fibroid diagnosis when women who used hormonally active pesticides were compared to those who used other pesticides (aOR = 1.02; 95% CI: 0.90, 1.15), either in uncorrected or corrected models.

5.4.4 Use of Specific Pesticides

Table 5.3 provides results for the association of specific pesticides and chemical class groupings within the subset of women who used agricultural pesticides. Of the four chemical classes investigated, ever use of organophosphates was weakly associated with fibroid diagnosis (aOR = 1.17; 95% CI: 1.05, 1.31). The association was only slightly higher (cOR = 1.21; 95% CI: 1.00, 1.45) after correcting for outcome misclassification (data not shown). Among the specific organophosphate pesticides, use of coumaphos (aOR = 1.41; 95% CI: 1.01, 1.96), malathion (aOR = 1.17; 95% CI: 1.05, 1.31), or parathion (aOR = 1.42; 95% CI: 1.00, 2.00) was significantly ($P < 0.05$) associated with elevated odds of fibroid diagnosis. Carbon tetrachloride showed the strongest association with fibroid diagnosis among the

pesticides examined (aOR = 1.70; 95% CI: 1.02, 2.85). Of the 10 pesticides identified *a priori* as possibly hormonally active, this was the only one significantly associated with fibroids. There was a suggestive inverse association with fibroid diagnosis among users of the carbamate aldicarb (aOR = 0.57; 0.32, 1.00). Spearman correlation coefficients among these five pesticides—coumaphos, malathion, parathion, carbon tetrachloride, and aldicarb—were all ≤ 0.1, indicating that observed associations were not confounded by other pesticide use (data not shown).

5.5 DISCUSSION

To our knowledge, this analysis is the first to explore the relationship between pesticide use and self-reported physician diagnosis of uterine fibroids. The Agricultural Health Study provided a unique opportunity to examine detailed information about specific pesticides among a large cohort of women who have higher exposures than the general population.

Although women who reported using pesticides to treat pests around their home or lawn/garden did not have increased odds of fibroids compared to women who did not use any pesticides, we observed a positive association among agricultural pesticide users. Evidence for an association with fibroids was found among ever users of the 10 pesticides we identified based on the toxicological evidence as possibly relevant to fibroid pathogenesis, when compared to women who did not use any pesticides. When compared to women who used other agricultural pesticides, however, the odds of fibroid diagnosis were similar. We therefore examined associations between fibroid diagnosis and specific pesticides among the subset of women who reported agricultural pesticide use. We found significantly elevated odds among organophosphate pesticide users and, specifically, among women who used

coumaphos, malathion, or parathion. None of these pesticides were identified as hormonally active as a result of our literature review, mainly because few studies have evaluated them for endocrine disrupting effects. The fumigant carbon tetrachloride, which was identified as hormonally active, had the strongest association with fibroid diagnosis among all individual pesticides examined. Early animal studies suggest that carbon tetrachloride acts indirectly, through hepatotoxic effects, to increase serum estradiol and progesterone levels, decrease estrone metabolism, and increase levels of these hormones in the uterus (164-166). There are no studies investigating whether carbon tetrachloride acts directly as an estrogen or progesterone mimic or agonist.

Although there are no epidemiologic data to compare to our results, there is a plausible biological mechanism for the role of hormonally active agents in fibroid pathogenesis. Results of clinical trials and experimental models, along with the observation that fibroids occur in women in their reproductive years and typically diminish after menopause, clearly indicate the role of both estrogen and progesterone in fibroid growth (reviewed by Marsh et al. (83)). *In vivo* and *in vitro* experiments have suggested that hormonally active agents can exert effects similar to ovarian hormones on fibroids. The most compelling evidence comes from the Eker rat, which spontaneously develops uterine fibroids with high frequency and many of the phenotypic characteristics of human fibroids. Diethylstilbesterol (DES), phytoestrogens, and some organochlorine pesticides (methoxychlor, kepone, endosulfan, toxaphene, and dieldrin) have been shown to have estrogenic activity in the Eker rat uterine leiomyoma (UL) cell line either by inducing a transcriptional response or cell proliferation *in vitro* (134). More recently, fenvalerate, a pyrethroid insecticide, was observed to increase the rate of growth *in vitro* of human UL as

well as Eker rat UL and uterine smooth muscle cells (9). None of the pesticides identified as being associated with fibroids in our analysis has been tested in the Eker rat.

Epidemiologic studies of the association between endocrine disruptors and uterine fibroids have been few. One study that examined DDT found significantly higher concentrations in leiomyomatous tissue compared to surrounding normal myometrium in a sample of 25 women (10). A prospective study of women living in the vicinity of a chemical plant explosion in Seveso, Italy found a statistically significant inverse association between serum levels of 2,3,7,8-tetrachlrodibenzo-p-dioxin (TCDD) and hazard of fibroid onset (137), in line with data that TCDD acts as an antiestrogen in the rat uterine myometrium (138). A cross-sectional analysis of women in the National Health and Nutrition Examination Survey found no association with self-reported fibroid diagnosis and blood levels of the endocrine disrupting heavy metals lead, cadmium, and mercury (139).

Because we used self-reported fibroid diagnosis to classify cases and non-cases, we likely misclassified a substantial proportion of true cases that had not come to clinical attention. In order to address this bias, we used a correction method in the logistic regression models to adjust for imperfect outcome measurement (12). Our assumptions regarding self-report accuracy were informed by an earlier analysis of data from the Uterine Fibroid Study (UFS) and Right from the Start (RFTS) which showed that, in particular, sensitivity of self-report was quite poor and differed with age (data not published). Because we assumed that misclassification was not dependent on exposure, results from models corrected for misclassification moved the odds ratio further away from the null than the (uncorrected) model which assumed perfect outcome measurement.

Although the AHS collected information on many different pesticides, our exposure information is limited in terms of actual levels, duration, frequency, and timing. The exposure measures were based on women's report of ever/never pesticide use, and may not reflect exposures that preceded fibroid diagnosis. Previous analyses of repeat interviews among a subset of farmers in the AHS indicated that agreement was relatively high (79-87%) for ever/never use of specific pesticides, but generally decreased with the amount of detail (e.g., duration , frequency) sought (167). It is not unreasonable to assume that farm women may have similarly high accuracy with respect to ever/never use of pesticides. It is unlikely that recall of pesticide use is different among women with and without fibroids because outcome data were gathered after information on pesticide use was collected. Information on other sources or modifying factors of pesticide exposure was not collected, and women who were exposed to pesticides through field work, spray drift, or carry-home contamination could have been erroneously classified as unexposed.

The focus of this analysis was on pesticide use patterns and hormonally active pesticides. The decision to label a pesticide hormonally active was based on a literature review that was limited to the published research. For example, a total of 156 citations were found related to endocrine-disrupting effects of organochlorine pesticides, compared to 59 for organophosphates. We found no toxicological publications that examined coumaphos as an endocrine disruptor. For this reason and because of the uniqueness of the AHS data set, we felt it important to present estimates for all pesticides with the caveat that some associations could have appeared by chance due to the number of exposures investigated.

Although retention rates were relatively good at the five-year follow-up, there were some differences between participants and nonparticipants which could have led to selection

bias. Women who did not participate in follow-up were slightly younger, less educated, more likely to be from North Carolina, and more likely to have reported never personally mixing or applying pesticides in the Enrollment Questionnaire. Follow-up participation rates were also lower among women who enrolled as applicators (and may have higher exposures) compared to applicators' wives. It is unclear whether these exclusions might have affected our results.

In conclusion, results of this analysis suggest that use of agricultural pesticides may be related to uterine fibroids in this population of farm women. Odds of fibroids were significantly elevated in women who used the five most common, less toxic pesticides and further elevated among users of additional pesticides. We illustrated the use of a relatively straightforward algorithm to assess the influence of bias arising from use of self-report to classify fibroid status. Unlike simpler approaches used previously (44, 158), this method accommodates the differential reporting accuracy according to age that was indicated in a separate analysis of self-report validity (data not published). Despite known problems with self-reported data, existing large cohorts such as the Agricultural Health Study provide important contributions to our understanding of fibroid risk factors and possible avenues for further investigation. *In vivo* or *in vitro* testing of the pesticides which we observed to be associated with fibroid diagnosis would be a good first step to confirm these associations and explore possible mechanisms of action.

Table 5.1 Selected characteristics of 16,526 women aged 21-59 in the Agricultural Health Study, by self-reported uterine fibroid status, 1993-2003

Characteristic	Fibroids *n* = 2,360	No fibroids *n* = 14,166	Crude odds ratio	95% confidence interval
State				
Iowa	1,687	10,825	1.00	Referent
North Carolina	673	3,341	1.29	1.17, 1.42
Age (years) at follow-up				
21 – 34	40	1,251	0.16	0.12, 0.23
35 – 39	154	2,195	0.36	0.30, 0.43
40 – 44	394	2,898	0.69	0.60, 0.79
45 – 49	572	2,899	1.00	Referent
50 – 54	607	2,596	1.19	1.05, 1.34
55 – 59	593	2,327	1.29	1.14, 1.47
Age (years) at menarche				
Less than 12	490	2,149	1.00	Referent
12	689	4,099	0.74	0.65, 0.84
13	666	4,245	0.69	0.61, 0.78
14	336	2,118	0.70	0.60, 0.81
15 or older	160	1,411	0.50	0.41, 0.60
Missing	19	144		
Timing of births				
No births	108	650	1.07	0.87, 1.32
All births <24 years maternal age	553	2,499	1.42	1.28, 1.58
Any birth ≥24 years maternal age	1,646	10,589	1.00	Referent
Missing	53	428		
Self-reported hysterectomy				
No	1,152	12,261	1.00	Referent
Yes	1,191	1,831	6.92	6.30, 7.61
Missing	17	74		
Grew up on farm				
No	995	6,029	1.00	Referent
Yes	1,316	7,859	1.02	0.93, 1.11
Missing	49	278		
Years lived/worked on farm				
Less than 5	61	561	1.00	Referent
5 – 10	149	1,339	1.02	0.75, 1.40
11 – 20	412	2,838	1.34	1.01, 1.77
21 – 30	527	3,031	1.60	1.21, 2.12
Over 30	1,085	5,589	1.79	1.36, 2.34
Missing	126	808		

Table 5.2 Association between use of hormonally active pesticides and self-reported uterine fibroids among 16,526 women aged 21-59 in the Agricultural Health Study, 1993-2003

Any possible hormonally- active pesticide[a]	Fibroids		No Fibroids		Adjusted[c]		Corrected-1[d]	
	Total No.	Exposed %[b]	Total No.	Exposed %[b]	OR	95% CI	OR	95% CI
Comparison group: never users of any agricultural pesticide	1,242	37	7,912	28	1.28	1.12, 1.45	1.48	1.20, 1.82
Comparison group: users of any agricultural pesticides other than hormonally active pesticides	1,533	30	8,197	27	1.02	0.90, 1.15	0.98	0.81, 1.20

Abbreviations: OR, odds ratio; CI: confidence interval.

[a] Pesticides identified *a priori* as hormonally active, estrus cycle disruptors, or utero- or ovotoxic: DDT, chlordane, lindane, dieldrin, toxaphene, maneb, atrazine, alachlor, carbon tetrachloride, and permethrin.

[b] A total of 462 women with fibroids and 2,210 women without fibroids were exposed to possible hormonally active pesticides.

[c] Adjusted for age (continuous), age squared, and state (IA/NC).

[d] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Women reporting hysterectomy: sensitivity=0.85. Sensitivity for women who did not have a hysterectomy, by age at follow-up 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity set at 0.95 for all women.

Table 5.3 Association between specific pesticide use and self-reported uterine fibroid diagnosis among 10,044 women aged 21-59 who mixed or applied agricultural pesticides in the Agricultural Health Study, 1993-2003

	Ever use in women with fibroids (n = 1,580)[a]		Ever use in women without fibroids (n = 8,464)[a]		Adjusted	
Pesticide name	No.	%	No.	%	OR[b]	95% CI
Organochlorines	221	14.5	918	11.3	1.05	0.90, 1.24
Aldrin	18	1.2	82	1.0	0.95	0.57, 1.60
Chlordane	133	9.0	520	6.5	1.11	0.91, 1.36
DDT	81	5.5	285	3.6	1.14	0.88, 1.48
Dieldrin[c]	8	0.5	34	0.4		
Heptachlor	22	1.5	86	1.1	1.11	0.69, 1.78
Lindane	53	3.5	269	3.3	0.90	0.67, 1.22
Toxaphene	18	1.2	82	1.0	0.96	0.57, 1.61
Organophosphates	827	52.7	3,871	46.0	1.17	1.05, 1.31
Chlorpyrifos	141	9.3	681	8.4	1.07	0.88, 1.30
Coumaphos	48	3.2	180	2.2	1.41	1.01, 1.96
Diazinon	353	23.2	1,613	19.9	1.11	0.97, 1.27
Dichlorvos	70	4.6	350	4.3	1.00	0.77, 1.31
Fonofos	62	4.1	270	3.3	1.13	0.85, 1.50
Malathion	639	41.2	2,844	34.4	1.17	1.05, 1.31
Parathion	44	2.9	153	1.9	1.42	1.00, 2.00
Phorate	58	3.8	310	3.8	0.92	0.69, 1.23
Terbufos	86	5.7	463	5.7	0.96	0.75, 1.22
Trichlorfon[c]	8	0.5	37	0.5		
Carbamates	913	58.3	4,564	54.2	1.00	0.90, 1.12
Aldicarb	14	0.9	116	1.4	0.57	0.32, 1.00
Carbofuran	66	4.4	273	3.4	1.14	0.87, 1.51
Carbaryl	891	57.5	4,454	53.8	0.99	0.88, 1.11
Benomyl	30	2.0	180	2.2	0.75	0.50, 1.11
Triazines	183	12.0	898	11.0	1.01	0.85, 1.20
Atrazine	151	9.9	706	8.7	1.05	0.87, 1.26
Cyanazine	95	6.3	457	5.6	1.06	0.84, 1.33
Metribuzin	56	3.7	291	3.6	0.90	0.67, 1.21
Other insecticides						
Permethrin	166	10.7	844	10.1	1.04	0.87, 1.25

table continues

Table 5.3 Association between specific pesticide use and self-reported uterine fibroid diagnosis among 10,044 women aged 21-59 who mixed or applied agricultural pesticides in the Agricultural Health Study, 1993-2003 (cont.)

	Ever use in women with fibroids (n = 1,580)[a]		Ever use in women without fibroids (n = 8,464)[a]		Adjusted	
Pesticide name	No.	%	No.	%	OR[b]	95% CI
Other herbicides						
2,4-D	428	28.0	2,242	27.4	0.98	0.87, 1.11
2,4,5 TP[c]	7	0.5	38	0.5		
2,4,5 T	26	1.7	92	1.1	1.29	0.83, 2.01
Alachlor	139	9.2	660	8.2	1.06	0.87, 1.29
Butylate	55	3.7	222	2.7	1.18	0.87, 1.60
Chlorimuron Ethyl	70	4.6	303	3.7	1.21	0.92, 1.58
Dicamba	129	8.5	660	8.1	1.00	0.82, 1.23
EPTC	42	2.8	230	2.9	0.90	0.64, 1.27
Glyphosate	1,019	65.4	5,333	63.9	1.07	0.96, 1.21
Imazethapyr	96	6.4	517	6.4	1.00	0.79, 1.26
Metolachlor	115	7.6	559	6.9	1.08	0.87, 1.34
Paraquat	45	3.0	192	2.4	1.13	0.81, 1.59
Pendimethalin	79	5.2	443	5.5	0.90	0.70, 1.15
Petroleum oil	131	8.7	598	7.4	1.15	0.94, 1.40
Trifluralin	176	11.7	835	10.3	1.07	0.90, 1.28
Other fungicides						
Captan	77	5.1	352	4.3	1.05	0.82, 1.36
Chlorothalonil	42	2.8	170	2.1	1.20	0.85, 1.71
Maneb	52	3.4	228	2.8	1.00	0.73, 1.37
Metalaxyl	51	3.3	305	3.7	0.77	0.56, 1.06
Ziram[c]	2	0.1	18	0.2		
Other fumigants						
Aluminum phosphide[c]	5	0.3	28	0.3		
Carbon tetrachloride	21	1.4	53	0.7	1.70	1.02, 2.85
Ethylene dibromide[c]	4	0.3	40	0.5		
Methyl bromide	39	2.5	210	2.6	0.87	0.61, 1.25

Abbreviations: DDT, dichlorodiphenyltrichloroethane; 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5 TP, (2,4,5-trichlorophenoxy)propionic acid; 2,4,5 T, (2,4,5-trichlorophenoxy)acetic acid; OR, odds ratio; CI, confidence interval.

[a] The total number of women with and without fibroids differs for each pesticide because of missing exposure data.

[b] Adjusted for age (continuous), age squared, and state (IA/NC). Comparison group consists of women who used other agricultural pesticides.

[c] Odds ratios not reported if fewer than 10 exposed cases.

Table 5.4 Sensitivity analysis of pesticide use patterns and self-reported uterine fibroids among 15,985 wives of private pesticide applicators in the Agricultural Health Study, 1993-2003

Pesticide use patterns[a]	Adjusted[b]		Corrected-1[c]		Corrected-2[d]		Corrected-3[e]		Corrected-4[f]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Never used	1.00	referent	1.00	referent	1.00	referent	1.00	referent	1.00	referent
Residential use only	0.92	0.76, 1.10	0.78	0.57, 1.07	0.76	0.53, 1.09	0.79	0.50, 1.25	0.28	0.04, 2.22
Commonly used	1.21	1.07, 1.35	1.42	1.16, 1.72	1.41	1.13, 1.76	1.60	1.20, 2.13	3.05	1.58, 5.91
1-2 agricultural pesticides	1.26	1.09, 1.45	1.43	1.13, 1.82	1.39	1.06, 1.81	1.70	1.20, 2.41	3.48	1.70, 7.15
≥ 3 agricultural pesticides	1.31	1.12, 1.53	1.44	1.11, 1.87	1.46	1.09, 1.94	1.80	1.22, 2.67	3.97	1.88, 8.38
Other pesticides	1.15	0.86, 1.53	1.30	0.79, 2.14	1.19	0.66, 2.15	1.43	0.71, 2.86	2.03	0.40, 10.31

Abbreviations: OR, odds ratio; CI: confidence interval.

[a] Never used: women who reported never mixing/applying any pesticide. Residential use only: did not use any of the 50 named pesticides, but reported personally treating home/lawn/garden for pests. Commonly used: used at least one of the five most frequently reported pesticides (glyphosate, carbaryl, malathion, 2,4-D, diazinon). 1-2 and ≥3 agricultural pesticides: used pesticides other than or in addition to the top five. Other pesticides: women who reported personally mixing/applying pesticides but used something other than 50 named. A total of 27 fibroid cases and 158 non-cases were missing data on pesticide use patterns.

[b] Adjusted for age (continuous), age squared, and state (IA/NC).

[c] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Women reporting hysterectomy: sensitivity=0.85. Sensitivity for women who did not have a hysterectomy, age at follow-up 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity set at 0.95 for all women.

[d] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Women reporting hysterectomy: sensitivity=0.85 and specificity by age at follow-up: 18-29: 0.99; 30-34: 0.98; 35-39: 0.95; 40-44: 0.95; 45-59: 0.95. Sensitivity for women who did not have a hysterectomy by age at follow-up 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity by age at follow-up, 18-29: 0.99; 30-34: 0.98; 35-39: 0.95; 40-44: 0.89; 45-59: 0.87.

[e] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Sensitivity by age at follow-up, 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity set at 0.95 for all women.

[f] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Sensitivity by age at follow-up, 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity by age at follow-up, 18-29: 0.99; 30-34: 0.98; 35-39: 0.95; 40-44: 0.89; 45-59: 0.87.

Table 5.5 Sensitivity analysis of the association between use of hormonally active pesticides and self-reported uterine fibroids among 16,526 women aged 21-59 in the Agricultural Health Study, 1993-2003

Any possible hormonally-active pesticide[a]	Adjusted[b]		Corrected-1[c]		Corrected-2[d]		Corrected-3[e]		Corrected-4[f]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Comparison group: never users of any pesticide	1.28	1.12, 1.45	1.48	1.20, 1.82	1.51	1.20, 1.90	1.71	1.26, 2.32	4.23	1.86, 9.61
Comparison group: users of other pesticides	1.02	0.90, 1.15	0.98	0.81, 1.20	1.02	0.81, 1.27	0.98	0.72, 1.35	1.07	0.65, 1.77

Abbreviations: OR, odds ratio; CI: confidence interval

[a] Pesticides identified *a priori* as hormonally active, estrus cycle disruptors, or utero- or ovotoxic: DDT, chlordane, lindane, dieldrin, toxaphene, maneb, atrazine, alachlor, carbon tetrachloride, and permethrin.

[b] Adjusted for age (continuous), age squared, and state (IA/NC).

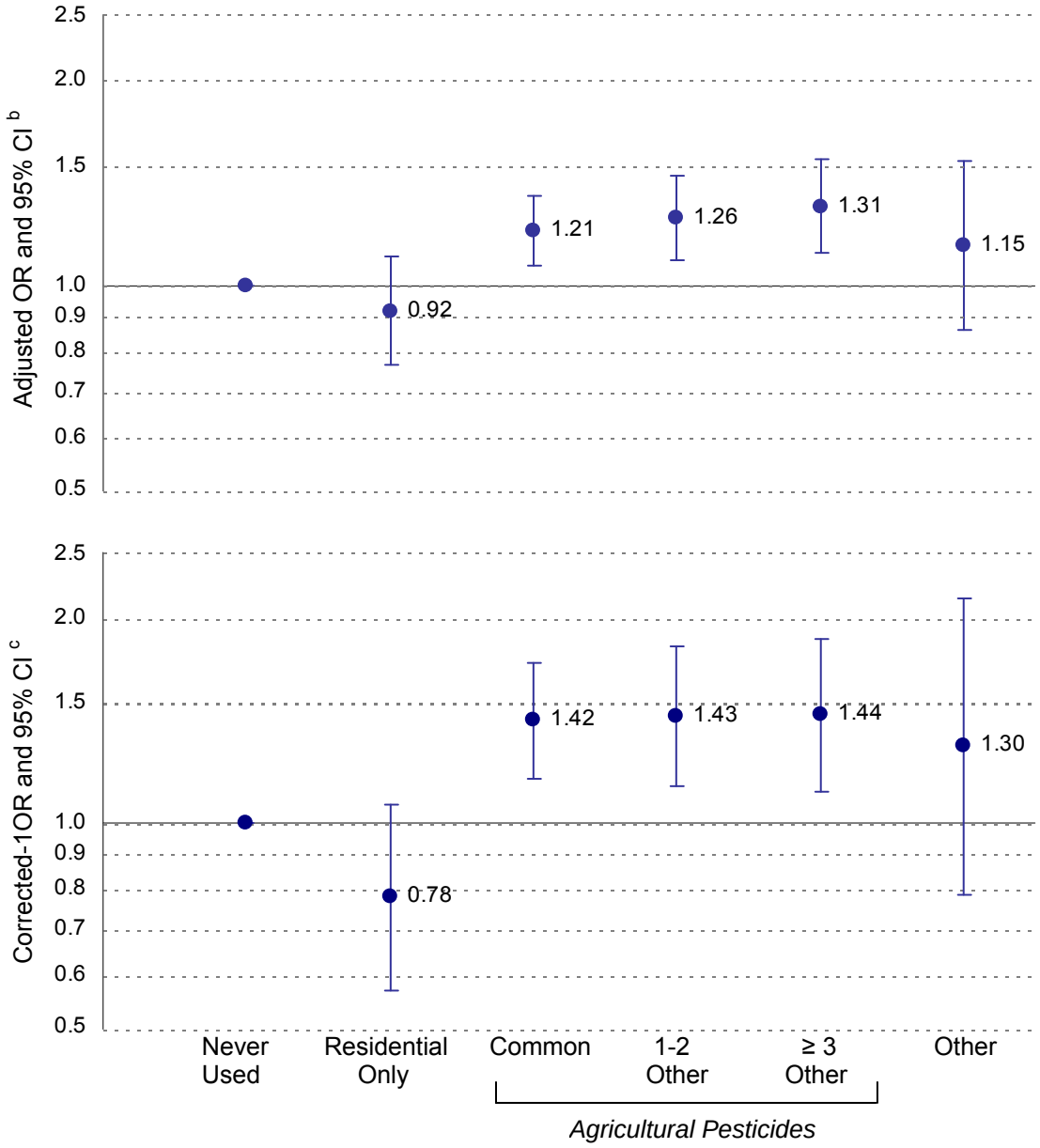
[c] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Women reporting hysterectomy: sensitivity=0.85. Sensitivity for women who did not have a hysterectomy, age at follow-up 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity set at 0.95 for all women.

[d] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Women reporting hysterectomy: sensitivity=0.85 and specificity by age at follow-up: 18-29: 0.99; 30-34: 0.98; 35-39: 0.95; 40-44: 0.95; 45-59: 0.95. Sensitivity for women who did not have a hysterectomy by age at follow-up 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity by age at follow-up, 18-29: 0.99; 30-34: 0.98; 35-39: 0.95; 40-44: 0.89; 45-59: 0.87.

[e] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Sensitivity by age at follow-up, 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity set at 0.95 for all women.

[f] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Sensitivity by age at follow-up, 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity by age at follow-up, 18-29: 0.99; 30-34: 0.98; 35-39: 0.95; 40-44: 0.89; 45-59: 0.87.

Figure 5.1 Association between pesticide use patterns[a] and self-reported uterine fibroids among 15,985 wives of private pesticide applicators in the Agricultural Health Study, 1993-2003



Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Never used (referent): never mixed/applied any pesticide. Residential only: did not use any of the 50 named pesticides, but personally treated home/lawn/garden for pests. Common: used any of the five most frequently reported agricultural pesticides (glyphosate, carbaryl, malathion, 2,4-D, diazinon). 1-2 and ≥ 3 other agricultural pesticides: used pesticides other than or in addition to the top five. Other pesticides: mixed/applied pesticides but used something other than 50 named.

[b] Adjusted for age (continuous), age squared, and state (IA/NC).

[c] Adjusted for age, age squared, and state and the following assumptions for outcome misclassification correction. Women reporting hysterectomy: sensitivity=0.85. Sensitivity for women who did not have a hysterectomy, age at follow-up 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Specificity set at 0.95 for all women.

CHAPTER 6: DISCUSSION

6.1 SUMMARY OF FINDINGS

This dissertation research accomplished the following aims: 1) to conduct a detailed assessment of self-reported uterine fibroid status and factors that might influence self-report validity and 2) to examine the association between pesticide use and uterine fibroid prevalence, using information obtained in Aim 1 to assess the degree to which parameter estimates are biased as a result of using self-reported fibroid diagnosis.

For Aim 1, the validity of self-reported uterine fibroid status was assessed in two different study populations: Right from the Start, which included pregnant women, most of whom were under 35, and the Uterine Fibroid Study, which included 35-49 year-old women randomly selected from among members of an urban health plan. Sensitivity (the proportion of women reporting fibroid diagnosis among those with fibroids detected at ultrasound) was low (< 0.50) in both study populations. In contrast, specificity (the proportion of women reporting no previous diagnosis among those with no fibroids at ultrasound) was moderate to high (0.86 to 0.98).

Among the possible determinants of validity that I investigated, race/ethnicity, age, and parity were associated with sensitivity and specificity of self-report. In both studies, self-report among black women was more sensitive but less specific compared to white women. Black women develop fibroids at a younger age and tend to have larger and more numerous tumors (20, 28, 30). Better reporting among black women with fibroids may be a result of

more severe symptoms or of increased awareness among medical professionals of black women as a "high-risk" group, leading to an increased likelihood of diagnosis. Age was positively associated with sensitivity and negatively associated with specificity. In white women, sensitivity was slightly lower among 45-49 year-olds compared to younger whites, for reasons that are not entirely clear. However, taken in light of the observation that the proportion of women with larger tumors increased with age among blacks but not whites, this finding suggests that there may be a real difference in the natural progression of these tumors among different racial/ethnic groups. In a study which tracked fibroid growth in tumors from 72 premenopausal women, older white women had a lower fibroid growth rate than younger whites, while this age difference was not seen in black women (31). It is unknown whether or not the inverse relationship between specificity and age truly reflects respondent error, but the finding that the proportion of apparent false-positives increased with duration between first diagnosis and ultrasound suggests that specificity may be affected by time since diagnosis as well as (or instead of) age at interview. Tumor regression has been observed to occur in a small percentage of premenopausal women with fibroids (22, 31, 154). Alternatively, pregnancies in the interval between previous diagnosis and study ultrasound may have eliminated fibroids (40, 41), which could also explain why parity was associated with lower specificity in this analysis. Parity was also positively associated with self-report sensitivity in this analysis, which may reflect an increased opportunity for diagnosis because of pregnancy-related ultrasound examinations.

For Aim 2, data from the Agricultural Health Study, a large cohort of pesticide applicators and their spouses, were used to investigate self-reported fibroid diagnosis and its relationship with different pesticide use patterns as well as its association with use of

hormonally active pesticides that were identified *a priori* based on a literature review. I hypothesized that there would be an increase in the odds of fibroids by pesticide type (residential; more common, lower toxicity agricultural; restricted use, higher toxicity agricultural) and number of pesticides used. Although women who reported residential pesticide use did not have increased odds of fibroids, there was a small positive trend across categories of agricultural pesticide use (compared to women who did not use any pesticides).

I was also interested in pesticides that showed evidence of endocrine-disrupting activity among prior *in vitro* or *in vivo* experiments. I hypothesized that use of pesticides which showed evidence of estrogenic/progestagenic activity, effects on follicle-stimulating or luteinizing hormones, direct effects on the ovary or uterus, or estrus cycle disruption would be positively associated with uterine fibroids. A review of the toxicology literature resulted in the selection of 10 hormonally active pesticides. I found slightly elevated odds of fibroids among women who used hormonally active pesticides compared to those who did not use any pesticides. However, the odds associated with the use of hormonally active pesticides were comparable when the referent group consisted of women who used agricultural pesticides other than those deemed hormonally active. I therefore investigated specific pesticides and chemical class groupings in an attempt to determine whether any of those not originally identified as hormonally active may be associated with fibroids. I found elevated odds among organophosphate pesticide users and specifically, among women who used coumaphos, malathion, or parathion.

None of these pesticides were identified *a priori* as hormonally active, in part because there were too few experimental studies of their endocrine disrupting effects to make a determination. Those that did examine endocrine disruption were limited mainly to effects

on estrogen. Coumaphos, malathion, and parathion are organophosphate insecticides that act through inhibition of the enzyme acetylcholinesterase (168). Because of their mechanism of action, the majority of research on organophosphates has focused on their possible neurotoxic effects. I did not find any published reports of studies examining endocrine disrupting effects of coumaphos. Malathion showed no evidence of estrogenicity *in vitro* (103, 169-171) nor in treated rats or cattle (172, 173), but did exhibit ovotoxic effects in rats (174). Similarly, parathion was not estrogenic in two *in vitro* assays (103, 170) but was found to have effects on ovarian morphology and estrus cycle disruption in rats (175-177). Only two published reports were found related to aldicarb, a carbamate pesticide that was negatively associated with odds of fibroids in this analysis. In one study of human breast and endometrial cancer cells, aldicarb inhibited estradiol and progesterone activity (178), which could explain our negative association of this pesticide with fibroids. However, aldicarb did not show estrogenic or anti-estrogenic activity in another study using estrogen-receptor responsive cell lines (108). Carbon tetrachloride, that was identified as hormonally active based on my review, had the strongest association with fibroid diagnosis among all individual pesticides. The evidence for possible hormonal activity comes mainly from studies in rats that demonstrated increased serum estradiol and progesterone levels, decreased estrone metabolism, and increased levels of these hormones in the uterus following exposure (164-166, 179, 180). However, many of these studies were conducted at doses that cause liver toxicity, raising the question of whether these findings can be extrapolated to humans with more chronic, low-level exposures.

I employed an outcome misclassification correction approach (12) to assess the degree to which effect estimates may have been biased by the use of self-report to identify

women with and without fibroids. The "base" model for outcome correction assumed moderate sensitivity for women who reported having had a hysterectomy, and age-varying sensitivity for those who did not. Specificity was assumed to be high for both groups. Although the base model correction resulted in odds ratios that were further away from the null than the uncorrected odds ratios, they were not drastically different. This is because half of the women who self-reported a fibroid diagnosis also reported having had a hysterectomy; the net result of setting a higher sensitivity value for these women is to lessen the degree of misclassification relative to models in which this distinction was not made. Underscoring this point is the fact that the correction model in which both sensitivity and specificity varied with age, regardless of hysterectomy history, yielded odds ratios that were 2 to 3 times higher than the uncorrected odds ratios (when odds ratios were above the null). Specificity seemed to be the most influential in determining the magnitude of the change in corrected compared to uncorrected odds ratios. Changing sensitivity estimates had less impact on resulting odds ratios when specificity was high (>0.95).

6.2 STRENGTHS AND LIMITATIONS

6.2.1 Aim 1

Only two studies have assessed the accuracy of self-reported fibroid diagnosis, but only focused on validating positive self-reports (37, 158). Results from the analysis of Right from the Start and the Uterine Fibroid Study represent the most detailed assessment of self-report validity to date. These studies provided relatively large, ethnically diverse study populations in which to examine possible determinants of reporting accuracy. Furthermore, the measurement of "true" fibroid status was based on results from ultrasound examinations performed by trained and certified sonographers. Ultrasound has high sensitivity (99%) and

specificity (91%) when compared to histological results, which are considered the gold standard (155).

There are special characteristics of RFTS and UFS populations that may limit their applicability to the AHS or other populations. RFTS includes volunteers who are pregnant or trying to conceive. These women may be more likely to have regular medical care or be more attentive of their reproductive health, which could improve their reporting accuracy over the general population. Similarly, participants in the UFS might be more likely to get regular medical care because they were members of a health plan.

Because fibroids can affect fertility, RFTS participants may have a lower prevalence of fibroids by virtue of the fact that they are pregnant. Resulting validity estimates may therefore not be representative of those in a study population with higher disease prevalence. It is possible that ultrasound examinations during pregnancy may not be as accurate because they might miss smaller fibroids, or mistake focal contractions for tumors. However, because one of the aims of RFTS2/3 was specifically focused on fibroids in pregnancy, sonographers were well-trained in interpreting ultrasound images and performed repeated measurements on each tumor to reduce measurement error. The increased hormone levels during pregnancy could cause fibroids to grow (156), in which case the pregnancy ultrasound results might detect fibroids that were too small to be detected prior to pregnancy. This is unlikely because the RFTS ultrasounds were performed early (6 to 13 weeks' gestation) in the first trimester, and there was no difference in fibroid prevalence by gestational age at ultrasound (28).

6.2.2 Aim 2

To my knowledge, this is the first epidemiologic investigation of the relationship between pesticide use and uterine fibroid prevalence. Pesticides are a source of exposure to potentially endocrine-disrupting chemicals, which could alter the hormonal milieu in a way that affects uterine fibroid development. The Agricultural Health Study provided an opportunity to examine specific pesticides and use patterns among a large cohort of women who, on average, have higher exposures than the general population. The fact that uterine fibroids affect such a large percentage of reproductive-age women requires both a large sample size and high exposure prevalence in order to detect statistically significant associations if they exist.

Because outcome status was ascertained by self-report, a substantial proportion of women with fibroids were likely misclassified as non-cases. In order to address this bias, I used a correction method in the logistic regression models to adjust for imperfect outcome measurement. Unlike simpler methods used previously in the Nurses' Health Study (44, 158), this approach allowed for varying sensitivity and specificity estimates (i.e., differential misclassification with respect to covariate patterns) that were evident in the analysis from Aim 1. Unlike Bayesian approaches, however, it assumes that sensitivity and specificity are known with certainty.

There are some considerations with regard to the applicability of the data used to inform assumptions about self-report sensitivity and specificity. There are likely differences between women who live and work on farms and women who enroll in a study of pregnancy (as in RFTS) or who are members of a health plan in an urban area (as in the UFS); to what extent these differences might influence the accuracy of women's self-reports cannot be

completely measured. Additionally, the AHS analysis population included older, as well as postmenopausal, women for whom information on validity of self-report was not available from Aim 1. Results of the sensitivity analyses conducted in the AHS provided some information about the extent to which assumptions about reporting accuracy in these women might influence the results, but did not clearly indicate one set of assumptions over another. The availability of hysterectomy data in the AHS helped in this respect, since it is a reasonable assumption that women who have had gynecological surgery are better reporters of fibroid status. In fact, the prevalence of self-reported fibroids was almost 40% among women who had hysterectomies, compared to 9% among women who did not have a hysterectomy.

Additional limitations of this analysis should be noted. Although the AHS collected information on many different pesticides, the exposure information was lacking in terms of magnitude, duration, frequency, and timing. The exposure measures were based on women's report of ever/never pesticide use, and may not reflect exposures that preceded fibroid diagnosis. The lack of a clear temporal relationship between exposure and outcome raises the possibility that any associations found in the analysis could be non-causal.

Recall of exposure history may be unreliable. It is unlikely that recall of pesticide use is different among women with and without fibroids because outcome data were gathered after information on pesticide use was collected. However, recall of lifetime exposure history may be progressively worse as women age, and age up to menopause is related to fibroid prevalence. Excluding older women in the AHS may have reduced errors, but the possibility of recall bias does exist. A recent reliability analysis of self-reported household pesticide use among participants of a case-control study in Italy showed good agreement—

approximately 75% for indoor pesticide use and over 90% for outdoor pesticide use—for both duration and frequency of lifetime pesticide use among a non-occupationally exposed group (181). Although there are no empirical data available, one would suspect that farm women are better reporters than the general population. Validation studies conducted among pesticide applicators in the AHS have demonstrated that they provide accurate information on pesticide use, farming activities, and lifestyle factors (167, 182, 183).

Information on other sources or modifying factors of pesticide exposure was not included in the proposed analysis, and could lead to exposure misclassification. Wives of farmers can be exposed to pesticides through spray drift or carry-home contamination from their husbands, among other sources (184). Women who engage in field work on the farm will also be indirectly exposed to pesticides even if they do not personally apply them. In addition, women who apply pesticides may have different behaviors that affect the amount of pesticide to which they are exposed. Information on the use of personal protective equipment, for example, was available for the female pesticide applicators but not for the farmers' wives. Although the AHS includes data on the husband's pesticide use and limited data on household hygiene factors that might impact residential contamination, the investigation of indirect exposures was not part of the proposed dissertation research. Women could therefore have been misclassified as unexposed when they may have been exposed through other routes, leading, in general, to an attenuation of any associations detected.

Finally, there were some differences between participants and nonparticipants in the Phase II follow-up which could lead to selection bias. Women who did not participate were slightly younger, less educated, from North Carolina, and more likely to have reported never

personally mixing or applying pesticides in the Enrollment Questionnaire. Follow-up participation rates were also lower among women who enrolled as applicators compared to applicators' wives. If pesticides are associated with fibroid diagnosis, the exclusion of pesticide applicators (who may have higher exposures) or of women who never mixed or applied pesticides could have resulted in attenuated estimates. Without knowing the prevalence of fibroids in follow-up nonrespondents, however, it is difficult to say with certainty how these exclusions may have affected our results.

6.3 IMPLICATIONS AND CONCLUSIONS

Uterine fibroids develop in the majority of reproductive-age women and "by the time of menopause in America, the presence of uterine fibroids seems to be the norm, not the exception" (185). Although these are benign tumors, they account for significant symptoms that affect women's quality of life; these include abnormal uterine bleeding, pain, frequent urination, infertility, and pregnancy complications (186). Despite the increasing availability of other treatment methods, fibroids remain the leading indication for hysterectomy in the United States. Over 200,000 hysterectomies for uterine fibroids are performed annually in this country, and between 3 to 5 billion dollars are spent in their diagnosis and treatment (11, 187).

Epidemiologic investigation of risk factors related to uterine fibroids is subject to challenges mainly related to disease detection and outcome misclassification. The ideal prospective design—one that enrolls a defined population of women determined to be fibroid-free at enrollment and then follows them over time—would be less prone to disease misclassification and better able to tie exposure histories to fibroid onset and development through regular screening of its study population. The gap between ideal designs and

practical approaches is a real one, however. A prospective cohort would have to be quite large to be adequately powered to examine factors such as exposures to agricultural pesticides, which are rare in the general population. The utility of ultrasound screening, although relatively non-invasive, could be costly (depending on the geographic dispersion of the cohort) and prone to loss to follow-up. Furthermore, established cohorts such as the Nurses' Health Study, the Black Women's Health Study, and the Agricultural Health Study have a wealth of information on potential risk factors and exposures. The reality of working within the limitations of existing, sometimes imperfect, studies underscores the importance of understanding how these limitations might affect the results. When researchers mention measurement error there is a tendency to assume first that it is nondifferential, and second that it will bias towards the null. The results presented here suggest that, at least for uterine fibroids, measurement error is related to factors that may be of interest in and of themselves or may be related to factors of interest, which would lead to biased estimates. The information on sensitivity and specificity provides a good starting point to at least assess the impact of measurement error on the magnitude and direction of bias. The findings also lend support to some of approaches that have been used in the past to minimize misclassification, for example, by limiting analyses to younger women (37, 157).

In conclusion, results of this dissertation research suggest that the use of agricultural pesticides may be related to uterine fibroids in this population of farm women. Significantly elevated odds were seen in women who used the five most common, generally less toxic pesticides (as classified by the US EPA) (188). Odds were further elevated among women who used less common, typically restricted-use, pesticides. I illustrated the use of a relatively easy-to-use algorithm to assess the influence of bias arising from use of self-report

to classify fibroid status. Whether or not this approach results in an actual "correct" effect estimate largely depends on if, and how, pesticides increase fibroid risk. There is little prior information to indicate how hormones, whether endogenous or exogenous, act to either initiate these tumors or cause them to progress. Given that the majority of reproductive-age women have fibroids, the more relevant question in terms of public health burden might be: What causes some women with fibroids to require treatment? The uncorrected odds ratio—using self-reported diagnosis to define outcome—may be "correct" if one assumes that pesticide exposure causes existing fibroids to grow to the point of becoming symptomatic, and that all symptomatic fibroids are diagnosed and all diagnosed fibroids are symptomatic. The difficulty arises from the fact that although symptoms such as heavy bleeding tend to increase with fibroid size, even small fibroids can cause heavy bleeding (189). Furthermore, a clinical diagnosis does not necessarily mean that a woman had large and/or symptomatic fibroids. In some women, fibroids are detected incidentally rather than because of symptoms (21, 69) and conversely, some women with symptoms do not necessarily get diagnosed (189, 190). The purpose of the outcome correction method was simply to obtain a better measure of fibroid prevalence (diagnosed or undiagnosed, large or small, symptomatic or asymptomatic). Due to the cross-sectional nature of the data, as well as limitations in our knowledge about fibroid etiology in general, it was impossible to speculate as to the relation between pesticides and fibroid onset or growth.

Pesticides are ubiquitous and many persist in the environment; several have been demonstrated to be endocrine disruptors (191). Women living on farms have increased opportunities for pesticide exposure through field work, personally mixing or applying pesticides, proximity to farming operations, and carry-home contamination by their husbands

(149). Unlike other established risk factors for fibroids that cannot be changed (e.g., age or race), pesticide use is a modifiable exposure. *In vivo* or *in vitro* testing for endocrine-disrupting effects of the pesticides associated with fibroid diagnosis in this analysis would be a first step to confirm these findings, explore possible mechanisms of action, and provide a basis for designing future epidemiologic research.

APPENDIX A

Literature Review on Endocrine Disrupting Activity of Pesticides in the Agricultural Health Study

In order to determine the specific pesticides of interest as hormonally active, I expanded on work previously performed by Sherry Farr (192), for which she conducted an extensive literature review. Between May and June of 2003, Farr searched the National Library of Medicine's PubMed database using each pesticide name entered singly along with the following keywords: hormone, hormone antagonist, ovary, ovarian disease, estrus cycle, uterus, uterine disease, genitalia, genital disease, reproduction, and endocrine disease. Results of this literature search were used in a weight of evidence review for endocrine disrupting effects of each pesticide. Articles considered were those reporting on *in vitro* assays or *in vivo* outcomes in animals, and results were grouped according to specific hormones (estrogen, androgen, thyroid, progesterone, FSH/LH) and outcomes (ovarian effects, estrus cycle effects). Within each of these groups, Farr assigned a category based on the weight of evidence, to create an "endocrine disruption profile" for each pesticide:

- Lack of data: only one or no published papers for a specific hormone/outcome
- No effect: majority of papers show no evidence of an effect on specific hormone or outcome
- Conflicting evidence: evidence for and against specific types of endocrine disruption
- Possible or probable: majority of studies indicate an effect on the specific hormone/outcome. The distinction between "possible" and "probable" was based on the weight and strength of evidence.

My approach to expanding on this work was to utilize the same search strategy while limiting the results to articles added to PubMed from 2003 to July 2009 (the date of my search). In assessing the weight of evidence, however, my goal was to answer the following:

1. For those pesticides previously classified as "possible" or "probable" disruptors of specific hormones or as "no effect," is there updated evidence which conflicts with this categorization? (For example, have there been additional reports on a "no effect" pesticide that would push it into the "conflicting evidence" category?)
2. For those pesticides previously classified as "conflicting evidence" or "lack of data," are there additional publications which would strengthen the weight of evidence either for or against endocrine disruption?
3. Finally, do changes in the weight of evidence regarding specific hormones or outcomes result in an overall endocrine profile that might be relevant in uterine fibroid development? These would be pesticides that increase estrogen or progesterone levels directly, or indirectly by affecting follicle-stimulating and luteinizing hormones or causing ovarian/estrus cycle effects.

The results of the literature search performed in 2003 and appended in 2009 are listed in Table A.1. Of the 49 unique pesticides asked about in the Agricultural Health Study, 10 did not have any published studies. As a group, organochlorines are the most well-researched, with a total of 156 citations to date. Pyrethroids seem to have received much more attention in recent years, with a total of 26 publications found between 2003 and 2009 (compared to only eight prior to 2003).

Results of the weight of evidence review and endocrine disruption profile appear in Table A.2. Based on the updated literature review, some changes were made to the overall

pesticide classifications with regard to specific hormones or outcomes. This table lists, for each endpoint, the number of publications from *in vitro* or *in vivo* studies. The "+" sign after each number indicates those studies that show evidence of any effect; those marked with "-" are studies that did not show any effect on the endpoint.

For purposes of this dissertation research, I examined in more detail the published results for any pesticide that had probable, possible, or conflicting data for estrogen, progesterone, or FSH/LH disruption or ovarian/uterine and estrus cycle effects. After reviewing all of the published results, I selected 10 pesticides to include as an exposure variable in my main analysis. These pesticides are listed in Table A.3, and sorted in order of the strength of the overall endocrine profile with regard to relevance for uterine leiomyomata. In this table, a "+" sign precedes results that suggest the pesticide is a hormone agonist or mimic, a "-" sign, those that suggest the pesticide is a hormone antagonist, and "ne" for no effect.

Table A.1 Results of literature search on endocrine disruption, ovarian, or estrus cycle effects of pesticides in the Agricultural Health Study

Pesticide Name	Year of first use	Year of last use	Number of relevant studies published: Up to June 2003[a]	Number of relevant studies published: June 2003-August 2009
Organochlorines				
Aldrin	1950	1974; 1987[b]	5	1
Chlordane	1948	1983; 1988[b]	7	2
DDT	1948	1972	34	17
Dieldrin	1951	1974; 1987[b]	22	3
Heptachlor	1952	1988[c]	9	3
Lindane	1947	[d]	35	5
Toxaphene	1948	1976; 1990[b]	12	1
Organophosphates				
Chlorpyrifos	1965		5	9
Coumaphos	1958		0	0
Diazinon	1948		4	3
Dichlorvos/DDVP	1948		2	3
Fonofos	1967	1998	0	2
Malathion	1955		9	3
Parathion	1954		7	5
Phorate	1959		0	2
Terbufos	1974		0	0
Trichlorfon	1954		2	3
Carbamates				
Aldicarb	1970		1	1
Carbaryl	1947		8	5
Carbofuran	1969	[e]	4	3
Benomyl	1969	2002	9	3
Thiocarbamates				
Butylate	1967		1	0
EPTC	1958		0	0
Mancozeb	1962		5	3
Maneb	1952		3	2
Ziram	1948		1	0
Phenoxy herbicides				
2,4-D	1948		3	3

table continues

Table A.1 Results of literature search on endocrine disruption, ovarian, or estrus cycle effects of pesticides in the Agricultural Health Study (cont.)

Pesticide Name	Year of first use	Year of last use	Number of relevant studies published: Up to June 2003 [a]	Number of relevant studies published: June 2003-August 2009
2,4,5-TP	1956	1984	0	0
2,4,5-T	1948	1985	1	1
Triazines				
Atrazine	1959		29	9
Cyanazine	1971	1999	2	0
Metribuzin	1972		2	0
Anilides				
Alachlor	1969		9	1
Metolachlor	1976		0	2
Dinitroanilines				
Pendimethalin	1974		1	0
Trifluralin	1963		3	2
Phthalimides				
Captan	1951		1	1
Chlorothalonil	1966		3	0
Others				
Aluminum phosphide	1958		0	0
Carbon tetrachloride	1948	1985	14	1
Chlorimuron ethyl	1985		0	0
Dicamba	1956		0	0
Ethylene dibromide	1948	1983	1	0
Glyphosate	1974		4	1
Imazethapyr	1989		0	0
Metalaxyl	1979		0	0
Methyl bromide	1947		4	0
Paraquat	1964		0	0
Permethrin / pyrethroids	1977		8	26
Petroleum oil/distillate	1947		2	0

[a] Table adapted from Farr (192), including results of previous literature search conducted between May and June 2003.

[b] First date is EPA partial ban; second date is comprehensive ban.

[c] Only currently approved commercial use is for fire-ant control around power lines.

[d] EPA cancelled in 2007; last year of use in 2009.

[e] EPA banned all but limited crop uses in 2006 with 4-year phase-out.

Table A.2 Classification of endocrine disrupting potential of pesticides in the Agricultural Health Study based on toxicological literature[a]

Pesticide Name and Classification	Estrogen	Androgen	Thyroid	Progesterone	FSH/LH	Ovarian/ Uterine	Estrus cycle
Organochlorines							
Aldrin	Conflicting *in vitro:* 2 - *in vivo:* 1 + 1 -	Possible *in vitro:* 0 *in vivo:* 2 +	No data	No data	Lack of data *in vitro:* 0 *in vivo:* 1 +	Lack of data 1 -	Conflicting 1 + 1-
Chlordane	Possible *in vitro:* 2 + 2 - *in vivo:* 2 +	Lack of data *in vitro:* 0 *in vivo:* 1 +	Lack of data *in vitro:* 0 *in vivo:* 1 +	No data	No data	Lack of data 1 -	Conflicting (from lack of data) 1 + 1-
DDT	Probable *in vitro:* 13 + 1 - *in vivo:* 5 +	Probable *in vitro:* 7 + 1 - *in vivo:* 7 + 2 -	Possible *in vitro:* 3 + *in vivo:* 0	Probable *in vitro:* 9 + *in vivo:* 2 +	Conflicting *in vitro:* 2 + *in vivo:* 2 + 2 -	Probable 4 + 1 -	Probable 5 + 1 -
Dieldrin	Conflicting *in vitro:* 5 + 5 - *in vivo:* 1 + 2 -	Possible *in vitro:* 3 + 1 - *in vivo:* 2 +	No data	No effect *in vitro:* 0 *in vivo:* 2 -	Lack of data *in vitro:* 0 *in vivo:* 1 +	Conflicting 1 + 1 -	No effect 2 -
Heptachlor	Conflicting (from possible) *in vitro:* 1 + 3 - *in vivo:* 2 + 1 -	Conflicting (from lack of data) *in vitro:* 0 *in vivo:* 1 + 1 -	Conflicting *in vitro:* 0 *in vivo:* 1 + 1 -	Possible *in vitro:* 0 *in vivo:* 3 +	Lack of data *in vitro:* 0 *in vivo:* 1 +	Conflicting 1 + 2 -	Conflicting (from possible) 2 + 1 -
Lindane	Conflicting *in vitro:* 3 + 5 - *in vivo:* 10 + 3 -	Probable *in vitro:* 1 + 1 - *in vivo:* 11 + 2 -	Conflicting *in vitro:* 0 *in vivo:* 3 + 3 -	Probable *in vitro:* 3 + *in vivo:* 3 +	Probable *in vitro:* 0 *in vivo:* 5 + 1 -	Conflicting (from probable) 3 + 2 -	Probable 6 +
Toxaphene	Possible *in vitro:* 9 + 3 - *in vivo:* 0	Lack of data *in vitro:* 0 *in vivo:* 1 +	Possible *in vitro:* 2 + *in vivo:* 0	No data	No data	No data	No data

table continues

Table A.2 Classification of endocrine disrupting potential of pesticides in the Agricultural Health Study based on toxicological literature[a] (cont.)

Pesticide Name and Classification	Estrogen	Androgen	Thyroid	Progesterone	FSH/LH	Ovarian/ Uterine	Estrus cycle
Organophosphates							
Chlorpyrifos	Conflicting (from no effect) *in vitro:* 3 + 4 - *in vivo:* 1+ 3 -	Conflicting (from lack of data) *in vitro:* 1 + *in vivo:* 1 + 1 -	Possible (from lack of data) *in vitro:* 1+ *in vivo:* 3 + 1 -	No data	Lack of data *in vitro:* 0 *in vivo:* 1 -	Conflicting (from no effect) 1 + 2 -	Lack of data 1 -
Diazinon	Conflicting (from lack of data) *in vitro:* 2 + 2 - *in vivo:* 0	Lack of data *in vitro:* 0 *in vivo:* 1 +	No data	No data	No data	No effect 2 -	No data
Dichlorvos	Lack of data *in vitro:* 1 - *in vivo:* 0	Conflicting *in vitro:* 1 + 1- *in vivo:* 2 -	No data	No data	Lack of data *in vitro:* 0 *in vivo:* 1 -	Lack of data 1 +	Lack of data 1 +
Fonofos	Lack of data *in vitro:* 1 + *in vivo:* 0	Lack of data *in vitro:* 1 + *in vivo:* 0	No data	No data	No data	No data	No data
Malathion	No effect *in vitro:* 4 - *in vivo:* 2 -	Conflicting *in vitro:* 0 *in vivo:* 2 + 2 -	Conflicting *in vitro:* 0 *in vivo:* 1 + 1 -	Lack of data *in vitro:* 0 *in vivo:* 1 +	Conflicting *in vitro:* 0 *in vivo:* 2 + 1 -	Conflicting (from no effect) 1 + 1 -	Lack of data
Parathion	No effect *in vitro:* 3 - *in vivo:* 0	Probable (from possible) *in vitro:* 2 + *in vivo:* 4 +	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	No data	Possible 3 +	Possible 2 +
Phorate	Lack of data *in vitro:* 1 + *in vivo:* 0	Lack of data *in vitro:* 1 + *in vivo:* 0	No data	No data	No data	No data	No data
Trichlorfon	No data	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	Possible (from lack of data) *in vitro:* 2 + *in vivo:* 0	No data	Possible 2 +	No data

table continues

Table A.2 Classification of endocrine disrupting potential of pesticides in the Agricultural Health Study based on toxicological literature[a] (cont.)

Pesticide Name and Classification	Estrogen	Androgen	Thyroid	Progesterone	FSH/LH	Ovarian/ Uterine	Estrus cycle
Carbamates							
Aldicarb	Conflicting (from lack of data) *in vitro:* 1 + 1 - *in vivo:* 0	No data	No data	Lack of data *in vitro:* 1 + *in vivo:* 0	No data	No data	No data
Carbaryl	Conflicting (from lack of data) *in vitro:* 2 + 1 - *in vivo:* 0	No effect *in vitro:* 1 - *in vivo:* 1 -	Possible (from lack of data) *in vitro:* 1 + *in vivo:* 1 +	Lack of data *in vitro:* 1 + *in vivo:* 0	Lack of data *in vitro:* 0 *in vivo:* 1 +	Possible 3 +	Lack of data 1 +
Carbofuran	No effect *in vitro:* 5 - *in vivo:* 0	Conflicting (from lack of data) *in vitro:* 1 + *in vivo:* 1 -	Conflicting *in vitro:* 0 *in vivo:* 1 + 1 -	No data	Lack of data *in vitro:* 0 *in vivo:* 1 -	Possible 2 +	Possible 2 +
Benomyl	Conflicting *in vitro:* 1 + 2 - *in vivo:* 2 + 1 -	Conflicting (from no effect) *in vitro:* 0 *in vivo:* 1 + 3 -	Lack of data *in vitro:* 0 *in vivo:* 1 -	No effect *in vitro:* 0 *in vivo:* 2 -	Conflicting *in vitro:* 0 *in vivo:* 1 + 3 -	Conflicting 2 + 2 -	No data
Thiocarbamates							
Butylate	No data	No data	No data	No data	No data	Lack of data 1 +	No data
Mancozeb	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	Conflicting (from probable) *in vitro:* 3 + 1 - *in vivo:* 0	No data	No data	Possible 3 +	Probable 4 +
Maneb	No data	No data	Conflicting (from possible) *in vitro:* 1 - *in vivo:* 3 +	No data	No data	Lack of data 1 +	No data

table continues

Table A.2 Classification of endocrine disrupting potential of pesticides in the Agricultural Health Study based on toxicological literature[a] (cont.)

Pesticide Name and Classification	Estrogen	Androgen	Thyroid	Progesterone	FSH/LH	Ovarian/ Uterine	Estrus cycle
Ziram	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	No data	No data	No data	No data	No data
Phenoxy Herbicides							
2,4-D	No effect *in vitro:* 3 - *in vivo:* 1 -	Conflicting (from lack of data) *in vitro:* 1 + 1 - *in vivo:* 0	Lack of data *in vitro:* 0 *in vivo:* 1 +	Lack of data *in vitro:* 0 *in vivo:* 1 +	Lack of data *in vitro:* 0 *in vivo:* 1 -	No data	No data
2,4,5-T	Lack of data *in vitro:* 1 + *in vivo:* 0	Lack of data *in vitro:* 0 *in vivo:* 1 +	No data	No data	No data	No data	No data
Triazines							
Atrazine	Conflicting *in vitro:* 1+ 10 - *in vivo:* 9 + 2 -	Probable *in vitro:* 4 + *in vivo:* 6 +	Conflicting *in vitro:* 2 + *in vivo:* 2 + 2 -	Probable *in vitro:* 0 *in vivo:* 4 + 1 -	Probable *in vitro:* 0 *in vivo:* 6 +	Probable 6 +	Probable 10 + 1 -
Cyanazine	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	No data	No data	No data	No data	Lack of data 1 +
Metribuzin	No data	No data	Possible *in vitro:* 0 *in vivo:* 2 +	No data	No data	No data	No data
Anilides							
Alachlor	Conflicting (from possible) *in vitro:* 3 + 2 - *in vivo:* 0	No data	Possible *in vitro:* 0 *in vivo:* 3 +	No data	No data	Possible 2 +	No data
Metolachlor	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	Lack of data *in vitro:* 0 *in vivo:* 1 -	No data	No data	No data	No data

table continues

Table A.2 Classification of endocrine disrupting potential of pesticides in the Agricultural Health Study based on toxicological literature[a] (cont.)

Pesticide Name and Classification	Estrogen	Androgen	Thyroid	Progesterone	FSH/LH	Ovarian/ Uterine	Estrus cycle
Dinitroanilines							
Pendimethalin	No data	No data	Lack of data *in vitro:* 0 *in vivo:* 1 +	No data	No data	No data	No data
Trifluralin	No effect (from Lack of data) *in vitro:* 2 - *in vivo:* 0	Lack of data *in vitro:* 1 - *in vivo:* 0	Conflicting *in vitro:* 0 *in vivo:* 2 + 1 -	No data	Lack of data *in vitro:* 0 *in vivo:* 1 +	No data	No data
Phthalimides							
Captan	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	No data	No data	No data	Lack of data 1 +	No data
Chlorothalonil	No effect *in vitro:* 2 - *in vivo:* 0	Lack of data *in vitro:* 1 - *in vivo:* 0	No data	No data	No data	Lack of data 1 -	No data
Others							
Carbon tetrachloride	Possible *in vitro:* 0 *in vivo:* 6 + 2 -	Conflicting *in vitro:* 0 *in vivo:* 1 + 3 -	Probable *in vitro:* 0 *in vivo:* 5 +	Probable *in vitro:* 0 *in vivo:* 4 +	Conflicting (from lack of data) *in vitro:* 0 *in vivo:* 1 + 1 -	Possible 2 +	Possible 2 +
Ethylene dibromide	No data	No data	No data	No data	No data	No data	Lack of data 1 +
Glyphosate	No data	Lack of data *in vitro:* 0 *in vivo:* 1 +	No data	Lack of data *in vitro:* 1 + *in vivo:* 0	No data	Conflicting 1 + 1 -	No data
Methyl bromide	No data	Lack of data *in vitro:* 0 *in vivo:* 1 +	No data	No data	No data	No effect 2 -	Lack of data 1 -

table continues

Table A.2 Classification of endocrine disrupting potential of pesticides in the Agricultural Health Study based on toxicological literature[a] (cont.)

Pesticide Name and Classification	Estrogen	Androgen	Thyroid	Progesterone	FSH/LH	Ovarian/ Uterine	Estrus cycle
Permethrin, cypermethrin, fenvalerate	Conflicting *in vitro:* 8 + 4 - *in vivo:* 3 -	Possible (from conflicting) *in vitro:* 5 + 1 - *in vivo:* 3 + 2 -	No data	Possible (from no effect) *in vitro:* 5 + 2 - *in vivo:* 1 + 1 -	Possible (from lack of data) *in vitro:* 0 *in vivo:* 2 +	Conflicting (from no effect) 3 + 1 -	Lack of data 1 +
Petroleum oil	No data	No data	No data	No data	No data	Possible 2 +	Lack of data 1 +

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone

[a] Table adapted from Farr (192), including results of previous literature search conducted between May and June 2003. Results shown display the classification based on the updated evidence, the previous classification based on the evidence up to June 2003 (if different), the type of test (*in vitro* / *in vivo*), and the number of publications indicating an effect (+) or no effect (-). There was no published literature for the following pesticides that are excluded from this table: coumaphos, terbufos, EPTC, 2,4,5-TP, aluminum phosphide, chlorimuron ethyl, dicamba, imazethapyr, metalaxyl, and paraquat.

Table A.3 Pesticides selected as candidates for assessing the association with uterine fibroid prevalence

Pesticide name	Reference	Evidence[a]	
DDT			
Mainly estrogenic, some metabolites anti-estrogenic, dependent on metabolite, dose, duration	(193)	+	o,p'-DDT activated E-sensitive gene expression
	(103)	+	*p,p'*-DDT, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, and technical-grade DDT mixture increased cell proliferation in E-SCREEN
	(114)	+	*o,p'*-DDT and technical-grade DDT uterotropic in several early studies (review)
	(114)	+	*o,p'*-DDT competes for binding to ER; *p,p'*-DDT relatively inactive in several studies (review)
	(114)	+/ne	*o,p'*-DDT advanced vaginal opening in rats exposed neonatally; DDT increased time-to-pregnancy; some studies showed no effect on fertility and fecundity (review)
	(110)	+	*o,p'*-DDT and *p,p'*-DDE showed estrogenic activity in YES, a competition binding assay, and the MCF-7 cell luciferase assay
	(115)	+	DDT increased uterine weight in mice
	(194)	+	*o,p'*-DDT initiated embryo implantation and maintained pregnancy; administration at mating time or during fertilization caused embryo loss
	(108)	+	ERα and ERβ agonist in HELN cell line
	(109)	+	*p,p'*-DDT & *p,p'*-DDE ERα agonists in yeast assay
	(107)	+	*p,p'*-DDE ↑ cell proliferation in CAMA-1 cells in presence of estrogen & androgens
	(195)	ne	no effect on cell proliferation of HEECs
	(111)	+	*p,p'*-DDT and *p,p'*-DDE ↑ estradiol secretion by granulosa and theca cells
	(196)	-	*p,p'*-DDT ↓ serum estradiol *in vivo*
	(197, 198)	+	*p,p'*-DDT & *p,p'*-DDE ER agonists in transgenic male mice
	(112, 113)	+/-	*o,p'*-DDT & metabolites showed antiestrogenic action in theca & granulosa cells; *p,p'*-DDT & metabolites showed estrogenic action; all except *o,p'*-DDT increased estradiol secretion
	(199)	-	*o,p'*-DDT & *p,p'*-DDT & their metabolites ↓ estradiol secretion in human placental explants
Progesterone agonist & antagonist effects, dependent on metabolite, dose, duration	(109)	-	*p,p'*-DDT & *p,p'*-DDE PR antagonists in yeast assay
	(196)	+	*p,p'*-DDT ↑ serum progesterone *in vivo*
	(200)	-	DDT ↓serum progesterone *in vivo*
	(113, 201)	+/-	*p,p'*-DDT & *o,p'*-DDT ↓ progesterone secretion in JEG-3 cells; *p,p'*-DDE & *o,p'*-DDE ↑ progesterone secretion
	(199, 202)	+/-	*p,p'*-DDE, *o,p'*-DDT & *o,p'*-DDE ↑ progesterone secretion in human placental explants, but ↓secretion after long-term exposure
	(170, 203)	-	DDE, *o,p'*-DDT & *p,p'*-DDT ↓progesterone synthesis in granulosa cells

table continues

Table A.3 Pesticides selected as candidates for assessing the association with uterine fibroid prevalence (cont.)

Pesticide name	Reference	Evidence [a]	
	(204)	-	various metabolites ↓ progesterone-induced reporter gene activity
	(205)	+/-	DDT ↑ progesterone production in rat granulose cells at low concentrations, ↓ production at high concentration
No apparent effect on FSH/LH	(203)	-	DDE ↓ FSH-stimulated cAMP synthesis in granulosa cells
	(206)	ne	*o,p'*-DDT ↓ serum LH in male rats but no effect in females
	(196)	ne	*p,p'*-DDT no effect on FSH/LH males or females
Disrupts estrus cycle and ovotoxic	(115, 207-209)	+	persistent vaginal estrus
	(210)	+/ne	*o,p'*-DDT altered estrus cycling; *p,p'*-DDT had no effect
	(196)	ne	no effect on estrus cycling
	(200, 207, 211, 212)	+	various ovarian effects: ↓ ovulation rate, absence of corpora lutea, ↑follicular cysts, ovarian hypertrophy
	(213)	ne	no change in ovarian weight
Toxaphene			
Estrogenic effects	(121)	+	increased cell proliferation in E-SCREEN
	(134)	+	increased proliferation of uterine leiomyoma cells *in vitro*
	(214)	+	induced BRCA1-gene expression in MCF-7
	(215)	+	increased cell proliferation in MCF7, but no effect on ER or PR levels
	(216)	ne	did not bind ER, increase cell proliferation, or alter E2 catabolism in MCF-7 focus assay
	(217)	-	ER antagonistic activity in MCF-7 cells
	(218)	~+	no competition with 17β-estradiol for human or alligator ER binding when used alone; some displacement when used in combination with other pesticides
	(219)	~+	did not bind to the mouse uterine ER; weakly estrogenic in MCF-7 cells and yeast-based reporter gene assays
	(108)	+	ERα and ERβ agonist in HELN cell line
Carbon tetrachloride			
In females, indirectly increases estrogen levels	(165)	+	↑serum estradiol levels in rats with CCL4-induced cirrhosis
	(166)	+	inhibition of 17β-estradiol and estrone metabolism by rat liver microsomes *in vitro* and *in vivo*, increased uterine weight, and increased levels of these compounds in uterus
	(220)	+	inhibition of 17β-estradiol metabolism in liver-damaged rats
	(164)	n.e.	no change in serum estradiol levels in rats

table continues

Table A.3 Pesticides selected as candidates for assessing the association with uterine fibroid prevalence (cont.)

Pesticide name	Reference	Evidence [a]	
In females, indirectly increases progesterone levels	(165, 221)	+	↑serum progesterone levels in rats with CCL4-induced hepatocellular carcinoma
	(164)	+	↑serum progesterone levels in liver-damaged rats
	(222)	-	↓ serum and liver progesterone levels
May disrupt estrus cycle; ovo- and utero-toxicity	(179, 180)	+	rats went into persistent diestrus phase; ovaries and uterus had less weight and less vascularized
Permethrin or other pyrethroids			
Some pyrethroids may be estrogenic	(223)	ne	no effect of permethrin on estradiol metabolism by HLM
	(224)	+	↑ MCF-7 cell proliferation in presence of estradiol (permethrin & cypermethrin)
	(108)	+	ERα and ERβ agonist (Fenvalerate only, no effect of permethrin)
	(171)	ne	no effect of various pyrethroids in E-CALUX
	(225)	ne	no effect of various pyrethroids in three assays measuring ERα-mediated mechanisms
	(226, 227)	ne	no effect in uterotrophic assay (permethrin, fenvalerate)
	(228)	+/-	↓ estradiol production & ↑ proliferation of rat granulosa cells (fenvalerate)
	(229)	-/ne	fenvaerate & permethrin inhibited MCF-7 cell proliferation in presence of estradiol; cypermethrin had no effect; none of them acted on ER
	(230)	ne	perinatal fenvalerate exposure had no effect on plasma estrogen in rats
	(231, 232)	+/-	bifenthrin enantiomers had differential effects; lambda-cyhalothrin had estrogenic activity in E-SCREEN
	(169, 233)	+	↑ proliferation of MCF-7 cells, competed for binding to ER, induced pS2 mRNA gene expression (various pyrethroids with differential responses)
	(234)	+	fenvalerate & sumithrin estrogenic in two E-responsive human cell lines
	NIEHS, unpublished	+	↑ proliferation of Eker rat uterine leiomyoma cells (fenvalerate)
Anti-progestagen	(235)	-	↓ serum progesterone *in vivo* (fenvalerate)
	(228, 236, 237)	-	↓ FSH-stimulated progesterone production in rat and human granulosa cells
	(234, 238)	-	antiprogestagen activity in T47D (permethrin, fenvalerate, d-trans allethrin)
	(230)	ne	perinatal fenvalerate exposure had no effect on plasma progesterone
	(239)	-	fenvalerate ↓ progesterone production in MLTC-1
	(240)	ne	no effect on PR binding, no progesterone agonist/antagonist activity in T47D (various pyrethroids)
Limited data on FSH/LH	(241)	-	esfenvalerate inhibited afternoon LH surge in female rats

table continues

Table A.3 Pesticides selected as candidates for assessing the association with uterine fibroid prevalence (cont.)

Pesticide name	Reference	Evidence [a]	
May effect estrus cycle, ovo-and utero-toxic	(235)	+	fenvalerate ovotoxic
	(242)	-	tetramethrin ↓ absolute and relative uterine weights
	(243)	+	permethrin ↑ uterine weights
	(230)	+	perinatal fenvalerate exposure resulted in disruptions in estrus cycling & interaction with uterine weight
Lindane			
Conflicting evidence of estrogenic and anti-estrogenic activity	(103, 244, 245)	ne	no cell proliferation in E-SCREEN or other E-sensitive cell lines
	(108, 245-247)	ne	no competitive binding to ER
	(244)	+	some estrogen response element transcription
	(246)	-	inhibited formation of estradiol-receptor complex in rat uterus
	(248)	+	↑ serum estradiol in ewes
	(109)	-	ERβ antagonist in yeast assay
	(249)	~+	weak estrogenic activity *in vivo* when combined with estradiol
	(250, 251)	+	↑ uterine epithelial height and vaginal epithelial thickness or ↑ uterine weight *in vivo*
	(252, 253)	ne	no effect on estrogen levels/number of ER *in vivo*
	(254, 255)	-	delay in vaginal opening and ↓ uterine weight
	(256)	-	lack of implantation and fetal loss, reversed by E administration
	(257)	ne	no change in estradiol metabolism from *in utero* exposure, ↑ uterine weight at weaning but no difference at maturity
Progesterone antagonist	(258)	-	↓ FSH and TGFβ1-stimulated progesterone production
	(109)	-	PR antagonist in yeast assay
	(259, 260)	-	↓ luteal progesterone levels in ewes and serum progesterone in mice
LH disturbances	(248)	-	↓ basal LH concentrations in ewes
	(255)	-	↓ serum and pituitary LH, ↑ pituitary FSH in rats
	(259, 261)	+/ne	increased LH pulse frequency in ewe lambs, but nothing in adults
Disruption of ovarian/estrus cycles	(259)		↓ number of corpora lutea in ewes
	(200)		reduced ovulation rates in rabbits
	(262)	ne	no effect on ovulation rates in pigs
	(257)	ne	no histological changes in ovary from *in utero* exposure
	(251, 255)		prolonged proestrus phase, delayed ovulation in rats
	(254)		decreased number of days in proestrus
	(259, 263, 264)		↑length of estrus phase in ewes, rats

table continues

Table A.3 Pesticides selected as candidates for assessing the association with uterine fibroid prevalence (cont.)

Pesticide name	Reference	Evidence [a]	
Dieldrin			
May have weak estrogenic activity	(108)	+/-	ERα agonist and ERβ antagonist in HELN cell line
	(265)	+	change in ERα and β mRNA steady state levels
	(121, 266)	+	↑ cell proliferation in E-SCREEN and other assays with MCF-7 cells
	(219, 267)	ne	no effect on cell proliferation in some assays
	(121, 268)	~+	weak competitive binding to the ER
	(269)	ne	no estrogen responsive reporter gene activation of HeLN cells
	(218, 219, 267, 270)	ne	no competitive binding to human or alligator ER, or in other estrogen receptor assays
	(116)	ne	no effect on uterine weight or timing of vaginal opening
	(271)	-	↑ 17β-estradiol metabolism
Limited data on progesterone, FSH/LH	(272, 273)	ne	no change in serum progesterone in mice or pigs
	(274)	-?	short-term ↓ of FSH/LH levels in rats
Limited data, but appears to have no effect on ovary or estrus cyle	(116)	ne	no change in ovulation in rats
Alachlor			
May be weak estrogen	(108)	ne	no significant transactivational activity in ERα or ERβ
	(103)	ne	non-estrogenic in E-SCREEN
	(110)	~+	weak estrogenic effects in YES, the competition binding assay, and the MCF-7 cell luciferase assay
	(218)	~+	weak estrogen in competition binding assay
	(275)	+	like 17β-estradiol, has the ability to suppress tumor necrosis factor alpha (TNF)-induced apoptosis in ER-positive MCF-7 cell line
May be ovotoxic	(276, 277)	+	decreased ovarian weight in rabbits
Chlordane			
Estrogen agonist & antagonist effects	(278)	-	stimulated estrone metabolism; inhibited an increase in uterine weight
	(279)	-	delayed vaginal opening in mice exposed neonatally
	(280)	+	increased cell growth in two estrogen-responsive cell lines (MCF-7 and GH3); addition of growth factors had a somewhat additive effect
	(103)	ne	non-estrogenic in E-SCREEN
	(218)	~+	no competition with 17β-estradiol for human or alligator ER binding when used alone; some displacement when used in combination with other pesticides
	(108)	~+	moderate ERα and ERβ agonist in HELN reporter cell line

table continues

Table A.3 Pesticides selected as candidates for assessing the association with uterine fibroid prevalence (cont.)

Pesticide name	Reference	Evidence [a]	
Conflicting results on estrus cycling	(281)	ne	no estrus cycle or ovarian effects
	(282)	+	exposure to chlordane constituent caused shift from primarily proestrus and estrus to primarily metestrus
Atrazine			
Either no effect or possibly anti-estrogenic	(108, 283)	ne	no activity in ERα or ERβ
	(284)	-	antiestrogenic in yeast screen
	(285)	ne	no effect on proliferation of rat pituitary cell line or E-dependent tumor growth
	(103, 245, 286)	ne	no proliferation or ER-transactivational activity in E-sensitive cell lines (MCF-7, HeLa, yeast cells)
	(287)	~-	reduced E-stimulated uterine weight gain *in vivo*; poor binding to ER, ↑plasma estradiol in one rat strain
	(288)	-	weak inhibition of estrogen-stimulated responses in the rat uterus
	(289-291)	-	delayed vaginal opening, inhibition of uterine weight gain
	(292)	ne	no binding to ER, no transactivational activity in yeast assay
	(293)	ne	non-estrogenic in E-CALUX
	(294)	+	↑plasma estradiol in one rat strain but not another
	(295)	+	↑plasma estradiol in pigs
Possible antiprogestagen	(286, 291)	-	decreases in cytosolic progesterone receptor (PR) binding levels *in vivo*
	(287, 296)	-	↓ plasma progesterone
LH/FSH effects strain-dependent	(296-300)	-	suppresses LH and/or FSH surge in certain strains of female rats
Disrupts estrus cycle; may also be ovotoxic, but effects are strain and duration dependent	(290, 295, 297, 301-303)		increased length of diestrus in pigs, rats
	(287, 294, 304)		prolonged estrus phase in rats
	(305)		short-term exposure prolonged diestrus; shift to prolonged estrus phase with longer term exposure
	(306)	ne	no effect when administered prior to mating
	(307)		morphological signs of ovotoxicity in subacute, but not subchronic, exposure
	(290, 294, 295, 297, 303, 308)		↓ ovarian weight, ↑ ovarian follicular cysts in rats and pigs

table continues

Table A.3 Pesticides selected as candidates for assessing the association with uterine fibroid prevalence (cont.)

Pesticide name	Reference	Evidence [a]	
Mancozeb			
May be ovotoxic, estrus cycle disruptor	(309-312)		↓ number of estrus cycles, shortened estrus phase, increased diestrus phase in rats; decreased diestrus and increased estrus in mice
	(309-311)		histopathologic changes in ovaries, decreased number of healthy follicles
	(116, 313)	ne	no effect on estrus cyclicity

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; E-CALUX, assay with human ovarian carcinoma cells transfected with estrogen-responsive luciferase reporter gene plasmid; ER, estrogen receptor; E-SCREEN, assay using ability to induce cell proliferation of estrogen-sensitive human breast cancer cells as screening tool for estrogenic compounds; GH3, rat pituitary cell line; HELN, HeLa (human cervical cancer) cells transfected with estrogen receptor; HLM, human liver microsomes; MCF-7, human breast cancer cell line; MLTC, mouse Leydig tumor cells; PR, progesterone receptor; T47D, human breast cancer cell line; YES, *in vitro* Yeast Estrogen System assay using human estrogen receptor to screen for estrogenicity.

[a] For hormonal endpoints, the short description of study results is preceded by a "+" to indicate that the general effect is to increase the hormone, a "-" to indicate the general effect is to decrease the hormone, or "ne" to indicate no effect on the hormone. For the ovarian and estrus cycle endpoints, only "ne" is used.

APPENDIX B

Estimates for the Association of Pesticide Use and Fibroids Using Different Referent Groups

Some studies of multiple pesticide exposures have categorized exposures to single pesticides (or groupings) as ever/never in which the referent group includes both those unexposed to any pesticide as well as those exposed to pesticides other than those of interest. This classification allows for a larger sample size and increased precision over excluding the "other" pesticide exposure group from the analysis. On the other hand, keeping the other exposure group in the referent category may attenuate the risk estimates for any given pesticide/group of interest, making it more difficult to detect an association if one exists.

Although analysis of the 50 individual pesticides was not part of the initial aims of this research, the associations between specific pesticides and odds of uterine fibroid diagnosis were examined in response to findings from the main analysis. I was specifically interested to see if there were any pesticides that were not classified as possibly hormonally active based on my literature review, but that were associated with uterine fibroids. To assess the degree to which estimates might be attenuated, I examined associations with the referent group both including and excluding never users of any pesticides. Of the 43 pesticides examined (some were excluded due to small numbers), fibroid diagnosis was statistically significantly associated with ever use of 10 pesticides—chlordane, coumpahos, diazinon, malathion, parathion, carbaryl, chlorimuron ethyl, glyphosate, petroleum oil, and carbon tetrachloride—when the referent group included never users of any pesticide. Odds ratios were modestly elevated (ORs around 1.2 to 1.5) among users of about one-third of the individual pesticides examined; the strongest association with fibroids was seen in users of

carbon tetrachloride (OR = 1.87; 95% CI: 1.12, 3.12). This association remained relatively strong when the referent group consisted only of other pesticide users (OR = 1.70; 95% CI: 1.02, 2.85). Odds ratios for organophosphates as a class and specific organophosphate pesticides remained statistically significant (though attenuated). Aldicarb use was inversely associated with fibroids in both analyses.

Table B.1 Association between specific pesticide use and self-reported uterine fibroid diagnosis among 16,526 women aged 21-59 in the Agricultural Health Study, 1993-2003

	Referent includes never users of any pesticide and users of other pesticides		Referent includes only users of other pesticides	
	OR[a]	95% CI	OR[a]	95% CI
Organochlorines	1.16	0.99, 1.36	1.05	0.90, 1.24
Aldrin	1.05	0.63, 1.76	0.95	0.57, 1.60
Chlordane	1.22	1.00, 1.49	1.11	0.91, 1.36
DDT	1.27	0.98, 1.63	1.14	0.88, 1.48
Dieldrin[b]				
Heptachlor	1.22	0.76, 1.96	1.11	0.69, 1.78
Lindane	0.99	0.73, 1.33	0.90	0.67, 1.22
Toxaphene	1.04	0.62, 1.75	0.96	0.57, 1.61
Organophosphates	1.26	1.15, 1.38	1.17	1.05, 1.31
Chlorpyrifos	1.16	0.96, 1.40	1.07	0.88, 1.30
Coumaphos	1.52	1.10, 2.11	1.41	1.01, 1.96
Diazinon	1.21	1.06, 1.37	1.11	0.97, 1.27
Dichlorvos/DDVP	1.09	0.84, 1.42	1.00	0.77, 1.31
Fonofos	1.23	0.93, 1.63	1.13	0.85, 1.50
Malathion	1.27	1.15, 1.40	1.17	1.05, 1.31
Parathion	1.53	1.08, 2.15	1.42	1.00, 2.00
Phorate	1.01	0.76, 1.34	0.92	0.69, 1.23
Terbufos	1.04	0.82, 1.33	0.96	0.75, 1.22
Trichlorfon[b]				
Carbamates	1.13	1.03, 1.24	1.00	0.90, 1.12
Aldicarb	0.61	0.35, 1.08	0.57	0.32, 1.00
Carbofuran	1.25	0.95, 1.64	1.14	0.87, 1.51
Carbaryl	1.12	1.02, 1.23	0.99	0.88, 1.11
Benomyl	0.81	0.55, 1.21	0.75	0.50, 1.11
Triazines	1.11	0.94, 1.31	1.01	0.85, 1.20
Atrazine	1.14	0.95, 1.38	1.05	0.87, 1.26
Cyanazine	1.15	0.92, 1.45	1.06	0.84, 1.33
Metribuzin	0.99	0.74, 1.32	0.90	0.67, 1.21
Other insecticides				
Permethrin	1.13	0.95, 1.35	1.04	0.87, 1.25
Other Herbicides				
2,4-D	1.09	0.97, 1.22	0.98	0.87, 1.11
2,4,5 TP[b]				
2,4,5 T	1.41	0.91, 2.20	1.29	0.83, 2.01
Alachlor	1.16	0.95, 1.40	1.06	0.87, 1.29
Butylate	1.29	0.95, 1.74	1.18	0.87, 1.60
Chlorimuron Ethyl	1.31	1.00, 1.71	1.21	0.92, 1.58

table continues

Table B.1 Association between specific pesticide use and self-reported uterine fibroid diagnosis among 16,526 women aged 21-59 in the Agricultural Health Study, 1993-2003 (cont.)

	Referent includes never users of any pesticide and users of other pesticides		Referent includes only users of other pesticides	
	OR[a]	95% CI	OR[a]	95% CI
Dicamba	1.10	0.90, 1.34	1.00	0.82, 1.23
EPTC	0.99	0.71, 1.38	0.90	0.64, 1.27
Glyphosate	1.18	1.08, 1.30	1.07	0.96, 1.21
Imazethapyr	1.09	0.87, 1.36	1.00	0.79, 1.26
Metolachlor	1.17	0.95, 1.44	1.08	0.87, 1.34
Paraquat	1.22	0.88, 1.71	1.13	0.81, 1.59
Pendimethalin	0.97	0.76, 1.25	0.90	0.70, 1.15
Petroleum oil	1.24	1.02, 1.51	1.15	0.94, 1.40
Trifluralin	1.17	0.98, 1.39	1.07	0.90, 1.28
Other Fungicides				
Captan	1.14	0.89, 1.47	1.05	0.82, 1.36
Chlorothalonil	1.29	0.91, 1.83	1.20	0.85, 1.71
Maneb	1.08	0.79, 1.48	1.00	0.73, 1.37
Metalaxyl	0.84	0.62, 1.15	0.77	0.56, 1.06
Ziram[b]				
Other Fumigants				
Aluminum phosphide[b]				
Carbon tetrachloride	1.87	1.12, 3.12	1.70	1.02, 2.85
Ethylene dibromide[b]				
Methyl bromide	0.94	0.66, 1.34	0.87	0.61, 1.25

Abbreviations: OR, odds ratio; CI, confidence interval.

[a] Adjusted for age (continuous), age squared, and state (IA/NC). Comparison group consists of women who used other agricultural pesticides.

[b] Odds ratios not reported if fewer than 10 exposed cases.

APPENDIX C

Sensitivity Analysis Results

Results of sensitivity analyses with varying assumptions regarding sensitivity and specificity of self-report are shown below. Three main scenarios were examined: 1) varying age-specific sensitivity and overall specificity; 2) varying assumptions about overall sensitivity and specificity among women reporting a hysterectomy; and 3) varying assumptions about age-specific sensitivity among women 50-59 years old (for whom validity analysis data were not available).

The greatest impact of changes in sensitivity and specificity were observed in scenario 1, where I did not differentiate between hysterectomy and non-hysterectomy records (Table and Figure C.1). In all instances, corrected odds ratios were further away from the null than the uncorrected odds ratios which assumed no misclassification. In general, changing sensitivity estimates when overall specificity was high (0.97) did not dramatically influence the odds ratios. As specificity decreased, the impact of increases or decreases to age-specific sensitivity was greater. The greatest change was seen when sensitivity was decreased by 0.05 for each age group and specificity was 0.90. Models in which sensitivity was decreased by 0.10 points did not converge, indicating that perhaps the maximum likelihood estimate of the odds ratio approached 0 or infinity because of negative numbers in either the numerator or denominator of the maximum likelihood estimation formula (12):

$$OR = \frac{p_{D+|E+} - (1 - spec)}{sens - p_{D+|E+}} \div \frac{p_{D+|E-} - (1 - spec)}{sens - p_{D+|E-}}$$

When women with hysterectomy were differentiated in terms of their assumed reporting accuracy, the relative difference between corrected and uncorrected odds ratios was smaller. When overall sensitivity and overall specificity were varied in this subgroup (Table and Figure C.2), the biggest change from the uncorrected odds ratio was seen in the lowest sensitivity and specificity cell. Similar patterns were observed as above: varying sensitivity values had the greatest impact when specificity was lower. For the pesticide use exposure metric, outcome misclassification correction resulted in a somewhat flatter trend in the odds ratios for agricultural pesticide users.

Finally, I examined corrected odds ratios when different assumptions were made about trends in self-report sensitivity among women not reporting a hysterectomy (Table and Figure C.3). Although corrected odds ratios were further from the null, varying assumptions did not change the effect estimates.

Table C.1 Association between use of hormonally active pesticides and fibroid diagnosis, varying assumptions for age-specific sensitivity and overall specificity

	Specificity[a]		
Sensitivity	0.90	0.95	0.97
+0.10	2.24	1.59	1.48
+0.05	2.40	1.64	1.52
Age-specific[b]	2.68	1.71	1.56
-0.05	3.19	1.78	1.61
-0.10	c	c	c

Note: uncorrected OR = 1.28; 95% confidence interval: 1.12, 1.45. Referent group is women who did not use any pesticides.

[a] Specificity set to this value for everyone, regardless of age.

[b] Sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30.

[c] No convergence.

Table C.2 Association between use of hormonally active pesticides and fibroid diagnosis, varying assumptions for women with hysterectomy

	Specificity[a]		
Sensitivity[b]	0.85	0.90	0.95
0.85	1.92	1.65	1.48
0.90	1.87	1.62	1.47
0.95	1.83	1.60	1.45

Note: uncorrected OR = 1.28; 95% confidence interval: 1.12, 1.45. Referent group is women who did not use any pesticides.

[a] Specificity is varied for women regardless of hysterectomy status, based on assumption that self-report accuracy in women with hysterectomy will never be lower than for those with no hysterectomy.

[b] Sensitivity is varied for women with hysterectomy only. For women without hysterectomy, sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30.

Table C.3 Association between use of hormonally active pesticides and fibroid diagnosis, varying assumptions for sensitivity among women aged 45-59

Assumptions	OR	95% CI
Uncorrected (no assumptions)	1.28	1.12, 1.45
Sensitivity stays level[a]	1.47	1.21, 1.80
Sensitivity continues increasing with age[b]	1.47	1.22, 1.79
Sensitivity continues decreasing with age[c]	1.46	1.19, 1.80

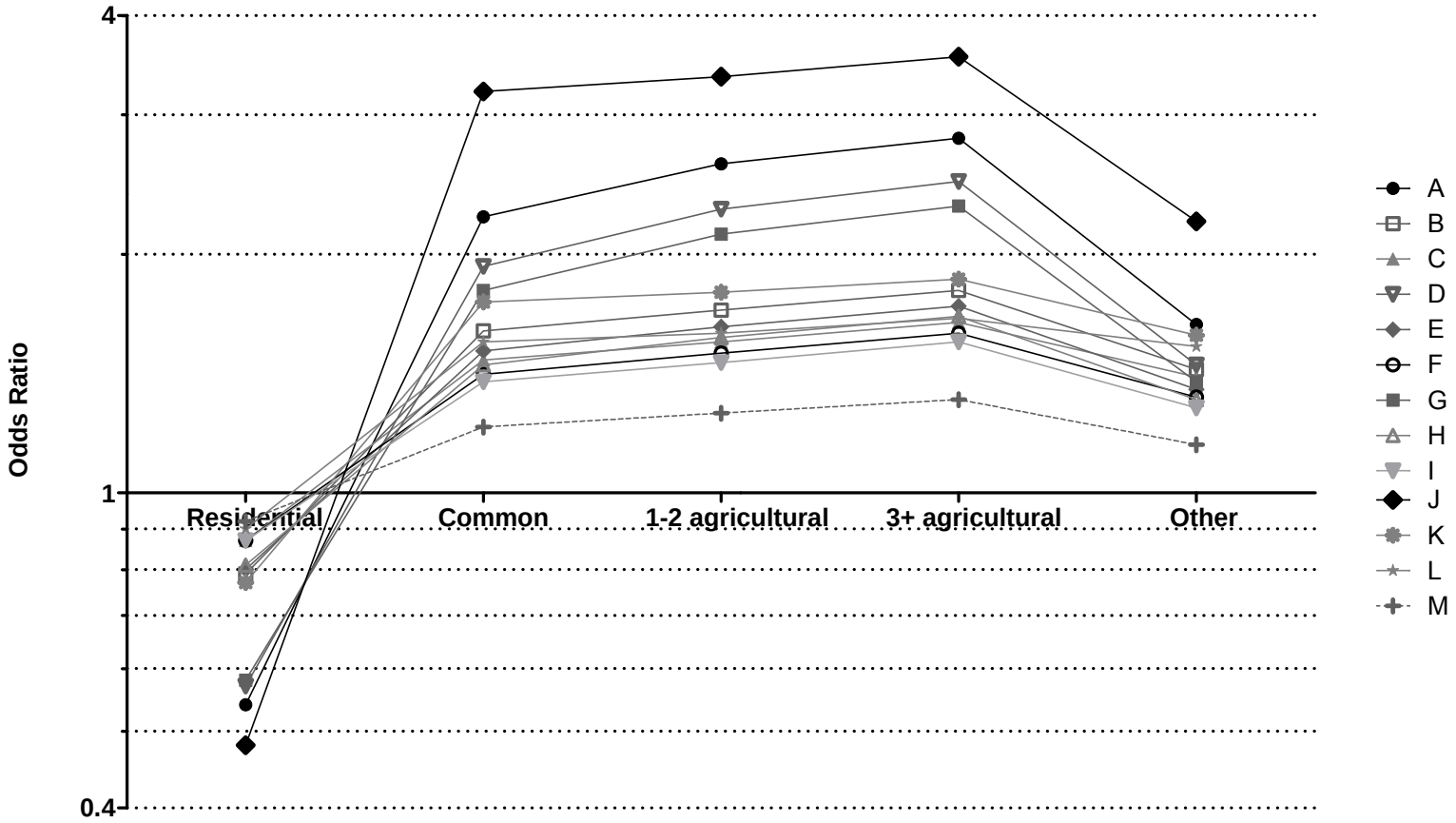
For women with hysterectomy, Se=0.85 and Sp=0.95. For women with no hysterectomy, Sp=0.95 and

[a] Sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.40.

[b] Sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-49: 0.45; 50-54: 0.50; 55-59: 0.55.

[c] Sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-49: 0.35; 50-54: 0.30; 55-59: 0.25.

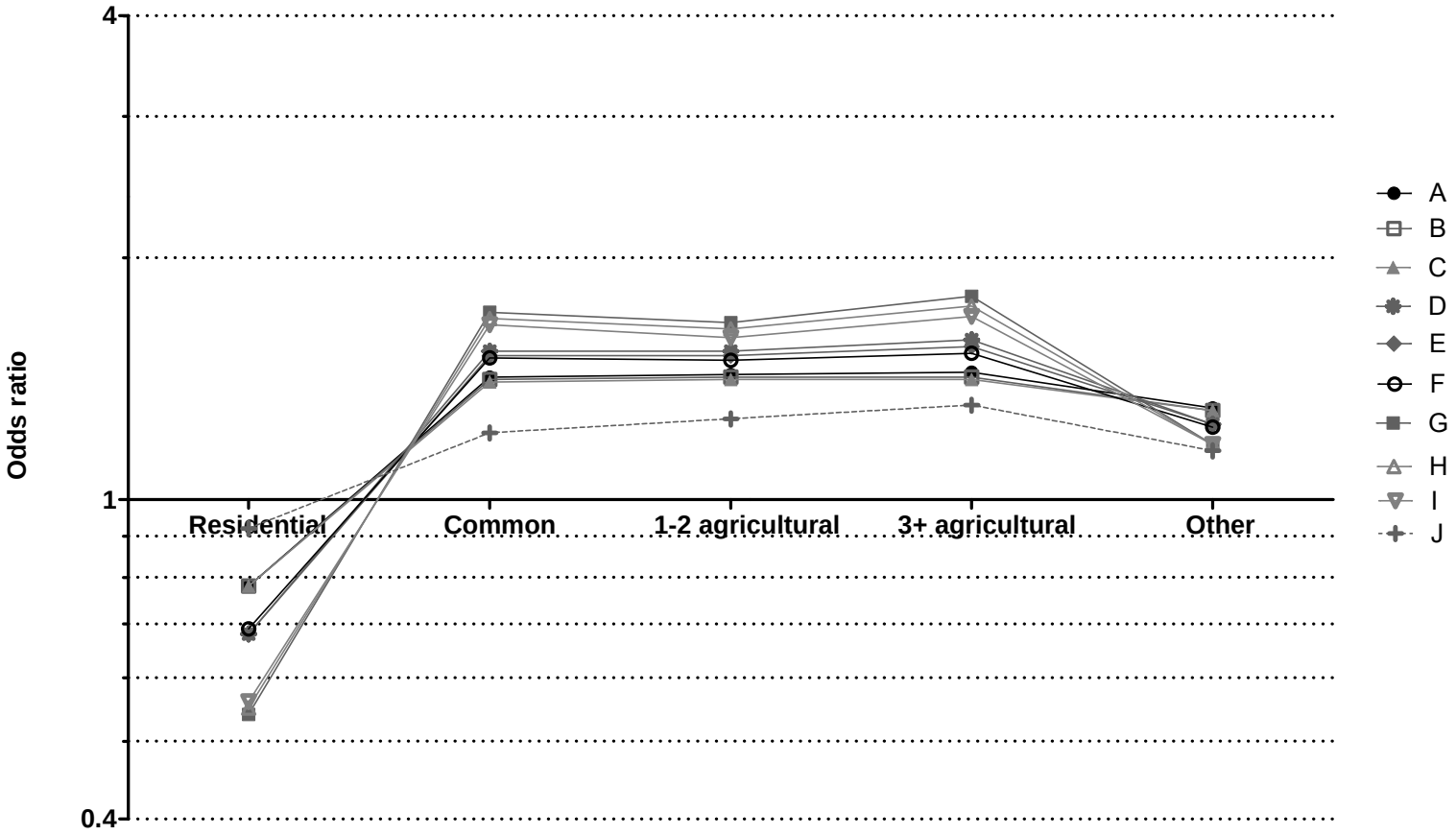
Figure C.1 Sensitivity analysis of the association between pesticide use patterns and self-reported uterine fibroid diagnosis, varying assumptions for age-specific sensitivity and overall specificity



M: Uncorrected odds ratio estimates

A:	Age-specific Se; Sp=0.90	D:	Se +0.05; Sp=0.90	G:	Se +0.10; Sp=0.90	J:	Se -0.05; Sp=0.90
B:	Age-specific Se; Sp=0.95	E:	Se +0.05; Sp=0.95	H:	Se +0.10; Sp=0.95	K:	Se -0.05; Sp=0.95
C:	Age-specific Se; Sp=0.97	F:	Se +0.05; Sp=0.97	I:	Se +0.10; Sp=0.97	L:	Se -0.05; Sp=0.97

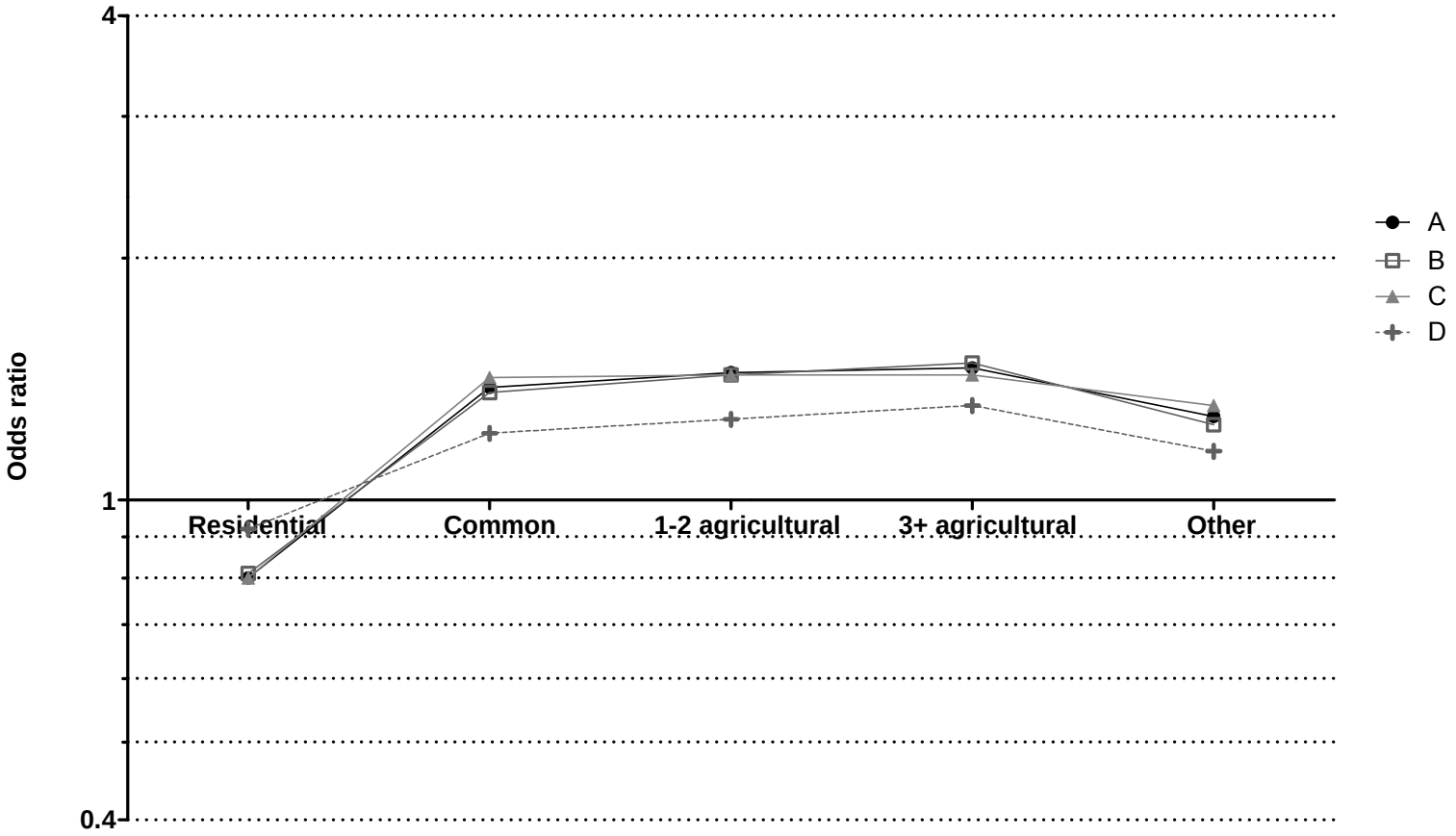
Figure C.2 Sensitivity analysis of the association between pesticide use patterns and self-reported uterine fibroid diagnosis, varying assumptions for women with hysterectomy



J: Uncorrected odds ratio estimates. Models below have the following Se values for non-hysterectomy women: 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.30. Sensitivity was varied for hysterectomy women, as was specificity (for hysterectomy and non-hysterectomy)

A: Se=0.85; Sp=0.95 D: Se=0.85; Sp=0.90 G: Se=0.85; Sp=0.85
B: Se=0.90; Sp=0.95 E: Se=0.90; Sp=0.90 H: Se=0.90; Sp=0.85
C: Se=0.95; Sp=0.95 F: Se=0.95; Sp=0.90 I: Se=0.95; Sp=0.85

Figure C.3 Sensitivity analysis of the association between pesticide use patterns and self-reported uterine fibroid diagnosis, varying assumptions for sensitivity among women aged 45-59



D: Uncorrected odds ratio estimates.
For women with hysterectomy, Se=0.85 and Sp=0.95. For women with no hysterectomy, Sp=0.95 and
A: Sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-59: 0.40.
B: Sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-49: 0.45; 50-54: 0.50; 55-59: 0.55.
C: Sensitivity by age 18-29: 0.15; 30-34: 0.20; 35-39: 0.35; 40-44: 0.40; 45-49: 0.35; 50-54: 0.30; 55-59: 0.25.

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