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OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

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Summary and Conclusions
This memo is the Pyrethroids: Tier II Epidemiology Report focused on carcinogenic and non-carcinogenic health effects in humans for the chemical class, pyrethroids, as well as select individual pyrethroid chemicals including allethrin, bifenthrin, cyfluthrin, gamma-cyhalothrin, alpha-cypermethrin,

beta-cyfluthrin, cypermethrin, zeta-cypermethrin, cyhalothrin, lambda-cyhalothrin, deltamethrin, esfenvalerate, etofenprox, cyphenothrin, fenpropathrin, flumethrin, tau-fluvalinate, imiprothrin, momfluorothrin, d-Phenothrin/sumithrin, prallethrin, tefluthrin, and tetramethrin. A Tier II report for permethrin, a specific pyrethroid, including epidemiologic studies as well as incidents and medical case studies was recently conducted in 2017 (E. Evans et al., D440947, 06/27/2017). As a result, permethrin-specific epidemiologic studies were excluded from this Tier II Epidemiology Report. In addition, epidemiologic studies on the pyrethrins were excluded from this Tier II Epidemiology Report as well as these are being considered separately.

For this Pyrethroids Tier II Epidemiology Report, a systematic literature review was conducted and epidemiological studies investigating the association between exposure to pyrethroids, the chemical class, as well as individual pyrethroids[1] and carcinogenic and non-carcinogenic human health effects were reviewed. The epidemiology review followed the outlines of OPP's Framework document[2] for incorporating epidemiological and incident data into OPP risk assessments. The review covered a broad range of health outcomes including several cancers, neurodevelopmental/neurobehavioral/neurocognitive effects, birth effects, male reproductive effects, and a variety of more general other effects (many from the Agricultural Health Study).

Overall, there is little substantive evidence to suggest a clear associative or causal relationship between exposure to pyrethroids and cancer and non-cancer health endpoints in the cohort, case-control, and cross-sectional studies, and studies including the Agricultural Health Study (AHS) publications reported here. The Agency will continue to monitor the epidemiology for pyrethroids, and if a concern is triggered, additional review and analysis will be conducted.

[1] Individual pyrethroids with EPA PC codes are as follows:

Chemical	PC Code	CAS No.
Allethrin	004005	584-79-2
Bifenthrin	128825	82657-04-3
Cyfluthrin	128831	68359-37-5
Cyfluthrin, beta-	118831	68359-37-5
Cyhalothrin	128867	68085-85-8
Cyhalothrin, lambda-	128897	91465-08-6
Cyhalothrin, gamma-	128807	76703-62-3
Cypermethrin	109702	52315-07-8
Cypermethrin, alpha-	209600	67375-30-8
Cypermethrin, zeta-	129064	52315-07-8
Cyphenothrin	129013	39515-40-7
Deltamethrin	097805	52918-63-5
Esfenvalerate	109303	66230-04-4
Etofenprox	128965	80844-07-1
Fenpropathrin	127901	39515-41-8
Flumethrin	036007	69770-45-2
Fluvalinate, tau-	109302	69409-94-5
Imiprothrin	004006	72963-72-5
Momfluorothrin	016331	609346-29-4
Tefluthrin	128912	79538-32-2
Tetramethrin	069003	7696-12-0
Tralomethrin	121501	66841-25-6
d-Phenothrin sumithrin	069005	26002-80-2
Prallethrin	128722	23031-36-9

[2] US EPA. December 28, 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides. https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf.

1 Statement of Objective

In this epidemiology literature review, the Agency undertook a systematic and formalized procedure to collect, evaluate, and integrate the epidemiological literature investigating potential associations between exposure to the chemical class, pyrethroids, as well as individual pyrethroids[3] relative to human health outcomes to assess whether the current scientific literature suggests an association between exposure to these chemicals and specific human health outcomes.[4]

2 Introduction

The National Academy of Sciences National Research Council (NRC) and the National Academy of Medicine (formerly the Institute of Medicine) define systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies", and NRC in a 2014 report identified systematic literature review strategies as "appropriate for EPA" and "specifically applicable to epidemiology and toxicity evaluations".[5]

To meet this charge, EPA's Office of Pesticide Programs (OPP) published a framework for incorporating epidemiological data in risk assessments for pesticides which described a systematic review process relying on standard methods for collecting, evaluating, and integrating the scientific data supporting Agency decisions.[6] The epidemiology framework characterized "fit for purpose" systematic reviews for incorporating human epidemiology data in OPP risk assessments for pesticides, meaning that the complexity and scope of each systematic review is tailored to a specific analysis and follows the key characteristics outlined in the Cochrane Handbook:[7]

- a clearly stated set of objectives with pre-defined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search to identify all relevant studies;
- an assessment of the validity of the findings from the identified studies; and
- a systematic presentation and synthesis of the characteristics and findings of the included studies.

Following the procedures described in the OPP epidemiology framework, the Agency conducted a formalized literature review to collect, evaluate, and integrate evidence from relevant epidemiological literature on the association between exposure to pyrethroids, as a chemical class, as well as individual pyrethroids and human health outcomes in order to evaluate whether chronic, low level exposure to these chemicals is associated with an increased (or decreased) risk of various cancer and non-cancer health effects. Chronic, low level exposure may arise from typical occupational or personal use of these

[3] Individual pyrethroids assessed were allethrin, bifenthrin, cyfluthrin, gamma-cyhalothrin, alpha-cypermethrin, beta-cyfluthrin, cypermethrin, zeta-cypermethrin, cyhalothrin, lambda-cyhalothrin, deltamethrin, esfenvalerate, etofenprox, cyphenothrin, fenpropathrin, flumethrin, tau-fluvalinate, imiprothrin, momfluorothrin, d-Phenothrin/sumithrin, prallethrin, tefluthrin, and tetramethrin.

[4] A Tier II report for permethrin, a specific pyrethroid, including epidemiology studies as well as incidents and medical case studies was recently conducted in 2017 (E. Evans et al., D440947, 06/27/2017). As a result, permethrin-specific epidemiology studies were excluded from this Tier II Epidemiology Report. See US EPA. June 27, 2017. Permethrin: Tier II Incident and Epidemiology Report. https://www.regulations.gov/document?D=EPA-HQ-OPP-2011-0039-0084. Epidemiology studies on the pyrethrins were excluded from this Tier II Epidemiology Report as well as these are being considered separately.

[5] NRC. 2014. Review of EPA's Integrated Risk Information System (IRIS) Process. Washington, DC: National Academies Press.

[6] US EPA. December 28, 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides. https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf

[7] Higgins, J. P., & Green, S. (Eds.). (2011). Cochrane handbook for systematic reviews of interventions (Vol. 4). John Wiley & Sons.

pesticides. Literature reviewed in this section investigated population-based exposure and human health effects.

3 Background on Pyrethroids

HED is conducting a re-evaluation of the toxicity, exposure, and risk profile pertaining to the chemical class, pyrethroids as well as individual pyrethroids under the Food Quality Protection Act (FQPA)-mandated Registration Review program. The registration review program is designed to ensure EPA evaluates new information regarding pesticides on a 15-year cycle, and to update the risk assessment and initiate new regulatory requirements, when appropriate, to ensure the protection of human health and the environment. Pesticides included in the registration review program are pesticides for which EPA completed a Re-registration Eligibility Decision (RED) under the FQPA.

To help support the FQPA evaluation of pyrethroids[8], the Agency in 2018 contracted with Westat to conduct an epidemiology systematic literature review to investigate evidence on the human health effects associated with exposure to the pyrethroid chemical class and select individual pyrethroids. Sixty-two publications from 2003 – 2017 were identified for inclusion in the epidemiology literature review. These publications investigated carcinogenic and non-carcinogenic effects. Various study designs, including cohort, case-control, and cross-sectional were represented in the epidemiology material. Included publications were restricted to English language articles that reported estimates of effect (*e.g.,* odds or other effect size ratios, p-trend, or regression or correlation coefficients) for pyrethroids and/or individual pyrethroids specifically. Retrieved articles included study populations from the USA, France, Poland, South Africa, and South America.

The epidemiology section of this report details the formalized procedure used to collect relevant epidemiology literature on the human health effects associated with the chemical class, pyrethroids, as well as individual pyrethroids exposure (**Section 4 Systematic Literature Review and Data Collection**). It describes the findings of the articles selected for inclusion in the epidemiology literature review and critically evaluates the articles (**Section 5 Data Evaluation**). Finally, evidence from the literature is integrated to arrive at conclusions regarding key epidemiological findings for health outcomes associated with pyrethroids and individual pyrethroids (see footnote above) exposure.

[8] This epidemiology review is also intended to assist any decisions with respect to retention of FQPA safety factors. In 2011, the Office of Pesticide Programs (OPP) re-evaluated the need of a 10X FQPA Safety Factor for human health risk assessments for pyrethroid pesticides and based on a review of the available guideline and literature studies, the Agency reduced the FQPA Safety Factor to 1X for adults, including women of child-bearing age, and children greater than 6 years of age. However, the Agency retained a 3X FQPA Safety Factor for children from birth to 6 years of age. The retention of the 3X factor was based on age-dependent-pharmacokinetics and was further supported by physiologically-based pharmacokinetic (PK) model predictions of a 3-fold increase of pyrethroid concentration in juvenile target tissues compared to adults. In addition, there was sufficient evidence that the pharmacodynamic (PD) responses [were] similar in adults and juveniles and therefore a full 10X safety factory [was] not warranted Also in 2011, the Agency began to work with the Council for the Advancement of Pyrethroid Human Health Risk Assessment (CAPHRA), to obtain additional data for the assessment of pyrethroid age-dependent sensitivity using PBPK models for two separate models: rats to human models, and adults to children models. See Scollon, E.; 6/27/2011;TRX#: 0056045. The Agency is currently assessing these PBPK models to determine if there is a difference in sensitivity to pyrethroids for children versus adults, and ultimately make a decision regarding the retention of the 3X for children < 6 years of age based on the pharmacokinetic model. Information relevant to any putative enhanced sensitivity to pyrethroids is contained in Section 7.1 of this document on neurodevelopmental, neurobehavioral, and neurocognitive effects.

4 Systematic Literature Review and Data Collection

4.1 Data Collection: Methods and Sources

4.1.1 Eligibility Criteria

Specific inclusion criteria were identified prior to collecting potentially relevant publications. Inclusion criteria required studies to meet population, exposure, comparator, and outcome of interest (PECO) criteria.[9, 10] The population of interest was humans with no restrictions, including no restrictions on age, life stage, sex, country of residence/origin, race/ethnicity, lifestyle, or occupation. Exposure was to pyrethroids or pyrethrins in any application via any route of exposure. The exposed or case population must have been compared to a population with low/no exposure or to non-cases to arrive at a risk/effect size estimate of a health outcome associated with pyrethroids, and pyrethrins[11] and individual pyrethroids. The outcome of interest was all reported human health effects, with no restrictions on human system affected. Additionally, study publications must have been full text articles from observational studies published in English language peer reviewed journals, and publications must have reported on original data. The terms "pyrethrins" and "permethrin" were included in the literature search for this memo; however, once the study abstracts were later reviewed and assessed, studies that dealt exclusively with pyrethrins and/or permethrin[12] – but and not specific pyrethroids or pyrethroids as a general class were excluded from this memo.

Exclusion criteria were also identified prior to collecting potentially relevant publications. Articles were excluded for the following reasons: not full text (*e.g.,* abstracts); not peer-reviewed; not in English; non-human study subjects; in-vitro studies; fate and transport studies; outcome other than human health effects (*e.g.,* environmental measures); experimental model system studies; no risk/effect estimate reported (*e.g.,* case studies/series); no original data (*e.g.,* review publications).

A key element of the inclusion/exclusion criteria hinged on the definition of "human health effect" outcomes. For the purposes of the epidemiology literature review, the Agency considered human health effects via the toxicological paradigm presented by the NRC as pathologies or health impairments subsequent to altered structure/function.[13] Thus, studies with outcomes of altered structure (*e.g.,* Deoxyribonucleic acid (DNA) alteration, sister chromatid exchange, cell proliferation) or biomarker or other exposure outcomes (*e.g.,* in breast milk, urine, cord blood, or plasma) that did not also include an associated health pathology (*e.g.,* cancer, asthma, birthweight) failed to meet the inclusion criteria for "human health effects" for the purposes of this epidemiology literature review.

4.1.2 Open Literature Search

To complete a thorough search of the published literature in peer-reviewed journals, the Agency employed the scientific contractor Westat to search the established literature databases PubMed, PubMed Central, Science Direct, Toxline, SCIELO (Scientific Electronic Library Online), and SciSearch (**Table 1**). Publication records captured by the open literature search were imported into the citation manager

[9] Woodruff, T. J., & Sutton, P. (2014). The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. *Environmental Health Perspectives (Online)*, *122*(10), 1007.

[10] Rooney AA, Boyles AL, Wolfe MS, Bucher JR, and Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environmental Health Perspectives.

[11] The keyword 'pyrethrins' was included in the search inclusion criteria in an effort to not exclude potential studies involving pyrethroids. This is because studies on pyrethrin may also report findings on pyrethroids.

[12] See US EPA. June 27, 2017. Permethrin: Tier II Incident and Epidemiology Report. https://www.regulations.gov/document?D=EPA-HQ-OPP-2011-0039-0084.

[13] Henderson, R., Hobbie, J., Landrigan, P., Mattisoti, D., Perera, F., Pfttaer, E., ... & Wogan, G. (1987). Biological markers in environmental health research. Environmental Health Perspectives, 7, 3-9.

software EndNote (Clarivate Analytics, version 7.0.1), which was used to organize the literature search results and to remove duplicate records.

Publications underwent a series of reviews to determine eligibility for inclusion in the epidemiology literature review. In order to be retained in the epidemiology literature review, study publications had to meet the specific inclusion/exclusion criteria described above. Publications started with a title review, then progressed to an abstract review, and finally to a full text review. In the full text review, Endnote was used to search article PDFs for "pyrethroid", or by an individual pyrethroid name to locate in-text references to these chemicals. If a publication failed to satisfy the inclusion/exclusion criteria at any point in the review process, it was excluded and did not move to the next review step. Articles that met inclusion criteria at every review step were retained for the epidemiology literature review.

4.1.3 Supplemental Literature Search

To supplement the open literature search conducted via PubMed, Toxline, SciSearch, SCIELO, PubMed Central, and ScienceDirect, the Agency reviewed publications resulting from the AHS for articles that satisfied the inclusion/exclusion criteria. The AHS is a high quality, federally-funded prospective epidemiologic study evaluating the association between pesticide use and various health outcomes including cancer. and represents a collaborative effort between the US National Cancer Institute (NCI), National Institute of Environmental Health Sciences (NIEHS), CDC's National Institute of Occupational Safety and Health (NIOSH), and the US EPA. The AHS includes information on use of 50 different pesticide active ingredients commonly used in agriculture including the pyrethroids. The AHS participant cohort includes more than 89,000 licensed commercial and private pesticide applicators and their spouses from Iowa and North Carolina. Enrollment occurred from 1993 – 1997, and data collection is ongoing. The AHS provides high quality data from a large participant cohort over time, with reliable data of pesticide usage and lifestyle factors and information on specific pesticides rather than simply pesticide groups.[14, 15]

The AHS maintains on its website an electronic list of publications resulting from AHS studies and using the AHS cohort.[16] These articles were imported into Endnote, and Endnote was used to run a full text search ("Any Field + PDF with Notes") for "pyrethroid", or by an individual pyrethroid name in order to capture AHS publications potentially relevant to the epidemiology literature review. The articles resulting from this full text search underwent title, abstract, and full text review. AHS articles that satisfied the inclusion/exclusion criteria as described above were selected for inclusion in the epidemiology literature review.

The final phase of data collection was a reference review of articles captured in the open literature search, the AHS publication search, and previously published Agency documents. References were examined to identify relevant publications that were not captured in either the open literature search or the AHS publication search. Resulting articles from this reference review that satisfied inclusion/exclusion criteria were selected for inclusion in the epidemiology literature review.

[14] Blair A, Tarone R, Sandler D, Lynch CF, Rowland A, Wintersteen W, et al. Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. Epidemiology. 2002; 13(1):94–9.
[15] Koutros, S., Andreotti, G., Berndt, S. I., Barry, K. H., Lubin, J. H., Hoppin, J. A., . . . Yuenger, J. (2011). Xenobiotic metabolizing gene variants, pesticide use, and risk of prostate cancer. Pharmacogenetics and genomics, 21(10), 615.
[16] Agricultural Health Study: Publications https://aghealth.nih.gov/news/publications.html.

4.2 Data Collection: Results

4.2.1 Open Literature Search and Study Selection

The literature search, run on January 25, 2018, resulted in 570 distinct (non-duplicate) articles returned from PubMed, PubMed Central, Toxline, SciSearch, SCIELO and Science Direct (**Table 1**). Of the 570 articles returned, 139 articles were identified as potentially relevant to the epidemiology literature review of pyrethroids and underwent title review for inclusion/exclusion criteria, which resulted in the removal of 79 articles and the retention of 60 articles for abstract review. A total of 41 articles passed the abstract review based on the inclusion and exclusion criteria and underwent full text review for inclusion in the epidemiology literature review.

Table 1: Literature databases, search strategies, search date(s), and articles returned [17, 18]*

Database	Search Date(s)	Articles Returned
PubMed	12/26/17-1/3/18	323
PubMed Central	1/3/18–1/4/18	84
Science Direct	1/8/18–1/10/18	69
Toxline	1/4/18–1/5/18	51
SciSearch	1/8/18–1/9/18	43
SCIELO	1/5/18	0

*The search queries used for each of these databases can be found in Appendix B, below.

4.2.2 Supplemental Literature Search and Study Selection

The AHS publication search returned 60 articles from a full text search for the keyword 'pyrethroid', and 5 articles from a full text search for the keyword 'pyrethrin'. Additionally, the following number of articles were returned while searching for individual pyrethroids: bifenthrin (2 studies), cyfluthrin (4 studies), lambda cyhalothrin (2 studies), zeta cypermethrin (1 study), esfenvalerate (3 studies), and tefluthrin (2 studies). A total of 16 of those AHS articles passed title, abstract, and full text review for inclusion/exclusion criteria, and were included within the epidemiology literature review.

The final phase of article selection, the reference review of articles selected for inclusion and previously published Agency documents, resulted in the addition of additional articles ($n \leq 10$) selected for inclusion in the epidemiology literature review.

A total of 62 articles were selected for inclusion in this epidemiology literature review (Figure 1) focused on the association between pyrethroids and human health outcomes, with some authors reporting more than one health effect within each study.

[17] Chemical synonyms were utilized in the PubMed and the Web of Science literature search to capture articles utilizing only these terms in the citation material and the abstract; since ScienceDirect searches full text, only the generic chemical names were searched in ScienceDirect to reduce false hits. Chemical synonyms obtained from the following manual: Roberts, James R., and John Routt Reigart. *Recognition and management of pesticide poisonings*. 6th edition. National Pesticide Telecommunications Network, 2013.

[18] The number of articles reported reflects a net return and does not consider duplicates (the same article returned in multiple databases and/or multiple times in one database).

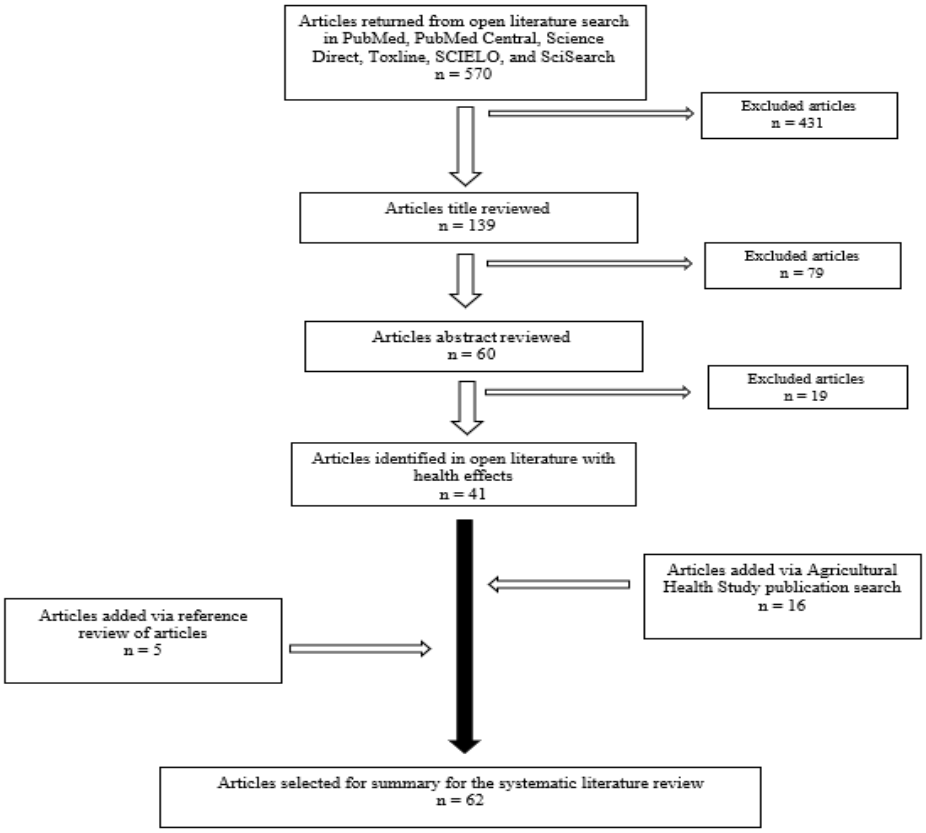

Figure 1: Selection of studies for literature review of pyrethroids and epidemiological health effects.

5 Data Evaluation

5.1 Overview of Publications Selected for Inclusion

The 62 studies selected for inclusion in the epidemiology literature review included cohort studies, case-control studies, and ecological studies (**Appendix A**).

5.2 Study Review and Quality Assessment

Data evaluation included a concise summary of the 62 publications found to be fit for purpose and thus included in the literature review of epidemiology investigations of health effects associated with pyrethroids, pyrethrins, or individual pyrethroids. Summaries provide information on study design, results, conclusions, and study quality, and recount details including the exposure measurement, outcome ascertainment, number of participants (n), number exposed/number of cases, number in reference (un-exposed/control) group, effect measure (*e.g.,* odds ratio (OR), relative risk (RR), hazard ratio (HR), regression coefficients) and associated estimate of uncertainty and/or statistical significance (*e.g.,* confidence interval (CI), p-value), confounders considered, and methods of analysis. The Agency considered these elements in assessing the quality of each publication and its applicability to an overall assessment of the health effects associated with pyrethroids and pyrethrins exposure.

The assessment of study quality followed the OPP epidemiology framework (US EPA, 2016). As shown in **Table 2** below, the study quality assessment considered aspects such as design, conduct, analysis, and interpretation of study results, including whether study publications incorporated a clearly articulated hypothesis, adequate assessment of exposure, critical health windows, valid and reliable outcome ascertainment, a sample representative of the target population, analysis of potential confounders,

characterization of potential systematic biases, evaluation and reporting of statistical power, and use of appropriate statistical modeling techniques.

Table 2: Summary of study quality considerations (Adapted from Table 2 of US EPA, 2016).

Parameter	High	Moderate	Low
Exposure assessment	Accurate and precise quantitative relationship with external exposure, internal dose, or target dose, possibly associated with a MOA/AOP. If questionnaire utilized, questionnaire and/or interview answered by subjects for chemical-specific exposure	Evidence exists for a relationship between biomarker in a specified matrix and external exposure, internal dose, or target dose. Questionnaire and/or interview for chemical-specific exposure answered by subjects or proxy individuals	Poor surrogate Low-quality questionnaire and/or interview; information collected for groups of chemicals rather than chemical-specific; no chemical-specific exposure information collected; ever/never use of pesticides in general evaluated
Outcome Assessment	Standardized tool, validated in study population; medical record review/diagnosis confirmation by trained staff; appropriate consideration of prevalence/incidence of cases	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated	Selected sections of test, or maternal report, other; or, maternal/paternal self-report; unclear/no consideration for whether prevalent or incident cases are appropriate
Confounder control	Good control for important confounders relevant to scientific question, and standard confounders	Moderately good control confounders, standard variables, not all variables relevant for scientific question	Multi-variable analysis not performed no adjustments; no stratification, restriction, or matching
Statistical Analysis	Appropriate to study question and design, supported by adequate sample size, maximizing use of data, reported well (not selective)	Acceptable methods, questionable study power (especially sub-analyses), analytic choices that lose information, not reported clearly	Minimal attention to statistical analyses, comparisons not performed or described clearly
Risk of (other) bias (selection, differential misclassification, effect size magnification, other)	Major sources of other potential biases not likely present, present but analyzed, unlikely to influence magnitude and direction of the risk estimate	Other sources of bias present, acknowledged but not addressed in study, may influence magnitude but not direction of estimate	Major study biases present, unacknowledged or unaddressed in study, cannot exclude other explanations for study finding

Study design generally influenced the assessment of study quality. Cohort studies, which enable researchers to assess the temporality of exposure in relation to health outcome and to consider multiple health outcomes, were generally considered higher quality than other study designs. Case-control studies, which can be susceptible to recall bias, were generally considered lower quality than nested case-control studies, which may be less susceptible to selection and recall bias. Cross sectional studies cannot distinguish temporality for exposure in relation to health outcomes; therefore, cross-sectional studies were generally considered lower quality than cohort or case-control studies and were regarded as hypothesis-generating in the absence of additional studies supporting an observed association. The lowest quality study design considered was ecologic studies, due to an inability to extrapolate observed associations from the group level to the individual level (ecological fallacy) inherent in the ecologic study design. This major limitation of ecological studies results in the tendency for some observed relations that appear on a group level to disappear when the effects are analyzed at the level of the individual. As a result, ecologic studies were also generally regarded as hypothesis-generating studies.

Studies that characterized the exposure-response relationship (*e.g.,* with a dose-response curve or trend statistic) were, in general, considered higher quality in EPA's evaluation of studies than studies that did not characterize exposure-response. Studies that specified temporality (*i.e.,* those that determined exposure preceded a health outcome) and studies that specified uncertainties in the analysis were, in

general, considered higher quality than studies that failed to specify temporality and studies that lacked an examination of uncertainty. Consistent results between study groups (*e.g.,* a significant and positive association seen for both farmers and commercial applicator study groups within a single study or studies showing similar results between farmers in different locations) bolstered the assessment of study quality.

It important to recognize that the considerations listed above in **Table 2** (and study quality categorizations in general) are not intended to be hard demarcations between these quality categorizations which are perhaps better considered as a continuum: in addition, it is possible and oftentimes likely that individual studies have characteristics that cross among these elements or vary depending on the several specific outcome and exposures being considered in the study.

Risk estimates (estimates of effect) reported in epidemiological studies were generally characterized as follows:

- no evidence of a positive association between exposure and outcome (*e.g.,* OR = 1.00; OR < 1.00);
- no evidence of a significant positive association (*e.g.,* OR > 1.00 but not significant);
- evidence of a slight positive association (*e.g.,* 1.00 < OR < 1.30 and significant);
- evidence of a positive association (*e.g.,* 1.30 ≤ OR < 2.0 and significant); and
- evidence of a moderately strong (*e.g.,* 2.0 ≤ OR < 3.0 and significant) or strong (*e.g.,* OR ≥ 3.0 and significant) positive association.[19]

However, we recognized that results that failed to attain statistical significance may still indicate clinical, biological, and/or public health importance and may warrant further exploration (US EPA, 2016). We particularly noted in this review observed associations with large effects sizes (*e.g.,* OR ≥ ~ 2.5) even in the absence of significance, perhaps indicating a smaller than optimal sample size or the potential for biases. Conversely, we also recognized that statistical significance does not necessarily imply clinical or biological importance, particularly with larger than necessary sample sizes and other study elements that influence the reliability of estimated effects.

5.3 Categories of Evidence

After assessing the quality of the relevant studies, an overall evaluation of the strength of the evidence for an association between exposure and each health effect is assessed. Based on this assessment, each health effect is assigned a category of evidence (**Table 3**). The categories of evidence are guided by several documents developed by EPA and others. The main reference is from the Institute of Medicine (now the Academy of Medicine)[20] which details various "Categories of Association" that guide strength of the evidence conclusions for any putative linkage between an exposure and a health effect. Also considered in developing OPP's categories of evidence were the National Toxicology Program's OHAT document

[19] For articles that reported ORs, RRs, and HRs, the confidence interval (CI) acted as a proxy for significance testing, with CIs that do not contain the null value (OR / RR / HR = 1.00) considered significant. P-value significance considered a critical value of α = 0.05 unless otherwise specified by the authors and noted in the summaries here.

[20]IOM (1998). Veterans and Agent Orange Update 1998. National Academy Press. Washington, DC. https://www.nap.edu/read/6415/chapter/1. Some of this material is derived from and/or consistent with U.S. Department of Health and Human Services. *The Health Consequences of Smoking: A Report of the Surgeon General*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2004 and its Chapter 1 " Introduction and Approach to Causal Inference", available at: http://www.surgeongeneral.gov/library. Much of this material is also presented in a more recent National Academies publication from 2018: National Academies of Sciences, Engineering, and Medicine 2018. *Gulf War and Health: Volume 11: Generational Health Effects of Serving in the Gulf War*. Washington, DC: The National Academies Press. https://doi.org/10.17226/25162

on systematic review and evidence integration,[21] OPP's epidemiologic framework document,[22] and EPA's Preamble to the Integrated Science Assessments (ISAs) which serves as a scientific foundation for the review of EPA's National Ambient Air Quality Standards (NAAQS).[23]

In this memorandum, each category is assigned based on a flexible case-by-case approach that considers the weight of the epidemiological evidence and expert judgement rather than a binding or inflexible formulaic approach in deciding the number and/or quality of studies that would be necessary to assign a specific evidence category. As described in OPP's epidemiologic framework document, when assigning a level of evidence category to an exposure and the body of evidence pertaining to that health effect, the following were considered:

- the level of quality of the studies available in the peer-reviewed literature for that health effect;
- the strength of the associations (effect sizes); and,
- the consistency of the association in magnitude and direction across available studies.

[21] Woodruff TJ and Sutton P. 2014. *The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes*. Environ. Health Perspect. Oct;122(10):1007-14. doi: 10.1289/ehp.1307175.

[22] US EPA. December 28, 2016. Office of Pesticide *Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides*. https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf.

[23] US EPA (U.S. Environmental Protection Agency). 2015. *Preamble to the Integrated Science Assessments.* National Center for Environmental Assessment, RTP Division, Office of Research and Development, USEPA. https://yosemite.epa.gov/sab/sabproduct.nsf/0/33E1AD305287588F85257D20006BE8CC/$File/ISA_PREAMBLE_FINAL2015.PDF

Table 3: Categories of Evidence

Evidence Category	Description
Sufficient Epidemiological Evidence of a Clear Associative or Causal Relationship	*Sufficient epidemiological evidence to suggest a clear associative or causal relationship between the exposure and the health outcome.* There is high confidence in the available evidence to suggest that a clear associative or causal relationship exists between the exposure and the health outcome of interest. Studies are minimally influenced by chance, bias, and confounding. Further, additional epidemiological data, evidence, or investigations are unlikely to substantively affect the overall magnitude or direction of the observed association or result in a meaningful change with respect to any conclusions regarding this association. This level of evidence might be met, for example, if several high- or moderate- quality studies on different study populations, by different authors, in different settings, and/or using different epidemiological study designs that are likely to be minimally influenced by bias and confounding show a clear associative or causal relationship; such observed effects should be consistent among studies with respect to magnitude and direction of effect sizes. Evidence is strengthened when one or more high- or moderate-quality studies also demonstrate dose-response trends, with the range of these doses (exposures) considered sufficient to cover the range of expected human exposure levels (including the high end) and the evidence base consists of a least one high-quality prospective cohort study.
Limited but Insufficient Epidemiological Evidence of an Association	*Limited but insufficient epidemiological evidence to conclude that there is a clear associative or causal relationship between the exposure and the health outcome.* There is some confidence that the available evidence accurately reflects a clear association between the exposure and the health outcome of interest, but the evidence is limited because the studies are of insufficient quantity, quality, (internal) validity, or consistency or because chance, bias, and confounding could not be ruled out with confidence. While the present body of evidence suggests that a relationship between exposure and disease outcome may possibly exist, additional high- or moderate-quality epidemiological data, evidence, or investigations could affect the overall magnitude or direction of the observed associations and might result in a meaningful change to this level of evidence category. This level of evidence category might be met, for example, if the body of evidence is: (1) based at least on at least one high-quality study suggesting a statistically significant relationship and the results of other high or moderate quality studies are mixed, contradictory, imprecise, ambiguous, or inconsistent; (2) based on several moderate-quality studies which show a relationship between exposure and outcome that is less pronounced than in (1); or (3) based on many studies (both moderate and possibly low-quality studies) showing a generally consistent direction and for which additional and more thorough analysis would be needed to make the determination of a relationship.
Insufficient Epidemiological Evidence of an Association	*Insufficient epidemiological evidence to conclude that there is a clear associative or causal relationship between the exposure and the health outcome.* There is minimal confidence in the available evidence that the findings accurately reflect an association between the exposure and the health outcome of interest because the studies are of insufficient quantity, quality, (internal) validity, consistency, or statistical power to permit a conclusion to be reached, and/or chance, bias, or confounding may play an important role and cannot be ruled out. Further, additional high- or moderate-quality epidemiological data, evidence, or investigations could substantively affect the overall magnitude or direction of any observed associations. This level of evidence category might be met, for example, if the body of evidence is: (1) too small to permit conclusions, such as when there are no available studies to validate or corroborate the findings of a single moderate- or low-quality study; (2) based entirely on one or more studies judged to be of low-quality; or (3) based on multiple moderate- or low-quality studies, but the heterogeneity of exposures, outcomes, and methods leads to mixed, conflicting, imprecise, ambiguous, or contradictory conclusions.

Evidence Category	Description
No Epidemiological Evidence of an Association	*No epidemiological evidence to conclude that there is a clear associative or causal relationship between the exposure and the health outcome.* There is no epidemiological evidence to suggest the presence of an association between the exposure and the health outcome of interest. While all available studies failed to observe a positive association, there is minimal confidence because the studies are of insufficient quantity, quality, (internal) validity, consistency, or statistical power to permit a conclusion to be reached, and/or chance, bias, or confounding may play an important role and cannot be ruled out. Further, additional high- or moderate-quality epidemiological data, evidence, or investigations could substantively affect the overall magnitude or direction of any observed associations. This level of evidence category might be met, for example, if the body of evidence consists of only studies that failed to show evidence of an association; however the studies may have small sample sizes or be insufficiently powered, and/or chance, bias, or confounding may play an important role.
Sufficient Evidence of No Causal Relationship	*Sufficient epidemiological evidence to suggest there is no causal relationship between the exposure and the health outcome.* There is high confidence in the available evidence to suggest there is no causal relationship between the exposure and the health outcome of interest. The studies are minimally influenced by chance, bias, and confounding, and it is unlikely that additional epidemiological data, evidence, or investigations would meaningfully affect the current overall magnitude, direction, or conclusions about the association. This level of evidence category might be met, for example, if at least one high-quality study with adequate power (e.g., ≥80%) to detect an effect size determined to be of substantive importance fails to show an effect and no other high or moderate quality studies provide affirmative evidence against this null result. In addition, data would also exist that suggests no significant dose-response trends are present with the range of these doses (exposures) considered sufficient to cover the range of expected human exposure levels (including the high end) and the evidence base consists of at least one high-quality prospective cohort study.

5.4 Common Study Considerations

This section describes more specific study considerations that were frequently identified when evaluating the available epidemiologic literature on pyrethroids. These general considerations include pyrethroid biomonitoring and the Agricultural Health Study (AHS).

5.4.1 Pyrethroid Biomonitoring

Many of the epidemiologic studies identified assessed pyrethroid exposure by measuring pyrethroid metabolite levels in urine samples obtained from study participants. Urinary biomonitoring can provide a quantified measurement of internal dose and has advantages over indirect methods that are also commonly used in the available literature, such as pesticide use questionnaires, environment monitoring, and historical records (US EPA, 2016). While this is the case, biomonitoring also has limitations that need to be considered when evaluating studies. These limitations are described in US EPA (2016) and include:

- Urine is often only sampled from a single point in time and may not reflect accurately reflect longitudinal patterns, particularly if exposures are highly variable and compounds are rapidly eliminated from the body.
- Evaluation of biomarkers requires an understanding of degradation and metabolism of chemicals in both the environment and human body. Differences in metabolism and uncertainty as to whether the biomarker measures exposure to the active ingredient or the environmental

degradates may all account for apparent differences in biomarkers of exposure among individuals, and possibly between comparison groups.

- Urinary output can be highly variable and requires that concentrations be normalized across and within study participants when making comparisons. A range of approach may be reported in the literature, but a commonly accepted approach for normalizing urinary concentrations is to adjust for creatinine output or by specific gravity adjustment. Further, differences may be attributable to time of urine collection (*e.g.,* first morning void, random void, after overnight fasting) or the collection mode (*e.g.,* spot urine sample, 24-hour collection)

In addition to the considerations above, it is also important that studies ensure that their sampling protocol and analytical methods minimize the potential for sample contamination and measurement error to the extent possible. Therefore, it is important that studies characterize their quality assurance and quality control (QA/QC) plan and use an established analytical method that provides unambiguous identification and quantitation of the biomarker with an acceptable level of sensitivity.

With regard to urinary biomonitoring of pyrethroids more specifically, the most commonly measured urinary biomarkers and their corresponding parent compounds are summarized in the **Table 4** below. As shown, *cis*- and *trans*-DCCA, CDCA, and 3-PBA are less specific biomarkers that may reflect exposure to multiple different parent compounds, where as DBCA and 4F3-PBA may be reflective of exposure to deltamethrin and cyfluthrin, respectively (CDC, 2009; Sudakin, 2006). While these biomarkers may be indicative of direct exposure, the same biomarkers are formed in the environment through natural degradation processes. As such, measurement of these pyrethroid urinary biomarkers may reflect exposure to pyrethroid insecticides and intake of the same compounds in the environment (Sudakin, 2006).

Table 4: Common pyrethroid urinary biomarkers and their corresponding parent compound (Adapted from CDC, 2009 and Sudakin, 2006).

Biomarker	Allethrin	Cyfluthrin	Cyhalothrin	Cypermethrin	Deltamethrin	Fenpropathrin	Fenvalerate	Permethrin	Resmethrin	Tetramethrin	tralomethrin
cis-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (***cis*-DCCA**)		●		●				●			
trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (***trans*-DCCA**)		●		●				●			
cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (**DBCA**)					●						
cis/*trans*-chrysanthemum dicarboxylic acid (**CDCA**)	●								●	●	
3-phenoxybenzoic acid (**3-PBA**)			●	●	●	●	●	●			●
4-fluoro-3-phenoxybenzoic acid (**4F3-PBA**)		●									

5.4.2 Agricultural Health Study (AHS)

A number of the identified epidemiologic studies were conducted as part of the AHS. As described earlier in this document, the AHS is a federally funded effort begun in the early 1990s that evaluates associations between pesticide exposures and cancer and other health outcomes. The participant cohort includes more

than 50,000 licensed private (farmer) and commercial pesticide applicators from Iowa and North Carolina in addition to their spouses (for a total of more than 90,000 participants). The AHS is a prospective cohort design in which enrollment occurred from 1993 – 1997; data collection is ongoing from both applicator and spousal participants. Because the AHS is a prospective cohort design, this means that much of the exposure information is collected *prior to* the diagnosis (or detection) of the disease, and this can potentially limit to a substantial degree issues related to (case) recall bias which is a serious methodological weakness of many case-control studies. Such recall bias can be common among case-control designs where individuals that are either diseased (cases) or not (controls) are asked about their exposure histories. To the extent that cases and controls can differentially recall such exposures, such case-control designs can potentially be subject to considerable biases. For the nested case- control studies within the AHS, this can potentially lead to recall biases depending on the degree to which either the study collects information from farmers (or next of kin) after the disease diagnosis and whether cases and controls are asked to provide supplemental information or more detailed questionnaires regarding exposure history or other practices. Cancer determination in the AHS is through cancer registries in the states of IA and NC and are considered reliable.

While the AHS provides generally high-quality information with reliable data regarding pesticide usage and lifestyle factors and information on specific pesticides rather than simply pesticide classes or groups, collecting such exposure information can be complex and it can be difficult to judge its validity or reliability. The AHS has been reviewed in this regard and has been found to be generally reliable:[24] the study design/questionnaire is particularly advantageous because it collects information on individual pesticides – and not just groups or classes of pesticides as is characteristic of several other epidemiologic studies. But individuals – particularly over several years or decades – are exposed to a number and variety of pesticides which can complicate epidemiological analyses by introducing confounders or sometimes "collinearity" whereby it can be difficult to isolate causal or suggestive factors contributing to disease. In addition, field studies have shown wide variation in work and hygienic practices among farmers (and farm workers) and exposures – and especially exposures over long time periods time - can thus be difficult to accurately assess. The AHS does have in place an algorithm that attempts to account for certain work or hygienic practices by adjusting estimated exposures to account for use by farmers of personal protective equipment and practices; this algorithm considered such work and hygienic practices, including the mix of activities performed (*e.g.,* mixing/loading vs. application) and provides exposure estimates on both a cumulated (lifetime day)- and intensity-weighted cumulated (intensity-weighted lifetime day)- basis.[25] Nevertheless, the AHS algorithms assume that total (cumulated) lifetime exposure depends on the multiplicative product of annual frequency of applications by a farmer and the association number of years of application and this may not be strictly true and could systematically overestimate or underestimate exposures. Too, use practices such as application equipment and methods for a given pesticide can change over time, in addition to formulations (and farming practices in general) which can add additional uncertainties with respect to any assessment of cumulated exposure.

[24] Blair A, Tarone R, Sandler D, Lynch CF, Rowland A, Wintersteen W, et al. Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. Epidemiology. 2002; 13(1):94–9.
[25] Dosemeci, M, Alavanja MCR, Rowland AS, Mage D, HoarZahm S, Rothman N, Lubin JH, Hoppin JA, Sandler DP, and Blair A. 2002. A Quantitative Approach for Estimating Exposure to Pesticides in the Agricultural Health Study. Ann. Occup. Hyg. 46(2): 245-260.

6 Carcinogenic Effects

A number of studies were reviewed which investigated carcinogenic effects. Specifically, one study investigated the relationship between pyrethroid exposure and childhood brain tumors in China (Chen et al., 2016), two studies (Ding et al. 2012 in China, Ferreira et al. 2013 in Brazil) investigated the relationship between pyrethroids and leukemia in children, and one study (Engel et al.,2005) conducted as part of the AHS investigated the relationship between pyrethroid exposure and breast cancer. These are further described below.

6.1 Childhood Brain Tumors (CBT)

One study investigated the relationship between childhood brain tumors (CBT) and pyrethroid exposure in East China. These study results are provided below.

Chen et al. (2016) conducted a case-control study to evaluate the association between pyrethroid exposure and CBT among children residing in East China, with a cross-sectional analysis of urinary biomarker data. Cases and controls were recruited from two medical centers located in Shanghai between 2012 to 2015, and cases included children aged 0 – 14 years, who were diagnosed with CBT (within the past 4 weeks). Controls were selected from the Departments of Child and Adolescent Healthcare and (frequency) matched to the cases by age (within 12 months), sex, and province of residence. In total, 172 cases and 189 controls met the eligibility criteria, but final study counts were based on 161 cases[26] and 170 controls after exclusions due to missing urinary samples or missing questionnaire data. Pyrethroid exposure was evaluated using a spot urine sample for each case and control to detect three nonspecific pyrethroid metabolites through gas chromatography-mass spectrometry (GC-MS): *cis*-DCCA, *trans*-DCCA, and 3-PBA. Detection rates ranged from 68.2% (for *cis*-DCCA in the control group) to 91.3% (for 3-PBA in the case group). Standard laboratory quality control protocols were followed and included freezing samples during storage at -80°C, determining intra-assay precision and mean recoveries, and use of blanks and QC (spiked) samples. Urinary creatinine corrections were made to account for differing urine dilutions. A study questionnaire was administered to the mothers of the cases and controls via trained nurses. This questionnaire acted as an additional tool for exposure assessment, and included questions detailing exposure histories during pregnancy and household usage of pesticides. An unconditional logistic regression was used to calculate ORs and 95% CIs, adjusting for sex, age, household income, maternal education level, and province of residence. Quartiles of pyrethroid metabolite levels were created for each of the three metabolites (*cis*-DCCA, *trans*-DCCA, and 3-PBA), and results indicated a positive association in the second highest quartile (Q3) of exposure for *trans*-DCCA (Q3: 0.44 - < 1.38 µg/g creatinine OR: 1.95; 95% CI: 1.04, 3.68 with 52 cases and 43 controls), and a strong positive association in the highest quartile (Q4) for *trans*-DCCA and 3-PBA (Q4: > 1.38 µg/g creatinine OR: 2.58; 95% CI: 1.38, 4.80 with 67 cases and 42 controls, p-trend = 0.025 for *trans*-DCCA; Q4: > 1.81 µg/g creatinine OR: 3.26; 95% CI: 1.73, 6.14 with 75 cases and 42 controls, p-trend = 0.013 for 3-PBA). A strong positive association was also observed for total metabolites[27] in the two highest quartiles (Q3: 1.65 - < 4.25 µg/g creatinine OR: 2.25; 95% CI: 1.14, 4.42 with 46 cases and 43 controls; Q4: > 4.25 µg/g creatinine OR: 3.60; 95% CI: 1.87, 6.93 with 72 cases and 42 controls, p-trend = 0.009). No evidence of a significant positive association was reported for *cis*-DCCA in any quartile; nor for Q2 for *trans*-DCCA and Q2, and Q3 for 3-PBA.

[26] The 161 cases were distributed among 8 brain cancer types: medulloblastoma (n = 37); choroid plexus papilloma (n = 19); craniopharyngioma (n = 17); ependymoma (n = 13); teratoma (n = 7); penioblastoma (n = 5); and primitive neuroectodermal tumor (n = 4).

[27] Total metabolites are the total concentrations of *cis*-DCCA, *trans*-DCCA, and 3-PBA.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids and brain tumors in children. The single study on CBT by Chen et al. (2016) was ranked to be low quality and had several limitations. Most importantly, the study's assessment of chronic pyrethroid exposure was based on analysis of a single, spot urine sample from each study subject and was obtained after CBT diagnosis. Pyrethroids have a relatively short biological half-life (on the order of hours) in the body, so it is not clear that this approach can reliably estimate chronic exposure over the long term during any time interval that preceded diagnosis of CBT. In addition, the approach used here measured three metabolites or degradates of pyrethroid pesticides and may simply be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites present in the environment and food and not pyrethroid pesticides *per se*. While the questionnaire attempted to evaluate active use or exposure to pyrethroid pesticides by households, the study authors noted the lack of association between self-reported household pesticide usage and the levels of urinary pyrethroid metabolites detected (this, despite the fact that pyrethroid metabolites in urine in the case group were higher than those in the control group, and the case group reported higher usage of mosquitocides and pesticides for cockroach control). Such lack of association could have been for a number of reasons including the possibility that pyrethroid (or pyrethroid degradate) exposure occurred from sources other than active near-term use of pyrethroid-containing household products. Lastly, a wide range of ages were used in this study for the cases and controls, which could potentially be an issue – particularly given the heterogeneity of the eight tumor types investigated here – if different age groups of children have different exposure levels to pyrethroids.

6.2 Childhood Leukemia

Two studies (Ding et al. 2012, Ferreira et al. 2013) investigated the relationship between pyrethroids and leukemia in children. These study results are provided below.

- Ding et al. (2012) conducted a case-control study to evaluate the association between urinary pyrethroid metabolites (*cis*-DCCA, *trans*-DCCA, and 3-PBA) and acute lymphocytic leukemia (ALL) among children residing in Shanghai, China, with cross-sectional analysis of urinary biomarker data. ALL cases were identified from four children's hospital within Shanghai during 2010 – 2011, and included children aged 0 – 14 years of age who had been recently diagnosed with ALL (less than two weeks). Controls were selected from the Departments of Child and Adolescent Healthcare and were matched to the cases via age (within 1 year), sex, and hospital at diagnosis. Based on this approach, a total of 213 cases and 235 controls were identified and – after excluding those with missing urine and/or questionnaire data – a total of 176 cases and 180 controls were included in the study and statistical analysis. Exposure was assessed by collecting spot urine samples from the study participants and measuring for the following three pyrethroid metabolites:[28] 3-PBA, *cis*-DCCA, and *trans*-DCCA using GC-MS detection with a limit of detection (LOD) of 0.1 µg/L, with < LOD measurements assigned a value of ½ LOD. Standard laboratory quality control protocols were followed and included freezing samples during storage at -80°C, determining intra-assay precision and mean recoveries, and use of blanks and QC (spiked) samples. Urinary creatinine corrections were made to account for differing urine dilutions. In addition, a study questionnaire was administered to the mothers of the cases (shortly after diagnosis) and to the mothers of controls via trained interviewers. This questionnaire acted as an additional tool for the exposure assessment, and included questions detailing demographic information and past household pesticide usage (from birth to cancer diagnosis of the child)

[28] These metabolites would be associated with exposure to, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, tralomethrin, and permethrin. See *Table 4: Common pyrethroid urinary biomarkers and their corresponding parent compound* in Section 5.3.1 for a cross-walk between parent pyrethroid pesticides and environmental degradates and metabolites.

obtained during an in-person interview with the children's mothers.[29] Only household pesticide usage was considered in this study; professional pest control or pesticides for lawn and garden were not used by any of the study families. An unconditional logistic regression analysis was conducted to determine ORs and 95% confidence intervals for individual pyrethroid metabolites, adjusting for sex, age, household income, place of residence, parent education level, and breast-feeding duration.[30] Quartiles were created for each urinary pyrethroid metabolite in children, and individual adjusted ORs were reported for each quartile. For *cis*-DCCA and *trans*-DCCA, evidence of a strong positive association at the two highest metabolite levels was observed relative to ALL in children (*cis*-DCCA 0.45 - < 1.26 µg/g creatinine OR = 2.23 95% CI: 1.18, 4.23 n = 55 cases, 45 controls; > 1.26 µg/g creatinine OR = 2.21 95% CI: 1.16, 4.19 n = 54 cases and 45 controls; *trans*-DCCA 0.54 - < 1.39 µg/g creatinine OR = 2.09 95% CI: 1.10, 3.97 n = 52 cases, 45 controls; > 1.39 µg/g creatinine OR = 2.33 95% CI: 1.23, 4.41 n = 57 cases, 45 controls). Significant exposure-response trends were observed for *cis*-DCCA, *trans*-DCCA, and 3-PBA (p-trend = 0.055, 0.024, 0.019, respectively). When the individual maternal urinary metabolites (3-PBA, *cis*-DCCA, and *trans*-DCCA) were summed and assessed, evidence of a strong positive association at the two highest metabolite levels was observed, along with a significant exposure-response trend (1.88 - < 4.25 µg/g creatinine OR = 2.21 95% CI: 1.14, 4.30 n = 50 cases and 45 controls; > 4.25 µg/g creatinine OR = 2.75 95% CI: 1.43, 5.29 n = 59 cases, 45 controls, p-trend = 0.010).

The study quality was ranked low. Study limitations included the study's assessment of chronic pyrethroid exposure which was based on analysis of a single, spot urine sample from each study subject. Pyrethroids have a relatively short biological half-life in the body, so it is not clear that this approach can reliably estimate chronic exposure during the time interval that preceded diagnosis. In addition, the study measured three metabolites or degradates of pyrethroid pesticides and may simply be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites present in the environment and food and not pyrethroid pesticides *per se*. In fact, the study authors noted (data not provided) that there was no correlation between self-reported use of household pesticides and the pyrethroid concentrations measured in urine. Importantly, too, the temporal relationship between the exposure and the outcome cannot be confirmed due to the cross-sectional study design. Lastly, a wide range of ages were used in this study for the cases and control, which could potentially become an issue if different age groups of children, have different exposure levels to pyrethroids.

- Ferreira et al. (2013) conducted a multicenter, hospital-based case-control study to evaluate the associations between perinatal pesticide exposure and leukemia among children less than 2 years of age in Brazil. Study participants were recruited from 13 states in all geographic areas of Brazil, with the exception of the Amazon. Cases (n = 252) were identified between 1999 and 2007 and were children less than 24 months of age with a conclusive diagnosis of acute lymphoid leukemia (ALL) (n = 193) or acute myeloid leukemia (AML) (n = 59) confirmed by morphology, immunophenotype, and standard cytogenetic-molecular methods. Controls (n = 423) were frequency-matched to cases by age and geographic area with non-malignant diseases who were

[29] This included information on age, educational level, household income, occupation, place of residence, maternal smoking, alcohol use, X-ray exposure and viral infection during pregnancy as well as family history of cancer and autoimmune diseases. Also included were questions relating to regular use of pesticides either by the mother herself or other family members and, if so, the types of pesticides used (mosquito repellent, cockroach killer, mothproofing agent, rodenticide, termite control, herbicide, or other pesticides).

[30] The regressions were repeated by including other commonly-considered confounders (maternal smoking, alcohol use, X-ray exposure, and viral infection during pregnancy, parental occupation, and family history of cancer autoimmune disease) but all p-values were > 0.10 and including them did not markedly alter the results.

either patients at the same Brazilian National Health System centers or patients of general hospitals in the same cities. Pesticide exposure was assessed through in-person interviews of mothers of cases and controls using a standardized questionnaire. Pesticide exposure (including pyrethroids and individual pesticides)[31] was evaluated based on the mother's report of any contact with pesticides (at least once) at home or in the workplace during the 3 months before pregnancy, throughout each pregnancy trimester, and during the 3 months after birth (breastfeeding). Unconditional logistic regression was used to estimate ORs and 95% CIs, adjusting for maternal age at birth, skin color, child's birth weight, maternal education, and oral contraceptive usage during pregnancy. Of the 193 ALL cases, 63 (32.60%) cases reported maternal exposure to pyrethroids during pregnancy and evidence of a positive association was reported for pyrethroid exposure during pregnancy and child ALL (adjusted OR 1.80; 95% CI: 1.10, 2.90). Of the 59 AML cases, 25 (42.40%) cases reported maternal exposure to pyrethroids during pregnancy and evidence of a strong positive association was reported for pyrethroid exposure during pregnancy and child AML (adjusted OR (aOR) 3.39; 95%CI: 1.72, 16.78). More detailed results for individual pyrethroid compounds are further summarized below:

- For the analysis of ALL cases and maternal exposure to individual pyrethroids, evidence of a moderately strong association was reported for exposure to the pyrethroid imiprothrin during pregnancy and child ALL cases at 0-11 months of age (adjusted OR 2.61; 95%CI: 1.06, 6.93, with n = 12 cases, 14 controls); No evidence of a positive association was reported for ALL cases at 0 - 11 months and 12 - 23 months and maternal exposure to any of the other pyrethroids prallethrin, tetramethrin, D-phenothrin, and D-allethrin or for imiprothrin and ALL cases at 12 - 23 ($0.69 \leq$ adjusted OR ≤ 4.16; all 95% CIs encompassed the null 1.0, with n = 1 - 19 cases, 3 - 24 controls).
- For the analysis of AML and maternal exposure to individual pyrethroids, among AML cases 0 - 11 months, strong associations were reported for maternal exposures to the pyrethroids prallethrin, tetramethrin, and D-allethrin and AML cases 0 - 11 months of age (prallethrin aOR = 8.06; 95% CI: 1.17, 55.65, with n = 2 cases, 3 controls; tetramethrin aOR = 6.19; 95% CI: 2.07, 18.56, with n = 8 cases, 23 controls; D-allethrin aOR = 6.19; 95% CI: 2.07, 18.56, with n = 8 cases, 24 controls) respectively. No evidence of significant positive associations was reported for the pyrethroids imiprothrin and D-phenothrin and AML cases 0 - 11 months of age ($1.64 \leq$ aOR ≤ 3.41; all 95% CIs encompassed the null value 1.0, n = 1 - 5 case, 3 - 14 controls).
- For AML cases 12 - 23 months of age, evidence of a strong association was reported for maternal exposure to D-phenothrin and child AML cases 12 - 23 months (aOR 8.43; 95% CI: 1.59, 44.75, with 4 cases, 3 controls); no evidence of significant positive associations were reported for other individual pyrethroids prallethrin (n = 0 cases), imiprothrin, tetramethrin, and D-allethrin for AML cases aged 12 - 23 months ($0.46 \leq$ aOR ≤ 2.85; all 95% CIs encompassed the null value 1.0, n = 2 - 7 cases, 3 - 26 controls).

The quality of the study was ranked moderate. Although evidence of a strong association was reported between pyrethroids (as a group) and AML and ALL, and for individual pyrethroids and AML and ALL, the findings were mixed across the different age groups, and numbers of cases exposed to individual pesticides were small ($n \leq 20$ cases). Study strengths included the use of a case-control study design to study a rare cancer outcome and a well-defined hospital-based case

[31] Risk estimates for permethrin and esbiothrin were included in this study but were not reported here for the following reasons a.) this entire epidemiology memo is exclusive of permethrin risk estimates as noted above in the 'Background' section and b.) for esbiothrin, this individual pyrethroid was not one of the selected pesticides included within the epidemiology literature search, as noted above in the 'Background' section of this memo.

definition confirmed by morphology, immunophenotype, and standard cytogenetic-molecular methods. However, recall bias was an important limitation in this study, as questionnaires were used to evaluate past exposures and the mothers of the children with leukemia may differentially recall past pesticide exposure, relative to the mothers of the children without leukemia. An additional limitation of the exposure assessment approach is that it assessed ever/never use of pyrethroid pesticides and did not consider the magnitude, duration, or frequency of exposure during the time-period of interest and thus no dose-response could be evaluated. Assessment of individual pyrethroid compounds was a strength of the study, but the study population included only 63 ALL cases and 25 AML cases that reported use of any pyrethroids, respectively. The results for the individual pyrethroid compounds are based on a smaller subset of these exposed cases (*i.e.,* n = 1 - 19 of 63 ALL exposed cases and n = 2 - 8 of 25 AML exposed cases for the individual pyrethroids considered) and may be less statistically reliable. Lastly, pesticide exposures were highly correlated within the specified times frames of the study (*i.e.,* 3 months prior to pregnancy, during pregnancy, and after pregnancy). Authors reported pesticide exposures were highly correlated, with statistically significant high Pearson's correlation coefficients, r > 0.77. As a result, this may have made it difficult to parse an individual pesticide's effects during a specified window of time.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroid exposure and childhood leukemia. Ding et al. (2012) investigated the association between pyrethroid metabolites and acute lymphocytic leukemia (ALL) in children in China. The study was ranked to be low quality. Most importantly, the study's assessment of chronic pyrethroid exposure was based on analysis of a single, spot urine sample from each study subject. Pyrethroids have a relatively short biological half-life in the body, so it is not clear that this approach can reliably estimate chronic exposure during the time interval that preceded diagnosis. In addition, the study measured three metabolites or degradates of pyrethroid pesticides and may simply be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites present in the environment and food and not pyrethroid pesticides *per se*. Ferreira et al. (2013) evaluated the association between exposure to pyrethroids and childhood leukemia in Brazil. The quality of the study was ranked moderate. Although a strong association was observed for ALL and AML cases relative to pyrethroid exposure, the study was limited by several factors that ultimately decreased the reliability of the study's results. Limitations included a small number of exposed cases, the lack of maternal cumulative exposure evaluation, exposure assessment subject to recall bias, and the fact that pesticide exposures were greatly correlated within the specified time frames of the study (*i.e.,* 3 months prior to pregnancy, during pregnancy, and after pregnancy), which may have made it difficult to parse an individual pesticide's effects during a specified window of time.

6.3 Breast Cancer

One study investigated the relationship between breast cancer and pyrethroid exposure. These study results are provided below.

Engel et al. (2005) conducted a prospective cohort study to evaluate the association between chemical classes of pesticides including pyrethroids and breast cancer incidence among farmers' wives as part of an AHS study. Pesticide exposure was assessed based on self-reported questionnaires completed by both the farmers' wives and the farmers during study enrollment (1993 – 1997); the questionnaire data obtained from the farmers was considered a proxy for their spouses' indirect pesticide exposure. Breast cancer cases were identified using cancer registries in Iowa and North Carolina. Of the 309 breast cancer cases identified within the cohort (n = 30,454) from study enrollment through 2000, a total of 9 (3.0%) women personally reported pyrethroid exposure, and of the controls (n = 30,145 controls, women not

diagnosed with breast cancer) 1,393 (4.80%) women personally reported pyrethroid use. Poisson regression was used to calculate rate ratios for individual pesticides, with each analysis adjusted for age, race, and state of residence. The authors reported no evidence of a positive association between breast cancer incidence and ever exposure to pyrethroids (RR = 0.60; 95% CI: 0.30, 1.10). A subset analysis conducted for wives who reported never using pyrethroids considered husbands' pyrethroid use (as indirect exposure) and similarly observed no evidence of a positive association between husband's pyrethroid use and wife's risk of breast cancer (RR = 1.10; 95% CI: 0.70, 1.70).

EPA Conclusion

Overall, there is no epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids and breast cancer. Study results from Engel et al. (2005) showed no evidence of a positive association for breast cancer. The study quality was ranked moderate. The prospective cohort study design of AHS was a strength of the study as was the determination of breast cancer outcome using cancer registry data. Study limitations included the potential for exposure misclassification from self-reported previous pesticide exposures by the study participants, including both the farmers' wives and the farmers.

7 Non-Carcinogenic Effects

A large number of studies were reviewed with covered non-carcinogenic effects that may be associated with pyrethroids. The included studies concerning neurodevelopmental, neurobehavioral and neurocognitive effects; birth effects, including birth defects; male reproductive effects with respect semen quality, male reproductive hormone levels, and sperm damage/genetic abnormalities. A variety of more general effects – many from the AHS – were also available and reviewed. These are discussed in detail below.

7.1 Neurodevelopmental, Neurobehavioral, and Neurocognitive Effects

A total of 16 epidemiologic studies were identified that examined the association between exposure to pyrethroids and adverse neurodevelopment in children.[32] An evaluation of these studies is provided below and is organized into separate sections on: (i) prenatal exposure and neurodevelopmental effects; (ii) pediatric exposure and neurobehavioral and neurocognitive effects; and (iii) Autism Spectrum Disorders (ASD). Each section provides a summary of each study, assessment of study quality, and overall conclusions on the available epidemiologic evidence.

7.1.1 Prenatal Exposure and Neurodevelopmental Effects

Seven cohort studies investigated the association between prenatal maternal exposure to pyrethroids and developmental delays in children. Five of these studies assessed prenatal exposure by measuring pyrethroid urinary metabolites in pregnant women and a single study used a geographic information system (GIS)-based approach that relied on California Pesticide Use Reports (PUR) and maternal residential addresses. Potential developmental delays were assessed using a range of different tests on children ranging from 18-months to nine-years-old. These studies are summarized below:

- Xue et al. (2013) conducted a prospective study to investigate the association between maternal exposure to pyrethroids during pregnancy and subsequent infant development in a cohort of 497 mother-infant pairs living in Henan, China. The study population was comprised of pregnant women who gave birth between July – December 2010 in a hospital located in a Chinese county (Jiaozuo Henan) and met none of the following criteria: a.) stillbirths b.) lack of a gravida

[32] Although 17 epidemiology studies were identified, two studies (Viel et al. 2015, Viel et al. 2017) were accounted for twice (once in the prenatal exposure and neurodevelopmental effects section, and once in the pediatric exposure and neurobehavioral effects section), making the total count 16 epidemiology studies.

questionnaire and birth information concerning newborns and c.) unqualified samples of urine. Pregnancy urine samples were also collected at this time and measured using GC-MS to determine pyrethroid metabolite concentrations including three main metabolites: 3-PBA, *cis*-DCCA, and *trans*-DCCA. The questionnaire included basic information on pregnancy, occupational history, spouse, family, living environment (including pesticide exposure), lifestyle, pregnancy history, history of diseases, medical history, and vitamin supplement intake. At follow-up, the child's intellectual development (including the mental and neuro-development) was measured using the Development Screen test[33] (which included 120 test items of movement, social adaptation, and intelligence), and a mental development index (MI) score and a development quotient (DQ) score were obtained from the test for each child. The mothers also completed a questionnaire about their infants, including the information on growth and development, feeding, diseases, and basic information of the family, environment, and lifestyle. The authors used ANOVA to compare the development quotient (DQ) and mental development index (MI) between three synthetic pyrethroid level groups (low exposure group: less than the 25th percentile, middle exposure group: between the 25th percentile and 75th percentile, and high exposure group: greater than the 75th percentile). The authors also used multiple linear regression with a stepwise procedure to evaluate the association between the standardized DQ data (*i.e.,* transformed data to have mean = 0 and standard deviation = 1) and the standardized maternal total SP exposure data, where the list of covariate candidates included maternal age, educational (below high school, high school and technical secondary school, and college and above), occupation (manual laborer, other work, and no jobs and at home), whether there were any abnormalities in pregnancy, whether pregnant women took medicine, residence (city, town, and rural), feeding, main caregivers of infants, physical development index of 1-year-old infants, whether infants suffered severe disease after being born, and whether infants were exposed to harmful substances. Besides standardized maternal pyrethroid exposure, four significant variables ($p < 0.05$) remained in the final model, including standardized maternal education, standardized residence of infant, standardized main caregiver of infant, and standardized infant suffering disease or not. The authors reported evidence of negative association between maternal SP exposure and infant DQ in the analysis of variance (ANOVA) analysis ($p = 0.047$) and in the analysis of multiple linear regression with stepwise procedure (standardized partial regression coefficient ($\beta = -0.1527$, $p < 0.05$).

The quality of the study was ranked low. While the study was done prospectively, enrolled a relatively large sample of 497 mother-infant pairs, and directly assessed exposure by measuring urinary pyrethroid metabolites, the study has several limitations. Importantly, pyrethroid exposure was assessed by measuring urinary pyrethroid metabolites in only a single spot-urine sample during pregnancy. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. The authors also report that they "summed" the individual metabolites but provide no indication how this was done or if this was done on a simple concentration or (a likely more appropriate) molar basis. Too, no information on laboratory QA/QC procedures or results was provided. In addition, the study provided limited details on the statistical approach, and it was unclear how the authors could standardize the categorical variables, such as education (below high school, high school and technical secondary school, and college and above), residence of infants (city, town, and rural), etc., to and develop a single estimated standardized regression coefficient for each covariate in the model. The poorly described their statistical methods (such as how categorical variables were standardized) and the decision to standardize both the dependent and independent variables in the

[33] Zheng MS, Feng LY, Liu XY, Xu X, Li HR, Wang KL. Standardization of the mental developmental screening test (DST) for children aged 0-6 years in China. Chin J Pediatr.1997;35:117-120.

linear regression analysis cause difficulties in interpreting the results. Further, the authors indicated only that stepwise regression analysis was used to select the statistical model described in the paper (*e.g.,* critical p-values for variables to be enter or leave the model were not provided). This type of automated stepwise variable selection approach is generally considered appropriate only for studies conducting exploratory analysis for purposes of hypothesis generation, and purported statistical significance arising from studies that use this technique is not valid and cannot be relied upon.[34]

- Viel et al. (2015) conducted a prospective study to investigate the association between prenatal exposure to pyrethroids (measured by urine pyrethroid metabolite concentrations) and cognitive developmental delays in children at follow-up at six years old. The study also performed a cross-sectional analysis to assess urinary pyrethroid metabolites levels in children and neurobehavioral effects observed, which is described here and further referenced in the next section on pediatric exposure. Using data from the Perturbateurs endocriniens: Etude Longitudinale sur les Anomalies de la Grossesse, l'lnfertilite et l'Enfance (PELAGIE) cohort in the Brittany region of France, a study sub-cohort of mother-child pairs participated in the cohort study. Mother-child pairs included women who were pregnant (< 19 weeks of gestation) between 2002 – 2006, delivered a liveborn singleton infant, and followed-up with their healthy child six years later for a neuropsychological assessment. A total of 287 mother-child pairs completed the study. Maternal exposure was measured via a urine sample collected at 6 – 19 weeks of gestation and using a self-administered questionnaire. At follow-up at six years of age, the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) was administered to children to assess their cognitive abilities in verbal comprehension and working memory and mothers completed the Home Observation for Measurement of the Environment (HOME) survey to evaluate quality and extent of stimulation available to the child in the home environment. Children also provided a urine sample at the 6-year follow-up visit. For both maternal and child urinary measurements, ultra-performance liquid chromatography and triple quadrupole mass spectrometry (UPLC/MS-MS) was used to detect concentrations of the following pyrethroid metabolites: 3-PBA, 4F3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA. A multiple linear regression model was used to regress each of cognitive scores on each of maternal prenatal metabolite concentrations (the coefficients and their 95% CIs of the maternal prenatal pyrethroid metabolite concentration levels were reported from the model). Metabolite levels were stratified into two groups for 4F3-PBA (< LOD and > LOD) and three groups (< LOD, and then into lower half and upper half of detectable concentrations) for 3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA. A reverse-scale Cox regression model was used to obtain the p-value of monotonic trend test between a metabolite levels and each of the cognitive scores. All the analyses adjusted for confounders based on specific pyrethroid exposures.[35] For

[34] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. Psychosomatic Med. 66:411-42.

[35] In the assessment evaluating maternal exposure concentrations of pyrethroid metabolites and cognitive abilities, *cis*-DCCA was adjusted for presence of acid-leachable lead in the living room, and *trans*-DCCA was adjusted for maternal fish consumption. In the children's exposure concentrations of pyrethroid metabolites, 3-PBA was adjusted for child duration of television watching and child extra-circular sport activities relative to the verbal compression index, and for child extra-curricular sport activities only for the working memory index; 4F3-PBA was adjusted for the presence of acid-leachable lead in the living room in the verbal comprehension index, and for maternal age and child sex, and the presence of acid-leachable lead in the living room in the working memory index; *cis*-DCCA was adjusted for child extra-curricular sport activities only in the verbal comprehension index, and for maternal age, tobacco, smoking at the beginning of pregnancy, number of siblings at age 6, child extra-circular sport activities and research psychologist in the working memory index; *trans*-DCCA was adjusted for length of pregnancy, child duration of television watching and child extra-circular sport activities in the verbal comprehension index, and for maternal age, number of siblings at age 6, child education, child duration of television watching and child extra-circular sport activities in the

maternal pyrethroid metabolite concentrations, evidence of a significant negative association was observed for only *trans*-DCCA relative to the *working memory* index in children at the highest prenatal metabolite concentration (≥ 0.210 µg/L β: -6.44; 95% CI: -11.92, -0.97 with n = 52 cases). No evidence of a significant exposure-response trend was observed for *working memory* indices of the Wechsler Intelligence Scale in children and any of the urinary metabolites tested – DBCA, 3-PBA, 4F3-PBA, *cis*-DCCA, and *trans*-DCCA (all p-trends > 0.05) and no statistically significant findings or trends were found for the *verbal comprehension* aspect of the Wechsler Intelligence Scale, less emphasis was placed on this singular finding due to the decreased reliability.

The quality of the study was ranked moderate. Study strengths included the prospective study design and the extensive measures used to determine the outcome. Study limitations included a spot-single urine sample collected from the mother at 6 – 19 weeks of gestation used to ascertain prenatal exposure in the study. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. In addition, we note that multiple comparisons were performed, and – of these – only one significant association was observed in the highest exposure group for only *trans*-DCCA and then only for working memory and not verbal comprehension. In addition, for working memory – and despite the one statistically significant result – no trend was seen. Finally, no information on laboratory or intelligence testing QA/QC procedures or results was provided.

- Viel et al. (2017) investigated the association between prenatal urinary pyrethroid metabolites and the risk of neurobehavioral disorders in children using data from the PELAGIE cohort. The study also performed a cross-sectional analysis to assess urinary pyrethroid metabolites levels in children and neurobehavioral effects observed which is described here and further referenced in the next section on pediatric exposure. Using data from the PELAGIE cohort in the Brittany region of France, a study sub-cohort of mother-child pairs participated in the cohort study. Mother-child pairs included women who were pregnant (< 19 weeks of gestation) between 2002 – 2006, delivered a liveborn singleton infant, and followed-up with their healthy child 6 years later for a neuropsychological assessment. A total of 282 mother-child pairs participated in the study. At follow-up, behavioral disorders were assessed in children age 6 years old, using three subscales (internalizing disorders, externalizing disorders, and prosocial behavior), all part of the Strengths and Difficulties Questionnaire (SDQ). Urine samples were also collected at follow-up from both the mother and child to determine metabolite concentrations of pesticides including the following five for pyrethroids: 3-PBA, 4F3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA. Metabolite levels in mothers were stratified into two groups for 4F3-PBA (< LOD and > LOD) and three groups (< LOD, and then into lower half and upper half of detectable concentrations) for 3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA. A logistic regression and reverse-scale Cox regression model were used to determine ORs and 95% CI and Cox p-values, adjusting for maternal tobacco smoking at the start of pregnancy, HOME score, child extra-curricular sport activities, child duration of television watching, and parity. The following variables were included within the model: child sex, maternal education, child urinary creatinine concentration, and detection of dimethyl and diethyl phosphates in child urine samples. For maternal urinary concentrations, no evidence of a significant positive association and no evidence of a significant exposure-respond trend was observed for any of the five urinary metabolites (3-PBA, 4F3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA) relative to any of the three subscales of the SDQ

working memory index; DBCA was adjusted for length of pregnancy, child duration of television watching and child extra-circular sport activities in the verbal comprehension index, and for child duration of television watch and child extra-circular sport activities in the working memory index.

(internalizing disorders, externalizing disorders, and prosocial behavior) ($p \geq 0.05$ in all instances).

The quality of the study was ranked moderate. Strengths of the study included the prospective cohort study design in measuring prenatal exposures in mothers relative to the neurobehavioral effects in children, and the extensive tools used to measure the outcome (neurobehavioral effects in children). The primary limitation of the study was that a single-spot urine sample, collected from the mother during 6 – 19 weeks of gestation, was used to ascertain prenatal exposure in the study. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants that may be present in the environment and food. Additionally, no information on laboratory QA/QC procedures or results was provided.

- Watkins et al. (2016) conducted a prospective study to investigate an association between maternal exposure to pyrethroids during pregnancy and subsequent neurodevelopmental effects in children in a cohort of 187 mother-infant pairs living in Mexico City. Using data from the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) cohort study, study participants included women who were pregnant between 1997 – 2001, delivered a liveborn singleton infant, followed-up with their healthy child at two to three years of age for a neurodevelopmental assessment, and did not meet any of the exclusion criteria.[36] Exclusion criteria for the children in the study were also outlined.[37] Maternal exposure was measured via a urine sample collected during the 3rd trimester.. Additional urine samples were collected from a subset of these mothers (n = 21) during the 1st and 2nd trimesters to determine if detection rates of pyrethroids varied during trimesters of pregnancy. Urine samples were transported on dry ice to another location where high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used to detect concentrations of 3-PBA pyrethroid metabolite in each sample. Variability testing confirmed detection rates were similar between trimesters of pregnancy. Maternal metabolite levels of 3-PBA were then stratified into the following: 3-PBA < LOD, medium 3-PBA metabolite concentrations, and high 3-PBA metabolite concentrations; however, study authors did not report specific concentration ranges for the medium and high metabolite 3-PBA levels, A total of 187 mother-child pairs completed the study and the LOD for 3-PBA was 0.25 ng/mL. At follow-up, the neurodevelopment of each child was assessed at 2 and 3 years of age using the Psychomotor Development Index (PDI) and the Mental Development Index (MDI), as part of the Bayley Scales for Infant Development. A multiple linear regression model was used to determine if an association was present between maternal pyrethroid exposure as measured by urine concentrations of 3-PBA from the mothers at the 3rd trimester and neurodevelopmental scores in their children at 2 and 3 years of age, for each neurodevelopment index (PDI and the MDI) of the Bayley Scales for Infant Development, adjusting for urinary specific gravity, blood lead, maternal IQ, child sex, socioeconomic status (SES) score, and education. The linear regression analysis was performed separately using the whole cohort, female sub-cohort, and male sub-cohort. For the MDI, evidence of a significantly negative association was reported for only the medium 3-PBA metabolite level during the 3rd trimester of pregnancy and MDI scores in female children at 24 months ($\beta = -6.20$; 95% CI: -12.30, -0.14 n = 23 females; p-trend = 0.05). However, since a statistically significant p-trend was not observed at 24 months, and since no evidence of a significant association was reported at 36 months for

[36] Exclusion criteria for mothers in the study included the following: daily consumption of alcoholic beverages during pregnancy, plans to leave the area within the next 5 years, addiction to illegal drugs, habitual use of prescription drugs, diagnosis of high risk pregnancy, pre-eclampsia, gestational diabetes, or renal or heart disease, a history of infertility, diabetes, psychosis, or suffering from seizures requiring medical treatment.

[37] Exclusion criteria for children from the neurodevelopmental assessments if they were very low birth weight (< 1.5 kg) or borne severely premature (< 32 weeks gestation).

females at any metabolite concentration in addition to a non-significant p-trend, less emphasis was placed on this singular finding due to the decreased reliability. No evidence of a significant association was observed between any level of 3-PBA metabolite concentrations during the 3rd trimester of pregnancy and MDI scores at 24 and 36 months when the data was further stratified into male children only or when all of the study participants were considered as a group(males at 24 months: $-3.43 \leq \beta \leq -2.58$; all 95% CIs encompassed the null value of 0.0 – all p-trends were ≥ 0.05, with n = 84; males at 36 months: $-2.28 \leq \beta \leq -1.25$; all 95% CIs encompassed the null value of 0.0 – all p-trends were ≥ 0.05, with n = 77; all study participants at 24 months: $-3.80 \leq \beta \leq -3.54$ – all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 172; all study participants at 36 months: $-3.13 \leq \beta \leq -0.49$; all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 159). Similarly, for PDI scores at 24 months and at 36 months, no evidence of a significant association was observed between 3-PBA metabolites during the 3rd trimester of pregnancy and PDI scores among all study participants when considered as a group, as well as among males and females when the data was further stratified (all study participants at 24 months: $-0.42 \leq \beta \leq 0.64$; all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 171; all study participants at 36 months: $-3.08 \leq \beta \leq -1.55$; all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 159; males at 24 months: $-0.59 \leq \beta \leq 2.95$; all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 83; males at 36 months: $-5.31 \leq \beta \leq -1.66$; all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 77; females at 24 months: $-3.32 \leq \beta \leq -3.14$; all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 88; females at 36 months: $-1.00 \leq \beta \leq -0.27$; all 95% CIs encompassed the null value of 0.0; all p-trends were ≥ 0.05, with n = 82).

The quality of the study was ranked moderate. Study strengths included the cohort study design, the care taken in transporting the collected urine samples (*i.e.,* transported on dry ice), and the neurodevelopment assessments used to determine potential developmental delays within the study. Study limitations included the measured 3-PBA metabolite concentrations being below the limit of detection for more than half of all study participants and the use of a single maternal spot-urine sample to assess long-term exposure. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants and metabolites that may be present in the environment and food. Furthermore, the medium and high 3-PBA metabolite levels were not reported by the study authors. Additionally, no information on laboratory QA/QC procedures or results was provided.

- Gunier et al. (2017) conducted a prospective cohort study to investigate the associations between prenatal residential proximity to agricultural use of pesticides and neurodevelopment in 7-year-old children that are part of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort. The study population (n = 283) consisted of children born to mothers recruited and enrolled between October 1999 and October 2000 from health clinics serving low-income residents of the Valley. Inclusion criteria for mothers included the following: ≥ 18 years of age, < 20 weeks of gestation, were eligible for MediCal, spoke English or Spanish, and were planning to deliver at the county hospital. Of the 601 mothers enrolled, 537 were followed to delivery. Twins and children with medical conditions that could affect their neurodevelopment assessment were excluded from the analysis. Though 330 participating children were followed through age seven, children were included for the present analysis only if maternal residence during pregnancy for a minimum of 75 days for each of two or more trimesters was known to the investigators, if the child completed all components of the neuro-developmental assessment at seven years old, and if they had prenatal dialkyl phosphate (DAP) measurements. The investigators used global positioning system (GPS), GIS, and PUR data to estimate potential exposure from pesticide use within 1 km of maternal residences during

pregnancy. The amount of pesticide active ingredient applied within each 2.59 square-km weighted by the proportion of agricultural land area within the 1 km buffer was used to determine exposure for each residence. Pesticide exposure during pregnancy was estimated by first determining reported pesticide use during each trimester. Trimester-specific estimates were summed, then averaged, across the entire pregnancy by dividing by the number of trimesters included in the assessment for each residence. A single bilingual psychometrician administered the WISC-IV to estimate participants' Full-Scale Intelligence Quotient (FSIQ) and domain-specific intelligence quotient (IQ) (Working Memory, Processing Speed, Perceptual Reasoning, Verbal Comprehension) at age 7. Assessments were administered in each participant's dominant language (68% completed the WISC-IV in Spanish; 32% completed the assessment in English). Raw WISC-IV scores were standardized against U.S. population-based norms for English- and Spanish-speaking children. After verifying the deviation from linearity was not significant by the generalized additive model (GAM), multiple linear regression model was used to regress the 7-year IQ (Full Scale and domain specific) on the $\log_{10}$-tranform of average trimester pesticide use. Covariates, selected both *a priori* and if found to be empirically associated with IQ, included child's age at IQ assessment, sex, language of assessment, maternal education, maternal intelligence, maternal country of birth, maternal depression at the 7-year visit, HOME score at the 7-year visit, household poverty level at the 7-year visit, and prenatal urinary DAP concentration. Results were presented as mean changes (regression coefficients) in FSIQ for a standard deviation increment in estimated pesticide exposures, along with corresponding 95% CIs and p-values. Evidence of a significantly negative association was reported relative to each standard deviation increase in pyrethroid use within residential proximity of 1 kilometer among pregnant mothers and their children's scores for FSIQ ($\beta = -2.0$; 95% CI: –3.70, –0.30, $p < 0.05$ with $n = 255$) Perceptual Reasoning ($\beta = -2.10$; 95% CI: –4.00, –0.20, $p < 0.05$ with $n = 283$) and Verbal Comprehension ($\beta = -1.80$; 95% CI: –3.40, –0.30, $p < 0.05$ with $n = 283$). No evidence of a significantly negative association was observed for Working Memory ($\beta = -1.50$; 95% CI: –3.20, 0.20 with $n = 256$) and Processing Speed scores ($\beta = -1.10$; 95% CI: –2.80, 0.60 with $n = 256$).

The quality of the study was ranked low. Strengths of the study included the cohort study design, and the recognized FSIQ cognitive assessment used to determine intelligence in children. Limitations include the use of California PUR data to measure potential pesticide use including pyrethroids within proximity to a pregnant woman's residence. This approach has not been fully validated and additional information is needed to characterize the relationship between PUR information and the actual exposure-levels experienced by individuals as a result of living in agricultural communities. An additional limitation of this approach is that the authors report a moderate to high correlation between the pesticide group evaluated, based on pesticide use within one kilometer of maternal residence. Specifically, the correlation coefficient between pyrethroid use and the pesticide groups organophosphates, carbamates, and manganese fungicides were 0.82, 0.68, and 0.79, respectively. As such, the approach may lack the specificity to assess pyrethroids and be unable to distinguish between factors associated with geographic proximity to agricultural land and pesticide use more generally. The potential for selection bias was also high: a substantial fraction of study participants was lost to follow-up relative to enrollment at the start of the study. Specifically, 601 mothers enrolled in the study, but only 537 were followed to delivery. Of these 537, only 330 were followed through to age 7; and finally, test scores from only 255 to 283 individuals were available (from the starting 601). It is not known if this degree of loss to follow-up was differential, but – if so – this could potentially lead to substantial issues in the study with selection bias. We note that the mothers of children included in the analyses were significantly more likely to be married, nonsmokers during pregnancy, and approximately two years older at delivery relative to mothers of children who dropped out.

- Furlong et al. (2017) conducted a prospective study to investigate associations between urinary pyrethroid metabolites in pregnancy and subsequent measures of children's behavior and executive function of children at 4, 6 and 7-9 years of age in a longitudinal birth cohort conducted in New York City. The study population consisted of 404 primiparous women recruited from the Mount Sinai Diagnostic and Treatment Center (serving the East Harlem population), or one of two private practices on the Upper East Side of Manhattan and their children. Women in late pregnancy were enrolled and delivered at Mount Sinai Hospital between May 1998 and July 2001. Participating women provided a spot urine sample and completed questionnaires about their home, demographics, and behavioral characteristics during their 3rd trimester of pregnancy. Maternal urine samples were analyzed for three pyrethroid metabolites 3-PBA, *trans*-DCCA, and *cis*-DCCA by the CDC using HPLC-MS/MS. The HOME scale was assessed at 1 and 2-year visits, and neurodevelopment was assessed at 4-5, 6, and 7–9-year visits. Two neurodevelopmental assessments, a parent-report assessment of children's adaptive and problem behaviors (the Behavior Assessment System for Children (BASC)) and a parent-report assessment of children's executive functioning problems over a 6-month period (the Behavior Rating Inventory of Executive Functioning (BRIEF)) were conducted. The BASC indices included Externalizing Behaviors (comprised of the subscales Aggression, Hyperactivity, Conduct Problems), Internalizing Behaviors (subscales include Anxiety, Depression, Somatization), Adaptive Skills (subscales include Adaptability, Leadership, Social Skills), and the Behavioral Symptoms Index (subscales include Aggression, Hyperactivity, Anxiety, Depression, Attention, Conduct Problems, Atypicality); BRIEF indices included the Behavioral Regulation Index (subscales include Inhibit, Shift, Emotional Control) and the Metacognition Index (subscales include Initiate, Working Memory, Plan/Organize, Organization of Materials, and Monitor). Scores for both assessments are age-normalized and reported as T-scores. The investigators constructed longitudinal mixed models of neurobehavioral and executive functioning to examine associations with prenatal urinary pyrethroid metabolite levels dichotomized to indicate values above or below the limit of detection. Associations were adjusted for a comprehensive set of confounders considered *a priori*, including: race/ethnicity (non-Hispanic white, Hispanic, black/other); HOME scores; PDMPs (logged and continuous sum of dimethyl-phosphate, dimethyldithiophosphate, and dimethylthiophosphate); maternal marital status at follow-up (married, single, living with partner); maternal education at follow-up (high school or less vs some college or higher); child sex; creatinine; and visit. Of the 361 women enrolled, 162 mother/child pairs with complete covariate data returned for at least one BASC follow-up at the 4–5, 6, or 7–9-year visit, and 163 mother/child pairs with the BRIEF. The proportion of participants with detectable prenatal urinary pyrethroid metabolites was less than 30%, at both enrollment and follow-up. Among the mother/child pairs at follow-up (n = 162 mother/child pairs), the following number of participants reported detectable levels of 3-PBA, *cis*-DCCA, and *trans*-DCCA: 39 (24.1%), 15 (9.3%), and 22 (13.6%). For the mother/child pairs (n = 162), evidence of a significant negative association with 3-PBA concentrations during pregnancy was associated with the following: worse Internalizing (β: -4.50, 95% CI: -8.05, -0.95), Depression (β -3.21, 95% CI: -6.38, -0.05), Somatization (β -5.07, 95% CI: -8.62, -1.51), Behavioral Regulation (β: -3.59, 95% CI: -6.97, -0.21), Emotional Control (β: -3.35, 95% CI: -6.58, -0.12), Shifting (β: -3.42, 95% CI: -6.73, -0.11), and Monitoring (β: -4.08, 95% CI: -7.07, -1.08) scales in children. For *cis*-DCCA, evidence of a statistically significant association was observed for: worse Externalizing (β: -4.74, 95% CI: -9.37, -0.10), Conduct Problems (β: -5.35, 95% CI: -9.90, -0.81), Behavioral Regulation (β: -6.42, 95% CI: -11.39, -1.45), and Inhibitory Control (β: -7.20, 95% CI: -12.00, -2.39) in children. No evidence of a statistically significant association was observed for any of the remaining composite indices of the BASC or BRIEF for 3-PBA and *cis*-DCCA, and no evidence of a statistically significant association was observed for *trans*-DCCA urinary levels during pregnancy and any of the childhood behavior and executive functioning indices.

The quality of the study was ranked low. The primary strengths of the study were its prospective study design, and the longitudinal assessment of neurobehavioral development. While the study had several strengths, a major limitation of the study was that pyrethroid exposure was assessed by measuring urinary pyrethroid metabolites in a single spot-urine sample during pregnancy. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. In addition, only 162 of the 361 enrolled study subjects remained in the study after follow-up, resulting in a relatively low participation rate of only 45%. The investigators also reported that study participants that remained at follow-up were more likely to have pyrethroid biomarker levels below the LOD. Specifically, participation rates stratified by biomarker and whether levels were above the LOD were: 3-PBA (36% > LOD vs 48% < LOD); *cis*-DCCA (29% > LOD vs 48% < LOD); and *trans*-DCCA (28% vs 49%). This suggests that selection bias may be present in the study because exposed subjects were more likely to drop out of the study. In addition, multiple comparisons were made with no provision for statistical adjustment in the reported findings. Finally, the investigators were also unable to evaluate dose-response relationships due to the small numbers of participants with detectable pyrethroid metabolite levels Finally, no laboratory or neurodevelopmental assessments QA/QC information was made available or evidenced.

- Hisada et al. (2017) evaluated the association between maternal urinary pyrethroid metabolites and infant development in Japan. Using data from a prospective cohort study, participants included pregnant women living in Tokyo, Japan during 2009 - 2011, aged 20 – 50 years, with no known diseases. Maternal blood and urine samples were collected from the cases at 10 – 12 weeks of gestation (1st trimester), and urine samples were measured for the pyrethroid metabolite 3-PBA using HPLC-MS/MS. A single spot urine sample was collected at 10 - 12 gestational weeks at a maternal health checkup. The mother and child were then followed and observed up to 18 months following birth. At 1 month prior to the infant turning 18 months of age, the mother completed both the Kinder Infant Development Scale (KIDS) that includes 9 subscales assessing the child's development and the Index of Child Care Environment (ICCE) questionnaire assessing the home environment of the child (validated by cross-check with the universally used HOME scale). The responses from the KIDS questionnaire were summed and divided by chronological age to calculate a DQ for each child. A stepwise multiple linear regression model with variable selection criteria $P_{in} = 0.05$ and $P_{out} = 0.1$ was conducted to determine if an association existed between log-transformed maternal urinary 3-PBA metabolite concentrations and DQ scores in children at 18 months among the 102 mother-infant pairs in this study. Except for the ICCE score (dichotomized as low or high levels) that was retained in the final model, the following variables were <u>not</u> selected for the final model by the automated stepwise regression procedure employed: maternal age, log-transformed maternal body mass index (BMI),), gestational week for the index pregnancy, parity, infant sex, infant birth body weight, log-transformed infant blood thyroid-stimulating hormone (TSH) five days postpartum, and breast feeding. Evidence of a significant *positive* association was observed between log-transformed maternal urinary 3-PBA concentrations and the DQ score among infants at 18 months of age (partial regression coefficient $\beta = 3.22$; 95% CI: 0.58, 5.85 points/unit of log-transformed 3-PBA concentration; $p = 0.017$); this result suggests that increased 3-PBA urinary measurements result in *increased* DQ scores in infants however, in an additional analysis (including 88 mother-infant pairs due to the lack of fish consumption information) where fish consumption (dichotomized as frequent eater/less frequent eater) was included in addition to the list of covariate candidates, log-transformed 3-PBA was no longer significant and replaced by the significant fish consumption variable in the final model. Therefore, overall, no evidence of a significant association between maternal exposure to 3-PBA and adverse neurodevelopmental effects in children was observed, and what evidence was

available, though not robust, appeared to suggest an *increase* in child DQ scores as result of exposure to pyrethroids.

The quality of the study was ranked low. The prospective study design was a study strength; however, limitations included the use of a single spot-urine sample to assess pyrethroid exposure for which no laboratory QA/QC information was provided. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants that may be present in the environment and food. A further limitation was the use of automated stepwise multiple regression in the author's statistical analysis which is generally appropriate for hypothesis-generating studies and for which p-values are unreliable.[38] In addition, the unexpected and a rare finding of significant positive association reported between maternal urinary 3-PBA metabolite concentrations and DQ scores in children at 18 months disappeared after adjusting for fish consumption, potentially reflecting instability in this finding. Finally, no laboratory or KIDS/ICCE testing QA/QC information was provided.

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between maternal exposure to pyrethroids and neurobehavioral/neurocognitive effects in children. Seven of the epidemiologic studies reviewed assessed maternal exposure by either measuring pyrethroid urinary metabolites during pregnancy or agricultural pesticide use in relation to maternal address. The findings from these studies were mixed: three studies reported evidence of a negative association with neurodevelopment in children aged from 1-year to 9-years-old (Xue et al. 2013, Gunier et al. 2017, and Furlong et al. 2017) with increased maternal urinary measurements of pyrethroid metabolites associated with decreased test scores; one study reported no evidence of a significant association with neurodevelopment (Viel et al., 2017); and two studies reported no association between pyrethroid exposure and neurodevelopment (Viel et al. 2015, Watkins et al. 2016). Finally, one study suggested there was with *improved* neurodevelopment/neurocognitive test scores associated with increased urinary pyrethroid metabolite concentrations, but this positive association disappeared when fish consumption was added to the model (Hisada et al. 2017).

Although the seven studies relied on cohort designs, important limitations were identified in all studies that may decrease their reliability and help explain the mixed findings across studies. With respect to assessment of pyrethroid exposure, six of the seven studies (Xue et al., 2013, Viel et al., 2015, Viel et al., 2017, Watkins et al., 2016, Furlong et al., 2017, Hisada et al. 2017) assessed exposure by measuring urinary pyrethroid metabolites. While this biomonitoring approach has many advantages over other methods, it also has important limitations with respect to the assessment of prenatal exposure to pyrethroids. Most importantly, all six studies collected only a single maternal spot-urine sample during pregnancy and may not accurately reflect longitudinal exposure patterns or exposure during critical periods of neurodevelopment, particularly since exposure to pyrethroids may be highly variable with respect to frequency, magnitude, and duration. Also, it is not known how accurate a single spot urine sample would be in predicting long-term, past, or average exposure, which is particularly relevant for the outcomes measured here. In addition, while the urinary pyrethroid metabolites that were measured are potentially indicative of exposure to (parent) pyrethroid pesticides *per se*, they may also be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. As such, measurement of urinary metabolites may not represent exposure to active pyrethroids at all but instead be indicative of exposure to non-active and non-toxic environmental degradants/metabolites of

[38] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. Psychosomatic Med. 66:411-42.

the pyrethroids to which individuals may have been exposed. An additional consideration when performing biomonitoring and in evaluating studies that rely on this medium to evaluate exposure is the reliability of analytical methods and establishment of procedures to ensure samples are contaminant-free at the time of measurement. While some studies provided more detailed information on their instrumentation, analytical methods, and QA/QC procedures (Viel et al., 2015, Viel et al., 2017; Furlong et al., 2017) than others, many of the studies provided only limited details (*e.g.,* no information on instrumentation, laboratory/field blanks, method and field recoveries, etc.), making it difficult to evaluate biomonitoring methods and procedures and their results.

In addition to the six studies that measured urinary maternal pyrethroid metabolites to determine prenatal exposures, another study (Gunier et al., 2017) evaluated residential prenatal pesticide exposure and children's intelligence based on a GIS-based approach that combined California PUR data and maternal residence during each trimester of pregnancy. In this study, the authors reported evidence of a significant negative association between exposure as measured by California PUR data and children's intelligence. This GIS approach to estimating exposure has not been fully validated and additional information is needed to characterize the relationship between California PUR information and the actual exposure-levels experienced by individuals as a result of living in agricultural communities or near agricultural application sites. An additional limitation of this approach is that the authors report a moderate to high correlation between the pesticide group evaluated, based on pesticide use within one kilometer of maternal residence. Specifically, the correlation coefficient between pyrethroid use and the pesticide groups organophosphates, carbamates, and manganese fungicides were 0.82, 0.68, and 0.79, respectively. As such, the approach may lack the specificity to assess pyrethroids and be unable to distinguish between factors associated with geographic proximity to agricultural land and pesticide use more generally.

With respect to statistical limitations, two studies (Hisada et al., 2017 and Xue et al., 2013) appear to have used automated stepwise regression methods to select their final regression model. This approach is generally considered appropriate only for studies conducting exploratory analysis for purposes of hypothesis generation, and purported statistical significance arising from studies that use this technique is not valid and cannot be relied upon. In addition, Furlong et al. (2017) reported a relatively low participation rate (45%) due to loss to follow-up. This loss to follow-up was more pronounced amongst exposed study subjects (3-PBA: 36% > LOD vs 48% < LOD; *cis*-DCCA: 29% > LOD vs 48% < LOD; and *trans*-DCCA: 28% vs 49%, suggesting that selection bias (through differential loss to follow-up) may be present in the study because exposed subjects were more likely to drop out of the study.

7.1.2 Pediatric Exposure and Neurobehavioral Effects

A total of eight epidemiologic studies were identified that assessed pediatric exposure (during childhood) to pyrethroids by measuring urinary metabolites in children, with two of the studies (Viel et al. 2015, Viel et al. 2017) included within this section since they each performed an additional analysis within their prospective cohorts investigating this same relationship. All of these studies used cross-sectional study designs (Oulhote and Bouchard, 2013; Quirós-Alcalá et al., 2014; Fiedler et al., 2015; Wagner-Schuman et al., 2015; van Wendel de Joode et al., 2016; Wang et al., 2016; Viel et al., 2015; Viel et al., 2017). These studies are summarized below:

- Oulhote and Bouchard (2013) conducted a cross-sectional study using the Canadian Health Measures Survey (CHMS), (2007-2009) to investigate the association between urinary pyrethroid metabolites and parentally-reported behavioral problems among a representative sample of Canadian children aged 6-11 years old (n = 1,081). A spot urine sample was collected from each child within two weeks of survey questionnaire completion (20mL samples). Five urinary metabolites of pyrethroids were measured via laboratory methods: 4F3-PBA, DBCA, *cis*-DCCA,

trans-DCCA, and 3-PBA.[39] Among the study participants (n = 779), exposure to *cis*-DCCA and 3-PBA were detected in > 97% of children's urine samples. The median concentrations for pyrethroid metabolites were 0.05 µg/L, 0.15 µg/L and 0.20 µg/L, respectively, for *cis*-DCCA, *trans*-DCCA, and 3-PBA. In addition, each parent was asked about pesticide use in the preceding month. Behavioral problems were assessed using the parent version of the SDQ, an instrument intended for use in population surveys. Logistic regression models were used to estimate associations between log-transformed (base 10) metabolite concentrations and high scores on the SDQ, considering the complex survey design. Models of exposure to *cis*- and *trans*-DCCA were adjusted for sex, age, race/ethnicity, income, parental education, blood lead levels, maternal smoking during pregnancy, birth weight, and urinary creatinine (indicator of urine dilution). Models of exposure to 3-PBA were further adjusted for BMI and fasting status. For *cis*-DCCA, evidence of a moderately strong association between a 10-fold increase in metabolite concentration (roughly equivalent to a shift from the 5th to the 75th percentile) and the SDQ overall score for Total Difficulties among children was observed (OR = 2.00; 95% CI: 1.10, 3.60, with n = 69/779 cases, p-value < 0.05). No evidence of a significant positive association was observed for 3-PBA and for *trans*-DCCA metabolites relative to the SDQ overall score for Total Difficulties in children (1.00 ≤ ORs ≤ 1.60; all CIs encompassed the null value of 1.0, with n = 69/779 cases, p-value ≥ 0.05).

The quality of the study was ranked low. CHMS provides comprehensive monitoring of the Canadian population's health and health habits, but is based on a cross-sectional study design. As such, CHMS cannot assess the temporal association between pyrethroid exposure and neurobehavior. In addition to this limitation, the CHMS only obtained a single spot urine sample collected throughout the study. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food.

- Quirós-Alcalá et al. (2014) conducted a cross-sectional study using National Health and Nutrition Examination Survey (NHANES) cycles 1999-2000 and 2001-2002 to investigate the relationship between urinary pyrethroid metabolites and parent-reported learning disability (LD) and/or attention deficit/hyperactivity disorder (ADHD) in a representative sample of U.S. children aged 6-15 years old. Pyrethroid exposure measurements were conducted on a random subsample of participants ≥ 6 years of age. At physical examination, single spot urine samples were collected and metabolite concentrations including *cis*-, *trans*-DCCA, and 3-PBA, resulting from the degradation of pyrethroids were determined. Parental report of a LD and/or ADHD, was based on parental/guardian response to two NHANES interview questions. Logistic regression models were used to determine ORs and corresponding 95% CIs for potential associations between concentrations of each metabolite and parent-reported LD, ADHD, and both, considering the complex survey design. Models were adjusted for sex, age, race/ethnicity, household reference education level, low birth weight status, maternal age at child's birth, neonatal intensive care unit admission, maternal smoking during pregnancy, day care/preschool attendance, and health insurance. Among the study participants, 1,680 children had complete exposure and outcome information for 3-PBA (maximum likelihood estimation was used to impute 23% of the data < LOD; log-transformed 3-PBA was used as continuous variable in the regression model), 1,659 for *cis*-DCCA (dichotomized by below/above LOD), and 1,669 for *trans*-DCCA (dichotomized by below/above LOD). No evidence of a significant positive association was observed between a 10-fold increase in 3-PBA concentration and parent-reported LD, ADHD, or both (LD + ADHD) in children (1.16 ≤ adjusted ORs ≤ 1.45; all CIs encompassed the null value of 1.00; p > 0.05).

[39] The study authors mentioned that associations were not reported for metabolites 4F3-PBA and DBCA in the study because more than 50% of children had urinary levels below the limit of detection.

Similarly, no evidence of a significant positive association was observed for *cis*-DCCA relative to parent-reported LD, ADHD, or both (LD + ADHD) in children ($1.00 \leq$ adjusted ORs ≤ 1.43; all CIs encompassed the null value of 1.00; $p > 0.05$), as well as for *trans*-DCCA ($1.29 \leq$ adjusted ORs ≤ 1.84; all CIs encompassed the null value of 1.00; $p > 0.05$).

The quality of the study was ranked low. An adequate sample size was considered a strength of this study. A major limitation of the study included the poor outcome ascertainment. The identification of cases was solely based on interviewed responses by the parents which might have resulted in outcome misclassification. Furthermore, the parental responses were based on only two interview questions that defined the outcome of interest. Additional limitations included the cross-sectional design of the study and the use of a single spot urine sample. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants that may be present in the environment and food.

- Fiedler et al. (2015) conducted a cross-section study to evaluate the association between pesticide exposures including pyrethroids and neurobehavioral effects among children from farming communities in Thailand. The study population included Thai children (n = 54) aged 6-8 years old, who were part of the rice (n = 24) or aquaculture (n = 29) farming communities located in the Bangkok, Thailand area. The children were randomly selected from a group of 200 volunteers, and were free of significant developmental delay, mental retardation, diabetes, neurologic disorder, significant head trauma, and lung, kidney and cardiac disease. The children from the aquaculture (shrimp farming) region of Thailand served as the control group in this study. Both farming regions used pyrethroids for mosquito control in the home and only rice farmers used pyrethroids on their farms. Although the year(s) the study was carried out and the child's role on the farm is unclear, the study consisted of three phases – Session I, II, and III – with Session I acting as preliminary orientation phase; the neurobehavioral data collected from Session I[40] was not evaluated in this study. The HOME was used during Session I initial visit to assess home environment and parental education and intelligence were included in the statistical analysis. Pyrethroid pesticide exposure was assessed during the high use (Session II) and low use farming seasons (Session III), and exposure to pesticides including pyrethroids was measured via metabolite concentrations found within the child's urine samples (first morning void) collected at the home on the morning of the neurobehavioral assessment (Session II and III). Samples were stored at -40 °C at the neurobehavioral testing center and were shipped on dry ice to the laboratory where they were tested for concentrations of two pyrethroid metabolites 3-PBA and *cis/trans*-DCCA, using HPLC-MS/MS at a laboratory in Atlanta, Georgia. Limited details were provided on QA/QC procedures. Neurobehavioral effects were analyzed using the Behavioral Assessment and Research System (BARS), a computerized test system that was translated into Thai, and administered to each child by a trained study examiner three times, once every six months (Sessions I, II, III). The BARS test incorporated nine specialized neurobehavioral tests[41] to determine the neurobehavioral performance of each study participant. At each assessment,

[40] During the preliminary phase (Session I), additional information regarding home environment and parental education levels and intelligence was obtained from the parents of the study participants, using the Home Observation for Measurement of the Environmental (HOME) scale and vocabulary tests. Both assessments were modified and translated to accommodate the cultural and language differences.

[41] Nine neurobehavioral assessment tests grouped into four domains included:

1) *Latency of Response Domain*: symbol-digit test (SDT) for information processing speed, match-to-sample (MTS) for visual memory, and continuous performance test (CPT) for sustained attention;
2) *Accuracy of Response Domain*: (MTS, CPT);
3) *Motor Speed Domain*: finger tapping (TAP) for response speed and coordination, divided attention test (DAT), and Purdue pegboard (PEG) for dexterity; and
4) *Learning Domain*: object memory test (OMT) for recall and recognition memory, visual motor intelligence (VMI) for hand-eye coordination, and digit span test (DST) for memory and attention.

parents completed a questionnaire about their child's activity and potential for pesticide exposure. Initially authors collapsed the nine neurobehavioral variables into four domains to account for multiple comparisons: latency of response, accuracy of response, motor speed, and learning and multivariate analyses; however, no statistical correction for multiple comparisons was conducted. Mixed linear multivariable regression models were used to estimate the association between pyrethroid exposure and neurobehavioral effects in children and seasonal variation in exposure. In addition, a mixed linear multivariable model was used to determine if an interaction between farming region and season and the neurobehavioral performance in children. All analyses were adjusted for age and HOME total score. Rice farm children had significantly higher DCCA levels in their urine than aquaculture farm participants during the high exposure season, but during the low exposure season, DCCA levels were similar (high use: $p = 0.04$, low use: $p = 0.45$). No other significant differences in either of the pyrethroid metabolites DCCA or 3-PBA were noted between the participant groups or seasons. No significant adverse neurobehavioral effects were observed between rice farm or shrimp farm children during either the high or low pesticide use season.

Overall, pyrethroid metabolites were not significant predictors of adverse neurobehavioral performance during either season. For the analysis within season, significant overall effects for the metabolites DCCA and 3-PBA were reported for the domain *learning,* during the low use season only (DCCA learning: $p = 0.04$; 3-PBA learning: $p = 0.005$) and for only one (OMT) of the three tests within the learning domain. Increasing DCCA and 3-PBA each predicted lower OMT recognition scores (DCCA: $p = 0.0001$; 3-PBA: $p = 0.0001$). Authors reported that values for OMT recognition memory were highly skewed with most values at the maximum (16), indicating a violation of testing assumptions. No significant associations were reported for the associations between pyrethroid metabolites DCCA and 3-PBA and the other domains: latency of response, accuracy of response, or motor speed. For the analysis across seasons, increasing average pyrethroid metabolite DCCA across seasons was significantly associated with improved average latency of response domain (p-value not reported) and for only one of the three tests within the latency of response domain (SDT: DCCA $p = 0.04$); no differences in performance were observed for any other domains for increasing average DCCA across seasons and for any neurobehavioral performance domains for increasing average 3-PBA across seasons. For the analysis to assess within participant changes of pyrethroid metabolites and effects on neurobehavioral measures, within participant increases in DCCA across seasons were significantly associated with lower scores on recognition memory, one of three indices within OMT (OMT: DCCA $p = 0.02$); no other indices of OMT, or any other tests were significantly associated with within participant increases in DCCA across seasons. Within participant changes in 3-PBA across seasons did not predict changes in neurobehavioral performance (data not reported). In a repeated analysis controlling for type of farm, to determine if the effect of working on different farming operations could influence neurobehavioral performance and overcome effects of pyrethroid metabolites, authors reported no difference in the results.

The quality of this study was ranked low. Study strengths included using urinary concentrations of pyrethroid metabolites as biomarkers for pyrethroid exposure and collecting samples from different seasons of pesticide use for each participant to assess differences in exposure, standardized testing methods and trained testers for the neurobehavioral performance assessments and providing day before training of parents on collection methods for urinary specimen. A major limitation was the cross-sectional study design and thus the inability to assess the temporal association between pyrethroid exposure and neurobehavioral effects in children. Additional limitations include using only a single spot-urine sample from the child to determine exposure over the 6-month period of the season. Additionally, authors provided minimal information about QA/QC procedures and analytical methods for pyrethroid metabolites. Further limiting the overall

utility of the study from a regulatory standpoint, authors did not report actual risk or effect size estimates or confidence intervals, but instead only reported p-values for the results of the analyses of pyrethroid metabolites and neurobehavioral performance indices. The authors performed a large number of comparisons and did not adjust for multiple comparisons using statistical methods, and the sample sizes were small (n = 54, total made of up of n = 24 who were part of the rice farming group or n = 29 as part of the aquaculture group.

- Wagner-Schuman et al. (2015) conducted a cross-sectional study to evaluate the association between pyrethroid exposure and ADHD among children. The study utilized data from the nationally representative NHANES 2001-2002. The study population consisted of children aged 8 to 15 years old (n = 687) who had urine samples analyzed for 3-PBA, a pyrethroid metabolite, and data available for ADHD assessment and covariates of interest. Cases were ascertained by two methodologies: 1) meeting DSM-IV criteria for ADHD assessed by the National Institute of Mental Health Diagnostic Interview Schedule for Children (DISC), which was completed by caregivers two to four weeks after the NHANES mobile-examination, or 2) caregiver report of an ADHD diagnosis during an NHANES interview. A total of 93 participants met the case-ascertainment criteria by one or both methods. Two logistic regression models were used to evaluate the association between ADHD and urinary 3-PBA: one treated urinary 3-PBA as a categorical variable (below/above LOD) and other as a continuous variable (log_{10}-transformed). Additionally, the authors also examined the dose-response relationship between the continuous log_{10}-transformed 3-PBA by fitting 3-knot restricted cubic polynomial splines (knots at 10^{th}, 50^{th}, and 90^{th} percentile of log_{10}-transformed 3-PBA) in the logistic regression model. Finally, the authors evaluated the effects of 3-PBA separately for males (n = 323) and females (n = 364) by adding the interactions of sex and 3-PBA variables to the logistic regression models. All the analyses were adjusted for sex, household income to poverty line ratio, age, race/ethnicity, health insurance status, prenatal tobacco exposure, log_{10}-transformed blood lead concentration, log_{10}-transformed urinary organophosphate pesticide metabolite concentration (DMAP), and log_{10}-transformed urinary creatinine (to adjust for urine dilution).). Among the children with detectable levels of 3-PBA urine concentrations (n = 556), evidence of a moderately strong association was found between 3-PBA metabolite concentrations and ADHD compared to those undetectable 3-PBA urinary (OR: 2.42; 95% CI: 1.06, 5.57 with n = 131 cases);); however, when the log_{10}-transformed 3-PBA was treated as continuous, the association between 3-PBA and ADHD was not significant (OR = 1.57, 95% CI = 0.88 – 2.78 for each 10-fold increase in urinary 3-PBA). When the data was further stratified by sex, evidence of a moderately strong association was found between detectable 3-PBA concentrations levels and ADHD <u>in males</u> compared to males with undetectable concentrations (OR: 2.95; 95% CI: 1.07, 8.08). No evidence of a significant positive association was found between detectable 3-PBA metabolite concentration levels and ADHD in females compared to females with non-detectable concentrations (OR: 1.54; 95% CI: 0.32, 7.33). The spline analyses also showed the log-odds of ADHD increased at higher 3-PBA in boys but not in girls.

  The quality of the study was ranked low. Strengths of the study included the structured diagnostic methods and tools used to measure exposure and outcome. The main limitation of the study was the cross-sectional study design. This design does not ensure that the exposure predates the outcome. An additional limitation was the use of a single spot-urine sample. The use of a spot-urine sample may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. In addition, approximately half of the cases were identified through report by the caregiver leading to the potential for recall bias, as well as use of a proxy (caregiver instead of the child) which may have decreased the reliability of the measured outcome.

- van Wendel de Joode et al. (2016) conducted a cross-sectional study to evaluate the association between pyrethroid exposure and several neurobehavioral impairment outcomes among children aged 6 to 9 years old in Costa Rica. The study area within Talamanca County, Costa Rica, consisted of three villages; one near banana plantations, one near plantain farms, and one near predominately organic growers. Among 190 eligible children, informed consent was obtained from 160 parents, and 140 subjects completed the study (*i.e.,* they provided at least one urine sample, completed parental interview, physical exam by physician, and underwent neurobehavioral assessments). Metabolite biomarkers of pesticide exposures including pyrethroids were quantified in urine samples provided by the participants. Urine samples were generally 'first morning' samples obtained on the day of the outcome assessment (six children provided spot samples at the outcome assessment), and the samples were stored at 4 °C during the assessment, and at -20 °C prior to shipment to a laboratory located in Sweden, where they were tested for concentrations of two pyrethroid metabolites 3-PBA, using HPLC-MS. Repeat urine samples were provided by 40 children (on average 3.3 months after the outcome assessment). Limited details were provided on QA/QC procedures. Urinary 3-PBA concentrations were quantified as biomarkers of pyrethroid pesticide exposure. The neurobehavioral outcomes were broadly categorized as intellectual ability, behavioral problems, sensory function, perception and memory, and motor function. More specifically: three psychometricians administered the WISC-IV (Mexican-Spanish version) to determine children's cognitive abilities, and scores for the three WISC-IV domains (perceptual reasoning, working memory, and processing speed) were calculated.[42] Additional tests were conducted in children to assess the following: behavioral problems, sensory function, perception and memory, and motor function.[43] Multivariable linear regression models, were used to evaluate the associations between most neurobehavioral outcomes and $\log_{10}$-transformed 3-PBA. Due to the violation of the normality assumption in the linear models of some neurobehavioral outcomes (Reaction Time Tests, Conner's Parent Rating Scale-Revised tests, and Lanthony Desaturated D-15 tests), the authors dichotomized these neurobehavioral outcomes at the 50^{th} and 75^{th} percentile, then used logistic regression models to evaluate the associations between these outcomes and $\log_{10}$-transformed 3-PBA. Results of linear models were presented by gender whenever the pesticide-by gender- interaction was notable (*i.e.,* $p < 0.20$ for interaction). All models adjusted for the following potential confounders; maternal education, child's gender, age, body mass index, number of siblings, if child had repeated a school year, and visual acuity impairment. All models also included log creatinine as a covariate, to correct for urinary dilution. Among intellectual ability assessments (working memory, processing speed, and perceptual reasoning), evidence of a significant negative association between 3-PBA metabolite levels and processing speed index in children was reported ($\beta = -5.30$; 95% CI: -10.30, -0.20 per 10-fold increase in 3-PBA urinary concentration, with $n = 140$). When the data was further stratified by gender, a significant negative association between working memory in girls per 10-fold increase in 3-PBA was reported (β: -8.80; 95% CI: -16.10, -1.40, with $n = 71$ girls; $\beta = -1.90$; 95% CI: -8.50, 4.60, with $n = 69$ boys). No statistically significant associations were observed with 3-PBA and behavioral problems (inattention, oppositional disorder, hyperactivity, and ADHD index), sensory function (color discrimination), any perception and memory assessment (visual copy and recall, verbal recall, and level of learning),

[42] In this study, full-scale IQ and verbal comprehension (additional indexes of the WISC-IV) were not assessed since the WISC-IV was not standardized in Costa Rica. As a result, the WISC-IV scores obtained in this study followed Mexican population-based norms.

[43] Additional tests included: the Conner's Parent Rating Scale – Revised Short Version to assess behavioral abnormalities, the Lanthony Desaturated D-15, test to determine sensory function, the Rey-Osterrieth Complex Figure to assess visuospatial abilities, the Children's Auditory Verbal Learning Test 2nd edition to test verbal and memory skills, the Eye-Hand Coordination subtest of the Frostig Developmental Test of Visual Perception, 2nd edition to determine visual-motor coordination, the Wide Range Assessment of Visual Motor Ability pegboard test to determine fine motor dexterity, and the Reaction Time Test to assess attention.

or motor function (eye-hand coordination, fine motor, and reaction time) ($0.40 \leq ORs \leq 1.70$; all CIs encompassed the null value of 1.0; n = 124 total exposed cases, individual number of pyrethroid exposed cases not provided).

The quality of the study was ranked low. A strength of the study included collecting repeat urine samples from some of the study participants, which the study authors mentioned allowed them to analyze if urinary concentrations could denote longer-term exposures; however, the study also had several limitations. Study limitations included the cross-sectional study design and the inability to assess the temporal association between pyrethroid exposure and neurodevelopment, as well as one of the administered tests, WISC-IV, not being standardized in Costa Rica at the time the study was conducted. In addition, the study authors performed a large number of comparisons and did not adjust for multiple comparisons using statistical methods. Lastly, no information on laboratory QA/QC procedures or results was provided.

- Wang et al. (2016) conducted a cross-sectional study to evaluate the association between exposure to pesticides, including pyrethroids, and neurobehavioral effects among children in China. The study targeted three kindergartens in Nanjing, China that covered both urban and rural areas and included 406 randomly selected children that were aged between 3 to 6 years old from the three kindergartens and had no reported diseases. Following study enrollment, a neurobehavioral assessment was conducted in these children using the following measures: Chinese Binet test, arithmetic test, maze test, cancellation test, and a picture completion test. A single morning urine sample from the child was collected by the parents on the day of the neurobehavioral tests and analyzed for a range of pesticide metabolites, including chlorpyrifos metabolite 3,5,6-trichloropyridinol and the pyrethroid metabolites 3-PBA, 4F3-PBA, and DBCA. Maternal questionnaires were also used to assess exposure characteristics, including washing fruits/vegetables, use of pesticides indoors, proximity to agricultural fields and parks, and frequency of smelling pesticides indoors. Bivariate analyses and multiple linear regression were used to screen and select potential covariates to include in the analysis models, and it is unclear how many variables were screening in this process. After the screening test, only age, sex, and outside school education expense, which were significantly associated with two or more test scores and also were suggested to be related to neurobehavioral outcomes in the literature, were included to analysis models as covariates. Two different multiple linear regression models were used to assess the associations between each of 5 neurobehavioral test scores with the 3-PBA exposure: one model incorporated 3-PBA as a dichotomized variable (below/above LOD) and other model incorporated log-transformed 3-PBA concentration as continuous variable with values < LOD imputed using multiple imputation procedures. While other pyrethroid urinary metabolites were measured, the investigators did not report how this additional exposure data on pyrethroids was considered in their analysis. The authors reported lower scores in Chinese Binet test and arithmetic test for subjects detected urinary 3-PBA ($\beta = -3.47$; 95% CI: -5.82, -1.12, $p \leq 0.01$ and $\beta = -1.09$; 95% CI: -1.78, -0.41, $p \leq 0.01$, respectively). However, 3-PBA was not significant factor on Chinese Binet test and arithmetic test scores when treated as continuous variable ($\beta = -3.47$, 95% CI = -8.21 – 1.26 and $\beta = -0.55$, 95% CI = -1.94 – 0.84 per 10-fold increase in 3-PBA urinary concentration, respectively). 3-PBA was significant effect on the cancellation test score when treated as a continuous independent variable ($\beta = -3.96$, 95% CI = -7.06 – -0.86 per 10-fold increase in 3-PBA) but was not significant when treated as a dichotomized (below/above LOD) independent variable ($\beta = 0.98$, 95% CI = -0.67 – 2.63). Neither $\log_{10}$-transformed 3-PBA nor dichotomized (below/above LOD) 3-PBA was significant factor of picture complete test score or maze test score as the 95% CIs of the estimates encompassed 0.

The study quality was ranked low. The primary limitation of the study was that its cross-sectional design was unable to assess the temporal association between exposure and neurobehavioral development. This limitation was further impacted by the fact that exposure was assessed using a single, spot-urine measurement of the non-specific biomarker 3-PBA. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants that may be present in the environment and food. In addition to this limitation, the statistical analysis did not account for sampling of children from 3 different schools located in urban and rural regions. Specifically, the investigators used multiple linear regression, which assumes that all study subjects are independent even though they are clustered within the 3 schools. Therefore, a mixed effects model may be more appropriate and would be able to properly account for these potential differences. Lastly, no information on laboratory QA/QC procedures or results was provided.

- As previously described in the section above, part of the Viel et al. (2015) study included conducting a cross-sectional analysis of urinary pyrethroid metabolites levels in children at age 6. Using data from the PELAGIE cohort in the Brittany region of France at follow-up at six years of age, the WISC-IV was administered to children to assess their cognitive abilities in verbal comprehension and working memory and the HOME survey to evaluate quality and extent of stimulation available to the child in the home environment was completed by their mothers. Children also provided a urine sample at the follow-up visit. UPLC/MS-MS was used to detect concentrations of the following pyrethroid metabolites: 3-PBA, 4F3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA in urine samples. A multiple linear regression model was used to regress each of cognitive scores on each of children's pyrethroid metabolite concentrations (the coefficients and their 95% CIs of the children's pyrethroid metabolite concentration levels were reported from the model). A reverse-scale Cox regression model was used to obtain the p-value of monotonic trend test between a metabolite levels and each of cognitive scores. All analyses adjusted for confounders based on specific pyrethroid exposures.[44] Metabolite levels were stratified into two groups for 4F3-PBA and three groups for 3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA. To preserve the sample size in the analyses, some missing values of covariates of 20 mother-infant pairs with were replace by the model values of non-missing values. Among 287 children included in the analysis, the missing WISC-WMI values of 4 children were replaced by the predicted value (WISC-WMI value = 12). Based on the cross-sectional analysis of children's pyrethroid metabolite concentrations, a significant negative association and significant exposure-response trend was reported at the highest metabolite concentration level for 3-PBA and for DBCA for the verbal comprehension index, part of the Wechsler Intelligence Scale (3-PBA β: -5.18; 95% CI: -9.25, -1.11 with n = 91 cases; p-trend = 0.04; DBCA β: -6.75; 95% CI: -11.17, -2.32 with n = 95 cases; p-trend < 0.01). Additionally, a significant exposure-response trend was observed between DBCA metabolite concentrations and the working memory index (p-trend < 0.01) ; however, neither the mid exposure group 0.134-0.345 μg/L nor the high exposure group ≥0.345 μg/L were

[44] In the children's exposure concentrations of pyrethroid metabolites, 3-PBA was adjusted for child duration of television watching and child extra-circular sport activities relative to the verbal compression index, and for child extra-curricular sport activities only for the working memory index; 4F3-PBA was adjusted for the presence of acid-leachable lead in the living room in the verbal comprehension index, and for maternal age and child sex, and the presence of acid-leachable lead in the living room in the working memory index; *cis*-DCCA was adjusted for child extra-curricular sport activities only in the verbal comprehension index, and for maternal age, tobacco, smoking at the beginning of pregnancy, number of siblings at age 6, child extra-circular sport activities and research psychologist in the working memory index; *trans*-DCCA was adjusted for length of pregnancy, child duration of television watching and child extra-circular sport activities in the verbal comprehension index, and for maternal age, number of siblings at age 6, child education, child duration of television watching and child extra-circular sport activities in the working memory index; DBCA was adjusted for length of pregnancy, child duration of television watching and child extra-circular sport activities in the verbal comprehension index, and for child duration of television watch and child extra-circular sport activities in the working memory index.

significantly different from the low exposure group < 0.134 μg/L (β = -3.56, 95% CI = -7.84 – 0.71 and β = -3.97, 95% CI = -8.33 – 0.45, respectively). No evidence of a significant association or significant exposure-response trend was observed for childhood levels of 4F3-PBA, *cis*-DCCA, and *trans*-DCCA.

The quality of the study was ranked low. While the overall study was done prospectively, the analysis that evaluated urinary levels in children was cross-sectional because both exposure and neurodevelopment were measured at the same point in time. As such, the analysis was unable to assess the temporal association between exposure and neurobehavior development. Additional limitations included the single urine sample collected from the children to determine long-term exposure. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. Lastly, no information on laboratory QA/QC procedures or results was provided.

- As previously mentioned in the section above, part of the Viel et al. (2017) study, investigated the association between childhood urinary pyrethroid metabolites and the risk of neurobehavioral disorders in children at follow-up, using data from the PELAGIE cohort. Similar study methods were used in the study mentioned above (Viel et al. 2015). At follow-up, behavioral disorders were assessed in children at 6 years of age, using three subscales (internalizing disorders, externalizing disorders, and prosocial behavior), part of the Strengths and Difficulties Questionnaire (SDQ). Urine samples were also collected at follow-up from both the mother and child to determine metabolite concentrations of pesticides including the following five pyrethroids: 3-PBA, 4F3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA. A logistic regression model and a reverse-scale Cox regression model were used to determine ORs and 95% CI and Cox p-values, with a long list of considered covariates[45] to adjust in the models. The following variables were included within the model: child sex, maternal education, child urinary creatinine concentration, and detection of dimethyl (DM) and diethyl (DE) phosphates in child urine samples. To preserve the sample size in the analyses, some missing values of covariates of 20 mother-infant pairs with were replace by the model values of non-missing values. A total of 282 mother-child pairs participated in the study. Metabolite levels in children were stratified into two groups (< LOD or > LOD) for 4F3-PBA and three groups (< LOD, below the median of detected values, or above the median of detected values) for 3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA. For 3-PBA, evidence of a moderately strong association for abnormal or borderline social behavior was observed at one concentration only (0.008 – 0.037 μg/L OR: 2.93; 95% CI: 1.27, 6.78) but not at a higher exposure group ≥ 0.038 μg/L (OR = 1.91, 95% CI = 0.80 – 4.57). A significant positive exposure-response trend was observed for 3-PBA and externalizing disorders based on the externalizing scores relative to the referent (p-value = 0.04) and a negative significant exposure-response trend was observed for *trans*-DCCA urinary concentrations of children and externalizing disorders based on the externalizing scores relative to the referent (p-value = 0.03). No evidence of a significant positive association was observed at any additional individual metabolite concentration levels for *trans*-DCCA and 3-PBA (0.57 ≤ OR ≤ 1.96; all CIs encompassed the null value of 1.0). Additionally, no evidence of a significant association at any

[45] List of considered covariates to adjust in the models: maternal variables including tobacco smoking (yes/no), age, BMI (≤ 25, > 25 kg/m^2), place of residence (rural, urban) parity(yes, no), education ((≤ 12, > 12), WAIS-III VIS, usual fishing consumption (< 2, ≥ 2 times a week), length of pregnancy, breastfeeding (none, ≤ 16, > 16 weeks) and children variables including sex, birth weight, education (nursery/primary school), number of siblings at age 6, sleep duration (< 10.5, 10.5-11, > 11 hours per day), duration of television watching (< 2.5, 2.5-4.5, > 4.5 hours per week), duration play video games (0, 0-1.5, > 1.5 hours per week), child extra-curricular sport activities (yes/no), and urinary cotinine concentration (< 6, ≥ 6 mg/L) and several environmental factors including HOME score, acid-leachable lead in the living room (≤ 1, 1-3, > 3 mg/m^2), number of smokers at home (0, 1, ≥ 2), and cigarettes smoked at home (0, 1-10, > 10 per day).

individual metabolite concentration level or a significant positive exposure-response trend was observed for 4F3-PBA, *cis*-DCCA and DBCA for the three subscales of the SDQ (internalizing disorders, externalizing disorders, and prosocial behavior) ($p \geq 0.05$).

The quality of the study was ranked low. While the overall study was done prospectively, the analysis that evaluated urinary levels in children was cross-sectional because both exposure and neurodevelopment were measure at the same point in time. As such, the analysis was unable to assess the temporal association between exposure and neurobehavioral development. Another issue was that the significant findings might be results of false findings from multiple comparisons and of statistical bias from overparameterized models. Additional limitations included the single urine sample collected from the children to determine long-term exposure. This approach may not accurately reflect longitudinal exposure patterns and may also be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food.

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between pediatric exposure to pyrethroids and neurobehavioral effects in children. A total of eight epidemiologic studies were identified that assessed pediatric exposure (during childhood) to pyrethroids by measuring urinary metabolites in children. Six of the eight studies reported statistically significant decrements in neurobehavioral function (Oulhote and Bouchard 2013, Wagner-Schulman et al. 2015, van Wendel de Joode et al. 2016, Viel et al. 2015, Viel et al. 2017, Wang et al. 2016) and the remaining two studies reported a non-significant association (Quirós-Alcalá et al. 2014, Fiedler et al. 2015). Two studies were performed in the U.S. using NHANES cycles 1999-2000 and 2001-2002, one used the CHMS and the six remaining studies were done in China, Costa Rica, Thailand, or France. While these associations were reported, all eight of these studies relied on lower quality cross-sectional designs (none used prospective cohort or case-control designs) and thus could not assess the temporal association between pyrethroid exposure and neurobehavioral effects in children. Additional limitations of these studies included the assessment of pyrethroid exposure using only a single spot-urine sample from the child and – given the generally intermittent and occasional nature of exposures and short half-lives in the body – it is difficult to evaluate its accuracy. Further, only minimal information (if any) was provided by authors with respect to analytical methods or QA/QC procedures and protocols. In addition, the statistical analyses performed by Wang et al. (2016) appears to have been not fully appropriate given the statistical treatment of the data from the three schools targeted in the study. Too, a number of the studies were exploratory in nature, performing a broad range of multiple comparisons with no corrections for multiple comparisons or false discovery rates.

7.1.3 Autism Spectrum Disorders (ASD)

Three studies were identified that evaluated the association between pyrethroids and ASD in children. Two of the three studies (Shelton et al. 2014, Schmidt et al. 2017) were conducted as part of the Childhood Autism Risks from Genetics and Environment (CHARGE) Study in California and investigated prenatal pyrethroid exposure to pyrethroids using a GIS-based approach that relied on California PUR and residential address information. The third study (Domingues et al. 2016) was a cross-sectional study that examined the association between the urinary metabolite 3-PBA and ASD in a study population of 40 Italian children. These studies are summarized below:

- Shelton et al. (2014) conducted a population-based case-control study to investigate the association between residential proximity to pesticides including pyrethroids in pregnant women and the risk of neurodevelopmental effects among their offspring. Using data from the CHARGE study, cases included children aged 2 – 5 years, living within the Sacramento area in California,

who were recruited from the Department of Developmental Services (DDS) in California, and had been diagnosed with either ASD or Developmental Delay (DD). Diagnoses were confirmed or reclassified as ASD using the Autism Diagnostic Observation Schedule or the Social Communications Questionnaire. Controls, those with Typical Delay (TD), were recruited from the general population using birth record data, and were frequency matched to the cases based on age, sex, and the DDS catchment area they would have been part of had they been a case. A wide range of exposure information was collected from the parents of participating children, as well as all residential addresses from 3 months prior to pregnancy to time of interview. However, rather than utilizing the exposure information collected from parents, potential exposures to pesticides were estimated by linking the locations of pesticide applications from the California PUR to the residential locations of mothers over the course of the entire pregnancy and preconception. A PUR record of any pyrethroid application within a 1.25, 1.50, or 1.75 km buffer of the mother's residence was considered an "exposure" for the respective buffer sizes. Due to differing chemical structures and observed behavioral effects in animal studies, pyrethroids were further stratified and reported as either a type 1 or type 2 pyrethroid in this study. The study authors reported risk estimates for type 2 pyrethroids only, due to the low prevalence of type 1 pyrethroid pesticide applications. A multinomial conditional multiple logistic regression was run to determine an OR and 95% CI controlling for home ownership, maternal place of birth, paternal education, child race/ethnicity, year of birth, and maternal prenatal vitamin intake (3 months prior to pregnancy to the first month of pregnancy). The time of maternal exposure during pregnancy was further stratified into the following exposure subcategories: pregnancy (overall), preconception, 1st trimester, 2nd trimester, and 3rd trimester.[46] Among the total ASD cases (n = 486), DD cases (n = 168), and TD cases (n = 316), 106 of the total ASD cases, 36 of the total DD cases, and 67 of the TD cases were classified as pyrethroid exposures for the 1.50 km buffer (*i.e.,* pesticide applications were made with 1.5 km of the mother's residence); similarly, 100 of the total ASD cases, 34 of the total DD cases, and 63 of the total TD cases were classified as type 2 pyrethroid exposure for the 1.5 km buffer. For ASD, evidence of a positive association was reported during the 3rd trimester among pregnant women living within 1.50 and 1.75 kilometers of pesticide applications of pyrethroids (OR = 1.87; 95% CI: 1.02, 3.43 and OR = 1.83; 95% CI: 1.04, 3.23, respectively). Additionally, evidence of positive association was observed during preconception and the 1st trimester of pregnancy among pregnant women who lived within 1.50 kilometers of pesticide applications for type 2 pyrethroids (preconception OR = 1.98; 95% CI: 1.06, 3.71; 1st trimester OR = 1.85; 95% CI: 1.01, 3.38). For DD, evidence of a moderately strong association was reported for pyrethroids and type 2 pyrethroids, respectively, during the 3rd trimester among pregnant women living within 1.75 kilometers of pesticide applications of pyrethroids (OR = 2.34; 95% CI: 1.18, 4.67; OR = 2.31; 95% CI: 1.15, 4.66), but effects sizes were lower at (the shorter) 1.25 km and 1.5 km distances and were not statistically significant.

The quality of the study was ranked low. Study strengths included ascertaining subject outcome, where diagnoses of study participants were confirmed or reclassified, which minimized case misclassification. One main study limitation was the limited exposure information reported or incorporated in the analyses. For example, the study did not consider various potential sources of exposure (*e.g.,* dietary intake, residential pesticide applications, etc.,) or the "wide range of environmental exposures" surveyed from parents. Instead, proximity to agricultural pesticide applications was assumed to result in an exposure. Additionally, quantitative information such as the pounds of pesticides applied was not incorporated into the analyses but was simply classified as exposure or no exposure over the entire pesticide class. Furthermore, there was no way to ascertain past exposures (*i.e.,* via biomonitoring) using this case-control study design. Another

[46] The periods of exposure during pregnancy were defined as the following in this study: preconception (90 days prior to conception). 1st trimester (0 – 90 days), 2nd trimester: (91 – 180 days), 3rd trimester (181 days – birth).

study limitation included the lack of report for the number of corresponding exposed cases and controls for each time period, buffer distance, and pesticide class when reporting the risk estimates for ASD and DD, relative to pyrethroids and type-2 pyrethroid exposures. Another shortcoming of the study was performing separate statistical analyses for each time period, buffer distance, and pesticide class rather than combining these analyses using additional covariates, which would, in part, result in CIs that better reflect significance of the ORs. Another statistical concern was the calculation of statistical weight for each case or control within each stratum in the analysis. Note that the controls were selected based on frequency-matching factors "DDS catchment area, age, and sex of case child." When the data were analyzed using PROC SURVEYLOGISTIC with STRATA statement, the proportions of cases and controls were not balanced across strata; there was a need to calculate the weight of each case or control within each stratum. The use of demographic factors (race, maternal age, maternal education, etc.) – which were not the frequency-matching factors but were included in the model as covariates – to calculate the weight of each case or control within each stratum, may not have been appropriate. Lastly, one would expect the magnitude of the ORs to increase with agricultural pesticide applications closer to subject homes (*i.e.,* ORs should be larger for smaller buffer distances) if outcomes were associated with higher exposures. However, this was not the case for all time periods with significant ORs.

- Domingues et al. (2016) conducted a cross-sectional study[47] to investigate the association between the pyrethroid urinary metabolite 3-PBA and ASD in Italian children. For the 3-PBA-specific analysis, the study population consisted of cases (n = 21) with ASD, aged 5-12 years old and of both sexes, recruited from the Neuropsychiatric Unit of the Bellaria Hospital of Bologna, Italy. Patients with any medical or neurological comorbidity were excluded from participation. ASD diagnoses were healthy children of both sexes and 5-12 years of age, recruited from the local community, with no sign of cognitive, learning, or psychiatric involvement. Urine samples were collected from all study subjects and frozen and stored at 20°C prior to extraction of 3-PBA and quantification using gas chromatography. Differences in urine 3-PBA concentrations between cases and controls were statistically compared using a non-parametric test (Mann-Whitney U). No evidence of a statistically significant correlation was observed between the Childhood Autism Rating Scale (CARS) score and 3-PBA among ASD cases ($R2 = 0.0554$; $p > 0.05$). Urinary concentrations of 3-PBA were slightly higher among ASD cases compared to urinary concentrations among controls (average or median levels were not presented; data were presented in figures only), but no evidence of a statistically significant difference ($p = 0.054$) was observed and only p-values – and no confidence intervals were presented. Further, no evidence of a correlation between CARS total score and 3-PBA in urine was observed in the ASD cases ($R2 = 0.0539$; $p > 0.05$).

  The quality of the study was ranked low. The primary strengths of this study, set in Bologna, Italy, were the strong case definition and characterization. Study limitations included the small sample size and the cross-sectional study design. This design lacks the ability to determine temporality. Other limitations the issues associated with the single assessment of the urinary pyrethroid metabolite as a biomarker for pyrethroid exposure at the biologically relevant time period in the development of ASD. Further limitations included the lack of control for potential confounders, and potential selection bias due to imperfect control subject selection. The statistical analysis was minimal, and no multivariate modeling was conducted to account for potential confounding. Lastly, no information on laboratory QA/QC procedures or results was provided.

[47] Although the study authors indicate the study design in Domingues et al. (2016) follows a case-control design, we believe the study follows the cross-sectional study design based on the methods used to measure exposure of the children.

- Schmidt et al. (2017) conducted a case-control study to examine combined prenatal exposure to pesticides and maternal folic acid intake (FA) in relation to autism spectrum disorder (ASD). The investigators analyzed a subset of the CHARGE case-control study. Cases were children, aged 2 to 5 years old, born in California between 2000 and 2007, with autism identified through the California Regional Center System. Controls were identified from state birth files and frequency matched to cases by age, gender, and catchment area (*i.e.,* areas of a specified list of the California Regional Centers). Maternal FA intake and household pesticide product use were retrospectively collected via interviews from 2003 to 2011. Interviews covered the period 3 months prior to conception through the interview date. The study focuses on FA intake during the first month of pregnancy. FA intake for the month was calculated from all reported supplements taken and fortified sources consumed by the mother and dichotomized as low (< 800 μg) and high (≥ 800 μg). The investigators summarized household pesticide exposure as 'none' or 'any' during the whole pregnancy period and by whether exposure was indoors, outdoors, or pet use. 'Any' was sometimes further categorized as 'some' (less than 6 months of pregnancy) and 'regular' (6 or more months of pregnancy). The interview also identified 11 mothers (6 cases and 5 controls) with occupational pesticide exposure during pregnancy. Agricultural pesticide exposures during the 6-month period (3 months prior to conception through the end of the 1st trimester) and within 1250 m of the home were dichotomized as 'exposed' and 'unexposed' using a linkage of geocoded mother's addresses with a statewide database of commercial pesticide applications. Other time periods and proximal distances were considered in supplemental analyses. Logistic regression models of ASD as the outcome were run and ORs with corresponding 95% CI were reported, adjusted for home ownership, child's year of birth, maternal vitamin B_6 intake, and maternal vitamin D intake during the first month of pregnancy. Maternal FA intake was stratified into two categories (≥ 800 μg or < 800 μg) and pesticide exposures including pyrethroid exposure, was also further stratified into no exposure vs. any exposure.

  No evidence of a significant positive association was observed between exposure to pyrethroids combined with <u>low FA intake</u> relative to ASD among offspring (OR: 2.10; 95% CI: 0.90, 4.80 with n = 25 ASD cases, 11 controls), and no evidence of a positive association was observed between exposure to pyrethroids combined with <u>high FA intake</u> relative to ASD among offspring (OR: 0.90; 95% CI: 0.50, 1.80 with n = 22 ASD cases, 19 controls), relative to the reference group of high FA intake and no pyrethroid exposure. Similarly, no evidence of a significant positive association was observed between no exposure to pyrethroids combined with low FA intake and ASD among offspring (OR: 1.20; 95% CI: 0.70, 2.10 with n = 153 ASD cases, 96 controls), relative to the reference group of high FA intake and no pyrethroid exposure.

  The study quality was ranked low. While the case-control design was efficient and availability of ASD and birth registries minimized selection bias, assessment of exposure retrospectively (up to 5 years or more) using a questionnaire potentially introduced recall bias. Folic acid intake was also based on the same questionnaire and subject to bias, making it difficult to assess the interaction between both exposures. In addition, the investigators separate analysis using California PUR data has not been validated and used a relatively large buffer distance (*i.e.,* 1.25 km), so it is unclear if this approach is reliable. Furthermore, there may be systematic differences between individuals living near agricultural areas and individuals living in non-agricultural areas, so residual confounding may be present. An additional limitation was that only 65% of participants had information on both FA intake and pesticide exposures, so a large proportion of study subjects had missing data.

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between urinary pyrethroids metabolites and ASD in children. Three studies examined the

association between exposure to pyrethroids and ASD in children. Shelton et al. (2014) and Schmidt et al. (2017) were both based on the same underlying cases-control study, CHARGE, and used a GIS-based approach that estimated pyrethroid exposure using California PUR data and residential address information provided by study subjects. While Shelton et al. (2014) reported evidence of a significant association between pyrethroid exposure and ASD, the use of PUR has important limitations. As described above, this approach has not been fully validated and additional information is needed to characterize the relationship between California PUR information and the actual exposure-levels experienced by individuals as a result of living in agricultural communities. As described above, this approach may lack the specificity to assess pyrethroids and be unable to distinguish between factors associated with geographic proximity to agricultural land and pesticide use more generally. Schmidt et al. (2017) further examined the CHARGE study cohort. The study does not provide additional information on the association between pyrethroid exposure and ASD, but rather examined the potential effect modification and reported no evidence of a significant positive association between exposure to pyrethroid combined with low maternal folic acid intake relative to ASD among offspring. The remaining study of ASD by Domingues et al. (2016) was a cross-sectional analysis of 40 children recruited from a hospital in Italy. The investigators' statistical analysis was extremely limited and consisted of a univariate analysis of the correlation between urinary 3-PBA levels in children and ASD. As such, the study did not adjust for confounders and was insufficient for purposes of evaluating the relationship between pyrethroid exposure and ASD. Further, only minimal information was provided by authors with respect to analytical methods, and no information on laboratory QA/QC procedures or results was provided.

7.2 Birth Effects

7.2.1 Anogenital Distance (AGD)

One study investigated the relationship between AGD and pyrethroid exposure in Denmark.

Dalsager et al. (2017) conducted a cohort study to evaluate the association between maternal exposure to pesticides including pyrethroids and anogenital distance (AGD) and gestational size among newborns at three months of age. The results for AGD are presented here, and those for gestational size are presented later in this memorandum under the gestational size endpoint.[48] Study participants included women who were part of the Odense Child Cohort (OCC), who lived within the Odense municipality in Denmark, were newly pregnant during the period from January 2010 to December 2012, and provided a single urine sample at 28 weeks of gestation after overnight fasting. Of the 6,707 eligible women asked to participate in the larger OCC, 2,874 (42%) agreed to participate in this sub-study. They were recruited either at a voluntary meeting regarding ultrasound examinations, at their first antenatal visit or at the ultrasound examination at the Odense University Hospital between gestational ages 10 and 16 weeks. Birth outcome information was available for 2,522 children, and 2,338 of them also completed the 3-month clinical examination in which AGD was measured. The sub-study population (n = 858 women with analyzed urine sample) had significantly fewer smokers, were significantly more likely of non-European origin, and significantly higher pre-pregnancy BMI when compared to all participating women in the OCC. Exposure was assessed using the collected urine sample and samples were stored frozen at -80°C until analysis. Urine concentrations were measured for pesticide metabolites including the common pyrethroid metabolite 3-PBA using LC-MS with an LOD of 0.03 µg/L. Concentrations of 3-PBA were detectable in 94.3% of the urine samples from the pregnant women and were expressed on a per gram creatinine basis to correct for urinary dilution. Median, 75th, and 95th percentile concentrations of 3-PBA were 0.23, 0.45, and 1.73 µg/g creatinine, respectively among the 857 available urine samples. Supplemental questionnaire data was collected twice during pregnancy from each child's mother and included questions regarding

[48] Results from Dalsager et al. (2017) on gestational size are summarized and evaluated in Section 7.2.2 of this report.

demographics, social factors, general health, and lifestyles. Birth outcomes were determined using birth records and AGD was measured at three months of age in newborns, and was comprised of two measurements (each measured separately for males and females).[49] Linear regression was done in two ways: one with creatinine-adjusted 3-PBA as a (grouped) categorical variable and the second with creatinine-adjusted 3-PBA considered as a continuous variable (and for which assumptions of linear regression relating to normality of residuals, as well as linearity and homogeneity were tested and satisfied). In the former analyses, maternal urinary 3-PBA levels obtained after overnight fasting were grouped into tertiles,[50] with tertile 1 used as the reference group. Based on this approach, the investigators reported no statistically significant findings among the eight comparisons performed for AGDas for boys, for AGDap for boys, for AGDaf for girls, and for AGDas for girls (-0.53 mm $< \beta < 0.96$ mm; all CIs encompassed the null value of 0; all p-trends > 0.05; n = 409 – 419 males, n = 322 – 325 females) nor any significant trends with increasing creatine-adjusted maternal 3-PBA concentration ($0.14 < p < 0.87$). Similarly, the investigators reported no evidence of an association between creatinine-adjusted maternal urinary 3-PBA levels and any of the (four) measured AGD values when maternal 3-PBA urinary concentration was instead considered as a $\log_2$-transformed continuous variable in a multiple linear regression, adjusting for weight-for-age z-score and age-at-three-months examination. In all final models, the residuals were checked for normal distribution as well as linearity and homogeneity.

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between exposure to pyrethroids and pyrethroid metabolites and anogenital distance in children. One study, Dalsager et al. (2017), examined the association between pyrethroids and pyrethroid metabolites and birth defects in children. The overall quality of the study was ranked moderate. Study strengths included the prospective study design and clinical measurement of AGD. While the study had these strengths, the exposure assessment was limited and based on measurement of the 3-PBA urinary metabolite in a single spot-urine measurement taken from mothers at 28 weeks of gestation after overnight fasting. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. Additionally, only 42% of the eligible women participated in the OCC, with participants better educated and more often of Danish origin than non-participants. Too, no information was provided by the study authors on laboratory QA/QC procedures or QA/QC results was provided.

7.2.2 Gestational Size (Birth Weight, Length, and Head Circumference)

Five studies investigated the relationship between maternal exposure to synthetic pyrethroids and pyrethroid metabolites with respect to gestational size in infants. This included two retrospective studies conducted in Poland (Dabrowski et al., 2003; Hanke et al., 2003), two prospective studies conducted in Tokyo and Denmark (Zhang et al., 2014 and Dalsager et al., 2017, respectively), and one cross-sectional study in rural China (Ding et al., 2015). These are described below:

- Dabrowski et al. (2003) conducted a hospital-based case-control study to evaluate the association between prenatal exposure to pesticides including synthetic pyrethroids in Polish women farmers and birthweight among infants. The study population included women living in Central Poland who had given birth on 70 randomly selected days between January 1998 – June 2001 at 25

[49] AGD (for males) = AGDas (measurement from the center of the anus to the posterior base of scrotum) + AGDap (measurement from the center of the anus to the cephalad insertion of the penile); AGD (for females) = AGDaf (measurement from the center of the anus to the posterior fourchette) + AGDac (measurement from the center of the anus to the top of the clitoris). The AGD was measured by expert-trained technicians in triplicate using a Vernier caliper and an arithmetic mean calculated. Four such trained technicians were used to minimize inter-observer variations.

[50] Tertiles of the urinary 3-PBA metabolite concentrations as follows: 0.007 to < 0.16 µg/g creatinine (1st tertile); 0.16 to < 0.35 µg/g creatinine (2nd tertile); and 0.35 to < 29.34 µg/g creatinine (3rd tertile).

selected rural district maternity hospitals. Cases (n = 117) included women who had delivered infants born with low birth weight (< 2,500 grams) and who reported being involved with farming activities for at least 7 days while pregnant. Controls (n = 377) included women who gave birth to a non-low birth weight infant and similarly reported being involved with farm activities for at least 7 days while pregnant. The study population was relatively homogenous with respect to socioeconomic status, age, and nutrition habits and the study was limited to uncomplicated pregnancies; mothers with diabetes, hypertension, infectious diseases, and other diseases which might increase the risk of intrauterine growth retardation or pre-term birth were excluded from the study. A questionnaire was administered to cases and controls 1 - 2 days after delivery to collect information on maternal anthropometrics, demographics, health status, job history, and smoking.[51] The maternal questionnaire also included questions on occupational hazards, including the use of pesticides in each trimester of pregnancy and frequency of heavy physical work on the farm. Based on maternal questionnaire responses, the investigators identified study subjects that reported pesticide exposure during the first two trimesters of pregnancy (n = 95 study subjects) and performed a follow-up interview conducted 6 - 12 months after delivery with husbands of women who reported pesticide use on their farms (n = 49 of 95 study subjects). This follow-up interview was done to confirm maternal pesticide exposure and obtain information on active ingredients/trade names of pesticides and the timing and duration of exposure. Based on this exposure assessment approach, 95 study subjects reported pesticide exposure during the first two trimesters of pregnancy and follow-up interviews were conducted with 49 husbands of women who reported pesticide use on their farms during pregnancy (51.6%); based on these interviews, the study identified six study subjects exposed to pyrethroids. Multiple regression was used to evaluate differences in pregnancy duration and birthweight at a given duration and logistic regression was used to model the impact of pesticide exposure on the occurrence of low birth weight (< 2,500 grams). The models included (general) pesticide exposure as well as infant sex, maternal pre-pregnancy weight, height, smoking during pregnancy, calendar year of birth, and involvement in fieldwork.[52] To determine if an association existed between prenatal exposure to specific pesticides including pyrethroids during the 1st and 2nd trimesters of pregnancy and low birth weight among infants, the expected birth weights of the infants of exposed mothers were compared to the observed birth weights, while adjusting only for the place of residence. No evidence of a significant difference was reported between prenatal exposure to synthetic pyrethroids for observed vs. expected birthweight for exposure in the 1st and 2nd trimester of pregnancy in infants (Difference = -154 grams, p-value = 0.286)[53] based on six pyrethroid-exposed cases. Similarly, no evidence of a significant difference was reported for pregnancy duration (Difference = 0 weeks, p-value = 0.451).

The quality of the study was ranked low. Strengths of the study included the hospital-based case-control design, clinical measurement of birth weight and pregnancy duration, and use of a follow-up interview with male spouses to confirm use of pesticides on farms during pregnancy which found an 85.7% confirmation rate among women who reported at least some pesticide exposure on the farm. Limitations included the potential for recall bias since mothers reported their pesticide exposure retrospectively 1-2 days after delivery and after birthweight was measured, and mothers who gave birth to infants with lower birthweights may have recalled their exposures

[51] Specific questions related to weight and height, and pre-pregnancy weight; job history; chronic diseases during pregnancy such as hypertension and diabetes; smoking and reproductive history such as spontaneous and elective terminations, still births, live births, and the number, duration, birthweight, and sex of each infant.
[52] The season of the year in which conception took place was strongly correlated with pesticide use and physical work, but the authors found that this variable did not significantly influence birth weight and this was thus not included in the multivariable analysis.
[53] No confidence interval was provided by study authors.

differently than those who gave birth to infants with higher birth weights.[54] Further, the exposure assessment only considered ever/never use of pesticides and did not consider the magnitude, frequency, or duration of exposure and thus measures with dose-response relationships could be determined In addition, the statistical methods in the study were not clearly described, and no rationale was provided for their selection and use only of "place of residence" – and not the remaining important determinants of birth weight and pregnancy duration (*e.g.,* infant sex, maternal pre-pregnancy weight, height, smoking during pregnancy, etc.) - as an adjustment. Further, the study identified 95 study subjects that reported pesticide exposure during the first two trimesters of pregnancy, but only six of these study subjects were determined to have been exposed to pyrethroids based on initial interview results of the women and follow-up interviews with spouses. Due to the small number of mothers exposed to pyrethroids (n = 6) and the limitations of the statistical analysis, the results for pyrethroids lack precision and may not be reliable in assessing the association between pyrethroid exposure and birthweight.

- Hanke et al. (2003) conducted a retrospective cohort study to evaluate the association between maternal exposure to pesticides including synthetic pyrethroids and birth weight among infants in Poland. The study population included women living within the Zadzim district (population = 2,200) in Poland who gave birth during January 1994 – December 2000. Maternity unit medical records were used to identify 123 women who met these criteria, and 104 were enrolled in the study after exclusion of 10 women with chronic health problems, six women who refused to participate, and three women who could not be contacted. The study population was homogenous, with respect to socio-economic status, age, and nutrition habits, and limited to uncomplicated pregnancies and mothers without diseases or other pathological conditions that increase the risk of intra-uterine growth retardation (*e.g.,* diabetes, hypertension, infectious disease). For each woman in the study, pregnancy duration (weeks), infant birth weight (grams), and infant sex data were obtained from these records. Pesticide exposure was assessed through completed questionnaires, and questions included maternal age and occupational histories of mothers three months prior to conception throughout the duration of pregnancy (all three trimesters). Data collected about each woman included anthropometric data such as weight and height, smoking status during pregnancy, job history (farming v. non-farming) and – for those that farmed - the main type of farming (orchards or other crop), and physical work in the field (not necessarily related to pesticides). In addition, questions regarding specific pesticide usage (along with trade names) and timing of application within the past seven years were included within the questionnaire (the woman was asked to verify this information with the pesticide applicator, often her husband), and based upon this obtained information, pesticide usage for each mother was reconstructed for the period from three months prior to pregnancy through the 3rd trimester of pregnancy. Since this study focused on small-for-gestational -age (SGA)-related measures and the 1st and 2nd trimesters are the target window for this endpoint, the study authors focused on exposures during this period. Among the total number of study subjects (n = 104), none reported direct spraying or mixing of pesticides but 60 reported (indirect) occupational exposures to pyrethroids during farm work, either via orchards (n = 18) or crop farming (n = 52); nine of these women reporting "orchard work" reported exposure to synthetic pyrethroids, and 17 of those reporting "crop farming" reported exposure to synthetic pyrethroids for a total of 26 women reporting exposure to synthetic pyrethroids. The distinction between "orchard work" and (other) "crop farming" was used as a surrogate for socio-economic class. A linear regression model was used to determine if an association existed between maternal pesticide exposure and birth weight among newborns using a regression coefficient and corresponding p-value and adjusting for exposure to other pesticides, pregnancy duration, infant sex, maternal pre-pregnancy weight,

[54] The authors, however, found that the confirmation rate of pesticide use -although limited - was similar between mothers of normal birth weight vs. low birth weight infants suggesting that such bias may not have been substantive.

smoking during pregnancy, involvement in field work, type of farming (orchard vs. other crop), calendar year of birth (to potentially account for time trends), and maternal age. The criteria for inclusion of these adjustments was based on findings on low birth weight risk factors found in another study conducted in the Lodz region of Poland. Significant variables for synthetic pyrethroids were pregnancy duration, maternal pre-pregnancy weight, year of birth, and type of farming (orchard vs. other crop/non-orchard). Evidence of an association between maternal exposure to synthetic pyrethroids and decreased birth weight among newborns was reported (β = -233.3 grams; 95% CI: -416.0, -50.6, p-value = 0.02).

The quality of the study was ranked moderate. Strengths included the hospital-based design, clinical measurement of birth weight, and assessment of maternal exposure that focused on the 3-month period prior to conception through the three trimesters of pregnancy. The exposure assessment also attempted to identify specific active ingredients using a Polish database of registered pesticides. With regard to limitations, the exposure assessment – while focused on use of specific active ingredients during the first two trimesters of pregnancy most relevant for birthweight outcomes – relied on self-report by study subjects after delivery. This approach could have introduced recall bias if mothers of infants with lower birthweight differentially recall their past use of pesticides. Additionally, the exposure assessment also only considered ever/never use of pesticides and did not consider the magnitude, frequency, or duration of exposure and thus measures with dose-response relationships could not be determined. Lastly, it should also be noted that the study consisted of only104 study subjects, of which only 26 reported use of pyrethroids during their 1st or 2nd trimester of pregnancy.

- Zhang et al. (2014) conducted a prospective cohort study to evaluate the association between maternal exposure to pyrethroid metabolites and thyroid hormone levels along with birth sizes in neonates. The results for birth sizes are presented here, and the results for thyroid hormone levels are reported later in this memorandum in **Section 7.2.4 on Thyroid Hormonal Levels in Neonates**. Study subjects included pregnant women in the 1st trimester of gestation during 2009 - 2011 who lived in Tokyo, were 20 - 50 years old, and who did not have any illnesses pertaining to thyroid function. A total of 424 women were originally approached to participate in the study, with 315 women in their 1st trimester of gestation agreeing to participate. Blood and urine samples were available from 231 of these women and – of these 231 women, 37 left the cohort by the time of delivery, seven due to spontaneous abortion, 12 due to change of hospital, eight due to multiple pregnancy, and 10 due to refusal for other reasons. The remaining 47 were not included due to the failure to sample blood from the baby, leaving a total of 147 mother-neonate pairs that were included within this study of the original 424 women that were approached (35%). There were no statistically significant differences between the mothers that were the subjects of the study, mothers that dropped out of from the original cohort (n = 37) and mothers whose neonates blood was not sampled (n = 47), with $p > 0.05$. In addition, there was no difference in birth size between the neonates that were part of the study from whom blood was not sampled ($p > 0.05$). Maternal exposure to pyrethroids was assessed using a single spot urine sample that was collected during pregnancy (11 – 14 weeks of gestation)[55] and measured for the pyrethroid metabolite 3-PBA. Concentrations of the pyrethroid metabolite 3-PBA in the maternal urine samples were measured using HPLC-MS, which achieved a specific gravity (SG)-adjusted[56] LOD of 0.02 ng/mL. Individual metabolite levels at less than this level were assigned a value of ½ LOD in the statistical analysis, and 97.8% of the measurements were above the LOD (226 of the 231

[55] A discrepancy in time of collection of maternal blood and urine samples was noted in the published study between the text which states samples were collected during the "10th-12th gestational weeks" and footnote a of table 2 which states that maternal blood and urine samples were collected at "11 – 14 weeks gestation.

[56] To account for urine dilution effects, concentration corrections were made using the Specific Gravity (SG) method, adjusting to a SG of 1.020.

subjects). Median and 75th percentile SG-adjusted 3-PBA concentrations were 0.380 and 0.793 ng/mL, respectively. Quality control procedures were carried out and included the use of procedural blanks as well as a quality control blank in each group of measurements. With respect to the birth size outcome variable, the following were measured immediately after birth: head circumference (cm), birth weight (grams), birth length (cm), and chest circumference (cm). A multivariable linear regression with automated stepwise regression (with $P_{enter} = 0.05$ and $P_{leave} = 0.1$) was used with birth sizes as the dependent variables and list of covariate candidates including log-transformed maternal urinary 3-PBA, log-transformed maternal urinary iodine, log-transformed maternal TSH, maternal age, maternal pregnancy BMI; parity (primiparous/multiparous), gestational weeks, cigarette smoke exposure (yes/no); drinking alcohol during pregnancy (yes/no); food frequency (every meal/not every meal), and neonatal sex (male/female). For birth sizes, evidence of positive associations were reported for birth weight and head circumference relative to maternal exposure to 3-PBA (birth weight: $\beta = 67.2$, 95% CI: 11.6 - 122.8 g/unit of log-transformed maternal urinary 3-PBA ng/ml, p-value = 0.017; head circumference: $\beta = 0.325$, 95% CI: 0.078 - 0.572 cm/unit of log-transformed maternal urinary 3-PBA ng/ml, p-value = 0.010), meaning both birth weight and head circumference measurements improved with increasing exposure. No evidence of a statistically significant association was reported between 3-PBA levels and birth length or chest circumference (p-values > 0.05). Besides multivariable linear regression analyses, the authors also performed ANOVA analyses to compare birth sizes between the quartiles of maternal urinary 3-PBA concentration and reported no significant differences in birth sizes between quartiles of maternal urinary 3-PBA concentration (birth weight p-value = 0.501, birth length p-value = 0.694, chest circumference p-value = 0.950, and head circumference p-value = 0.897).

The quality of the study was ranked low. The prospective study design was a study strength as well as the quality control procedures utilized for urine sampling and the use of hospital-based outcome measure of birth weight, birth length, head circumference and chest circumference. While the study had several strengths, the statistical methods used in the study relied on automated stepwise selection to identify covariates in their statistical model. This approach is generally considered appropriate only for studies conducting exploratory analysis for purposes of hypothesis generation; purported statistical significance arising from studies that use this technique is not valid and cannot be relied upon to suggest causal relationships.[57] In addition to limitations in their statistical methods, the study obtained only a single urine sample to determine the pyrethroid metabolite concentrations over time. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food.

- Ding et al. (2015) conducted a cross-sectional study to evaluate the association between maternal exposure to pesticides including pyrethroid metabolites and birth outcomes in rural northern China (Shandong province). The women worked in agricultural fields. Study participants included pregnant women who had visited the Binhai Hospital, and eligible cases included pregnant women (between September 2010 to September 2012), who were older than 18 years of age, were involved in a singleton pregnancy, did not report assistance with pregnancy or co-morbidities defined by the study,[58] and lived within the area for three or more years. A total of

[57] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. Psychosomatic Med. 66:411-42.

[58] Comorbidities included pre-gestational or gestational diabetes, chronic or pregnancy-associated hypertension, HIV infection or AIDS, and illicit drug use.

636 women met the eligibility criteria with 568 agreeing to participate (89.3% response rate). Of these 568 women, 63 women were excluded for not having urine samples, 32 for missing values of major confounders, two women with urinary creatinine concentrations < 0.1 g/L, and 17 infants with missing neonatal anthropometric data, leaving 454 mother-infant pairs in the final analysis (71% of those approached). Exposure was assessed using spot urine samples collected from the mother at the hospital prior to delivery (up to 3 days before; samples were stored at -80°C until shipment to the laboratory) as well as questionnaire data obtained during an in-person interview by trained nurses. Questionnaires collected information regarding demographic and socioeconomic data as well as pregnancy data[59] and also included questions on prior pesticide use. With respect to this latter topic, nearly two-thirds (63.9%) of the families did not report using pesticides in the household during pregnancy and nearly one-fifth (18.3%) reported using household pesticides frequently. More than two-thirds (68.7%) of the women reported washing fresh fruits and vegetables before eating or cooking, with only one-eighth (12.6%) reporting seldom or never washing their fresh fruit or vegetables. All of the women attended at least one prenatal visit to the Binhai Hospital during the current pregnancy, but no additional pre-natal details were available. Infant sex, birth date, body weight, birth length, and head circumference were obtained from medical records as well as data on prior medical history, current health status, and clinical estimate of gestational age (ultrasound and timing of last menstrual period). Urine concentrations were measured for pyrethroid metabolites 3-PBA, *cis*-DCCA, and *trans*-DCCA, using GC-MS detection which achieved an LOD of 0.1 g/L. Individual metabolite levels at less than this level were assigned $\frac{1}{\sqrt{2}}$ LOD; approximately 70 - 80% of the measurements were above the LOD. Median and 75th percentile concentrations, respectively, of *cis*-DCCA, *trans*-DCCA and 3-PBA were 0.51, 0.65, and 0.68 µg/g and 0.91, 1.10, and 1.24 µg/g, adjusted for creatinine.[60] Laboratory quality control procedures were carried out and included intra- and inter-assay precision testing, and the use of laboratory blanks. Birth outcomes were determined using birth records and were defined as the following: birth weight (grams), birth length (cm), head circumference (cm), and gestational length (weeks). Children were evaluated at birth in addition (as stated by the study authors) to a follow-up period of up to two years. A linear regression was used to determine if an association existed between creatinine-adjusted and log10-transformed pyrethroid metabolite concentrations and birth outcomes using regression coefficients and 95% CIs, adjusting for parity, infant sex, pre-pregnancy BMI, household monthly income, maternal age, and passive smoking.[61] These were selected based on being at least marginally significantly associated ($p < 0.10$) with at least two of the outcomes in the present study. The analyses of birth weight, birth length, and head circumference were also adjusted for length of gestation.[62] When birthweight, birth length, head circumference, and length of gestation were assessed separately, no evidence of an association was observed for 3-PBA, *cis*-DCCA, and *trans*-DCCA ($-37.67 < \beta < 22.60$; all CIs encompassed the null value of 0; all p-trends > 0.05). When the individual maternal urinary metabolites (3-PBA, *cis*-DCCA, and *trans*-DCCA) were summed and assessed, however, evidence of a negative association was observed only for infant birthweights (β: -96.76 95% CI: -173.15, -20.37, p-trend = 0.013), indicating that a log10 increase in summed pyrethroid concentrations was predicted to result in a 96.76-gram reduction in infant birth weight after

[59] Demographic and socioeconomic information included maternal age, height, pre-pregnancy weight, education level, household income, and address. Maternal characteristics included cigarette smoking, alcohol use, dietary habits and employment. Other information obtained by interview and verified by medical records included previous pregnancies, current pregnancy complications, weight gain and self-reported last menstrual period.

[60] The median (non-creatinine adjusted) concentrations (IQR) for *cis*-DCCA, *trans*-DCCA, and 3-PBA, respectively, were 0.50, 0.57, and 0.62 µg/L for the 50th percentile, and 0.79, 0.96, and 1.17 µg/L for the 75th percentile.

[61] Although few mothers reported smoking themselves or consuming alcohol, almost one-third (31.3%) reported living with a smoker during pregnancy.

[62] For the gestational length birth outcome, the linear regression was adjusted for parity, infant sex, household monthly income, maternal age, and passive smoking.

adjusting for confounders. No associations were seen for birth length, head circumference, or length of gestation. Total (*i.e.,* summed) metabolite levels were not found to be associated with birth length, head circumference, or length of gestation.

The study quality was ranked low. Study strengths the hospital-based measurement of the birthweight, birth length, head circumference, and gestational duration outcome measures. Study limitations included the single urine sample collected to determine the pyrethroid metabolite concentrations. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. We note, too, along these lines that despite the fact that more than 82% of the samples contained detectable levels of pyrethroid metabolites, only about one-third of families reported using household pesticides during pregnancy. Given the generally low concentrations found at the 75th percentile, this suggest that much of the pyrethroid metabolites found were likely due to background exposures to non-toxic environmental degradates. Importantly, no single metabolite was statistically-significantly associated with any of the four birth outcomes measured (birth weight, birth length, head circumference, and gestational duration), and it was only the *summed* metabolite measure that was determined to be statistically significantly associated with (only) birth weight and not with the remaining three outcome measures. Lastly, the study authors mentioned a follow-up period as part of this study, but no study results were provided for the follow-up period.

- As previously described, Dalsager et al. (2017) conducted a prospective cohort study to evaluate the association between maternal exposure to pesticides including pyrethroids and anogenital distance (AGD) and gestational size among newborns at three months of age. This was done as part of the Odense Child Cohort in the Odense region in Denmark. The results for gestational size are presented here while the results for AGD were reported earlier in this memorandum under that endpoint and under which additional details regarding the study can be found. Gestational size was obtained from hospital-birth records and included birthweight, head circumference, abdominal circumference, and gestational age. The investigators then used multiple regression to assess the relationship between maternal urinary 3-PBA levels (log_2-transformed) obtained at gestational age 28 weeks with these measures of gestational size stratified by sex and adjusting for maternal education, pre-pregnancy body mass index, smoking, and gestational age (for birth weight, and head and abdominal circumference) or by maternal educational level, pre-pregnancy BMI and smoking (for gestational age) Linear regression was done in two ways: one with 3-PBA as a (grouped) categorical variable and the second with 3-PBA considered as a continuous variable (and for which assumptions of linear regression relating to normality of residuals, as well as linearity and homogeneity were tested and satisfied). In the former analyses, maternal urinary 3-PBA levels obtained after overnight fasting were grouped into tertiles,[63] with tertile 1 used as the reference group. Based on this approach, the investigators reported only one statistically significant finding among the sixteen comparisons performed: for boys (and not girls), they reported a statistically significant decrease in abdominal circumference of 1.4 cm (95% CI: -2.5, -0.4) in the second (but not the third) tertile.[64] The investigators reported no evidence of an association between maternal urinary 3-PBA levels and birthweight, head circumference, and gestational age ($-50.10 < \beta < 20.80$; all CIs encompassed the null value of 0; all p-trends > 0.05; n = 411 – 414 males, n = 364 – 366 females). When maternal 3-PBA urinary concentration was instead considered as a log_2-transformed continuous variable, the investigators reported that a

[63] Tertile 1: 0.007 to < 0.014 µg/g creatinine; tertile 2: 0.014 to < 0.24 µg/g creatinine; and tertile 3: 0.24 to < 5.52 µg/g creatine.
[64] For girls only, the reported a marginally significant decrease of 1.0 cm (95% CI: -1.9, -0.0) in abdominal circumference in the third tercile only. The p-trend value was reported as 0.05.

doubling of the maternal 3-PBA urinary concentration was associated with a statistically significant 0.3 cm decrease (95% CI: -0.5, -0.003) in abdominal circumference.

The overall quality of the study was ranked moderate. Study strengths included the prospective study design, and hospital-based clinical determination of birthweight, head circumference, abdominal circumference, and gestational age outcome measures. While the study had these strengths, the exposure assessment was limited and based on measurement of the 3-PBA urinary metabolite in a single spot-urine measurement from mothers at 28 weeks of gestation. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. Additionally, no information on laboratory QA/QC procedures or results were provided.

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between maternal exposure to pyrethroids and gestational size. A total of five studies were identified, including two studies that assessed exposure retrospectively using maternal questionnaires on pesticide use during pregnancy (Dabrowski et al. 2003, Hanke et al. 2003) and three prospective studies that measured pyrethroid metabolite levels in spot-urine samples collected during pregnancy (Zhang et al., 2014; Ding et al., 2015; Dalsager et al., 2017). The two retrospective studies were both conducted in Poland and focused on women living in rural areas: Dabrowski et al. (2003) reported no evidence of a significant association between pyrethroids exposure during the first two trimesters of pregnancy and birthweight (Difference = -154, p-value = 0.286) and Hanke et al. (2003) reported evidence of a significant inverse association (β = -233.3 grams; 95% CI: -416.0, -50.6, p-value = 0.02). Both studies relied on relatively small study samples and included only six and 26 subjects reporting pyrethroid exposure, respectively. The studies also both relied on questionnaires to assess ever/never pyrethroid exposure. This approach could have introduced recall bias and did not allow the investigators to assess magnitude, duration, or frequency of exposure during pregnancy or evaluate dose-response trends.

Results from the three prospective studies were mixed with respect to the association between measured maternal urinary pyrethroid metabolite levels during pregnancy and adverse effects on various aspects of gestational size. Zhang et al. (2014) in Tokyo reported evidence of a positive association between maternal 3-PBA and birth weight and head circumference – meaning both measures of gestational size improved with increasing exposure – with no evidence of a statistically significant association reported for birth length and chest circumference. Ding et al. (2015) in a rural region in Shandong province in northern China reported no evidence of an association between any of 3-PBA, *cis*-DCCA, or *trans*-DCCA with birthweight, birth length, head circumference, and length of gestation assessed separately. However, when Ding et al. (2015) summed the three pyrethroid metabolites in a separate analysis, they reported evidence of a negative association between total urinary metabolite levels and birthweight (β: -96.76 95% CI: -173.15, -20.37, p-trend = 0.013). Finally, Dalsager et al. (2017) as part of the Danish OCC study reported no evidence of an association between maternal urinary 3-PBA levels and birthweight, head circumference, and gestational age. Dalsager et al. (2017) was also the only one of the studies to examine abdominal circumference at three months of age and reported evidence of a negative association between the upper tertile of maternal 3-PBA levels and abdominal circumference amongst girls; however, no association was observed in the middle tertile of maternal 3-PBA levels for girls and a similar association was not observed in their separate analysis of boys. When an alternate analysis was performed, and maternal 3-PBA urinary concentration was instead considered as a $\log_2$-transformed continuous variable, the Danish investigators reported that a doubling of the maternal 3-PBA urinary concentration was associated with a statistically significant 0.3 cm decrease (95% CI: -0.5, -0.003) in abdominal circumference for females only. All three studies generally had strong study designs, but it is unclear if measurement of pyrethroid metabolite levels in spot-urine samples provides a reliable estimate of

exposure during pregnancy and several studies involved multiple comparisons for which corrections or adjustments for multiple comparisons or false discovery rates were not made. In addition, Zhang et al. (2014) also used automated stepwise regression in their statistical analysis. This variable selection method is considered unreliable with -p-values that are not correctly calculated and the potential for biased parameter estimates with 95% confidence intervals that are too narrow due to underestimation of standard errors.

7.2.3 Birth Defects

Three studies investigated the relationship between specific pyrethroids and birth defects, including an Arkansas case-control on hypospadias (Meyer et al. 2006) and two California cases-control studies that relied on similar methods and focused on congenital heart defects (Carmichael et al., 2014) and five types of birth defects (anotia/microtia, anorectal atresia/stenosis, transverse limb deficiency, craniosynostosis, diaphragmatic hernia) (Carmichael et al. 2016). These are described below:

- Meyer et al. (2006) conducted a population-based case-control study to evaluate potential associations between hypospadias (male birth defect in which the opening of the urethra is located on the underside of the penis instead of the tip) and exposure to bifenthrin and other pesticides by investigating the geographical proximity between areas of pesticide application and residences of hypospadias cases. The study population consisted of subjects previously involved in a urogenital birth defects study, and cases were defined as male children living in eastern Arkansas who were born from 1998 through 2002, and whose maternal residence was a geo-codable address at their time of birth. Cases were identified using the Arkansas Reproductive Health Monitoring System, and the controls were determined from the state's Department of Health vital records department via birth certificates. Two controls were selected for each case, and the controls included the next two males born directly after each case without congenital malformations identified on their birth certificate; each control was frequency-matched based on maternal race. Exposure was assessed using pesticide usage data from agricultural databases within the state, and pesticides were categorized based on the pounds (lbs) applied or persisting within a 500-meter proximity of each case's maternal residence (at time of birth), during the 70-day critical period for *in vivo* male external genitalia development - gestation weeks 6 to 16. The estimated use for individual pesticides was calculated using an exposure metric.[65] A multivariable unconditional logistic regression model using categorical variables based on observed cut points in the data to calculate ORs and 95% CIs for individual pesticide exposures. This was adjusted for the main effects of paternal education level, maternal age, weight gain during pregnancy, gestational age at birth, timing of first prenatal care visit, parity, and number of cigarettes smoked per day during pregnancy. Backward elimination was used to identify additional potential confounders. Variables that were found to be associated with hypospadias with $p < 0.05$ (*i.e.,* month of pregnancy in which prenatal care began, number of previous births, and the exposure metric representing total pesticide use) were added to the final model in addition to statistically significant first-order interaction terms. For the categorical treatment broken down into the reference, < 0.02 lbs and ≥ 0.02 lbs categories, 46 cases reported exposure to bifenthrin of the 354 total hypospadias cases, and 95 controls reported exposure to bifenthrin of the 727 total controls. No evidence of a significant positive association was observed between hypospadias and bifenthrin exposure: (> 0 to < 0.02 lbs OR: 1.11; 95% CI: 0.62, 1.97 with 35 cases, 64 controls; ≥ 0.02 lbs OR: 0.86; 95% CI: 0.37, 2.02 with 11 cases and 31 controls) In a second analysis by the

[65] ArcGIS (ESRI Redlands, CA) software was used to determine acres of crops cultivated within a 500-m buffer around each home. Dates containing exposure period for each subject were then linked with estimated dates of crop specific pesticide applications and field dissipation half-lives. Authors cross referenced pesticide use data for each application with acres grown for each crop type within the 500-m buffer and calculated an estimated use (pounds of active ingredient) for each pesticide during the exposure period for each subject.

authors, the unconditional logistic regression model treated the exposure metric as continuous and the (non-significant) odds ratio was found to be 0.98 (95% CI: 0.92-1.04) per 0.005 lbs bifenthrin applied.

The quality of the study was ranked moderate. Strengths included the case-control study design, case ascertainment using a medical birth defect registry, and objective measure of exposure thus removing potential for recall bias. Study limitations included potential misclassification of outcome of the controls. The birth certificates used to identify the controls did not indicate any information regarding birth defects, but authors did not validate that controls were truly free of congenital malformations (*i.e.,* birth defect registry or medical record review). Birth certificates have been shown to underreport birth defects and data quality is known to vary between hospitals and states.[66] This potentially could have caused some of the controls to have been misclassified. Additionally, the study did not account for possible residential mobility of the study participants between pregnancy and childbirth with residency coded only for maternal address at delivery. As a result, the maternal residential addresses during the exposure period may have differed from the reported addresses at childbirth that were geocoded and used to determine exposure at 6 - 10 weeks gestation, possibly causing exposure misclassification. Lastly, only a small number (n = 11) of exposed cases in the high exposure category for bifenthrin exposure were observed in this study.

- Carmichael et al. (2014) conducted a population-based case-control study to evaluate the relationship between exposure to pyrethroids (among other pesticides) during the peri-conception period and risk of specific congenital heart defects (CHD) among infants born to women whose residence at the time of delivery was one of eight counties in the San Joaquin Valley of California. The study population consisted of cases (n = 569) with any of eight CHD phenotypes (heterotaxia, tetralogy of Fallot, D-transposition of the great arteries, hypoplastic left heart syndrome, coarctation of the aorta, pulmonary valve stenosis, perimembranous ventricular septal defect, atrial septal defect secundum) and randomly selected non-malformed live-born infant controls (n = 785) identified from birth hospitals. CHD cases (infants or fetuses) were identified as part of the California Center of the National Birth Defects Prevention Study (NBDPS) and confirmed by echocardiography, cardiac catheterization, surgery, or autopsy reports. Cases (n = 569 total) had estimated dates of delivery between October 1997 and December 2006. Excluded cases from the study included those that arose from or were strongly suspected to have arisen from single - gene disorders, chromosomal aneuploidy, or identifiable syndromes. Controls included non-malformed infants randomly selected from birth hospitals from which the cases arose. Maternal interviews were done primarily by telephone using a standardized computer-based questionnaire between six weeks and 24 months after the date of delivery. Interviews were conducted with mothers of 70% of the eligible cases (n = 704) and 69% of controls (n = 974) and were completed with an average of 12 months and 18 months of the estimated date of delivery for cases and controls, respectively. In the interview, maternal residential history was reported three months prior to conception throughout pregnancy up to delivery for residences occupied for more than one month and these were geocoded. Exposure assignments were made for those mothers who lived at the geocoded addresses for at least 75% of the 3-months window[67] from one month prior to two months following the date of conception which covers the time period of heart development using the geocoded subjects' residences and linking residence with pesticide use reporting records from the California Department of Pesticide Regulation's PUR data. Geocoding

[66] National Birth Defects Prevention Network (NBDPN). *Guidelines for Conducting Birth Defects Surveillance.* Sever, LE, ed. Atlanta, GA: National Birth Defects Prevention Network, Inc., June 2004.
[67] For those mothers that reported multiple address, days at each address were used to weight the exposure assignment.

was successful for 87% of cases and 83% of controls (585 of 647 and 807 of 967, respectively). Pesticide use in this study was considered to be a proxy for exposure via spray drift or off-site movement through volatilization.[68] Reported pesticide use, including pyrethroids, within a 500 m radius of the mother's address during the periconceptual period was classified as 'any use' versus 'no use'. The investigators evaluated 53 pesticide groups and 248 specific pesticides. Overall, 38.1% of control mothers and 38.0% of case mothers (299/785 and 216/569) were predicted to have had any periconception pesticide exposure, with 14% of control mothers exposed to one or more pyrethroid pesticides. Multivariable logistic regression models were used for each of the eight CHD phenotypes that included more than 50 cases total and pesticide exposures with five or more exposed cases and controls. The potential confounders selected for this model were chosen based on prior reported risk factors for CHDs, as mentioned by the study authors. As such, adjustments were made for ethnicity (non-Hispanic white, US-born Hispanic, foreign-born Hispanic, other), education (less than high school, high school, more than high school), maternal age at delivery, intake of folic acid supplements, alcohol use, and smoking during the month before or the first two months of pregnancy in the analysis. Other potential confounders[69] were evaluated using a bivariate analysis based on ever/never use, showed no significant association, and were not included in final models. Based on this approach, the authors only reported results for which the OR was ≤ 0.5 or ≥ 2.0 or for which the confidence interval excluded 1.0, indicating that the other results were available from the authors upon request. For pyrethroids as a group, no evidence of an association was reported. For individual pyrethroid pesticides for which the OR was ≤ 0.5 or ≥ 2.0 or for which the confidence interval excluded 1.0, only two observed associations met this criteria;[70] evidence of a moderately strong association was reported between lambda-cyhalothrin and atrial septal defect secundum (OR: 2.90; 95% CI: 1.10, 7.90, n = 6 exposed cases/126 nonexposed cases, 15 exposed controls/770 nonexposed controls) While not statistically significant, an OR of 2.60 was observed and reported between cyfluthrin and atrial septal defect secundum (OR: 2.60; 95% CI: 0.90, 7.10 with n = 6 exposed cases/126 nonexposed cases, 13 exposed controls/772 nonexposed controls).[71]

The overall quality of the study was ranked moderate. Strengths of the study included the population-based case-control design, the assessment of residential pesticide exposure that was "spatially and temporally specific" and targeted at the relevant embryonic time period. The investigators also conducted maternal interviews to confirm maternal residence during the entire relevant exposure window during pregnancy (and not just address at delivery) and accounted for it in an appropriate way. Additionally, study strengths included the well-defined birth defect outcomes obtained from a birth defect registry. Limitations in the exposure assessment included

[68] Off-site transport of pesticides can occur in a variety of ways. These include airborne drift of aerosols and dust particles from spray applications (spray drift); post-application volatilization drift from evaporation from leave and soil surfaces for volatile and semi-volatile pesticides; and from incidental oral exposure from contaminated house dust.

[69] The following potential confounders were considered in this study but were not included within the model: maternal education, pre-pregnancy body mass index, use of folic acid-containing supplements, smoking, alcohol consumption, parity, and plurality.

[70] The authors report that limiting reporting to only those cases which were statistically significant (*i.e.,* odds ratios that excluded 1.0) or for which odds ratios were less than or equal to 0.5 or greater than or equal to 2.0 was done to "guard against multiple testing error". Such a practice does not protect one against the multiple comparisons (not "multiple testing") issue and, further, leads to issues associated with effect size magnification when power is low as is likely here. Given that the number of exposed cases were only 6 for both the lambda cyhalothrin and cyfluthrin estimates, such low power and the resultant effect size magnification issue also likely biases these results away from the null and produce inflated odds ratios. See Ioannidis, J. P. A. 2008. Why most discovered true associations are inflated. Epidemiology 19: 640-648. [accessed 24 February 2019 at http://www.dcscience.net/ioannidis-associations-2008.pdf].

[71] The study authors mentioned that specific congenital heart defect AORs were reported for individual pesticides that observed at least 5 exposed cases and controls (in addition to 1.) having a confidence interval not including 1.0 and 2.) the odds ratio is ≤ 0.5 or ≥ 2.0. For pyrethroids, AORs were reported for atrial septal defect, but AORs were not provided for other congenital heart defects including heterotaxia, Tetralogy of Fallot, hypoplastic left heart syndrome, coarctation of the aorta, pulmonary valve stenosis, and ventricular septal defect perimembranous due to the practice of only reporting effects that passed a given threshold.

the fact that using only the 500 m distance from an application as a criterion to determine if exposure occurred is only an indirect measure of exposure for which little evidence or support exists and does not consider a variety of other factors that would determine the likelihood and degree of exposure such as chemical half-lives and vapor pressures, wind patterns, time spent at home, or other potential sources of exposure such as household uses or occupation. Too, pyrethroids in general have low vapor pressure, low volatility, and short half-lives in the environment, further limiting the ability of a 500 m zone to accurately serve as a surrogate for exposure to pyrethroids. In addition, the authors investigated many birth defects, none of which were corrected for multiple comparisons which would lead to an elevated false discovery rate. Finally, the reporting of only results which passed some statistical (*e.g.,* $p < 0.05$) or overall magnitude (*e.g.,* OR < 0.5 or > 2) will lead to a recognized issue of effect size inflation whereby reported results will likely be inflated (and to a potentially sizable degree). Lastly, it should be noted that a relatively small number of exposed cases and controls (n < 20) were observed for cyfluthrin and lambda-cyfluthrin relative to the select types of congenital heart defects.

- Carmichael et al. (2016) conducted a population-based case-control study to evaluate the relationship between exposure to the pyrethroid cyfluthrin (among many other pesticides) during the peri-conception period and risk of birth defects among infants born to women whose residence at the time of delivery was one of eight counties in the San Joaquin Valley of California. The study design and conduct are the same as that described above in Carmichael (2014). Briefly, the study population consisted of cases (n = 367) with any of the five types of birth defects (anotia/microtia, anorectal atresia/stenosis, transverse limb deficiency, craniosynostosis, and diaphragmatic hernia[72]) and randomly selected non-malformed live-born infant controls (n = 785) identified from birth hospitals. Study subjects had dates of delivery between October 1997 and December 2006. Birth defect cases were identified from all hospitals with obstetric or pediatric services, cytogenetic laboratories, and all clinical genetics prenatal and postnatal outpatient services in the region as part of the NBDPS. Birth defects experts reviewed the abstracted medical records of potential cases to ensure that inclusion criteria were met; potential cases with recognized or strongly suspected single-gene disorders, chromosomal aneuploidy, or identifiable syndromes were excluded from the study. Controls were selected such that the control distribution by hospital was proportional to the underlying birth population. Maternal interviews were conducted via telephone six weeks to 24 months following pregnancy, and these interviews were used to assess history of residence during three months before conception and throughout pregnancy. Interviews were conducted with mothers of 72% of eligible cases (n = 480) and 69% of eligible controls (n = 974), with median time to interview of 11 months for cases and 6 months for controls. Poorly managed Type I or Type II diabetes is associated with increased risk of many birth defects so mothers of cases (n = 18) and controls (n = 7) with these conditions were excluded from analyses. The investigators estimated pesticide exposure for the period from one month before, to two months after, each mother's reported date of conception by geocoding subjects' residences and linking residence with the California Department of Pesticide Regulation PUR data, for pesticides applied (in a quantity > 100 lbs) in any of the eight San Joaquin Valley counties in any year during the study period. Except for limb deficiency defects and craniosynostosis for which the developmental time period may extend beyond the first two months following conception, this time period over which exposure was assessed covers the time during which these remaining birth defects would have developed. Pesticide use, including pyrethroids, within a 500 m radius of the mother's address during the

[72] Specifically, these represent: anotia/microtia = reduction or absence of the external portion of the ear and atretic ear canal; anorectal atresia/stenosis = absence, closure, or constriction of the rectum or anus; transverse limb deficiency = limbs with absent distal segments and intact proximal structures; craniosynostosis = premature fusion of one or more cranial sutures; diaphragmatic hernia = an opening in the diaphragm through which a portion of the abdominal contents protrudes into the thoracic cavity

periconceptual period was classified as any use versus no use and it was found that 46% of mothers of cases and 38% of mothers of controls lived within this 500 m zone of pesticide applications during the periconceptual period. The investigators estimated ORs and corresponding 95% CIs from multivariable logistic regression models for each birth defect, adjusting for race/ethnicity (non-Hispanic white, US-born Hispanic, foreign-born Hispanic, and other), education (less than high school, high school, more than high school), and maternal age at delivery (years, as continuous).[73] Case mothers were determined to be more likely foreign-born Hispanic, to be older than 30 years, and to have lower education compared with control mothers. Other potential confounders were evaluated but not included in final models. The investigators estimated associations for 85 groups and 95 chemicals with five or more exposed case mothers and five or more exposed control mothers. Based on this approach, the authors only reported results for which the OR was ≤ 0.5 or ≥ 2.0 or for which the confidence interval excluded 1.0. For pyrethroids as a group, no evidence of an association was reported. For individual pyrethroid pesticides, evidence of strong association was reported between cyfluthrin and craniosynostosis (birth defect) among offspring (OR: 4.60; 95% CI: 1.50, 14.0 with n = 5 exposed cases/74 non-exposed cases, 13 exposed controls/766 non-exposed controls).

The overall quality of the study was ranked moderate. Study strengths included the case-control study design, the close tracking of material address through pregnancy, and the well-defined birth defect outcomes obtained from a birth defect registry. The investigators also conducted maternal interviews to confirm maternal residence during pregnancy. Limitations in the exposure assessment included the fact that using only the 500 m distance from an application as a criterion to determine if exposure occurred is only an indirect measure of exposure for which little evidence or support exists and does not consider a variety of other factors that would determine the likelihood and degree of exposure such as chemical half-lives and vapor pressures, wind patterns, time spent at home, or other potential sources of exposure such as household uses or occupation. In addition, a large number of statistical comparisons and no multiple comparison for false discovery rate corrections were applied. Additionally, a small number of exposed cases and controls were observed for cyfluthrin (n = 5 exposed cases and 13 exposed controls), generating an imprecise odds ratio and one that is likely to be artificially inflated.[74]

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids and pyrethroid metabolites and birth defects in children. Three studies evaluated the potential association between maternal exposure to pyrethroids and different birth defects in children. Meyer et al. (2006) reported no evidence of a significant positive association between bifenthrin exposure and the birth defect hypospadias. The two remaining studies – Carmichael et al. (2014) and Carmichael et al. (2016) – were conducted in California using similar methods and data sources to identify birth defect cases and ascertain pesticide exposure. Carmichael et al. (2014) focused on eight congenital heart defects and examined associations with pyrethroids, both considered as a group and then also as individual pyrethroids. The study reported no evidence for pyrethroids as a group and the eight congenital heart defects but reported evidence of a moderately strong association between lambda-

[73] The following covariates were considered in this study; however, no substantial associations were reported for each: education, age, maternal race/ethnicity, smoking, drinking, plurality, parity, pre-pregnancy body mass index, and use of folic acid-containing supplements. However, due to known variability in prevalence of sociodemographic variables, the study adjusted for the mentioned covariates above.

[74] Given that the number of exposed cases were only 5, such low power and the resultant effect size magnification issue likely biases these results away from the null and produced inflated odds ratios. See Ioannidis, J. P. A. 2008. Why most discovered true associations are inflated. Epidemiology 19: 640-648. [accessed 24 February 2019 at http://www.dcscience.net/ioannidis-associations-2008.pdf]

cyhalothrin and atrial septal defect secundum and no evidence of a significant positive association between cyfluthrin and atrial septal defect secundum. Carmichael et al. (2016) focused on five other birth defects and examined specific pesticides with five or more exposed cases. As with Carmichael et al. (2014), the study reported no evidence for pyrethroids as a group but reported evidence of a strong association between cyfluthrin and craniosynostosis. The findings of both Carmichael et al. (2014) and (2016), particularly with respect to individual pyrethroids, should be interpreted with caution because both studies performed a large number of statistical comparisons and no multiple comparison for false discovery rate corrections were applied. In addition, both studies were small (n = 5 or n = 6 exposed cases) and would likely be subject to effect size magnification, reporting only those effect sizes that were < 0.5 or > 2 or those that were statistically significant). The authors of both studies highlight this limitation in their discussion and indicates that studies were "hypothesis-generating" in nature.

7.2.4 Thyroid Hormonal Levels in Neonates

One study investigated the association between maternal exposure to pyrethroid metabolites and thyroid hormone levels in neonates (Zhang et al., 2014).

As previously summarized, Zhang et al. (2014) conducted a prospective cohort study to evaluate the association between maternal exposure to pyrethroid metabolites and thyroid hormone levels along with birth sizes in neonates. The results for thyroid hormone levels in neonates are presented here, and the results for birth sizes are reported earlier in this memorandum under the gestational size endpoint. Study subjects included pregnant women in the first trimester of gestation during 2009 - 2011 who lived in Tokyo, were 20 - 50 years old, and who did not have any illnesses pertaining to thyroid function. A total of 424 women were originally approached to participate in the study, with 315 women in their first trimester of gestation agreeing to participate. Blood and urine samples were available from 231 of these women and – of these 231 – 37 left the cohort by the time of delivery: 7 due to spontaneous abortion, 12 due to change of hospital, 8 due to multiple pregnancy, and 10 due to refusal for other reasons. The remaining 47 were not included due to the failure to sample blood from the baby, leaving a total of 147 mother-neonate pairs that were included within this study of the original 424 women that were approached (35%). For the thyroid hormone analysis, concentrations of the TSH and free thyroxine (fT4) were measured from maternal and neonatal blood samples using electrochemiluminescence immunoassay (ECLIA) and enzyme-linked immunosorbent assay (ELISA) methods, respectively. There were no statistically significant differences between the mothers that were the subjects of the study, mothers that dropped out of from the original cohort (n = 37) and mothers whose neonates blood was not sampled (n = 47), with $p < 0.05$. In addition, there was no difference in birth size between the neonates that were part of the study from whom blood was not sampled. Maternal exposure to pyrethroids was assessed using a spot urine sample that was collected during pregnancy (11 – 14 weeks of gestation) and measured for the pyrethroid metabolite 3-PBA. Concentrations of the pyrethroid metabolite 3-PBA in the maternal urine samples were measured using HPLC-MS, which achieved a SG-adjusted[75] LOD of 0.02 ng/mL. Individual metabolite levels at less than this level were assigned a value of ½LOD in the statistical analysis, and 97.8% of the measurements were above the LOD (226 of the 231 subjects). Median and 75th percentile SG-adjusted 3-PBA concentrations were 0.380 and 0.793 ng/mL, respectively. A maternal blood sample was also taken by hospital staff the same day for isolation of the serum for the thyroid hormone analysis (consisting of TSH and fT4). Neonatal blood samples were taken at about the 5th day postpartum by a heel prick. Maternal serum and urine and the filter paper with neonatal blood spots were stored frozen at -20°C until analysis. All neonatal fT4 were within the normal range (> 0.9 ng/dl) and all but one neonatal TSH value were within normal range (9 µIU/mL). Quality control procedures were carried out and included the use of procedural blanks as well as a quality control blank in each group of measurements. A multivariable linear regression with stepwise regression was used with neonatal fT4 and log-transformed neonatal TSH concentrations as the dependent variables and list of covariate candidates

[75] To account for urine dilution effects, concentration corrections were made using the SG method, adjusting to a SG of 1.020.

including log-transformed maternal urinary 3-PBA, log-transformed maternal urinary iodine, log-transformed maternal TSH, maternal age, maternal pregnancy BMI; parity (primiparous/multiparous), gestational weeks, cigarette smoke exposure (yes/no); drinking alcohol during pregnancy (yes/no); food frequency (every meal/not every meal), and neonatal sex (male/female) with $P_{enter} = 0.05$ and $P_{leave} = 0.1$ as stepwise variable selection setting. None of the independent variables, including log-transformed maternal urinary 3-PBA, were significant in the automated stepwise regression analyses. Besides multivariable linear regression analyses, the authors also performed ANOVA analyses to compare neonatal fT4 and log-transformed neonatal TSH concentrations between the quartiles of maternal urinary 3-PBA concentration and reported no significant differences in neonatal fT4 and log-transformed neonatal TSH concentrations between quartiles of maternal urinary 3-PBA concentration (neonatal fT4 p-value = 0.090, log-transformed neonatal TSH concentrations p-value = 0.693).

The quality of the study was ranked low. The prospective study design was a study strength as well as the quality control procedures utilized for urine sampling. While the study had several strengths, the statistical methods used in the study relied on automated stepwise selection to identify covariates in their statistical model. This approach is generally considered appropriate only for studies conducting exploratory analysis for purposes of hypothesis generation; purported statistical significance arising from studies that use this technique is not valid and cannot be relied upon to suggest causal relationships.[76] In addition to limitations in their statistical methods, the study obtained only a single urine sample to determine the pyrethroid metabolite concentrations over time. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between exposure to pyrethroid metabolites and thyroid hormone levels in neonates. One low quality study, Zhang et al. (2014), reported no evidence of a significant positive association between maternal 3-PBA and thyroid hormone levels in neonates. Although Zhang et al. (2014) had a strong study design, it is unclear if measurement of pyrethroid metabolite levels in spot-urine samples provides a reliable estimate of exposure during pregnancy. In addition, Zhang et al. (2014) also used stepwise regression in their statistical analysis. This variable selection method is considered unreliable and can result in biased parameter estimates with 95% confidence intervals that are too narrow due to underestimation of standard errors.

7.3 Male Reproductive Effects

7.3.1 Reproductive Hormone Level Effects

Four studies investigated the association between exposure to pyrethroid metabolites and reproductive hormone levels in men (Han et al., 2008; Meeker et al., 2009; Radwan et al., 2014; Yoshinaga et al., 2014).

- Han et al. (2008) conducted a cross-sectional study to evaluate associations between urinary levels of the pyrethroid metabolite 3-PBA and serum reproductive hormone levels among a study population of adult Chinese men diagnosed with unexplained infertility (n = 212) and with no history of occupational pesticide exposure. The study population was sampled from affiliated

[76] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. Psychosomatic Med. 66:411-42.

hospitals of Nanjing Medical University, and eligible men identified between March 2004 and March 2006 were recruited to participate in the study. Men taking hormonal medications, those with known causes of male endocrine dysfunction, and those with occupational pesticide exposure were excluded from the study. A single spot urine sample and a single venous blood sample were obtained from each participant on the same day. Gas chromatography-mass spectroscopic methods were used to quantify 3-PBA levels in the urine samples adjusted for urinary creatinine concentration. Urine samples from 10 subjects with creatinine concentrations above 300 mg/dl (considered too concentrated) or below 30 mg/dl (considered too dilute) were excluded from primary analysis, as recommended by the NIOSH Manual of Analytical Method, 4th Edition.[77] All subjects who provided urine and blood samples (n = 199) had detectable levels of urinary 3-PBA ranging from 0.162 μg/g to 21.46 μg/g, with the median concentration of 3-PBA as 0.815 μg/g creatinine pyrethroid metabolites. Levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), testosterone (T), and prolactin (PRL) were quantified by radioimmunoassay in serum from blood samples collected from each participant. In addition to these biomarkers, a detailed physical examination was performed on each subject, and a questionnaire was administered to collect information on demographics, lifestyle factors, occupational and environmental exposures, genetic risk factors, sexual and procreate state, medical history, and physical activity. Multiple linear regression models were used to estimate associations between log-transformed serum hormone concentrations and urinary 3-PBA levels. Age and BMI were included as covariates given previous studies demonstrating a relationship between these two variables and hormone level, with all other possible covariates among smoking status (current and former versus never), passive smoking (1 h/d, 0.5–1 h/d, < 0.5 h/d, 0 h/d), drinking status (> 3 times per week, 1–3 times per week, former drinking, and never drinking), history of operation (yes/no), medicine intake (yes or no), psychologic tension (most nervous, more nervous, normal, relaxed), and work type (daily relay, night shift, inverse shift) selected as covariates using backward stepwise linear regression (p-stay: 0.05). The final regression models included the following covariates: age, body mass index, drinking status, passive smoking, regular medicine intake, ever history of (surgical) operation, as well as psychologic tension. Evidence of a statistically significant, positive association was observed for creatinine-adjusted 3-PBA levels and (log-transformed) serum LH (β-coefficient: 0.209; 95% CI: 0.045, 0.373; p = 0.013), and a statistically significant, negative (inverse) association was observed between (log-transformed) E2 and 3-PBA level (β-coefficient: -0.274; 95% CI: -0.508, -0.041; p = 0.022). No evidence of a statistically significant association was observed between log-transformed values of FSH, T, and PRL and creatinine-adjusted 3-PBA concentrations (FSH β-coefficient: -0.066 95% CI: -0.217, 0.086; T β-coefficient: -0.010; 95% CI: -0.819, 0.062; PRL β-coefficient: 0.048; 95% CI: -0.099, 0.195).

The overall quality of the study was ranked low. Study limitations included the cross-sectional study design and use of a single urine sample and a single blood sample to quantify pesticide exposure and serum hormone levels, respectively. Since the pesticide exposure marker and the hormone levels were measured contemporaneously in this cross-sectional study, the associations observed do not provide direct evidence of a temporal relationship between pesticide exposure and hormone levels, and this approach may not accurately reflect longitudinal or longer-term exposure patterns. Further, this also may simply be reflective of exposure to pre-formed non-toxic, environmental degradates/metabolites that may be present in the environment and food. Finally, the automated stepwise regression approach is generally considered appropriate only for

[77] Eller, P. M. (1994). *NIOSH manual of analytical methods* (Vol. 94). Diane Publishing.

conducting exploratory analysis for purposes of hypothesis generation, and purported statistical significance arising from studies that use this technique is not valid and cannot be relied upon.[78]

- Meeker et al. (2009) conducted a cross-sectional study to evaluate the association between pyrethroid exposures and effects in serum thyroid and reproductive hormone levels in 161 men. Study participants consisted of men aged 18 to 54 years of age who were recruited from the andrology lab located at Massachusetts General Hospital from April 2000 through April 2003. Approximately 65% of eligible men agreed to participate, and an anonymized retrospective review of clinic records showed that there were no differences between participants and non-participants with respect to age or semen parameters. Exclusion criteria included men with prior vasectomy or those currently using exogenous hormones. A brief questionnaire regarding health information was administered by a nurse, and height and weight were measured. A single urine sample was collected to determine by HPLC-MS/MS specific gravity-adjusted concentrations of the following pyrethroid metabolites: 3-PBA, *cis*-DCCA, and *trans*-DCCA. The median SG-adjusted concentrations for 3-PBA, *cis*-DCCA, and *trans*-DCCA were 0.15, 0.16 and 0.11 ng/mL, respectively. The 75th and 90th percentile SG-adjusted concentrations for 3-PBA, *cis*-DCCA and *trans*-DCCA were 0.47 and 1.31 ng/mL, 0.30 and 0.60 ng/mL, and 0.38 and 1.35 ng/mL, respectively, with 95th percentile respective concentrations of 2.68, 1.64, and 4.07 ng/mL. Forty-six percent of 3-PBA, 47% of *cis*-DCCA, and 49% of *trans*-DCCA were below the detection limit of 0.1 µg/L for all metabolites. Additionally, a non-fasting blood sample was collected at this time and serum samples were used to measure the following reproductive and thyroid hormone levels: FSH, LH, inhibin B, testosterone, sex hormone binding globulin (SHBG), free androgen index (FAI), estradiol, prolactin, free T4, T:LH ratio, total T3, and TSH. A multivariable linear regression model was used to determine if an association was present between pyrethroid exposures and specific reproductive hormone levels using regression coefficients and 95% CIs and adjusting for BMI, age, smoking (current vs never/former) and time of day (morning vs. afternoon) at blood draw. Serum concentrations of testosterone, estradiol, inhibin B, free T4, and total T3 were approximately normally distributed and not transformed, but distributions of FSH, LH, SHBG, FAI, prolactin and TSH were skewed left and log-transformed based on statistical and biological considerations. Due to the high proportion of samples that were below the LOD, categories of metabolite exposure concentrations were created as per the following percentiles: < 50th (control), 50th – 75th, and > 75th percentile. Among the total number of men (n = 161), evidence of a significant exposure-response trend was observed with increasing levels of FSH following exposure to 3-PBA, *cis*-DCCA, and *trans*-DCCA (p-trend = 0.002; p-trend = 0.02; p-trend = 0.02, respectively). Additionally, evidence of a significant exposure-respond trend was observed for LH, inhibin B,[79] and T:LH ratio relative to *cis*-DCCA (p-trend = 0.01; p-trend = 0.03; p-trend = 0.04), and evidence of a positive exposure-response trend for inhibin B relative to *trans*-DCCA was observed (p-trend = 0.02).

  The overall quality of the study was ranked low. The main limitations of the study were the cross-sectional design which does not ensure that the exposure predates the outcome, the single spot urine sample collected, and the lack of ancillary information regarding the history and/or recent pyrethroid usage within the questionnaire data. A single urine sample may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. Finally, there were multiple comparisons made (120 regression coefficients comparing

[78] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. *Psychosomatic Med.* 66:411-421.

[79] In this study, Inhibin B (a specific reproductive hormone) was further adjusted for season in the statistical analysis.

effects of five different measures of pyrethroid metabolite exposure at various quantiles with 12 outcome measures related to hormone levels) and no indication that the confidence intervals or p-values were adjusted for these multiple comparisons or false discovery rates.

- Radwan et al. (2014) conducted a cross-sectional study to evaluate association between pyrethroid exposures and semen quality and reproductive hormones among non-farmers in Poland. The results for reproductive hormones are presented here, and the results for semen quality in **Section 7.3.2**. Briefly, study subjects included men (n = 344) aged < 45 years of age who had visited an infertility clinic in Lodz, Poland between 2008 and 2011 for diagnostic purposes, and who had a normal semen concentration.[80] Fifty-nine percent of the men agreed to participate. Exposure was assessed using a questionnaire which collected information on demographics, lifestyle factors, and medical history during an interview in addition to collecting blood, urine and semen. A total of 334 (of the 344 enrolled, or 97.1%) men provided sufficient samples to be included in the study. Urine samples were measured for the following pyrethroid metabolites using GC-MS: *cis*-DCCA, *trans*-DCCA, 3-PBA and DBCA. The limit of detection (LOD) for all metabolites was 0.1 ng/mL and proportions of samples below LOD for 3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA were 28.4%, 42.0%, 34.5%, and 83.2%, respectively. Geometric mean concentrations for 3-PBA, *cis*-DCCA, *trans*-DCCA and DBCA were 0.17, 0.11, 0.15, and 0.05 µg/L, respectively; and 50th, 75th, and 95th percentile concentrations for 3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA were, respectively, as follows: 0.16, 0.24, and 0.51 µg/L; 0.13, 0.17, and 0.44 µg/L; 0.15, 0.23, and 0.60 µg/L; and 0.05, 0.05 and 0.26 µg/L Creatinine-adjusted pyrethroid metabolites levels in urine were reported as ≤ 50th percentile level or > 50th percentile level for all the pyrethroid metabolites except DBCA which were reported as ≤ LOD or > LOD due to the high proportion of samples that were below the LOD. In addition, the sum of pyrethroids was presented and consists of specific metabolites – *cis*-DCCA + *trans*-DCCA + DBCA. Plasma samples were assessed for the following three reproductive hormones: FSH, estradiol, and testosterone. Internal quality control procedures were performed to ensure precision of daily assessments of semen sample concentrations, in addition to external quality control measures. Multiple linear regression models were run to evaluate the associations between pyrethroids (log-transformed) and reproductive hormones using regression coefficients and 95% CIs while adjusting for age (years), abstinence (days), smoking (yes/no), alcohol consumption (none or < 1 drink/week; 1 to 3 drinks/week; and 4 to 7 drinks/week), and past diseases (yes/no). Evidence of a significant negative association was observed for *trans*-DCCA at the > 50th metabolite level in the urine and the level of testosterone hormone (β = -0.52; 95% CI: -0.99, -0.03, p = 0.04). No evidence of a significant association was observed for *cis*-DCCA and 3-PBA relative to any reproductive hormones tested (FSH, estradiol, testosterone) as all CIs encompassed the null value of 0, and p-trend were ≥ 0.05.

  The overall quality of the study was ranked low. Strengths included the quality control procedures incorporated as part of the methods as well as the fact that smoking status was confirmed by analysis of cotinine. Additionally, laboratory QA/QC procedures were reported. Study limitations included the cross-sectional study design, and the single spot urine sample collected for the duration of the study. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. Further, a substantial fraction of the concentrations were < LOD, which led to an apparent dichotomization of all results into ≤ 50th percentile and > 50th percentile. With respect to the statistics, we note that 30 different comparisons were performed with no multiple comparisons or false discovery rate corrections done which increases the likelihood of spurious findings. We note, too, our confusion

[80] In this study, normal semen quality was defined as 20 – 300 mln/mL.

with respect to the statistics that were performed on the data: specifically, the authors did not make clear what the regression coefficient represent and appear to have developed coefficient for what should be baseline (dummy) comparisons (here, the $\leq 50^{th}$ percentile) when none should exist.

- Yoshinaga et al. (2014) conducted a cross-sectional study to evaluate the relationship between pyrethroid exposure and serum concentrations of reproductive hormones in male university students. The study utilized data from a larger cross-sectional, multi-center study of Japanese men aged 18 to 24 years, who were university students from four cities in Japan. The sub-population for this current study included subjects recruited from the western part of the Tokyo metropolitan area between April 2002 and May 2003 (n = 322). Study participants completed a self-administered questionnaire about lifestyle information and diet, provided semen, serum, and urine samples and underwent a physical examination by urologists whose examination and measurements were cross-validated. Exposure was determined by analysis of urine samples for the pyrethroid metabolite 3-PBA. Urinary 3-PBA concentration was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and dilution effect was corrected by specific gravity of urine sample. Concentrations of reproductive hormones - LH, FSH, SHBG, T, and inhibin B were measured in serum samples using time-resolved immunofluorometric and two-sided enzyme immunometric (for inhibin B) assays. A total of 294 subjects (91%) had urinary 3-PBA concentrations above the detection limit of 0.07 ng/mL. Stepwise multiple regression was used to obtain β coefficients and p-values for log-transformed serum hormone concentrations as a function of log-transformed 3-PBA concentration for each outcome, adjusting for potential confounders age, BMI, presence/absence of varicocele, season of blood sampling, smoking, and frequency of soy product consumption. No significant associations between pyrethroid exposure as measured by urinary 3-PBA and the reproductive hormone levels LH, FSH, SHBG, T, and Inhibin B in men aged 18 to 24 years were reported based on variables selected by stepwise multiple regression (P_{in} and P_{out} set at p = 0.05 and 0.1), respectively.

  The overall quality of the study was ranked low. The primary limitations of the study were the cross-sectional design, the lack of ancillary information regarding the history and/or recent pyrethroid usage within the questionnaire data, and inability to assess the temporal associations between pyrethroid exposure and hormone levels. The study also assessed exposure by measuring 3-PBA levels in a single spot urine sample collected from each study participant. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. Finally, the study relied on stepwise selection when performing multiple regression analysis of the association between hormone levels and urinary 3-PBA. This variable selection method is considered unreliable and can result in biased parameter estimates with 95% confidence intervals that are too narrow due to underestimation of standard errors.[81]

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and male reproductive hormone levels. The four available studies assessed exposure by measuring pyrethroid urinary metabolites and considered a wide range of different hormones. All of the studies relied on cross-sectional designs and were not able to assess the temporal association between pyrethroid exposure and hormone levels, especially since the

[81] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. Psychosomatic Med. 66:411-42.

pyrethroid metabolites have a relatively short biological half-life and likely represent only a short time period of exposure. The studies were also exploratory in nature and evaluated a large number of associations between different urinary metabolites and a number of different hormone measurements. In addition, the findings were mixed across the four studies with no single pyrethroid metabolite-hormone association or relationship duplicated in any second study. For example, Meeker et al. (2009) found FSH statistically elevated for all pyrethroid metabolites investigated in that study (3-PBA, *cis*-DCCA, *trans*-DCCA, *cis*- + *trans*-DCCA, and sum pyrethroids), but Radwan et al. (2014) – looking for many of the same metabolites – found this for none. Similarly, Han et al. (2008) and Yoshinaga et al (2014) failed to find a significant association between FSH and the 3-PBA pyrethroid metabolite they investigated. The findings across the four studies are summarized in the **Table 5** below.

Table 5: Summary of reported findings on the association between pyrethroid exposure and male hormone levels.

Study Authors	Study Design [1]	Exposure Metric	Male Hormone Levels							
			FSH	*LH*	*E2*	*T*	*PRL*	*Inhibin B*	*SHBG*	*FAI (T:SHBG)*
Han et al. (2008)	CS	3-PBA	⦸	↑●	↓●	⦸	⦸			
Meeker et al. (2009)	CS	3-PBA	↑●	⦸	⦸	⦸	⦸	⦸	⦸	⦸
		cis-DCCA	↑●	↑●	⦸	⦸	⦸	↓●	⦸	⦸
		trans-DCCA	↑●	⦸	⦸	⦸	⦸	↓●	⦸	↓●
		cis- + *trans*-DCCA	↑●	↑●	⦸	⦸	⦸	⦸	⦸	⦸
		Sum Pyrethroids	↑●	⦸	⦸	↓●	⦸	⦸	⦸	↓●
Radwan et al. (2014)	CS	3-PBA	⦸		⦸	⦸				
		cis-DCCA	⦸		⦸	⦸				
		trans-DCCA	⦸		⦸	↓●				
		DBCA	⦸		⦸	⦸				
		Sum Pyrethroids	⦸		⦸	⦸				
Yoshinaga et al. (2014)	CS	3-PBA	⦸	⦸		⦸		⦸	⦸	

Legend:
⦸ - No evidence of an association ($p > 0.05$).
● - Evidence of a significant association ($p < 0.05$).
↑ - Positive association. ↓ - Negative association.
[1.] Study Design –*CS* = Cross-Sectional

7.3.2 Semen Quality

Six studies evaluated the association between exposure to pyrethroids and semen quality, including one study that specifically assessed the pyrethroid fenvalerate (Lifeng et al., 2006) and five studies focused on pyrethroid urinary metabolites (Meeker et al., 2008; Xia et al., 2008; Ji et al., 2011; Imai et al., 2014; Radwan et al., 2014). These study results are provided below:

- Lifeng et al. (2006) conducted a cross-sectional study of male workers in a pesticide factory located in a suburban area of southeast China. A total of 100 workers, age 21-42 years, participated in the study. Thirty-two of the participants were fenvalerate exposed in the production area of the pesticide manufacturing facility, 46 were from office areas of that same facility and were considered to be a non-exposed, internal comparison group, and 22 were officers in a CDC facility in an urban distribution of the same city and served as the external control group. All participants provided semen samples and completed a questionnaire during a face-to-face interview that included questions regarding occupational history and lifestyle factors. On three consecutive days, air samples were collected from the fenvalerate production area, the

factory office, and the CDC office. Concentrations of fenvalerate in these air samples were quantified by vapor phase chromatography. The geometric mean (range; n) air concentration for the exposure group was 21.55 x 10^{-4} mg/m3 (6.76 x 10^{-4} to 797.54 x 10^{-4} mg/m3; n = 18), for the internal control group was 1.19 x 10^{-4} mg/m3 (1.13 x 10^{-4} to 1.58 x 10^{-4}; n = 9). Fenvalerate was not detected in the external control air. Individual personal air and dermal samples were also collected from three randomly selected participants in both the exposed and external comparison groups for three consecutive days; dermal contamination was measured by attaching fibrous filter membranes to 10 unspecified body areas. Semen samples were obtained from participants after 3 days of recommended sexual abstinence, and semen indices (liquefaction time, pH value, viscidity, sperm volume, sperm motility, percent motile sperm, sperm density, and sperm count per ejaculum) were quantified using World Health Organization (WHO) guidelines (2010). Sperm motility parameters included curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), beat cross frequency (BCF), straightness (STR), and linearity (LIN) were analyzed using the HST computer-assisted sperm motility analysis (CASA) system. Continuous semen parameters were analyzed using one-way ANOVA with (unspecified) post hoc analysis, and data were presented as means ± SD or geometric means and ranges. The proportion of participants with "abnormal" semen parameters in each of the exposure groups were also presented, though the statistical test used to compare these proportions was not specified. When semen parameters in the fenvalerate exposure group were compared to the internal and external control groups, evidence of a statistically significant decrease in mean progression grade ($p < 0.05$ compared with external control, but no significant difference between internal control) and geometric mean sperm count[82] ($p < 0.05$ compared with external and internal controls) were observed. No other differences between any of the three groups were seen in volume, pH, motility, or sperm concentration. Additionally, there was evidence of a statistically significant increase in the proportions of exposed participants with abnormal viscidity, progression, coagulation, and sperm count relative to at least one of the "unexposed" groups (viscidity: $p < 0.01$ compared to external control, $p < 0.05$ n = 9 compared with internal control group; progression: $p < 0.05$ n = 15 compared with external control group; coagulation: $p < 0.05$ n = 13 compared with internal control group, $p < 0.05$ compared with external control group; sperm count: $p < 0.05$ n = 12 compared with internal control group, $p < 0.05$ compared with external control group). No evidence of a statistically significant increase or decrease relative to the proportions of participants with abnormal liquefying time, volume, pH, motility, concentration, and morphology was observed between groups ($p \geq 0.05$). However, when comparing sperm motility parameters between the exposure group and the control groups, statistically significant decreases in BCF ($p < 0.05$ compared with external control group), LIN ($p < 0.05$ compared to external and internal control groups), and STR ($p < 0.05$ when compared to external and internal control groups) were observed. Differences in other measures of sperm motility, VCL, VAP, and VSL were not statistically significant. Finally, no statistically significant differences were found in the abnormality rate of sperm morphology in each of the three exposure groups (exposure, internal control, and external control) in any of the abnormality measures that were evaluated (total, head, neck, tail, or mixed).

The overall quality of the study was ranked low. Strengths of the study included quantitative evaluation of the outcome (various sperm quality and motion parameters). However, major study limitations included the lack of temporality due to the cross-sectional study design, and no consideration of the potential effects on sperm quality based on the documented exposure of study participants to other chemicals with reproductive effects (specifically toluene and xylene). Although potentially of lesser concern given the occupational nature of the environment and a

[82] Sperm count is a derived quantity and represents the product of ejaculate volume (mL) and sperm concentration. Note that there were no significant differences between the volume and the concentration among any of the three groups.

reasonable presumption of regular and routine exposures, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis which would have occurred weeks or up to a couple months prior to the measured exposure period. Furthermore, additional study limitations included the lack of discussion and detail regarding the statistical analyses employed (including perhaps an assumption of a normal distribution required for ANOVA as well as no details with respect to the "post-hoc" testing adjustment claimed) and the lack of adjustment for potential confounders (*e.g.,* smoking status, alcohol consumption status, length of service, etc., although the study authors stated that "the populations were similar" in these regards) and no indication of whether differences in age between the groups was considered or evaluated. Finally, multiple comparisons were performed (not all of them independent) and there was no adjustment of the p-values or other considerations made to account for the large number of outcome measures which were evaluated.

- Meeker et al. (2008) conducted a cross-sectional study to evaluate the association between pyrethroid exposure and semen quality and sperm DNA damage among non-farmers within the US. The results for semen quality are presented here, and the results for sperm DNA damage are reported in **Section 7.3.3**. Study participants included men aged 18 to 54 years of age who were recruited from the andrology lab located at Massachusetts General Hospital during January 2000 to April 2003. Exclusion criteria included prior vasectomy, self-reported medical risk factors for infertility, or current use of exogenous hormones. Sixty-five percent of men agreed to participate, with the primary reason given for non-participation being lack of time. A retrospective analysis of anonymized clinic records of non-participants revealed no substantive differences among age or semen parameters between participants and non-participants. A single urine sample was collected to determine the following pyrethroid metabolites: 3-PBA, *cis*-DCCA and *trans*-DCCA and analyzed using HPLC-MS/MS, with an LOD of 0.1 µg/L for all three metabolites. SG was used to adjust urine samples for dilution. The 50th, 75th, and 95th percentiles for SG-adjusted 3-PBA were 0.14, 0.45, and 2.48 ng/mL; for *cis*-DCCA and *trans*-DCCA, corresponding concentrations were 0.14, 0.29, and 1.55 ng/mL and 0.12, 0.39, and 4.07 ng/mL, respectively. Semen samples were collected on the same day as the urine sample following 48 hours of abstinence and used to assess semen quality parameters including semen concentration, motility, and morphology in addition to sperm motion parameters (*i.e.,* VSL, VCL, and LIN).[83] Multiple linear regression was used to assess the potential association between categorized pyrethroid exposure as measured by pyrethroid metabolites in the urine (< 50th (reference), 50th – 75th, and > 75th percentile) and semen quality parameters (*i.e.,* concentration, motility, and morphology) and sperm motion parameters (*i.e.,* VSL, VCL, and LIN), adjusting for abstinence period and age.[84] Sperm concentrations were natural log-transformed in this regression. In addition to multiple linear regression, multiple logistic regression analysis was also performed on the relationship between the three urinary pyrethroid metabolite concentration categories and sperm motility and morphology in which subjects were categorized into either above or below the reference sperm concentration level set by the World Health Organization (1999)[85] at 20 million sperm/ml, by motility (50% motile sperm), and also by morphology (4% normal); reference subjects here were selected to be those that were above the reference level for all three parameters.

  In the semen quality parameters analysis (concentration, motility, and morphology) looking at single (unsummed) metabolites using multiple linear regression, only motility displayed a

[83] VSL, VCL, and LIN refer, respectively to straight line velocity; curvilinear velocity and linearity (defined as VSL/VCL)
[84] BMI, race, and smoking were additionally considered as covariates and excluded based on statistical or biological considerations
[85] World Health Organization (WHO). WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction. New York: Cambridge University Press, 1999.

statistically significant relationship and then only for *trans*-DCCA at the highest grouped concentration (*i.e.,* > 75th percentile coefficient = -9.96, 95% CI: -18.8, -1.06), with a p-trend of 0.06. Neither 3-PBA or *cis*-DCCA displayed statistically significant semen quality parameters and none showed statistically significant p-trends although 3-PBA did approach statistical significance for semen concentration (> 75th coefficient = -0.37, 95% CI: -0.78, 0.04) albeit with a non-significant p-for-trend of 0.14. For the motion parameters (VSL, VCL and LIN) analyzed by multiple linear regression, none of the nine comparisons performed showed statistical significance either with respect to their coefficient or as part of a p-trend calculation. When the same sperm quality parameters were dichotomized (concentration < 20 mln/ml; motility < 50%; morphology > 4% normal) and investigated by logistic regression, the only single metabolite comparisons that produced statistically significant odds ratios were for *trans*-DCCA for sperm concentration < 20 mln/ml (OR = 2.72, 95% CI: 1.07, 6.92; n = 16) with a p-trend of 0.06. We note the relatively small numbers of cases (n = 16) from which this odds ratio is derived. No statistically significant p-trends were observed for *trans*-DCCA using logit regression for semen motility and morphology, and none of the other 8 comparisons (3 metabolites *x* 3 quality parameters) were statistically significant or displayed significant p-trends. We note the relatively small numbers of cases (n = 16) from which this odds ratio is derived. More specifically, for *cis*-DCCA and 3-PBA, no evidence of a significant positive association was observed in any exposure tertile (0.38 < OR < 1.95; all CIs encompassed the null value of 1, n = 6 - 30) and no statistically significant p-trends were observed.

The overall quality of the study was ranked low. Strengths of the study included quantitative evaluation of measured exposure (urine concentration) and outcome (various sperm quality and motion parameters). The main limitation of the study was the cross-sectional study design, which does not ensure that the exposure predates the outcome. Other limitations included the lack of ancillary information regarding the history and/or recent pyrethroid usage within the questionnaire data and the single spot urine sample collected in the study, which may not accurately reflect longitudinal or longer-term exposure patterns as the exposure measurements may simply reflect exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. In addition, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis which would have occurred weeks or up to a couple months prior to the measured exposure period. Further, although semen sample collection and analysis seem to follow the WHO guidelines (2010), no information on standard laboratory QA/QC procedures (*e.g.,* blinded study design and replicate sample scoring) or results was provided to ensure interlaboratory variability was minimized. Finally, multiple comparisons were performed by the study authors and none of the confidence intervals or p-values of the several outcome measures across several exposure measures were adjusted.

- Xia et al. (2008) conducted a cross-sectional study to evaluate the relationship between exposure to pyrethroids and semen quality in Chinese men. Eligible participants included men diagnosed with unexplained male factor infertility and no known medical history of risk factors for infertility, from affiliated hospitals of the Nanjing Medical University between March 2004 and March 2006. Of those eligible, 92% consented to participate (n = 376). Participants completed a physical examination, a questionnaire about lifestyle factors, occupational and environmental exposures and medical history, and provided a single spot urine sample and semen sample on the same day. Exclusion criteria included a medical history of risk factors for infertility, abnormal sexual and ejaculatory functions, and men receiving treatment for infertility. All participants reported that their lifestyles and environments had not been changed in the several months before sample collection. Urinary concentrations of 3-PBA were detected by a sensitive and selective capillary gas chromatographic procedure with mass-spectrometric detection. Creatinine-corrected

concentrations of 3-PBA were found to follow a log-normal distribution with a geometric mean of 1.019 µg/g CR and 50th, 75th, and 95th percentile concentrations of 0.593, 1.501, and 4.301 µg/g CR, respectively. Calibration with pooled urine of known amount of 3-PBA, and quality control samples were analyzed in parallel with unknown samples in each analytical series. Creatine correction was used to account for urine dilution and samples with creatinine concentrations greater than 300 mg/dL or less than 30 mg/dL were excluded from analyses as being too concentrated or too dilute to provide valid results. Semen analysis was conducted in accordance to WHO 2010 guidelines and outcome measures included semen volume, sperm concentration, sperm number, motility, progression and motion parameters were determined using Micro-cell slide and computer-aided semen analysis (CASA). Nine sperm progression and motion parameters were determined for sperm tracks: beat cross frequency (BCF), amplitude of lateral head displacement (ALH), curvilinear velocity (VCL) as a measure of sperm vigor, average path velocity (VAP), straight line velocity (VSL) as a measure of sperm progression, linearity (LIN) equal to VSL/VCL x 100%, straightness (STR) equal to VSL/VAP x 100%, mean angular deviation (MAD), and wobble (WOB) equal to VAP/VCL, and all measured in um/s or as a fraction. Quality control measures were enforced throughout, each sample was detected twice consecutively, observation and counting were automatic, and backgrounds of samples were blinded to avoid bias. Concentrations of 3-PBA were used as both a continuous and also categorized into four quartiles according to interquartile range. Semen parameters were dichotomized based on WHO reference values. The comparison group were defined as men with all four semen parameters at or above the WHO reference value. Individuals whose values were more than the WHO reference value on the semen parameter of interest, but less than the WHO reference value on one, two, or both of the other three semen parameters, were excluded from the analysis of the semen parameter of interest. Forward stepwise multiple linear regression was used calculate adjusted ORs and 95% CIs for the associations between dichotomized semen parameters and CR-adjusted urinary 3-PBA quartiles, controlling for age, abstinence time, smoking, and alcohol consumption. Age and abstinence time were included in all multivariable models based on published evidence of an association with semen quality. Parameters not included for statistical and/or biological but considered were BMI and certain lifestyle factors. Scatterplots and multiple linear regression models were used to estimate associations between log-transformed semen parameters and urinary 3-PBA levels. Age, BMI, smoking status, drinking status, and abstinence time were included as covariates. For the analysis of the continuous semen parameters with continuous CR-adjusted 3-PBA levels, the CR-adjusted 3-PBA did not show any association with altered semen parameters (data not reported). Multivariate linear regression analyses for CASA motion parameters showed significant positive associations for VCL and VSL with increased CR-adjusted 3-PBA levels ($p = 0.039$ and $p = 0.003$), data not reported. Conditional logistic regression was also performed for which semen parameters were dichotomized. For the analysis of the relationship between dichotomized semen parameters and CR-adjusted urinary 3-PBA quartiles, men in the highest quartile of 3-PBA concentrations (1.501-21.279 µg/g of CR) was significantly associated with below reference sperm concentration (OR 2.04; 95% CI: 1.02, 4.09, $p < 0.05$, with $n = 36$ cases below-reference semen parameters), and the exposure-response trend was significant (p-trend = 0.027) with quartile 1 3-PBA concentration (0.100-0.593 µg/g of CR) as the referent. However, no other 3-PBA quartiles and sperm concentration were significant ($1.31 < OR < 1.73$; all 95% CIs encompassed the null value of 1.0, with $n = 27$-33 cases below-reference semen parameters). For the analysis of semen volume, the association between only quartile 3 3-PBA and semen volume was significant (OR = 3.62; 95% CI 1.04, 12.54, p-value < 0.05, with $n = 12$ cases below-reference semen parameters). No other associations between any quartile of 3-PBA and any other semen parameters were significant.

The overall quality of the study was ranked low. Strengths of the study included quantitative evaluation of measured exposure (urine concentration) and outcome (various sperm quality and motion parameters) and use of quality control procedures consistent with WHO guidelines (2010). The primary limitation of the study was that it relied on a cross-sectional design and was unable to assess the temporal associations between pyrethroid exposure and hormone levels. The study also assessed exposure by measuring 3-PBA levels in a single spot urine sample collected from each study participant. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. Additionally – and even though study participants indicated that there were no major lifestyle or environmental changes in the several prior months prior to study participation/sample collection – semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis which would have occurred weeks or up to a couple months prior to the measured exposure period. The study claims to have used conditional logistics regression methods, but it is not clear this is true: conditional logistic regression methods are often appropriate for matched data and this is not the case here.

- Ji et al. (2011) conducted a cross-sectional study to evaluate the association between pyrethroid exposures and semen quality and sperm DNA damage among 240 men in China. The results for semen quality are presented here, and the results for sperm DNA damage are reported later in this memorandum under a separate sperm damage and genetic abnormalities endpoints. Using data from the Nanjing Medical University (NJMU) Infertile Study, eligible men without any known infertility issues at the time, who were volunteers with the NJMU affiliated hospitals between April 2005 – March 2007 and recruited from an NJMU infertility clinic, participated in the study. Men with abnormal sexual and ejaculatory functions, immune infertility, semen non-liquefaction, medical history of risk factors for infertility, and receiving treatment for infertility were excluded from the study. Other exclusions included men with other known factors related to male infertility including genetic disease, infection, occupational exposure to PAHs or suspected other agents as well as subjects with Y-chromosome microdeletions for the azoospermia factor region. Study participants completed a questionnaire regarding past exposures, lifestyle factors, occupational and environmental exposures, medical history, genetic risk factors, sexual and reproduction status, and physical activity, and also underwent a physical examination and provided a single urine sample to measure the urinary concentration of the pyrethroid metabolite, 3-PBA. Semen samples were also collected on that same day, and semen quality parameters including sperm concentration, and motility, as well as total sperm count, and seminal volume were assessed. Analysis of the urine samples for 3-PBA was by GC-MS methods with a median concentration of 0.79 µg/g creatinine; no direct reporting was provided in the publication about LODs, but the article suggests that all were above detection limits with the following 3-PBA urinary concentrations (µg/g creatinine) quartiles reported, Q1: 0.19 – 0.53; Q2: 0.53 – 0.79, Q3:0.80 – 1.49, and Q4: 1.50 – 10.37. Linear regression was used to determine the potential association between pyrethroid exposure (as the 3-PBA metabolite) via urinary concentrations and semen quality parameters, adjusting for smoking (current and former vs. never), age, BMI, and abstinence time (0-3 days, 4-5 days, 6-7 days and ≥8 days). Some of the outcome variables (*e.g.,* sperm concentration, total sperm count) had skewed distributions and natural log transformations were used to determine if these resulted in improved model fit which was evaluated by residual analysis. The regression analysis was conducted in two ways, by first considering the 3-PBA metabolite concentrations as a continuous variable and then by stratifying the 3-PBA concentration data by the quartiles indicated above; natural log transformation of some semen parameters were performed to improve the model fit. Among the total number of study participants (n = 240 men), a significant negative regression coefficient was reported between sperm concentration and 3-PBA urinary concentrations based on the linear regression coefficient

considering 3-PBA concentrations as continuous input (β = -0.27; 95% CI: -0.41, -0.12 p-value < 0.001) and adjusting for age, smoking, abstinence time, and BMI. No evidence of a significant association was observed between 3-PBA concentration and seminal volume, sperm motility, or total sperm count,[86] again considering these independent variables on a continuous basis. When the data was instead analyzed by quartiles of 3-PBA using categorical regression, similar evidence of a significant decrease was observed for the natural log-transformed sperm concentration and total sperm count, relative to increasing 3-PBA concentrations (p-trend = 0.001 and p-trend = 0.002, respectively), adjusted for age, smoking, abstinence time and BMI).

The overall quality of the study was ranked low. Potential strengths of the study included ensuring strict quality control procedures (*e.g.,* replicate sample scoring and blinding of exposure status of corresponding semen samples) were followed to minimize interlaboratory variability, and an objective, quantitative evaluation of measured exposure on various outcome measures. Another strength of the study was the inclusion of a residual analysis to ensure adequate model fit. The primary limitation of the study was its inability to determine temporality due to the cross-sectional study design. The single spot urine and semen sample collected for the duration of the study was another limitation, since a single urine sample may not accurately reflect longitudinal or longer-term exposure patterns and the exposure measurements may simply reflect exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. Additionally, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis which would have occurred weeks or up to a couple of months prior to the measured exposure period. Lastly, multiple comparisons were made among the numerous outcome measures, but the confidence intervals or p-values were not adjusted to account for this leading to increased chances for spurious findings.

- As previously described, Radwan et al. (2014) conducted a cross-sectional study to investigate the association between pyrethroid exposures and semen quality and reproductive hormone effects in men in Poland. The study evaluated both the association between pyrethroid exposures and semen quality, and the association between pyrethroids and reproductive hormones, among non-farmers in Poland; the results for semen quality are presented here, and the results for reproductive hormones are reported under a separate health endpoint. Briefly, study subjects included men (n = 344) < 45 years of age who had visited an infertility clinic in Lodz, Poland between 2008 and 2011 for diagnostic purposes, and who had a normal semen concentration.[87] Fifty-nine percent of the eligible men agreed to participate. A questionnaire which collected information on demographics, lifestyle factors, and medical history during an interview was administered, and a single spot urine sample and a single semen sample were collected. A total of 334 (of the 344 enrolled, or 97.1%) men provided sufficient samples to be included in the study. After collection, urine samples were stored frozen at -20°C prior to analysis. Urine samples were measured for the following pyrethroid metabolites using GC-MS: *cis*-DCCA, *trans*-DCCA, 3-PBA, and DBCA. In addition, the sum of pyrethroids was presented and consisted of specific metabolites – *cis*-DCCA + *trans*-DCCA + DBCA. Creatinine-adjusted pyrethroid metabolites levels in urine were reported as either ≤ 50th percentile level or > 50th percentile level for all the pyrethroid metabolites except DBCA.[88] For DBCA, levels were reported as ≤ LOD or > LOD due to the high proportion of samples that were below the level of detection (LOD). The LOD for all metabolites was 0.1 ng/mL and proportions of samples below LOD for 3-PBA, *cis*-DCCA, *trans*-

[86] Sperm count was considered to be marginally statistically significant with a coefficient of -0.05, a 95% CI of (0.10, 0.01), and a p-value of 0.098**.**

[87] In this study, normal semen quality was defined as 20 – 300 mln/mL.

[88] Creatine adjusted geometric mean concentrations for *cis*-DCCA, *trans*-DCCA, and 3-PBA were 0.11, 0.15, and 0.17 µg/gram creatinine. Corresponding 50th, 75th, and 95th percentiles for the *cis*-DCCA, *trans*-DCCA, and 3-PBA metabolites were, respectively: 0.13, 0.17, and 0.44 µg/g creatinine; 0.15, 0.23, and 0.60 µg/g creatinine; and 0.16, 0.24, and 0.51 µg/g creatinine.

DCCA, and DBCA were 28.4%, 42.0%, 34.5%, and 83.2%, respectively. Collected semen samples were assessed based on the following criteria: sperm concentration, motility, and morphology in terms of main semen parameters and straight-line velocity (VSL), curvilinear velocity (VCL), and linearity (LIN) in terms of direction of motion parameters. Internal quality control procedures were performed to ensure precision of daily assessments. A multiple linear regression was performed to determine the potential association between pyrethroids and sperm quality using regression coefficients and 95% CIs, while adjusting for age, abstinence (days), smoking (yes/no), alcohol consumption (none or less than 1 drink per week, 1 to 3 drinks per week, and 4 to 7 drinks per week), and past diseases that may have had an impact on semen quality (yes/no).[89] Natural log transformation was used on sperm concentration to obtain a normal distribution which was evaluated by visual inspection of a quantile-normal plot as well as on pyrethroid metabolite concentrations. For sperm concentrations, evidence of a significant negative correlation was observed only for *trans*-DCCA at the > 50th metabolite level in the urine (β = -0.33; 95% CI: -0.64, -0.02, p = 0.04). For abnormal sperm morphology, evidence of a significant positive correlation was observed with urine levels of *trans*-DCCA at both the ≤ 50th level (β = 2.79; 95% CI: 1.58, 5.00, p = 0.01) and > 50th level (β = 2.80; 95% CI: 1.56, 5.04, p = 0.02), urine levels of *cis*-DCCA at the > 50th level (β = 1.22; 95% CI: 1.10, 1.92, p = 0.05), and urine levels > LOD for the sum of the *cis*-DCCA, *trans*-DCCA, and DBCA metabolites (β = 0.40; 95% CI: 0.17, 0.84, p = 0.04). For DBCA, evidence of a significant negative correlation was observed for VCL at the > LOD metabolite level in the urine (β = -4.06; 95% CI: -8.74, -0.63, p = 0.04), VSL at the > LOD level (β = -2.73; 95% CI: -5.72, -0.01, p = 0.05), and LIN at both metabolite levels in the urine (≤ LOD β = -1.13; 95% CI: -2.19, -0.08, p = 0.04; > LOD level β = -1.32; 95% CI: -2.36, -0.28, p = 0.01). For 3-PBA, evidence of a significant negative correlation was observed for VCL at the > LOD metabolite level (β = -0.92; 95% CI: -1.89, -0.01, p = 0.05).

The overall quality of the study was ranked low. Strengths included the extensive semen quality measurements, the extensive internal quality control procedures incorporated as part of the semen sampling and analysis methods to minimize intra-laboratory variability. The main limitation of the study was the cross-sectional study design. This design does not ensure that the exposure predates the outcome. Other study limitations included the single spot urine and semen samples collected for the duration of the study. This approach may not accurately reflect longitudinal or longer-term exposure patterns and the exposure measurements may also simply be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. Similarly, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis (which would have occurred weeks or up to a couple months prior to the measured exposure period). Finally, confidence intervals or p-values were not adjusted for numerous multiple comparisons of several semen quality measures across several exposure measures.

- Imai et al. (2014) conducted a cross-sectional study to evaluate the relationship between pyrethroid exposure and semen quality in Japanese male university students. The study utilized data from a larger cross-sectional, multi-center study of Japanese men aged 18 to 24 years, who were university students from four cities in Japan. Eligibility criteria required that the student and his mother to have been born in Japan. The subjects were a subpopulation of a larger cross-section study recruited at four study centers and included subjects recruited from the western part of the Tokyo metropolitan area between April 2002 and May 2003 (n = 322). Recruitment was by leaflet to which 323 subjects responded to the 2,034 leaflets distributed. Study participants completed a self-administered questionnaire about lifestyle information and diet, provided semen, serum, and urine samples and underwent a physical examination by urologists whose examination

[89] These include mumps, cryptorchidism, testes surgery, and testes trauma.

and measurements were cross-validated. The lifestyle information included smoking and alcohol consumption, and the diet information included questions about the frequency of consumption of selected foods (fish, dairy, vegetable and fruits). The physical examination included measurement of testis size and determination of the presence or absence of varicoceles as well as measures of height, weight, and BMI. Exposure was determined by analysis of urine samples for the pyrethroid metabolite 3-PBA. Urinary 3-PBA concentration was measured by LC-MS/MS and dilution effect was corrected by specific gravity of urine sample. A total of 294 subjects (91%) had urinary 3-PBA concentrations above the detection limit of 0.08 ng/mL. SG-adjusted urinary 3-PBA concentrations ranged from < 0.07 to 12.9 ng/mL, with mean, geometric mean, and median concentrations of 1.01, 0.588, and 0.641 ng/mL, respectively. For every batch of urine samples undergoing pretreatment, a procedural blank and an in-house control urine sample were included for internal analytical quality control and mean and standard deviation of repeated measurements of in-house control urine was reliable. Analysis of semen were categorized into four motility classes according to WHO guidelines, and semen volume, sperm concentration, motility, and number of total and motile of sperm were calculated. Duplicated measurements were conducted and quality control of semen analysis of the testing laboratory was rigorously assessed by cross-validation with external laboratories. Stepwise multiple regression was used to obtain β coefficients and p-values for cubic root transformed semen parameters (except sperm motility which was normally distributed) as a function of log-transformed 3-PBA concentration for each outcome, adjusting for potential the confounders of age, BMI, season of sampling, abstinence period, presence of varicocele, testis size, and frequency of cheese, soy, and non-oily white fish consumption. No significant associations were reported between pyrethroid exposure as measured by urinary 3-PBA concentration and variation in any of the semen parameters examined in the present study (*i.e.,* sperm concentration, motility semen volume, and total number of sperm or number of motile sperm).[90] Variables were selected by stepwise multiple regression (P_{in} and P_{out} set at $p = 0.05$ and 0.1), respectively.

The overall quality of the study was ranked low. The primary limitation of the study was that it relied on a cross-sectional design and was unable to assess the temporal associations between pyrethroid exposure and hormone levels. The study also assessed exposure by measuring 3-PBA levels in a single spot urine sample collected from each study participant. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. Additionally, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis which would have occurred weeks or up to a couple months prior to the measured exposure period. Finally, the study relied on automated stepwise selection when performing multiple regression analysis of the association between hormone levels and urinary 3-PBA. This variable selection method is considered unreliable and can result in biased parameter estimates with 95% confidence intervals that are too narrow due to underestimation of standard errors.[91]

[90] Variables selected by the automated selection procedure that were associated with one or more of sperm concentration, motility, semen volume, total number of sperm and number of motile sperm were: age, abstinence period; presence of varicocele, season of sampling (summer), consumption of soy > 2-3 times per week and > 1 time per week; and consumption of cheese > 1 time per week and 2-3 times per month.

[91] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. Psychosomatic Med. 66:411-421.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and adverse effects on semen quality parameters. There were six available studies that examined semen quality parameters. As with the studies on male hormone levels, these studies were cross-sectional, assessed exposure by measuring pyrethroid urinary metabolites (with the exception of one study that focused on fenvalerate), and considered a range of semen quality parameters. The studies also evaluated a large number of associations between different urinary metabolites and a number of different semen quality parameters and reported mixed findings across the studies. Specifically, a negative association between 3-PBA and sperm concentration was reported in two studies, but not in the other three studies that investigated the same association. Similarly, no other pyrethroid metabolite-semen parameter association was reported in more than a single study. The findings across the studies are summarized in the **Table 6** below.

Table 6: Summary of reported findings on the association between pyrethroid exposure and semen parameters.

Study Authors	Study Design [1]	Exposure Metric	Semen Parameters							
			Concentration	***Motility***	***Morphology***	***VSL***	***VCL***	***LIN***	***Volume***	***Count***
Lifeng et al. (2006)	CS	Fenvalerate (air)	⦸	⦸	⦸	⦸	⦸	↓●	⦸	↓●
Meeker et al. (2008)	CS	3-PBA	⦸	⦸	⦸	⦸	⦸	⦸		
		cis-DCCA	⦸	⦸	⦸	⦸	⦸	⦸		
		trans-DCCA	⦸	⦸	⦸	⦸	⦸	⦸		
		cis- + *trans*-DCCA	⦸	⦸	⦸	⦸	⦸	⦸		
		Sum Pyrethroids	⦸	⦸	⦸	⦸	⦸	⦸		
Xia et al. (2008)	CS	3-PBA	↓●	⦸					⦸	⦸
Ji et al. (2011)	CS	3-PBA	↓●	⦸					⦸	↓●
Imai et al. (2014)	CS	3-PBA	⦸	⦸					⦸	⦸
Radwan et al. (2014)	CS	3-PBA	⦸	⦸	⦸	⦸	⦸	↓●		
		cis-DCCA	⦸	⦸	↑●	⦸	⦸	⦸		
		trans-DCCA	↓●	⦸	↑●	⦸	⦸	⦸		
		DBCA	⦸	⦸	⦸	↓●	↓●	↓●		
		Sum Pyrethroids	⦸	⦸	↑●	⦸	⦸	⦸		

Legend:
⦸ - No evidence of an association ($p > 0.05$).
● - Evidence of a significant association ($p < 0.05$).
↑ - Positive association. ↓ - Negative association.
[1] Study Design –*CS* = Cross-Sectional

7.3.3 Sperm Damage and Genetic Abnormalities

Six cross-sectional studies investigated the association between exposure to pyrethroids via urinary metabolites and sperm damage and genetic abnormalities (Meeker et al., 2008; Ji et al., 2011; Young et al. 2013; Jurewicz et al., 2015; Radwan et al., 2015; Jurewicz et al., 2016). These study results are provided below:

- As previously described in **Section 7.3.2**, Meeker et al. (2008) conducted a cross-sectional study to evaluate the association between pyrethroid exposures and semen quality and sperm DNA damage among non-farmers within the US. The results for DNA damage are presented here while the results for semen quality were reported earlier in this memorandum under the semen quality endpoint. Detailed background information on the study can be found under that previously-reported endpoint. For the present endpoint, a Neutral Comet Assay was used to determine sperm

DNA damage, and measurements were based on three sperm characteristics including the Comet extent, tail distributed moment (TDM), and percent DNA located in the tail (tail %).[92] A multiple linear regression was used to determine if an association between exposure to pyrethroids as measured by the 3-PBA, *cis*-DCCA, and *trans*-DCCA metabolites and sperm DNA damage existed using regression coefficients and 95% CIs, adjusting for smoking and age. Exposure was assessed using categories of each of the three metabolite concentrations and consisted of the following percentiles: < 50th, 50th – 75th, and > 75th percentile. Sperm DNA damage analysis was available for only 143 individuals and evidence of a positive exposure-response trend was observed between exposure to the 3-PBA metabolite and sperm DNA damage, measured as the percent DNA located in the tail (tail %) at > 75th percentile compared to the less than 50th percentile median (β = 6.45, 95% CI: 0.86, 12.0; p-trend = 0.02). No other pyrethroid metabolite (or combination of metabolites) was significant or revealed a significant p-trend for the tail % measure. For Comet extent, evidence of a positive association was observed only in the 50th - 75th percentile for the 3-PBA metabolite (50th – 75th β = -14.4; 95% CI: -27.9, -0.92) but the p-trend was not significant (p = 0.81). For the sum of pyrethroids (3-PBA + *cis*-DCCA + *trans*-DCCA), statistically significant findings were found for both Comet Extent (50th – 75th β = -16.2; 95% CI: -29.7, -2.62) and TDM (50th – 75th β = -7.63; 95% CI: -13.3, -1.93), but again only for the 50th - 75th percentile and <u>not</u> for the higher > 75th percentile grouping; p-trend in neither case, however, was significant (p-trend = 0.94 and 0.55, respectively). No other finding among the total of 30 comparisons performed in the study (3 outcomes x 5 chemicals x 2 concentration levels) or for any of the metabolites or their sums were significant.

The overall quality of the study was ranked low. Potential strengths of the study included a quantitative objective laboratory-based evaluation of exposure and outcome. The main limitation of the study was the cross-sectional study design, which does not ensure that the exposure predates the outcome. The single spot urine and semen sample collected for the duration of the study was another major limitation since samples collected at different time points during the study could have potentially provided more information and contributed to both the exposure and outcome. For example, a single urine sample may not accurately reflect longitudinal or longer-term exposure patterns and the exposure measurements may simply reflect exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. Additionally, semen analysis was limited to only a single sample collected during the examination at a time point which would not be temporally concordant with spermatogenesis (which would have occurred weeks or up to a couple months prior to the measured exposure period). Although semen sample collection and analysis appeared to follow the WHO guidelines (2010), no information on standard laboratory QA/QC procedures (*e.g.,* blinded study design and replicate sample scoring) or results was provided. Finally, study authors performed many comparisons considering both outcome (6 outcomes total) and exposure (5 chemical entities total) – not all of which were independent – which limits the ability to infer statistical significance since there was no multiple comparison adjustment made to the confidence intervals or p-values.

- As previously described in **Section 7.3.2**, Ji et al. (2011) conducted a cross-sectional study to evaluate the association between pyrethroid exposures and both semen quality and sperm DNA damage among men in China. The results for semen quality are reported earlier under that health endpoint and the results for sperm DNA damage are presented here. Detailed background information on the study can be found under the previously-reported endpoint under the semen quality section of this memorandum. For the DNA damage endpoint considered here, semen

[92] Comet extent measures total comet length from the head to the tail. Tail % is a measure of the proportion of total DNA that is present in the tail. And TDM is a measure that takes into account the distance and intensity of the comet fragment.

samples were collected, and a TUNEL[93] assay was used to assess DNA fragmentation. DNA fragmentation was not normally distributed, so a natural log transformation was used prior to a multiple regression analysis to determine the potential association between pyrethroid exposure (as measured by the 3-PBA metabolite urinary concentrations) and sperm DNA damage, adjusting for smoking, age, BMI, and abstinence time. A second analysis was conducted further stratifying the data by quartiles of 3-PBA urinary concentrations (described previously) relative to sperm DNA damage, using the natural log transformation of sperm DNA fragmentation to improve model fit. Among the total number of study participants (n = 240 men), a significant positive association was observed between sperm DNA fragmentation and 3-PBA urinary concentrations ($\beta = 0.27$; 95% CI: 0.15, 0.39, p-value < 0.001). In a second analysis in which 3-PBA concentration was divided into quartiles, evidence of a significant increase in DNA fragmentation with increasing 3-PBA concentrations was also observed (p-trend < 0.001).

The overall quality of the study was ranked low. Potential strengths of the study included strict quality control procedures (*e.g.,* replicate sample scoring and blinding of exposure status of corresponding semen samples) and a quantitative and objective laboratory-based evaluation of exposure and various outcome measures. Another strength of the study was the inclusion of a residual analysis to ensure adequate statistical model fit. A major limitation of the study was its cross-sectional design whereby exposure and outcome were measured at the same time which limits the ability to determine temporality. The single spot urine and semen sample collected for the duration of the study was another limitation: a single urine sample may not accurately reflect longitudinal or longer-term exposure patterns and the exposure measurements may simply reflect exposure to non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. In addition, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis (which would have occurred weeks or up to a couple of months prior to the measured exposure period).

- Young et al. (2013) conducted a cross-sectional study to evaluate the association between pyrethroid exposure and sperm sex chromosome disomy using a subset of data from a parent study of 341 men aged 20 - 54 years old seeking an infertility assessment recruited from the Massachusetts General Hospital Fertility Center during January 2000 – May 2003. Of those approached for this sub-study, 75 (65%) eligible men agreed to participate, with those declining participation most likely citing lack of time as the reason. A self-administered questionnaire provided demographic information, lifestyle factors, and medical and fertility history. Environmental (non-occupational) exposure to pyrethroids including 3-PBA, *cis*-DCCA and *trans*-DCCA, was assessed using urine samples collected during the study, and analyzed by HPLC/MS. The median, 75th percentile and 90th percentile creatine-adjusted concentrations for 3-PBA were 0.11, 0.36, and 0.94 µg/g creatine. For *cis*-DCCA, and *trans*-DCCA, corresponding concentrations were 0.09, 0.20, and 0.37 µg/g creatinine and 0.13, 0.34, and 0.90 µg/gram creatinine, respectively. The LODs for 3-PBA, *cis*-DCCA, and *trans*-DCCA were 0.10 µg/L, 0.23 µg/L, 0.35 µg/L, respectively and the percentage above the LOD was 56% for 3-PBA, 29% for *cis*-DCCA, and 25% for *trans*-DCCA. To detect sperm sex chromosome disomy (the outcome measure from this study), a fluorescence in situ hybridization (FISH) analysis was performed on the collected semen sample. Based on the general shape of the histograms constructed for each (count) outcome variable, Poisson regression was used to determine if an association existed between pyrethroid metabolite concentrations (3-PBA, *cis*-DCCA, and *trans*-DCCA) and disomic

[93] The TUNEL assay was defined as: Terminal deoxynucleotidyl transferase (TdT) duTP Nick-End Labeling assay. This is considered to be a sensitive method of detecting single and double-stranded DNA fragmentation in sperm. Results from the TUNEL assay are generally strongly correlated with other techniques for assessing DNA damage such as the Comet assay and the sperm chromatin structure assay.

cells including XX18, YY18, XY18, 1818, and total sex chromosomes by calculating incidence rate ratios (IRRs) and 95% CIs, adjusting for age, smoking, sperm motility, specific gravity, and log of sperm concentration. Due to the relatively low proportion of urine samples containing detectable levels of pyrethroid metabolites and limited distribution of values above the LOD, urine concentrations were dichotomized into < LOD and > LOD values and an imputed value equal to ½ LOD was used when the machine did not detect a metabolite. Evidence of a slight positive association was observed above the limit of detection for 3-PBA, *cis*-DCCA, and *trans*-DCCA relative to the disomic cell YY18 (3-PBA IRR: 1.28; 95% CI: 1.15, 1.42, n = 42; *cis*-DCCA IRR: 1.18; 95% CI: 1.05, 1.31, n = 22; *trans*-DCCA IRR: 1.19; 95% CI: 1.06, 1.34, n = 19). Evidence of a slight positive association was observed above the limit of detection for *cis*-DCCA and *trans*-DCCA relative to the disomic cell XX18 (*cis*-DCCA IRR: 1.22; 95% CI: 1.09, 1.36, n = 22; *trans*-DCCA IRR: 1.30; 95% CI: 1.17, 1.46, n = 19) and total disomy (*cis*-DCCA IRR: 1.12; 95% CI: 1.06, 1.17, n = 22; *trans*-DCCA IRR: 1.09; 95% CI: 1.04, 1.15, n = 19). No evidence of a significant positive association was observed relative to disomic cell 1818 for 3-PBA, *cis*-DCCA, and *trans*-DCCA.

The overall quality of the study was ranked low. Study strengths included the quantitative and objective laboratory measures associated with both the exposure and outcome measures. The main limitation of the study was the cross-sectional study design, which does not ensure that the exposure predates the outcome. Other key limitations included the single urine sample collected during the study due to the rapid metabolization of pyrethroids in the urine, and that semen analysis was limited to only a single sample collected during the examination at a time point which would not be temporally concordant with spermatogenesis. We note too, that for 3-PBA, the direction of disomy was inconsistent, with some changes reflecting increased IRRs and some reflecting decreased IRRs. In addition, we note that a sizable fraction of the analytical results (44% for 3-PBA, 71% for *cis*-DCCA and 75% for *trans*-DCCA) were below the analytical detection limits reported by the study.

- Jurewicz et al. (2015) conducted a cross-sectional study to evaluate the association between pyrethroids exposure and DNA sperm damage among men living in Lodz, Poland. Study participants included men recruited from an infertility clinic in Central Poland between January 2008 and April 2011, aged ≤ 45 years old who were determined to have a normal sperm concentration of 15 – 300 10^6/mL, following the standard of the WHO.[94] Study participants were a subset of participants from a larger study of 344 men designed to assess the impact of environment, lifestyle, and occupation on semen quality. Fifty-nine percent of men who were asked to participate agreed, with the principal cited reason for not participating being lack of time. A total of 194 men provided sufficient samples for analysis. Participants answered a detailed questionnaire covering demographic, medical, and lifestyle risk factor. Saliva samples (for cotinine measurement) were collected from the study participants along with occupational exposure information obtained during an interview. Environmental (non-occupational) exposure to pyrethroids including 3-PBA, *cis*-DCCA, *trans*-DCCA, and DBCA was assessed via urine samples analyzed for pyrethroid metabolites using GC/MS methods. Measurable levels of *cis*-DCCA, *trans*-DCCA, and 3-PBA were found in approximately 65%, 57%, and 72% of the samples, respectively, with a limit of detection of 0.1 ng/mL for all analytes. Creatinine-adjusted geometric mean concentrations for *cis*-DCCA, *trans*-DCCA, and 3-PBA were 0.12, 0.16, and 0.15 µg/g creatinine, respectively; the median, 75th, and 95th percentile concentrations, respectively, were 0.11, 0.14, and 0.42 µg/g creatinine for *cis*-DCCA; 0.14, 0.20, and 0.56 µg/g creatinine for *trans*-DCCA; and 0.13, 0.19; and 0.46 µg/g creatinine for 3-PBA. The outcome

[94] WHO (2010) WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th edn. World Health Organization, Cambridge University Press; Cambridge, UK.

measure, sperm DNA damage from sperm collected on the same day as the urine sample, was evaluated and measured by the sperm chromatin structure assay (SCSA), as per with the following criteria measures: DNA fragmentation index (DFI), medium DFI, high DFI, and high DNA stainability (reflective of the number of immature sperm). A multiple regression model was used after dichotomizing each urinary pyrethroid metabolite as either > 50th or < 50th percentile to determine if an association between specific urinary pyrethroid metabolites and sperm DNA damage in males was present, while adjusting for sexual abstinence (days), age (years), smoking (yes/no), past diseases (yes/no), and alcohol consumption (none or < 1 drink per week; 1-3 drinks per week; or 4-7 drinks per week). Among the total number of study participants (n = 286), evidence of a slight positive association was observed for *cis*-DCCA > 50th percentile and M DFI and HDS (coefficient: 1.10; 95% CI: 1.04, 1.21, p = 0.04; coefficient: 1.09; 95% CI: 1.04, 1.55, p = 0.04). For 3-PBA, evidence of an association was observed with H DFI (coefficient: 1.07; 95% CI: 1.04, 1.52, p = 0.03). No evidence of a significant positive association was observed for *trans*-DCCA and DBCA pyrethroid metabolites or any of DFI, MDFI, HDFI or HDS or for DFI or HDS for *cis*-DCCA for either the ≤ 50th percentile or > 50th percentile comparisons performed.

The overall quality of the study was ranked low. Study limitations included the cross-sectional study design created a temporality issue, and the single urine and semen samples collected from the study participants throughout the duration of the study. Since pyrethroids are rapidly metabolized and excreted, urinary metabolite concentrations likely reflect exposure over hours or days preceding the single urine sample collection and may not be temporally relevant with respect to the sperm DNA damage observed in the study. Further, confidence intervals or p-values were not adjusted for numerous multiple comparisons of several sperm damage measures across several exposure measures. Specifically, we note that of 40 comparisons made, only 3 were judged significant and even then, these were not consistent for either exposure or outcome measure. It is likely that any reasonable adjustment for multiple comparisons (*e.g.,* Benjamini-Hochberg FDR) would have resulted in none of these three observed associations being significant. Finally, while the authors indicated that multiple linear regression was performed on dichotomized pyrethroid exposure measures (≤ 50th percentile and > 50th percentile), it is not clear from the description of the statistics what exactly was done and it appears that non-standard statistical methods may have been used.

- Radwan et al. (2015) conducted a cross-sectional study to evaluate the association between pyrethroid exposures and male sperm chromosome disomy in men in Poland. Cases were drawn from participants of a parent study (n = 344) that included men aged < 45 years of age who had visited an infertility clinic in Lodz, Poland between 2008-2011 for diagnostic purposes, and who had a normal semen concentration. Among the 344 men who enrolled in the parent study, 59% agreed to participate and 195 men had sufficient urine and semen samples available as samples from the parent study had been used for other semen analysis research and were eligible for this study. Exposure was assessed using a questionnaire which collected lifestyle factors and medical history during an interview in addition to collecting a single spot urine and semen samples. Urine samples were measured for the following four pyrethroid metabolites using a validated gas chromatography ion-trap mass spectrometry (GC-IT/MS) method: *cis*-DCCA, *trans*-DCCA, 3-PBA, and DBCA. Creatinine-adjusted geometric mean concentrations of *cis*-DCCA, *trans*-DCCA, 3-PBA, and DBCA were 0.10, 0.15. 0.16, and 0.04 µg/g creatine. The 50th 75th, and 95th percentile concentrations for creatinine-adjusted *cis*-DCCA, *trans*-DCCA, 3-PBA, and DBCA were as follows: 0.11, 0.15, and 0.43 µg/g creatine for *cis*-DCCA; 0.15, 0.20, and 0.58 µg/g creatine for *trans*-DCCA; 0.14, 0.19, and 0.47 µg/g creatine for 3-PBA; and 0.03, 0.04, and 0.25 µg/g creatine for DBCA. The LOD for all of the pyrethroid metabolites was 0.1 ng/mL and the percentages of participants' urine samples below the limit of detection for 3-PBA, *trans*-DCCA, *cis*-DCCA, and DBCA, were 28.41%, 34.49%, 42.03%, and 83.19%, respectively. Because of the

high proportion of samples with creatinine-adjusted pyrethroid metabolites below the (LOD, 3-PBA, *trans*-DCCA, and *cis*-DCCA were categorized into low (≤ 50th percentile) and high (> 50th percentile) groups; DBCA was dichotomized into categories below the LOD or above the LOD because this metabolite was detected in only 16.81% samples. For the outcome measure, a single semen sample was obtained from each participant, and sperm aneuploidy was measured by multicolor fluorescence in-situ hybridization (FISH) using DNA probes specific for chromosomes X, Y, 18 (centromeric probes), and 13 and 21 (locus specific probes). After sample preparation, the aneuploidy frequency was evaluated in two hybridization areas, with 1,000 cells evaluated in each area. Normal sperm were defined as having one signal from chromosome 18 and one signal from chromosome X or chromosome Y in the first area and having a single signal from both chromosomes 13 and 21 in the second area. Aneuploidy was considered present when the number of fluorescent signals specific for the analyzed chromosomes was different in comparison with normal cells and when the size and intensity of the signal was similar to those detected in normal nuclei. Diploidy was defined by the presence of two signals for each of the studied chromosomes in the presence of the sperm tail and an oval head shape. Two technicians scored half of the total number of slides each. For quality control, six slides were scored by both technicians and no significant differences in scoring were found ($p > 0.05$). The investigators evaluated six types of sperm disomy: XY sperm (sperm FISH genotype: X-Y-18), disomy X (X-X-18), disomy Y (Y-Y-18), disomy 18, 21 and 13 (X-18-18 or Y-18-18, X-21-21 or Y-21-21 and X-13-13 and Y-13-13, respectively). Sperm aneuploidy outcomes were expressed as percentages and were log-transformed for regression analysis. The authors constructed multivariate negative binomial regression models for disomy X and disomy 13 as continuous aneuploidy outcomes (dependent variables), and each of the pyrethroid metabolites and potential confounders as independent variables. For disomy Y, XY, 18, 21, total sex chromosome disomy, and total chromosome disomy, the investigators constructed generalized linear mixed models with a Poisson distribution to model covariate-adjusted associations between urinary pyrethroid metabolite levels and each type of sperm aneuploidy. Models were adjusted for sexual abstinence, age, smoking, alcohol consumption, past diseases, sperm concentration, and motility. No significant associations between urinary *cis*-DCCA and any sperm aneuploidy were reported. A significant positive association was reported between *trans*-DCCA at the > 50th percentile and XY disomy ($\beta = 1.20$; 95% CI: 1.01, 1.44, $p = 0.04$). All other associations between urinary *trans*-DCCA levels and all other sperm aneuploidy were not statistically significant. The authors report that urinary 3-PBA was significantly associated with XY disomy at the > 50th percentile (> 50th: $\beta = 1.27$; 95% CI: 1.05, 1.54, $p = 0.02$), Y disomy at both < 50th and > 50th percentiles (< 50th: $\beta = 1.35$; 95% CI: 1.02, 1.78, $p = 0.04$; > 50th: $\beta = 1.41$; 95% CI: 1.06, 1.88, $p = 0.02$), disomy of chromosome 18 (> 50th: $\beta = 1.31$; 95% CI: 1.03, 1.68, $p = 0.03$), disomy of chromosome 21 at both < 50th and > 50th percentiles (< 50th: $\beta = 1.30$; 95% CI: 1.01, 1.66, $p = 0.04$; > 50th: $\beta = 1.28$; 95% CI: 1.01, 1.63; $p = 0.04$), and total disomy at both < 50th and > 50th percentiles (< 50th: $\beta = 1.14$; 95% CI: 1.02, 1.47, $p = 0.03$; > 50th: $\beta = 1.30$; 95% CI: 1.10, 1.59, $p = 0.04$). All other associations between urinary 3-PBA and other sperm aneuploidy were not significant. No significant associations were observed between urinary DBCA levels and any of the analyzed chromosome disomy outcomes. In an additional analysis in which the sum of all four urinary pyrethroid biomarkers were modeled as a continuous exposure variable, no significant associations with any of the chromosome disomy outcomes were reported.

The overall quality of the study was ranked low. Strengths included the quantitative, objective, and laboratory-based measures of exposure and outcome. The main limitation of the study was the cross-sectional study design. This design does not ensure that the exposure predates the outcome. Other study limitations included the single spot urine and semen samples collected for the duration of the study. This approach may not accurately reflect longitudinal or longer-term exposure patterns and the exposure measurements may also simply be reflective of exposure to

non-toxic, pre-formed environmental degradants/metabolites that may be present in the environment and food. Similarly, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis (which itself would have occurred weeks or up to a couple months prior to the measured exposure period). Statistical confidence intervals or p-values were not adjusted for numerous multiple comparisons of several DNA aneuploidy/disomy measures across the several exposure measures thus permitting many chance results to appear (particularly given that the pyrethroid metabolite levels are highly correlated). Finally, the authors did not make clear exactly what statistical measures were used except to refer to it. Specifically, they refer to "negative binomial" and "generalized linear mixed model" but it is unclear how the regression coefficients for the "$\leq 50^{th}$" and "$> 50^{th}$" percentiles were derived or how they should be interpreted. It appears that no dummy/indicator variables were used which we would have expected. As a result, the two regression coefficients that were generated and presented by the authors as principal findings of their research for each of the aneuploidy conditions – one for $\leq 50^{th}$ percentile and one for $> 50^{th}$ percentile – are not readily interpretable and their importance or relevance is not clear. Additionally, a substantial fraction of the urinary pyrethroid metabolite measurement were in many cases < LOD, which results in additional concerns about reaching conclusions based on these findings.

- Jurewicz et al. (2016) conducted a cross-sectional study on a subset of men (n = 194) from a parent study of men (the "Environmental factors and male infertility" study, n = 344) under 45 years of age who visited an infertility clinic in Lodz, Poland for diagnostic purposes between 2008 and 2011. The purpose of their study was to evaluate whether urinary pyrethroid metabolite concentrations (along with phthalate and polycyclic aromatic hydrocarbon metabolites) are associated with the proportion of Y/X chromosome bearing sperm (*i.e.,* the Y:X ratio). Approximately 59% of eligible men agreed to participate, with many of those declining participation indicating lack of time as the reason. Among the biospecimens (urine and semen samples) that had been used for prior research, eligibility for this study was based on availability of urine and semen samples. Of the 344 men enrolled in the original (prior) study, 194 provided sufficient samples (56%). Urinary concentrations of three pyrethroid metabolites, 3-PBA, *cis*-DCCA, and *trans*-DCCA, were quantified using (GC-IT/MS) methods. The geometric mean concentrations for 3-PBA, *cis*-DCCA, and *trans*-DCCA were 0.16, 0.17, and 0.15 µg/L, respectively. The limit of detection (LOD) for all of the pyrethroid metabolites was 0.1 ng/mL, with the percentages of participants' urine samples with detectable levels of 3-PBA, *cis*-DCCA, and *trans*-DCCA of 73.3%, 61.5%, and 68.2%, respectively. Demographic information, lifestyle factors, and medical history were collected using a questionnaire completed by all participants. The Y:X ratio of sperm in a semen sample collected from each participant was assessed by fluorescent in situ hybridization (FISH). The authors constructed multiple linear regression models of the Y:X ratio, with metabolite concentrations entered as continuous, log-transformed variables, and also as the sum of the pyrethroid metabolites, and the ratio of *cis*-DCCA and *trans*-DCCA. Concentrations below the lower limit of detection were assigned a value equal to ½ LOD. Covariates were included in the regression models based on "biological and statistical consideration". Age, smoking, sperm concentration, sperm motility, sperm morphology, and urinary creatinine concentration were included as covariates based on their associations with Y chromosome fraction observed in previous studies. Other covariates (past diseases, abstinence, alcohol consumption) were included if their unadjusted associations with the Y chromosome fraction had an associated p-value less than 0.2. Associations between the Y chromosome fraction and urinary metabolite levels were presented as β coefficients and corresponding 95% confidence intervals and p-values. Because pyrethroid metabolite levels and concentration of phthalate metabolites were highly correlated, the investigators separated models for each. Before correction for multiple comparisons, their findings were as follows: The sum of 3-PBA, *cis*-DCCA and *trans*-DCCA was negatively associated with the Y:X ratio, $\beta_{sum} = -0.016$; 95% CI: -0.032, -0.001;

p = 0.039. In a multivariate model including *cis*-DCCA and *trans*-DCCA, both were significantly associated with Y:X ratio (β_{CDCCA} = -0.018; 95% CI: -0.029, -0.006; p = 0.002 and β_{TDCCA} = 0.017; 95% CI: 0.006, 0.027; p = 0.003). The authors reasoned that the opposite signs observed in the two regression models suggests the appropriateness of a model for the ratio of *cis*-DCCA and *trans*-DCCA, rather than sum of the two pyrethroid pesticide metabolites. The ratio of *cis*-DCCA to *trans*-DCCA was negatively associated with the proportion of Y-bearing sperm, $\beta_{CDCCA:TDCCA}$ = -0.019; 95% CI: -0.028, -0.010; p < 0.001. The association remained after additional adjustment for the phthalate and PAH metabolite concentrations, $\beta_{CDCCA:TDCCA\ ratio}$ = -0.018; 95% CI: -0.029, -0.008; p < 0.001 and the observed association with Y:X ratio remained statistically significant after applying an FDR correction for multiple comparisons.

The overall quality of the study was ranked low. Study limitations included the cross-sectional study design created a temporality issue, the use of non-specific and non-persistent pesticide exposure biomarkers to measure pyrethroids, and the single urine and semen samples collected from the study participants throughout the duration of the study, since pyrethroids are rapidly metabolized and excreted, and urinary metabolite concentrations likely reflect exposure over hours or days preceding the single urine sample collection. Additionally, semen analysis was limited to only a single sample collected at a time point which would not be temporally concordant with spermatogenesis which would have occurred weeks or up to a couple months prior to the measured exposure period. However, a limitation of the regression analysis was its entirely exploratory nature and the multitude of models that were attempted, none which appeared to be *a priori* and many of which appeared to be *ad hoc.*

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and sperm damage and genetic abnormalities. There were six available studies that examined sperm damage and genetic abnormalities. As with the studies on male hormone levels and semen quality, these studies were cross-sectional, assessed exposure by measuring pyrethroid urinary metabolites, and considered a range of different genetic parameters. Similarly, the studies also evaluated a large number of associations and generally reported mixed findings across the six studies with only one positive association reported in more than one study: both Ji et al. (2011) and Jurewicz et al. (2015) reported positive associations between urinary 3-PBA levels and DNA fragmentation. The findings across the studies are summarized in the **Table 7** below.

Table 7: Summary of reported findings on the association between pyrethroid exposure and sperm damage and genetic abnormalities.

Study Authors	Study Design [1]	Exposure Metric	Sperm Genetic Abnormality					
			Comet Extent	*TDM*	*Tail %*	*Y:X ratio*	*DNA Fragmentation*	*chromosome disomy*
Meeker et al. (2008)	CS	3-PBA	⊘	⊘	↑●			
		cis-DCCA	⊘	⊘	⊘			
		trans-DCCA	⊘	⊘	⊘			
		cis- + *trans*-DCCA	⊘	⊘	⊘			
		Sum Pyrethroids	⊘	⊘	⊘			
Ji et al. (2011)	CS	3-PBA					↑●	
Young et al. (2013)	CS	3-PBA						↓●
		cis-DCCA						↑●
		trans-DCCA						↑●

Study Authors	Study Design [1]	Exposure Metric	Sperm Genetic Abnormality					
			Comet Extent	*TDM*	*Tail %*	*Y:X ratio*	*DNA Fragmentation*	*chromosome disomy*
Jurewicz et al. (2015)	CS	3-PBA					↑●	
		cis-DCCA					↑●	
		trans-DCCA					⦸	
		DBCA					⦸	
		Sum Pyrethroids					⦸	
Radwan et al. (2015) [2]	CS	3-PBA				⦸		↑●
		cis-DCCA				⦸		⦸
		trans-DCCA				⦸		⦸
		DBCA				⦸		⦸
Jurewicz et al. (2016)	CS	3-PBA				⦸		
		cis-DCCA				↓●		
		trans-DCCA				↑●		
		cis- + *trans*-DCCA				⦸		
		Sum Pyrethroids				↓●		

Legend:
⦸ - No evidence of an association ($p > 0.05$).
● - Evidence of a significant association.
↑ - Positive association. ↓ - Negative association.
[1] *Study Design –CS = Cross-Sectional;*
[2] Study also included 6 measures of sperm aneuploidy (XY18, XX18, YY18, %1818, %1313, and %2121). No other study evaluated these measures, so the results are not summarized in the table.

7.3.4 Fecundability

One study investigated the association between fecundability and exposure to pesticides including pyrethroids.

Sallmén et al. (2003) used information available for all employees working in agriculture in Finland and the nationwide database on medically diagnosed pregnancies in 1973-1990 to investigate whether the work of men in greenhouses and exposure to pesticides was associated with time to pregnancy in a synthetic prospective study. The study period was 1980 to 1990 and data on the cultivated plants and pesticides used for each plant were collected using questionnaires administered to enterprises. The exposure assessment was conducted by an experienced occupational hygienist from 1994 to 1995, 4 to 15 years after the studied pregnancy and included: questionnaire data, data on cultivated plants and pesticides used as reported by the employers, as well as results of previous studies on exposure conditions in Finnish greenhouses. The authors acknowledged a low participation rate (43%) and noticed that participation was to some extent related to women's reproductive history. The calendar months with pesticide exposures were also taken into account. Male exposures to other occupational factors and female exposures were defined on the basis of the questionnaire information. Three categories of exposure were defined: having applied pesticides at least once a week or having worked with treated plants on three days/week (High); applying pesticides 2-3 times a month or handling treated plants 1-2 days/week (Moderate); and spraying pesticides only once a month or working with treated plants less than once a week (Low). Pregnancy was defined as the first eligible pregnancy (either a birth or a spontaneous abortion) during the study period. Data on time to pregnancy and related factors were ascertained using questionnaires administered in two stages in 1994 to 1995. The study was restricted to couples (n = 588) with information on time to pregnancy from either the second or the first inquiry. Time-to-pregnancy data were analyzed with a discrete proportional hazards regression analysis and the outcome parameter was a HR, but in the context of this study the authors use the phrase "fecundability density ratio" (FDR).

Models were adjusted for male participation in the study, female age, previous pregnancies, last contraceptive method, marital status, smoking, interaction between drinking coffee and smoking, use of alcohol, employment, work in greenhouses or gardens, spraying of pesticides, and missing information. The FDR was further stratified based on the type of exposure – either not protected efficiently or protected efficiently – and separate FDRs were reported for each type of exposure. For pyrethroids, evidence of a statistically significant association was observed between exposure to pyrethroids among males who reported not being protected efficiently and decreased fecundability (FDR: 0.40; 95% CI 0.19, 0.85 with n = 15). For men who were protected efficiently when exposed to pyrethroids, no evidence of a statistically significant decrease in fecundability was reported (FDR: 1.02; 95% CI: 0.62, 1.66 with n = 31).

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroid exposure and fecundability. The overall quality of the study was ranked low. A major study limitation included the self-reported exposure and outcome. Although some historical occupational records were consulted, the exposure measurement for this study was primarily based on self-report and not supported by any measurement of biomarkers. In regard to the outcome, pregnancy data was also self-reported. Furthermore, data on time to pregnancy and related factors were asked in questionnaires 4 to 15 years after the studied pregnancy, so recall bias could have affected the accuracy of such reports. In addition, only a small number of cases were observed among males who reported decreased fecundability who were not efficiently protected. Finally, the authors acknowledged a low participation rate (43%) and noted that participation was to some extent related to women's reproductive history, all of which might have resulted in selection bias.

7.4 Other Non-Carcinogenic Effects

7.4.1 Neurologic Effects

Two AHS studies, Kamel et al. (2005, 2007), investigated the association between pyrethroid exposures and neurologic symptoms in adults, both using cross-sectional study designs. A third study (Motsoeneng and Dalvie 2015), a cross-sectional study not part of the AHS, evaluated the association between urinary pyrethroid metabolites and neurologic symptoms among female farmers.

- Kamel et al. (2005) conducted a cross-sectional study to evaluate the association between exposure to pesticides including pyrethroids and neurologic symptoms among pesticide applicators enrolled in the AHS. The study population (n = 18,782) consisting of male pesticide applicators in the AHS living in Iowa and North Carolina. Pesticide exposure was assessed for 50 different pesticides, including pyrethroids, using self-administered questionnaires completed at study enrollment and at home. Cases were defined as pesticide applicators who had experienced ≥ 10 neurologic symptoms[95] (*i.e.,* many symptoms) commonly associated with pesticide intoxication within the year prior to enrollment, and controls included individuals who had indicated they had experienced < 10 neurologic symptoms. ORs and 95% CIs were calculated using a logistic regression model adjusting for state, age, education, cigarette smoking, and alcohol consumption. For each exposure level, individual ORs were reported for lifetime days of pesticide use, with or without use in the past year. Among the total AHS applicators (n = 18,782), 5,258 (28%) reported exposure to pyrethroids and 13,523 (72%) reported no exposure to pyrethroids. Exposure was further stratified into low exposure (1 – 50 lifetime days of use) and

[95] Neurologic symptoms included: headache, fatigue, tension, insomnia, irritability, dizziness, numbness in hands or feet, depression, nausea, absent mindedness, difficulty concentrating, loss of appetite, excessive sweating, twitches in arms or legs, fast heart rate, weakness in arms or legs, poor balance, poor night vision, tremor in hands, blurred/double vision, changes in smell or taste, difficulty speaking, and loss of consciousness.

high exposure (> 50 lifetime days of use) categories, and for each exposure category, pesticide use was also further stratified as use or no use in the past year. For the low exposure category (1 – 50 lifetime days of use), for no use reported within the past year, evidence of a positive association for neurologic symptoms (OR = 1.31; 95% CI: 1.17, 1.47 with 13% of exposed cases and 10% of exposed controls) was observed; no evidence of a significant positive association was observed for this low exposure category for use reported within the past year (OR = 1.16; 95% CI: 0.99, 1.37 with 6% of exposed cases and 5% of exposed controls). For the high exposure category (> 50 lifetime days of pesticide use) for no use and use reported within the past year, respectively, evidence of a positive association between pyrethroid exposure and neurologic symptoms was observed (OR = 1.24; 95% CI: 1.09, 1.58 with 4% of exposed cases and 3% of exposed controls without use in the past year; OR = 1.31; 95% CI: 1.09, 1.58 with 5% of exposed cases and 3% of exposed controls with use in past year).

- Kamel et al. (2007) conducted a cross-sectional study to evaluate the association between pyrethroid exposure and neurologic symptoms among pesticide applicators enrolled in AHS. Using data from the AHS, Kamel et al. (2007) applied similar study methods used by Kamel et al. (2005) including the design, and the study population remained the same (*i.e.,* male pesticide applicators). Among the total AHS study participants (n = 18,782), 992 (28% of cases) cases and 3,204 (22% of controls) controls reported exposure to pyrethroids. Evidence of a positive association was observed between pyrethroid use and neurologic symptoms among male applicators within the AHS (adjusted OR = 1.30; 95% CI: 1.20, 1.40), based on ever/never use.

- Motsoeneng and Dalvie (2015) conducted a cross-sectional study to evaluate the association between pyrethroid exposure and neurotoxic symptoms among female farmers in Western Cape, South Africa. Study participants included women who were either part of the farm group (farm group n = 121) or the town group (town group n = 90). The farm group consisted of women who worked and lived on the farm, women who lived but did not work on the farm, and women who only worked on the farm; the town group included women living in towns located 5 to 10 km away from agricultural areas (town group n = 90). All participants were between 18 and 70 years old. All participants answered a questionnaire (Q16) in their preferred language and provided a urine sample on the same day. Data collection occurred during the pesticide spraying season. The Q16 questionnaire consisted of 16 questions that focused on 16 specific neurotoxic symptoms, with yes/no responses corresponding to symptoms associated with neurotoxicity. Five pyrethroid metabolites 3-PBA, 4F3-PBA, DBCA, and *cis*- and *trans*-DCCA were quantified in urine samples. Laboratory quality control procedures were carried out including the use of spiked pooled urine. Eleven samples were judged insufficient for analysis. Participants with urinary creatinine outside the WHO range were excluded (n = 18). Multiple logistic regression models were used to evaluate associations between pyrethroid urinary biomarker levels and dichotomous outcomes, and linear regression was used for the analysis of Q16 score. The investigators adjusted for age, education, household income as well as for drug use, alcohol usage, current smoking, language, and previous poisoning (the last four variables were selected based on bivariate testing). Evidence of a moderately strong association was observed between DBCA exposure and neurotoxic symptoms among women in the farm group from the Q16 questionnaire including difficulties with reading, buttoning, and notes,[96] relative to women in the town group (reading OR: 2.95; 95% CI: 1.16, 7.54 with n = 31 farm women, 16 town women; buttoning OR: 8.93; 95% CI: 1.71, 46.5 with n = 6 farm women and 4 town women; OR notes: 2.82; 95% CI: 1.04, 7.63 with n = 36 farm women and 14 town women). For *cis*-DCCA, evidence of a strong

[96] In this study, the Q16 questionnaire defined reading, buttoning, and notes based on positive responses to the following questions: a) Do you generally find it hard to get the meaning from reading newspapers and books? b) Do you have any problems with buttoning and unbuttoning? c) Do you often have to make notes about what you must remember?

association was observed only for buttoning among women in the farm group, relative to women residing in town (buttoning OR: 3.03; 95% CI: 1.22, 7.50 with n = 6 farm women, and 4 town women). For *trans*-DCCA, 4F3-PBA, and 3-PBA, no evidence of a significant positive association was observed for any of the 16 neurotoxic symptoms among the farm group women ($0.62 \leq ORs \leq 1.82$; all CIs encompassed the null value of 1.0; with n = 30 – 105 farm women and 15 – 42 town women) compared to women living in town.

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and neurologic symptoms. Kamel et al. (2005) reported evidence of a positive association between self-reported neurologic symptoms and exposure to pyrethroids at 1 - 50 days of cumulative lifetime exposure days (with no prior use in the past year observed), and at > 50 days of cumulative lifetime exposure days, without and with prior use in the past year. For ever/never pyrethroid use, Kamel et al. (2007) reported evidence of a positive association between pyrethroid use and self-reported neurologic symptoms among male applicators within the AHS. Both of these studies used data from the AHS, which was considered a study strength; however, the cross-sectional study design of the studies made it impossible to determine temporality. Furthermore, additional weaknesses included case ascertainment that relied on self-report that may result in misclassification of the health condition. More specifically, case ascertainment was self-reported by study participants and did not include clinical confirmation and/or medical report. Due to these limitations, both Kamel et al. (2005) and (2007) were ranked low quality. Motsoeneng and Dalvie (2015) further examined association between pyrethroid metabolites and neurologic symptoms in women in South Africa and reported evidence of a moderately strong association. The overall quality of the study was ranked low and had several important limitations. Most importantly, the study used a cross-sectional design and was unable to assess temporal relationship between pyrethroid exposure and neurological symptoms. The use of non-specific biomarkers as a means to measure direct exposure to pyrethroids, and a single urine sample collected throughout the entire study was a study limitation. The small sample size was also a limitation of the study. Specifically, only six women in the farm group and four women in the town group reported difficulties with buttoning and unbuttoning, resulting in odd ratio estimates that lack precision and may be unreliable.

7.4.2 Coronary Heart Disease in Adults

One study evaluated the association between exposure to pyrethroid urinary metabolites and coronary heart disease (CHD) risk in adults.

Han et al. (2017) conducted a cross-sectional study to evaluate the association between pyrethroid exposure and coronary heart disease (CHD) risk among people living in Xin Zhou City of Shanxi province in China. The study population consisted of CHD cases (n = 72) and healthy age- and gender-matched controls (n = 136) identified between October 2013 and January 2014. Potential study participants with a family history of coronary heart disease, adverse medical history, and occupational chemical exposures were excluded from the study. The specifics of CHD case identification and control selection were not presented in further detail. Study subjects provided fasting urine samples, which were stored at −80°C until shipment to the China Agricultural University for analysis. Urinary levels of three pyrethroid metabolites – urinary *cis*-CDDA, *trans*-CDDA, and 3-PBA – were quantified using GC-MS and categorized by tertile for analysis. Potential covariate information was collected by trained interviewers at the time of urine sample collection, and included sex, smoking status (never, occasional, often), alcohol consumption (never, occasional, often), education (< 9 years, 9 years < n < 13 years, > 13 years), place of residence (area or city), age (continuous), and BMI (continuous). Among the healthy control subjects, the median concentrations of urinary *cis*-CDDA, *trans*-CDDA, and 3-PBA were 1.03, 0.42, 0.74 µg/L respectively. Based on the metabolite concentration of the control group, individually measured pyrethroid metabolites (3-PBA, *cis*-CDDA, and *trans*-CDDA) were categorized into tertiles,

with the 1st tertile used as a reference to signify no/low exposure. The investigators then estimated odds ratios (OR) and corresponding 95% CIs from multivariable logistic regression models with stepwise variable selection used to select covariates that were significantly associated with case/control status (< 0.1). For 3-PBA, evidence of a strong association was observed for CHD in both the middle and upper tertile metabolites concentrations relative to the control group (middle tertile – OR: 3.52; 95% CI: 1.43, 8.62; upper tertile – OR: 3.62; 95% CI: 1.48, 8.88, with n = 32 cases and 45 – 46 controls/concentration level, p-trend = 0.009). For *cis*-CDDA and for *trans*-CDDA, evidence of a strong association was observed for CHD at the high metabolite concentration level only relative to the control group (*cis*-CDDA upper tertile – OR: 6.86; 95% CI: 2.76, 17.06 with n = 55 cases and 46 controls, p-trend = 0.00; *trans*-CDDA upper tertile – OR: 6.94; 95% CI: 2.80, 17.19 with n = 58 cases and 46 controls, p-trend = 0.00). No evidence of a significant positive association was observed at the low metabolite concentration level for *cis*-CDDA and *trans*-CDDA relative to CHD.

EPA Conclusion

Overall, there is insufficient evidence at this time to conclude that there is a clear associative or causal relationship between exposure to pyrethroid urinary metabolites and coronary heart disease (CHD) risk. The overall quality of Han et al. (2017) was ranked low. While the study reported evidence of a strong association between CHD and pyrethroid urinary metabolite levels, the study relied on a weaker cross-sectional design that could not assess the temporal association between pyrethroid exposure and congenital heart defects in adults. The assessment of exposure by measuring urinary metabolite levels in a single spot-urine sample from each subject may also accurately reflect longitudinal or longer-term exposure patterns and may be reflective of exposure to non-toxic, environmental degradants/metabolites that may be present in the environment and food. The study also reported very limited information on its design, so it is not clear that the investigators had clear case and control selection criteria. This could have introduced selection bias because it is not clear that cases and controls were recruited from the same general study population. An additional limitation of the study was that the statistical approach relied on stepwise variable selection. This variable selection method is considered unreliable and can result in biased parameter estimates with 95% confidence intervals that are too narrow due to underestimation of standard errors.[97] Finally, only minimal information was provided by authors with respect to analytical methods or QA/QC procedures and protocols.

7.4.3 Depression

Three studies (Beard et al. 2013, Beard et al. 2014, Campos et al. 2016) evaluated the relationship between exposure to pyrethroids and depression in female spouses and male pesticide applicators.

- Beard et al. (2013) conducted a prospective cohort study to evaluate the association of exposure to pyrethroids and other pesticides and depression among farmers' wives enrolled in the AHS. The study population consisted of female spouses (n = 16,893) in the AHS living in Iowa and North Carolina, and pesticide exposure was assessed during study enrollment for 50 different pesticides including pyrethroids using self-administered questionnaires. Cases included farmers' wives who self-reported physician-diagnosed depression between the time of study enrollment (1993-1997) to study follow-up (2005-2010), and cases were confirmed through responses to questions during the telephone follow-up interview. The controls included study participants who did not report incident depression. RRs and 95% CIs were calculated using a log-binomial regression model to determine if an association between ever-use of a pesticide and depression

[97] See for example Flom, P. L., Cassell, D. L. (2007). Stopping stepwise: Why stepwise and similar selection methods are bad, and what you should use. Statistics and Data Analysis. NESUG 2007 and Babyak, Michael A. (2004). What You See May Not Be What You Get: A Brief, Non-technical Introduction to Overfitting in Regression-Type Models. Psychosomatic Med. 66:411-421.

existed. Inverse probability weights were applied to adjust for education level, age at enrollment, ever diagnosed with diabetes, state of residence, and drop out, as well as account for the substantial number of study subjects (n = 10,639) who did not complete the follow up interview (1,342 due to death). Among the total 1,054 cases, 55 (5%) reported exposure to pyrethroids. No evidence of a significant positive association was observed between exposure to pyrethroids and incident depression among farmer's wives (RR = 1.07; 95% CI: 0.81, 1.40) based on ever/never use. A further analysis considered husbands' use of specific pesticides based on ever/never use and the risk of depression among their wives who had reported never using pesticides. Among the total 444 cases, 107 (26%) reported exposure to pyrethroids. No evidence of a significant positive association was for pyrethroids (RR = 1.08; 95% CI: 0.87, 1.36).

The quality of the study was ranked moderate. Strengths of the study included the AHS cohort study design, and the large sample size. Self-report of depression was a study limitation due to due to its non-standardized and non-clinically-based diagnosis. In this study, exposure misclassification likely occurred among women self-reporting pesticide exposure relative to incident depression, especially since pesticide exposure was only considered as a binary variable (ever/never exposure) and additional exposure study details such as frequency and duration were not asked at study enrollment for individual pesticides. Misclassification of exposure is more likely to have occurred in the subset analysis since it relied on self-report of the husband's pesticide usage to assess indirect exposure for their wives.

- In a separate study, Beard et al. (2014) conducted a prospective cohort study to evaluate the association between exposure to pyrethroids and other pesticides and depression among male pesticide applicators enrolled in AHS. Participants self-reported physician diagnoses of depression prior to enrollment only (defined as 'PRE-E' in the study), at both enrollment and follow-up (defined as 'PRE-B' in the study), or at follow-up only (defined as 'POST' in the study). Pesticide exposure (ever/never) was assessed via two self-administered questionnaires, one during study enrollment and a second follow-up questionnaire five years after enrollment. Polytomous logistic regression was used to calculate ORs and 95% CIs for individual pesticides. Inverse probability weighting adjusted for confounders including age, diabetes diagnosis, education level, and state of residence as well as missing covariate data for subjects and study drop-outs. Among the study population (n = 21,208), 1,702 (8%) reported receiving a diagnosis of depression (cases). Of those 1,702 cases, 474 reported depression diagnoses at enrollment but not follow-up, and 128 (28%) of those cases reported exposure to pyrethroids. A total of 540 individuals of the 1,702 cases reported depression diagnosis at both enrollment and follow-up, and 146 (28%) of those cases reported exposure to pyrethroids. Finally, 688 individuals of the 1,702 cases reported depression diagnosis at follow-up only, and 164 (25%) of those cases reported exposure to pyrethroids. There were 19,506 study participants who reported no physician diagnosis of depression (controls), and 4,805 (26%) of those controls reported exposure to pyrethroids. Results suggested no evidence of a significant positive association between pyrethroids exposure and risk of depression for those who reported depression at enrollment only (OR = 1.20; 95% CI: 1.00, 1.50); for those who reported depression at both enrollment and follow-up (OR = 1.10; 95% CI: 0.90, 1.40); and for those who reported depression at follow-up only (OR = 0.90; 95% CI: 0.80, 1.10). A Wald chi-square tests found no significant difference in the ORs between these groups for pyrethroids exposure (p = 0.17).

  The quality of the study was ranked moderate. The study leveraged the AHS to prospectively evaluate the relationship between depression and pesticide exposure in a large, well characterized agricultural cohort. Limitations for this study included misclassification of the outcome among the cases. For example, some of the cases who reported a previous diagnosis of depression at study enrollment failed to report depression at follow-up. As a result, these cases were classified

incorrectly as part of the 'PRE-E' group within the study, and instead should have been classified as part of the 'PRE-B' group. The study authors mentioned that the methods used to confirm the cases differed at study enrollment and at follow-up (self-report at study enrollment vs. in-person interview at follow-up), and perhaps may have contributed to the incorrect classification. Second, the limited information collected during the study regarding depression made it difficult to assess the association between depression and pesticide exposure long-term. Also, the study was unable to analyze current depression among study participants since the study questionnaires asked about depression diagnosis on an ever/never basis. Third, confounding was another potential study limitation since study participants were asked about exposures to several different pesticides. Although the study authors mentioned they performed inverse probability weighting in analyzing the study to adjust for potential confounding, they realized a small amount of confounding may still have been present. Additionally, missing information reported during the study from dropout and missing questionnaires may have also led to missing data bias and selection bias. Lastly, the study authors acknowledged that some misclassification of pesticide exposures inevitably occurred within this study, even though sensitivity analyses performed in previous studies had shown that self-reported exposures were reliable and accurate.

- Campos et al. (2016) conducted a population-based cross-sectional study to evaluate the association between pyrethroid and other pesticide exposures and common mental disorders including depression. The study population (n = 869) consisted of a representative sample of adults (> 18 years of age) of both genders sampled from the agricultural population of Dom Feliciano, Brazil. Between May 2011 and March 2012, the investigators conducted an in-home interview, during which time questionnaires were administered to ascertain self-reported pesticide exposure (history of acute pesticide intoxication, exposure to pesticides by chemical group, years of pesticide exposure, age at onset of pesticide exposure; history of illness after use of pesticides and use of personal protection equipment). During the same interview, self-reported history of symptoms consistent with common mental disorders was ascertained using the Self-Reporting Questionnaire (SRQ-20); the SRQ-20 is composed of 20 questions aimed at identifying psychosomatic symptoms associated with non-psychotic disorders. Using the SRQ-20, the investigators asked respondents to report symptoms present during the previous three months; they considered a symptom score ≥ 8 to be positive for their "common mental disorders" outcome. Self-reported history of depression diagnosis was assessed using a general health questionnaire. The authors estimated odds ratios (OR) and corresponding 95% CIs and p-values from multivariable logistic regression models adjusting for gender, age, SES indicators (food security and level of schooling), osteoarticular disease, and age began smoking. Among the study population, 298 participants (60.8%) reported pyrethroid use, and the prevalence of the common mental disorders outcome was 23% (n = 194) among the 840 participants who fully completed the SRQ-20; the prevalence of self-reported depression was 21% (n = 179). Although evidence of a positive association was observed between exposure to pyrethroids and self-reported depression (OR: 1.80; 95% CI: 1.01, 3.21; p = 0.047), when the Bonferroni correction was applied and the data was further stratified by time of use (time use categories: ≤ 5 years of use (control) and ≥ 6 years of use), no evidence of a significant positive association was reported for self-reported depression (≥ 6 years of use: OR: 0.79; 95% CI: 0.38, 1.64, p = 0.520). Furthermore, no evidence of a significant positive association between pyrethroid exposure prevalence of a common mental disorder (≥ 6 years of use: OR: 1.70; 95% CI: 0.34, 8.48, p = 0.519) was observed.

  The quality of the study was ranked low. A study strength included the large sample size. As for study limitations, the cross-sectional design did not permit discerning the temporality of observed relationships and the self-reported exposures and outcomes decreased the validity of the reported results. In the study, the SRQ-20 questionnaire was used to evaluate common mental disorders and should be assumed more of a screening instrument instead of a diagnostic tool, due to the

questionnaire's sensitivity to a wide range of affections that trigger positive mental suffering. Furthermore, the use of self-report instead of a definitive tool to measure exposure was another limiting factor of the study, that could have led to exposure misclassification.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and depression. Two prospective studies were conducted in AHS and reported no evidence of a significant positive association between exposure to pyrethroids and incident depression among farmer's wives (Beard et al., 2013) or male pesticide applicators (Beard et al., 2014). Both studies leveraged AHS to evaluate the relationship between depression and pesticides in a large, well characterized agricultural study population, but relied on self-report of physician-diagnosed depression. This approach may not systematically identify depression cases and introduce misclassification of depression in the study. An additional limitation of Beard et al. (2013) is that more limited questionnaire information was collected to assess the pyrethroid exposure of farmer's wives. As a result, the study only assessed ever/never exposure using their male spouse's pesticide use history as a proxy. In addition to the two AHS studies, Campos et al. (2016) reported no evidence of a significant positive association between pyrethroid exposure and both depression in rural Brazil. The study relied on a more limited cross-sectional design and was unable to assess the temporal relationship between pyrethroid exposure and depression. Additionally, the study assessed exposure using a questionnaire and may be subject to recall bias.

7.4.4 Suicide

One study investigated the association between pyrethroids exposure and suicide.

Beard et al. (2011) conducted a prospective cohort study to evaluate the association between exposure to a number of pesticides including pyrethroids and suicide among commercial applicators and farm owners/operators enrolled in AHS. Pesticide exposure was assessed via a self-administered questionnaire at enrollment. Cases (suicides after enrollment) were identified by linking the AHS cohort to state mortality files and the National Death Index through 2009. The Cox proportional hazards model was used to analyze the association between pesticide exposure and suicide risk and calculate HRs and 95% CIs, adjusting for age, sex, number of children, frequency of alcohol consumption within the past year, and smoking. Among the study population (n = 81,998), 13,326 reported pyrethroids exposure. There were 110 suicides (cases) occurring between enrollment in the AHS (from 1993 to 1997) and May 2009. The study results suggested no evidence of a significant positive association between suicide and pyrethroids exposure (HR = 1.09; 95% CI: 0.68, 1.74 with n = 23 exposed cases) based on ever/never use

EPA Conclusion

Beard et al. (2011) provides no epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and suicide. The quality of the study was ranked moderate. Study strengths include the prospective design of AHS and exposure assessment approach. The study was also able to identify suicide cases using the National Death Index. This approach may be comprehensive for suicide cases resulting in mortality but provides incomplete characterization of suicidal behavior because cases of suicide attempt and ideation cannot be identified using the National Death Index and addition information to help characterize suicidal behavior was not obtained via questionnaire. Lastly, healthy worker effect bias may have been present and contributed to the small number of cases observed in this study, assuming people considered to be depressed who potentially became suicidal may have been less likely to enroll in this AHS study.

7.4.5 Hearing Loss

One study investigated the association between pyrethroids exposure and hearing loss.

Crawford et al. (2008) conducted a prospective cohort study to evaluate the association between pyrethroid exposure and hearing loss among white male pesticide applicators[98] enrolled in AHS. The study population consisted of white male pesticide applicators living in Iowa and North Carolina, who indicated experiencing hearing loss during a follow-up interview. Pesticide exposure was assessed via a self-administered questionnaire at study enrollment and during a follow-up interview conducted five years later. Investigators then used this information to estimate intensity-weighed cumulative days of use for individual pesticides. Logistic regression analysis was used to calculate ORs controlling for age, state, solvent exposure, metal exposures, and noise exposure. Among the study population (n = 14,229), 4,926 hearing loss cases were reported and 9,303 controls were identified. Missing data was reported for 341 cases and 657 controls. Two levels of exposure (excluding the referent (no exposure)) were constructed based on non-intensity-adjusted cumulative lifetime days of use and included the following: low exposure (2.5 – 17.5), and high exposure (> 17.5), and ORs were reported for each group. No evidence of a significant positive association between hearing loss and exposure to pyrethroids was reported in either the low and high exposure groups (low exposure group OR: 1.06; 95% CI: 0.94, 1.19 with 595 (13%) cases and 1,085 (12%) controls; high exposure group OR: 1.03; 95% CI: 0.92, 1.16 with 611 (13%) cases and 1,155 (13%) controls, with a p-trend = 0.69).

EPA Conclusion

Overall, there is no epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between exposure to pyrethroids and hearing loss. Crawford et al. (2008) reported no evidence of a significant positive association between hearing loss and exposure to pyrethroids in either the low and high exposure groups. The overall study quality was ranked moderate. Study strengths include the prospective design of AHS and exposure assessment approach. The self-reported diagnosis (poor case ascertainment) was a limitation and may have been underreported in the study due to societal stigmas associated with hearing loss.

7.4.6 Amyotrophic lateral sclerosis (ALS)

One study investigated the association between pyrethroids exposure and ALS.

Kamel et al. (2012) conducted a nested case control study to evaluate the association between exposure to pesticides including pyrethroids and ALS among participants of the AHS. Exposure to pyrethroids and other pesticides, defined as ever use, was ascertained by self-reported questionnaires at study enrollment in the AHS (1993 – 1997). Cases of ALS were identified by state mortality files and the National Death Index and were defined as ALS listed as an underlying or contributing cause of death. Of the n = 84,739 AHS participants, 41 cases of ALS were identified by death certificate from enrollment through February 7, 2010, and 6 (15%) of these cases were identified as having been exposed to pyrethroids, and of the 84,698 non-cases, 12,471 (15%) reported exposure to pyrethroids. Unconditional logistic regression models were adjusted for age and gender because ALS incidence is greater in men and risk of ALS increases with increased age. Results suggested no evidence of a significant positive association between exposure to pyrethroids and ALS (OR = 1.40; 95% CI: 0.60, 3.40).

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids and ALS. Kamel et al. (2012) reported no evidence of a significant positive association between exposure to pyrethroids and ALS. The quality of the study was ranked moderate. Study strengths included its prospective design, and the use of AHS cohort data although the study was limited by reliance on the National Death Index to identify ALS cases. This

[98] The study authors indicate that cases included white male pesticide applicators only, as there were too few non-white male pesticide applicators along with female pesticide applicators who met the case definition, to be included within the analysis.

approach will miss subjects who currently have ALS or died because of another cause. Additionally, a small number of reported cases exposed to pyrethroids (n = 6) was observed.

7.4.7 End-Stage Renal Disease (ESRD)

One study investigated the relationship between pyrethroid exposure and ESRD in the AHS cohort.

Lebov et al. (2015) conducted a prospective cohort study to evaluate the association between exposure to pyrethroids and end-stage renal disease (ESRD) among female spouses of pesticide applicators enrolled the AHS. ESRD cases were ascertained through linkage with the U.S. Renal Data System (USRDS). Of the 31,142 study participants, 98 ESRD cases were identified. Pesticide exposure was assessed by information obtained via self-administered questionnaires completed at enrollment and at home, with this information used to assess both direct exposure (wives' personal use of pyrethroids) and indirect exposure (husbands' use of pyrethroids). The Cox proportional hazards model was used to calculate HRs for ESRD, controlling for age. For direct exposure (pesticide use by female spouses), of the total 34 cases of ESRD that were observed, 3 (10.3%) reported exposure to pyrethroids, and of the total 17,391 non-cases, 1,423 (8.9%) reported pyrethroids exposure. No evidence of a significant positive association was observed between direct pyrethroids exposure and ESRD (HR: 1.57; 95% CI: 0.50, 4.91). For the indirect exposure analysis (husbands' use of pesticides among wives' who reported no personal use of pesticides), 64 confirmed cases of renal disease were identified among study participants, and 7 (13.0%) of those cases reported husbands' ever use of pyrethroids. Among the 13,653 non-cases, 2,798 (23.1%) controls reported husbands' ever use of pyrethroids. Results suggested no evidence of a positive association between indirect pyrethroids exposure and ESRD (HR = 0.68; 95% CI: 0.31, 1.49).

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and end-stage renal disease. Lebov et al. (2015) reported no evidence of a significant positive association between direct pyrethroids exposure and ESRD in farmers' wives and no evidence of a positive association between indirect pyrethroid exposure and ESRD in farmer's wives. The quality of the study was ranked moderate. As part of the AHS, this study benefited from the study design and the strengths of the AHS study cohort. However, the study was only able to consider pesticide exposures before study enrollment because of the low response rate follow-up AHS questionnaires. The small number of exposed cases (< 5) created wide confidence intervals and decreases the reliability of the results in this study.

7.4.8 Diabetes and Glucose Abnormalities

Diabetes

One study (Montgomery et al. 2008) investigated the relationship between diabetes incidence and pyrethroid exposure in the AHS cohort.

Montgomery et al. (2008) conducted a prospective cohort study to evaluate the association between pyrethroids exposure and diabetes among pesticide applicators enrolled in the AHS. The study population consisted of participants in the AHS (n = 33,457)[99], and incident diabetes was identified via self-report at either enrollment, on the take-home questionnaire and during a follow-up interview completed five years after enrollment in the AHS (1999 – 2003). A questionnaire detailing pesticide usage was used to determine lifetime exposure. Logistic regression was used to determine the association between diabetes and pyrethroids exposure. Among the 1,176 diabetic cases, 235 (20%) reported ever use of pyrethroids.

[99] The study population included only applicators who completed questionnaires at enrollment and follow-up.

Among the 30,611 non-diabetic controls with complete data, 7,006 (23%) reported ever use of pyrethroids. Reported results provide no evidence of a positive association between ever use of pyrethroids and diabetes (OR = 0.97; 95% CI: 0.84, 1.13), and when adjusting for BMI and state of residence in addition to age, no evidence of a significant positive association was observed (OR = 1.07; 95% CI: 0.92, 1.25).

EPA Conclusion

Overall, there is no epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between exposure to pyrethroids and diabetes. In Montgomery et al. (2008), no evidence of a positive association between ever use of pyrethroids and diabetes, and when adjusting for BMI and state of residence in addition to age, no evidence of a significant positive association was observed. The quality of the study was ranked moderate. Self-reported diagnosis of diabetes among the study participants and the inability to control for diet and exercise were considered study limitations and may have resulted in misclassification of some of the observed results and/or errors induced by confounding, respectively. The potential for selection bias was also present since a large number of participants who did not complete a follow-up questionnaire might have been diabetic at study enrollment.

Glucose Abnormalities

Two studies (Wang et al. 2011, Hansen et al. 2014) investigated the association between pyrethroid exposure and abnormal glucose regulation among factory workers in China and in Bolivia.

- Wang et al. (2011) conducted a cross-sectional study to evaluate the association between pyrethroid pesticide exposure and abnormal glucose regulation among factory workers in China. In this study, abnormal glucose regulation included diabetes and impaired glucose regulation. Cases included males and females, aged 20 – 60 years old, who reported exposure of 8 hours/day for > 1 year while working in one of the two defined pesticide factories in China between March to June 2009. Controls included factory workers who did not report any exposure to pyrethroid pesticides. Blood samples were collected from the cases and controls to measure blood glucose levels, in addition to administering the oral glucose tolerance test to determine glucometabolic state. Medical and occupational histories were also obtained. A logistic regression was used to determine ORs and 95% CIs, adjusted for age, gender, smoking, and drinking. Among the total number of study participants (n = 3,080), 1,347 reported exposure to pyrethroids and 1,733 reported no exposure to pyrethroids. Evidence of a positive association was observed between exposure to pyrethroids and abnormal glucose regulation in pesticide factory workers (OR: 1.48; 95% CI: 1.24, 1.77).

  The quality of the study was ranked low. Study limitations included the cross-sectional study design and inability to determine temporality due to the cross-sectional study design. Additional limitations included no information regarding how many blood samples were collected from each of the study participants, and limited information on the exposure to pyrethroids (*i.e.,* study did not define any specific details on pyrethroids and exposure to pyrethroids was based on 'yes/no' response only from study participants).

- Hansen et al. (2014) conducted a cross-sectional study to evaluate the association between occupational pesticide exposure including pyrethroids and abnormal glucose regulation among male pesticide sprayers employed in public vector control programs (n = 108) and a reference group of non-exposed referent subjects (n = 89). Sprayers were recruited from among 160 current and former sprayers working in vector control centers located in three large Bolivian cities (La Paz, Santa Cruz, and Cochabamba). Non-exposed participants were recruited from the non-

spraying employees working at the same vector control centers, including university students taught by some of the investigators (n = 41), and some others (n = ~ 26). Study evaluations were conducted in June and July of 2012 several months after the intensive pesticide application season. To ascertain demographic information, health status, and pesticide exposure, an occupational medicine specialist administered an interview questionnaire. The primary exposure metric was being a pesticide sprayer (yes/no). Based on their survey responses, sprayers were additionally characterized according to three additional pesticide exposure metrics: duration of pesticide spraying (total number of years working with pesticides), intensity of pesticide spraying (number of hours of spraying per week in the weeks with actual spraying), and cumulative pesticide exposure (total number of hours sprayed). These continuous exposure variables were then collapsed into quintiles. Blood specimens were collected at the time of the interview, then processed, frozen, and shipped to a private laboratory for analysis, using high-performance liquid chromatography. Participants were categorized as normal (HbA1c < 5.6%) or prediabetic/diabetic (HbA1c ≥ 5.6%). Covariates to be included in multivariable models were selected *a priori* and included body mass index, age, educational level (less than primary school, primary school, secondary school or technical education, university), use of antidiabetics (yes/no), family history of diabetes (yes/no), location (La Paz, Santa Cruz, or Cochabamba (as a proxy for ethnicity), and smoking status (never smoker, ex-smoker, or current smoker). Separate logistic regression models were used for the abnormal glucose regulation and symptom-based outcomes and presented as odds ratios and corresponding 95% CIs. The authors also evaluated dose response trends in risk of HbA1c ≥ 5.6% and the symptoms of glucose dysregulation by quintiles of exposure metrics. Analyses were performed for all sprayers (n = 108) and controls (n = 89) and among the subset of pesticide sprayers who reporting having only used pyrethroids (n = 52). Among sprayers exposed to only pyrethroids, evidence of a strong association was observed for abnormal glucose regulation (HbA1c ≥ 5.6%) relative to non-sprayer referents (OR: 18.50, 95% CI: 5.50, 62.50 with n = 32 cases, 7 controls), however the small number of cases and corresponding wide confidence intervals, decreases the reliability of the results. For the dose-response analysis, none of the quintile-specific odds ratios were statistically significant (1.0 ≤ adjusted OR ≤ 14.70; all CIs encompassed the null value of 1.0, with n = 21 – 23 exposed cases/quintile), and there were insufficient data to report associations for the two highest quintiles. Nevertheless, evidence of a statistically significant p-trend (0.014) was reported in the adjusted analysis between self-reported cumulative exposure and abnormal glucose regulation among sprayers, Similar positive trends were not observed for duration or intensity of spraying pyrethroid pesticides.

The quality of the study was ranked low. Study strengths included the means used to determine the exposure and outcome in the study (*i.e.,* conducting interviews and measuring the outcome via HPLC); however, the study was limited by the cross-sectional study design and lack of temporality, small number of cases, as well as the differences between the sprayers and non-sprayer groups. For example, the sprayers were substantially older, had higher BMI, were more likely to be current or former smokers, had higher prevalence of family history of diabetes, and were less educated, relative to the referent participants. As a result, residual confounding was possible.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a causal or clear associative relationship between pyrethroids exposure via collected blood samples and glucose abnormalities. Although two studies (Wang et al. 2011, Hansen et al. 2014) reported evidence of a positive association between pyrethroids and abnormal glucose abnormality, the quality of each study was ranked as low due to several study limitations. A major study limitation in both studies included the cross-sectional study design and the inability to determine temporality. Additionally, limited exposure information was provided in each study. Furthermore, Hansen et al. (2014) reported a small number of

cases. As a result, these mentioned study results decreased the reliability of the provided results, and we consider the epidemiological evidence as insufficient at this time.

7.4.9 Myopia

One study evaluated the association of pyrethroid urinary metabolites and myopia in adolescents and young adults.

Migneron-Foisy et al. (2017) conducted a cross-sectional study to evaluate the relationship between pyrethroid exposure and myopia among adolescents (12-19 years-old) and young adults (20-40 year-old) participating in NHANES, 1999-2008. The study population (n = 2,911) focused on individuals aged 12 to 40 years to exclude development of cataracts and other age-related ocular factors that could impact refraction. A NIDEK ARK-760 autorefractor was used to measure the refractive error of each eye after removing corrective lenses. The outcome variable in this study had two categories: moderate myopia (Spherical equivalent (SphEq) ≤ -1.00 and > -5.00 Diopter (D)) and high myopia (SphEq ≤ -5.00 D). The reference group consisted of subjects presenting with emmetropia/low hyperopia (SphEq > -1.00 and less than 3.00 D). At physical examination, one-spot urine sample was collected and metabolite concentrations resulting from the degradation of pyrethroids were determined. Multiple logistic regression models stratified by age (12 - 19 years old (adolescents) and 20 - 40 years old (young adults) and sex were used to assess the relation between log10-transformed urinary levels of pesticide metabolites and the risk of moderate and high myopia, taking into account the complex survey design. Models were additionally adjusted for sex, age, ethnicity, diabetes, creatinine, cadmium and lead concentrations, and income in both age groups, and also for education level and cigarette and alcohol consumption in the adult group. 3-PBA values above the lower detection limit were found in 74.6% of the analyzed samples. No evidence of a significant positive association was observed between a 10-fold increase of 3-PBA metabolites relative to moderate myopia and high myopia among adolescents (0.74 ≤ OR ≤ 1.19; all CIs encompassed the null value of 1.0 with n = 1,523 samples) and among young adults (0.87 ≤ OR ≤ 1.35; all CIs encompassed the null value of 1.0 with n = 1,388 samples).

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroid urinary metabolites and myopia. The quality of the study was ranked low. While the study was able to examine the association between pyrethroid urinary metabolites and myopia in a large, nationally representative sample of the U.S. population, the study was unable able to assess temporality because of the cross-sectional design of NHANES. Additionally, NHANES only measures pyrethroid metabolite levels in a single, spot-urine measurement from each participant. This approach may not accurately reflect longitudinal or longer-term exposure patterns and may also simply be reflective of exposure to non-toxic, environmental degradates/metabolites that may be present in the environment and food.

7.4.10 Sleep Apnea

One study investigated the relationship between pyrethroid exposure – specifically bifenthrin, cyfluthrin, lambda cyhalothrin, and tefluthrin – and sleep apnea in the AHS cohort.

Baumert et al. (2018) conducted a nested case-control study to evaluate the association between exposure to individual pyrethroids and sleep apnea among male pesticide applicators enrolled in the AHS, using data from the Agricultural Lung Health Study (ALHS). Cases included asthmatic pesticide applicators (97% males) who self-reported physician-diagnosed sleep apnea via an ALHS telephone interview. AHS exposure questionnaires (one at enrollment (1993 – 1997), two at follow-up (1999 - 2003, 2000 – 2010)) were used to assess ever/never exposure for select pyrethroid pesticides. Controls were randomly selected from the AHS cohort and included study participants who did not self-report sleep apnea. Logistic regression was used to calculate ORs and 95% CIs adjusting for state, age, diabetes, BMI, asthma,

hypertension, and cardiovascular disease. Among the total cases (n = 234) and controls (n = 1,335), the following number of cases reported, respectively, exposure to bifenthrin, cyfluthrin, lambda cyhalothrin, and tefluthrin: 5 (2.1%), 28 (12.0%), 18 (7.7%), and 19 (8.1%), and the following number of controls reported exposure to bifenthrin, cyfluthrin, lambda cyhalothrin, and tefluthrin: 11 (0.82%), 137 (10.3%), 87 (6.5%), and 76 (5.7%). Although evidence of a strong association was observed between bifenthrin and sleep apnea in male pesticide applicators (OR: 4.39; 95% CI: 1.33, 14.50 with n = 5 cases, 11 non-cases, p = 0.015), due to the small number of cases reported and since no evidence of a positive association was observed for the other individual pyrethroids cyfluthrin, lambda cyhalothrin, and tefluthrin exposure relative to sleep apnea in male pesticide applicators ($1.24 \leq ORs \leq 1.38$; all CIs encompassed the null value of 1.0; p-values > 0.05 n = 18 – 28 cases, 76 – 137 non-cases), we consider these study results to be less reliable.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids and sleep apnea in male pesticide applicators. The quality of the study was ranked moderate. Although evidence of a positive association was observed between bifenthrin and sleep apnea in male pesticide applicators, due to the small number of cases reported and since no evidence of a positive association was observed for the other individual pyrethroids, we consider these study results to be less reliable. Study strengths included the case-control study design, and the extensive amount of AHS data, including individual pyrethroid data. Study limitations included recall bias due to the self-reported exposure that potentially led to exposure misclassification. Furthermore, ascertainment relied on a self-reported diagnosis of sleep apnea, instead of a clinical confirmation, and may have resulted in outcome misclassification.

7.4.11 Respiratory Effects

Wheeze

One cross-sectional study (Hoppin et al. 2016) investigated the association between allergic and non-allergic wheeze and individual pyrethroid pesticide exposures among male farmers.

Hoppin et al. (2016)[100] conducted a cross-sectional study to investigate the association between exposure to individual pyrethroid pesticide and allergic and non-allergic wheeze among male farmers enrolled in the AHS. The study population consisted of male participants in the AHS (n = 22,134) who completed a self-reported questionnaire at enrollment (1993 – 1997) detailing pesticide usage and symptoms of wheeze. Cases were subdivided into allergic wheeze (n = 1,310), defined as at least one episode of wheeze or whistling in the chest in the past year with a physician-diagnosis of hay fever, and non-allergic wheeze (n = 3,939), defined as at least one episode of wheeze or whistling in the chest in the past year without a physician diagnosis of hay fever. Survey information was used to assess specific pesticide exposure (current, past, or never use) and to assess frequency and duration of use. Among the 1,310 allergic wheeze cases, 2% (n ~ 26 - 27) reported current use of reported current use of bifenthrin 1% (n ~ 13), 2% (n ~ 26) reported current use of esfenvalerate, and zeta cypermethrin, 3% (n ~ 39) reported current use of tefluthrin, 5% (n ~ 66) reported current use of lambda cyhalothrin, and 8% (n ~ 105) reported current use of cyfluthrin. Among the 3,939 non-allergic wheeze cases, 1% (n ~ 39) reported current use of bifenthrin and zeta cypermethrin, 2% (n ~ 79) reported current use of esfenvalerate, 4% (n ~ 159) reported current use of lambda cyhalothrin and tefluthrin, and 9% (n ~ 355) reported current use of cyfluthrin. Of the 16,885 non-case subjects, 1% (n ~ 169) reported current use of bifenthrin, and zeta cypermethrin, 2% (n ~ 338) reported current of esfenvalerate, 3% (n ~ 507) reported current use of

[100] Hoppin et al. 2016 is not a strict update to Hoppin et al. 2006a/2006b or 2002. We can assume overlap in participants, but publications do not summarize the overlap.

tefluthrin, 4% (n ~ 675) reported current use of lambda cyhalothrin, and 8% (n ~ 1,351) reported current use of cyfluthrin. Polytomous logistic regression was used to determine the association between wheeze and ever exposure to each pesticide individually (compared to never exposed), and allergic and non-allergic wheeze were investigated separately. Models were adjusted for age, body mass index (BMI), state, smoking, and current asthma, as well as for days applying pesticides and days driving diesel tractors.

For the individual pyrethroids, evidence of a moderately strong association was observed for zeta cypermethrin exposure and allergic wheeze (OR: 2.02; 95% CI: 1.24, 3.30); no evidence of a significant positive association was observed for the other individual pyrethroids including bifenthrin, cyfluthrin, esfenvalerate, lambda cyhalothrin, and tefluthrin ($0.99 \leq ORs \leq 1.19$); all CIs encompassed the null value of 1.0). For non-allergic wheeze, no evidence of a significant positive association was observed for any of the individual pyrethroids ($0.88 \leq ORs \leq 1.13$; all CIs encompassed the null value of 1.0).

Exposure was further analyzed by frequency of current use (days per year used), with categories of exposure created by tertiles of the distribution of users' frequency of the individual pyrethroids cyfluthrin, lambda cyhalothrin, esfenvalerate, tefluthrin, and zeta cypermethrin, with the top tertile further divided into thirds, to create five days-per-year of use categories; frequency data for bifenthrin was not provided. For each category, ORs and 95% CIs were estimated, comparing the exposure category to subjects never exposed.

For the individual pyrethroids, evidence of a moderately strong association for allergic wheeze and zeta cypermethrin, specifically at 5 – 90 days per year of use only, was observed (OR = 2.16; 95% CI: 1.01, 4.65 with n = 8 cases). Due to the small number of cases and because no evidence of a positive association was observed for any other exposure category for allergic wheeze and for non-allergic wheeze for zeta cypermethrin, we consider these study results to be less reliable. For cyfluthrin, only the second highest exposure category (10 - 11 days per year of use) showed evidence of positive association with non-allergic wheeze (OR = 1.42; 95% CI: 1.02, 1.98 with n = 50 cases); this was not seen in other categories (past use, 1 – 3 days per year of use, 4 – 6 days per year of use, 7 – 9 days per year of use, and 12 – 200 days per year of use), so we similarly consider these study results to be less reliable. Additionally, no evidence of a significant positive association was observed for allergic wheeze for cyfluthrin. For lambda cyhalothrin, esfenvalerate, and tefluthrin, no evidence of a significant positive exposure-response relationship (*i.e.,* evidence of higher prevalence with increasing frequency of use) for both allergic and non-allergic wheeze was observed ($0.57 \leq ORs \leq 1.73$ for allergic wheeze; $0.78 \leq ORs \leq 1.09$; all of the CIs encompassed the null value of 1.00 in each exposure category.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids and allergic and non-allergic wheeze among male farmers. One study, Hoppin et al. (2016), examined the association between wheeze and pyrethroid exposure and was ranked low quality. Hoppin et al. (2016) benefited from the large AHS participant cohort with data collected on specific pesticide usage, demographics, and lifestyle factors. Weaknesses of the Hoppin et al. (2016) study included the cross-sectional study design and thus lack of relative temporal information on exposure and outcome, the potential for the healthy worker effect confounding the results poor case ascertainment from relying on self-reported outcome of the study participants instead of clinical and/or medical record the potential for recall bias and exposure misclassification.

Asthma

Two cross-sectional studies (Hoppin et al. 2008, Mwanga et al. 2016) investigated the association between exposure to pyrethroids and pyrethroid metabolites and asthma among farm women. These study results are provided below:

- Hoppin et al. (2008) conducted a cross-sectional study to evaluate the association between pesticide exposure including pyrethroids and adult-onset asthma among farm women enrolled in the AHS. The study population consisted of female participants in the AHS (n = 25,814) who completed a self-reported questionnaire or telephone interview at study enrollment (1993 – 1997), detailing pesticide usage and whether they had received a physician's diagnosis of asthma. This information was used to assess pesticide exposure, to determine lifetime total years of pesticide use, and to assess frequency of application. Asthma cases were then subdivided into atopic or non-atopic asthma, where atopic asthma was based on self-reported eczema and/or hay fever. A polytomous logistic regression model was used to determine the association between asthma and pesticide exposure including pyrethroids controlling for age, state, smoking status, BMI, and whether or not the subject had grown up on a farm. A total of 702 adult-onset asthma cases were identified. Among the 282 atopic asthma cases among females, 18 (7%) reported ever use of pyrethroids. Among the 420 non-atopic asthma cases, 25 (6%) reported ever use of pyrethroids; of the 25,112 control subjects, 1,222 (5%) reported ever use of pyrethroids. For atopic asthma, no evidence of a significant positive association for pyrethroids exposure was observed (OR = 1.46; 95% CI: 0.90, 2.37). For non-atopic asthma, no evidence of a significant positive association for pyrethroids exposure was observed (OR = 1.34; 95% CI: 0.88, 2.02).

  The overall quality of the study was low. As part of the AHS, this study benefited from the strengths of the AHS study. Study limitations included the cross-sectional study design and thus lack of relative temporal information on exposure and outcome, the small number of cases, poor case ascertainment from relying on self-reported outcome of the study participants instead of clinical and/or medical record confirmation, exposure misclassification from the self-reported exposure, and the inability to analyze dose-response trends due to the lack of frequency and duration exposure details.

- Mwanga et al. (2016) conducted a cross-sectional study to evaluate the association between pyrethroid exposures and serum cytokine patterns as well as asthma-related outcomes among female farm workers and resident women living a rural area of the Western Cape province of South Africa. Although this study investigated other relationships, only the asthma-related findings from this study are summarized. The study sample (n = 211) was comprised of 113 women currently living on a farm (89 farm workers, and 24 farm residents not working on the farm), as well as 98 residents of neighboring towns, 8 of whom worked on farms. The women were categorized as being either farm dwellers (n = 121) or town dwellers (n = 90). Spot urine samples were collected during the working week at the end of the work day and kept on dry ice before to being stored at -20°Cprior to analysis. The following pyrethroid metabolites were quantified in the urine samples: *cis*- and *trans*-DCCA, DBCA, 4F3-PBA, and 3-PBA, using GC-MS/MS. All urinary pesticide metabolite levels were adjusted for urinary creatinine concentration; 18 samples with either very high, or very low, creatinine concentrations were excluded from the analysis. Urinary pyrethroid pesticide metabolite levels were dichotomized into high- and low-exposure groups. An exposure variable defined as the sum of the five pyrethroid pesticide concentrations was also calculated. Prevalence of asthma and asthma-related outcomes was assessed using the European Community Respiratory Health Survey (ECRHS) questionnaire. Sensitization to house dust mite, grass pollen, cat, dog, and cockroach allergens was determined using a Phadiatop® test (Thermo Fisher Scientific, Uppsala, Sweden). A blood

sample was also collected from each participant at the end of the work day. For the asthma, the main outcome variables - all dichotomous - were physician -diagnosed asthma, adult-onset asthma, and current asthma – were entered into logistic regression models to evaluate associations with the biomarkers of pesticide exposure. A logistic regression was performed to determine ORs and 95% CIs for individual pyrethroid metabolites relative to the outcome, adjusting for current smoking, atopy, having been born on a farm, and level of education. The prevalence of physician-diagnosed asthma was (n = ~23 - 24 cases, (11%)), adult-onset asthma (n ~ 18 – 19 cases, (9%)), and current asthma (n = ~ 12 – 13 cases (6%)) in this study. For *cis*-DCCA, *trans*-DCCA, DBCA, 4F3-PBA, and 3-PBA, no evidence of a significant positive association was observed for physician-diagnosed asthma, for adult-onset asthma, or for current asthma among farm women ($0.16 \leq$ ORs ≤ 2.64; all CIs encompassed the null value of 1.0), relative to women who resided in town. Further, for all the pyrethroids combined (*cis*-DCCA + *trans*-DCCA + DBCA + 4F3-PBA + 3-PBA), no evidence of a significant positive association was observed for doctor diagnosed asthma, for adult-onset asthma, or for current asthma among farm women compared to women who resided in town ($0.62 \leq$ ORs ≤ 2.04; all CIs encompassed the null value of 1.0).

The quality of the study was ranked low. Study limitations included the cross-sectional study design. Due to the urinary pesticide metabolites and study endpoints were assessed at the same point in time in this cross-sectional study, the associations observed do not provide direct evidence of a temporal relationship between pesticide exposure and the asthma-related outcomes. Additionally, the non-specific nature of the urinary metabolites and their quantification in only a single urine sample may have contributed to the substantial misclassification of typical pesticide exposures among the study participants. No information on laboratory QA/QC procedures or results was provided. Lastly, the small number of cases reported decreased the reliability of the study.

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and asthma among farm women. The AHS study, Hoppin et al. (2008), reported no evidence of a significant positive association between pyrethroids exposure and asthma among farm women. Similarly, for urinary pyrethroid metabolites, Mwanga et al. (2016) reported no evidence of a significant positive association among women farmers. Study strengths included the use of AHS data (in the case of the Hoppin (2018) data and the various measurements used to determine exposure. Both studies were limited by the cross-sectional study design and small number of cases reported.

Chronic Bronchitis

One cross-sectional study evaluated the association between pyrethroids exposure and chronic bronchitis in non-smoking farm women.

Valcin et al. (2007) conducted a cross-sectional study to evaluate the association between exposure to pesticides including pyrethroids and chronic bronchitis among farm women enrolled in the AHS. The 21,541 study participants were non-smoking female spouses of pesticide applicators. Participant-administered questionnaires determined pesticide exposures including pyrethroids, health outcome (self-report of physician-diagnosed chronic bronchitis), and potential confounders. A logistic regression was used to calculate individual ORs and 95% CIs for specific, controlling for age and state. Of the 583 cases, 6% reported exposure to pyrethroids, defined as total years and days of use. Of the 20,958 controls, 5% reported exposure to pyrethroids. Results showed no evidence of a significant positive association between chronic bronchitis and pyrethroids exposure, using a logistic regression model (OR = 1.33; 95% CI: 0.91, 1.93) when adjusted for age and state. Additionally, further adjusting the model to account for variable within each functional group similarly resulted in no evidence of a significant positive

association between chronic bronchitis and pyrethroids exposure, respectively (OR = 1.13; 95% CI: 0.76, 1.68).

EPA Conclusion

Overall, there is insufficient epidemiological evidence at this time to conclude that there is a clear associative or causal relationship between pyrethroids exposure and chronic bronchitis among farm women. One study, Valcin et al. (2007), reported no evidence of a significant positive association between exposure to pyrethroids and chronic among farm women. The study was of cross-sectional design and quality was ranked low. A strength of the study included use of the AHS data. Study limitations included the cross-sectional study design, which prevented the study from asking the study participants about respiratory signs and symptoms during enrollment.

8 Overall Conclusion

A total of 62 published epidemiologic studies on the association between exposure to pyrethroids, as either a chemical class or individual pyrethroids, and adverse health outcomes were reviewed in this Tier II Epidemiology Review. Based on review of these studies, there is insufficient evidence to suggest a clear associative or causal relationship between exposure to pyrethroid pesticides and the carcinogenic and non-carcinogenic health outcomes examined in the epidemiologic literature.

The reviewed studies were derived from almost 600 that were originally identified by a systematic literature review and include, particularly, large numbers of articles investigating pyrethroid exposure and the outcomes of (i) neurobehavioral, neurodevelopmental, and neurocognitive effects in children; (ii) male reproductive effects including semen quality, reproductive hormone levels, and sperm damage and genetic abnormalities; and (iii) birth effects, including birth defects.

In general, many of the published studies were of low quality and relied on cross-sectional designs in which exposure measures and outcome measures are determined at the same time. Such designs are generally considered inferior to cohort or case-control studies in which a temporal sequence is actually established (*i.e.,* exposure happens before the outcome). In addition, the Agency found that:

- Many of these pyrethroid studies used urine biomonitoring data to assess exposure, analyzing for common pyrethroid metabolites/environmental degradates (3-PBA and *cis*- and *trans*-DCCA). Virtually all of these urine biomonitoring studies relied on only a single urine sample. A key criticism of this approach is that such measurements from a single urine sample reflect recent (at best, last several days) exposure and may not be reflective of preceding putative initiating events that would lead to the observed health outcomes. Further, such measures do not distinguish between (recent) exposure to active parent pyrethroid or what may be exposure to long ago pre-formed, non-toxic degradates found in food or the environment. Questionnaires in most cases – when used – were used to collect demographic, anthropometric, and similar data and did not inquire about use of pesticides. In those that did, results from the (again, single sample urine) biomonitoring did not seem to match well or necessarily correspond with the questionnaire responses (*e.g.,* reported high exposed users did not necessarily show high urine concentrations).

- There were a number of studies where the statistical analyses were either not optimal or were inappropriate, and multiple comparisons (uncorrected for multiple comparisons) were a large concern. In a number of cases, the numbers of comparisons done were numerous (dozens) such that statistical significance/p-values at this stage become close to meaningless. Several studies used automated stepwise regression techniques that similarly produced p-values which did not mean what they purport to mean and could introduce significant bias into the analyses.

- Many of the studies relied on very small counts of exposed cases with the outcome (*e.g.,* <5 to 10) or were otherwise generally quite small (*e.g.,* <20). And few studies – partly as a result – were able to assess exposure (dose)-response, which is an important component in the Bradford Hill considerations for causality.

- In a number of cases, concentrations of pyrethroid metabolites/degradates were less than the instrument LOD, which meant that suboptimal statistical procedures and/or default assumptions needed to be made (*e.g.* concentrations below the LOD were assigned a value of LOD/2).

- When groups of studies investigated ostensibly similar related outcomes, there were often differences in methods used to quantify those outcomes. Frequently, too, results from these

studies were seen to be discordant or mixed, and did not seem to necessarily point in the same direction or toward the same overall consistent conclusion.

- While the Agency found in a few instances strong (*e.g.,* odds ratios >3) or moderately strong (*e.g.,* odds ratios >2) associations between pyrethroid exposure and a given health outcome, these were in generally weak, low-quality studies with small counts and were either singular (only one study) or were not replicated in similar studies which did not find these relationships.

A more detailed review and summary of the findings organized by health outcome is provided below.

Carcinogenic Effects

- A total of 4 studies investigated the relationship between exposure to pyrethroids and carcinogenic outcomes including childhood brain tumors, childhood leukemia, and breast cancer.

  - For childhood brain tumors and childhood leukemia, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposures. While available studies on these childhood cancers reported some positive findings, there was only a single study on childhood brain tumors (Chen et al., 2016) and two studies on childhood leukemia (Ding et al., 2012 and Ferreira et al., 2013). Several limitations were noted for each of these studies and these were of low quality.
  - For breast cancer, there is ***no evidence*** at this time to conclude that there is a clear associative or causal relationship between pyrethroid exposure and breast cancer. This determination was based on a single, prospective study of farmers' wives within the AHS study population that reported no positive findings (Engel et al., 2005).

Neurodevelopmental, Neurobehavioral, and Neurocognitive Effects in Children

- A total of 16 studies investigated the relationship between exposure to pyrethroids and neurodevelopmental, neurobehavioral, and neurocognitive effects in children. These studies focused on prenatal exposure and neurodevelopmental effects (7 of 16 studies), pediatric exposure and neurobehavioral effects (8 of 16 studies), and Autism Spectrum Disorders (ASD) (3 of 16 studies).[101]

  - For prenatal exposure and neurodevelopment, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship between maternal exposure to pyrethroids and neurobehavioral/neurocognitive effects in children. Seven of the epidemiologic studies reviewed assessed maternal exposure by either measuring pyrethroid urinary metabolites during pregnancy or agricultural pesticide use in relation to maternal address. The findings from these studies were mixed: three studies reported evidence of a negative association with neurodevelopment in children aged from 1-year to 9-years-old (Xue et al., 2013, Gunier et al., 2017, and Furlong et al., 2017) with increased maternal urinary measurements of pyrethroid metabolites associated with decreased test scores; one study reported no evidence of a significant association with neurodevelopment (Viel et al., 2017); and two studies reported no association between pyrethroid exposure and neurodevelopment (Viel et al., 2015, Watkins et al. 2016). Finally, one study suggested *improved* neurodevelopment/neurocognitive test scores was associated with increased urinary

[101] Two studies (Viel et al., 2015, Viel et al., 2017) were accounted for twice (once in the prenatal exposure and neurodevelopmental effects section, and once in the pediatric exposure and neurobehavioral effects section) making the total count 16 epidemiology studies reviewed in the Neurodevelopmental, Neurobehavioral, and Neurocognitive Effects section.

pyrethroid metabolite concentrations; however, this positive association disappeared when fish consumption was added to the model (Hisada et al., 2017).

- For pediatric exposure and neurobehavior, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship between pediatric exposure to pyrethroids and neurobehavioral effects in children. Six of the eight studies reported statistically significant decrements in neurobehavioral function (Oulhote and Bouchard 2013, Wagner-Schulman et al., 2015, van Wendel de Joode et al., 2016, Viel et al., 2015, Viel et al. 2017, Wang et al., 2016) and the remaining two studies reported a non-significant association (Quirós-Alcalá et al., 2014, Fiedler et al., 2015). However, all studies relied on lower quality cross-sectional designs and thus could not assess the temporal association between pyrethroid exposure and neurobehavioral effects in children. Additional limitations of these studies included the assessment of pyrethroid exposure using only a single spot-urine sample from the child and – given what may be generally intermittent and occasional nature of exposures and short half-lives in the body – it is difficult to evaluate its accuracy. Further, only minimal information (if any) was provided by authors with respect to analytical methods or QA/QC procedures and protocols. In addition, the statistical analyses performed by Wang et al. (2016) appears to have not been fully appropriate given the statistical treatment of the data from the three schools targeted in the study. Additionally, a number of the studies were exploratory in nature, performing a broad range of multiple comparisons with no corrections for multiple comparisons or false discovery rates.
- For ASD, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship between pyrethroid exposure and ASD in children. Two of the three available articles (Shelton et al., 2014, Schmidt et al., 2017) were based on the same study population and assessed exposure using a GIS-based approach that was based on California PUR that has not been fully validated. As such, additional information is needed to characterize the relationship between California PUR information and the actual exposure-levels experienced by individuals as a result of living in agricultural communities. The remaining study (Domingues et al., 2016) was a cross-sectional study of 40 children that performed only a univariate analysis of the correlation between urinary 3-PBA levels in children and ASD. As such, the study did not adjust for confounders and was insufficient for purposes of evaluating the relationship between pyrethroid exposure and ASD.

Birth Effects

- A total of 8 studies investigated the relationship between pyrethroids and adverse birth outcomes, including anogenital distance (1 of 8 studies), gestational size (*e.g.,* birthweight and head circumference, 5 of 8 studies), birth defects (3 of 8 studies), and neonatal thyroid hormone level (1 of 8 studies).[102]

  - For anogenital distance, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure. This determination was based on a single study (Dalsager et al., 2017) that reported no evidence of a positive association between maternal urinary metabolite concentrations and anogenital distance in newborns.
  - For gestational size, there is ***insufficient*** at this time to conclude that there is a clear associative or causal relationship with maternal pyrethroid exposure. Overall findings were mixed, with one study reporting a positive association between urinary pyrethroid metabolite

[102] Two studies were accounted for twice in the Birth Effects section. Dalsager et al. (2017) was accounted for once in the anogenital distance effect section and once in the gestational size effects section, and Zhang et al. (2014) was accounted for once in the gestational size effect section and once in the thyroid hormonal levels in neonates effects section, making the total count eight epidemiology studies reviewed in the Birth Effects section.

levels and increased bodyweight (Hanke et al. 2003), three studies reporting no evidence of an association (Dabrowski et al., 2003; Ding et al., 2015; Dalsager et al., 2017), and one study reporting a negative association between 3-PBA and bodyweight (Zhang et al., 2014). Dalsager et al. (2017) was the only study to examine the effect of maternal pyrethroid exposure on abdominal circumference at three months of age and reported evidence of negative association between upper tertile of maternal 3-PBA levels and abdominal circumference amongst girls; however, no association was observed in the middle tertile of maternal 3-PBA levels for girls and a similar association was not observed in their separate analysis of boys.

- For birth defects, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure. Three studies (Meyer et al., 2006; Carmichael et al., 2014; Carmichael et al., 2016) investigated the relationship between maternal exposure to pyrethroids – specifically for bifenthrin, lambda-cyhalothrin, and cyfluthrin – and birth defects among children. Meyer et al. (2006) reported no evidence of a significant positive association between bifenthrin exposure and the birth defect, hypospadias. Carmichael et al. (2014) reported evidence of a moderately strong association between lambda-cyhalothrin exposure and atrial septal defect secundum ($n = 6$) and Carmichael et al. (2016) reported evidence of strong association between cyfluthrin and craniosynostosis ($n = 5$); however, both studies were determined to lack reliability because of the small number of cases ($n \leq 6$). Additionally, both Carmichael et al. (2014) and Carmichael et al. (2016) were exploratory and performed a large number of statistical comparisons, may have been subject to selection bias, and relied on a GIS-based approach to assess exposure that has not been validated.
- For thyroid hormonal levels in neonates, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure. This determination was based on a single study (Zhang et al., 2014) that reported no evidence of a positive association between maternal urinary pyrethroid metabolite concentrations and thyroid hormone levels in newborns.

Male Reproductive Health Outcomes

- A total of 13 studies investigated the relationship between pyrethroids and adverse effects on reproductive hormone levels (4 of 13 studies), semen quality (6 of 13 studies), and sperm damage and genetic abnormalities (6 of 13 studies).[103] These studies all relied on cross-sectional designs that were unable to assess the temporal association between pyrethroid exposure and male reproductive health outcomes and considered a wide range of biologic parameters that may influence male fertility. In addition to these studies, a single study investigated the relationship between pyrethroid exposure and fecundability.

  - For reproductive hormone levels, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure. Findings were mixed across the 4 available studies with no single pyrethroid metabolite-hormone association reported in more than a single study. For example, Meeker et al. (2009) found FSH statistically elevated for all pyrethroid metabolites investigated in that study (3-PBA, *cis*-DCCA, *trans*-DCCA, *cis*- + *trans*-DCCA, and sum pyrethroids), but Radwan et al. (2014) – looking for many of the same metabolites – found this for none. Similarly, Han et al. (2008)

[103] Three studies were accounted for twice in the Male Reproductive Effects section: Ji et al. (2011) was accounted for in both the semen quality effects and the sperm damage and genetic abnormalities effects; Radwan et al. (2014) was accounted for in both the reproductive hormone level effects and the semen quality effects; and, Meeker et al. (2008) was accounted for in both semen quality effects and sperm damage and genetic abnormalities, making the total count of 14 studies reviewed in the Male Reproductive Effects section.

and Yoshinaga et al. (2014) failed to find a significant association between FSH and the 3-PBA pyrethroid metabolite.

- For semen quality, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure. The studies evaluated a large number of associations between different urinary metabolites and a number of different semen quality parameters and reported mixed findings across the 6 studies. A negative association between 3-PBA and sperm concentration was reported in two studies (Xia et al., 2008, and Ji et al., 2011), but not in the other three studies that investigated the same association. No other pyrethroid metabolite-semen parameter association was reported in more than a single study.
- For sperm damage and genetic abnormalities, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure. As with other male reproductive outcomes, the studies evaluated a large number of associations and generally reported mixed findings across the 6 studies with only one positive association reported in more than one study: both Ji et al. (2011) and Jurewicz et al. (2015) reported positive associations between urinary 3-PBA levels and DNA fragmentation.
- For fecundability, there is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure. This determination was based on a single study (Sallmén et al., 2003) of low quality that reported decreased fecundability in males who were not efficiently protected from pyrethroid exposure.

Other Non-Carcinogenic Health Outcomes

- The remaining studies investigated the relationship between pyrethroids and a wide range of other health outcomes. These health outcomes were typically only examined in a small number of studies – often only a single study, but no more than 3 studies.

  - There is ***no evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure for the following health outcomes: neurologic effects, , suicide, hearing loss, and diabetes. .
  - There is ***insufficient evidence*** at this time to conclude that there is a clear associative or causal relationship with pyrethroid exposure for the following health outcomes: coronary heart disease in adults, depression, amyotrophic lateral sclerosis, end-stage renal disease, glucose abnormalities, myopia, sleep apnea, and the respiratory effects wheeze, asthma, and chronic bronchitis.

Overall -and in summary - the epidemiology review found that there is insufficient evidence to suggest a clear associative or causal relationship between exposure to pyrethroids and the carcinogenic and non-carcinogenic health outcomes investigated in the studies reported here. The Agency will continue to monitor the epidemiology data, and – if a concern is triggered – additional analysis will be conducted.

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Appendix A: Summary of Articles Selected for Inclusion

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
Baumert et al., 2018	Nested case-control study Male pesticide applicators	High-quality questionnaire answered at enrollment and follow-up	Self-reported responses by cases during follow-up telephone interview	Adjusted for state, age, diabetes, BMI, asthma, hypertension, and cardiovascular disease	Logistic regression was used to obtain OR and 95% CI	Recall bias, exposure misclassification	Moderate
Beard et al., 2011	Population-based case-control study Males only	Self-administered questionnaire requesting occupational history, smoking history, alcohol consumption, and demographic information in conjunction with job exposure matrix to estimate lifetime cumulative exposure	Cancer registry with histopathological verification	Adjusted for alcohol consumption, cigarette year, education level, pipe years, and respondent	Conditional logistic regression to obtain HR and 95% CI; a small number of exposed cases ($n \leq 12$)	Recall bias, exposure misclassification, lack of extensive medical histories of study participants	Moderate
Beard et al., 2013	Retrospective cohort study Males only	Employment history records were used to categorize level of exposure (*i.e.,* time-weighted average of exposure). Cumulative exposure was determined by multiplying work time by a weight assigned to its corresponding time-weighted average	Death certificates ascertained via the Social Security Administration and National Death Index. Expected death rates were determined via the National Center for Health Statistics	Moderate control for confounders. Adjusted for age, pay status, and calendar year	Miettinen method to obtain RR and	Employee records only used for exposure assessment, frequency and duration data for individuals was unknown, no demographic data provided; missing data; misclassification of exposure and outcome due to self-report	Moderate
Beard et al., 2014	Case-control study Males only	Two self-administered questionnaires at enrollment and follow-up regarding	Cases were ascertained by physicians	Adjusted for confounders including age, diabetes diagnosis, education level,	Polytomous logistic regression was used to obtain OR and 95% CI	Exposure and outcome misclassification from self-reported exposures	Moderate

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
		past pesticide exposure		and state of residence, as well as missing covariate data and study drop-outs			
Campos et al., 2016	Population-based cross-sectional study	In-home interview using questionnaires to document self-reported pesticide usage	Using a self-reporting questionnaire (SRQ-20), study participants were rated based on their responses to the questionnaire and participants with a symptom score of ≥ 8 were considered positive for "common mental disorders" outcome	Adjusted for gender, age, SES indicators (food security and/or level of schooling) and additional covariates	Multivariate logistic regression models were used to calculate an OR and 95% CI	Self-reported exposure, questionnaire used was not considered robust, exposure misclassification, temporality issue, lack of outcome ascertainment by physician or medical records	Low
Carmichael et al., 2014	Population-based case-control study	Participants' residences were geocoded and linked with pesticide use reporting records from the California Department of Pesticide Regulation, one month before, to two months after, each mother's reported date of conception	Participants of the California Center of the National Birth Defects Prevention Study (NBDPS) and ascertained by echocardiography, cardiac catheterization, surgery, or autopsy reports	Adjusted for ethnicity, education, maternal age at delivery, intake of folic acid supplements, alcohol use, and smoking during the month before or the first two months of pregnancy	Multivariate logistic regression models were used to calculate an OR and 95% CI	Exposure misclassification, small sample size, selection bias	Moderate
Carmichael et al., 2016	Population-based case-control study	Participants' residences were geocoded and linked with pesticide use reporting records from the California Department of Pesticide Regulation, one month before, to two months after, each mother's	Cases were identified via the California Center of the National Birth Defects Prevention Study (NBDPS) and ascertained via medical experts	Adjusted for birth defect, adjusting for ethnicity, education, and maternal age at delivery	Multivariate logistic regression models were used to calculate an OR and 95% CI	Exposure misclassification, selection bias, small sample size	Moderate

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
		reported date of conception					
Chen et al., 2016	Hospital-based case control study in China Children aged 0 – 14 years controls were recruited from two medical centers located in Shanghai between 2012 to 2015	Single spot urine sample and questionnaire data	A diagnosis of CBT (within the past 4 weeks)	Adjusted for sex, age, household income, maternal education level, and province of residence	Unconditional logistic regression was used to calculate OR and 95% CI	Single spot urine sample; information bias and recall bias were likely present; maternal exposure during pregnancy was not considered; a wide range of ages were used in this study for the cases and control	Low
Crawford et al., 2008	Cohort study Male pesticide applicators	High-quality questionnaires answered by subjects in AHS cohort regarding occupational pesticide use histories	Self-reported hearing loss	Adjusted for age, state, solvent exposure, metal exposures, and noise exposure	Logistic regression to obtain OR and 95% CI	Self-reported diagnosis (lack of case ascertainment) may have led to under-reporting	Moderate
Dabrowksi et al. 2003	Population-based case-control study; Polish women farmers and their offspring	Questionnaire on occupational histories and previous pesticide usage during pregnancy, in addition to surveying husbands on pesticide exposures, at 6 – 12 months following birth	Mothers who delivered children and completed a questionnaire via interview	Adjusted for place of residence	Linear regression to obtain OR and 95% CI	Self-reported exposure; recall bias, exposure misclassification	Low
Dalsager et al., 2017	Cohort study Pregnant women who were part of the Odense Child Cohort, In Denmark and their offspring	Single spot urine sample and questionnaire data	Birth records	For birth outcomes, adjusted for smoking, education, BMI, and gestational age For AGD, adjusted for weight for age z-score, and age at three months	Linear regression to obtain regression coefficients and 95% CI	No laboratory QA/QC reported; single urine sample collected	Moderate
Ding et al., 2012	Case-control study in China Children only	Questionnaire data obtained during an in-person interview as well as urine samples	Cases were identified from four children's hospital within Shanghai who had	Adjusted for sex, age, household income, place of residence, parent	Unconditional logistic regression analysis was conducted to	Single spot urine sample, recall bias, exposure misclassification	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
		using gas chromatography-mass spectrometry detection	been recently diagnosed with ALL (less than two weeks)	education level, and breast-feeding duration	determine ORs and 95% CIs		
Ding et al., 2015	Prospective cohort study in China Pregnant women	Questionnaire data obtained during an in-person interview as well as urine samples using GC-MS detection	Birth outcomes were determined using birth records	Adjusted for parity, infant sex, pre-pregnancy BMI, household monthly income, maternal age, passive smoking, and length of gestational length	Linear regression was used using regression coefficients and 95% CIs	Single spot urine sample	Moderate
Domingues et al., 2016	Population-based case-control study	Urine samples were collected and measured using gas chromatography	Cases were identified via the Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV) criteria, the Autism Diagnostic Observation Schedule, and Childhood Autism Rating Scale (CARS)	No study details provided	Non-parametric test (Mann-Whitney U) was used to statically compare urine concentrations between cases and controls and obtain correlation coefficient and 95% CI	Small sample size; no control for confounders; selection bias	Low
Engel et al., 2005	Case-control study Women only	Take-home questionnaire from spouses of enrolled applicators obtaining farm exposures, general health information, and reproductive health history; information obtained from applicators used as measure of possible indirect exposure to spouses	State cancer registries identifying malignant breast cancer	Adjusted for age, race and state; evaluated BMI, age at menarche, parity, age at first birth, menopausal status, age at menopause, family history of breast cancer, physical activity, smoking, alcohol consumption, fruit and vegetable consumption and education but none found to be significant	Poisson regression to obtain RR and 95% CI	Recall bias, exposure misclassification, lack of information on length of marriage could result in overestimating exposure based on husband	Moderate

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
Ferreira et al., 2013	Hospital-based case-control study in children in Brazil	Questionnaire data obtained during an in-person interview	Cases confirmed by morphology, immunophenotype, and standard cytogenetic-molecular methods	Adjusted for maternal age at birth, skin color, child's birth weight, maternal education, and oral contraceptive usage during pregnancy	Unconditional logistic regression was used to estimate OR and 95% CIs; small number of exposed cases	No measure of maternal urinary pyrethroid urinary biomarkers; recall bias; no correction for multiple comparisons	Moderate
Fiedler et al., 2015	Cross-sectional study in Thai children	Urine samples collected from children (first morning void) were measured for pyrethroid metabolites after being shipped to a laboratory	Neurobehavioral and Executive Functioning (Behavioral Assessment and Research System for Children); HOME scale was used to assess home environment	Adjusted for age and HOME total score	Mixed linear multivariate regression models	Temporality issue due to study design, minimal information about QA/QC procedures & analytical methods; no actual risk estimates reported (only reported p-values); no adjustment for multiple comparisons	Low
Furlong et al., 2017	Prospective birth cohort study	Maternal urinary pyrethroid biomarkers during late pregnancy (3-PBA, *trans*-DCCA, and *cis*-DCCA) dichotomized to indicate values above or below the limit of detection	Neurobehavioral and Executive Functioning (Behavioral Assessment System for Children, the Behavior Rating Inventory of Executive Function)	Adjusted for race/ethnicity, Adjusted for HOME scores, PDMPs, maternal marital status at follow-up, maternal education at follow-up, child sex, creatinine, visit	Longitudinal mixed models of neurobehavioral and executive functioning by prenatal urinary pyrethroid metabolite levels (dichotomized to indicate values above or below the limit of detection)	Selection bias due to substantial loss to follow-up; large proportion (>70%) of subjects with levels of urinary metabolite concentrations below the lower limit of detection; one spot urine sample measured; non-specific nature of the metabolites measured; testing scores/outcome assessment based on parental reporting of behavior	Low
Gunier et al., 2017	Longitudinal birth cohort study; Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, California Salinas Valley	GPS, GIS, and Pesticide Use Report data	Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) to determine FSIQ	Adjusted for child's age at IQ assessment, sex, language of assessment, maternal education, maternal intelligence, maternal country of birth, maternal	IQ modeled as a continuous variable using generalized additive models (GAMs); evaluated non-linearity with cubic splines; pesticide use expressed linearly (on log10 scale) in	Potential for selection bias (differential loss to follow-up), and non-differential measurement error for both pesticide exposures and the IQ outcome	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
				depression at 7-year visit, Home Observation for Measurement of the Environment (HOME) score at 7-year visit, household poverty level at 7-year visit, and prenatal urinary dialkyl phosphate (DAPs) concentration	final regression models		
Han et al., 2008	Cross-sectional study Males only	Single spot urine sample and questionnaire data, physical examination	Single blood sample	Adjusted for age, body mass index, drinking status, passive smoking, medicine intake, and history of operation	Multiple linear regression models to obtain correlation coefficient and 95% CI	Cross-sectional study design; temporality unknown	Low
Han et al., 2017	Case-control study	Urine samples were collected and measured using GC-MS	Not provided	Cases and controls matched on gender and age; Models adjusted for age and location of residence	Multivariate logistic regression	Selection bias; single urine sample collected; temporal variability; exposure misclassification; no ascertainment of cases	Low
Hanke et al., 2003	Retrospective case-control study in Poland	Questionnaire data on occupational histories of mothers three months prior to conception throughout the duration of pregnancy (all three trimesters)	Medical records were used to ascertain births	Adjusted for pesticide exposure, pregnancy duration, infant gender, pre-pregnancy weight, smoking during pregnancy, involvement in field work, type of farming, calendar year of birth, and maternal age	Linear regression model to obtain beta regression coefficient and 95% CI	Recall bias; small number of exposed cases; self-reported exposure	Moderate
Hansen et al., 2014	Cross-sectional study Males only	Interview questionnaire	HPLC method	Adjusted for BMI, age, educational level, use of	Multivariate logistic regression	Study design; lack of temporality; residual confounding (due to	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
				antidiabetics, family history of diabetes, location, smoking status		unmeasured confounders and/or confounder misclassification); no QA/QC information provided	
Hisada et al., 2017	Prospective cohort study in Tokyo, Japan Mothers and children	Maternal blood and urine samples using HPLC method	Kinder Infant Development Scale (KIDS)and Index of Child Care Environment (ICCE) questionnaire	Adjusted for a variety of covariates	Stepwise multiple regression model to obtain regression coefficient and 95% CI	Statistical method; single urine sample; no laboratory QA/QC information provided	Low
Hoppin et al., 2008	Cross-sectional study Females only	High-quality questionnaires answered by subjects in AHS cohort regarding past pesticide use	Self-reported physician diagnosed asthma	Adjusted for age, state, smoking status, BMI, and whether or not the subject had group up on a farm	Polytomous logistic regression to obtain OR and 95% CI	Small number of cases; Lack of case ascertainment and lack of exposure details (no frequency and duration provided); temporality issue due to study design	Low
Hoppin et al. 2016	Cross-sectional study Males only	Self-reported questionnaires at enrollment	Whistling in the chest (at least one episode within the past year) and physician diagnosis of hay fever (allergic wheeze only)	Adjusted for age, body mass index (BMI), state, smoking, and current asthma, as well as for days applying pesticides and days driving diesel tractors	Polytomous logistic regression was used to calculate OR and 95% CIs	Cross-sectional study design; temporality unknown; potential for the healthy worker effect confounding the results; recall bias; exposure misclassification	Low
Imai et al., 2014	Cross-sectional study Population based exploratory Males only	Single spot urine sample collected and measured via LC/MS/MS and questionnaire data, physical examination	Single semen sample	Adjusted for age, BMI, season of sampling, abstinence period, presence of varicocele, testis size, and frequency of cheese, soy, and non-oily white fish consumption	Stepwise multiple regression to determine regression coefficients	Cross-sectional study design; temporality unknown	Low
Ji et al., 2011	Cross-sectional study Males only	Self-reported questionnaire and underwent a physical examination	Semen samples were collected, and a	Adjusted for smoking, age, BMI, and abstinence time	Logistic regression was used to determine correlation	Cross-sectional study design; temporality unknown; single spot urine sample collected;	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
			TUNEL[104] assay assessed semen quality parameters including seminal volume, sperm concentration, motility, total count, and DNA fragmentation		coefficients and 95% CIs	no QA/QC procedures or results reported	
Jurewicz et al., 2015	Cross-sectional study Males only	Interview was conducted to determine occupational exposure(s)	Collected urine, salvia, and semen samples	Adjusted for sexual abstinence, age, smoking, past diseases, and alcohol consumption	Multiple logistic regression to obtain coefficients and 95% CI	Study design; temporality unknown men recruited from a fertility clinic, likely does not represent the general population; the use of non-specific biomarkers; single urine sample collected	Low
Jurewicz et al., 2016	Cross-sectional study Males only	Questionnaire data was used to access exposure	Collected urine and semen samples	Adjusted for sexual abstinence, age, smoking, past diseases, alcohol consumption	Multiple logistic regression was used to determine regression coefficient and 95% CI	Study design; temporality unknown men recruited from a fertility clinic, likely does not represent the general population; the use of non-specific biomarkers; single urine sample collected	Low
Kamel et al., 2005	Cross-sectional study Male pesticide applicators	High-quality questionnaires answered by subjects in AHS cohort regarding occupational pesticide use histories	Self-reported neurologic symptoms	Appropriate. Adjusted for state, age, education, cigarette smoking, and alcohol consumption	Logistic regression was used to calculate OR and 95% CI	Lack of case ascertainment; temporality issue; recall bias	Low
Kamel et al., 2007	Cross-sectional study	Self-administered questionnaires at study enrollment and during the follow-up	Self-reported PD	Adjusted for state, age, and type of participant	Hierarchical regression model was used to	Temporality issue, no case ascertainment; recall bias	Low

[104] The TUNEL assay was defined as: Terminal deoxynucleotidyl transferase (TdT) duTP Nick-End Labeling assay.

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
		telephone interview (5 years later) regarding past pesticide use		(applicator or spouse)	calculate OR and 95% CI		
Kamel et al., 2012	Nested case-control study	Self-administered questionnaires completed at home regarding past pesticide usage	State mortality files and the National Death Index	Adjusted for age and gender	Unconditional logistic regression model was used to calculate OR and 95% CI; small number of exposed cases (n = 6)	Exposure based on ever/never	Moderate
Lebov et al., 2015	Cohort study Females spouses of pesticide applicators	Self-administered questionnaires completed at enrollment and at home regarding past pesticide usage to assess both direct exposure and indirect exposure (from husbands' pesticide use)	Cases were ascertained via a national database	Adjusted for age	Cox proportional hazards model to obtain HR and 95% CI	Self-reported recall	Moderate
Lifeng et al., 2006	Cross-sectional study; males only	Self-reported occupational history; Air sampling for 3 days quantified by vapor phase chromatography; Personal air and dermal samples collected from three randomly selected participants in both the exposed and external comparison groups	Semen indices (liquefaction time, pH value, viscidity, sperm volume, sperm motility, percent motile sperm, sperm density, and sperm count per ejaculum) were quantified using WHO guidelines	No study details provided	One-way ANOVA	Lack of temporality due to study design; no control for confounding; small sample size	Low
Meeker et al., 2008	Cross-sectional study; males only recruited from andrology lab in Massachusetts General Hospital	Collected single urine sample	For the semen quality analysis, semen samples were collected to determine semen quality parameters	Adjusted for abstinence period and age in the semen quality analysis; adjusted for smoking and	Multivariate linear regression was used to determine OR and 95% CI (semen quality analysis);	Lack of temporality due to study design; single spot urine sample to determine long-term exposure; no information	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
			For the sperm DNA analysis, a Neutral Comet Assay was used to detect sperm damage	age in the sperm DNA damage analysis	correlation coefficients and 95% CIs (sperm DNA damage analysis)	on QA/QC procedures or results; selection bias	
Meeker et al., 2009	Cross-sectional study; males only recruited from andrology lab in Massachusetts General Hospital	Single urine sample	Collected blood samples	Adjusted for BMI, age, smoking and time of day at blood draw	Multivariate linear regression was used to determine correlation coefficients and 95% CIs	Lack of temporality due to study design; single spot urine sample to determine long-term exposure; no information on QA/QC procedures or results; selection bias	Low
Meyer et al., 2006	Case-control study Male children	Agricultural databases within the state were used to assess exposure	Cases and controls were identified via the state Reproductive Health Monitoring System and via the state's health vital records department via birth certificates	Adjusted for paternal education level, maternal age, weight gain during pregnancy, gestational age at birth, timing of first prenatal care visit, parity, number of cigarettes smoked per day during pregnancy	Multivariate unconditional logistic was used to calculate OR and 95% CI	Lack of racial diversity; controls potentially misclassified	Moderate
Migneron-Foisy et al., 2017	Cross-sectional study	One-spot urine sample was collected and metabolite concentrations were determined	Myopia - Two categories: Moderate (SphEQ ≤1.00 and >-5.00 D); High (SphEQ ≤-5.00 D)	Adjusted for sex, age, ethnicity, diabetes, creatinine (indicator of urine dilution), cadmium and lead concentrations, and income in both age groups, but also for education level and cigarette and alcohol consumption in the adult group	Multiple logistic regression models	Lack of temporality due to study design; several samples below limit of detection; one urine sample collected throughout testing	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
Montgomery et al., 2008	Prospective cohort study	Questionnaires provided at enrollment, take-home, and during the follow-up interview regarding past pesticide exposures	Self-reported incident diabetes	Adjusted for age, BMI, and state of residence	Logistic regression was used to calculate OR and 95% CI	No case ascertainment; lack of control for potential confounders; selection bias	Moderate
Motsoeneng and Dalvie 2015	Cross sectional study; Women only	Single urine sample	Q16 questionnaire made up of 16 questions, with yes/no responses as a means to assess symptoms associated with neurotoxicity	Adjusted for age, education, household income, drugs, alcohol usage, current smoking, language and previous poisoning	Logistic regression model was used to obtain OR and 95% CI	Lack of temporality due to study design; one urine sample collected throughout the study; small sample size	Low
Mwanga et al., 2016	Cross sectional study; Women only	Single urine sample	European Community Respiratory Health Survey (ECRHS) questionnaire was used to assess prevalence of asthma	Adjusted for current smoking, atopy, born on a farm, and level for education.	Logistic regression model was used to obtain OR and 95% CI	Lack of temporality due to study design; one urine sample collected throughout the study; small number of cases	Low
Oulhote and Bouchard 2013	Cross sectional study; Canadian Health Measures Survey (2007-2009)	Spot urine sample collected within 2 weeks of survey questionnaire completion	Parentally reported behavioral problems, using the parent version of the Strengths and Difficulties Questionnaire (SDQ) intended for use in population surveys	Adjusted for sex, age, race/ethnicity, income, parental education, blood lead levels, maternal smoking during pregnancy, birth weight, and urinary creatinine. Models for 3-PBA and ΣDAP, were further adjusted for BMI and fasting status.	Logistic regression was used to calculate OR and 95% CI	Lack of temporality due to study design; one urine sample collected throughout the study	Low
Quirós-Alcalá et al., 2014	Cross sectional study; based on NHANES cycles 1999-2000 and 2001-2002	Spot urine sample collected and measured at physical examination	Parent-reported	Adjusted for sex, age, race/ethnicity, education level (household), low birth weight status, maternal age at child's birth,	Multiple logistic regression models	Outcome misclassification; lack of temporality due to cross-sectional study design; single urine sample collected throughout study	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
				neonatal intensive care unit admission, maternal smoking during pregnancy, day care/preschool attendance, and health insurance			
Radwan et al., 2014	Cross-sectional study; participants recruited from an infertility clinic in Lodz, Poland Males only	Single urine sample using GC-MS and questionnaire data	For the semen quality analysis, collected semen samples were analyzed for semen morphology parameters For the reproductive hormone analysis, plasma samples were analyzed for reproductive hormones	Adjusted for age, abstinence, smoking, alcohol consumption, and past diseases	Multiple linear regression was run to determine regression coefficients and 95% CIs	Single spot urine sample collected; lack of temporality due to study design; selection bias	Low
Radwan et al., 2015	Cross-sectional study; participants recruited from an infertility clinic in Lodz, Poland Males only	Single urine sample using GC-MS and questionnaire data	Semen samples were analyzed for sperm aneuploidy	Adjusted for sexual abstinence, age, smoking, alcohol consumption, past diseases, sperm concentration, and motility.	Generalized linear mixed models with a Poisson distribution to determine regression coefficients and 95% CI	Cross-sectional study design; temporality unknown	Low
Sallmén et al., 2003	Synthetic Prospective Study, Conditional on Pregnancy, as stated by the authors	Self-reported exposure	Medically diagnosed pregnancies database	Adjusted for male participation in the study, female age, previous pregnancies, last contraceptive method, marital status, smoking, interaction of coffee and smoking, use of alcohol,	Proportional hazards regression models to obtain FDR and 95% CI	Selection bias; self-reported exposure and outcome; low participation rate; recall bias	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
				employment, work in greenhouses or gardens, spraying of pesticides, and missing information			
Schmidt et al., 2017	Case-control study; children aged 2 to 5 years Childhood Autism Risk from Genetic and Environmental (CHARGE) study	Retrospective self-report interview on folic acid intake and household pesticide exposure; statewide database of commercial pesticide applications to determine proximal agricultural pesticide exposures	Autism spectral disorder (ASD) identified from California Regional Center System	Adjusted for home ownership, child's year of birth, maternal vitamin B6 intake, and maternal vitamin D intake	Logistic regression to obtain OR and 95% CI	Potential missing data; self-report data	Low
Shelton et al., 2014	Case-control study; children aged 2 to 5 years Childhood Autism Risk from Genetic and Environmental (CHARGE) study	Linked California Pesticide Use Report data with maternal residential locations	Autism spectral disorder (ASD) confirmed using the Autism Diagnostic Observation Schedule or the Social Communications Questionnaire	Adjusted for home ownership, maternal place of birth, paternal education, child race/ethnicity, year of birth, and maternal prenatal vitamin intake (3 months prior to pregnancy to the first month of pregnancy)	Multinomial conditional multiple logistic regression to obtain OR and 95% CI	Limited exposure information reported or incorporated in the analyses; statistical analyses shortcomings;	Low
Valcin et al., 2007	Cross-sectional study Female spouses of pesticide applicators	High-quality questionnaires answered by subjects in AHS cohort regarding past pesticide exposures	Self-reported physician diagnosis	Adjusted for age and state	Logistic regression to obtain OR and 95% CI	Lack of case ascertainment; temporality issue due to study design; study failed to ask about signs and symptoms at enrollment	Low
van Wendel De Joode et al., 2016	Cross-sectional study; children aged 6 to 9 years in Costa Rica	Urinary samples to measure pesticide exposure	Neurobehavioral outcomes were determined using three	Adjusted for maternal education, child's gender, age, body mass index, number of siblings,	Linear regression to obtain coefficients and 95% CI	Temporality due to cross-sectional study design; multiple comparisons; no information on laboratory QA/QC procedures or	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
			psychometricians using well established neurobehavioral assessment instruments.	if child had repeated a school year, and visual acuity impairment.		results was provided; one of the administered tests, WISC-IV, was not standardized in Costa Rica at the time the study was conducted	
Viel et al., 2015	Prospective cohort study with cross-sectional analysis in Brittany, France; used data from the PELAGIE cohort Mother-child pairs	Urinary samples to measure pesticide exposure and self-administered questionnaires	At follow-up, Wechsler Intelligence Scale for Children was administered to children to assess cognitive abilities in verbal comprehension and working memory; mothers completed the HOME (Home Observation for Measurement of the Environment) survey; children also provided a urine sample which was measured by UPLC/MS-MS	Adjusted for several confounders for each pyrethroid, see text.	Reverse-scale Cox regression model to obtain regression coefficients and 95% CIs	Single urine sample collected; no information on laboratory QA/QC procedures or results was provided	Moderate - Neurodevelopmental Effects Low – Neurobehavioral Effects
Viel et al., 2017	Prospective cohort study with cross-sectional analysis in Brittany, France; used data from the PELAGIE cohort Mother-child pairs	Urinary samples to measure pesticide exposure and self-administered questionnaires	At follow-up, behavioral disorders were assessed in children age 6 years old, using three subscales (internalizing disorders, externalizing disorders, and prosocial behavior), all part of the Strengths and Difficulties Questionnaire (SDQ)	Adjusted for maternal tobacco smoking at the start of pregnancy, HOME score, child extra-curricular sport activities, child duration of television watching, and parity	Logistic regression and reverse-scale Cox regression model were used to determine OR and 95% CI	Single urine sample collected; no information on laboratory QA/QC procedures or results was provided	Moderate - Neurodevelopmental Effects Low – Neurobehavioral Effects
Wagner-Schuman et al., 2015	Cross-sectional study;	Single spot urine collection	Meeting DSM-IV criteria for ADHD using the National	Adjusted for child sex, household income to poverty	Logistic regression to obtain OR and 95% CI	Temporality due to cross-sectional study design; single spot urine sample	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
	8 to 15-year-old children, NHANES 2001-2002		Institute of Mental Health Diagnostic Interview Schedule for Children (DISC) or caregiver report of a prior ADHD diagnosis	line ratio, age, race/ethnicity, health insurance status, prenatal tobacco exposure, blood lead concentration, urinary organophosphate pesticide metabolite concentration (DMAP), urinary creatinine (to adjust for urine dilution)		collected throughout study	
Wang et al., 2011	Cross-sectional study; Pesticide factory workers in China	Blood samples collected (frequency not reported)	Abnormal glucose regulation	Adjusted for age, gender, smoking, and drinking	Logistic regression to obtain OR and 95% CI	Temporality due to cross-sectional study design; limited exposure information and number of blood samples collected; no QA/QC information provided	Low
Wang et al., 2016	Cross-sectional study; School-aged children in Nanjing, China	Single morning urine sample collected from child, along with maternal questionnaires	Neurobehavioral assessment was conducted using the following measures: Chinese Binet test, arithmetic test, maze test, cancellation test, and a picture completion test	Adjusted for age, sex, and outside school education expense	Multiple linear regression to obtain correlation coefficients and 95% CIs	Temporality due to cross-sectional study design; statistical analysis did not account for sampling from 3 different schools; no QA/QC information provided	Low
Watkins et al., 2016	Prospective cohort study in Mexico; using data from the ELEMENT cohort study Mother-child pairs	Maternal urine samples collected and measured via HPLC/MS-MS, along with a self-administered questionnaire	At follow-up, the neurodevelopment of each child was assessed at 2 and 3 years of age, using the Psychomotor Development Index (PDI) and the Mental Development Index	Adjusted for urinary specific gravity, blood lead, maternal IQ, child sex, SES score, and education	Logistic regression to obtain regression coefficients and 95% CIs	Single spot-urine sample to determine long-term exposure; measured metabolites concentrations were <LOD; missing data being reported; no QA/QC information provided	Moderate

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
			(MDI), as part of the Bayley Scales for Infant Development				
Xia et al., 2008	Cross-sectional study Males only	Single spot urine sample collected and measured via GC-MS and questionnaire data, physical examination	Single semen sample	Adjusted for age, body mass index, alcohol consumption, smoking status, and abstinence time	Conditional logistic regression to obtain ORs and 95% CI Multiple linear regression models to obtain regression coefficient and 95% CI	Cross-sectional study design; temporality unknown	Low
Xue et al., 2013	Prospective study in China Mother-child pairs	Blood and urine samples were also collected at this time, and measured using gas chromatography-mass spectrometry, along with questionnaires at follow-up	At follow-up, the child's intellectual development was measured using the Development Screen test scale	Adjusted for age; educational background; maternal occupation; presence or absence of any abnormalities in pregnancy; whether the mother took medicine; residence; feeding; main caretakers of infants, physical development index of 1-year old infants; presence or absence of severe disease; and presence or absence of exposure to harmful substances in addition to level of exposure to synthetic pyrethroids	Stepwise regression analysis to obtain correlation coefficient and 95% CI	Statistical approach used; single spot-urine sample to determine long-term exposure; no QA/QC information provided	Low

Journal Article	Study Design	Exposure Assessment	Outcome Assessment	Confounder Control	Statistical Analyses	Risk of (Other) Bias	Study Quality
Yoshinaga et al., 2014	Cross-sectional study Males only	Single spot urine sample collected and measured via LC-MS/MS, questionnaire data, physical examination	Single serum sample analyzed for reproductive hormone concentration	Adjusted for age, BMI, presence/absence of varicocele, season of blood sampling, smoking, and frequency of soy product consumption	Stepwise multiple regression to determine regression coefficients	Cross-sectional study design; temporality unknown	Low
Young et al., 2013	Cross-sectional study Men recruited from the Massachusetts General Hospital Fertility Center	Urine samples using HPLC-MS to determine pesticide metabolites	FISH analysis was preformed to detect sperm sex chromosome disomy	Adjusted for age, smoking, sperm motility, specific gravity, and log of sperm concentration	Poisson regression was used to determine IRR values and 95% CIs	Single spot-urine sample; temporality issue due to study design; potential selection bias; no mention of adjusting the confidence intervals or p-values due to multiple comparisons	Low
Zhang et al., 2014	Prospective cohort study in Tokyo	Maternal blood and a spot urine samples using HPLC-MS, along with neonatal blood samples postpartum	TSH blood levels were measured using the ELISA method; for birth size, the following variables were measured: head circumference, birth weight, birth length, and chest circumference	Adjusted for gestational weeks and BMI in mothers	Stepwise linear regression	Single spot-urine sample to determine long-term exposure	Low

Appendix B: Search Queries Used for Each Database Involved in Pyrethrins/Pyrethroids Human Health Systematic Literature Review

*In the table below, the Results Saved column indicates the total references saved from each database. The Unique Results column reflects the total number of references from each database after duplicates were removed.

Database	Query	Results Saved	Unique Results
PubMed	(pyrethrins[mh] AND (epidemiology OR epidemiologic OR case-control OR "case control" OR retrospective OR cohort OR prospective OR follow-up OR "follow up" OR longitudinal OR cross-sectional OR incidence OR prevalence OR mortality OR "occupational studies" OR "community study" OR "health surveys") AND (exposure OR exposures OR poisoning OR toxicity OR intoxication OR accidents OR accident OR accidental OR accidentally OR contamination OR inhalation OR inhaled OR absorb OR absorption OR ingest OR ingestion OR consume OR consumption OR drinking OR water)) NOT (treatment OR therapy OR case reports[publication type] OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		

	((allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrin OR cyhalothrin OR cypermethrin OR cyphenothrin OR deltamethrin OR d-phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin OR gamma cyhalothrin OR imiprothrin OR lambda cyhalothrin OR momfluorothrin OR permethrin OR prallethrin OR pyrethrins OR pyrethroids OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin) AND (epidemiology OR epidemiologic OR case-control OR "case control" OR retrospective OR cohort OR prospective OR follow-up OR "follow up" OR longitudinal OR cross-sectional OR incidence OR prevalence OR mortality OR "occupational studies" OR "community study" OR "health surveys") AND (exposure OR exposures OR poisoning OR toxicity OR intoxication OR accidents OR accident OR accidental OR accidentally OR contamination OR inhalation OR inhaled OR absorb OR absorption OR ingest OR ingestion OR consume OR consumption OR drinking OR water)) NOT (treatment OR therapy OR case reports[publication type] OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		
	(pyrethrins[mh] AND (herbicides/poisoning[mh] OR pesticides/poisoning[mh]) NOT (case reports[publication type] OR treatment OR therapy OR prognostic OR prognosis OR suicide)		
	(pyrethrins/adverse effects[mh] OR pyrethrins/poisoning[mh] OR pyrethrins/toxicity[mh]) AND (environmental illness[mh] OR occupational illness[mh] OR agricultural workers' diseases[mh] OR pregnancy outcome[mh] OR birth weight[mh] OR “health effects” OR “health impacts” OR illness OR illnesses OR disease OR diseases OR medical OR hospitalization OR hospitalizations OR “birth defects” OR “pregnancy outcome” OR “pregnancy outcomes” OR “birth weight” OR Parkinson OR parkinson’s OR “paralysis agitans” OR neurological OR neurologic OR neurodegenerative OR neurotoxic OR neuromuscular OR neurobehavioral OR behavioral OR developmental OR “attention deficit” OR cognitive OR cognition OR neoplasm OR neoplasms OR cancer OR carcinogen* OR leukemia OR myeloma OR lymphoma OR hodgkins OR “amyotrophic lateral sclerosis” OR mortality OR death OR diabetes OR myopia OR “eye disease*” OR “skin disease*” OR allergy OR allergic OR allergies OR respiratory OR gastrointestinal OR lung OR pulmonary OR thyroid) NOT (treatment OR therapy OR case reports[publication type] OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		
	((allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrin OR cyhalothrin OR cypermethrin OR cyphenothrin OR deltamethrin OR d-phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin OR gamma cyhalothrin OR imiprothrin OR lambda cyhalothrin OR momfluorothrin OR permethrin OR prallethrin OR pyrethrins OR pyrethroids OR pyrethrin OR pyrethroid OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin) AND ("health effects" OR "health impacts" OR illness OR illnesses OR disease OR diseases OR medical OR hospitalization OR hospitalizations OR "birth defects" OR "pregnancy outcome" OR "pregnancy outcomes" OR "birth weight" OR parkinson OR parkinson's OR "paralysis agitans" OR neurological OR neurologic OR neurodegenerative OR neurotoxic OR neuromuscular OR neurobehavioral OR behavioral OR developmental OR "attention deficit" OR cognitive OR cognition OR neoplasm OR neoplasms OR cancer OR carcinogen* OR leukemia OR		

	myeloma OR lymphoma OR hodgkins OR "amyotrophic lateral sclerosis" OR diabetes OR prediabet* OR myopia OR "eye disease*" OR "skin disease*" OR allergy OR allergic OR allergies OR respiratory OR gastrointestinal OR lung OR pulmonary OR asthma* OR thyroid OR semen OR fertility OR estrogen* OR sperm* OR cardiac OR mortality OR death) AND (Epidemiology OR epidemiologic OR case-control OR "case control" OR retrospective OR cohort OR prospective OR follow-up OR "follow up" OR longitudinal OR cross-sectional OR incidence OR prevalence OR mortality OR "occupational studies" OR "community study" OR "health surveys")) NOT (treatment OR therapy OR case reports[publication type] OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		
	((pyrethrins[mh] OR allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrin OR cyhalothrin OR cypermethrin OR cyphenothrin OR deltamethrin OR d-phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin OR gamma cyhalothrin OR imiprothrin OR lambda cyhalothrin OR momfluorothrin OR permethrin OR prallethrin OR pyrethrins OR pyrethroids OR pyrethrin OR pyrethroid OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin) AND (eye diseases[mh] OR gastrointestinal diseases[mh] OR nervous system diseases[mh] OR respiratory tract diseases[mh] OR skin diseases[mh] OR neoplasms[mh] OR pregnancy outcome[mh] OR birth weight[mh] OR prenatal exposure, delayed effects[mh] OR environmental illness[mh] OR occupational illness[mh] OR agricultural workers' diseases[mh] OR food contamination[mh])) NOT (treatment OR therapy OR case reports[publication type] OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		
	((pyrethrin*[title] OR pyrethroid*[title]) AND (diet OR consume OR consumption OR ingest*)) NOT (case reports[publication type] OR treatment OR therapy OR prognostic OR prognosis OR suicide)		
	PubMed total (searches conducted 12/26/17-1/3/18)	**509**	**323**
PubMed Central	((pyrethrins[mh] OR allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrin OR cyhalothrin OR cypermethrin OR cyphenothrin OR deltamethrin OR d-phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin OR gamma cyhalothrin OR imiprothrin OR lambda cyhalothrin OR momfluorothrin OR permethrin OR prallethrin OR pyrethrins OR pyrethroids OR pyrethrin OR pyrethroid OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin) AND ("health impact*" OR :health assessment*" OR "health effect*"OR illness OR illnesses OR disease OR diseases OR medical OR hospitalization OR hospitalizations OR "birth defects" OR "pregnancy outcome" OR "pregnancy outcomes" OR "birth weight" OR parkinson OR parkinson's OR "paralysis agitans" OR neurological OR neurologic OR neurodegenerative OR neurotoxic OR neuromuscular OR neurobehavioral OR behavioral OR developmental OR "attention deficit" OR cognitive OR cognition OR neoplasm OR neoplasms OR cancer OR carcinogen OR carcinogens OR carcinogenic OR leukemia OR myeloma OR lymphoma OR hodgkins OR "amyotrophic lateral sclerosis" OR diabetes OR prediabet* OR myopia OR "eye disease*" OR "skin disease*" OR allergy OR allergic OR allergies OR respiratory OR gastrointestinal OR lung OR		

	pulmonary OR asthma OR asthmatic OR thyroid OR semen OR fertility OR estrogen OR sperm OR cardiac OR mortality OR death)) NOT (treatment OR therapy OR “case reports” OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		
	((pyrethrins[mh] OR allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrin OR cyhalothrin OR cypermethrin OR cyphenothrin OR deltamethrin OR d-phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin OR gamma cyhalothrin OR imiprothrin OR lambda cyhalothrin OR momfluorothrin OR permethrin OR prallethrin OR pyrethrins OR pyrethroids OR pyrethrin OR pyrethroid OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin) AND **(**epidemiologic studies[mh] OR epidemiologic OR epidemiology OR "case control" OR retrospective OR cohort OR prospective OR "follow up" OR follow-up OR longitudinal OR cross-sectional OR "cross sectional" OR "mortality studies" OR incidence OR "occupational study" OR "occupational studies" OR "environmental study" OR "environmental studies" OR "community study" OR "community studies") AND (exposure OR exposed OR absorption OR absorb OR contamination OR accident OR accidents OR ingest OR ingestion)) NOT (treatment OR therapy OR “case reports” OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		
	((pyrethroid OR pyrethroids OR pyrethrin OR pyrethrins) Field: Title) NOT (treatment OR therapy OR “case reports” OR prognostic OR prognosis OR suicide OR drosophila OR rat OR rats OR mice OR mouse OR rodent OR rodents OR monkey OR monkeys OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterfl* OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR bee OR bees OR beetle OR beetles OR mayfl* OR sandfl* OR ticks OR dogs OR moth OR moths OR mosquito* OR aegypti OR gambiae OR anopheles)		

	PubMed Central Total (searches conducted 1/3/18–1/4/18)	**158**	**84**
Science Direct	((pyrethrum OR pyrethroid OR pyrethrin OR pyrethroids OR pyrethrins) AND ("health effect" OR "health impact" OR "health hazard" OR “health effects” OR “health impacts” OR “health hazards” OR illness OR illnesses OR disease OR diseases OR medical OR hospitalization OR hospitalizations OR “birth defects” OR “pregnancy outcome” OR “pregnancy outcomes” OR “birth weight” OR parkinson OR parkinson’s OR “paralysis agitans” OR neurological OR neurologic OR neurodegenerative OR neurotoxic OR neuromuscular OR neurobehavioral OR behavioral OR developmental OR “attention deficit” OR cognitive OR cognition OR neoplasm OR neoplasms OR cancer OR carcinogen* OR leukemia OR myeloma OR lymphoma OR hodgkins OR “amyotrophic lateral sclerosis” OR diabetes OR prediabet* OR myopia OR “eye disease*” OR “skin disease*” OR allergy OR allergic OR allergies OR respiratory OR gastrointestinal OR lung OR pulmonary OR asthma* OR thyroid OR semen OR fertility OR estrogen* OR sperm* OR cardiac OR mortality OR death))) AND NOT (Treatment OR therapy OR prognosis OR prognostic OR suicide OR “case report” OR rat OR rats OR mice OR mouse OR rodent OR rodents OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterflies OR amphibians OR clams OR marine OR aquatic OR shrimp OR earthworm OR mosquitos OR mosquitoes OR mosquito OR anopheles OR gambiae OR aegypti OR drosophila OR bee OR bees OR beetle OR beetles OR mayfly OR sandflies OR ticks OR dogs)		
	((Allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrins OR cyhalothrins OR cypermethrin OR cyphenothrin OR deltamethrin OR d-Phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin OR gamma cyhalothrin OR imiprothrin OR lamda cyhalothrin OR momfluorothrin OR permethrin OR prallethrin OR pyrethrins OR pyrethrin OR pyrethroids OR pyrethroid OR pyrethrum OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin) AND ("health impact" OR "health hazard" OR "health effect" OR “health impacts” OR “health hazards” OR “health effects” OR “health risk” OR “health risks” OR “human risk” OR “human risks”))		
	((pyrethrum OR pyrethroid OR pyrethrin OR pyrethroids OR pyrethrins) AND (epidemiology OR epidemiologic OR “case control” OR case-control OR retrospective OR cohort OR prospective OR follow-up OR longitudinal OR cross-sectional OR ”occupational studies”) AND NOT (Treatment OR therapy OR		

	prognosis OR prognostic OR suicide OR “case report” OR rat OR rats OR mice OR mouse OR rodent OR rodents OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterflies OR amphibians OR clams OR marine OR aquatic OR shrimp OR earthworm OR mosquitos OR mosquitoes OR mosquito OR anopheles OR gambiae OR aegypti OR drosophila OR bee OR bees OR beetle OR beetles OR mayfly OR sandflies OR ticks OR dogs)		
	Science Direct Total (searches conducted 1/8/18–1/10/18)	**69**	**69**
Toxline	((pyrethrins OR 121-21-1 [rn]) OR pyrethroid OR (pyrethrum OR "pyrethrins bsi iso " OR pyrethrins OR 8003-34-7 [rn]) AND ("health impacts" OR illness OR illnesses OR disease OR diseases OR medical OR pregnant OR pregnancy OR birth OR neurodegenerative OR neurotoxic OR neurobehavioral OR leukemia OR fertility OR semen OR sperm OR diabetes OR asthma OR cancer OR respiratory) AND ("environmental exposure" OR "occupational exposure")) AND NOT (treatment OR therapy OR prognosis OR prognostic OR suicide OR “case report” OR rat OR rats OR mice OR mouse OR rodent OR rodents OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterflies OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR mosquitos OR mosquitoes OR mosquito OR anopheles OR gambiae OR aegypti OR drosophila OR bee OR bees OR beetle OR beetles OR mayfly OR sandflies OR ticks OR dogs)) AND NOT PubMed [org] AND NOT pubdart [org]		
	((pyrethrins OR 121-21-1 [rn]) OR pyrethroid OR (pyrethrum OR "pyrethrins bsi iso " OR pyrethrins OR 8003-34-7 [rn]) AND ("health impacts" OR illness OR illnesses OR disease OR diseases OR medical OR pregnant OR pregnancy OR birth OR neurodegenerative OR neurotoxic OR neurobehavioral OR leukemia OR fertility OR semen OR sperm OR diabetes OR asthma OR cancer OR respiratory) AND ("epidemiology OR epidemiologic OR "case control" OR retrospective OR cohort OR prospective OR "follow up" OR follow-up OR longitudinal OR cross-sectional OR "cross sectional" OR "mortality studies")) AND NOT PubMed [org] AND NOT pubdart [org]		

	(pyrethrins OR pyrethrum OR "pyrethrins bsi iso " OR "pyrethrin i") AND NOT (rat OR rats OR mice OR mouse OR rodent OR rodents OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterflies OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR mosquitos OR mosquitoes OR mosquito OR anopheles OR gambiae OR aegypti OR drosophila OR bee OR bees OR beetle OR beetles OR mayfly OR sandflies OR ticks OR dogs)) AND 1900:2017 [yr] AND (eng [la]) AND (BIOSIS[org]) AND NOT PubMed[org] AND NOT pubdart[org]		
	Toxline Total (searches conducted 1/418–1/5/18)	**62**	**51**
SCIELO	(pyrethrins OR pyrethrin OR pyrethroids OR pyrethroid OR pyrethrum) NOT ((treatment OR therapy OR prognosis OR prognostic OR suicide OR "case report" OR rat OR rats OR mice OR mouse OR rodent OR rodents OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterflies OR amphibians OR clams OR marine OR aquatic OR shrimp OR earthworms OR mosquitos OR mosquitoes OR mosquito OR anopheles OR gambiae OR "aedes aegypti" OR drosophila OR bee OR bees OR beetle OR beetles OR mayfly OR sandflies OR ticks OR tick OR dogs))		
	(pyrethrum OR pyrethroid OR pyrethroids OR pyrethrin OR pyrethrins) AND ("environmental exposure" OR "occupational exposure" OR "health effects" OR "health hazards" OR "health impacts" OR illness OR illnesses OR disease OR diseases OR medical OR pregnant OR pregnancy OR birth OR neurodegenerative OR neurotoxic OR neurobehavioral OR leukemia OR fertility OR semen OR sperm OR diabetes OR asthma OR cancer OR respiratory)		
	(pyrethrum OR pyrethroid OR pyrethroids OR pyrethrin OR pyrethrins) AND ("epidemiology OR epidemiologic OR "case control" OR retrospective OR cohort OR prospective OR "follow up" OR follow-up OR longitudinal OR cross-sectional OR "cross sectional" OR "mortality studies")		
	(ti:(Allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrins OR cyhalothrins OR cypermethrin OR cyphenothrin OR deltamethrin ORd-Phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin ORgamma cyhalothrin OR imiprothrin OR lamda cyhalothrin OR momfluorothrin ORpermethrin OR prallethrin OR pyrethrins OR pyrethrin OR pyrethroids OR pyrethroid OR pyrethrum OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin))		
	SCIELO Total (searches conducted 1/5/18)	**0**	**0**

SciSearch	(all(pyrethrin+) OR all(pyrethroid+) OR all(pyrethrum)) AND (all("health hazard+") OR all("health impact+") OR all("health effect+") OR all("health hazard+")) NOT ((all(Treatment) OR all(therapy) OR all(prognosis) OR all(prognostic) OR all(suicide) OR all("case report") OR all(rat) OR all(rats) OR all(mice) OR all(mouse) OR all(rodent) OR all(rodents) OR all(zebrafish) OR all(trout) OR all(fish) OR all(bird) OR all(birds) OR all(frog) OR all(frogs) OR all(sheep) OR all(butterflies) OR all(amphibian*) OR all(clams) OR all(marine) OR all(aquatic) OR all(shrimp) OR all(earthworm*) OR all(mosquitos) OR all(mosquitoes) OR all(mosquito) OR all(anopheles) OR all(gambiae) OR all(aegypti) OR all(drosophila) OR all(bee) OR all(bees) OR all(beetle) OR all(beetles) OR all(mayfly) OR all(sandflies) OR all(ticks) OR all(dogs))		
	(((Allethrin OR beta-cyfluthrin OR bifenthrin OR cyfluthrins OR cyhalothrins OR cypermethrin OR cyphenothrin OR deltamethrin OR d-Phenothrin OR esfenvalerate OR etofenprox OR fenpropathrin OR flumethrin OR gamma cyhalothrin OR imiprothrin OR lamda cyhalothrin OR momfluorothrin OR permethrin OR prallethrin OR tau-fluvalinate OR tefluthrin OR tetramethrin OR zeta-cypermethrin)) AND (illness OR illnesses OR disease OR diseases OR medical OR hospitalization OR hospitalizations OR "birth defects" OR "pregnancy outcome" OR "pregnancy outcomes" OR "birth weight" OR parkinson OR parkinson's OR "paralysis agitans" OR neurological OR neurologic OR neurodegenerative OR neurotoxic OR neuromuscular OR neurobehavioral OR behavioral OR developmental OR "attention deficit" OR cognitive OR cognition OR neoplasm OR neoplasms OR cancer OR carcinogen* OR leukemia OR myeloma OR lymphoma OR hodgkins OR "amyotrophic lateral sclerosis" OR diabetes OR prediabet* OR myopia OR "eye disease*" OR "skin disease*" OR allergy OR allergic OR allergies OR respiratory OR gastrointestinal OR lung OR pulmonary OR asthma* OR thyroid OR semen OR fertility OR estrogen* OR sperm* OR cardiac OR mortality OR death) AND ((Exposure OR exposures OR poisoning OR toxicity OR intoxication OR accidents OR accident OR accidental OR accidentally OR contamination OR inhalation OR inhale OR absorb OR absorption OR ingest OR ingestion OR consume OR consumption OR drinking OR water)) AND (epidemiology OR epidemiologic OR case-control OR "case control" OR retrospective OR cohort OR prospective OR follow-up OR "follow up" OR longitudinal OR cross-sectional OR incidence OR prevalence OR mortality OR "occupational studies" OR "health surveys")) NOT (Treatment OR therapy OR prognosis OR prognostic OR suicide OR "case report" OR rat OR rats OR mice OR mouse OR rodent OR rodents OR zebrafish OR trout OR fish OR bird OR birds OR frog OR frogs OR sheep OR butterflies OR amphibian* OR clams OR marine OR aquatic OR shrimp OR earthworm* OR mosquitos OR mosquitoes OR mosquito OR anopheles OR gambiae OR aegypti OR drosophila OR bee OR bees OR beetle OR beetles OR mayfly OR sandflies OR ticks OR dogs)) and (at.exact("Article" OR "Review")		
	SciSearch Total (searches conducted 1/8/18–1/9/18)	51	43
	All Database Total (searches conducted 12/26/17–1/10/18)	847	570*