Lifetime Pesticide Use and Telomere Shortening among Male Pesticide Applicators in the Agricultural Health Study

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Introduction

Pesticides are widely used in the United States and worldwide and are pervasive in our environment. Several pesticides have been associated with various cancers in experimental studies (summarized by the U.S. Environmental Protection Agency (U.S. EPA 2012)) and in epidemiologic studies of farmers (Blair and Freeman 2009) and pesticide manufacturing workers (Acquavella et al. 1996; Fryzek et al. 1997; Kogevinas et al. 1997; Leet et al. 1996) and among register pesticide applicators, including the Agricultural Health Study (AHS) cohort, one of the largest prospective studies of pesticide applicators (Alavanja et al. 1996).

The mechanisms by which pesticides may be linked to cancers in humans are unclear. Potential mechanisms include oxidative stress, DNA damage, chromosome aberration, immune response abnormality, and chronic inflammation (Casale et al. 1998; Hooge et al. 2000; Stiller-Winkler et al. 1999; Underge and Basaran 2005). These biological processes are also involved in telomere shortening (von Zglinicki 2002). The genetic integrity of the genome is maintained, in part, by the architecture of telomeres (Arindani et al. 2000; Meeker et al. 2004). Telomeres typically shorten with each cell division. When telomeres reach a critical length, cellular apoptosis or senescence is triggered. Cancer cells bypass these pathways and continue to divide despite the presence of chromosomal instability (Blasco 2005). Human epidemiologic investigations, mostly from case–control studies, have suggested that telomere length (TL) in surrogate tissues (i.e., blood or buccal cells) is associated with some but not all cancers (Broberg et al. 2005; Hou et al. 2009; Jang et al. 2008; McGrath et al. 2007; Risques et al. 2007; Shao et al. 2007; Widmann et al. 2007). While most studies have reported that shorter telomeres in surrogate tissue are positively associated with cancer (as reviewed by Ma et al. (2011) and Wenzens et al. (2011)), longer telomeres have been associated with cancer in some studies and reviews (Gramatges et al. 2010; Han et al. 2009; Lan et al. 2009; Shen et al. 2011; Svenson et al. 2009).

The potential effects of pesticide exposure on TL in surrogate tissues have not been well characterized, although associations with telomere shortening have been reported for occupational exposures (Eshkoor et al. 2011) and persistent organic pollutants (Shin et al. 2010). In the present study, we examined whether lifetime use of 48 pesticides is associated with telomere shortening in buccal cell DNA from 1,234 white male cancer-free licensed pesticide applicators participating in the AHS.

Materials and Methods

Study population. A detailed description of the AHS has been published (Alavanja et al. 1996). Briefly, 57,310, or 82%, of pesticide applicators seeking pesticide licensing in the U.S. states of Iowa and North Carolina were enrolled between 13 December 1993 and 31 December 1997. All data used in these analyses were based on AHS data release PIREL0506.01. Enrolled participants were licensed private pesticide applicators (mostly farmers) residing in Iowa and North Carolina.

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Address correspondence to M.C. Alavanja, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd., Rm. 8000, North Bethesda, MD 20892 USA. Telephone: (301) 435-4720. E-mail: alavanj@mail.nih.gov.

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and commercial applicators residing in Iowa. All pesticide applicators completed an enrollment questionnaire that inquired about ever/never use of 50 pesticides (National Institutes of Health 2013) as well as the duration (years) and frequency (average days per year) of use for 22 of these pesticides. In addition, 44% of the participants completed a second take-home questionnaire at enrollment that inquired about the duration and frequency of use of the remaining 28 pesticides. Approximately 5 years after enrollment, participants completed a follow-up phone interview to collect additional pesticide use and medical history information and were asked to provide a mouthwash rinse sample for extraction of DNA from buccal cells. Participants who agreed to provide buccal cells were sent a buccal cell collection kit and a postage-paid, padded envelope. Informed consent for buccal cell collection and buccal cell analyses associated with potential carcinogenic risk assessment was obtained at the time of collection, and the study protocol and informed consent was reviewed by all relevant institutional review boards. A total of 36,342 (63%) participants completed the follow-up interview, and 20,421 (56%) returned buccal cells. No meaningful difference in demographics was observed between those who donated buccal cells and those who did not (Engel et al. 2002).

As part of a nested case–control study of prostate cancer, 1,372 participants who had no history of prostate cancer (controls) and were > 40 years of age at the time of buccal cell collection were initially selected for TL measurement. Of these, 1,234 met inclusion criteria for the TL analysis. Participants excluded from the analysis were 115 men who had been diagnosed with any cancer prior to or within 3 years of buccal cell collection, 14 men who donated a buccal cell sample but did not provide a completed written consent at the time of the present analysis, and 9 men who were nonwhite. The present analysis was limited to white participants because of the small numbers of nonwhite applicators in the AHS cohort (Alavanja et al. 1996).

Pesticide exposure. Two pesticide exposure metrics were used. Pesticide use was evaluated as both lifetime days of pesticide use (years of use × days per year) and lifetime intensity-weighted days of pesticide use (lifetime exposure days × intensity score). The intensity score was computed from an algorithm that took into account exposure-modifying factors such as application method and protective equipment use (Coble et al. 2011).

Buccal cell collection. Buccal cells were collected from 1999 through 2006 using a mouthwash “swish and spit” collection technique (García-Closas et al. 2001). Buccal cells collection vials were returned to the National Institute on Aging Institute (NCI) repository via a postage-paid, padded envelope. All samples were stored at the NCI repository at −80°C. DNA from buccal cells was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI, USA).

TL measurements. Relative telomere length (RTL) in buccal DNA was measured at the Laboratory of Environmental Epigenetics, Center of Molecular and Genetic Epidemiology, Milan University, Italy, using quantitative real-time polymerase chain reaction (RT-qPCR) as described previously (Cawthon 2002). Briefly, this method measures RTL in genomic DNA by determining the ratio of telomere repeat copy number (T) to single copy gene (S) (36B4 gene located on chromosome 12, which encodes acidic ribosomal phosphoprotein P0) copy number (T/S ratio) in individual samples relative to a reference pooled DNA (Boulay et al. 1999). The reference pooled DNA was created using samples from 60 participants randomly selected from the population sample selected for this study and was used to generate a fresh standard curve, ranging from 0.25 to 8 ng/µL. In every T and S RT-qPCR run (see Supplemental Material, Figure S1), all samples were successfully run in duplicate with a 100% completion rate. The interbatch variability [coefficient of variation (CV)] in this study was 8.1%. The primer sequences and concentrations were GGTT TTTGAGGGTTAGGGTGGAGTGG GTT (270 nM) and TCCCCGACTATCC TATCTCCAATCCCTATCCCTATCC CTA (900 nM) for telomere; and CAGC AACGGGAAAGGTGATACCC (300 nM) and CCAATTCTATCATCAACGGGTACA (500 nM) for human beta-globin. The T (telomere) RT-qPCR mix was iQ SYBR Green Supermix (Bio-Rad, Hercules, California, USA) 1×, tel1b 100 nM, tel2b 900 nM, DMSO 1%, EDTA 1×. The S (human beta-globin) RT-qPCR mix was iQ SYBR Green Supermix (Bio-Rad) 1×, hbg1 300 nM, hbg2 700 nM, DMSO 1%, DTT 2.5 mM, EDTA 1×. We used the RT-qPCR primer sets previously described by McGrath et al. (2007). We used pooled DNA from 20 referents (500 ng for each sample), randomly selected from samples of this same study, to create a fresh standard curve, ranging from 8 ng/µL to 0.5 ng/µL, at every T and S RT-qPCR run. All samples contained Escherichia coli DNA heated at 96°C for 10 min and cooled at room temperature. 15 ng of DNA samples was added to each reaction (final volume, 20 µL). All RT-qPCRs were performed on a DNA Engine thermal cycler (Bio-Rad). The thermal cycling profile for both amplicons started with a 95°C incubation for 3 min to activate the hot-start iTaq DNA polymerase. The T RT-qPCR continued with 25 cycles at 95°C for 15 sec, and anneal/extend at 60°C for 15 sec. The S RT-qPCR continued with 35 cycles at 95°C for 15 sec, anneal at 58°C for 15 sec and extension at 72°C for 15 sec. At the end of each reaction, a melting curve was used for both T and S RT-qPCRs. All samples were run in triplicates.

Statistical analysis. The means of all three RTL measurements were used in the statistical analysis. Mean RTL values reported in Table 1 are the arithmetic mean stratum-specific estimates of RTL ± SD. Because RTL and both pesticide exposure metrics (i.e., lifetime-days and lifetime intensity-weighted days) had right-skewed frequency distributions, a natural logarithm transformation was applied to RTL and to both exposure metrics. Linear regression models were used to estimate the change in RTL with increasing pesticide use on a continuous scale (i.e., lifetime-days and lifetime intensity-weighted days). For presentation purposes, we also calculated the mean RTL for the reference group (i.e., no use of the pesticide) and each tertile of lifetime intensity-weighted days of pesticide use; however, the continuous measure was used in the analysis for testing statistical significance. Two pesticides (trichlorofon and ziram) had small numbers of exposed participants (< 20) and were dropped from the analyses. p-Values for the linear regression coefficient between each pesticide and RTL, showing the continuous change in RTL with increasing days of use, were adjusted for age at buccal cell sample collection (as a continuous variable), state of residence (Iowa vs. North Carolina), applicator license type (private, commercial), use of chewing tobacco regularly for 6 months or longer (yes vs. no) and total lifetime-days of all pesticide use (continuous). Further adjusting the linear regression model for body mass index (BMI) (continuous), alcohol consumption (none vs. any; or none, < 3, or ≥3 drinks per week), smoking (pack-years, current vs. never, ever vs. never), and self-reported cardiovascular disease, diabetes, and high blood pressure produced comparable results (data not shown), and these factors were not included in our final models. All tests were two-sided and p ≤ 0.05 was considered significant. All statistical analyses were conducted using AHS Data Release, version PIREL0506.01, and SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results Regression analysis p-values for model coefficients of ln-transformed pesticide exposures adjusted for age at buccal cell collection are shown according to selected characteristics shown according to selected characteristics.
in Table 1. RTL was negatively associated with age at buccal cell sample collection \((p = 0.003)\). Commercial pesticide applicators had shorter RTL \((\text{mean} = 1.08)\) than private applicators \((\text{mean} = 1.21, p = 0.01)\). The mean RTL in Iowa applicators \((1.19)\) was significantly shorter than the mean value in North Carolina applicators \((\text{mean} = 1.24, p = 0.03)\). The mean RTL was significantly longer for men who used chewing tobacco for \(\geq 6\) months \((\text{mean} = 1.27)\) than men who did not \((\text{mean} = 1.19, p = 0.01)\). Self-reported education, BMI, smoking status, pack-years smoked, alcohol consumption amount, family history of any cancers, and cardiovascular disease, diabetes, and high blood pressure were not significantly associated with RTL. It is worth noting that although RTL among current smokers is not statistically significant \((\text{overall} p > 0.05)\), both current smokers and those chewing tobacco had longer telomeres. All study participants applied some pesticides, but a comparison of those with more than the median number of applications days of total pesticide use versus those with less than the median number did not show any difference in RTL.

Among the 48 pesticides examined, increasing lifetime-days of pesticide use for 6 pesticides \([\text{alachlor}, \text{metolachlor}, \text{trifluralin}, 2,4\text{-dichlorophenoxyacetic acid} (2,4\text{-D}), \text{permethrin}, \text{and toxaphene}]\) were significantly \((p < 0.05)\) associated with decreases in RTL after adjusting for age at buccal cell collection, state of residence, license type, use of chewing tobacco, and total pesticide-application days \([\text{Table 2}; \text{see also Supplemental Material, Table S1}]\). Of these, four were herbicides \([\text{alachlor} (p = 0.002), \text{metolachlor} (p = 0.01), \text{trifluralin} (p = 0.05), \text{and} 2,4\text{-D} (p = 0.004)]\), and two were insecticides \([\text{permethrin} (p = 0.02) \text{and toxaphene} (p = 0.04)]\). No significant RTL lengthening was observed for any pesticide \([\text{Table 2}; \text{see also Supplemental Material, Table S1}]\).

Comparable results were observed with lifetime intensity-weighted days of pesticide use \([\text{Table 2}]\) for \(\text{alachlor} (p = 0.005), \text{metolachlor} (p = 0.01), \text{permethrin} (p = 0.02), \text{and} 2,4\text{-D} (p = 0.05)\). RTL also decreased with increasing use of trifluralin \((p = 0.06)\), but the association was not statistically significant. A statistically significant negative association with RTL was estimated for intensity-weighted lifetime-days of dichlorodiphenyltrichloroethane (DDT) use \((p = 0.03)\), but not for lifetime-days of DDT use \((p = 0.08)\). Adjustment for total days of pesticide use and for use of chewing tobacco did not affect the associations with pesticides in any meaningful way.

### Discussion

Seven pesticides were negatively associated with RTL in buccal cell DNA among cancer-free pesticide applicators > 40 years of age. Increasing lifetime-days of use of six pesticides used in agriculture \([\text{alachlor}, \text{metolachlor}, \text{trifluralin}, 2,4\text{-D}, \text{permethrin}, \text{and toxaphene}]\) were associated with significantly shorter telomeres after controlling for age at buccal cell collection, state of residence, license type, use of chewing tobacco, and total pesticide use days. Associations were similar between lifetime-days and intensity-weighted days of use of \text{alachlor}, \text{metolachlor}, 2,4-D, \text{permethrin}, and toxaphene. RTL also decreased with increasing lifetime intensity-weighted days of use of trifluralin, but the association was not statistically significant. For DDT, RTL shortening was significant in association with lifetime intensity-weighted days, but not lifetime-days of use.

The only study reporting data on pesticides and TL found that high levels of exposure to persistent organic pollutants (POPs), including organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenylethers (PBDEs) were associated with decreased RTL in peripheral blood leukocyte DNA, yet low levels of exposure were associated with increased RTL in an apparently healthy Korean population \([\text{Shin et al. 2010}]\). However, because of the limited sample size, and the very small numbers of observations at higher levels of exposure, more studies are needed to confirm the observation.

We observed that use of seven pesticides, as estimated by one or more exposure metrics, was associated with significantly decreasing RTL. Pesticide use has been noted to cause oxidative stress in humans \([\text{Honda et al. 2004; Houben et al. 2008; Meeker et al. 2004; von Zglinicki 2002}]\), and telomere shortening has been associated with cumulative oxidative stress \([\text{Houben et al. 2008; von Zglinicki 2002}]\). Telomeres are remarkably sensitive to damage by oxidative stress because of the high guanine content in specific telomere sequences and the deficiency in the repair of single-strand breaks \([\text{Honda et al. 2001; Meeker et al. 2004; von Zglinicki 2002}]\). Pesticide exposure may also lead to telomere shortening by causing inflammation \([\text{Figg et al. 2000}]\) reported that urinary 2,4-D concentration was associated with an increased lymphocyte

### Table 1. Mean RTL by selected characteristics of the cancer-free study population \((n = 1,234)\).

| Characteristic                  | Group          | n   | RTL (mean ± SD)       | p-Value |
|--------------------------------|----------------|-----|-----------------------|---------|
| Age at buccal cell collection  |                |     |                       |         |
| (years)                        | 41–62          | 312 | 1.25 ± 0.34           |         |
| 63–67                          | 296            |     | 1.21 ± 0.39           |         |
| 68–72                          | 333            |     | 1.18 ± 0.33           |         |
| 73–79                          | 294            |     | 1.18 ± 0.38           | 0.003   |
| BMI                            |                |     |                       |         |
| 18.65–25.66                    | 392            |     | 1.20 ± 0.34           |         |
| 25.67–28.60                    | 394            |     | 1.21 ± 0.38           |         |
| 28.61–46.98                    | 385            |     | 1.21 ± 0.36           | 0.93    |
| Applicator type                |                |     |                       |         |
| Private                        | 1,184          |     | 1.21 ± 0.36           |         |
| Commercial                     | 50             |     | 1.08 ± 0.43           | 0.01    |
| State of residence             |                |     |                       |         |
| Iowa                           | 921            |     | 1.19 ± 0.32           |         |
| North Carolina                 | 403            |     | 1.24 ± 0.43           | 0.03    |
| Education                      |                |     |                       |         |
| ≤ High school                  | 805            |     | 1.20 ± 0.37           |         |
| > High school                  | 406            |     | 1.21 ± 0.36           | 0.71    |
| Smoking status                 |                |     |                       |         |
| Never                          | 563            |     | 1.19 ± 0.32           |         |
| Former                         | 562            |     | 1.20 ± 0.38           |         |
| Current                        | 97             |     | 1.25 ± 0.42           | 0.63    |
| Pack-years of smoking          |                |     |                       |         |
| No                             | 563            |     | 1.19 ± 0.32           |         |
| < 20                           | 372            |     | 1.20 ± 0.32           |         |
| ≥ 20                           | 268            |     | 1.21 ± 0.47           | 0.90    |
| Cheewing tobacco use           |                |     |                       |         |
| No                             | 1,087          |     | 1.19 ± 0.36           |         |
| Yes                            | 147            |     | 1.27 ± 0.34           | 0.01    |
| Alcohol drinking               |                |     |                       |         |
| No                             | 516            |     | 1.22 ± 0.39           |         |
| Yes                            | 663            |     | 1.19 ± 0.31           | 0.10    |
| Cardiovascular disease         |                |     |                       |         |
| No                             | 346            |     | 1.17 ± 0.34           |         |
| Yes                            | 371            |     | 1.20 ± 0.34           | 0.25    |
| Diagnosis of diabetes          |                |     |                       |         |
| No                             | 1,167          |     | 1.20 ± 0.36           |         |
| Yes                            | 66             |     | 1.20 ± 0.33           | 0.78    |
| High blood pressure            |                |     |                       |         |
| No                             | 607            |     | 1.17 ± 0.32           |         |
| Yes                            | 225            |     | 1.19 ± 0.33           | 0.22    |
| Family history of any cancers  |                |     |                       |         |
| No                             | 548            |     | 1.20 ± 0.33           |         |
| Yes                            | 613            |     | 1.24 ± 0.39           | 0.88    |
| Use of any pesticides          |                |     |                       |         |
| Low (< 236 days)               | 562            |     | 1.21 ± 0.35           |         |
| High (> 236 days)              | 608            |     | 1.20 ± 0.37           | 0.48    |

\(\text{p}\)-Values from linear regression on In-transformed RTL \((\text{continuous})\) adjusted for age at buccal cell sample collection \((\text{continuous})\) for all characteristics other than age. \(\text{Arithmetic mean for the stratum-specific estimate of RTL} \pm \text{SD.}\)
replicative index, a cell proliferation biomarker. The replicative index for lymphocytes was higher among applicators than non-applicators and higher among applicators after spraying than before spraying. Telomeric DNA is dynamic, and TL typically shortens with each cell division (Hou et al. 2012). Therefore, the increased lymphocyte replicative index associated with the use of 2,4-D could be associated with inflammation because extensive cell proliferation and clonal expansion is an essential part of an inflammatory response (Hodes et al. 2002).

Of the seven pesticides associated with telomere shortening, four were herbicides and three were insecticides. The use of these chemicals was not strongly correlated with each other in our sample (the range of correlation coefficients between the seven pesticides varied from 0.1 to 0.24). These pesticides belong to different chemical classes and there were no chemical or functional classes where all pesticides were linked to TL shortening.

Alachlor has been shown to induce chromosomal aberrations in mouse bone marrow cells (Meisner et al. 1992) and to cause chromosomal damage in vitro experimental studies using Chinese hamster ovary cells (Lin et al. 1987), which may be related to TL shortening. In the AHS, a positive association between alachlor and the incidence of lymphohematopoietic cancers was found among applicators (Lee et al. 2004), but no excess cancer risk was observed in a study of alachlor manufacturing workers (Acquavella et al. 2004).

Metolachlor has been associated with lung cancer in the AHS (Alavanja et al. 2004), and the U.S. EPA (1995) classifies metolachlor as a possible human carcinogen (U.S. EPA group C). Trifluralin has been associated with colon cancer in the AHS (Kang et al. 2008), and the U.S. EPA (1996) classifies trifluralin as a possible human carcinogen (U.S. EPA group C). 2,4-D has been associated with prostate cancer in a large case–control study in British Columbia (Band et al. 2011), but not in the AHS cohort (Koutros et al. 2015), and with non-Hodgkin lymphoma (NHL) in a number of studies (Burns et al. 2011; Hoar et al. 1986; McDuffie et al. 2001; Miligi et al. 2006). The U.S. EPA (2005) classifies 2,4-D as not classifiable (U.S. EPA group D) with regard to human carcinogenicity, and the International Agency for Research on Cancer (IARC 2001) classifies the chlorophenoxy herbicide group as possibly carcinogenic to humans (IARC group 2B). Permethrin has been associated with multiple myeloma in the AHS (Rusiecki et al. 2009). In laboratory studies, treatment with a high dose of permethrin induced significant lymphocyte DNA damage in a rat model (Gabbianelli et al. 2004). The IARC (2001) considers DDT and toxaphene possible human carcinogens (IARC group 2B). In a recent Danish study conducted within a prospective cohort, pre-diagnostic adipose concentrations of DDT demonstrated a significant positive monotonic dose–response trend with NHL incidence (Brauner et al. 2012).

The present study is unique in that we have a relatively large population of licensed pesticide applicators who provided reliable information regarding their pesticide application history (Blair et al. 2002; Coble et al. 2011). We examined the relationship between cumulative lifetime use of specific pesticides and RTL in a cancer-free population (i.e., the study participants had no cancer diagnosis). Furthermore, we were able to adjust for potential confounding factors related to RTL. In the AHS, a priori–derived algorithm scores that incorporated several exposure determinants were used to predict pesticide exposure intensity. These algorithm scores have been shown to predict urinary pesticides levels (Coble et al. 2011; Thomas et al. 2010). The significant decrease in RTL with age that we observed was expected from 0.1 to 0.24). These pesticides belong to different chemical classes and there were no chemical or functional classes where all pesticides were linked to TL shortening. In Table 2, we present the results of this analysis for three pesticide groups: Herbicides, Insecticides, and Pesticides that are not classified as Herbicides or Insecticides.

### Table 2. Lifetime pesticide-use days, intensity-weighted lifetime pesticide-use days, and RTL

| Pesticide use (continuous) | Pesticide use (continuous) | n | RTL mean ± SD | $P_{\text{int}}$ (β ± SE) |
|---------------------------|---------------------------|---|---------------|--------------------------|
| **Herbicides**             |                           |   |               |                          |
| Alachlor (chloroacetanilide) | No use                 | 466 | 1.24 ± 0.41 |                           |
|                           | Low                    | 225 | 1.18 ± 0.31 |                           |
|                           | Medium                 | 215 | 1.18 ± 0.32 |                           |
|                           | High                   | 219 | 1.16 ± 0.33 | 0.011 ± 0.010            |
| Metolachlor (acetamide)    | No use                 | 622 | 1.23 ± 0.38 |                           |
|                           | Low                    | 223 | 1.17 ± 0.31 |                           |
|                           | Medium                 | 112 | 1.18 ± 0.31 |                           |
|                           | High                   | 157 | 1.16 ± 0.37 | 0.003 ± 0.004            |
| Trifluralin (dinitroaniline) | No use                 | 515 | 1.23 ± 0.36 |                           |
|                           | Low                    | 249 | 1.20 ± 0.39 |                           |
|                           | Medium                 | 191 | 1.17 ± 0.33 |                           |
|                           | High                   | 159 | 1.16 ± 0.33 | 0.008 ± 0.004            |
| 2,4-D (phenoxyacid)        | No use                 | 194 | 1.27 ± 0.48 |                           |
|                           | Low                    | 366 | 1.21 ± 0.36 |                           |
|                           | Medium                 | 320 | 1.21 ± 0.31 |                           |
|                           | High                   | 318 | 1.16 ± 0.32 | 0.012 ± 0.004            |
| **Insecticide**            |                           |   |               |                          |
| DDT (organochlorine)       | No use                 | 428 | 1.21 ± 0.34 |                           |
|                           | Low                    | 153 | 1.13 ± 0.33 |                           |
|                           | Medium                 | 97  | 1.13 ± 0.32 |                           |
|                           | High                   | 121 | 1.16 ± 0.30 | 0.002 ± 0.006            |
| Permethrin (poultry/livestock) (pyrethroid) | No use                 | 1,021 | 1.21 ± 0.37 |                           |
|                           | Low                    | 36  | 1.17 ± 0.27 |                           |
|                           | Medium                 | 40  | 1.16 ± 0.28 |                           |
|                           | High                   | 28  | 1.10 ± 0.26 | 0.018 ± 0.008            |
| Toxaphene (organochlorine mixture) | No use                 | 679 | 1.18 ± 0.33 |                           |
|                           | Low                    | 60  | 1.13 ± 0.39 |                           |
|                           | Medium                 | 31  | 1.15 ± 0.28 |                           |
|                           | High                   | 37  | 1.14 ± 0.27 | 0.017 ± 0.009            |

*p* Value of regression coefficient for ln-RTL (continuous) regressed on ln-transformed lifetime days of pesticide use (continuous) or ln-transformed lifetime intensity weighted days of pesticide use (continuous), adjusted for age at buccal collection (continuous), state (Iowa vs. North Carolina), license types (private vs. commercial), regular use of chewing tobacco for ≥ 6 months (yes vs. no), total pesticide exposure days (continuous), RTL mean is the arithmetic mean for the stratum-specific RTL by pesticide-use group.
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