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# Incident thyroid disease in female spouses of private pesticide applicators

Srishti Shrestha[a], Christine G. Parks[a], Whitney S. Goldner[b], Freya Kamel[a], David M. Umbach[c], Mary H. Ward[d], Catherine C. Lerro[d], Stella Koutros[d], Jonathan N. Hofmann[d], Laura E. Beane Freeman[d], Dale P. Sandler[a,*]

[a] Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
[b] University of Nebraska Medical Center, Omaha, NE, USA
[c] Biostatistics and Computational Biology Branch, Research Triangle Park, NC, USA
[d] Occupational and Environmental Epidemiology Branch, National Cancer Institute, Rockville, MD, USA

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ABSTRACT

*Background:* Little is known about modifiable risk factors for thyroid disease. Several pesticides have been implicated in thyroid disruption, but clinical implications are not clear.
*Objective:* We assessed associations between pesticide use and other farm exposures and incident hypothyroidism and hyperthyroidism in female spouses of farmers in the Agricultural Health Study (AHS).
*Methods:* We used Cox proportional hazards models to estimate hazard ratios (HR) and 95% confidence intervals for risk of thyroid disease in 24,092 spouses who completed at least one follow-up questionnaire.
*Results:* We identified 1627 hypothyroid and 531 hyperthyroid cases over 20 years of follow-up. The fungicides benomyl, maneb/mancozeb, and metalaxyl, the herbicide pendimethalin, and among those over 60 years of age the insecticides parathion and permethrin (applied to crops) were associated with elevated hypothyroidism risk, with HR ranging from 1.56–2.44. Conversely, the insecticide phorate, and the herbicides imazethapyr and metolachlor were associated with decreased risk (HR ranging 0.63–0.73), as were long-term farm residence and other farm-related activities (HR ranging 0.69–0.84). For hyperthyroidism, the insecticide diazinon, the fungicides maneb/mancozeb, and the herbicide metolachlor were associated with increased risk (HR ranging 1.35–2.01) and the herbicide trifluralin with decreased risk (HR: 0.57).
*Conclusions:* Several individual pesticides were associated with increased risk of hypothyroidism and hyperthyroidism, although some pesticides were associated with decreased risk. Some of the findings, specifically associations with fungicides, are consistent with results from an earlier analysis of prevalent diseases in AHS spouses.

## 1. Introduction

Optimal levels of thyroid hormones (THs) are critical to many physiological processes. Excessive as well as decreased TH levels outside the optimal range may predispose individuals to adverse health outcomes including cardiovascular diseases, poor reproductive health, and impaired neurocognitive function (Cooper and Biondi, 2012; Klein and Ojamaa, 2001; Whybrow and Bauer, 2005a, 2005b). Some pesticides may alter thyroid function; for example, in humans, the organochlorine insecticides dichlorodiphenyltrichloroethane (DDT) and aldrin, the fungicide maneb, and metabolites of organophosphate insecticides have been linked with changes in circulating levels of thyroid-stimulating hormone (TSH) and THs (Blanco-Munoz et al., 2016; Campos and Freire, 2016; Freire et al., 2013; Lerro et al., 2017; Steenland et al., 1997). However, clinical implications remain unclear as findings from human studies are inconclusive, with null, inverse, as well as positive associations between specific thyroid function biomarkers and pesticides. While these biomarker-based studies provide some insight on the associations, these studies were generally limited due to smaller sample sizes, and mostly focused on euthyroid populations with inadequate power to look at clinical thyroid disease. Further, nearly all studies were cross-sectional, limiting causal inferences. Furthermore, only a limited number of pesticides were studied and data on many commonly used pesticides are lacking.

Pesticides are extensively used in the United States (US) (Atwood and Paisley-Jones, 2017). People may be exposed to pesticides via direct use or indirectly from environmental sources. Biomonitoring data from nationally representative surveys indicate that the general US

* Corresponding author at: Epidemiology Branch, National Institute of Environmental Health Sciences, 111 T.W. Alexander Dr – MD A3-05, Research Triangle Park, NC 27709, USA.
*E-mail address:* sandler@niehs.nih.gov (D.P. Sandler).

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population is exposed to numerous pesticides; some pesticides can be detected in > 50% of the population (Barr et al., 2005; Barr et al., 2010; CDC, 2009). Therefore, identifying links between pesticides and clinical thyroid conditions may have important implications.

The Agricultural Health Study (AHS), an ongoing prospective cohort study of private pesticide applicators (predominantly farmers) and their spouses, was conducted to examine health effects of pesticides and other farm-related exposures (Alavanja et al., 1996). Earlier AHS investigations have found that farmers exposed to pesticides, specifically organochlorines, had an elevated prevalence and incidence of hypothyroidism (Goldner et al., 2013; Shrestha et al., Submitted). Female spouses exposed to fungicides including maneb/mancozeb also had a higher prevalence of hypothyroidism and hyperthyroidism (Goldner et al., 2010).

The previous study of AHS spouses evaluated associations between ever-use of pesticides assessed at enrollment and thyroid diseases (mostly prevalent) identified from the enrollment and first follow-up surveys (Goldner et al., 2010). Since then, the AHS has collected information on thyroid diseases at two additional follow-up surveys. With newly identified cases during this extended follow-up as well as incident cases from the first follow-up, here we examine pesticide use in relation to incident thyroid disease in female spouses of farmers in the AHS. We also explored associations with other farm-related exposures.

## 2. Methods

### 2.1. Study population

Between 1993 and 1997, 52,394 farmers from North Carolina and Iowa enrolled in the study by completing an enrollment questionnaire (Alavanja et al., 1996). At enrollment (Phase 1), farmers were given a questionnaire that asked about socio-demographics, pesticide use, and medical history, to be filled out by their spouse; 32,345 spouses (75% of married spouses, 219 male and 32,126 female) returned the questionnaire. Female spouses also provided information on reproductive history. Follow-up interviews were conducted in 1999–2003 (Phase 2), 2005–2010 (Phase 3), and 2013–2016 (Phase 4) to update information on pesticide use, socio-demographics, and health. We restricted the current analysis to the female spouses. All questionnaires can be accessed from the study website (https://aghealth.nih.gov/collaboration/questionnaires.)

### 2.2. Pesticide, farm exposures, and thyroid disease

At Phase 1, spouses were asked if they ever personally mixed or applied any pesticides in their lifetime; numbers of years and days per year they mixed or applied pesticides; and when pesticides were used, the percent of the time they personally spent mixing and applying them. Further, spouses were asked about ever-use of 50 named pesticides, with questions “In your lifetime, have you mixed or applied the following…” (questions presented in eFig. 1). Spouses were also asked about farm-related exposures including living on a farm, applying fertilizers, tilling soil, and sun exposure. Participants were asked about doctor-diagnosed thyroid diseases at all phases (eTable 1). At Phase 1, participants were asked if they had goiter, thyrotoxicosis/Grave's disease, or other thyroid disease. In Phases 2, 3 and 4, participants were asked if they had hypothyroidism and/or hyperthyroidism, age at diagnosis, and if they ever received treatment for either condition.

### 2.3. Hypothyroidism and hyperthyroidism

Given that thyroid disease may have a varying natural history/disease course (for instance, hypothyroidism can develop after hyperthyroidism as a natural course of disease or after treatment, details in Supplemental Methods), we employed several decision rules to define “hypothyroidism” and “hyperthyroidism” when participants reported multiple thyroid disease types (eTable 2). For age at diagnosis, when participants provided different ages across the surveys, we used the age provided at the earliest follow-up survey, assuming reduced recall over time. For participants who did not provide age at diagnosis, we used the mid-point between the last disease-free phase and when they first reported disease to estimate age at diagnosis (estimated for 110 hypothyroidism and 34 hyperthyroidism cases).

Of the 28,046 female spouses who completed at least one follow-up survey (eFig. 2), we excluded participants with missing or inconsistent thyroid disease responses, thyroid cancer cases, prevalent disease, or unspecified thyroid disease. Of the 24,598 remaining disease free individuals at enrollment, 1627 (6.6%) participants developed (age at diagnosis > age at enrollment) hypothyroidism, 531 (2.2%) developed hyperthyroidism, 506 (2.1%) developed other or unknown thyroid conditions, and 21,934 did not report any thyroid disease.

### 2.4. Thyroid disease validation

To evaluate the quality of self-reported diagnoses, we re-contacted participants who had reported incident thyroid disease in Phases 3 and 4 to confirm their diagnosis, obtain details of medication use and treatments, and obtain consent for retrieval of medical records. We received confirmation questionnaires from 1174 participants (applicators and spouses). Of the 819 with self-reported hypothyroidism who completed the questionnaire, 82% confirmed their diagnosis; whereas of the 216 with self-reported hyperthyroidism (or both) who completed the questionnaire, only 51% confirmed their diagnosis. We have obtained medical records for 186 self-reported hypothyroidism and 43 hyperthyroidism (or both) cases to date. About 91% of self-reported hypothyroidism was confirmed by physicians/medical staff. We found low agreement for hyperthyroidism, however, with only 32% confirmed by medical records. This poor agreement may be because we did not reach the diagnosing physician or because, for some participants who were currently being treated for hypothyroidism after therapy for hyperthyroidism, records may have been incomplete or not thoroughly searched by the medical staff we reached.

### 2.5. Statistical analysis

We estimated odds ratios and 95% confidence intervals (CIs) for associations between covariates and hypothyroidism and hyperthyroidism using polytomous logistic regression. We used Cox proportional hazards models, separately for hypothyroidism and hyperthyroidism, to estimate hazard ratios (HRs) and 95% CIs for associations with pesticides and farm exposures. We used attained age as the time scale, with left-truncation at enrollment; the models were adjusted for state, education, and smoking status. For farm exposures, we additionally adjusted for ever-use of any pesticides. Time-at-risk was accrued until hypothyroidism or hyperthyroidism diagnosis, death, loss- or end- of follow-up. When proportional hazard assumptions were violated for covariates ($p_{\text{-interaction-attained-age-and-covariates}} < 0.10$), we used covariate-stratified Cox models, whereas for exposures, we allowed the HR to vary by median attained age (i.e., 60 years). Further, as 1273 spouses were missing information on smoking status and 3106 on education (2189 reported “something else” for education which was treated as a missing covariate), we used multiple imputation with the fully conditional specification method to impute these missing covariates (Lee and Carlin, 2010). We created five imputed datasets, performed regression analysis in each dataset, and obtained the pooled parameter estimates.

We performed several sensitivity analyses. For pesticide exposures, we adjusted for the top four pesticides (if more than four) whose Spearman correlation coefficient with the pesticide of interest was ≥0.40. For farm exposures, we performed two additional adjustments in separate models – adjusting for correlated farm exposures (≥0.40) and for all pesticides associated with the thyroid disease of interest (farm exposures were not correlated with any pesticides with