HHS Public Access

Author manuscript

Occup Environ Med. Author manuscript; available in PMC 2020 July 14.

Published in final edited form as:

Occup Environ Med. 2019 July ; 76(7): 467–470. doi:10.1136/oemed-2018-105559.

Longitudinal investigation of haematological alterations among permethrin-exposed pesticide applicators in the Biomarkers of Exposure and Effect in Agriculture study

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Abstract

Objectives—Permethrin use has been associated with an increased risk of multiple myeloma (MM) among pesticide applicators. However, the biological plausibility and mechanisms underlying this association are not fully understood. The aim of this study was to assess whether exposure to permethrin is related to hematologic alterations among occupationally exposed pesticide applicators.

Methods—We conducted a longitudinal study among 33 pesticide applicators in the Biomarkers of Exposure and Effect in Agriculture study comparing haematological parameters in the offseason with the day after permethrin exposure and, for 27 participants, approximately three weeks post-exposure. Complete blood counts with white blood cell differential and lymphocyte subsets were measured at each visit. Multivariate linear mixed effects models were used to assess the relationship between natural log-transformed hematologic parameters and exposure to permethrin.

Results—The adjusted geometric mean immature granulocyte count was elevated among pesticide applicators following permethrin exposure compared with their offseason levels (37% increase, 95% confidence interval 6%−76%). Modest but statistically significant ($P<0.05$) alterations in red blood cell parameters (e.g. decreased RBC count and haemoglobin and increased mean corpuscular volume and RBC distribution width-SD) were also observed the day after permethrin use compared with offseason levels; decreases in RBC count and haemoglobin and increases in RBC distribution width-SD persisted approximately three weeks after permethrin use.

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Contributors

JJS, DL, JeH, JuH, CFL, and JNH contributed to the design of the study, analysis of the data, and manuscript preparation. LBF, GA, and MA contributed to the design of the study and manuscript preparation.

Competing Interests

None declared.

Conclusions—Altered haematological parameters could be indicative of disrupted haematopoiesis, providing insights into the biological plausibility of the observed association between permethrin use and MM risk among pesticide applicators.

INTRODUCTION

Permethrin is the most commonly used synthetic pyrethroid insecticide in the United States, with over two million pounds applied annually.[1] Permethrin acts to control insects by disrupting sodium channels causing paralysis and eventual death, but this mechanism is largely absent in mammalian species.[2] However, concerns remain regarding the health effects associated with chronic exposure to permethrin.

The United States Environmental Protection Agency has classified permethrin as "likely to be carcinogenic".[1] In the Agricultural Health Study, a prospective cohort study that includes over 57,000 pesticide applicators, permethrin use has been associated with an increased risk of multiple myeloma (MM).[3] MM is a plasma cell malignancy that is expected to be newly diagnosed in over 30,000 individuals in the United States during 2018.[4] Plasma cells are fully differentiated B-cells that share a common progenitor lineage with other major types of white blood cells (WBCs), platelets, and red blood cells (RBCs). To gain a better understanding of how permethrin exposure may impact hematopoiesis and possible development of MM, we examined hematologic parameters following permethrin use in participants enrolled in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study.[5]

MATERIALS AND METHODS

Study population

A detailed description of the BEEA study, a molecular epidemiologic effort within the Agricultural Health Study, has been published.[5] Briefly, the current longitudinal investigation included 33 participants from Iowa enrolled in BEEA between 2014–2017 that were active permethrin users who had serial sample collections that coincided with the application of permethrin. Each participant had a reference visit collected during the offseason, when permethrin was not being actively used, and another within a day after permethrin use. Of the 33 participants, 27 had an additional follow-up visit approximately three weeks following permethrin use. All participants were male private pesticide applicators over the age of 50 in the Agricultural Health Study who had completed three previously administered questionnaires (1993–1997, 1998–2003, and 2005–2010),[6] had never been diagnosed with cancer (excluding non-melanoma skin cancer) or a blood clotting disorder, and still resided in Iowa. Upon enrollment in BEEA and at each subsequent visit, each participant completed a questionnaire to ascertain information on demographics and individual pesticide use.

Blood count quantification

During each visit a non-fasting blood sample was collected and delivered on the same day to the University of Iowa Hospitals and Clinics Emory Warner Clinical Laboratories (Iowa City, IA), which is accredited by the College of American Pathologists and is certified by the

Clinical Laboratory Improvement Amendments. An automated complete blood count with WBC differential was performed using a Sysmex XN-9000 analyzer, and lymphocyte subsets were measured using a Becton Dickinson FACSCanto™ flow cytometer. All analyses were performed within 24 hours of the blood sample collection.

Statistical analysis

Multivariate linear mixed effects models were used to assess the relationship between natural log-transformed hematologic parameters and time since exposure to permethrin (e.g., on the day after exposure and at the follow-up visit approximately three weeks later) relative to offseason levels. All models were adjusted for age (continuous), body mass index (continuous), season of blood draw (September-February or March-August), recent cold/infection (≤7 days), and use of either glyphosate or 2,4-D (≤7 days, 8 to 30 days, or no use in the last 30 days or since the prior visit). We controlled for these two herbicides because, besides permethrin, they were the most frequently used chemicals at the time of the visits (Table 1); recent use of other pesticides was minimal (i.e. <10% use). The potential confounding effects of alcohol and nonsteroidal anti-inflammatory drug use were assessed, as well as sensitivity analyses pertaining to current tobacco use and smoking status. These additional covariates did not substantively change our results and thus were not included in the final models. Based on these models, we estimated visit-specific adjusted geometric means (95% confidence interval [CI]) for all hematologic parameters. $P<0.05$ was considered statistically significant when performing pairwise comparisons between visits. All statistical analyses were performed using SAS v. 9.4 (Cary, NC).

RESULTS

At the time of permethrin exposure, participants had a median age of 61 years, 48% were overweight and 45% were obese, 48% did not consume alcohol in the past week, and 91% reported no current tobacco use (Table 1). These values were similar for the offseason and follow-up visits. Use of nonsteroidal anti-inflammatory drugs in the last 7 days was reported by 52% and 55% of participants at the offseason and exposure visits, respectively, and by 70% at the follow-up visit. Per the design of the study, no permethrin use within the last 7 days was reported at the time of the offseason visit and all participants used permethrin on the day prior to the exposure visit. A few participants (N=2) reported additional permethrin use within the last 7 days leading up to follow-up visit.

The adjusted geometric mean immature granulocyte count was significantly higher (37%, 95% CI 6% to 76%) at the exposure visit compared with the offseason visit (Table 2). Immature granulocytes counts remained slightly elevated three weeks after exposure to permethrin relative to offseason levels, although the difference was no longer statistically significant (8%, 95% CI −18% to 42%). We also observed non-significant increases in specific mature granulocyte counts in the exposure visit compared with the offseason visit. No differences were observed in any other WBC parameters including lymphocyte subsets.

We observed modest but statistically significant ($P<0.003$) decreases in RBC count and hemoglobin for the exposure and follow-up visits, respectively, compared with the offseason visit (Table 2). Decreases in hematocrit levels were borderline statistically significant for the

exposure visit (P=0.051) and significant (P=0.0008) for the follow-up visit compared to the offseason visit. We also observed a statistically significant increase in mean corpuscular volume (P=0.0004) and RBC distribution width-SD (P=0.02) when comparing the exposure visit with the offseason visit. The adjusted geometric mean RBC distribution width-SD remained elevated at a statistically significant level (P=0.04) while the mean corpuscular volume was no longer significant in the follow-up visit (P=0.36) when compared with the offseason visit. We further evaluated potential factors that may influence the intensity of permethrin exposure in relation to the hematologic parameters that were significantly altered in the overall study population (Supplemental Table 1).

DISCUSSION

Analyses of longitudinally collected samples timed specifically in relation to permethrin use suggest that recent permethrin exposure is associated with altered hematologic parameters, in particular for cells of myeloid lineage including immature granulocytes and RBCs. The observed alterations to myeloid-derived cell parameters may be indicative of stress hematopoiesis.[7] Similar effects have been observed among individuals with occupational exposure to benzene,[8] which has been associated with hematologic malignancies including MM.[9] In contrast, no statistically significant alterations in cells of lymphoid lineage were observed following permethrin exposure.

This is, to our knowledge, the first study to investigate hematologic alterations in permethrin-exposed pesticide applicators. The increase in immature granulocytes during the exposure visit compared to the offseason visit may be indicative of an inflammatory response or disruption of normal hematopoiesis in the bone marrow; the absence of a concomitant increase in mature granulocytes accompanying the observed increase in immature granulocytes may be due to rapid turnover or a delay or lack of granulocyte maturation. The accumulation of immature granulocytes has also been shown to suppress anti-tumor immune response and promote tumor angiogenesis.[10]

Regarding the observed alterations in RBC-related parameters, *in vitro* studies have shown that permethrin exposure affects morphology and can induce cell damage in RBCs.[11] In our study, occupational exposure to permethrin was associated with decreased RBC count and hemoglobin levels, as well as increased RBC distribution width-SD and mean corpuscular volume, a measure of the size of RBCs that can increase in response to low levels of hemoglobin. These findings may reflect permethrin-induced oxidative stress,[12] which has been associated with a decreased capacity of RBCs to mature and function properly.[13] Loss of RBCs is frequently observed among patients diagnosed with MM,[4] suggesting that erythrocytic toxicity may be one of the early signs of disease. However, none of the participants had RBC counts outside of the clinical reference range (4.50–6.20 10^6 cells/μL); as such, the observed associations may reflect early subclinical hematologic alterations that could influence the development of MM and its precursor monoclonal gammopathy of undetermined significance with continued permethrin exposure over time.

A major strength of this study was the availability of serial samples that coincided with the use of permethrin among occupationally exposed pesticide applicators. We also had detailed

information on permethrin use as well as co-exposures and other pertinent confounding factors While the overall sample size was relatively small and may be susceptible to sampling error, the longitudinal design of this study allowed us to efficiently evaluate hematologic alterations related to permethrin exposure, and we had sufficient power to detect statistically significant associations with several parameters.

The results of this investigation provide evidence supporting the potential biological plausibility of the association between permethrin use and MM previously observed in the Agricultural Health Study.[3] Future studies evaluating immunologic and hematologic biomarkers related to MM will be critical in evaluating the functional impact associated with the alteration of these hematologic parameters. Given the widespread use of permethrin in both residential and commercial settings, our findings may have public health implications beyond occupationally exposed pesticide applicators.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to acknowledge the participation of the Biomarkers of Exposure and Effect in Agriculture Study participants that made this work possible. Furthermore, we would like to thank Amy Miller, Kate Torres, Emily Tristani, Himanshi Singh, Marsha Dunn (Westat, Rockville, Maryland) and Debra Podaril and Debra Lande (University of Iowa) for study coordination, data management, and field research efforts. We also thank Peter Hui and Lonn Tremblay (Information Management Services, Rockville, Maryland) for additional data management support.

Funding

This work was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute (Z01CP010119) and the United States Environmental Protection Agency through an inter-Agency agreement (XCP13001-001-0003).

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Key Messages

What is already known about this subject?

- Several epidemiological studies have observed an increased risk of multiple myeloma (MM) among agricultural workers including pesticide applicators who reported high lifetime use of permethrin.
- The biological plausibility and mechanisms through which permethrin exposure is associated with MM are not fully understood.

What are the new findings?

- Permethrin use may be associated with haematological alterations including elevated levels of immature granulocytes and altered red blood cell parameters.

How might this impact on policy or clinical practice in the foreseeable future?

- Understanding the biological plausibility and mechanisms through which permethrin exposure is associated with the development of MM can inform assessments of the carcinogenic potential of this widely used pyrethroid insecticide.

Table 1.

Selected Characteristics of Recent-Permethrin Applicators

Characteristics	Offseason n (%)	Exposure Visit n (%)	Follow-Up Visit n (%)
Participants (n)	33	33	27
Δ Days, median (range)[a]	135 (13 to 840)		
Δ Days, median (range)[b]			21 (16 to 25)
Age at Visit, median (range)	61 (51 to 88)	61 (51 to 89)	62 (51 to 89)
BMI			
Normal	2 (6)	2 (6)	1 (4)
Overweight	16 (48)	16 (48)	14 (52)
Obese	15 (45)	15 (45)	12 (44)
Season of Blood Draw			
September-February	14 (42)	8 (24)	12 (44)
March-August	19 (58)	25 (76)	15 (56)
Current Use of Tobacco Products			
No	30 (91)	30 (91)	25 (93)
Yes	3 (9)	3 (9)	2 (7)
Weekly Alcohol Servings			
0	18 (55)	16 (48)	12 (44)
<5	8 (24)	10 (30)	10 (37)
≥5	7 (21)	7 (21)	5 (19)
Recent Infection (Last 7 Days)			
No	29 (88)	31 (94)	22 (81)
Yes	4 (12)	2 (6)	5 (19)
NSAID Use (Last 7 Days)			
No	16 (48)	15 (45)	8 (30)
Yes	17 (52)	18 (55)	19 (70)
Permethrin Use (Last 7 Days)			
No	33 (100)	0 (0)	25 (93)
Yes	0 (0)	33 (100)	2 (7)
Recent Glyphosate Use[c]			
Did Not Use	30 (91)	22 (67)	21 (78)
7 or Less Days Ago	2 (6)	5 (15)	4 (15)
8–30 Days Ago	1 (3)	6 (18)	2 (7)
Recent 2,4-D Use[c]			
Did Not Use	32 (97)	24 (73)	23 (85)
7 or Less Days Ago	0 (0)	4 (12)	3 (11)
8–30 Days Ago	1 (3)	5 (15)	1 (4)

[a]Days between the offseason and exposure visit.

[b] Days between the exposure and exposure follow-up visit.

[c] Within the last 30 days or since the last visit, whichever is less.

BMI, body mass index; NSAID, non-steroidal anti-inflammatory drugs; 2,4-D, 2,4- dichlorophenoxyacetic acid.

Table 2.

Hematologic Profile of Recent-Permethrin Applicators[a]

Component	Offseason	Exposure Visit	Follow-Up Visit
White Blood Cells (1000/μL)	6.0 (5.3 to 6.8)	6.2 (5.6 to 6.9)	6.1 (5.4 to 6.8)
Neutrophils (per μL)	3626 (3096 to 4246)	3745 (3264 to 4296)	3675 (3176 to 4252)
Eosinophils (per μL)	126 (91 to 174)	149 (112 to 199)	137 (101 to 186)
Basophils (per μL)	30 (22 to 40)	32 (25 to 42)	31 (23 to 41)
Immature granulocytes (per μL)	**17 (12 to 22)**	**23 (18 to 29)** *	**18 (14 to 23)** #
Monocytes (per μL)	501 (415 to 605)	491 (415 to 581)	484 (406 to 578)
Lymphocytes (per μL)	1527 (1308 to 1782)	1531 (1328 to 1765)	1486 (1282 to 1722)
CD19 (per μL)	178 (141 to 225)	179 (144 to 223)	178 (142 to 224)
CD3+CD4+ (per μL)	771 (636 to 934)	766 (643 to 913)	761 (632 to 918)
CD3+CD4-CD8- (per μL)	29 (19 to 45)	33 (23 to 48)	27 (18 to 40)
CD3+CD8+ (per μL)	278 (207 to 372)	257 (197 to 336)	262 (198 to 348)
CD3+CD16+CD56+ (per μL)	40 (21 to 76)	48 (28 to 85)	48 (26 to 87)
CD4+CD8+ Ratio	2.8 (2.2 to 3.7)	2.9 (2.3 to 3.7)	2.9 (2.3 to 3.8)
Neutrophil Lymphocyte Ratio	2.3 (1.9 to 2.9)	2.4 (2.0 to 2.9)	2.4 (2.0 to 3.0)
Red Blood Cells (10^6/μL)	**4.97 (4.80 to 5.16)**	**4.83 (4.67 to 4.99)** *	**4.79 (4.63 to 4.96)** *
Hemoglobin (g/dL)	**15.1 (14.6 to 15.6)**	**14.6 (14.2 to 15.1)** *	**14.4 (14.0 to 14.9)** *
Hematocrit (%)	**44.5 (43.0 to 46.0)**	43.6 (42.3 to 45.0)	**43.0 (41.7 to 44.4)** *
MCV (fL)	**89.4 (87.8 to 91.0)**	**90.4 (88.9 to 91.9)** *	**89.6 (88.1 to 91.2)** #
MCH (pg)	30.3 (29.7 to 30.9)	30.3 (29.7 to 30.9)	30.3 (29.6 to 30.9)
MCHC (g/dL RBC)	33.4 (32.6 to 34.3)	33.6 (32.8 to 34.3)	33.4 (32.6 to 34.2)
RDW (%)	13.0 (12.7 to 13.3)	13.0 (12.8 to 13.3)	13.1 (12.8 to 13.3)
RDW-SD (fL)	**42.3 (41.2 to 43.5)**	**42.9 (41.8 to 44.0)** *	**42.8 (41.7 to 43.9)** *
Platelets (1000/μL)	214 (195 to 234)	218 (201 to 238)	216 (198 to 235)
MPV (fL)	10.7 (10.3 to 11.0)	10.5 (10.2 to 10.9)	10.6 (10.2 to 10.9)

[a] adjusted geometric means (95% CI) were estimated using linear mixed models adjusted for age, body mass index, season of blood draw, recent infection or cold, and other recent pesticide use.

*, P<0.05 when compared to offseason visit (denoted in bold)

#, P<0.05 when compared to exposure visit (denoted in bold)

CD: cluster of differentiation; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MPV: mean platelet volume; RDW: red blood cell distribution width; RDW-SD: red blood cell distribution width SD.