Author’s response to reviews

Title: A prospective study of cancer risk among Agricultural Health Study farm spouses associated with personal use of organochlorine insecticides.

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REVIEWER #1:

This prospective cohort study of cancer and pesticide exposures in farm spouses fills an important knowledge gap. Much of what we know about chemicals and cancer comes from occupational studies, but women's employment histories mean they have not historically been included, so less is known about chemicals and cancer in women. This study of wives of pesticide applicators is a creative way to fill that gap.

It adds to the evidence that OC use contributes to some cancers.

The detailed self-report of pesticide use represents a good exposure assessment strategy. Compared with biological measures, it may better represent direct pesticide application exposures rather than exposures to breakdown products from multiple sources (e.g., in the case of DDE as an indicator of DDT exposure). Extensive information was collected on potential confounders.
COMMENTS AND RESPONSES:

1. **COMMENT**: Line 160-161 and throughout. The statement that most studies of OCs and breast cancer are null is outdated. Most studies of OC residues in older adults are null. However, several reports on early life exposure to OCs provide evidence of an effect on breast cancer from exposure during breast development, which is not complete until the end of the first full-term pregnancy. An important prospective cohort study - the Child Health and Development Studies - has reported higher breast cancer risk associated with certain OCs when exposure was prenatal or under age 14 years (Cohn et al.). The Long Island Breast Cancer Study (LIBCSP) (White et al.) and Sister Study (NIEHOFF et al.) have reported elevated risk in women who recalled seeing or running in the pesticide mist behind fogger trucks as girls. The LIBCSP also reported reduced five-year survival associated with higher OC blood levels at diagnosis (Parada et al.).

**RESPONSE**: We have revised the Introduction and Discussion sections of the paper.

Lines 118-119: However, several reports provide evidence for an increased risk of breast cancer in adulthood with early life exposure to OCs.

Lines 266-275: Although some studies have found an increased risk of breast cancer among women exposed to OCs during critical developmental windows [41-43], our findings are consistent with most studies of breast cancer among women exposed to OCs in adulthood.

2. **COMMENT**: Line 171-172. Pesticide applicators were recruited in 1993-1997 when they applied for a license. Would this generally be when they first began applying pesticides? This matters, because the OCs were banned before that time, so this design may not be recruiting highly exposed people. What are the expectations about the life experiences of these women that would have resulted in OC use and exposure during the years of OC use?

**RESPONSE**: Of course, OCs were unlikely to be used by our study population at the time of study enrollment (1993-1997) due to their U.S. ban during the 1970s and 1980s. As indicated originally in the paper, the AHS spousal enrollment questionnaires asked about the life-time personal use of OC exposures. Therefore, our analyses presented in the paper examining associations between the personal use of OCs and cancers of specific sites are based on the spouses’ self-reported life-time use of OCs not their use of OCs at the time of study enrollment. (Lines 147-156)

3. **COMMENT**: Line 302. Please revise. E.g., This is consistent with most studies of residues in older adults.
RESPONSE: Addressed in paper as per suggestion

Lines 266-275: Although some studies have found an increased risk of breast cancer among women exposed to OCs during critical developmental windows [41-43], our findings are consistent with most studies of breast cancer among women exposed to OCs in adulthood [7, 29, 30, 32-39].

4. COMMENT: Line 308. However, please add reference to associations with early life exposure.

RESPONSE: References have been added accordingly (Line 268-269).

5. COMMENT: Line 312. Why do you think the evidence differs for dieldrin?

RESPONSE: As stated in the paper, while it appears that in vitro and animal studies have suggested that dieldrin, and other OCs, elicit tumor promoting effects via induction of endocrine receptor activities, few epidemiologic analyses of breast cancer have shown evidence for associations with dieldrin exposures in adulthood. Given our small number of dieldrin exposed ER-/PR- breast cancer cases (n=3), we do not see strong evidence of an association between use of dieldrin and breast cancer. (Lines 290-300)

6. COMMENT: Limitations. The discussion of limitations is generally good. However, I am concerned about an additional limitation - complex confounding by age cohort that may result from the timing of the adoption and then banning of the chemicals. For example, the youngest women in this cohort have potential exposure beginning in utero and extending until each chemical was banned. The oldest women do not have prenatal exposure. Table 1 shows that the ever-users of OCs are older, grew up on a farm, and have higher parity. These differences are expected, since OC use occurred earlier in history. Interestingly, more of the ever-users reached menarche at 12 years or less and more have a family history of cancer. Please discuss the interaction of cohort effects and exposure and how this might play out in the analysis. In addition, do you think that growing up on a farm is a good-enough proxy for OC exposure during the years of use? Consider whether there is a natural experiment within this cohort, as in CHDS, comparing women who grew up on a farm and were born before 1948 (about when DDT came into use) or who reached age 14 before that date with those who are younger.

RESPONSE: Thank you for your comments. While we agree that “growing up on a farm” is not a good-enough proxy for early life OC exposures, we were limited by the lack of available
questionnaire information to examine early life exposures and the its possible effects on cancer risk among our study population. In response to your inquiry, we reran models for risk of all cancer sites and breast cancer overall -- stratified by birth year:

(Lines 366-375)

Although some studies have reported an increased risk of breast cancer among women exposed to OCs during critical developmental windows in early life [41-43], our findings are consistent with most other studies that did not evaluate timing of exposure. [7, 29, 30, 32-39]. Although we did not have information on timing of exposure, we used year of birth as a surrogate for the opportunity for exposure during critical developmental periods. OCs were first registered in 1948, therefore we assumed women born before 1936 would not have any OC exposures prior to menarche. When we restricted analyses to women born after 1936, the RR for breast cancer and any OC use was 1.22 (0.94-1.59), compared to 0.84 (0.60-1.18) among women who were born prior to 1936, furthermore we did not observe a significant interaction between any OC use and year of birth (Pint = 0.11).

7. COMMENT: Typo: line 114 change between to with

RESPONSE: Addressed in paper as per suggestion (Line 114).

REVIEWER #2:

The article entitled "A prospective study of cancer risk among Agricultural Health Study farm spouses associated with personal use of organochlorine insecticides" is interesting but some aspects can be improved as following:

COMMENTS AND RESPONSES:

1. COMMENT: Lines 177-180. Add the response rate of the "female and family health questionnaire" for the 28,909 spouses that participated in the study, instead of giving the response for the initial population (n=32,345).

RESPONSE: The response rate of the Female and Family Health Questionnaire was also 60.0% among our study population of 28,909. This has been revised accordingly
Lines 136-137: In addition, 60.0% of the spouses in this analysis also completed the Female and Family Health questionnaire which focused on reproductive health histories. The study protocol was approved by all relevant institutional review boards.

2. COMMENT: Lines 210-211, page 7. State the statistical method used to decide to include or not include covariates in the final models instead of only stating that "these covariates did not substantially affect our results and were not included in the final models"

RESPONSE: We added the additional comments to the Methods section of the paper

Line 174-179: We considered the following additional confounders: BMI, race, family history of cancer, and ever use of any pesticide, but did not include them in our final models as they did not appreciably alter our results by ≥ 10%.

3. COMMENT: In page 8 lines 217-224- It does not seem a very good method not to include pesticides (both non-OC and OC pesticides) that were correlated. Combine pesticides that have similar biological action and have the same target organs in terms of toxicity based on experimental animals studies. For example combine Aldrin and Dieldrin and re-run the models.

RESPONSE: As indicated in the original manuscript, Poisson regression models were used to assess the effects of correlated pesticides and pesticides with similar biological action in our main effect models. These included Aldrin and Dieldrin, and Aldrin and Heptachlor, and demonstrated no significant effects (see attached results). In addition, the aforementioned pesticides were not highly correlative, ρ=0.43 for aldrin and dieldrin, and ρ=0.42 for aldrin and heptachlor.

As an example, we have included the results of analyses in which we reran our models adjusting for dieldrin use with aldrin exposure (For your review, see attached SUPPLEMENTAL TABLE 1. (DO NOT PUBLISH - FOR REVIEWER #2 ONLY))

4. COMMENT: Line 224. Instead of using sun protection as a potential confounder for melanoma, sunburns should be used or at least skin colour if you do not have sunburns as a variable.

RESPONSE: Thank you for this comment. We revised our analyses and reran our models for melanoma adjusting for a sun sensitivity variable indicative of the sensitivity of participants’ skin to sunburns if exposed to strong sunlight for more than an hour. In doing so, we found no appreciable difference (> 10%) in our risk values for melanoma and OC use.
Line 188: “sun protection” has been replaced with “sun sensitivity”

5. COMMENT: State in the limitations of the study, the low response rate of the "female and family health questionnaire".

RESPONSE: Addressed in paper as per suggestion

Lines 347-350: While we had a low response rate of the female and family health questionnaire, our reported results and final models were based solely on information collected from the spousal enrolment questionnaire.