UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: March 30, 2021

SUBJECT: **Malathion**: Tier II Incident and Epidemiology Report

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EXECUTIVE SUMMARY FOR MALATHION

Malathion is a non-systemic, wide spectrum organophosphorus (OP) insecticide that is currently undergoing Registration Review by the Office of Pesticide Programs. It is used in the agricultural production of a wide variety of food/feed crops to control insects such as aphids, leafhoppers, and Japanese beetles. This pesticide has been reviewed in the past by CEB on three separate occasions in 2009, 2014, and 2016.[1]

As part of the Registration Review, OPP's Health Effects Division (HED) conducted a Tier II evaluation of incident data and epidemiology research to assess the potential association between malathion exposure and adverse human health effects. This evaluation considered a range of incident data sources that included adverse incidents reported to OPP's Incident Data System (IDS), NIOSH's Sentinel Event Notification System for Occupational Risk (SENSOR) Program, and the National Pesticide Information Center (NPIC). In order to evaluate the relevance of published epidemiology research, HED conduced a systematic review of the epidemiologic literature on malathion using methods described in OPP's "Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides" and generally followed the guidance provided in the National Toxicology Program/Office of Health Assessment and Translation (NTP/OHAT).[2] This systematic literature review considered studies available in peer-reviewed literature databases (e.g., PubMed, PubMedCentral, Scopus, and Science Direct) and a HED-maintained electronic library of published articles from the Agricultural Health Study (AHS), and aimed to identify original, peer-reviewed publications on epidemiologic studies. Key findings from HED's Tier II evaluation of incident data and epidemiology research are summarized below.

Incident Summary

For this Malathion Tier II Incident and Epidemiology Report, HED reviewed malathion incidents from four sources: the OPP Incident Data System, the NIOSH SENSOR-Pesticides Program, the California PISP (Pesticide Illness Surveillance Program) and the USEPA-funded National Pesticide Information Center (NPIC). In IDS, SENSOR-Pesticides and NPIC, HED found the majority of malathion incidents were low in severity (78% in IDS, 73% in SENSOR-Pesticides, NPIC 79%). In addition, in IDS and SENSOR, malathion incidents appear to be decreasing over time. From January 1, 2014 to February 25, 2021, there were 66 incidents reported to Main IDS and 194 incidents reported to Aggregate IDS that involved the active ingredient malathion. In addition, there were 193 cases reported to SENSOR-Pesticides (2010-2017), 115 human incidents reported to CA PISP (2012-2017) and 172 human incidents reported to NPIC (2014-2020) involving malathion. In Main IDS, most individuals reported being exposed to malathion during application and indoor exposure. NPIC found that most malathion cases were related to spills, primarily indoors.[3] SENSOR-Pesticides (from 2010-2017) found the main contributing factor in malathion case reports involved pesticide user spills or splashes (both for occupational and residential users). Of the occupational malathion cases reported in SENSOR-Pesticides, nearly 75% involved agricultural workers exposed to pesticide residues while working in treated fields. The California PISP (from 2012-2017) found that most malathion incidents involved fieldworkers

[1] M. Hawkins and J Cordova, *Updated Review of Malathion Incident Reports*, 2/26/2009; S. Recore et al., D423155, 9/30/2014; and C. Williams et al. D426077, 3/10/2016

[2] See *Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration,* January 9, 2015. https://ntp niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf

[3] The findings from IDS and NPIC are often similar and compliment the other because most NPIC cases are reflective of homeowner reports, and IDS is reflective of Registrant incident reports that primarily stem from homeowner calls

exposed to either pesticide residue or from off-site movement of the pesticide. Reported symptoms continue to include mostly neurological, gastrointestinal and respiratory effects.

In summary with respect to incident data, CEB concludes that CEB did not identify any aberrant effects outside of those anticipated and documented as a result of general OP toxicity. In addition, the majority of malathion incidents were low in severity and all death and most major severity incidents reported involved misuse (usually ingestion) of a malathion product.

Epidemiology Summary

HED conducted a systematic review of the epidemiologic literature and identified a total of 109 epidemiological articles that investigated the relationship between malathion and a range of health outcomes, consisting of 42 publications on carcinogenic health outcomes and 67 on the non-carcinogenic health outcomes. For the majority of these cancer outcomes, the available evidence was limited to only 1-2 published articles. There were a larger number of published articles for breast cancer (5 articles), NHL (11 articles), and prostate cancer (8 articles). Respiratory effects, Parkinson disease, and thyroid effects were the three largest non-carcinogenic health endpoints in this memo.

For each health endpoint for malathion, individual epidemiological studies were summarized along with a strengths and limitations characterization. The overall weight of the epidemiological evidence was then assessed by considering the level of quality of the studies available in the peer-reviewed literature for each health effect, the strength of the associations (effect sizes), and consistency of the association in magnitude and direction across available studies was considered, as described in OPP's epidemiologic framework document and detailed here in Tables 6 (describing study quality considerations) and Table 7 (describing five "Levels of Evidence" ranging from "*Sufficient Evidence of No Causal Relationship"* up to *"Sufficient Epidemiological Evidence of a Clear Associative or Causal Relationship"*).

Twenty-four cancer outcomes were examined in 42 epidemiologic studies, with most cancer outcomes investigated in only one or two studies. Based on our review of these carcinogenic outcomes, we concluded:

- there was *no epidemiological evidence* of a clear associative or causal relationship between malathion exposure and three cancer outcomes: colon cancer, esophageal cancer, and rectal cancer. This conclusion was based on evidence that was limited to studies on each cancer outcome that reported no evidence of a positive association between malathion exposure and the cancer outcome (e.g., all reported OR effect estimates were ≤ 1.0).

- there was *insufficient epidemiological evidence* of a clear associative or causal relationship between malathion exposure and eighteen cancer outcomes: all cancers, bladder cancer, brain and spinal cancer (glioma), breast cancer, colorectal cancer, childhood cancer, gastric cancer, kidney cancer, lung cancer, leukemia, lymphatic-hematopoietic cancer, Hodgkin lymphoma, melanoma, multiple myeloma, non-Hodgkin lymphoma (NHL), ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, soft tissue carcinoma, and uterine cancer. The majority of these cancer outcomes were also only investigated in a single study population (the AHS study cohort) and thus do not provide independent information in different study populations. Breast cancer was investigated in five studies, prostate cancer examined in eight studies, and NHL in eleven studies. However, given the limited number of study populations available and mixed results observed for each health outcome, there was minimal confidence in the available evidence so additional epidemiological evidence could substantively affect the overall magnitude or direction of any observed associations.

Thirty-four non-carcinogenic health outcomes were examined in 67 epidemiologic studies. Based on our review of these non-carcinogenic outcomes, we concluded:

- there was *no epidemiological evidence* of a clear associative or causal relationship between malathion exposure and the following outcomes: amyotrophic lateral sclerosis (ALS), autoimmune disease (antinuclear antibodies), dream enacting behavior, fatal injury, kidney function, monoclonal gammopathy of undetermined significance, neurodevelopmental/neurobehavorial effects in children, recurrent pregnancy loss, sleep apnea, stroke, suicide, testosterone level effects, and other thyroid disease. This conclusion was based on evidence that was limited to one or two studies on each health outcome that reported no evidence of a positive association between malathion exposure and the health outcome of interest (e.g., reported OR effect estimates were ≤ 1.0).

- there was *insufficient epidemiological evidence* of a clear associative or causal relationship between malathion exposure and the remaining health effects: autism spectrum disorder, birth defects, birth effects, birthweight, cerebral palsy, depression, diabetes, end stage renal disease, endometriosis, eye disorders, gestational hypertension, hearing loss, myocardial infarction (MI), nervous system function (neonatal, central, and peripheral nervous system in adults), olfactory impairment, Parkinson's disease (PD), respiratory effects (asthma, chronic bronchitis, rhinitis, wheeze), rheumatoid arthritis, hyperthyroid disease, hypothyroid disease, and weight gain in adults. The majority of these effects were also only investigated in a single study population (the AHS cohort) and frequently reported no evidence of a significant positive association (e.g., OR > 1.00 but not significant). Given the limited number of studies available for each outcome, there was generally minimal confidence in the available evidence since additional epidemiological evidence could substantively affect the overall magnitude or direction of any observed associations.

Additional details regarding these studies are presented in the main text, with summary information presented in Section 4 (Conclusions) and in Appendix B (Summary of Epidemiologic Studies and Study Quality Assessment).

In summary with respect to epidemiology data, CEB concludes that while individual epidemiology studies were identified that reported a positive association between malathion exposure and some adverse health effects, the overall evidence was mostly based on a small body of studies (i.e., typically only one or two study populations per health outcome) that often had substantive limitations with respect to their study design, exposure assessment approach, and/or outcome assessment approach. As such, HED concluded that overall, there was no or insufficient epidemiologic evidence to suggest that a clear associative or causal relationship exists between malathion exposure and the adverse health effects examined in the available epidemiologic literature for both carcinogenic and non-carcinogenic outcomes. The Agency will continue to monitor the epidemiology data and -- if a concern is triggered -- additional analysis will be conducted.

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1 BACKGROUND

Malathion is a non-systemic, wide spectrum organophosphorus (OP) insecticide. It is used in the agricultural production of a wide variety of food/feed crops to control insects such as aphids, leafhoppers, and Japanese beetles. Malathion is also used in the Cotton Boll Weevil Eradication Program, Fruit Fly (Medfly) Control Program, and for mosquito-borne disease control. It is also available to the home gardener for outdoor residential uses which include vegetable gardens, home orchards, and ornamentals. Malathion is formulated as a technical, a dust, an emulsifiable concentrate (EC), a ready-to-use (RTU) product, a pressurized liquid, and a wettable powder (WP). Several of the 95% liquids are intended for ultra-low-volume (ULV) applications. Malathion can be applied using ground or aerial equipment, thermal and non-thermal fogger, ground boom, airblast sprayer, chemigation, and a variety of hand-held equipment such as backpack sprayers, low pressure handwands, hose-end sprayers, and power dusters.

This Malathion Tier II Incident and Epidemiology Report reviews human observation data from a variety of sources including:

- Human incident (poisoning) data from the following sources:
    - Office of Pesticide Program's (OPP) Incident Data System (IDS) database.
    - National Institute of Occupational Safety and Health (NIOSH) SENSOR-Pesticides.
    - National Pesticide Information Center (NPIC) (Agency Sponsored); and
    - California's Pesticide Incident Surveillance Program (PISP); and,
- Epidemiological studies from the open literature.

A Tier II incident and epidemiology report, as compared to a Tier I incident and epidemiology report, provides additional details and greater depth in scope of review of information relating to human exposure. Utilization of these data will aid HED in better defining and characterizing the potential risk of malathion pesticide products to the U.S. population and particular sub-groups such as workers and children.

Incident data are collected systematically, but differently, across the different databases used by the Agency with respect to such issues as coverage, certainty/confidence, fields/parameters reported, and usability. The three pesticide incident data sources (IDS, NIOSH SENSOR-Pesticides, and NPIC) were used in this malathion report since they provide useful content and historical perspective. Various other comparable sources of data are available (e.g., the Bureau of Labor Statistics, emergency room outpatient surveillance, National Poison Data System (NPDS), etc.) but are not included in this review. By looking across the five data sources which were used, the Agency is confident that we are considering adequate and appropriate information to recognize and discern trends and patterns in permethrin-associated acute pesticide poisonings, or "incidents."

It is important to recognize, however, that reports of adverse health effects allegedly due to a specific pesticide exposure (i.e., an "incident") are largely self-reported and therefore, generally speaking, neither exposure to a pesticide nor reported symptoms (or the connection between the two) are validated. Therefore, only rarely can causation be determined or definitively identified based on incident data. However, incident information can provide important feedback to the Agency. Human incident data, in concert with other human observational studies (biomonitoring and epidemiological studies) and the human health risk assessment, can assist the Agency in determining potential risks of pesticides/pesticide product exposure, and can help characterize that risk. This review assesses acute pesticide poisoning incidents and published epidemiology studies to inform the preliminary risk assessment for malathion.

2 REVIEW OF INCIDENT ANALYSIS

2.1 Incident Data System (IDS) (2014-2021)

OPP's IDS includes reports of alleged human health incidents from various sources, including mandatory Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Section 6(a)(2) reports from registrants, other federal and state health and environmental agencies, and individual consumers. Since 1992, OPP has compiled these reports in IDS. IDS contains reports from across the U.S. and most incidents have all relevant product information recorded. Reports submitted to the IDS represent anecdotal reports or allegations only, unless otherwise stated in the report.

IDS records incidents are stored in one of two modules: Main IDS or Aggregate IDS:

- Main IDS generally contains incidents resulting in higher severity outcomes and provides more detail with regard to case specifics.[4] This system stores incident data for death, major and moderate incidents, and it includes information about the location, date, and nature of the incident. Main IDS incidents involving only one pesticide are considered to provide more certain information about the potential effects of exposure from the pesticide.

- Aggregate IDS contains incidents resulting in less severe human incidents (minor, unknown, or no effects outcomes). These are reported by registrants only as counts in what are aggregate summaries.

For Main IDS from January 1, 2014 to February 25, 2021, there were 66 incidents reported that involved the active ingredient malathion. Of these 66 incidents, 56 incidents involved the single active ingredient malathion (only). The other 10 malathion incidents reported involved multiple active ingredients. Of these 66 total incidents, four incidents were classified as major severity, 52 incidents were classified as moderate severity, six incidents were classified as minor severity and four incidents were classified as having no or unknown symptoms. Two of the major severity incidents involved ingestion of the product, one incident occurred following spilling the product and the final major severity incident was determined by the medical professional not to be related to OP toxicity.

Twenty-eight single ai incidents from 2016 to 2020 were reviewed for exposure scenario and health effects. Most individuals reported being exposed during application, and indoor exposure. Most people reported exposure during application of the product (n=12) including those who reported exposure due to spills, leaks, and blowback during use. Other exposures included ingestion (three unintentional, one intentional), exposures to spills or leaks (not during application), and off-site movement of the product. All twenty-eight incidents are described in Appendix A, Table A-1.

Individuals most often reported neurological symptoms, including dizziness, confusion, headache, and numbness followed next by respiratory and gastrointestinal symptoms, including shortness of breath, coughing, nasal discharge, throat irritation, nausea, vomiting, and diarrhea. Other less-reported symptoms were cardiovascular and dermal symptoms which included chest pain, hypertension, rash, redness, itching and swelling.

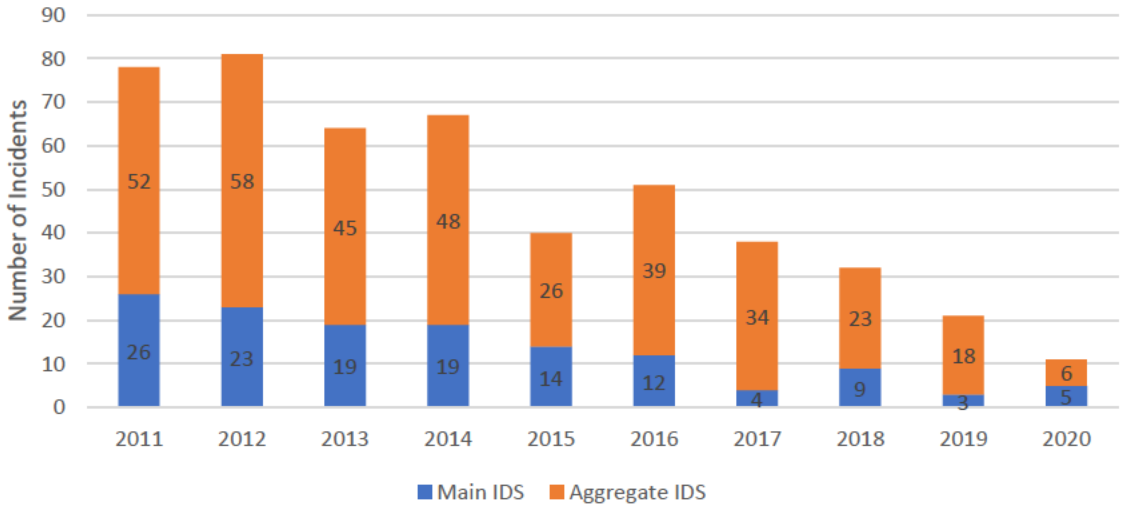
For Aggregate IDS from January 1, 2014 to February 25, 2021, there were 194 incidents reported involving malathion. Two incidents had no or unknown effects and 192 incidents were classified as minor

[4] Occasionally, low severity incidents are self-reported by the consumer directly to Main IDS.

severity. Minor severity means that a person alleged or exhibited some symptoms, but they were minimally traumatic, the symptoms resolved rapidly and usually involved skin, eye, or respiratory irritation. Because these incidents are in Aggregate IDS and thus fall within the categories reported as counts (which includes minor, unknown or no effects), there is no unique report that provides details about the incident.

The malathion incidents trend over time from 2011 to 2020 was reviewed. **Figure 1** shows that there has been a steady decrease in malathion incidents reported to Main and Aggregate IDS over time.

Figure 1. Malathion Incidents Reported Main and Aggregate IDS (2011-2020)



2.2 SENSOR-Pesticides (2010-2017)

The Center for Disease Control's National Institute for Occupational Safety and Health (CDC/NIOSH) manages a pesticide surveillance program and database entitled the Sentinel Event Notification System for Occupational Risk (SENSOR)-Pesticides.[5] All cases must report at least two adverse health effects. Evidence for each case is evaluated for its causal relationship between exposure and illness based on the NIOSH case classification index.[6] Using standardized protocol and case definitions, SENSOR-Pesticides state coordinators, operating out of the state's department of health, receive state pesticide incident reports from local sources, then follow up with case sources to get incident scenario to obtain medical records and verify exposure scenario information.[7] This database includes pesticide illness case reports from multiple states from 1998-2017.[8]

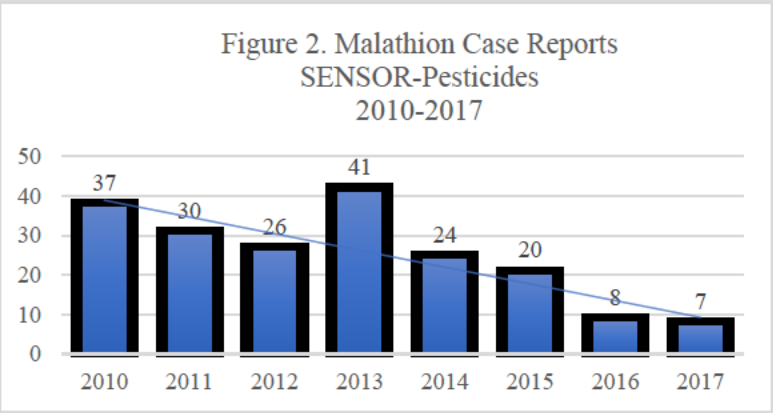
A query of SENSOR-Pesticides from 2010-2017 identified a total of 193 cases involving malathion. Of these, 40 cases were low in severity, 40 were moderate severity, and 10 were high in severity; including

[5] SENSOR-Pesticides webpage: http://www.cdc.gov/niosh/topics/pesticides/overview.html.
[6] https://www.cdc.gov/niosh/topics/pesticides/pdfs/casedef.pdf
[7] https://www.cdc.gov/niosh/topics/pesticides/pdfs/pest-sevindexv6.pdf
[8] Currently participating states are: California, Florida, Illinois, Louisiana, Michigan, Nebraska, New Mexico, North Carolina, Oregon, Texas and Washington. The participating states for a given year vary depending on state and federal funding for pesticide surveillance.

three fatalities.[9] EPA notes that all three fatal malathion case reports involved intentional ingestion of a highly concentrated (all three ingested products containing over 50% malathion) malathion product. Two of these malathion ingestion fatalities were reported in North Carolina, and one case was reported in Florida. Further, all seven of the high severity cases were also due to the intentional ingestion of malathion for the purpose of self-harm. A total of 14 cases, including all fatal and high severity cases, involved the individual's intentional ingestion of a malathion product.

Table 1. Malathion Case Severity SENSOR-Pesticides (2010-2017)	
Fatal	3 (1.6%)
High	7 (3.6%)
Moderate	40 (20.7%)
Low	143 (74.1%)

Malathion incidents reported to SENSOR-Pesticides have declined over the past decade (Figure 2). Of the 193 malathion case reports, 38% were occupational in nature and 62% were residential exposures. The main factor that contributed to 31% of the malathion cases in SENSOR-Pesticides was pesticide user spills or splashes (that did not involve equipment malfunction). Of the 73 occupational case reports, 74% were agricultural fieldworkers exposed to pesticide residues in the field. Another 19% of occupational cases were applying (or handling, n=2) the pesticide at the time of their exposure. The product most often implicated in the malathion cases (n=39%) is EPA Reg. No. 4-99 (Malathion 50% EC).



Inhalation was the most common route of exposure (60%) in the malathion cases (**Table 2**).

Table 2. Case Exposure Routes* SENSOR-Pesticides (2010-2017)	
Inhalation	116
Dermal	63
Ingestion	31
Ocular	24
***cases may report more than one exposure route**	

[9] SENSOR-Pesticides illness severity is categorized into four groups using standardized criteria (CDC, 2005). In low severity cases, two or more adverse health effects must be reported. The low severity illness usually resolves without treatment and there are fewer than 3 days lost from work. In moderate severity cases, the illness is non-life threatening, but requires medical treatment. No residual impairment is expected, and time lost from work is five days or fewer. In high severity cases, illness is life threatening, requires hospitalization, often has greater than 5 days lost from work, and may result in permanent impairment. Finally, fatal cases of pesticide poisoning were placed in a separate category.

Adverse health effects were primarily reported for the nervous system, followed by the gastrointestinal system and the respiratory system (**Table 3**). The top ten most frequently reported symptoms among the malathion incident reports in SENSOR-Pesticides, starting from the top symptom reported, were: nausea, headache, eye pain/irritation, dizziness, vomiting, upper respiratory pain/irritation, shortness of breath, diarrhea, abdominal pain/cramping, and cough.

Table 3. Malathion Adverse Health Effects by Body System* SENSOR-Pesticides (2010-2017)	
Nervous System	127
Gastrointestinal	97
Respiratory	80
Cardiovascular	39
Dermal	37
Renal	5
***cases may report adverse health effects for multiple body systems**	

2.3 California's Pesticide Incident Surveillance Program (PISP) (2012-2017)

The Pesticide Illness Surveillance Program (PISP) maintains a database of pesticide-related illnesses and injuries in California. Case reports are received from physicians and via workers' compensation records. The local County Agricultural Commissioner investigates circumstances of exposure. Medical records and investigative findings are then evaluated by DPR technical experts and entered into an illness registry.

PISP contains both residential and occupational pesticide incidents. PISP has limited coverage (only California) and is therefore not used for identifying national trends over time. However, the incident information is entered by professionals with expertise in pesticides who extensively follow-up on each reported incident, establishing a high degree of confidence in the information provided for each reported incident.

In PISP from 2012-2017 there were 115 incident reports involving malathion. Of these, 58 cases were agricultural, and 57 cases were non-agricultural. The majority of PISP malathion case reports (43%) were agricultural fieldworkers exposed to either pesticide residue in treated fields or off-site movement of the pesticide, which includes both spray drift and volatilization.

A number of incidents involved homeowners or workplace bystanders who were exposed to malathion products that were stored in and around the home and were later spilled, leaked, or splashed after pesticide product container was accidentally broken or was thrown away. Some examples of such incidents are as follows:

- A janitor threw a malathion container into the dumpster outside the backdoor of a Post Office. Three postal workers complained about the odor that came in through the back door and developed symptoms including: shortness of breath, chest tightness, mouth numbness, tachycardia (rapid heartbeat) and throat irritation.

- A man was vacuuming his garage shelving. The vacuum hose caught on a glass bottle which contained a half cup of malathion, causing it to fall and break. He used gloves to clean it up. His wife insisted hazmat be called, and he was taken for care.

- An old container of malathion leaked in the garage of an elderly man's home and was absorbed into the drywall, creating a strong odor. He went to stay with his daughter and hazmat contained the spill. A few days later he sought care.

Table 4 provides a complete listing of the incident case's activity at the time of their exposure.

Table 4. Malathion Incidents Reported CA PISP 2012-2017	
Activity	**Count**
Applicator	6
Field Worker	50
Mixer/Loader	2
Other	15
Routine (Other or Unspecified)	2
Routine Indoor	25
Routine Outdoor	11
Unknown	4
Total	**115**

Regarding case exposure scenarios, 25% of PISP malathion cases were related to off-site movement of the pesticide; 22% of cases were related to residue exposures, 11% were ingestions of the pesticide (eight ingestion cases were self-harm attempts and five were accidental ingestions).

While PISP does not include a severity code for their cases, they do include information on whether the case was hospitalized. Fourteen malathion incident cases were hospitalized; these were primarily those who intended to commit self-harm; primarily via ingestion. **Appendix A, Table A-2** provides details on malathion cases who were hospitalized.

Symptoms commonly reported among malathion case exposures included: headache, dizziness, nausea, vomiting, diarrhea, and respiratory irritation.

2.4 National Pesticide Information Center (NPIC) (2014-2020)

The National Pesticide Information Center or NPIC is a cooperative effort between Oregon State University and EPA which is funded by EPA to serve as a source of objective, science-based pesticide information and to respond to inquiries from the public and to incidents. NPIC functions nationally during weekdays from 8:00 am to 12:00 pm Pacific Time through a toll-free telephone number in addition to the internet (www.npic.orst.edu) and email. Similar to Poison Control Centers, NPIC's primary purpose is not to collect incident data, but rather to provide information to inquirers on a wide range of pesticide topics, and direct callers for pesticide incident investigation and emergency treatment. Nevertheless, NPIC does collect information about incidents (approximately 4000 incidents per year) from the public and records that information in a database. While NPIC is a source of national incident information, it generally receives fewer reports than IDS. Regardless, if a high frequency is observed in IDS, NPIC provides an additional source of information to see whether there is evidence of consistency across national data sets or possibly duplication and additional information about the same incident(s).

From January 1, 2014 to November 14, 2020, 172 human incidents involving malathion were reported to NPIC. NPIC estimates a certainty index that classifies an incident (including reported symptoms) as consistent or inconsistent with expected exposure to a pesticide, or whether the incident was unclassifiable. Of the 172 reported incidents, 68 were classified as consistent.[10] Of the 68 incidents that were classified as consistent, 54 incidents were classified as minor severity and 14 incidents were

[10] Consistent means that the majority of reported symptoms are consistent with exposure to the active ingredient according to published information, and the time course between exposure, onset, and duration of symptoms could be conceivably consistent with the toxicology of the active ingredient and the reported exposure pathway is conceivably plausible based on the history provide

classified as moderate severity. Of the remaining 104 incidents, 20 incidents were classified as inconsistent and/or unlikely related to malathion exposure, 75 were asymptomatic and considered unclassifiable and nine were classified as unknown.

The 68 incidents that were classified as consistent were further reviewed for exposure scenario and symptoms. Most people reported exposure due to the product being spilled either indoors (n=22) or outdoors (n=4), followed by applicators (n=15) who were most often reported exposure due to spills, leaks, and blowback while use. Other exposures included indoor application (n=9), exposure to off-site movement of the product (n=6), and while handling a leaky container (n=4).

Individuals reported neurological, respiratory, gastrointestinal, ocular, dermal and cardiovascular symptoms, including dizziness, brain fog, headache, lightheadedness, numbness, sore throat, difficulty breathing, nose burning, coughing, wheezing, skin irritation, eye irritation and burning, lacrimation, cramping, nausea, stomach ache, vomiting, diarrhea, chest pain and tightness.

2.5 Tier II Acute Incident Report Review Summary and Conclusions

Overall, the majority of malathion incidents were low in severity (78% in IDS, 73% in SENSOR-Pesticides, NPIC 79%). In both IDS and SENSOR, malathion incidents appear to be decreasing over time. In IDS (Main), most individuals reported being exposed to malathion during application, and indoor exposure. NPIC found that most malathion cases were related to spills, primarily indoors.[11] SENSOR-Pesticides (from 2010-2017) found the main contributing factor in malathion case reports involved pesticide user spills or splashes (both for occupational and residential users). Of the occupational malathion cases reported in SENSOR-Pesticides, nearly 75% involved agricultural workers exposed to pesticide residues while working in treated fields. The California PISP (from 2012-2017) found that most malathion incidents involved fieldworkers exposed to either pesticide residue or from off-site movement of the pesticide.

HED's review of reported symptoms indicate acute exposure to malathion results in predictable and documented organophosphate acute effects. These include neurological, gastrointestinal and respiratory effects, primarily. HED did not identify any aberrant effects outside of those anticipated and documented as a result of general OP toxicity. Acute adverse health effects due to OP/malathion exposure are generally mild to moderate and are reversible with primary medical intervention.

3 TIER II EPIDEMIOLOGY REVIEW

3.1 Introduction

OPP conducted a systematic review of peer reviewed epidemiology studies that examined the association between malathion and adverse health effects. The specific aims of the systematic review of the epidemiology literature were to:

- Conduct a literature search and assemble a database of epidemiological studies examining the human health effects associated with malathion exposure; and,
- Review, summarize, and assess the quality of the assembled literature.

[11] The findings from IDS and NPIC are often similar and compliment the other because most NPIC cases are reflective of homeowner reports, and IDS is reflective of Registrant incident reports that primarily stem from homeowner calls

This report describes the systematic review approach and results of OPP's evaluation of epidemiology literature. This evaluation focused on characterizing results with respect to health outcomes evaluated in the literature and identifying strengths and limitations and overall quality of the study in the regulatory context.

3.2 Review Framework

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. In a 2014 report, NRC identified systematic literature review strategies as "appropriate for EPA" and "specifically applicable to epidemiology and toxicity evaluations"[12] for regulatory purposes.

In 2016, EPA OPP published a framework for incorporating epidemiological data into 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.[13] The epidemiology framework characterized "fit for purpose" systematic reviews for incorporating human epidemiology data into 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:[14]

- Clearly stated set of objectives with pre-defined eligibility criteria for studies.
- Explicit, reproducible methodology.
- Systematic search to identify all relevant studies.
- Assessment of the validity of the findings from the identified studies.
- Systematic presentation and synthesis of the characteristics and findings of the included studies.

Following the procedures described in the OPP epidemiology framework, OPP conducted a formalized literature review to collect, evaluate, and integrate evidence from relevant epidemiological literature on the association between malathion exposure and human health outcomes to evaluate whether exposure to this chemical is associated with an increased (or decreased) risk of adverse health outcomes.

3.3 Methods

3.3.1 Systematic Literature Search

The literature search methodology followed the guidance provided in the National Toxicology Program/Office of Health Assessment and Translation (NTP/OHAT*) Handbook for Conducting a*

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

[13] 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

[14] Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., and Welch, V.A. (Eds.) (2019). Cochrane Handbook for Systematic Reviews of Interventions. 2nd edition. Chichester (UK): John Wiley & Sons.

Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration, January 9, 2015. For the search, the following population, exposure, comparator, and outcome of interest (PECO) criteria below guided the inclusion/exclusion criteria and selection of terms:

- **Population** of interest: Population studied must be humans with no restrictions, including no restrictions on age, life stage, sex, country of residence/origin, race/ethnicity, lifestyle, or occupation

- **Exposure**: Exposure studied must be to malathion in any application via any route of exposure.

- **Comparator**: Exposed or case populations must be 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 malathion exposure.

- **Outcome**: All reported human health effects, with no restrictions on human system affected (effects could be based on survey or other self-report, medical records, biomarkers, publicly available health data, or measurements from human sample populations).

Based on these PECO criteria, inclusion/exclusion terms were identified, and a literature search was conducted in PubMed, PubMed Central, Scopus, and Science Direct. The search included all published articles from January 1, 1980 through February 16, 2021. Database searches were conducted in "All" fields, except in Scopus. Database searches were conducted in "All" fields, except in Scopus. Scopus is an abstract and citation database and therefore searches the abstract and references sections but not the remaining text. A search in Scopus conducted in "All" fields retrieves many irrelevant articles; thus, the search was done using the "Title-Abstract-Keyword" fields.

A complete listing of PECO search terms is provided in **Table 5**. Each specific pesticide search term was combined with search terms. Terms within each row were combined with "OR", and terms between rows were combined with "AND", except for the Excluded Terms, which were combined with either "NOT" or "AND NOT."

Table 5: Malathion Literature Search Terms

Malathion Chemical Terms	(malathion[MeSH]) OR (malathion)
Exposure Terms	absorb*; Accident, occupational (MeSH); accident*; consum*; contamin*; drink*; Environmental exposure (MeSH); Expos*; Food contamination (MeSH); Fungicides, Industrial (MeSH); Herbicides (MeSH); Ingest*; Insecticide (MeSH); Intoxication*; Occupational exposure (MeSH); Pesticides (MeSH); poison*; Poisoning (subheading); Prenatal exposure, delayed effects (MeSH); skin absorption (MeSH); toxic*; Toxicity (subheading); water
Epidemiologic Methods Terms	case-control; cohort; community stud*; cross-sectional; environmental stud*; epidemiolog*; Epidemiologic methods (MeSH); Epidemiologic Studies (MeSH); Epidemiology (subheading); Health survey*; Incidence (MeSH); longitudinal; occupational stud*; prospective; retrospective

Malathion Chemical Terms	(malathion[MeSH]) OR (malathion)
Health Effects/Disease Terms	Adverse effects (subheading); Agricultural workers' disease (MeSH); allergy; Allergy and Immunology (MeSH); arthritis; asthma; birth defect*; Birth weight (MeSH); cancer*; carcinogen*; cardiac; confusion; death; disease*; dizziness; emetic*; Environmental illness (MeSH); Health effect*; Health impact*; hepat*; Hodgkin's; hospital*; illness*; kidney; leukemia; liver (MeSH); lung; lymphatic system (MeSH); lymphoma; medical; morbidity; mortality; myeloma; myocardial; nausea; Neoplasm (MeSH); nervous system (MeSH); neurodegenerat*; Neurodegenerative disease (MeSH); neurologi*; neuromuscular*; neurotoxi*; Occupational illnesses (MeSH); Pregnancy outcome (MeSH); Pregnancy outcome*; pulmonary; renal; respirat*; respiratory paralysis; sarcoma; thyroid; thyroid (MeSH); thyroid hormones (MeSH)
Excluded Terms	Avian; bee / bees; beetle*; bird*; case report*; drug*; fish; invertebrate*; leaf; leaves; medicine*; mites; monkey* ; mouse/mice; prognosis; prognostic; rat/rats; rodent*; sheep; suicide; therap*; treatment*; trout; zebrafish

[MeSH] = Medical Subject Headings, which is a National Library of Medicine (NLM) controlled vocabulary thesaurus used for indexing articles for PubMed.
[subheading]=a qualifier used to describe a specific aspect of a MeSH heading
* indicates truncation (i.e., that alternate endings were searched)

Based on the PECO criteria and search terms described above, the literature search aimed to identify original, peer-reviewed publications on epidemiologic studies. Exclusion criteria were also identified prior to collecting potentially relevant publications. Publications 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 malathion-specific investigation (*e.g.,* general insecticide); no risk/effect estimate reported (*e.g.,* case studies/series); and no original data (*e.g.,* review publications).[15] In addition, the review focused on epidemiology studies and excluded publications on acute poisonings and overexposure.

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, OPP considered human health effects via the toxicological paradigm presented by the NRC as pathologies or health impairments subsequent to altered structure/function.[16] Thus, studies with outcomes of altered structure (*e.g.,* 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.

[15] While the search focused on original peer-reviewed publications, OPP does seek out and consider other sources of information that are not peer-reviewed (e.g., letters to the editor, corrections, commentary) on a case-by-case basis when this information provides clarification or other material findings or information of relevance to our evaluation of the literature.

[16] Goldstein, B., Gibson, J., Henderson, R., Hobbie, J., Landrigan, P., Mattison, D., Perera, F., Pfitzer, E., Silbergeld, E., Wogan, G. (1987). Biological markers in environmental health research. *Environmental Health Perspectives*, *7* (3-9).

3.3.2 Supplemental Literature Search

To supplement the open literature search described above, OPP reviewed publications resulting from the Agricultural Health Study (AHS) for publications that satisfied the inclusion/exclusion criteria. The AHS is a federally funded study that evaluates associations between pesticide exposures and cancer and other health outcomes 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 maintains on its website an electronic list of publications resulting from AHS studies using the AHS cohort.[17] These publications were imported into Endnote, and Endnote was used to run a full text search ("Any Field + PDF with Notes") for "malathion" to ensure all AHS publications relevant to the epidemiology literature review were identified. AHS publications 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 publications captured in the open literature search, the AHS publication search, and previously published OPP documents. References were examined to identify relevant publications that were not captured in either the open literature search or the AHS publication search. Resulting publications from this reference review that satisfied inclusion/exclusion criteria were selected for inclusion in the epidemiology literature review.

3.4 Literature Search Results

The search of the open literature returned 4,296 publications (2,958 unique publications) across PubMed, PubMed Central, Science Direct, and Scopus and these publications were assembled into an EndNote Library (version x9) (1,335 duplicates were removed).

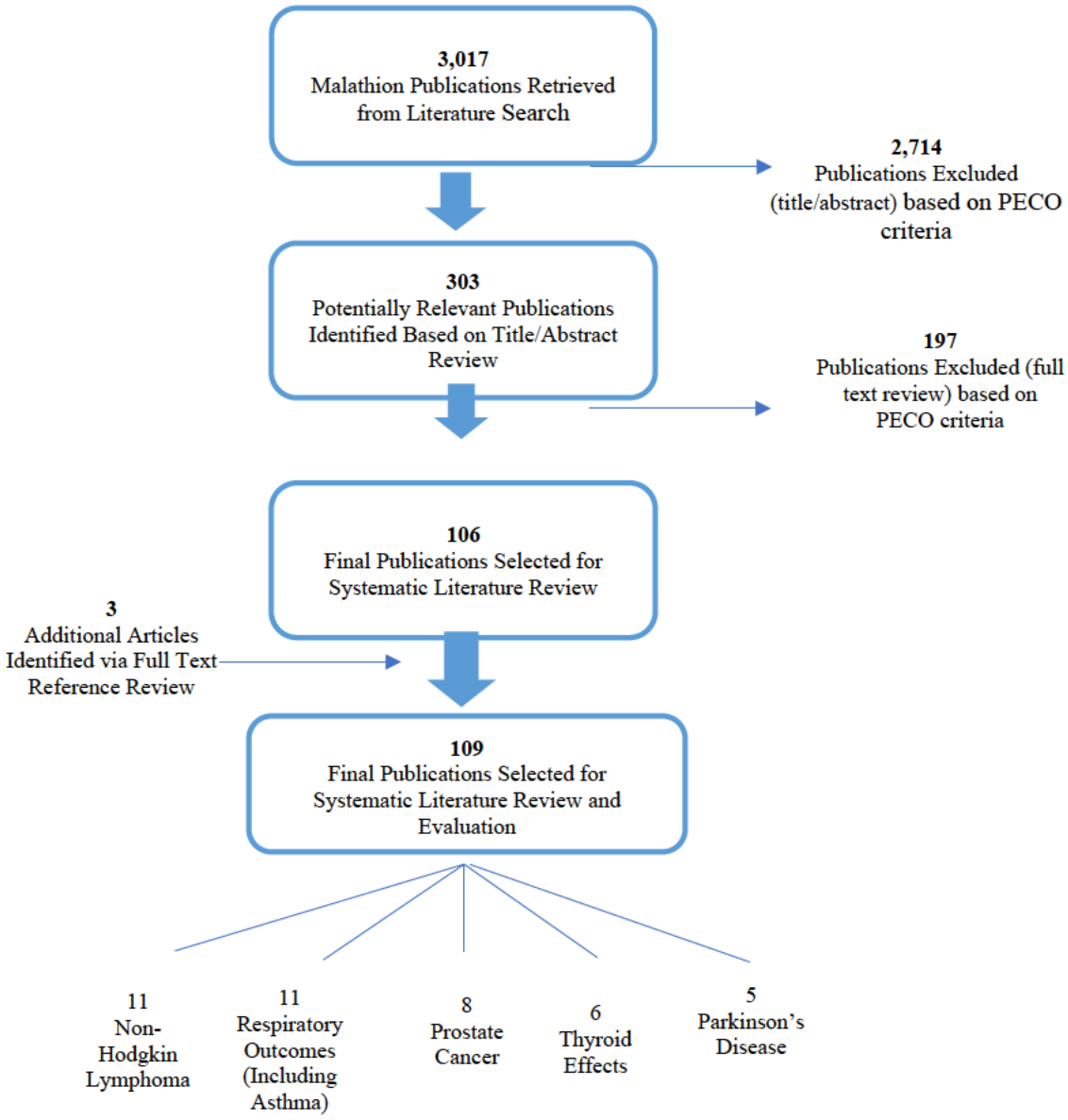
One hundred and ten AHS publications that included the terms "malathion" in the text were identified in a supplemental search of the AHS EndNote library (51 of these were also identified in the open literature search and these were removed from the open literature total articles retrieved).

A total of 3,017 publications (2,907 open literature, 110 AHS) underwent title and abstract screening for potential relevance using the PECO criteria and exclusion criteria described in the **Systematic Literature Search** section.

Of these, 303 (193 open literature, 110 AHS) publications were selected for full text review based on this approach and of these, 197 (156 open literature, 41 AHS) were excluded because they did not include malathion-specific analyses and three additional articles were identified through reference review. A total of 109 publications were selected for literature review and evaluation. A summary of the literature search and supplemental AHS search is provided in **Figure 3** below.

[17] Agricultural Health Study Publications: https://aghealth nih.gov/news/publications.html

Figure 3. Summary of Literature Search Results



3.5 Literature Review and Evaluation Approach

3.5.1 Study Review and Quality Assessment

A total of 109 peer-reviewed epidemiologic publications were identified for OPP's literature review and evaluation. Each publication was reviewed and relevant information was summarized on study design, results, conclusions, the strengths and weaknesses of each study per the epidemiology framework (US EPA, 2016), 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), beta coefficient (β) and associated estimates of uncertainty and/or statistical significance (*e.g.,* confidence interval (CI), p-value),

confounders considered, and methods of analysis. OPP considered these elements in assessing the quality of each publication and its applicability to an overall assessment of the health effects associated with malathion exposure in terms of usefulness for regulatory purposes.

The assessment of study quality followed the OPP Framework. As shown in **Table 6**, the study quality assessment for regulatory purposes 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 6. Epidemiology Study Quality Considerations for Regulatory Purposes (Adapted from Table 2 in US EPA, 2016)

Parameter	High	Moderate	Low
Exposure assessment	Exposure assessment includes information on malathion or metabolite in the body, quantitative air sample data, or high-quality questionnaire on chemical-specific exposure assessment during relevant exposure window	Questionnaire based individual level information on malathion	Low quality questionnaire-based exposure assessment, or ecologic exposure assessment, with or without validation
Outcome Assessment	Standardized tool, validated in study population; or, medical record review with trained staff	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated	Subject report, without additional validation
Confounder control	Good control for important confounders relevant to malathion study question, and standard confounders	Moderately good control of confounders, standard variables, not all variables for malathion study question	Multi-variable analysis not performed, no adjustments
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 (esp. 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, 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

Note: Overall study quality ranking based on comprehensive assessment across the parameters.

Study design 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 are susceptible to recall bias, were generally considered to be of 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, due to an inability to extrapolate observed associations from the group level to the individual level (ecological fallacy) inherent in the ecologic study design. Ecologic studies were generally regarded as hypothesis-generating studies (US EPA, 2016).

Studies that characterized the exposure-response relationship (*e.g.,* with a dose-response curve or trend statistic) were, in general, considered higher quality 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 and explored 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) bolstered the assessment of study quality.

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

- No evidence of a positive association between exposure and outcome (e.g., 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.[18]

However, we recognize that results that fail 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 large observed associations (*e.g.,* OR ≥ ~2.5) even in the absence of significance, perhaps indicating a smaller than optimal sample size.

3.5.2 Categories of Evidence

The categories of evidence described in **Table 7** are guided by several documents that have been developed by EPA and others. These include as a main reference a document developed by the Institute of Medicine (now the Academies of Science, Engineering, and Medicine)[19] which detailed various "Categories of Association" which describes guidance for drawing conclusions regarding the overall strength of the evidence that exists regarding any putative linkage between an exposure and a health effect

[18] Although listed as OR (odds ratios) here, these characterizations are also applicable to risk ratios (RRs) and hazard ratios (HRs). For publications 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.

[19] 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: https://www ncbi.nlm.nih.gov/books/NBK44695/. 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.

(IOM, 1998). Also considered in developing OPP's categories of evidence were the NTP's OHAT document on systematic review and evidence integration (Woodruff and Sutton, 2014), OPP's epidemiologic framework document (US EPA, 2016), and EPA's Preamble to the Integrated Science Assessments which serve as a scientific foundation for the review of EPA's National Ambient Air Quality Standards (US EPA, 2016).

In this memorandum, each category is assigned based on a case-by-case approach that considers the weight of the epidemiological evidence and expert judgement and not 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. When assigning a level of evidence category to an exposure and the body of evidence pertaining to that health effect, 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 consistency of the association in magnitude and direction across available studies was considered, as described in OPP's epidemiologic framework document.

Table 7. Tier II Epidemiology Studies 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 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 that is consistent among studies with respect to magnitude and direction of effect sizes. Such 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.

Evidence Category	Description
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 outcome.* There is some confidence that the available evidence accurately reflects a clear association between the exposure and the outcome, 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 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 outcome.* There is minimal confidence in the available evidence that the findings accurately reflect an association between the exposure and the outcome 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.
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 outcome.* There is no epidemiological evidence to suggest the presence of an association between an exposure and outcome. This level of evidence category might be met, for example, if the body of evidence consists of high- or moderate-quality studies that show no evidence of a statistically significant association and generally appear to have small effect sizes, and/or for which chance, bias, or confounding may play an important role.

Evidence Category	Description
Sufficient Evidence of No Causal Relationship	*Sufficient epidemiological evidence to suggest there is no causal relationship between the exposure and the outcome.* There is high confidence in the available evidence to suggest there is no causal relationship between the exposure and the outcome. 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 a meaningful 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 a least one high-quality prospective cohort study.

3.5.3 Common Study Considerations

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

Malathion Biomonitoring

Some of the epidemiologic studies identified assessed malathion exposure by measuring malathion 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 malathion, the specific urinary biomarker is malathion dicarboxylic acid (MDA). This memo focused on exposure to the specific metabolite for malathion, MDA, instead of exposure to non-specific malathion metabolites such as dialkyl phosphate (DAPs). Since the purpose of this memo was intended to assess exposure to the parent compound, and less specific biomarkers may reflect exposure to *multiple* different parent compounds, this memo solely considered epidemiological studies involving the specific malathion metabolite (MDA) and did not consider studies involving non-specific malathion metabolites.

Agricultural Health Study

Many studies reviewed in this memorandum are part of the Agricultural Health Study (AHS). 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 potentially related to (case) recall bias which can be a serious methodological weakness of many case-control studies. Such recall biases 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 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 generally provides 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: 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 a number of other epidemiology studies. But individuals -- particularly over a number of 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 – especially exposures over long time periods time – and 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. 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 associated 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.

3.6 Literature Review and Evaluation

This section presents a review and evaluation of the epidemiologic literature on the potential association between malathion exposure and carcinogenic and non-carcinogenic adverse health outcomes. The review and evaluation of the available literature is organized by carcinogenic and non-carcinogenic adverse health outcomes. For each of the health outcome sections, individual study publications are summarized and then an overall evaluation of findings is characterized. **Appendix B** provides a tabular summary of all the studies reviewed, with respect to their design, methods, results, and study quality organized by health outcome.

3.6.1 Carcinogenic Health Outcomes

For carcinogenic health outcomes, EPA conducted a review of 42 publications that investigated the relationship between malathion exposure and carcinogenic effects including: all cancers combined, bladder cancer, brain and spinal cancer, breast cancer, cancers of the large intestines, childhood cancer, esophageal cancer, gastric cancer, kidney cancer, lung cancer, lymphohematopoietic cancers, ovarian cancer, pancreatic cancer, prostate cancer, soft tissue sarcoma, thyroid cancer, and uterine cancer. The 42 studies for these health outcomes are described below.

Cancer (all sites)

Two publications (Bonner et al., 2007; Lerro et al., 2015) evaluated the relationship between malathion exposure and all cancers in adults.

- The association between cancer (all sites) and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[20]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. In the all cancer analysis for IWLD with the

[20] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile of exposure (T1 – T3: 0.93 ≤ RRs ≤ 1.10; all CIs encompassed the null value of 1.0, with n = 207 – 218 exposed cases in each tertile, with 349 cases in the non-exposed group)[21]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.60). A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present and the study authors concluded this bias had no effect on the reported risk estimates.

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including all cancers combined among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997 living in Iowa and North Carolina who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD – O – 3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history, and other covariates that may be potential confounders. The association between malathion exposure and all cancers was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application, and correlated/associated pesticide use. Models for cancers of the ovaries, breast, uterus, and all sites combined (all cancers) were additionally adjusted for number of live births, menopause status at enrollment, and oral contraceptive use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 2,712 total cancer cases identified during the study period, 558 cases reported direct exposure to malathion. No evidence of a significant positive association was reported between malathion exposure and total cancer risk (RR = 1.03; 95% CI: 0.92, 1.15; with n = 558 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and -- similarly -- no evidence of a significant positive association was reported for malathion exposure and all cancers (RR = 1.04; 95% CI: 0.91, 1.18; with n = 411 exposed cases).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the

[21] Risk estimates for intensity-weighted lifetime days of exposure for cancer (all sites) reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.85 ≤ RRs ≤ 1.05; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and cancer (all sites) among pesticide applicators and their spouses. This determination is based on a limited body of evidence that consisted of the AHS cohort involving pesticide applicators and their spouses. Bonner et al. (2007) was ranked high and Lerro et al. (2015) was ranked moderate quality for regulatory purposes. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and cancer (all sites) among pesticide applicators enrolled in the AHS prospective cohort. Several strengths were noted including the prospective cohort study design as part of the AHS, the ascertainment of cancer using established cancer registries, and the strengths of the AHS exposure assessment approach. Lerro et al. (2015) reported no evidence of a significant positive association between malathion ever exposure and cancers (all types) among female spouses of pesticide applicators in the AHS prospective cohort. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate statistical methods used. Additional details regarding the duration of time for pesticide usage (e.g., days, months, years) was not provided but would have been helpful.

Bladder cancer

Two publications (Bonner et al., 2007, Koutros et al., 2016) examined the association between malathion exposure and bladder cancer among pesticide applicators.

- The association between bladder cancer and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Additionally, risk ratios were determined in a separate analysis for frequency (days of use per year), intensity (intensity score), and duration (years of use) of malathion exposure. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[22]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. Risk estimates for frequency, intensity, and duration of exposure for bladder cancer were also calculated by the study authors. A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present and the study authors concluded this bias had no effect on the reported risk estimates. In the bladder cancer

[22] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

analysis using IWLD exposure with the non-exposed group as the referent, no evidence of a positive association was observed in any tertile (T1 – T3: 0.85 ≤ RRs ≤ 0.91; all CIs encompassed the null value of 1.0, with n = 8 – 9 exposed cases per tertile, with 14 cases in the non-exposed group)[23]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.91). Additionally, no evidence of a positive association between bladder cancer for frequency, intensity, and duration[24] of malathion exposure was observed (0.65 ≤ RRs ≤ 1.00; all CIs encompassed the null value of 1.0, with n = 7 – 19 exposed cases, with 15 cases in the non-exposed group, p-trends > 0.05).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

- Koutros et al. (2016) investigated the association between malathion and other pesticides and bladder cancer incidence among study participants in the prospective AHS cohort. The study population consisted of male pesticide applicators in Iowa and North Carolina with incident bladder cancer cases identified through cancer registry files in Iowa and North Carolina through 2011. Pesticide exposure was assessed via two self-administered questionnaires, one administered during study enrollment and a second follow-up questionnaire administered five years after enrollment. Investigators used this questionnaire data to estimate intensity-weighed lifetime days of use,[25] and Poisson regression analysis was used to calculate RRs adjusting for age, race, state of residence, pack-years of cigarettes, and pipe smoking. Among the study population (n = 54,344), 321 bladder cancer cases were reported, with 223 of the cases reporting exposure to malathion. The study results suggested no evidence of a significant positive association between malathion exposure and risk of bladder cancer (RR = 1.01; 95% CI: 0.65, 1.58) based on ever/never use. Further analyses considered intensity-weighted lifetime days of malathion use. Low and high exposure categories were created, split at the median exposure value based on cumulative intensity-weighted days, and RRs were reported for each category. Adjusting for the aforementioned factors, the researchers observed no evidence of a significant positive association between malathion exposure and bladder cancer in the first three categories (RR = 1.00; 95% CI: 0.62, 1.59 for the 1st quartile median category, RR = 1.15; 95% CI: 0.71, 1.86 for the 2nd quartile median category, RR = 1.14; 95% CI: 0.71, 1.83 for the 3rd quartile median category, with n = 27-29 exposed cases in all categories and n = 49 unexposed cases), no evidence of a positive association between malathion exposure and bladder cancer in the last exposure category (RR = 0.95; 95% CI: 0.60, 1.52 for the 4th quartile median category, with n=29), and there was no evidence of increasing risk of bladder cancer with increased use of malathion (p-trend = 0.73). Additional analyses of intensity-weighted lifetime days of malathion use stratified the data by smoking status (never, former, and current smoker strata), and found no evidence of a positive association between malathion exposure and bladder cancer risk among never or former smokers and no evidence of a

[23] Risk estimates for intensity-weighted lifetime days of exposure for bladder cancer reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.71 ≤ RRs ≤ 1.40; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

[24] In a separate analysis, frequency of use was defined as: <5 or ≥5 days of use per year, duration of use was defined as : ≤10 years of use or >10 years of use, and intensity was defined by tertiles; however, the tertiles were not specified in the table by the study authors.

[25] Cumulative lifetime days of use is the product of years of use and the number of days used per year. Intensity-weighted lifetime days of use is defined as the product of exposure intensity (based on mixing status, application method, equipment repair, and use of personal protective equipment) and lifetime days of use.

trend of increasing risk with increased use (never smoking stratum: p-trend = 0.63; former smoking stratum: p-trend = 0.85; current smoking stratum: p-trend=0.82). Likelihood ratio tests to assess the differences between the never, former, and current smoking strata found no evidence of effect modification by smoking on the relationship between malathion exposure and bladder cancer (p-interaction = 0.44).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the pesticide exposure assessment.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and bladder cancer. This determination was based on two publications (Bonner et al., 2007, Koutros et al., 2016) that investigated the potential association among the AHS pesticide applicators. Bonner et al., 2007 reported no evidence of a significant positive association between malathion exposure and bladder cancer among pesticide applicators enrolled in the AHS prospective cohort, based on lifetime days and intensity-weighted lifetime days of use. Additionally, no evidence of a positive association for frequency, intensity, and duration of malathion exposure was observed. The study quality was ranked high quality for regulatory purposes and several strengths were noted including the prospective cohort study design as part of the AHS, the ascertainment of cancer using established cancer registries, and the strengths of the AHS exposure assessment approach. The second publication, Koutros et al. (2016), reported no evidence of a significant positive association between malathion exposure and bladder cancer among AHS pesticide applicators based on ever/never use. Further analyses that considered intensity-weighted lifetime days of malathion use and intensity-weighted lifetime days of malathion use stratified by smoking status (never, former, and current smoker strata), reported no evidence of a significant positive association between malathion exposure and bladder cancer, with no evidence of statistically significant p-trends. The quality of the study was ranked high for regulatory purposes.

Brain and Spinal Cancer (Glioma)

Three studies (Lee et al., 2005; Yiin et al., 2012; Lerro et al., 2015) evaluated the association between malathion exposure and glioma.

- Lee et al. (2005) investigated the association between farming and agricultural pesticide use, including malathion, and glioma in the Nebraska Health Study II, a case-control study of adults in eastern Nebraska. The study population included white residents of eastern Nebraska, ≥21 years old. Cases of incident primary glioma diagnosed between 1 July 1988 and 30 June 1993 with histological confirmation were identified using the Nebraska Cancer Registry and participating hospitals in Lincoln and Omaha. Controls for the current study were randomly selected from the control group of a previous study covering the same base population and were frequency matched by age, sex, and vital status to the combined distribution of the glioma, stomach, and esophageal cancer cases. Demographic, medical and family history, occupational, and, pesticide exposure information (for those who lived or worked on farm) was collected via telephone interview conducted during 1992-1994. Pesticide exposure information was limited to use prior to 1985, the time period of the previous study. Interviews were conducted for 251 cases and 498 controls; however, most interviews were conducted via proxy (76% of cases, 60% of controls) who were primarily spouses (45%) or other primary relatives (46%). Unconditional logistic regression was used to calculate ORs and 95% CIs for farming activity and for individual pesticide use, adjusted for age, respondent type, and sex,

with the non-farmers as a reference group. Among the 251 cases and 498 controls included in the final analysis, 16 cases and 29 controls reported malathion exposure. No evidence of a significant positive association was reported for malathion ever use and glioma among farmers in Nebraska (OR = 2.00; 0.90, 4.20; with n = 16 exposed cases). When stratified by type of respondent (self or proxy), evidence of a strong association was reported among cases who completed the interview themselves. We note the small number of exposed cases and corresponding wide confidence interval (OR = 3.40; 95% CI: 1.20, 9.30; with n = 11 exposed cases and n = 17 exposed controls). No evidence of a positive association was reported between malathion ever use and glioma among those cases who had a proxy respondent (OR= 0.80; 95% CI: 0.20, 2.80; with n = 5 exposed cases and n = 12 exposed controls).

The overall quality of the study was ranked low quality based on the study quality criteria provided in the OPP framework. The study had several limitations related to its design, exposure assessment approach, statistical analysis, and ability to control for confounding. With regard to study design, Lee et al. (2005) used a case-control approach and may have introduced selection bias when recruiting their control group. Differences between the results for the self-reporting respondents and the proxy respondents illustrate the possible problem, as the control groups for each of these respondents were constructed differently and each could be biased in a different way. In the analysis, the reference group for the statistical tests was non-farmers, even though the pesticide use questions were not asked of non-farmers. As a result, the results for pesticides are confounded with farmer versus non-farmers and control groups with different proportions of farmers will result in different statistical results. The use of respondent-reported malathion use to ascertain exposure introduced further uncertainty because it is not possible to attribute the increased odds of glioma to malathion exposure alone since the self-reporting and proxy respondents have different levels of knowledge about pesticide use and possibly different motives for responding. Moreover, self-reported exposure assessment is likely to be subject to differential misclassification because study participants may incorrectly recall previous pesticide usage. In addition to these limitations, we note the number of exposed cases was small (10 < exposed cases < 19).

- Yiin et al. (2012) investigated the association between pesticide exposure, including malathion, and glioma among rural pesticide applicators enrolled in the Upper Midwest Health Study (UMHS), a population-based case-control study. The study population included participants residing in four states (Michigan, Iowa, Wisconsin, and Minnesota), aged 18 – 80 (between ascertainment/diagnosis in 1995 through January 1997). Cases of histologically confirmed glioma were identified via participating medical facilities, oncologists, and neurosurgeons in the area, and glioma registrations in state cancer registries were reviewed to capture any missed diagnoses. Controls included participants aged 18 – 80 years old, with or without a self-reported history of cancer other than glioma who were randomly selected from state driver's license/nondriver ID records and from Health Care Financing Administration's Medicare data within a 10-year age group as determined by the age distribution of glioma cases in that state from 1992 – 1994. Controls were frequency-matched to cases by state. Pesticide exposure (cumulative use and lifetime intensity weighted) through 1992 was assessed using information collected on an interviewer-administered questionnaire. Demographic information and occupational and medical histories were also collected via the questionnaire. Proxy respondents were used in this study in the event the cases were deceased or impaired and unable to participate in the study. A separate analysis was conducted with and without proxy respondents in an effort to examine any differences that may exist. Unconditional logistic regression was used to estimate ORs and 95% CIs for the association between cumulative years and estimated lifetime cumulative exposure of farm pesticide use and glioma, adjusted for the 10-year age group, sex, age, and education (less than high school, high school graduate, college graduate). Among the 778 and 1,175 total cases and controls, respectively, 228 (29%) glioma cases and 417 (35%) controls reported exposure to pesticides while being on a farm. A further analysis within this study then looked at the relationship between pesticide

exposure including malathion among study participants whose occupation was not farm-related. Of the total 65 cases and 34 controls who reported pesticide exposure in non-farm jobs, 9 (13.8%) cases and 18 (52.9%) controls reported malathion exposure among the sample population that included proxy respondents. No evidence of a positive association was reported between malathion ever use and glioma among non-farm applicators (OR=0.69; 95% CI: 0.30, 1.56; with n=9 exposed cases and n=18 exposed controls). No evidence of a significant positive association was reported between malathion exposure in non-farm jobs and glioma among non-farm applicators, *when proxy respondents were excluded* (OR=1.04; 95% CI: 0.45, 2.40; with n=9 exposed cases and n=18 exposed controls). An additional analysis evaluated pesticide use among cases who reported home and garden pesticide usage. Forty-five (11.3%) of the 399 cases with proxy respondents and 84 (41.2%) of the 204 cases without proxy respondents who reported pesticide exposure throughout the home and through gardening also reported exposure to malathion. No evidence of a positive association was reported between malathion exposure and glioma in either analysis (*Proxy respondents included* – OR=0.82; 95% CI: 0.56, 1.20 with n=45 exposed cases, 84 controls; *Proxy respondents excluded* – OR=0.72; 95% CI: 0.44, 1.18 with n=24 exposed cases, 84 controls).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP framework. The study had several limitations related to its design, exposure assessment approach, statistical analysis, and ability to control for confounding. With regard to study design, Yiin et al. (2012) used a case-control approach and may have introduced selection bias when recruiting their control group. A further limitation of the study was the large number of proxy respondents used to complete interviews for the cases (45% of case interviews), relative to the controls (3% of control interviews). Although the study authors mentioned they tried offsetting the number of proxies used by conducting two separate analyses (with and with proxy respondents), inaccurate information obtained from the proxy respondents was still a strong possibility, potentially interfering with estimates of some of the observed outcomes. The use of respondent-reported malathion use to ascertain exposure introduced further uncertainty because it is not possible to attribute the increased odds of glioma to malathion exposure alone since the self-reporting and proxy respondents have different levels of knowledge about pesticide use and possibly different motives for responding.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including brain cancer among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997 living in Iowa and North Carolina who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD – O – 3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history and other covariates that may be potential confounders. The association between malathion exposure and brain cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 38 brain cancer cases identified during the study period, 11 cases reported

direct exposure to malathion. No evidence of a significant positive association was reported between malathion exposure and brain cancer (RR = 1.57; 95% CI: 0.65, 3.78; with n = 11 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment. We note the number of exposed cases was small (10 < exposed cases < 19).

EPA Conclusion

Overall, there is *insufficient epidemiological* evidence at this time to conclude that there is a clear associative or causal relationship between malathion exposure and brain cancer including glioma. This determination was based on three publications (Lee et al., 2005; Yiin et al., 2012; Lerro et al., 2015) that assessed the association between malathion exposure and brain cancer among adults living in Nebraska, among pesticide applicators living in the Midwest, and among women in the AHS prospective cohort. Lee et al. (2005) reported no evidence of a positive association between malathion exposure and glioma in a case-control study in Nebraska, but when the data was further stratified by type of respondent (self or proxy), evidence of a strong association was reported among cases who completed the interview themselves, among a small number of exposed cases (n = 11) and corresponding wide confidence interval. No evidence of a positive association was reported between malathion ever use and glioma among those cases who had a proxy respondent. The study was ranked low quality for regulatory purposes. Proxy respondents were used suggesting the possibility of meaningful information (recall) bias. Further, in order to obtain sufficient younger controls for comparison purposes, they were required to add more controls to the study using random digit dialing and death certificates. These control selection methods may have resulted in a reference population that was not appropriate for this study. We also note the number of exposed cases was small which limits the ability to interpret with confidence the observed odds ratios. The second study, Yiin et al. (2012), reported no evidence of a positive association between malathion exposure and glioma among applicators, and no evidence of a significant positive association when the proxy respondents were excluded, in a case-control study involving four Midwest states. The study was ranked moderate quality for regulatory purposes, and study imitations included the large proportion of proxy respondents, self-report of exposure, selection-bias, and recall-bias. The third publication, Lerro et al. (2015), reported no evidence of a significant positive association between malathion exposure and brain cancer among female spouses in the AHS cohort. The overall quality of the study was ranked moderate for regulatory purposes. Additional details regarding the duration of time for pesticide usage (e.g., days, months, years) was not provided but would have been helpful.

Breast cancer

Five publications (Engel et al., 2005; Mills and Yang, 2005; Lerro et al., 2015; Engel et al., 2017; Golmohammadzadeh et al., 2019) examined the association between malathion exposure and breast cancer.

- Engel et al. (2005) evaluated the association between breast cancer incidence among farmers' wives and specific pesticides including malathion. The study population consisted of female spouses of pesticide applicators enrolled in the AHS living in Iowa and North Carolina. Breast cancer cases were identified using cancer registries in Iowa and North Carolina from enrollment (1993-1997) through 2000. Pesticide exposure was assessed based on self-reported questionnaires completed by the AHS participants during study enrollment. Of the 309 breast cancer cases identified within the cohort (n = 30,454), 63 (20.8%) women reported malathion use. Of the 30,145 non-cases (women not diagnosed

with breast cancer) with complete data, a total of 5,706 (19.2%) women reported malathion use. Poisson regression was used by the authors to calculate RRs and 95% CIs for individual pesticides, including malathion, and each analysis was adjusted for age, race, and state of residence. The authors reported no evidence of a positive association between ever use of malathion and breast cancer incidence among all wives in the cohort (RR = 0.90; 95% CI: 0.70, 1.20; with 63 exposed cases). A subset analysis conducted for wives who reported no prior pesticide use (n = 13,449) considered husbands' malathion use and no evidence of a significant positive association was similarly reported between husband's malathion use and wife's risk of breast cancer (RR = 1.40; 95% CI: 1.00, 2.00; with 101 cases indirectly exposed).

A second subset analysis -- by state -- also found no evidence of a positive association between ever exposure to malathion and breast cancer incidence among all women in the cohort in Iowa (RR = 0.80; 95% CI: 0.60, 1.20 with n = 43 cases (21.3% cases exposed), and no evidence of a significant positive association between husband's malathion use and wife's risk of breast cancer among wives who reported never using malathion in Iowa (RR= 1.40; 95% CI: 0.90, 2.20 with n = 63 cases (68.5% husbands exposed). Similarly, in North Carolina, no evidence of a positive association was observed between ever exposure to malathion and breast cancer incidence among all women in the cohort (RR = 1.00; 95% CI: 0.60, 1.70 with n = 20 cases (19.8% cases exposed), and no evidence of a significant positive association between husband's malathion use and wife's risk of breast cancer among wives who reported never using malathion (RR= 1.50; 95% CI: 0.80, 2.70 with n = 38 cases (71.7% husbands exposed). A separate subset analysis by menopausal status at enrollment also found no evidence of a positive association between ever exposure to malathion and breast cancer incidence for women who were either pre-menopausal or post-menopausal at enrollment among all women in the cohort (RR pre-menopausal = 0.90; 95% CI: 0.50, 1.50 with 16 exposed cases out of n = 87 total cases (19.1% exposed) and 13,087 non-cases (18.0% exposed); RR post-menopausal = 0.90; 95% CI: 0.60, 1.20 with n = 41 cases out of n = 192 total cases (21.7% exposed) and 10,736 non-cases (23.1% exposed)). Among wives who reported never personally using malathion themselves, no evidence of a significant positive association was found between husband's malathion use and wife's risk of breast cancer for women who were either pre- or post-menopausal at enrollment (RR pre-menopausal = 1.50; 95% CI: 0.70, 3.00 with n = 25 exposed-through-husbands-use cases (71.4% husbands exposed)), and RR post-menopausal = 1.50; 95% CI: 1.00, 2.30 with n = 69 exposed-through-husband's-use cases (69.7% husbands exposed)).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. As part of the AHS, this study benefited from the strengths of the AHS study cohort as described above. However, the investigators assessed indirect exposure based on self-reported pesticide use from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses. In fact, more cases reported indirect exposure to malathion (n = 101) from the wives' husbands than direct exposure (n = 63) from the wives themselves.

- Mills and Yang (2005) conducted a nested case-control study to investigate the association between breast cancer and pesticide exposures, including malathion, using a prospective cohort study of farmers who were part of the United Farm Workers of America (UFW). The study population consisted of Hispanic women who were members of the UFW at any time between 1973 and 2001. Incident cancer cases were identified by linking the UFW cohort to the California Cancer Registry during the years 1988 through 2001. Controls consisted of Hispanic females within the UFW cohort who had never been diagnosed with cancer and were randomly selected from the remaining UFW cohort and age-matched (5:1) to the cases based on age of cancer diagnosis. Pesticide exposure for the cases and controls was assessed using three different types of records/databases: UFW records to verify occupational history; grower's contracts to establish the crop/commodity the member was

exposed to; and the California Department of Pesticide Regulation to determine specific pesticide usage. A total of 128 cases of breast cancer were identified between 1988 and 2001 in the UFW cohort, including 39 (81%) cases that reported any use of malathion out of 48 cases with information on malathion use/no use between 1988-1994, and 49 cases (61%) that reported any use of malathion out of 80 between 1995-2001. Conditional logistic regression was used to determine ORs and 95% CIs, adjusting for age, sex, duration of union affiliation, and start date of first union affiliation, fertility, and socioeconomic level. Categories of exposure for malathion (low, medium, and high use) were created using tertiles as the cutoff for each category. Mills and Yang (2005) stratified their analysis of the relationship between malathion use and breast cancer by year of diagnosis (1988-1994 and 1995-2001). Evidence of a moderately strong association was reported for only the medium exposure level of malathion use and breast cancer diagnosed from 1988 to 1994 (OR: 2.95; 95%CI: 1.07, 8.11 with n = 16 exposed cases) among a small number of cases. No evidence of a significant positive association was reported at the low or high exposure levels ($1.68 \leq$ all ORs ≤ 1.89; all 95% CIs encompassed 1.0; n=9-14 malathion exposed cases per exposure category). No evidence of a positive association was reported between any exposure level of malathion use and breast cancer that was diagnosed from 1995 to 2001 ($0.50 \leq$ all ORs ≤ 0.79; all 95% CIs encompassed 1.0; n=14-18 malathion exposed cases per exposure category).

The overall quality of the Mills and Yang (2005) study was ranked low based on the study quality criteria provided in the OPP Framework. The studied leveraged an existing prospective cohort of Hispanic farm worker women based on membership of the UFW during the years 1973 and 2001 and was able to systematically ascertain year of cancer diagnosis using the California cancer registry. The nested case-control study design also helped ensure that controls were systematically identified within the same target study population of Hispanic women farm workers. While these design features were important strengths of the study, the exposure assessment approach used to assess malathion exposure was more limited and relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. No information was provided to demonstrate that this ecologic, county-level pesticide use information can reliably estimate individual-level exposure. The investigators acknowledge this limitation in discussing their results and indicate that ecologic-level exposure assessments can lead to exposure misclassification that may "create spurious associations" that magnify or diminish the underlying true exposure-response relationship. We note the number of exposed cases was small ($10 <$ exposed cases < 19) which limits the ability to interpret with confidence the observed odds ratio.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints including breast cancer among female participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina and who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n = 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD – O – 3 codes from date of enrollment through whichever event was earlier - date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history and other covariates that may have been potential confounders. The association between malathion exposure and breast cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Models were additionally adjusted for number of live births, menopause status at enrollment, and oral contraceptive use. Among the 32,345 spouses of private

applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (220 males were excluded, 907 women with cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure all were excluded) and 5,704 reported lifetime ever use of malathion. Of the 1,059 breast cancer cases identified during the study period, 223 cases reported direct exposure to malathion. No evidence of a significant positive association between malathion exposure and breast cancer risk was reported among AHS spouses (RR = 1.05; 95% CI: 0.88, 1.26). When breast cancer was analyzed based on estrogen receptor (ER) or progesterone receptor (PR) status, no evidence of a positive association was identified for *ER+PR+* (*ER+PR+* - RR = 1.00; 95% CI: 0.79, 1.26; with n = 124 exposed cases out of n = 595 total cases) and no evidence of a significant positive association was identified for *ER-PR-* (*ER-PR-* - RR = 1.17; 95% CI: 0.77, 1.78; with n = 40 exposed cases) based on ever use. When breast cancer was stratified by menopausal status at enrollment, no evidence of a significant positive association was reported between malathion exposure and breast cancer for either pre- or postmenopausal participants (*Premenopausal* - RR = 1.04; 95% CI: 0.78, 1.38 with n = 80 exposed cases; *Postmenopausal* - RR = 1.03; 95% CI: 0.81, 1.30 with n = 132 exposed cases). In an additional sensitivity analyses that investigated associations between ever/never malathion exposure and breast cancer diagnosed five or more years after study enrollment among AHS spouses, no evidence of a significant positive association was reported between malathion ever exposure and breast cancer (RR = 1.10; 95% CI: 0.89, 1.35 with n = 156 exposed cases) and between malathion ever exposure and breast cancer diagnosed five or more years after enrollment, based on ER/PR status (*ER+PR+* - RR = 1.11 95% CI: 0.85, 1.45; with n = 95 exposed cases; *ER-PR-* - RR= 1.04 95% CI: 0.62 to 1.74).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

- Engel et al. (2017) examined the association between insecticide exposure, including malathion, and breast cancer among female spouses of private pesticide applicators enrolled in the AHS prospective cohort in a follow-up study to Engel et al. (2005) that included 10 – 11 additional years of follow-up time and additional incident breast cancer cases. The study population included wives of private pesticide applicators living in Iowa and North Carolina who enrolled in the AHS cohort between 1993 and 1997 and completed both enrollment questionnaires on farm exposures, general health, and reproductive health history and the five-year follow-up telephone interview. Incident cases of breast cancer were ascertained through state cancer registries in Iowa and North Carolina, and vital status was ascertained through state death registries and the National Death Index through 2010 (North Carolina) and 2011 (Iowa). Cases with breast cancer diagnosis prior to enrollment were excluded. Among the 30,595 women included in this analysis, 1,081 incident breast cancer cases were diagnosed during the follow-up period and 226 cases and 5,561 non-cases reported malathion exposure at enrollment. Exposure was assessed using data collected at enrollment on ever/never use of 50 pesticides including malathion and on the five-year follow-up interview that asked about frequency of use and pesticide handling practices. Lifetime pesticide use data collected from farmers on the enrollment questionnaire, and data from the farmer's five-year follow-up telephone surveys were also used to assess wives' indirect pesticide exposure. The association between direct and indirect malathion exposure and breast cancer among wives was assessed using Cox proportional hazards regression to estimate HRs and 95% CIs for ever use, intensity-weighted days of use in the previous growing season by the woman, and cumulative potential exposure from husband's use from enrollment through follow-up interview, adjusting for race (white, other), menopausal status, state, parity/age at first birth, and other pesticides. Several other potential confounders were considered but were not included in the final models because they did not change the risk estimates. Missing

covariate data were imputed and results were similar to those that included only available data. For the association between wives' reported ever use of malathion at enrollment and incident breast cancer, no evidence of a positive association was reported (HR = 1.00; 95% CI: 0.90, 1.20; with n = 226 exposed cases and 5,561 exposed non-cases)[26] and similar results were reported when further adjusted for other pesticides associated with breast cancer in the current analysis[27] (HR = 1.00; 95% CI: 0.80, 1.20; with n = 226 exposed cases and 5,561 exposed non-cases). In table 4 of the study, associations between the husbands' use of individual insecticides and risk of breast cancer among farmers' wives who never used pesticides in the AHS were reported. Both adjustments, without[28] and with pesticides associated with breast cancer[29], encompassed the null value ($0.60 \leq HR \leq 1.40$; all 95% CIs encompassed the null value of 1.0; with 216 exposed cases and 100 exposed non-cases). In the analysis that included ever use of malathion by both the women and their husbands where exposure to malathion was attributed to the wife if both husband and wife reported malathion use, no evidence of a positive association was reported for wives' reported use, or husbands' reported use (wives' indirect exposure) for either adjustment without or with specific pesticides ($0.70 \leq HR \leq 0.90$; all 95% CIs encompassed the null value of 1.0; with n = 4,191-5,907 exposed cases and 137 – 164 exposed non-cases). Similarly, no evidence of a positive association was reported for several other sensitivity analyses that examined the association between malathion exposure (direct or indirect) and breast cancers stratified by state of residence, menopausal status at diagnosis, or tumor hormone receptor status ($0.90 \leq HR \leq 1.00$; all 95% CIs encompassed the null value of 1.0; with 20 – 206 exposed cases per category).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. While this study benefited from the strengths of the AHS cohort as discussed previously, namely, the prospective cohort design, collection of exposure data before diagnosis, and extensive covariate information, there were a few limitations noted. Investigators were able to assess malathion direct exposure (ever use) among wives based on self-reported pesticide use data; however, the study was not able to examine lifetime cumulative pesticide exposure as the data was not available for wives of pesticide applicators. Additionally, the indirect exposure assessment was based on the self-reported pesticide use data from wives' husbands which has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses.

- Golmohammadzadeh et al. (2019) examined the association between malathion exposure and breast cancer among women. Using data from a case-control analysis conducted within the Mazandaran province in Iran, cases included women newly diagnosed with breast cancer, aged 20 – 75 years old, who had been histologically diagnosed at Imam hospital located in Sari, Iran between March and May of 2018. Controls were randomly selected from a group of women who were blood donors aged 23 – 66 years old who had visited patients at the same hospital, and had no family history or previous cancer or other illnesses. A structured questionnaire was given by trained interviewers to obtain demographic data on study participants as well as health backgrounds, past occupational pesticide exposures, and residential histories. Pesticide exposure including malathion was measured through blood serum from blood samples (5 mL) collected from the cases and controls and were kept frozen at -20°C. Pesticide residues were first extracted using the hexane and acetone (1:1) technique and later quantified using gas chromatography. Laboratory QA/QC methods were used in this study. An

[26] Adjusted for menopausal status, race, state, and combined parity/age at first birth.
[27] Adjusted for menopausal status, race, state, and parity/age at first birth and use of the following pesticides: benomyl, metribuzin, butylate, and toxaphene.
[28] Adjusted for menopausal status, race, state, and combined parity/age at first birth.
[29] Adjusted for menopausal status, race, state, and parity/age at first birth and use of the following pesticides: benomyl, metribuzin, butylate, toxaphene and additional pesticides: 2,4,5-trichlorophenozyacetic acid, 2-(2,4,5-trichlorophenozy)propanoic acid, trifluralin, aldicarb, and dieldrin.

unconditional logistic regression was used to calculate ORs and 95% CIs for pesticides including malathion. No adjustment for potential confounders was mentioned by the study authors in either statistical model. A total of 123 study participants were included within this study, with 72 cases and 51 controls. The mean ± SD serum levels measured for malathion were 79.60 ± 101.0 and 65.70 ± 12.80 among cases and controls, respectively. No evidence of a statistically significant difference in the mean serum levels measured for malathion between cases and controls was observed (estimated mean difference: 79.60 – 65.70 = 13.9; 95% CI: -45.80, 46.60, p = 0.98). For the unconditional regression analysis, it was unclear which subjects (cases only or both cases and controls) were used in the calculation when the authors reported a mean ± SD serum level measured for malathion as 79.4 ± 85.98; however, no evidence of a significant positive association was reported between malathion exposure and breast cancer among women (OR: 1.0; 95% CI: 0.99, 1.01 per 1 unit increase in serum level, p = 0.98). An additional analysis that evaluated malathion mean serum levels relative to the different stages of breast cancer (stages 1 – 4) provided no evidence of a statistically significant difference (p = 0.23), using a Kruskal-Wallis test.

The overall quality of the study was ranked low based on the study quality criteria outlined in the OPP framework. Golmohammadzadeh et al. (2019) relied on a case-control study design that assessed the relationship between breast cancer and pesticide exposure. Study strengths included case ascertainment and the use of laboratory QA/QC methods. Several issues with the statistics used in this study was considered a primary limitation. The study authors did not indicate that they adjusted for potential confounders within the statistical models, and thus the reported values were presumably unadjusted and did not include potential confounders within their statistical models. Second, the degrees of freedom in the t-test were denoted inaccurately. Specifically, the total number of subjects with malathion serum data was 57 subjects, not 123 subjects (51 controls + 72 cases). Additionally, although the authors reported a mean ± SD serum level measured for malathion, it was unclear in the unconditional logistic regression which subjects (cases only or both cases and controls) were used in this calculation. Two additional study limitations include potential selection bias and recall bias, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and breast cancer. Five publications (Engel et al., 2005; Mills and Yang, 2005; Lerro et al., 2015; Engel et al., 2017; Golmohammadzadeh et al., 2019) examined the association between malathion exposure and breast cancer among women in the AHS prospective cohort, among women in California, and among women in Iran. Engel et al. (2005), Engel et al. (2017), and Lerro et al. (2015) examined the association between malathion exposure and breast cancer among female spouses of pesticide applicators in the AHS prospective cohort, each with additional cases and longer follow-up time than the prior publication. All three publications reported no evidence of a significant positive association between malathion use and breast cancer and all three studies were ranked moderate quality. Mills and Yang (2005) investigated the association between malathion exposure and breast cancer among Hispanic women in California using pesticide use data and geospatial analysis in a case-control study, and reported evidence of a moderately strong association for malathion use (at the mid exposure level only) and breast cancer diagnosed from 1988 to 1994 among a small number of cases (n = 16). No evidence of a significant positive association was reported at the low or high exposure levels, and no evidence of a positive association was reported between any exposure level of malathion use and breast cancer that was diagnosed from 1995 to 2001. The study was ranked low quality for regulatory purposes. The exposure assessment approach relied on county-level pesticide use record information as a surrogate measure of exposure to estimate individual-level exposure. This may have caused exposure misclassification and affected the underlying true exposure-response relationship. We

also note the number of exposed cases was small which limits the ability to interpret with confidence the observed odds ratios. The fifth publication, Golmohammadzadeh et al. (2019), examined the association between malathion exposure and breast cancer among women living in Iran and reported no evidence of a statistically significant difference in mean serum levels measured for malathion between cases and controls. The study was ranked low quality for regulatory purposes, and limitations included the statistical analysis methods and reporting.

Cancers of the Large Intestine

Three publications (Bonner et al., 2007; Lee et al., 2007; Lerro et al., 2015) examined the relationship between malathion exposure and cancers of the large intestine including colorectal, rectal, and colon cancers.

Colon Cancer

Two publications (Lee et al., 2007; Lerro et al., 2015) examined the relationship between malathion exposure and colon cancer.

- Lee et al. (2007) investigated the association between pesticide exposure, including malathion, and cancers of the large intestine including colorectal, colon, and rectal cancers using data from the AHS prospective cohort. The study population (n = 56,813) consisted of male pesticide applicators and their spouses living in Iowa and North Carolina who were enrolled in the AHS cohort. Incident cases were identified using state cancer registry files from enrollment (1993-1997) through December 31, 2002 and International Classification of Diseases for Oncology (ICD-O-2) codes. Vital status was confirmed annually through state death registries and the National Death Index. Ever/never exposure to malathion was assessed through an initial enrollment questionnaire followed by a more detailed self-administered questionnaire filled out at home as part of study enrollment. Unconditional multivariable logistic regression was used to calculate ORs and 95% CIs for malathion, adjusting for age, smoking, state, and total lifetime days of pesticide application. Among the 212 colon cancer cases identified in the study, 112 reported ever-exposure to malathion, and 59 reported never-exposure to malathion (not all cases reported exposure status for malathion). No evidence of a positive association was reported between exposure to malathion and colon cancer, based on ever use (OR = 0.80; 95% CI: 0.50, 1.10; with n = 112 exposed cases and n = 59 unexposed cases).

  The overall study quality was ranked high based on the study quality criteria provided in the OPP Framework. The prospective cohort study design as part of the AHS, the ascertainment of cancer cases using established registries, and the pesticide exposure assessment were considered strengths of the study.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including colon cancer, among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina, who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classifications according to ICD – O – 3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history and other covariates that may be potential confounders. The association between malathion exposure and breast

cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 204 colon cancer cases identified during the study period, 38 cases reported direct exposure to malathion. No evidence of a positive association was reported between malathion exposure and colon risk (RR = 0.89; 95% CI: 0.58, 1.37; with n = 38 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and -- similarly -- no evidence of a positive association was reported for malathion exposure and colon cancer (RR = 0.95; 95% CI: 0.58, 1.57; with n = 29 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and colon cancer. Two publications (Lee et al., 2007; Lerro et al., 2015) examined the relationship between malathion exposure and colon cancer. Lee et al. (2007) reported no evidence of a positive association between malathion ever use and colon cancer. The study was determined to be high quality for regulatory purposes. Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including colon cancer among participants in the prospective AHS cohort. No evidence of a positive association was reported between malathion exposure and colon risk. Additional analyses in Lerro et al. (2015) investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and colon cancer. The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate statistical methods used.

Colorectal Cancer

Two publications (Bonner et al., 2007; Lee et al., 2007) examined the relationship between malathion exposure and colorectal cancer.

- The association between colorectal cancer and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries

and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. A Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[30]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. Risk estimates for frequency, intensity, and duration of exposure for colorectal cancer were also calculated by the study authors. A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present and the study authors concluded this bias had no effect on the reported risk estimates. In the colorectal cancer analysis for IWLD with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile of exposure (T1 – T3: $0.58 \leq RRs \leq 1.21$; all CIs encompassed the null value of 1.0, with n = 15 – 28 exposed cases, with 40 cases in the non-exposed group)[31]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.81). Additionally, no evidence of a significant positive association between colorectal cancer for frequency, intensity, and duration[32] of malathion exposure was observed ($0.83 \leq RRs \leq 1.27$; all CIs encompassed the null value of 1.0, with n = 18 – 45 exposed cases, with 40 cases in the non-exposed group, p-trends > 0.05).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

- Lee et al. (2007) investigated the association between pesticide exposure, including malathion, and cancers of the large intestine including colorectal, colon, and rectal cancers using data from the AHS prospective cohort. The study population (n = 56,813) consisted of male pesticide applicators and their spouses living in Iowa and North Carolina who were enrolled in the AHS cohort. Incident cases were identified using state cancer registry files from enrollment (1993-1997) through December 31, 2002 and International Classification of Diseases for Oncology (ICD-O-2) codes. Vital status was confirmed annually through state death registries and the National Death Index. Ever/never exposure to malathion was assessed through an initial enrollment questionnaire followed by a more detailed self-administered questionnaire filled out at home as part of study enrollment. Unconditional multivariable logistic regression was used to calculate ORs and 95% CIs for malathion, adjusting for age, smoking, state, and total lifetime days of pesticide application. Among the 305 colorectal cases identified in the study, 169 reported ever exposure to malathion, and 80 reported never exposure to malathion (not all cases reported exposure status for malathion). No evidence of a positive association was reported between exposure to malathion and colorectal cancer based on ever use (OR = 0.80; 95% CI: 0.60, 1.10; with n = 169 exposed cases and n = 80 unexposed cases).

[30] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[31] Risk estimates for intensity-weighted lifetime days of exposure for colorectal cancer reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.49 \leq RRs \leq 1.06$; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

[32] In a separate analysis, frequency of use was defined as: <5 or ≥5 days of use per year, duration of use was defined as : ≤10 years of use or >10 years of use, and intensity was defined by tertiles; however, the tertiles were not specified in the table by the study authors.

The overall study quality was ranked high based on the study quality criteria provided in the OPP Framework. The prospective cohort study design, the ascertainment of cancer cases using established registries, and the pesticide exposure assessment were considered strengths 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 malathion exposure and colorectal cancer. Two AHS publications (Bonner et al., 2007; Lee et al., 2007) examined the relationship between malathion exposure and colorectal cancer. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and colorectal cancer among pesticide applicators enrolled in the AHS prospective cohort, based on lifetime days and intensity-weighted lifetime days of use. Additionally, no evidence of a significant positive association for frequency, intensity, and duration of malathion exposure was observed. The study quality was ranked high for regulatory purposes, as several strengths were noted including the prospective cohort study design, the ascertainment of cancer using established cancer registries, and the strengths of the AHS exposure assessment approach. A second study, Lee et al. (2007), reported no evidence of a positive association between malathion exposure and colorectal cancer based on ever use. Several strengths were noted including the prospective cohort study design, the ascertainment of cancer using established cancer registries, and the strengths of the AHS exposure assessment approach. The study quality was ranked high for regulatory purposes.

Rectal Cancer

Two publications (Lee et al., 2007; Lerro et al., 2015) examined the relationship between malathion exposure and rectal cancer.

- Lee et al. (2007) investigated the association between pesticide exposure, including malathion, and cancers of the large intestine, including rectal cancer, using data from the AHS prospective cohort. The study population (n = 56,813) consisted of male pesticide applicators and their spouses living in Iowa and North Carolina who were enrolled in the AHS cohort. Incident cases were identified using state cancer registry files from enrollment (1993-1997) through December 31, 2002 and International Classification of Diseases for Oncology (ICD-0-2) codes. Vital status was confirmed annually through state death registries and the National Death Index. Ever/never exposure to malathion was assessed through an initial enrollment questionnaire followed by a more detailed self-administered questionnaire filled out at home as part of study enrollment. Unconditional multivariable logistic regression was used to calculate ORs and 95% CIs for malathion adjusting for age, smoking, state, and total lifetime days of pesticide application. Among the 93 cases of rectal cancer identified in the study, 57 reported ever exposure to malathion, and 21 reported never exposure to malathion (not all cases reported exposure status for malathion). No evidence of a positive association was reported between exposure to malathion and rectal cancer based on ever use (OR = 1.00; 95% CI: 0.60, 1.70; with n = 57 exposed cases and n = 21 unexposed cases).

  The study quality was ranked high based on the study quality criteria provided in the OPP Framework. The prospective cohort study design as part of the AHS, the ascertainment of cancer cases using established registries, and the pesticide exposure assessment were considered strengths of the study.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including rectal cancer among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina, who completed the

enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD-O-3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history and other covariates that may be potential confounders. The association between malathion exposure and rectal cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 61 rectal cancer cases identified during the study period, 12 cases reported direct exposure to malathion. No evidence of a positive association was reported between malathion exposure and rectal cancer (RR = 0.92; 95% CI: 0.43, 1.97; with n = 12 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and rectal cancer. Two publications (Lee et al., 2007; Lerro et al., 2015) examined the relationship between malathion exposure and rectal cancer. Both studies reported no evidence of a positive association between ever use of malathion and rectal cancer. Lee et al., (2007) study quality was ranked high for regulatory purposes, and the study quality for Lerro et al. (2015), was ranked moderate.

Childhood cancer

Two publications (Flower et al., 2004 and Park et al., 2020)[33] evaluated the relationship between malathion exposure and cancer in children.

[33] We became aware of a third publication, Lombardi et al. (2021), that reported on the association between prenatal malathion exposure and CNS tumors in children in California. Prenatal malathion exposure was determined using a geospatial analysis that assessed exposure to pesticides used within a 4,000m buffer around the maternal residence in a case-control analysis. Authors reported no evidence of a significant positive association between prenatal malathion exposure and astrocytoma (all types) (OR = 1.17; 95% CI: 0.96, 1.44; with n = 226 exposed cases) and no evidence of a positive association when further adjusted for other carcinogenic pesticides (OR = 0.98; 95% CI: 0.75, 1.29; with n = 226 exposed cases). No evidence of a positive association was reported for diffuse astrocytoma subtype and when further adjusted for other pesticides (0.70 < ORs < 1.00; with n = 65 exposed cases). No evidence of a significant positive association was reported for any of the other astrocytoma subtypes (0.90 < ORs < 1.21; all 95% CIs encompassed the null value of 1.00; with n = 63 - 150 exposed cases). This study was published and came to our attention after our search of the available literature was completed. As such, we did not include the findings in our overall weight of evidence for this health effect but wanted to mention it here. The inclusion of this study in our WOE assessment would not change our overall determination that overall, there is *insufficient epidemiological* evidence at this time to conclude that there is a clear associative or causal relationship between malathion exposure and brain and spinal cancer in children and adults.

- Flower et al. (2004), investigated the association between malathion exposure and childhood cancer (any cancer) as a result of previous parental occupational exposures to pesticides including malathion using data from the AHS. Parents participating in the AHS were identified via enrollment questionnaires (1993-1997), and cases were defined as children of AHS study participants in Iowa, who were born in 1975 or after, and were diagnosed with cancer according to the Surveillance, Epidemiology, and End Results (SEER) childhood cancer classification at the age of ≤ 19 years.[34] Childhood cancer cases were determined both retrospectively and prospectively following their parents' enrollment within the AHS (1993-1997), and each case was ascertained using birth certificates and linkage to the state cancer registry. A self-reported questionnaire detailing pesticide usage during study enrollment was completed by AHS farmers and their wives, including the application and mixing of 50 specific pesticides. Logistic regression was used to calculate ORs and 95% CIs for malathion ever exposure among parents and all childhood cancers in their offspring, adjusted for child's age at enrollment. Of the 17,280 children included in the analysis, 3,273 (19%) were exposed to malathion through parental (mother and/or father) malathion ever use while pregnant. Of the total 50 childhood cancer cases identified in the study, eleven cases reported parental malathion exposure and subsequent childhood cancer in their offspring. No evidence of a significant positive association was observed between parental malathion exposure and childhood cancer among a small (n = 11) number of cases (OR = 1.12; 95% CI: 0.57, 2.20).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the study design, the ascertainment of cases using the cancer registry and birth certificate data, and the retrospective and prospective means used to identify cases. A main limitation of the study included the indirect exposure measurement (parental self-reported malathion use). Study authors reported that exposure did precede all childhood cancer cases, however the time frame between parental self-reported malathion exposure and duration of use extended up to ten years, making it difficult to identify the true window of malathion exposure – e.g., prior to conception or prior to the birth of the child. We note the number of exposed cases was small (10 < exposed cases < 19).

- Park et al. (2020) evaluated the association between prenatal pesticide exposure, including malathion, and childhood cancer in a case-control study in California. This record-based study used the California Cancer Registry to identify all cases of childhood acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), diagnosed from 1986 to 2012, prior to the children turning six years of age. Controls were randomly selected from California birth records, and frequency matched to the cases by birth year. Cases and controls born between 1998 – 2011 and living in rural areas (non-urban areas) only were considered in this study, using rural-urban commuting area codes (RUCA) determined through Census Tract information. Among those eligible for the analysis, 132 ALL cases, 30 AML cases, and 9,805 controls were reported. Of these, 78 ALL cases, 15 AML cases, and 4,515 matched controls reported exposure to malathion. To estimate pesticide applications, the study obtained statewide pesticide use reporting records from the California Department of Pesticide Regulation Pesticide Use Reporting (PUR) data, in addition to land-use surveys in respect to crop cover reported by the California Public Land Survey System (PLSS). This combined data was collected to more accurately determine pesticide applications. The study authors initially considered 133 pre-selected pesticides including malathion classified by the EPA as possible, likely, or probable carcinogens, in addition to select pesticides that were widely used in the state of California. For each case/control, pesticide exposure (monthly and annual application rates- total pounds applied/acre over

[34] Ries LAG, Smith MA, Gurney JG, Linet M, Tamra T, Young JL, et al., eds. 1999. Cancer Incidence and Survival among Children and Adolescents: United States SEER Program 1975–1995. Bethesda, MD: National Cancer Institute, SEER Program.

a time period) was determined based on residential proximity to agricultural pesticide applications during pregnancy and the beginning of childhood based on a 4,000m buffer zone around the residential address listed on the birth certificate. Birth records, containing residential address information at time of birth, were linked to the cases and controls. Unconditional logistic regression was used to assess the relationship between ever/never use of select pesticides, including malathion, and childhood ALL and AML, and odds ratios (ORs) were calculated with corresponding 95% confidence intervals. Variables were selected based on literature review and 10% change in estimate criterion. Adjusted ORs were adjusted for birth year, mother's race, and SES-index variable, and the hierarchical logistic model (HLM) ORs were adjusted for birth year, mother's race, SES-index variable, and overall pesticide effect. Evidence of a positive association for ALL in children who resided in a 4,000 m buffer zone of malathion applications was observed in a single pesticide model; however, when further adjusted using the hierarchical statistical model, no evidence of a significant positive association was observed for ALL relative to malathion (adjusted OR: 1.54; 95% CI: 1.06, 2.22 with n = 78 exposed cases, 4,515 exposed controls; HLM OR: 1.16; 95% CI: 0.73, 1.85 with n = 78 exposed cases, 4,515 exposed controls). No evidence of a positive association for AML in children who resided in a 4,000 m buffer zone of malathion applications was reported (adjusted OR: 0.99; 95 CI: 0.46, 2.12 with n = 15 exposed cases, 4,515 exposed controls; HLM OR: 0.79; 95% CI: 0.42, 1.48 with n = 15 exposed cases, 4,515 exposed controls).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Strengths included the case-control study design, case ascertainment using cancer registry and birth certificate data, and objective measure of exposure thus removing potential for recall bias. The primary limitation of the study was that it relied on a geospatial approach to assess pesticide exposure based on residential address and land use data on malathion. While this approach helps minimize recall bias, the method relied on a 4,000 m buffer to assign exposure based on distance to agricultural land where malathion was reported to have been applied instead of relying on study participants or other measures to provide exposure data. Additionally, the study did not account for possible residential mobility[35] of mothers between pregnancy and childbirth with residency geocoded 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, possibly causing 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 malathion exposure and cancer in children. This determination is based on a limited body of evidence that consisted of two case-control studies in children. Flower et al. (2004) leveraged the AHS cohort to examine the association between parental exposure to malathion and childhood cancer and reported no evidence of a significant positive association, among a small number (n = 11) of potentially exposed cases based on paternal self-report of malathion use. As such, the effect estimate was relatively imprecise and based on an indirect exposure assessment approach that may not fully reflect the exposure experience by children. In addition to the small number of cases with indirect malathion exposure, the study was unable to assess direct malathion exposure among the children; instead, the study relied on father's (pesticide applicator's) self-reported malathion use. Furthermore, for the father's self-reported exposure, the time frame of pesticide exposure and duration of use extended up to ten years, making it difficult to identify the specific window of time when exposure to specific pesticides actually occurred – e.g., prior to conception or prior to the birth of the child. However, the

[35] Past studies have indicated that around 11 – 32 % of pregnant women move their residence at least one time throughout pregnancy, and the median move distances were between 4.2 – 10 km (Lupo et al., 2010; Strickland et al., 2017; Pereira et al., 2016)

study authors reported that exposure did precede all childhood cancer cases. The study quality was moderate for regulatory purposes. In the second study, Park et al. (2020), reported evidence of a positive association for ALL in children who resided in a 4,000 m buffer zone of malathion applications; however, when further adjusted using the hierarchical statistical model, no evidence of a significant positive association was observed for ALL relative to malathion. For AML in children, no evidence of a positive association was observed in children who resided in a 4,000 m buffer zone of malathion applications. Park et al. (2020) was ranked moderate quality for regulatory purposes due to the study's reliance on a geospatial approach to assess maternal pesticide exposure based on the residential address provided at birth and land use data on malathion, as well as not accounting for residential mobility of mothers.

Esophageal Cancer

One publication (Lee et al., 2004) examined the association between malathion and esophageal cancer.

Lee et al. (2004) investigated the association between farming and agricultural pesticide use, including malathion, and stomach and esophageal cancers in the Nebraska Health Study II, a case-control study of adults in eastern Nebraska. The study population included white residents of eastern Nebraska, ≥21 years old. Cases of incident stomach and esophageal adenocarcinoma were identified using the Nebraska Cancer Registry (1988 – 1990) and discharge and pathology records from 14 participating hospitals Nebraska. Controls for the current study were randomly selected from the control group of a previous study covering the same base population investigating lymphohematopoietic cancers (<65 years – random digit dialing, ≥65 years – Medicare files, for deceased cases – Nebraska mortality records) and were frequency-matched by age, gender, and vital status to the combined distribution of the glioma, stomach, and esophagus cancer cases. Demographic, medical, and family history as well as occupational and pesticide exposure information (for those who lived or worked on farm) was collected via telephone interview conducted during 1992-1994. Pesticide exposure information was limited to use prior to 1985, the time period of the previous study. Interviews were conducted for 137 esophageal cancer cases and 502 controls; however, most interviews were conducted via proxy (76% of esophageal adenocarcinoma cases, 61% of controls) who were primarily spouses or other primary relatives. Unconditional logistic regression was used to calculate ORs and 95% CIs for farming activity and for individual pesticide use, adjusted for age and gender, with the non-farmers as a reference group. Among the 137 esophageal cancer cases and 502 controls included in the final analysis, 12 esophageal cancer cases reported malathion exposure. No evidence of a positive association was reported for malathion ever use and esophageal cancer among farmers in Nebraska (OR = 0.70; 95% CI: 0.40, 1.50; with n = 12 exposed cases).

The overall quality of the study was ranked low quality based on the study quality criteria provided in the OPP framework. Study limitations were related to its design, exposure assessment approach, statistical analysis, and ability to control for confounding. With regard to study design, Lee et al. (2004) used a case-control approach and may have introduced selection bias when recruiting their control group. Differences between the results for the self-reporting respondents and the proxy respondents illustrate the possible problem, as the control groups for each of these respondents were constructed differently and each could be biased in a different way. The use of respondent-reported malathion use to ascertain exposure introduced further uncertainty because it is not possible to attribute the increased odds of esophageal cancer to malathion exposure alone. In particular, the self-reporting and proxy respondents have different levels of knowledge about pesticide use and possibly different motives for responding. Moreover, self-reported exposure assessment is likely to be subject to differential misclassification because those that are exposed or non-exposed may differentially recall previous pesticide usage.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a causal or clear associative relationship between malathion exposure and esophageal cancer. One study (Lee et al., 2004) examined the association between malathion exposure and esophageal cancer. Lee et al. (2004) reported no evidence of a positive association among farmers in Nebraska and was determined to be low quality for regulatory purposes due to several limitations including the study design, control selection, and the large number of proxy respondents.

Gastric Cancer

Two publications (Lee et al., 2004; Mills and Yang, 2007) examined the association between malathion and gastric cancer among residents in Nebraska.

- Lee et al. (2004) investigated the association between farming and agricultural pesticide use, including malathion, and gastric and esophageal cancers in the Nebraska Health Study II, a case-control study of adults in eastern Nebraska. The study population included white residents of eastern Nebraska, ≥21 years old. Cases of incident gastric and esophageal adenocarcinoma were identified using the Nebraska Cancer Registry (1988 – 1990) and discharge and pathology records from 14 participating hospitals Nebraska. Controls for the current study were randomly selected from the control group of a previous study covering the same base population investigating lymphohematopoietic cancers (<65 years – random digit dialing, ≥65 years – Medicare files, for deceased cases – Nebraska mortality records) and were frequency-matched by age, gender, and vital status to the combined distribution of the glioma, gastric, and esophageal cancer cases investigated. Demographic, medical and family history, occupational, and pesticide exposure information (for those who lived or worked on farm) was collected via telephone interview conducted during 1992-1994. Pesticide exposure information was limited to use prior to 1985, the time period of the previous study. Interviews were conducted for 170 gastric cancer cases and 502 controls (with response rates of 79% and 83% respectively); however, most interviews were conducted via proxy (80% of gastric cancer cases, 61% of controls); proxies were primarily spouses or other primary relatives. Among the 170 gastric cancer cases and 502 controls included in the final analysis, 14 gastric cancer cases (8.2%) reported malathion exposure. Unconditional logistic regression was used to calculate ORs and 95% CIs for farming activity and for individual pesticide use, adjusted for age and gender, with the non-farmers as a reference group. No evidence of a positive association was reported between malathion ever use and gastric cancer (OR = 0.80; 95% CI: 0.40, 1.60; with n=14 exposed cases). The quality of the study was ranked low based on the study quality criteria provided in the OPP framework. Study limitations were related to its design, exposure assessment approach, statistical analysis, and ability to control for confounding. With regard to study design, Lee et al. (2004) used a case-control approach and may have introduced selection bias when recruiting the control group. Differences between the results for the self-reporting respondents and the proxy respondents illustrates the possible problem, as the control groups for each of these respondents were constructed differently and each could be biased in a different way. The use of respondent-reported malathion use to ascertain exposure introduced further uncertainty because it is not possible to attribute the odds of esophageal cancer to malathion exposure alone. In particular, the self-reporting and proxy respondents have different levels of knowledge about pesticide use and possibly different motives for responding.

- Mills and Yang (2007) conducted a nested case-control study to investigate the association between malathion exposure and gastric cancer in a prospective cohort of farmers who were part of the United Farm Workers of America (UFW). Incident cancer cases were identified by linking the UFW cohort to state cancer registry files between 1988 and 2003, and the controls (no stomach cancer) from the

cohort were frequency-matched to the cases via birthdate, gender, and ethnicity. All of the controls selected were then required to provide proof of residence in the state of California during the time of the corresponding case's diagnosis. Exposure for the cases and controls was assessed using three different types of records/databases: (i) UFW records to verify occupational history; (ii) grower's contracts to establish the crop/commodity the member was exposed to; and (iii) the California Pesticide Use Record (PUR) data on specific pesticide usage at the county-level. Individual exposure to pesticides for the cases and controls was determined by taking the amount of pesticides including malathion applied in a given area at a certain time, and multiplying that by the amount of time spent by each of the cases and controls in that specified area. Among the 103 cases that had information on malathion, 69 (67%) reported exposure to malathion. Age-adjusted ORs and multivariable-adjusted ORs (and their corresponding 95% CIs) were then each calculated separately using the Mantel-Haenszel method and unconditional logistic regression.[36] No evidence of a significant positive association was reported between malathion ever use and gastric cancer (OR = 1.43; 95% CI: 0.84, 2.44; n = 69 exposed cases) based on ever/never use. Malathion exposure was further stratified into quartiles, based on pounds of use, and the following quartiles were reported for malathion: 0 lbs, 1-11 lbs, 12-42 lbs, and 43-8,164 lbs. Evidence of a moderately strong association was observed in the highest exposure quartile in the multivariable-adjusted analysis with the low exposure quartile as the referent (OR: 2.61; 95% CI:1.18; 5.76 with n = 30 exposed cases). No evidence of a significant positive association was reported in the mid exposure quartile (12-42 lbs) with the low exposure quartile as the referent (OR: 1.96; 95% CI: 0.88, 4.38), and no evidence of a significant positive association was reported in any exposure quality with the no exposure quartile as the referent ($0.72 \leq OR \leq 1.49$, all 95% CIs encompassed the null value of 1.00; with n = 14-30 cases per exposure category). Additionally, no evidence of a significant positive association was reported between malathion and gastric cancer (in any quartile) in the age-adjusted analysis ($0.50 \leq OR \leq 1.28$, all 95% CIs encompassed the null value of 1.00; with n = 14-30 cases per exposure category).

The overall quality of the Mills et al. (2007) was low based on the study quality criteria provided in the OPP Framework. The studied leveraged an existing prospective cohort of Hispanic farmworkers based on membership of the UFW and was able to systematically ascertain year of cancer diagnosis using the California cancer registry. While these design features were important strengths of the study, the exposure assessment approach used to assess malathion exposure was more limited and relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. No information was provided to demonstrate that this ecologic, county-level pesticide use information can reliably estimate individual-level exposure. The investigators acknowledge this limitation in discussing their results and indicate that ecologic-level exposure assessments can lead to exposure misclassification that may "create spurious associations" that magnify or diminish the underlying true exposure-response relationship.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and gastric cancer. Two publications (Lee et al., 2004; Mills and Yang, 2007) examined the association between malathion exposure and stomach cancer. Lee et al. (2004) reported no evidence of a positive association between malathion exposure and gastric cancer in a case-control study of residents in Nebraska and was low quality for regulatory purposes. Several limitations were noted including selection bias, recall bias, comparison of farmers to non-farmers, and a large number of proxy respondents compared to self-respondents. Both groups could have different levels of pesticide use knowledge, memory of use, and different motives for responding.

[36] The unconditional logistic regression controlled for age, sex, duration of union affiliation, and start date of first union affiliation.

Mills and Yang (2007) used a geospatial method to estimate malathion exposure based on residential proximity to agricultural malathion use and risk of gastric cancer among a nested cases-control of Hispanic farm workers. No evidence of a significant positive association was reported for malathion ever vs. never use. For the exposure-response analysis, evidence of a moderately strong association was observed in the highest exposure quartile in the multivariable-adjusted analysis with the low exposure quartile as the referent. No evidence of a significant positive association was reported in the mid exposure quartile with the low exposure quartile as the referent, and no evidence of a significant positive association was reported in any exposure quartile with the no exposure quartile as the referent. Additionally, no evidence of a significant positive association was reported between malathion and gastric cancer (in any quartile) in the age-adjusted analysis. The publication was low quality for regulatory purposes due to the exposure assessment approach.

Kidney Cancer

Two publications (Bonner et al., 2007; Andreotti et al., 2020) examined the relationship between malathion exposure and kidney cancer.

- Bonner et al. (2007) examined the association between kidney cancer and specific pesticides including malathion. The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. A Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[37]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. In the kidney cancer analysis for IWLD with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile of exposure (T1 – T3: $0.78 \leq RRs \leq 1.53$; all CIs encompassed the null value of 1.0, with n = 4 – 10 exposed cases, with 8 cases in the non-exposed group)[38]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.68). A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present and the study authors concluded this bias had no effect on the reported risk estimates.

  The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its

[37] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[38] Risk estimates for intensity-weighted lifetime days of exposure for kidney reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.92 \leq RRs \leq 2.00$; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

- Andreotti et al. (2020) investigated the association between exposure to malathion and other pesticides and renal cell carcinoma using data from the AHS prospective cohort. The study population (n=55,873) consisted of pesticide applicators living in Iowa and North Carolina who were enrolled in the AHS cohort and who did not have a history of cancer at enrollment (1,096 participants reporting cancer at enrollment were excluded and 341 not living in either Iowa or North Carolina were excluded). Incident cancer cases were identified using Iowa and North Carolina state cancer registry files from enrollment (1993-1997) through December 2014 in North Carolina and December 2015 in Iowa. International Classification of Diseases for Oncology (ICD-O-3) codes were used to classify cancer sites. Malathion exposure was assessed through the enrollment questionnaire (1993 – 1997) and the first follow-up interview five years after enrollment (1999 – 2005). Among the 308 renal cell carcinoma cases 150 (48.7%) had information on malathion exposure. Of these, 106 (70.7%) renal cell carcinoma cases reported malathion exposure. Multiple imputation was used to estimate pesticide exposures after enrollment for individuals who did not complete the interview (37%). Poisson regression was used to calculate incidence rate ratios and 95% confidence intervals for each category of intensity-weighted days of malathion use and lifetime days of malathion use compared with no use, adjusting for age (age at end of follow-up), state of enrollment, cigarette smoking status (never, former, current, missing), body mass index (<25, 25–30, >30 kg/m^2, missing), and ever use of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Authors reported adjustment for 2,4,5-T as an association between the pesticide and renal cell carcinoma was observed in this analysis and there was *a priori* evidence of an association. Cumulative intensity-weighted days of exposure and lifetime days of exposure for each pesticide were divided into quartiles, tertiles, or the median creating categories with at least 10 exposed cases in each category and were compared to no exposure group in the analysis. Specifically, for malathion, three tertile of exposure were created. No evidence of a significant positive association was reported for any exposure quartile of malathion and renal cell carcinoma (0.93 < rate ratios <1.08; all 95% CIs encompassed the null value of 1.0; with n=34-37 exposed cases per exposure category; p-trend=0.69), with the no exposure group as the referent, and no evidence of a significant exposure-response trend. Similar results were reported for lifetime days of malathion exposure and renal cell carcinoma.

  The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The general strengths of the publication included the underlying prospective design of AHS, the exposure assessment, and the availability of a U.S. registry to comprehensively identify cancer cases.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and kidney cancer. Two publications (Bonner et al., 2007; Andreotti et al., 2020) evaluated the association between malathion exposure and kidney cancer and renal cell carcinoma, a type of kidney cancer among pesticide applicators in the AHS cohort. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and kidney cancer among pesticide applicators, based on lifetime days and intensity-weighted lifetime days of use. The study quality was ranked high for regulatory purposes. A second study, Andreotti et al. (2020) similarly reported no evidence of a significant positive association for any exposure quartile of malathion and renal cell carcinoma among pesticide applicators, with the no exposure group as the referent, and no evidence of a significant exposure-response trend. Similar results

were reported for the lifetime days measure of malathion exposure and renal cell carcinoma. The study was determined to be high quality for regulatory purposes.

Lung Cancer

Four studies (Pesatori et al., 1994; Bonner et al., 2007; Lerro et al., 2015; Bonner et al., 2017) examined the potential association between malathion exposure and lung cancer.

- Pesatori et al. (1994) examined the association between lung cancer and exposure to pesticides, including malathion, in a nested case-control study among a cohort of licensed pest control workers in Florida. The 4,411 cohort participants were identified from Florida Department of Health and Rehabilitative Services pest control worker licensing records from 1965-1966. Cases included those members of the cohort with lung cancer listed as the underlying or contributing cause of death on the death certificate and two cases that were identified after the closing date of the cohort (1982). Controls were selected from the cohort by year of death and randomly matched to each case by race, gender, age, and vital status at time of case's death. Three living controls and two deceased controls were matched to each case and cause of death for deceased controls included heart disease, emphysema, cancer, and other causes. Vital status for participants was determined through January 1, 1977 and deaths through January 1, 1982 were identified using the National Death Index and Social Security mortality files. Trained interviewers, blinded to case-control status, conducted interviews with next-of-kin, regardless of vital status of participants, to gather information on lifestyle, dietary habits, occupational exposures and work practices of the participants. Living controls (n = 16) for whom next-of-kin could not be located completed the interview. Pesticide exposure information was collected between the date of application for the pest control licenses (1965-1966) through the closing date of the study (Jan 1, 1982), lost to follow-up, or date of death, whichever occurred first). Interviews were completed for 65 (83%) of the 78 lung cancer cases, 122 (80%) of the 152 deceased controls, and 172 (75%) of the 229 living controls. Of these, 11 (17%) cases, 13 (11%) deceased controls, and 29 (17%) living controls included in the final analysis reported malathion exposure. Unconditional logistic regression was used to calculate ORs and 95% CIs to estimate the association between malathion and lung cancer, adjusted for year of birth (1909, 1910-29, 1930-49) and cigarette pack-years ($\leq$30, 30.1-53, >53 pack-years). No evidence of a significant positive association was reported between malathion ever exposure and lung cancer among pest control operators when compared to dead controls (OR = 1.6; 95% CI: 0.5, 4.6; with n = 11 cases, n = 13 deceased controls) and no evidence of a positive association when compared to living controls (OR = 1.0; 95% CI: 0.4, 2.6; with n = 11 cases, n = 29 living controls). We note the small number of cases exposed to malathion ($10 < n \leq 20$).

  The study was ranked low quality based on the study quality criteria provided in the OPP Framework. The exposure assessment was the main limitation as pesticide exposure was reported by proxy for most of the participants (all except 16 living controls) and proxy respondents may not recall job related details as well as the actual participant especially the longer the timespan between date of pesticide use and date of interview occur. This may have led to recall bias. Additionally, the cases were compared to two control populations (deceased and living) which complicates the interpretation of the findings. We note also that the number of cases of lung cancer with malathion exposure were small ($10 < n \leq 20$).

- The association between lung cancer and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an

intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. A Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[39]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. In the lung cancer analysis for IWLD of exposure with the non-exposed group as the referent, no evidence of a positive association was observed in any tertile (T1 – T3: $0.78 \leq RRs \leq 1.00$; all CIs encompassed the null value of 1.0, with n = 14 – 21 exposed cases, with 31 cases in the non-exposed group)[40]. Further, there was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.42 for IWLD). A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present, and the study authors concluded this bias had no effect on the reported risk estimates.

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including lung cancer, among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina, who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD-O-3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history, and other covariates that may be potential confounders. The association between malathion exposure and lung cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105

[39] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[40] Risk estimates for intensity-weighted lifetime days of exposure for lung cancer reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.75 \leq RRs \leq 1.46$; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 165 lung cancer cases identified during the study period, 30 cases reported direct exposure to malathion. No evidence of a positive association was reported between malathion exposure and lung cancer risk among female spouses (RR = 1.00; 95% CI: 0.60, 1.65; with n = 30 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a significant positive association was reported for malathion exposure and lung cancer (RR = 1.11; 95% CI: 0.63, 1.93; with n = 26 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

- Bonner et al. (2017) investigated the potential association between pesticides including as malathion and incident lung cancer using data from the AHS prospective cohort. The study population (n=57,310) consisted of pesticide applicators living in Iowa and North Carolina, and pesticide exposure was assessed through two self-administered questionnaires completed at study enrollment and at home (1993 – 1997). Exposure information was updated through a follow-up questionnaire using a computer-assisted telephone interview between 1999 – 2005. The authors imputed the missing exposure data for subjects who did not complete the follow-up interview using multiple imputation, (37% of the participants did not complete the follow-up interview). Cases included AHS study participants with incident lung cancer between study enrollment up to December 31, 2010 in North Carolina and December 31, 2011 in Iowa. Cases were ascertained through cancer registries in Iowa and North Carolina, and vital status was confirmed using the state and national death databases. Cox proportional hazards regression was used to calculate HRs and 95% CIs for malathion and incident lung cancer, adjusting for smoking status and pack-years, age, sex, and total lifetime pesticide use. Tertiles were constructed based on lifetime days and intensity-weighted lifetime-days of exposure, and HRs were reported for each tertile. No evidence of a significant positive association was reported between malathion and lung cancer at any exposure level for lifetime or intensity weighted lifetime days of exposure (0.98 <all HRs <1.37; all CIs encompassed the null value of 1.0; with n = 28 – 76 exposed cases per exposure category; p-trends >0.05).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify and ascertain cancer cases through linkage to cancer registries, and exposure assessment approach which examined lifetime days of exposure and intensity-weighted lifetime days of exposure to malathion. One of the study limitations included a large percentage of missing exposure data during the follow-up period (37% of the participants did not complete the follow-up interview). The authors imputed the missing exposure data for subjects who did not complete the follow-up interview using multiple imputation, which is the considered state-of-the-science for dealing with missing data. The authors did not mention nor discuss any sensitivity analyses where only the data of completed subjects (i.e., not imputed) were analyzed.

EPA Conclusion

Overall, there is *insufficient epidemiological* evidence at this time to conclude that there is a clear associative or causal relationship between malathion exposure and lung cancer. Three AHS publications (Bonner et al., 2007; Lerro et al., 2015; Bonner et al., 2017) and one non-AHS study (Pesatori et al., 1994) were used to examine the association between malathion exposure and lung cancer among male

pesticide applicators and their female spouses. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and lung cancer among pesticide applicators, based on lifetime days and intensity-weighted lifetime days of use. The second study, Lerro et al. (2015), reported no evidence of a positive association between malathion exposure and lung cancer risk for female spouses in the AHS cohort. Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a significant positive association was reported for malathion exposure and lung cancer. In Bonner et al. (2017), no evidence of a significant positive association was reported between malathion exposure and lung cancer among pesticide applicators for intensity-weighted days of exposure. Bonner et al. (2007) was ranked high quality, and Lerro et al. (2015) and Bonner et al. (2017) were determined to be of moderate quality for regulatory purposes. In Bonner et al. (2017), there was a high percentage (37%, n = 20,968) of missing data reported from the enrollment and follow-up questionnaires. The fourth study, Pesatori et al. (1994), reported no evidence of a significant positive association between malathion ever exposure and lung cancer. The study was low quality for regulatory purposes and had substantive limitations in its exposure assessment. We also note the number of exposed cases in this study was small ($10 < n \leq 20$) which limits the interpretability of the observed ORs.

Lymphohematopoietic Cancers

Seventeen publications (Brown et al., 1990; Cantor et al., 1992; Brown et al., 1993; McDuffie et al., 2001; Waddell et al., 2001; De Roos et al., 2003; Mills et al., 2005; Bonner et al., 2007; Karunanayake et al., 2012; Hohenadel et al., 2011; Pahwa et al., 2012; Alavanja et al., 2014; Lerro et al., 2015; Presutti et al., 2016; Koutros et al., 2019; Leon et al., 2019; Latifovic et al., 2020) investigated the potential association between malathion exposure and lymphohematopoietic cancers including leukemia, Hodgkin lymphoma (HL), non-Hodgkin lymphoma, and multiple myeloma. Each of these are described, in turn, below.

Leukemia

Four studies (Brown et al., 1990; Mills et al., 2005; Bonner et al., 2007; Lerro et al., 2015) assessed the association between exposure to malathion and leukemias in pesticide applicators.

- Brown et al. (1990) evaluated the association between several pesticides, including malathion, and leukemia among male farmers using data from population-based case-control interview studies conducted by the National Cancer Institute in Iowa and Minnesota between 1981-1984. Cases of leukemia were determined either by a tumor registry database or a special surveillance network including hospital and pathology records in Iowa and Minnesota. Eligibility criteria for cases included Caucasian males aged ≥ 30 years old who were diagnosed with leukemia both retrospectively (one year prior to the start of the study) and prospectively (2 years following the start of the study). In Iowa, eligibility criteria were restricted to cases who were diagnosed between March 1981 and October 1983 and resided in any part of the state, and in Minnesota, a diagnosis period between October 1980 through September 1982 was required, with residence in cities besides Minneapolis, St. Paul, Rochester, or Duluth at the time of diagnosis. Pathology slides were used to ascertain cases by a group of trained pathologists. Controls consisted of Caucasian males *not* diagnosed with hematopoietic or lymphatic cancer who were part of a population-based sample and frequency-matched to the cases based on vital status at the time of the interview, state of residence, and age group (within 5 years). Controls were identified through a separate population-based case-control for this study through a) random digit dialing; b) Medicare files; or c) state death certificates. An initial in-person interview was conducted by a trained professional for the cases and controls during August 1981 to March 1984, and information including study participant demographics, medical histories, occupational histories (both farming and nonfarming jobs), sources of drinking water, smoking and

alcohol use, use of unpasteurized dairy products, and past farming practices was obtained via a standardized questionnaire at this time. For farming practices, detailed questions included the types of crop grown, and – for specific pesticides -- gathered information included the duration of pesticide use and if the respondent had personally mixed or applied the pesticide. Proxy respondents were used in place of the actual case or control due to death or incompetency during the interview portion of this study. During the initial interview, a total number of 578 cases were interviewed, with 340 of the cases living and 238 of the cases deceased; for the controls (n=1,245) 820 of the controls were living, and 425 were deceased. A second interview was carried out in 1987 via telephone to supplement the initial interview. Trained interviewers contacted study participants in Iowa to gather information concerning the usual number of days per year that each pesticide was used prior to and after 1960. For the supplemental interview, 86 of the 90 total cases completed the telephone interview (23 living, 63 deceased), and all 203 controls completed the interview (146 living, 57 deceased). Unconditional logistic regression was used to estimate the ORs and corresponding 95% CIs for the association between malathion exposure and leukemia among male farmers, adjusting for state, age, tobacco use, high-risk occupations, vital status, family history of lymphopoietic cancer, and high-risk exposures. Among the 578 cases and 1,245 controls, 335 cases and 698 controls reported ever farming; the remaining cases and controls reported never farming (n=243 cases, 547 controls). No evidence of a positive association was reported between malathion ever use and leukemia among farmers compared to nonfarmers (OR=0.90; 95% CI: 0.40, 1.90; with n=10 exposed cases and n=30 exposed controls). When the data was further stratified by exposure (days of use per year) for crops and animals by the following categories: 1 – 4 days of use/year, 5 – 9 days of use/year, 10+ days of use/year, and unknown, no evidence of a significant positive association and no evidence of a positive association was reported between malathion use on crops and leukemia among farmers (1 – 4 days of use/year OR: 1.20; 95% CI: 0.30, 3.90 with n = 4 cases, 9 controls; 5 – 9 days of use/year OR: 0.80; 95% CI: 0.20, 4.40 with n = 2 cases, 6 controls). Zero cases were reported by the study authors for the 10+ days of use/year and unknown exposure categories. For animals use, no evidence of a positive association was observed in the lowest exposure category (1 – 4 days of use/year OR: 0.50; 95% CI: 0.10, 1.30 with n = 5 cases, 25 controls), and an elevated but non-statistically significant association was reported in the highest exposure category (10+ days of use/year OR: 3.20; 95% CI: 1.00, 10.00 with n = 7 cases, 6 controls). Zero cases were reported by the study authors in the 5 – 9 days of use/year, and for the unknown exposure category, an odds ratio was not reported likely due to the very small number of exposed cases and controls observed ($n \leq 2$). When the data was further stratified based on pesticide use at least 20 years prior to the interview,[41] no evidence of a significant positive association was reported for leukemia among farmers (OR: 1.50; 95% CI: 0.80, 2.90; with n = 15 cases and n = 29 controls). We note the small number of exposed cases reported in this study.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included case ascertainment and in-person interviews conducted by trained interviewers. A study limitation included the use of proxy respondents to collect pesticide exposure information for cases and controls. This was especially a concern during the supplemental interview, as the study reported that only 23 of the 86 respondents were living cases. This limitation may have contributed to information bias and led to exposure misclassification Another study limitation included potential recall bias, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls. As a result, this recall bias may have led to exposure misclassification as well. Authors matched cases and controls on vital status to attempt to minimize bias. Finally, authors compared the farmers to nonfarmers, instead of exposed farmers to

[41] The study made no specific mention of why they chose 'at least 20 years prior to the interview', but one can interpret that the analysis that stratified the data based on pesticide handled at least 20 years ago, may have been relevant to the supplemental interview that asked farmers who reported applying pesticides, specifically, if they had applied pesticides prior to and after 1960. Perhaps, this 20-year time period was to allow for a latency period following exposure (malathion) before the outcome (leukemia) was diagnosed.

unexposed farmers, and any effects found from these comparisons might not be due to the chemical exposure but instead due to different risk of disease between two different subpopulations (farmers vs. nonfarmers). Lastly, we note the small number of cases reported in this study.

- Mills et al. (2005) conducted a nested case-control study to investigate the association between lymphohematopoietic cancers and pesticide exposures, including malathion, from farm work in a prospective cohort study of farmers who were part of the United Farm Workers of America (UFW). The study population consisted of members of the UFW at any time between 1973 and 2001. Incident cancer cases were identified by linking the UFW cohort to the California Cancer Registry during the years 1988 through 2001. Controls consisted of farm workers within the UFW cohort who had never been diagnoses with cancer and were randomly selected from the remaining UFW cohort and matched (5:1) to the cases based on age of cancer diagnosis, Hispanic ethnicity, and gender. Pesticide exposure for the cases and controls was assessed using three different types of records/databases: UFW records to verify occupational history, grower's contracts to establish the crop/commodity the member was exposed to, and the California Department of Pesticide Regulation to determine specific pesticide usage. Unconditional logistic regression was used to calculate ORs and 95% CIs, controlling for age, sex, duration of union affiliation, and start date of first union affiliation. Based on this approach, a total of 131 cases of lymphohematopoietic cancers were identified between 1988 and 2001 in the UFW cohort, including 60 cases of non-Hodgkin's lymphoma, 20 cases of multiple myeloma, and 51 cases of leukemia. The investigators analysis of the relationship between malathion use and lymphohematopoietic cancers compared high and low exposed groups and performed additional analyses stratified by cancer type and gender. Based on this approach, the investigators reported no evidence of a significant positive association between high malathion use and total leukemia (OR = 1.83; 95% CI: 0.91, 3.67; n = 51 total cases). When further stratified by type of leukemia, although elevated, the investigators reported no evidence of a significant positive association between high exposure to malathion and lymphocytic leukemia in the total study population (OR=2.88; 95% CI 0.94, 8.80; n = 23 cases) and no evidence of a significant positive association between high exposure to malathion and granulocytic leukemia in the total study population (OR=1.79; 95% CI: 0.63, 5.08; n = 20 cases). The analysis of total leukemia was also stratified by gender and it was reported that there was evidence of a strong association between high exposure to malathion and total leukemia among females (OR=4.91; 95% CI: 1.21-19.89; n = 16 female cases), and no evidence of a significant positive association between high exposure to malathion and total leukemia among males (OR=1.19; 95% CI: 0.51, 2.76; n = 35 male cases).

  The overall quality of the Mills et al. (2005) was ranked low based on the study quality criteria provided in the OPP Framework. The study leveraged an existing prospective cohort of farm workers based on membership of the UFW during the years 1973 and 2001 and was able to systematically ascertain year of cancer diagnosis using the California cancer registry. The nested case-control study design also helped ensure that controls were systematically identified within the same target study population of Hispanic women farm workers. While these design features were important strengths of the study, the exposure assessment approach used to assess malathion exposure was more limited and relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. No information was provided to demonstrate that this ecologic, county-level pesticide use information can reliably estimate individual-level exposure. The investigators acknowledge this limitation in discussing their results and indicate that ecologic-level exposure assessments can lead to exposure misclassification that may "create spurious associations" that magnify or diminish the underlying true exposure-response relationship. The investigators also reported that the statistical power of the study was low and ranged from 15% to 44% depending on prevalence of exposure to pesticides included in the study. Lastly, we note the strong association reported in this study for total leukemia among females was among a small number of exposed cases ($10 < \text{exposed cases} \leq 19$), with wide corresponding confidence intervals.

- The association between leukemia and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. A Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[42]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. In the leukemia analysis with IWLD of exposure with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile (T1 – T3: $0.84 \leq RRs \leq 1.45$; all CIs encompassed the null value of 1.0, with n = 6 – 10 exposed cases, with 11 cases in the non-exposed group)[43]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.25). A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present, and the study authors concluded this bias had no effect on the reported risk estimates.

  The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including leukemia, among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997 living in Iowa and North Carolina who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD-O-3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history, and other covariates that may be potential confounders. The association between malathion exposure and leukemia was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of

[42] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[43] Risk estimates for intensity-weighted lifetime days of exposure for leukemia reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.74 \leq RRs \leq 2.07$; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 63 leukemia cases identified during the study period, 14 cases reported direct exposure to malathion. No evidence of a positive association was reported between malathion exposure and leukemia risk among AHS spouses (RR = 0.73; 95% CI: 0.34, 1.54; with n = 14 exposed cases), based on ever/never use. Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and leukemia (RR = 0.79; 95% CI: 0.34, 1.85; with n = 11 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and leukemia in adults. Four studies (Brown et al., 1990; Mills et al., 2005; Bonner et al., 2007; Lerro et al., 2015) were identified that assessed the association between malathion exposure and leukemia among study populations in Minnesota and Iowa, California, and the AHS cohort. Brown et al. (1990) reported no evidence of a positive association between malathion exposure and leukemia among adult males in Minnesota and Iowa, and similarly, when the data was further stratified based on pesticide use at least 20 years prior to the interview, no evidence of a significant positive association was reported.[44] The study was ranked moderate quality for regulatory purposes and limitations included the use of proxy respondents and recall bias which likely led to exposure misclassification and the comparison of two different subpopulations (farmers vs. nonfarmers) who have a different risk of disease. We note the small number of exposed cases reported in this study. Mills et al. (2005) reported no evidence of a significant positive association between high malathion use and total leukemia among California farmworkers. When further stratified by type of leukemia, although elevated, the investigators reported no evidence of a significant positive association between high exposure (compared to low exposure) to malathion and lymphocytic leukemia in the total study population, and no evidence of a significant positive association between high exposure to malathion and granulocytic leukemia in the total study population. The analysis of total leukemia was also stratified by gender and evidence of a strong association was reported between high exposure to malathion and total leukemia among females, but no evidence of a significant positive association between high exposure to malathion and total leukemia among males. The study was ranked low quality for regulatory purposes due to a number of study limitations. The exposure assessment approach used to assess malathion exposure was more limited and relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. No information was provided to demonstrate that this ecologic, county-level pesticide use information can reliably estimate individual-level exposure. The investigators acknowledge this limitation in discussing their results and indicate that ecologic-level exposure assessments can lead to exposure misclassification that may "create spurious associations" that magnify or diminish the underlying true exposure-response relationship. The

[44] See Footnote 37.

investigators also reported that the statistical power of the study was low and ranged from 15% to 44% depending on prevalence of exposure to pesticides included in the study. Lastly, for the strong association reported for total leukemia in females, we note that the number of exposed cases was small and the corresponding confidence intervals were wide, which limits the ability to interpret with confidence the observed odds ratios. The two additional studies, Bonner et al. (2007) and Lerro et al. (2015), were both part of the AHS cohort. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and leukemia among pesticide applicators based on lifetime days and intensity-weighted lifetime days of use, and the study quality was ranked high for regulatory purposes. Lerro et al. (2015) reported no evidence of a positive association between malathion exposure and leukemia risk among AHS spouses based on ever/never use. The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Additional details regarding the duration of time for pesticide usage (e.g., days, months, years) was not provided but would have been helpful.

Lymphatic-hematopoietic cancers (all)

Two studies (Bonner et al., 2007; Alavanja et al., 2014) examined the potential association between malathion exposure and lymphatic-hematopoietic cancers (all).

- The association between lymphatic-hematopoietic cancers and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. A Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two-exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[45]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. In the lymphatic-hematopoietic cancers (all) analysis for IWLD with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile (T1 – T3: $0.81 \leq RRs \leq 1.25$; all CIs encompassed the null value of 1.0, with n = 16 – 24 exposed cases, with 34 cases in the non-exposed group)[46]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.25). A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present, and the study authors concluded this bias had no effect on the reported risk estimates.

[45] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[46] Risk estimates for intensity-weighted lifetime days of exposure for lymphatic-hematopoietic cancers (all) reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.87 \leq RRs \leq 1.49$; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and lymphatic-hematopoietic cancers (all). Two studies, (Bonner et al., 2007; Alavanja et al., 2014), investigated the relationship between malathion exposure and lymphatic-hematopoietic cancers (all) among pesticide applicators enrolled in the AHS prospective cohort and both studies reported no evidence of a significant positive association, based on lifetime days and intensity-weighted lifetime days of use. The Bonner et al. (2007) study quality was ranked high for regulatory purposes and several strengths were noted including the prospective cohort study design as part of the AHS, the ascertainment of cancer using established cancer registries, and the strengths of the AHS exposure assessment approach. The Alavanja et al. (2014) study was quality was ranked high for regulatory purposes based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure to malathion.

Hodgkin Lymphoma

Two studies (Karunanayake et al., 2012; Latifovic et al., 2020) examined the association between malathion exposure and Hodgkin Lymphoma (HL).

- Karunanayake et al. (2012) investigated the association between pesticide exposure, including malathion, and HL by conducting a population-based case-control study among men living in Canada known as the Cross-Canada Study of Pesticides and Health Study (CCSPH). The study population included males ≥19 years old who lived in one of six Canadian provinces and completed the postal questionnaire. Deceased participants were excluded from this analysis of the CCSPH data. Cases of HL included adult males diagnosed with HL between September 1991 to December 1994 and were ascertained via provincial cancer registries or hospital ascertainment (Quebec only). Cases were validated by a pathologist who reviewed pathology slides. Controls were randomly selected males from either health insurance records, telephone directories (Ontario) or voters lists (British Columbia), who resided in the same Canadian provinces as cases, and were matched to cases via age (± 2 years). A postal questionnaire was mailed to cases and controls to assess pesticide exposure, and follow-up telephone interviews regarding detailed pesticide use were conducted for each subject who reported more than 10 hours per year of pesticide use. The response rates for cases and controls were 67.1% and 48.0%, respectively.[47] Exposure to malathion included pesticides with malathion as the main active ingredient and mixtures of herbicides including malathion as one of multiple active ingredients. Conditional logistic regression was used to calculate ORs and 95% CIs for malathion and malathion containing mixtures and HL, adjusting for age and province of residence. Among the total HL cases (n=316), 27 reported exposure to any malathion containing herbicide, and 127 of the 1,506 controls reported exposure to any malathion containing herbicide. No evidence of a significant positive association was reported between malathion exposure and HL adjusted for age group and province of residence (OR=1.07; 95% CI: 0.65, 1.74; with n=27 exposed cases and n=127 exposed

[47] McDuffie, H. H., Pahwa, P., Robson, D., Dosman, J. A., Fincham, S., Spinelli, J. J., & McLaughlin, J. R. (2005). Insect repellents, phenoxyherbicide exposure, and non-Hodgkin's lymphoma. J Occup Environ Med, 47(8), 806-816. https://doi.org/10.1097/01.jom.0000167260.80687.78

controls), and when adjusted for medical variables that were statistically significant in bivariable analysis ($p < 0.20$) including history of measles, acne, hay-fever, shingles, and a positive family history (1st degree relative) of cancer, no evidence of a positive association was reported (OR=0.97; 95% CI: 0.58, 1.63). In an additional analysis where individual malathion exposure was considered a fumigant, no evidence of a significant positive association was reported for the association between malathion exposure and HL, adjusted for age group and province of residence (OR=1.09; 95% CI: 0.35, 3.39; with n=4 exposed cases and n=23 exposed controls), and when adjusted for history of measles, acne, hay fever, shingles, and a positive family history (1st degree relative) of cancer, no evidence of a significant positive association was reported for the association between malathion and HL (OR=1.22; 95% CI: 0.39, 3.81; with n=4 exposed cases and n=23 exposed controls). We note the very small number of cases in this analysis.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included age matching of the cases to the controls, and case ascertainment. Additionally, authors conducted a pilot study prior and a validation exercise for the study questionnaire as means to assess exposure accurately. Study limitations were related to the case-control study design and consisted of the potential for selection bias and recall bias. The cases living with the outcome may have remembered certain past exposures more accurately than the controls and this recall bias may have led to exposure misclassification. Another limitation of the study was the low response rate to the mailed questionnaires. Only 67.1% of the contacted cases and 48.0% of the contacted controls responded to the questionnaire and were included in the analysis. There may have been differences between those who responded and those who did not respond.

- Latifovic et al. (2020) investigated the association between pesticide exposure, including malathion, and the risk of HL among male farmers in the United States and Canada. The study population in the pooled analysis (cases of HL=507, controls=3,886) included participants enrolled in three of the four case-control studies that compose the North American Pooled Project (NAPP) in Nebraska, Kansas, and six Canadian provinces.[48] Cases of HL (n=507) were identified through the state cancer registry (Kansas, enrolled 1976 – 1982) and special surveillance of hospital and pathology records or study groups (Nebraska, enrolled 1983 – 1986) and cancer registries of the six Canadian provinces and hospital ascertainment in Quebec (enrolled 1991 – 1994). Population-based controls (n=3,886) were selected through random digit dialing, Medicare, or from state mortality records (deceased controls), provincial health insurance records, telephone listings, and voter's lists. Within each study, there were differences in matching of controls to cases including: age ($\pm$2 years) and vital status in Kansas; frequency-matched 3:1 by race, age ($\pm$2 years) sex, and vital status in Nebraska; and cases and controls were stratified by age ($\pm$2 years) and province in Canada. Controls were matched to the age groupings of all cancer cases recruited by the NAPP and not specifically to HL cases. Pesticide exposure was assessed using questionnaires administered via telephone in Kansas and Nebraska and from a mailed questionnaire to all participants and a follow-up telephone interview for those who reported $\geq$10 hours per year of pesticide use in Canada. Participants in Canada and Nebraska received a list of chemicals and trade names for their questionnaires, participants in Kansas did not.

[48] Three population-based case-control studies included in the Latifovic et al. (2020) analysis:

1. Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF Jr (1986) Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 256(9):1141–1147
2. Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A (1990) A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4D) in eastern Nebraska. Epidemiology 1(5):349–356
3. McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW (2001) NonHodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev 10(11):1155–1163

Questionnaires also collected demographic, lifestyle, and occupational characteristics, and cancer risk factors including medical history. To validate pesticide use, Kansas and Canada compared a subset of respondents self-reported pesticide use to pesticide suppliers' records of purchase and 60% agreement was reported in Kansas and agreement in Canada was reported as excellent. In Nebraska and Kansas response rates for the study populations ranged from 69.9% - 94% (response rates for HL in Canada were not reported) and proxy respondents were used for those unable to complete questionnaires in Kansas and Nebraska (Cases: 22.9 % - 26.5%; Controls: 43.6% - 52.3%). Logistic regression was used to estimate ORs and 95% CIs for the association between malathion exposure and HL, adjusted for age, sex, state or province of residence, and respondent type (self, proxy). Covariates were selected using theoretical consideration of the relationships determined using the directed acyclic graph approach, and a change in estimate method (10% change in the coefficient estimate) was used to create the final model. Study participants missing covariate data were excluded from the analysis, leaving 496 cases and 3,789 controls. No evidence of a significant positive association was reported between malathion exposure and overall HL (OR=1.21; 95% CI: 0.81, 1.81; with n=36 exposed cases and n=198 exposed controls), based on ever use. An additional analysis considered duration of use (number of years used) and frequency of use (days/year used) for malathion separately, using the following exposure categories: 0 (referent), 1-5 years used, ≥ 6 years used for duration of use, and 0, 1-2 days/year, ≥ 3 days/year. No evidence of a significant positive association was reported between malathion exposure and overall HL for duration of use ($0.92 \leq OR \leq 1.71$; all CIs encompassed the null value of 1; n = 10 – 22 exposed cases, 73 – 77 exposed controls, p-trend > 0.05) and for frequency of use ($0.94 \leq OR \leq 1.28$; all CIs encompassed the null value of 1; n = 7 – 19 exposed cases, 51 – 79 exposed controls, p-trend > 0.05).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the validation of HL diagnosis. Recall bias was a potential study limitation, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls and this may have contributed to exposure misclassification as well. Case and control selection methods differed between each study which may have led to selection bias, and different methods were used to collect pesticide use information (postal vs. telephone) potentially causing some misclassification of exposure. Certain participants who were prompted with a list of pesticide names may have remembered their pesticide exposures more accurately than those who were not prompted with pesticide names. Additionally, a large percentage of proxy respondents was reported by the study authors (~31%) which could have contributed to information bias and led to exposure misclassification; however, we note the study authors performed sensitivity analysis with proxy respondents excluded and reported that results were qualitatively similar.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and HL. Two studies (Karunanayake et al. (2012); Latifovic et al., 2020) were identified that assessed the association between malathion exposure and HL among residents of Canada and the United States. Karunanayake et al. (2012) reported no evidence of a significant positive association between malathion exposure and HL, when adjusted for age group and province of residence, and no evidence of a positive association when adjusted for medical variables that were statistically significant in bivariable analysis including history of measles, acne, hay-fever, shingles, and a positive family history (1st degree relative) of cancer. The study was ranked moderate for regulatory purposes and several study limitations were noted including selection bias, recall bias, and the low response rate to the mailed questionnaires (67.1% cases and 48% controls) as there may have been differences between those who responded and those who did not respond. The second study, Latifovic et al. (2020), reported no evidence of a significant positive association between malathion

exposure and overall HL based on ever/never use, as well as for duration of use and frequency of use, in a pooled analysis of three case-control studies in Nebraska, Kansas, and six Canadian provinces. This study quality was ranked moderate for regulatory purposes. Limitations included potential recall bias due to cases potentially remembering exposure differently than controls, different selection methods used for cases and controls, and different exposure assessments across studies.

Non-Hodgkin Lymphoma

Eleven publications were identified (Cantor et al., 1992; McDuffie et al., 2001; Waddell et al., 2001; De Roos et al., 2003; Mills et al., 2005; Bonner et al., 2007; Hohenadel et al., 2011; Alavanja et al., 2014; Lerro et al., 2015; Koutros et al., 2019; Leon et al., 2019) among various populations of male farmers in the United States and Canada including three publications (Bonner et al., 2007; Alavanja et al., 2014; Lerro et al., 2015) that evaluated NHL risk in the AHS prospective cohort.

- Cantor et al. (1992) investigated the association between malathion exposure and NHL among male farmers in Iowa and Minnesota. Using data from two population-based case-control studies, cases were determined either by the state health registry database or a special surveillance network including hospital and pathology records in Iowa and Minnesota. Eligibility criteria for cases included males aged ≥ 30 years old who were recently diagnosed with NHL. In Iowa, eligibility criteria were restricted to cases who were diagnosed between March 1981 and October 1983 and resided in any part of the state, and in Minnesota a diagnosis period between October 1980 through September 1982 was required, with residence in cities besides Minneapolis, St. Paul, Rochester, or Duluth at the time of diagnosis. NHL cases were confirmed by four pathologists by morphology, and the NHL subtype was determined when three of the four pathologists were in agreement with the subtype during the histopathologic review;[49] the subtypes included follicular, diffuse, small lymphocytic, and "other" NHL. Controls consisted of Caucasian white males, who had *not* been diagnosed with hematopoietic or lymphatic cancer and were randomly selected and frequency-matched to the cases based on vital status at the time of the interview, state of residence, and age group (within 5 years). Controls were identified through a separate population-based case-control for this study through a) random digit dialing; b) Medicare files; or c) state death certificates. In-person interviews were conducted by a trained professional for the cases and controls during August 1981 to March 1984 to obtain information about study participant demographics, medical history, occupational history (both farming and nonfarming jobs), past farming practices, and pesticide exposures (type and duration of use, and application method). Non-farmers (those who had never lived or worked on a farm as an adult) served as the reference population. Of the 622 cases interviewed, 184 (30%) of the cases were interviewed via proxy due to death or incompetency and, of the 1,245 controls, 425 (34%) controls were interviewed via surrogate. Unconditional logistic regression was conducted to determine ORs and corresponding 95% CIs for the association between malathion exposure and NHL among male farmers, adjusting for age, state, cigarette smoking status, high-risk occupations (e.g., nonfarming job related to NHL in this study), family history of lymphopoietic cancer, and high-risk exposures (e.g., exposure to hair dyes). For NHL subtypes, polychotomous logistic models were run using software created by the National Cancer Institute. Among the total cases (n = 622), the following cases of NHL subtypes were reported: 198 (31.8%) diffuse, 195 (31.4%) follicular, 85 (13.7%) small lymphocytic cell, and 144 (23.2%) other and undefined lymphomas. When the NHL cases and controls were further stratified by occupation, specifically farming, 356 of the 622 total cases (57%) and 698 of the total 1,245 controls) reported ever farming; the remaining cases and controls reported never farming (n = 266 cases, 547 controls). For malathion, when the data was stratified by pesticide applications

[49] The study mentioned that cases were considered "unclassifiable" if the panel of pathologists (three of the four) were not in agreement with the specific subtype of NHL, or if a specific subtype could not be determined from the provided tissue sample.

(to crops or to animals), no evidence of a significant positive association was reported between malathion exposure and NHL among farmers based on ever/never use from animal applications or from crop applications (animal applications OR: 1.30; 95% CI: 0.90, 2.10 with n = 43 cases, 67 controls; crop applications OR: 1.50; 95% CI: 0.80, 2.70 with n = 21 cases, 30 controls). Additionally, when malathion exposure was limited to pesticide use prior to 1965 (chosen because 15-18 years prior to diagnosis was a reasonable minimal latency period), a moderately strong association was reported for malathion use before 1965 and NHL for crop applications among a small number (n = 11) of cases with wide confidence intervals (crop OR: 2.90; 95% CI: 1.10, 7.40 with n = 11 exposed cases, n = 9 exposed controls); no evidence of a significant positive association was observed for animal applications (animal OR: 1.80; 95% CI: 1.00, 3.30 with n = 25 exposed cases, 30 exposed controls). Furthermore, when the state of residence among the study participants was considered, no evidence of a significant positive association was reported among a small number of cases in *Minnesota* from animal or from crop applications (animal OR = 2.00; 95% CI: 0.70, 5.30, with n = 9 exposed cases and n = 9 exposed controls; crop OR = 4.10; 95% CI: 0.90, 18.60, with n = 5 exposed cases and n = 3 exposed controls) and no evidence of a significant positive association was reported for malathion exposure and NHL among a small number of cases in *Iowa* from animal or from crop applications (animal OR = 1.50; 95% CI: 0.70, 3.10, with n = 16 exposed cases and n = 21 exposed controls; crop OR = 2.10; 95% CI: 0.60, 7.00, with n = 6 exposed cases and n = 6 exposed controls). Additionally, no evidence of a significant positive association was reported between malathion and NHL when malathion was handled without protective equipment for either animal and crop applications (animal OR: 1.40; 95% CI: 0.80, 2.20 with n = 33 cases, 52 controls; crop OR: 1.90; 95% CI: 0.90, 4.10 with n = 14 cases, 16 controls).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the study design, the adequate statistical methods, the measures taken to ascertain the study cases, and the in-person interviews conducted. A main study limitation included the use of proxy respondents among the cases and controls during the exposure assessment. The study indicated that 30% and 34% of the total cases and controls used proxy respondents to report their exposure which may have contributed to information bias and led to exposure misclassification. Another study limitation included potential recall bias, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls. As a result, this recall bias may have led to exposure misclassification as well. Another limitation of the study is that it appears the authors compared the odds of cases of exposed farmers to nonfarmers, instead of exposed farmers to unexposed farmers, and any effects found from these comparisons might not be due to the chemical exposure but instead due to different risk of disease between two different subpopulations (farmers vs. nonfarmers). Another study limitation included the fact that case and control selection methods differed between each study and likely led to selection bias, and different methods were used to collect pesticide use information (list of pesticides vs. voluntary recall). We note the number of exposed cases was small (10 < exposed cases < 19).

- In another study, McDuffie et al. (2001) evaluated the potential association between pesticides, including malathion and NHL by conducting a population-based case-control study among men living in Canada, known as the Cross-Canada Study of Pesticides and Health Study (CCSPH). Incident NHL cases included males who were: ≥ 19 years of age, diagnosed with NHL between September 1991 to December 1994, and who resided in either Quebec, Ontario, Alberta, Saskatchewan, Manitoba, or British Columbia. Cases were ascertained using cancer registries or hospital ascertainment (Quebec only), and pathology slides were reviewed by pathologists for validation. Authors reported that 84 % (436 of 517) of the NHL tumors were validated. Controls were randomly selected males from either health insurance records, telephone directories (Ontario) or voters lists (British Columbia), who resided in the same Canadian provinces, and were matched to the cases via age (± 2 years). A postal questionnaire was mailed to the confirmed cases to assess pesticide

exposure, and follow-up telephone interviews regarding detailed pesticide use were conducted for each subject who reported more than 10 hours/year of pesticide use. The response rates for the cases and controls were 67.1% and 48.0%, respectively. A conditional logistic regression was used to calculate ORs and 95% CIs for individual pesticide exposures including malathion, adjusting for age and province of residence. Among the total NHL cases (n = 517), 72 reported exposure to malathion, and 127 of the 1,506 controls reported malathion exposure. Evidence of a positive association was reported for any malathion exposure when the model was adjusted for age and province of residence (OR = 1.77; 95% CI: 1.28, 2.46; with n = 72 exposed cases and n = 127 exposed controls), and when the model was further adjusted for additional medical variables[50] (OR = 1.83; 95% CI: 1.31, 2.55; with n = 72 exposed cases and n = 127 exposed controls). In an additional analysis that analyzed frequency of exposure to malathion (as an individual compound) that divided days per year of exposure into two categories of lifetime exposure (>0 and ≤ 2 days per year of exposure vs. ≥ 2 days per year of exposure), evidence of a positive association was reported for >0 and ≤ 2 days per year of malathion exposure and ≥ 2 days per year of malathion exposure and NHL (>0 and ≤ 2 days OR = 1.82; 95% CI: 1.25, 2.68; with n = 50 exposed cases and n = 88 exposed controls; ≤ 2 days per year OR = 1.75; 95% CI: 1.02, 3.03; with n = 22 exposed cases and n = 39 exposed controls), with the no exposure group as the referent. In an additional analysis that analyzed the frequency of malathion exposure as an indoor fumigant relative to NHL, no evidence of a significant positive association was reported when the model was adjusted for province of residence and age (OR = 1.49; 95% CI: 0.72, 3.11; with n = 12 exposed cases and n = 23 exposed controls) and when the model was further adjusted for medical variables (OR = 1.54; 95% CI: 0.74, 3.22; with n = 12 exposed cases and n = 23 exposed controls). We note the number of exposed cases was small (10 < exposed cases < 19).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the study design, age matching the cases to the controls, the adequate statistical methods, and the measures taken to ascertain the study cases. A main study limitation included the use of proxy respondents among the cases and controls during the exposure assessment even though authors attempted to minimize the number of proxy respondents by making deceased ineligible to participate. Authors did not specify the percentage of the total cases and controls that used proxy respondents to report their exposure. Use of proxy respondents may have contributed to information bias and led to exposure misclassification. Another study limitation included potential recall bias, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls. As a result, this recall bias may have led to exposure misclassification as well. Another limitation of the study was the response rate. Only 67.1% of the contacted cases and 48% of the contacted controls responded to the mailed questionnaire and were included in the analysis. There may have been differences between those who responded and those who did not respond. We note the number of exposed cases was small (10 < exposed cases < 19).

[50] Medical variables included the following: history of measles, mumps, cancer, allergy, desensitization shots, and a positive family history of cancer in a first-degree relative.

- Waddell et al. (2001) investigated the association between exposure to malathion and other organophosphate pesticides and the risk of NHL among male farmers in the United States. The study population included white male farmers living in Iowa, Minnesota, Kansas, and Nebraska pooled from three case-control studies conducted by the National Cancer Institute with collaborators (including Cantor et al., 1992 above).[51] A total of 748 cases and 2,236 controls were included in the analysis. Cases (n = 748) were identified through state cancer or health record registries (Iowa, Kansas) and special surveillance of hospital and pathology records or study groups (Minnesota, Nebraska) and were enrolled between 1981 and 1986.[52] Expert pathologists reviewed tumor tissue from all eligible cases and classified each case by NHL subtype using the Working Formulation (National Cancer Institute).[53] Population-based controls (n = 2,236) were selected through random digit dialing (living controls < 65 years old), from Health Care Financing Administration Records (controls ≥ 65 years old), and from state mortality records (deceased controls). Cases and controls were frequency-matched based on race, state, 5-year age group (except deceased controls who were matched to deceased cases based on year of death), and vital status at time of interview. Study participants missing critical data or unsure of organophosphate exposure were excluded from the analysis, leaving 748 out of 780 cases and 2,236 of the 3,379 controls. Authors reported that for those with organophosphate use, the risk of NHL among farmers excluded from the analysis because of missing data were similar to the risk of NHL among non-farmers. Exposure was assessed through interviews (telephone – Kansas and Nebraska; in person – Iowa and Minnesota) that gathered data on demographic information (not specified), occupation, agricultural practices, hobbies, medical conditions, tobacco and alcohol use, family history of cancer, dietary history, and pesticide use (personal ever use of specific pesticides, lifetime use including days per year of use and years of use) information was collected to varying degrees of detail and methods in each case-control population. Response rates for each of the three study populations ranged from 76% - 96% with higher response rates among cases than controls. In addition, participants in Iowa, Nebraska, and Minnesota were asked about pesticide use based on a specific list of pesticides whereas participants in Kansas were asked to volunteer pesticide use information.[54] Non-farmers (those who had never lived or worked on a farm as an adult) served as the reference population. Farmers without exposure to organophosphate pesticides were not included in the control population even though they had a relative risk of 1.0 when compared to non-farmers. Authors reported this was to prevent the possibility of

[51] Three population-based case-control studies included in the Waddell et al. (2001) pooled analysis:
1) Cantor, K. P., Blair, A., Everett, G., Gibson, R., Burmeister, L. F., Brown, L. M., Schuman, L., Dick, F. R. (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Research*, 52(9), 2447-2455.
2) Zahm, S. H., Weisenburger, D. D., Babbitt, P. A., Saal, R. C., Vaught, J. B., Cantor, K. P., Blair, A. (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) in eastern Nebraska. *Epidemiology*, 349-356; and,
3) Hoar, S. K., Blair, A., Holmes, F. F., Boysen, C. D., Robel, R. J., Hoover, R., Fraumeni, J. F. (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256(9), 1141-1147.

[52] Eligibility criteria of cases varied across all three case-control studies. In Iowa, cases were eligible if they were diagnosed between March 1981-October 1983; in Minnesota cases were eligible if they were diagnosed between October 1980 and September 1982; cases in Kansas were diagnosed between 1979 and 1981; and, cases in Nebraska were diagnosed between July 1983 and June 1986.

[53] The Working Formulation was developed by the National Cancer Institute (part of the National Institutes of Health of the U.S. Department of Health and Human Services) in 1982 as a way to translate NHL diagnoses across the many recognized NHL classification systems with major groups identified by letters (A-J) and grouped according to prognosis. https://training.seer.cancer.gov/lymphoma/abstract-code-stage/morphology/formulation.html

[54] Lifetime pesticide use information: Nebraska – days per year of use and years of use were asked about for each pesticide; Kansas- days per year of use and years of use were not collected for individual pesticides; Iowa and Minnesota – days per year of use were not collected initially and a later attempt to capture these data was not successful.

misclassification (impact of false-negatives) in analysis of specific pesticides with small numbers of participants reporting exposure. Among the cases and controls that reported use of organophosphates in general, 117 direct and 41 (26%) proxy respondents among the 158 cases; and 224 direct and 55 (20%) proxy respondents among the 279 controls reported any organophosphate use. The majority of proxy respondents were spouses and authors reported that ORs from proxy respondents were larger than ORs for direct respondents. Among farmers reporting organophosphate use, proxy respondents contributed to 26% of case responses and 20% of control responses, for non-farmers reporting organophosphate use, percentages of proxy respondents were higher – 33% of cases, 43% of controls. Among the total study population included in the analysis, 91 (12.2%) of 748 cases and 147 (6.6%) of 2,236 controls reported exposure to malathion. The association between malathion exposure and NHL was assessed using logistic regression to estimate ORs and 95% CIs, adjusted for age, state of residence (Iowa, Kansas, Minnesota, Nebraska), and survey respondent type (direct, proxy). Authors reported that adjustment for other potential risk factors for NHL (not specified) had no effect on the outcome estimates and thus were not included in the final models. Evidence of a positive association was reported between malathion ever use and NHL among farmers relative to non-farmers (OR = 1.60; 95% CI 1.20, 2.20; with n = 91 exposed cases and n = 147 exposed controls). No evidence of a significant positive association was reported between malathion use and NHL among direct respondent farmers (proxy respondents excluded), relative to non-farmers (OR=1.20; 95% CI: 0.90, 1.80; with n = 68 exposed cases, 121 exposed controls).

Additional analyses were conducted for malathion exposure and NHL among direct respondent farmers (proxy respondents excluded) relative to non-farmers when stratified by several categories: 1) state of residence – Iowa, Kansas, Minnesota, Nebraska; 2) Age at first use of malathion (<20 years ago, ≥20 years ago); 3) Years of malathion use - <10 years, 10-19 years, ≥20 years; 4) days per year of malathion use - < 5 days, ≥5days; 5) protective gear – used, not used. For the analysis by *age at first use of malathion*, evidence of a positive association was reported between malathion use and NHL among direct respondent farmers who were ≥ 20 years of age, relative to non-farmers (≥ 20 years of age– OR = 1.70; 95% CI: 1.10, 2.90; with n=35 exposed cased). No evidence of a positive association was reported between malathion use and NHL among direct respondents < 20 years of age, relative to non-farmers (OR: 0.90; 95% CI: 0.50, 1.60 with n = 22 exposed cases). No evidence of a significant positive association was reported for *state of residence, years of malathion use, days per year of malathion use,* or *use of protective gear* (1.00< ORs < 2.70; 95% CIs encompassed the null value 1.0; with n = 3 - 43 exposed cases per category). An additional analysis of the association between malathion use and NHL among direct respondents relative to non-farmers was adjusted for use of other organophosphates fonofos and diazinon (in addition to age and state of residence). No evidence of a significant positive association was reported for the association between malathion use and any NHL subtype when adjusted for either fonofos or diazinon (1.10 < OR < 1.20; all 95% CIs encompassed the null value of 1.0; with n = 68 exposed cases).

For the analyses of malathion exposure and NHL subtypes, no evidence of a significant positive association was observed for follicular lymphoma, diffuse, small lymphocytic, and other types of NHL among direct respondents relative to non-farmers (0.90 < ORs <1.90; all other 95% CIs encompassed the null value 1.0; with n = 10-29 exposed cases per histologic type). When the association between malathion exposure and NHL subtypes was further adjusted for fonofos and diazinon, no evidence of a significant positive association was reported between malathion use and any of the NHL subtypes when adjusted for either fonofos or diazinon among direct respondents relative to non-farmers (0.90< ORs<1.90; 95%CIs encompassed the null value 1.0; with n = 10-29 exposed cases per NHL type).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Strengths included the case-control study design, histological confirmation of

tumor tissue by trained pathologists, and a relatively high survey response rate (~>80%) in all three of the pooled studies. The use of proxy respondents (up to 33% of cases and 43% controls) to capture pesticide use information of deceased was considered a study limitation as recall by proxy respondent may not be as accurate as from the actual pesticide user and authors reported higher ORs for proxy respondents than for direct respondents. Authors restricted the control population to include only non-farmers, and these were compared to farmer cases. An additional study limitation included the potential for recall bias, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls and this may have contributed to exposure misclassification as well. Case and control selection methods differed between each study, and different methods were used to collect pesticide use information (list of pesticides vs. voluntary recall). Certain participants who were prompted with a list of pesticide names may have remembered their pesticide exposures more accurately than those who were not prompted with pesticide names.

- De Roos et al. (2003) investigated the association of NHL and specific pesticides including malathion, using a pooled analysis of three case-control studies (Cantor et al., 1992; Hoar et al., 1986; Zahm et al., 1990). These three studies were performed by the National Cancer Institute to evaluate pesticide exposures and NHL in four Midwestern states within the United States, Iowa, Nebraska, Kansas, and Minnesota. The recruitment phase of each study differed. For Nebraska, cases were defined as Caucasian male subjects, diagnosed with NHL between July 1983 and June 1986, who lived in eastern Nebraska (one of the 66 counties) and were aged ≥ 21 years old.[55] Cases in Nebraska were identified through the Nebraska Lymphoma Study Group and local hospitals. In Kansas, cases were randomly selected from the state cancer registry, were Caucasian male subjects, diagnosed with NHL during 1979 and 1981, and were aged ≥ 21 years old.[56] In Minnesota and Iowa, cases were recently diagnosed with NHL, Caucasian male subjects, and aged ≥ 30 years old.[57] These cases were ascertained using records from the state cancer registry between 1981 to 1983 in Iowa, and from a surveillance program in hospitals and pathology laboratories in Minnesota during 1980 to 1982. Controls were randomly selected from a population of people living within a similar geographic location as the cases through Medicare records, random digit dialing, and state mortality files (deceased only). Also, the controls were frequency-matched to cases through race, sex, age, and vital status. Pesticide exposure was assessed through questionnaires administered by interviewers to study participants or proxy respondents (if respondents were deceased or incapacitated), using a series of exposure-related questions asked in various ways (e.g., directly vs. open-ended questions) depending on the state. A logistic regression and a hierarchical regression were used to calculate odds ratios and 95% confidence intervals for individual pesticide exposures, and each was adjusted for all of the other 46 pesticides assessed in this study, age, and study location. Among the total number of cases (n = 870) and controls (n = 2,569), 545 (62.6%) of the cases self-reported exposure and 325 (37.4%) exposure was reported via proxy respondent. For the controls, 1,413 (55.0%) self-reported exposure and 1,156 (45.0%) reported exposure via proxy respondent. When missing data variables were excluded from the analyses, 53 (8.10%) of 650 cases and 100 (5.20%) of 1,933 controls reported malathion exposure. No evidence of a significant positive association was reported between malathion exposure and NHL for either the logistic and hierarchical regressions (OR = 1.10; 95% CI: 0.60, 1.80; OR = 1.10; 95% CI: 0.70, 1.70), respectively.

[55] Zahm SH, Weisenburger DD, Babbitt PA, et al. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1990; 1:349–56.

[56] Hoar SK, Blair A, Holmes FF, et al. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA,1986;256:1141–7.

[57] Cantor KP, Blair A, Everett G, et al. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res 1992; 52:2447–55.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The pooled study design enabled the investigators to combine data from three population-based case-control studies which increased the number of exposed subjects and made it possible to include assessment of malathion, even though malathion use was relatively rare in both cases and controls (8.10% and 5.20% of cases and controls, respectively). Another strength of the study was that all cases were identified through established cancer registries and were clinically confirmed. With regard to limitations, recall bias was likely if the cases were more likely to recall past pesticide use than control subjects. The use of proxy respondents (up to 37% of cases and 45% of controls) to capture pesticide use information was considered a study limitation as recall by proxy respondents may not be as accurate as from the actual pesticide user. Authors reported higher ORs for proxy respondents than for direct respondents. Additionally, the case selection methods differed between each study which may have led to selection bias and different methods used to collect pesticide use information between studies potentially led to misclassification of exposure. Certain participants who were prompted with a list of pesticide names may have remembered their pesticide exposures more accurately than those who were not prompted with pesticide names.

- Mills et al. (2005) conducted a nested case-control study to investigate the association between NHL and pesticide exposures, including malathion, from farm work in a prospective cohort study of farmers who were part of the United Farm Workers of America (UFW). The study population consisted of members of the UFW at any time between 1973 and 2001. Incident cancer cases were identified by linking the UFW cohort to the California Cancer Registry during the years 1988 through 2001. Controls consisted of farm workers within the UFW cohort who had never been diagnoses with cancer and were randomly selected from the remaining UFW cohort and matched (5:1) to the cases based on age of cancer diagnosis, Hispanic ethnicity, and gender. Pesticide exposure for the cases and controls was assessed using three different types of records/databases: UFW records to verify occupational history, grower's contracts to establish the crop/commodity the member was exposed to, and the California Department of Pesticide Regulation to determine specific pesticide usage. Unconditional logistic regression was used to calculate ORs and 95% CIs, controlling for age, sex, duration of union affiliation, and start date of first union affiliation. Based on this approach, a total of 131 cases of lymphohematopoietic cancers were identified between 1988 and 2001 in the UFW cohort, including 60 cases of non-Hodgkin's lymphoma, 20 cases of multiple myeloma, and 51 cases of leukemia. The investigators analysis of the relationship between malathion use and NHL compared high and low exposed groups and performed additional analyses stratified by cancer type and gender. For <u>total NHL</u> the investigators reported no evidence of a significant positive association between high exposure to malathion and NHL in the total study population (OR=1.77; 95% CI: 0.99, 3.17; n = 60 cases). The analysis stratified by nodal and extranodal NHL showed evidence of a strong association between high exposure to malathion and NHL-extranodal (OR=3.52; 95% CI: 1.24, 10.0; n = 22 cases) and no evidence of a significant positive association between high exposure to malathion and NHL-nodal (OR=1.25; 95% CI: 0.60, 2.64; n = 38 cases). When stratified by gender, although elevated, the investigators reported no evidence of a significant positive association between high exposure to malathion and NHL among males (OR=2.01; 95% CI: 0.99, 4.1; n = 45 cases) and no evidence of a significant positive association between high exposure to malathion and NHL among females (OR=1.92; 95% CI: 0.60, 6.18; n = 15 cases).

  The overall quality of the Mills et al. (2005) was ranked low based on the study quality criteria provided in the OPP Framework. The studied leveraged an existing prospective cohort of farm workers based on membership of the UFW during the years 1973 and 2001 and was able to systematically ascertain year of cancer diagnosis using the California cancer registry. The nested case-control study design also helped ensure that controls were systematically identified within the same target study population of Hispanic women farm workers. While these design features were

important strengths of the study, the exposure assessment approach used to assess malathion exposure was more limited and relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. No information was provided to demonstrate that this ecologic, county-level pesticide use information can reliably estimate individual-level exposure. The investigators acknowledge this limitation in discussing their results and indicate that ecologic-level exposure assessments can lead to exposure misclassification that may "create spurious associations" that magnify or diminish the underlying true exposure-response relationship. The investigators also reported that the statistical power of the study was low and ranged from 15% to 44% depending on prevalence of exposure to pesticides included in the study. Lastly, we note the number of exposed female NHL cases was small (10 < exposed cases ≤ 19).

- Bonner et al. (2007) investigated the association between NHL and specific pesticides including malathion in a prospective study of the AHS cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. The response rate of the contacted study participants who completed the take-home questionnaire was 44% (n = 25,291). Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. A Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, education, and lindane use[58]. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were calculated for two exposure metrics (lifetime exposure-days and intensity-weighted lifetime-days of exposure[59]) and two reference groups consisting of non-exposed and the low-malathion exposed applicators were used to make statistical comparisons. In the NHL analysis for IWLD of exposure with the non-exposed group as the referent, no evidence of a positive association was observed in any tertile (T1 – T3: 0.53 ≤ RRs ≤ 0.83; all CIs encompassed the null value of 1.0, with n = 5 – 9 exposed cases, with 14 cases in the non-exposed group)[60]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.92). The authors also conducted a sensitivity analysis to assess selection bias and concluded that selection bias had no effect on the reported risk estimates.

  The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

[58] In the NHL analysis for malathion, the study authors further adjusted for lindane use since lindane has been associated with NHL.

[59] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[60] Risk estimates for intensity-weighted lifetime days of exposure for NHL reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.62 ≤ RRs ≤ 1.30; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

- In another analysis, Hohenadel et al. (2011) evaluated the potential association between both the combined and separate effects of pesticides including malathion and NHL among men. Using data from the same population-based case-control study (the CCSPH) as McDuffie et al. (2001) (see pg. 64 above), similar methods were used to obtain the cases and controls, as well as conduct the exposure assessment. An unconditional logistic regression model was used to calculate ORs and 95% CIs for pesticides including malathion, controlling for province, age, and use of a proxy respondent. For the individual and joint effects of pesticides model relative to NHL, malathion along with other common pesticides including 2,4-D, glyphosate, DDT, mecoprop, and carbaryl were considered. For individual pesticide effects in the malathion and DDT exposure group, evidence of a moderately strong association was reported between malathion exposure and NHL (OR = 2.03; 95% CI: 1.41, 2.94 with 52 cases and 95 controls). For individual pesticide effects, in the malathion and carbaryl exposure group evidence of a positive association between malathion exposure and NHL was reported (OR = 1.75; 95% CI: 1.22, 2.52 with 52 cases and 106 controls). For individual pesticide effects in the malathion and glyphosate exposure group evidence of a positive association between malathion exposure and NHL was reported (OR = 1.95; 95% CI: 1.29, 2.93 with 41 cases and 72 controls). For individual pesticide effects in the malathion and mecoprop exposure group, evidence of a positive association between malathion exposure and NHL was reported (OR = 1.76; 95% CI: 1.20, 2.60 with 44 cases and 92 controls). For individual pesticide effects, no evidence of a significant positive association between malathion exposure and NHL was reported among men in the malathion and 2,4-D exposure group (OR = 1.73; 95% CI: 0.81, 3.66 with 11 cases and 21 controls) among a small number of cases ($n \geq 10 \leq 20$).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the study design, age matching the cases to the controls, the adequate statistical methods, and the measures taken to ascertain the study cases. A main study limitation included the use of proxy respondents among the cases and controls during the exposure assessment. Use of proxy respondents may have contributed to information bias and led to exposure misclassification. We note the number of exposed cases was small ($10 <$ exposed cases ≤ 19).

- Alavanja et al. (2014) investigated the association between pesticide exposure, including malathion and NHL and NHL-subtypes among pesticide applicators in the AHS prospective cohort. The study population included private and commercial pesticide applicators enrolled in the AHS living in Iowa and North Carolina with no history of cancer reported at enrollment (1993-1997) and who had complete information on potential covariates. Tumor information was obtained through state cancer registry files in Iowa and North Carolina. Among the study population (n = 54,306), 523 total NHL cases were reported through 2010 (North Carolina) and 2011 (Iowa) and 332 cases reported exposure to malathion. Malathion exposure was assessed via self-administered questionnaires, one during study enrollment and a second follow-up questionnaire five years after enrollment (1999–2005). Investigators used this questionnaire data to estimate lifetime-days and intensity-weighted lifetime days of pesticide use. Poisson regression models and polytomous logistic regression models were used to calculate RRs for NHL and subtypes adjusting for age, race, state of residence, and herbicide use. No evidence of a positive association was reported between ever malathion exposure and overall risk of NHL (RR = 0.90; 95% CI: 0.80, 1.10; with n = 332 exposed cases). Further analysis by the authors considered lifetime days and intensity-weighted lifetime days of malathion use. Categories of exposure for malathion were created based on exposure tertiles separated at the median exposure level and included: low (< 8.75 lifetime days), medium (> 8.85 – 38.75 lifetime days), and high (> 38.75 – 737.5 lifetime days), and RRs were reported for each category. No evidence of a significant positive association was reported in any exposure category for lifetime days of exposure ($0.70 \leq RR \leq 0.97$; all 95% CIs encompassed the null value 1.0, with n = 47 – 75 cases per exposure category; p-

trend = 0.63). Similarly, no evidence of a significant positive association was reported for any exposure category for intensity-weighted lifetime days of exposure and all NHL cases ($0.80 \leq RR \leq 1.00$; all 95% CIs encompassed the null value 1.0, with n = 59 – 60 cases per exposure category; p-trend = 0.46). When the association between malathion and each of the NHL subtypes: small B-cell lymphocytic lymphomas (SLL)/ chronic B-cell lymphocytic lymphomas (CLL)/ mantle-cell lymphomas (MCL), diffuse large B-cell lymphomas, follicular lymphomas, other B-cell lymphomas, and multiple myeloma; was investigated, no evidence of a significant positive association was reported between malathion ever exposure and any of the NHL subtypes (*SLL/CLL/MCL*-RR=1.00; 95%CI: 0.70,1.40; with n=99 exposed cases; *Diffuse Large B-Cell* -RR=0.90; 95%CI: 0.60, 1.40; with n=72 exposed cases; *Follicular B-Cell*-RR=1.30; 95%CI: 0.70, 2.40; with n=46 exposed cases; *Other B-cell* – RR= 0.60; 95%CI: 0.30,1.00; with n=30 exposed cases; *Multiple Myeloma* – RR=0.90; 95%CI:0.60, 1.50; with n=61 exposed cases).

In the analyses of lifetime malathion use and NHL subtypes, with low and high exposure categories compared to the no exposure category as reference, no evidence of a significant positive association was reported for lifetime malathion exposure at any exposure category and any NHL subtype ($0.30<RR<1.60$; all other 95% CIs encompassed the null value of 1.0; with n=6-29 cases per exposure category; p-trends>0.05).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure to malathion.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and a range of cancer of various endpoints that included NHL in a prospective study of the AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997 living in Iowa and North Carolina who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD-O-3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history and other covariates that may be potential confounders. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 194 NHL cases identified during the study period, 34 cases reported direct exposure to malathion. No evidence of a positive association was reported between malathion exposure and NHL risk (RR = 0.64; 95% CI: 0.41, 0.99; with n = 34 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and NHL (RR = 0.60; 95% CI: 0.37, 0.98; with n = 27 exposed cases).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was

limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

- Koutros et al. (2019) evaluated the association between malathion exposure and NHL in men in the US and Canada by conducting a pooled analysis of four population-based case-control studies conducted in Kansas, Iowa, Minnesota and Nebraska in the 1980s by the National Cancer Institute and in the Canadian cities of Ontario, Quebec, Manitoba, Saskatchewan, British Columbia, and Alberta by the Cross Canada Study of Pesticides and Health between 1991 and 1994.[61] The study population in the pooled analysis included 1,690 NHL cases and 5,131 controls. All NHL cases were originally confirmed by pathology review as part of each individual study. In this analysis, incident NHL cases, aged ≥ 19 years old, were classified by NHL overall and subtypes based on the International Classification of Diseases for Oncology First Edition (ICD-O-1) coding system using their original NHL histology codes. Classifications included NHL (overall) or NHL subtype: diffuse large B-cell lymphoma, small lymphocytic lymphoma, follicular lymphoma, or other subtype. Controls were identified from the general population through various methods depending on the study population and included: random digit dialing, health insurance records, voter lists, state mortality files (for deceased cases), and Medicare listings from those 65 years or older. Controls were frequency matched to cases based on age (± 2 years or 5 years)[62] and location (state/province), and in some states matching also included race, vital status, sex, and year of death for deceased cases (Iowa, Minnesota, Nebraska and Kansas). Pesticide exposure was assessed through questionnaires administered by interviewers (in-person or telephone) or sent via mail for study participants and proxy respondents (if respondents were deceased or incapacitated). The study indicated that exposure data were provided via proxy respondents for 31.5% of cases and 33% of controls. Data on demographics, occupational, and medical history were also obtained from questionnaires. A linear trend test was also conducted using a Wald test. All models were adjusted for gender, study, age, and family history of lymphohematopoietic cancer. Additionally, a sensitivity analysis that was run to evaluate risk measures relative to duration of use with and without imputed duration, reported similar results. Unconditional logistic regression was used to estimate the association between ever/never malathion exposure and NHL (overall) and NHL subtypes (when select pesticide had ≥ 10 NHL cases), adjusting for gender, study, age, and family history of lymphohematopoietic cancer. Proxy respondent status was also considered as a potential covariate and effect modifier using a likelihood ratio test but proxy status was not included in the final model as it did not materially impact point

[61] The following four population-based case-control studies were included in this study (Koutros et al., 2019) and of these studies (Cantor et al., 1992, Zahm et al., 1990, and Hoar et al., 1986) were part of the pooled analysis mentioned in the Waddell et al. (2001) study above:
1) Cantor, K. P., Blair, A., Everett, G., Gibson, R., Burmeister, L. F., Brown, L. M., Schuman, L., Dick, F. R. (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Research*, 52(9), 2447-2455;
2) Zahm, S. H., Weisenburger, D. D., Babbitt, P. A., Saal, R. C., Vaught, J. B., Cantor, K. P., Blair, A. (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) in eastern Nebraska. *Epidemiology*, 349-356;
3) Hoar, S. K., Blair, A., Holmes, F. F., Boysen, C. D., Robel, R. J., Hoover, R., Fraumeni, J. F. (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256(9), 1141-1147; and,
4) McDuffie, H. H., Pahwa, P., McLaughlin, J. R., Spinelli, J. J., Fincham, S., Dosman, J. A., Robson, D., Skinnider, L. F., Choi, N. W. (2001). Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiology and Prevention Biomarkers*, 10(11), 1155-1163.

[62] The study indicated the controls were frequency-matched to the cases based on a range (± 2 years or 5 years) for the age variable. This range included the ages of the controls matched to the cases in the four, individual case-control studies (Hoar et al., 1986; Zahm et al.,1990; Cantor et al., 1992; McDuffie et al., 2001) that are part of this pooled analysis (Koutros et al., 2019).

estimates (by > 10%) and a significant effect modification by proxy status was not observed. A Phi coefficient was calculated for individual pesticides including malathion to determine the correlation among co-exposures (based on ever/never) to pesticides, and the median Phi coefficient for malathion was 0.19 (range 0.01 - 0.27), based on values reported in Supplemental Table 1 in the Appendix to Koutros et al. (2019), suggesting a weak correlation across pesticides was present in their study; pesticide co-exposures were not adjusted within the model for phi coefficients that were < 0.35. Lastly, test for heterogeneity among NHL subtypes using a polytomous logistic regression via a Wald test, indicated no statistical significance (all p-values > 0.05). Among the total cases and controls in this pooled analysis (n = 1,690 cases, 5,131 controls), 172 cases and 292 controls reported malathion exposure and 1,518 cases and 4,839 control reported no malathion exposure (based on ever/never exposure). Evidence of a positive association was reported between malathion and NHL among the study participants (OR: 1.63; 95% CI: 1.33, 2.0). When the data was further adjusted for correlated pesticides, evidence of a positive association was reported between malathion and NHL among the study participants (OR: 1.43; 95% CI: 1.14, 1.81). When the data was stratified into tertiles based on duration of pesticide use (low: < 6 years of malathion use vs. high: ≥ 6 years of malathion use), evidence of a borderline positive association was observed between malathion and NHL in the low and high categories of use (< 6 years adjusted OR: 1.40; 95% CI: 1.01, 1.92 with n = 65 cases, 128 controls; ≥ 6 years adjusted OR: 1.79; 95% CI: 1.38, 2.32 with n = 103 cases, 152 controls), relative to the unexposed group (n = 1,352 cases, 3,903 controls). A statistically significant exposure-response trend was observed (p < 0.0001). When the data was further adjusted for correlated pesticides, evidence of a positive association was observed between malathion and NHL in the high category of use (≥ 6 years adjusted OR: 1.57; 95% CI: 1.18, 2.01 with n = 103 cases, 152 controls,) with a statistically significant exposure-response trend (p < 0.01). No evidence of a significant positive association was observed between malathion and NHL in the low exposure of use (< 6 years adjusted OR: 1.25; 95% CI: 0.90, 1.75 with n = 65 cases, 128 controls).

- For the NHL subtypes, authors estimated the association between malathion ever use and individual NHL subtypes. The following results were reported:

    - For small lymphocytic lymphoma, no evidence of a significant positive association was reported for malathion use (OR: 1.50; 95% CI: 0.89, 2.54) and when further adjusted for correlated pesticides, no evidence of a significant positive association was reported (*additionally adjusted for pesticides*- OR: 1.04; 95% CI: 0.57, 1.91 with n=17 exposed cases), among a small number of exposed cases (>10-≤20).

    - For follicular lymphoma, evidence of a positive association was reported for malathion use (OR: 1.80; 95% CI: 1.31, 2.46) and when further adjusted for correlated pesticides, evidence of a positive association was reported (*additionally adjusted for pesticides*- OR: 1.58; 95% CI: 1.11, 2.27 with n = 55 exposed cases).

    - For diffuse large B cell lymphoma, evidence of a positive association was reported for malathion use (OR: 1.77; 95% CI: 1.33, 2.35) and when further adjusted for correlated pesticides, evidence of a positive association was reported (*additionally adjusted for pesticides*- OR: 1.61; 95% CI: 1.16, 2.22 with n = 68 exposed cases).

    - For other NHL subtypes, no evidence of a significant positive association was reported for malathion use (OR: 1.27; 95% CI: 0.86, 1.87) and when further adjusted for correlated pesticides, no evidence of a significant positive association was reported (*additionally adjusted for pesticides*- OR: 1.18; 95% CI: 0.77, 1.83 with n = 32 with n = 32 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths include the case-control study design, and validation of NHL diagnosis using pathology in each of the studies. Recall bias was a potential study limitation, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls and this may have contributed to exposure misclassification as well. Case and control selection methods differed between each study which likely led to selection bias, and different methods were used to collect pesticide use information (postal vs. telephone vs. in-person interviews) potentially causing some misclassification of exposure. Certain participants who were prompted with a list of pesticide names may have remembered their pesticide exposures more accurately than those who were not prompted with pesticide names. Additionally, a large percentage of proxy respondents was reported by the study authors (32% and 33% of the total cases and controls) which could have contributed to information bias and led to exposure misclassification; however, we note the study authors tested proxy respondent status as a potential covariate and effect modifier using the likelihood ratio statistical test. Since no statistical significance was observed for proxy respondent status as a covariate or effect modifier and ultimately was not considered to have an effect on point estimates (by > 10%), it was not included within the final models.

- Leon et al. (2019) examined the association between pesticide exposure and cancer in agricultural workers, including malathion and NHL, in a pooled analysis of data from three agricultural cohort studies, including AHS, as part of the AGRICOH. The AGRICOH is an international consortium of agricultural cohort studies that pool data to investigate health outcomes. The three cohorts included in this meta-analysis investigating effects of pesticide exposure on NHL were: (i) the AHS (data from private pesticide applicators only, commercial applicators excluded) of the United States; (ii) the Agriculture and Cancer (AGRICAN) cohort of France; and (iii) the Cancer in the Norwegian Agricultural Population (CNAP) cohort of residents of Norway. The three prospective cohorts assessed all incident cases of NHL and subtypes self-reported during follow-up (the date of enrollment for AHS and AGRICAN participants and 1993 for CNAP, the earliest year of follow-up) and through periodic data linkages to cancer and mortality registries. Specifically, for the AHS, this meta-analysis includes data from the AHS private pesticide applicators (commercial applicators were excluded), who enrolled between 1993 – 1997, with registry linkages until December 31, 2010 (North Carolina) and December 30, 2011 (Iowa). Malathion exposure was assessed through self-report of ever exposure to pesticide active-ingredients (AHS) and self-report of crops cultivated combined with country-specific crop-exposure matrices (AGRICAN and CNAP); enrollment for the AGRICAN was 2005 – 2007 and for CNAP, owners and non-owners using a farm ("farm holders") and their families were included in at least one of five national agricultural and horticultural censuses performed during 1969, 1974, 1979, 1985, and 1989 by Statistics Norway. Cohort members were linked with appropriate cancer and mortality registries and the U.S National Death Index (AHS and CNAP only) to identify cases of NHL. Cox proportional hazard regression models were used to estimate the association between ever use of malathion and incident NHL for each cohort, with never exposure as the referent. The AHS cohort specific regression model was adjusted for sex, state of residence, livestock (animal production), and pesticides terbufos, lindane, DDVP, permethrin, malathion, parathion, and carbaryl.[63] Resulting individual cohort estimates for malathion were then combined using random effects meta-analysis. Among the 316,270 agricultural workers included in the combined study population, 2,430 were cases of NHL (493 cases were participants of the AHS cohort). The AHS cohort-specific risk estimate for the association between malathion exposure and NHL was not reported. The authors reported no evidence of a positive association for malathion ever

[63] Each cohort Cox regression was adjusted for slightly different covariates: AGRICAN: sex, livestock, retirement status, number of selected types of crops for which pesticide treatment personally applied. CNAP: sex, livestock, dichlorvos, aldicarb, lindane, DDT, deltamethrin, mancozeb, linuron, glyphosate. AHS: sex, state, livestock, terbufos, lindane, DDT, permethrin, dicamba, parathion, carbaryl.

exposure and overall NHL (i.e., all subtypes considered together) (OR = 0.98; 95% CI: 0.82, 1.16, with n = 1,208 exposed cases), and no evidence of a significant positive association for malathion and any of the NHL subtypes in the meta-analysis (0.84 <HR <1.18; all 95% CIs encompassed the null value of 1.0; with n= 114 – 1208 exposed cases per category, p-trend >0.05).

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. Strengths of the study included the combination of three, very large international prospective cohort studies which increased the ability to detect epidemiological associations. Study limitations included differing exposure measurement methods used within the three studies, and potential exposure misclassification since the analysis of the combined cohort did not consider re-entry tasks through which contact with previously applied pesticides may have occurred. For example, only one of the two cohorts, the AHS cohort, uses actual exposure information collected by individuals through self-administered questionnaires; the French AGRICAN study and the Norwegian CNAP study instead rely on information from a crop-exposure matrix (CEM) to derive estimates of ever-exposure to glyphosate (among other pesticides). No actual pesticide exposure measurements were made in the AGRICAN or CNAP studies nor were specific questions about specific pesticide applications or application practices asked; instead, a variety of very general and very generic assumptions were made which likely led to what might be a substantial degree of exposure misclassification. In addition, the study protocol was such that exposure misclassifications may have been exacerbated since analysis of the combined cohort did not consider re-entry tasks through which contact with previously applied pesticides may have occurred and which may equal or exceed pesticide exposure through application. An additional complication was that such re-entry work was not evenly distributed through the cohort. For example, 73% of the males and 56% of the females in AGRICAN reported performing re-entry work in vineyards which is a rarely reported crop in the US AHS (1%) -- and consisted itself of 97% male farmers. An additional limitation included the fact that the three cohorts differed in fundamental ways including the age of the participants (the AHS members tended to be younger at the start of follow-up) and there was a larger percentage of AGRICAN women participants. Further, different statistical adjustments were made depending on what covariates were measured in each of the individual cohorts: The AGRICAN study did not adjust for cigarette smoking, alcohol intake, or family history of cancer as the US AHS did but did adjust for animal production and for different pesticide active ingredients from those adjusted for and published in the US AHS study. Study authors did state that improvements were planned, specifically indicating that the specificity of the exposure assignments will be improved by incorporating the probability of pesticide use and adding parameters reflecting duration, frequency, and use intensity. The AGRICAN study did not adjust for cigarette smoking, alcohol intake, or family history of cancer as the US AHS did, but did adjust for animal production and for different pesticide active ingredients from those adjusted for and published in the US AHS study.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and NHL among men and women in various study populations in the United States. EPA notes that among these many different investigations, there are essentially only four study populations in which the studies are based: an NCI pooled dataset of case-control studies in the U.S. Midwest, a nested case-control study of California farm workers (part of UFW prospective cohort study), the AHS study population, the cross-Canada case-control study series (Cross-Canada Study in Pesticide and Health, or CCSPH), and the international pooled study performed by the AGRICOH consortium. The key findings from these studies in addition to a few studies external are described below.

Wadell et al. (2001) and DeRoos et al. (2003) pooled three NCI-sponsored studies (Hoar et al. (1986), Zahm et al. (1990) and Cantor et al. (1992) that were conducted in the U.S. Midwest (IA, MN, KS, and NE). Results in Wadell et al (2001) were mixed across multiple analyses. There were two primary analyses that were presented: one reported significant evidence of a positive association between malathion-ever use and NHL among farmers relative to non-farmers (OR = 1.60 95% CI: 1.20, 2.20) when proxy respondents *were* included; the other found no evidence of a significant positive association among farmers (OR= 1.20 95% CI: 0.90, 1.80) when proxy respondents were excluded. DeRoos (2003) similarly pooled these three NCI studies, but found no evidence of a significant positive association between malathion exposure and NHL (OR 1.10; 95% CI: 0.70, 1.70 for their hierarchical regression model[64]).

Koutros et al. (2019) incorporated the three NCI studies reported above but also pooled this with one additional study from the CCSPH cohort (McDuffie et al., 2001): a variety of cohort groups were examined in Koutros et al. (2019) with the authors reporting evidence of a positive association between malathion exposure and NHL among study participants (odds ratios ranged from about 1.7 to 2.0 based on ever/never use) and evidence of a significant exposure-response trend for years of malathion use and NHL. Although not incorporated into Koutros et al (2019) pooled analysis, Hohenadel et al. (2011) also used the Canadian CCSPH cohort and found similar evidence of a positive to moderately strong association for malathion exposure and NHL.

All of the above publications were judged to be of moderate quality for regulatory purposes and had similar limitations. Limitations included recall-bias, a very large percentage of proxy respondents, and comparison of farmers to non-farmers. Additionally, the case selection methods differed between studies in the pooled analyses, which likely led to selection bias and the different methods were used to collect pesticide use information between studies potentially led to misclassification of exposure. Certain participants who were prompted with a list of pesticide names may have remembered their pesticide exposures more accurately than those who were not prompted with pesticide names.

Two publications (Bonner et al., 2007; Alavanja et al., 2014) examined the association between malathion exposure and NHL among pesticide applicators in the AHS prospective cohort and one publication (Lerro et al., 2015) looked at this relationship in spouses. Neither Bonner et al. (2007) and the later Alavanja et al. (2014) publications reported no evidence of a significant positive association between malathion exposure and NHL among AHS pesticide applicators, based on ever exposure, lifetime days exposure, and intensity-weighted lifetime days of exposure and no trends were seen with increasing exposures. (Lerro et al., (2015) similarly reported no evidence of a positive association among female spouses of pesticide applicators. All three publications on the AHS cohort were ranked high (Bonner et al. (2007) and Alavanja et al. (2014)) or moderate (Lerro et al. (2015)) quality and benefited from the general strengths of the AHS, including the prospective study design, case ascertainment via linkage to cancer registries, and exposure assessment.

Leon et al. (2019) took advantage of the international AGRICOH consortium and examined the association between malathion exposure among agricultural workers and non-Hodgkin lymphoma in a pooled international analysis of data from three agricultural cohort studies, including the AHS cohort. The study reported no evidence of a positive association for malathion exposure and NHL. The quality of the study was ranked low for regulatory purposes. Study limitations included differing exposure measurement methods used within the three studies, and potential exposure misclassification since the analysis of the combined cohort did not consider re-entry tasks through which contact with previously applied pesticides may have occurred. Additionally, the three cohorts differed in fundamental ways including the age of the

[64] Similar quantitative results were found in their non-hierarchical logistic regression model.

participants (the AHS members tended to be younger at the start of follow-up) and by the different statistical adjustments made within the individual cohorts depending on what covariates were measured.

The last publication, Mills et al. (2005), conducted a nested case-control study to investigate the association between NHL and pesticide exposures, including malathion, from farm work in a prospective cohort study of California farmers who were part of the United Farm Workers of America (UFW). The investigators analysis of the relationship between malathion use and NHL compared high and low exposed groups and performed additional analyses stratified by cancer type and gender. For total NHL, no evidence of a significant positive association between high exposure to malathion and NHL was reported in the total study population. The analysis stratified by nodal and extranodal NHL showed evidence of a strong association between high exposure to malathion and NHL-extranodal and no evidence of a significant positive association between high exposure to malathion and NHL-nodal. When stratified by gender, although elevated, the investigators reported no evidence of a significant positive association between high exposure to malathion and NHL among males and no evidence of a significant positive association between high exposure to malathion and NHL among females. We also note that the number of exposed female cases was small which limits the ability to interpret with confidence the observed odds ratios The study quality was ranked low for regulatory purposes as the exposure assessment approach used to assess malathion exposure was more limited and relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. The investigators also reported that the statistical power of the study was low and ranged from 15% to 44% depending on prevalence of exposure to pesticides included in the study. Lastly, we note that the number of female NHL exposed cases was small which limits the ability to interpret with confidence the observed odds ratios

Overall, the results of the studies investigating the association between malathion exposure and NHL were mixed: the two high quality studies from the AHS (Bonner et al. (2007) and Alavanja et al. (2014) were prospective cohort studies and did not find any evidence of an association between exposure to malathion and NHL using a variety of metrics, nor were any trends seen with increasing exposure. Similar findings were present in the moderate quality rated AHS Lerro et al. (2015) study conducted with AHS spouses as well as the most recent study, that of Leon et al. (2019); this latter study, however, was rated as low quality for regulatory purposes. The study by Koutros et al. (2019) was a pooled analysis of four population-based case-control studies conducted in Kansas, Iowa, Minnesota, and Nebraska in the 1980s by NCI and in the various Canadian locations (Ontario, Quebec, Manitoba, Saskatchewan, British Columbia, and Alberta) by the CCSPH between 1991 and 1994. The authors reported several moderately strong ORs resulting from a number of different analyses. While the overall quality of the pooled CCSPH study was ranked moderate, there were a number of limitations. Recall bias was a potential study limitation as was the fact that case and control selection methods differed between each study and may have led to selection bias. Further, different methods were used to collect pesticide use information (postal vs. telephone vs. in-person interviews) potentially causing some misclassification of exposure. Additionally, proxy respondents accounted for a large percentage of respondents. The last publication, Mill et al., 2005, reported no evidence of a significant positive association for total NHL among farm workers in California, and evidence of a strong association between high exposure to malathion and NHL-extranodal and no evidence of a significant positive association for NHL-nodal, in a stratified analysis. However, the study was ranked low quality for regulatory purposes since the exposure assessment relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. Additionally, the investigators reported that the statistical power of the study was low. Given the two high quality prospective cohort studies from the AHS that showed no association between malathion and NHL and the limitations cited regarding the several moderate quality studies that appeared to show an association, we conclude that the evidence is mixed and that there is insufficient epidemiological evidence of a clear associative or causal relationship between malathion exposure and NHL.

Multiple Myeloma

Four publications *(*Brown et al., 1993*;* Pahwa et al., 2012; Presutti et al., 2016; Leon et al., 2019) assessed the association between exposure to malathion and multiple myeloma (MM).

- Brown et al. (1993) investigated the association between malathion exposure and MM among men using data from three concurrent case-control studies conducted between 1981 – 1984 among MM cases in Iowa and non-Hodgkin lymphoma (NHL), and leukemia in Minnesota. MM cases included Caucasian men ≥ 30 years old who were diagnosed with MM between 1981 and 1984 and who lived in Iowa. Cases of MM were identified via the Iowa Health Registry and confirmed by a pathologist using pathology and laboratory reports. Controls were identified through random digit dialing, Medicare records, and state death certificates and included Caucasian men who did not have lymphatic or hematopoietic cancer. Controls were frequency-matched to the cases by age (within 5 years) and vital status (living or deceased) at time of interview. Exposure was assessed using a self-administered questionnaire; in-person interviews were conducted with next-of-kin if the study participant was deceased. Proxy respondents were used to complete in-person interviews for deceased cases (41%) and controls (30%). Logistic regression was used to calculate the OR and 95% CI for the association between malathion ever use and MM, adjusting for age and vital status, with nonfarmers as the referent group. Education and smoking were considered but found not to be confounders. For malathion, the data was further stratified based on exposure to animal insecticides or crop insecticides, and risk estimates were reported for both means of exposure. Of the total 173 MM cases and 650 controls, 6 cases and 44 controls reported exposure to malathion through animal insecticides, and 8 cases and 24 controls reported exposure to malathion through crop insecticides. No evidence of a significant positive association was reported between malathion ever use through crop insecticides and MM (OR = 1.90; 95% CI: 0.80, 4.60), and no evidence of a positive association was reported for malathion ever use through animal insecticides and MM (OR = 0.80; 95% CI: 0.30, 1.90).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the frequency matched cases to the controls, case ascertainment and the in-person interviews. A main study limitation included the use of proxy respondents (41% of cases and 30% of controls) to collect pesticide exposure information. This limitation may have contributed to information bias and led to exposure misclassification. Another study limitation included potential recall bias, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls. As a result, this recall bias may have led to exposure misclassification as well. Finally, authors compared the odds of cases of exposed farmers to nonfarmers, instead of exposed farmers to unexposed farmers, and any effects found from these comparisons might not be due to the chemical exposure but instead due to different risk of disease between two different subpopulations (farmers vs. nonfarmers). Lastly, we note the number of exposed cases was very small (< 10).

- Pahwa et al. (2012) investigated the potential association between exposure to pesticides, including malathion, and MM in a population-based case-control study among men in six Canadian provinces.[65] Incident cases of MM included males >19 years old with a first-time diagnosis of multiple myeloma (ICD-O M 9732/3) between September 1, 1991 and December 31, 1994. Cases were identified using provincial cancer registries, with the exception of Quebec where cases were ascertained based on hospital records. Study pathologists confirmed 36.5% of these cases using available pathology materials. Controls included males ≥ 19 years old who were randomly selected from either health insurance records (Alberta, Saskatchewan, Manitoba, and Quebec), telephone listings (Ontario), or

[65] The six Canadian provinces were Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia.

voter's lists (British Columbia) and were matched to cases based on age and residence. Pesticide exposure was assessed using a self-administered questionnaire that also included questions about demographic information, medical history, smoking history, and lifetime occupational and non-occupational (hobbies etc.) history and pesticide exposure. An additional telephone interview was administered to all participants with ≥10 hours of reported lifetime pesticide use and a 15% random sample of the remaining population who completed the self-questionnaire. Overall, participation rates were 58% for contacted cases and 48% for contacted controls, yielding 342 cases and 1,506 controls. Conditional logistic regression was used to determine ORs and 95% CIs for individual pesticides including malathion, adjusted for age, province of residence, and medical history variables (history of the following: measles, mumps, allergies, arthritis, shingles, and a positive family history of cancer in a first-degree relative). No evidence of a positive association was observed between exposure to malathion and MM (OR = 0.97; 95% CI: 0.62, 1.53; with n = 32 exposed cases and n = 127 exposed controls). No evidence of a significant positive associaton was observed between exposure to malathion as a fumigant and MM (OR = 1.16; 95% CI: 0.44, 3.11; with n = 6 exposed cases and n = 23 exposed controls).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the study design, age matching the cases to the controls, and case ascertainment. Additionally, authors conducted a pilot study prior and a validation exercise for the study questionnaire as means to assess exposure accurately. Study limitations were related to the case-control study design and consisted of the potential for selection bias and recall bias as the cases living with the outcome may have remembered certain past exposures more accurately than the controls. As a result, this recall bias may have led to exposure misclassification as well. Another limitation of the study was the response rate to the mailed questionnaires. Only 67.1% of the contacted cases and 48% of the contacted controls responded to the questionnaire and were included in the analysis. There may have been differences between those who responded and those who did not respond. Lastly, we note the number of exposed cases was very small (n<10).

- Presutti et al. (2016) investigated the association between pesticide exposure, including malathion, and the risk of MM among male farmers in the United States and Canada. The study population in the pooled analysis (cases of MM=547, controls=2,700) included participants enrolled in three of the four case-control studies that compose the North American Pooled Project (NAPP) in Nebraska, Iowa, and six Canadian provinces.[66] Cases of MM (n=547) were identified through the state cancer registry (Iowa, enrolled 1981 – 1984) and special surveillance of hospital and pathology records or study groups (Nebraska, enrolled 1983 – 1986) and cancer registries of the six Canadian provinces and hospital ascertainment in Quebec (enrolled 1991 – 1994). Population-based controls (n=2,700) were selected through random digit dialing, Medicare, or from state mortality records (deceased controls), provincial health insurance records, telephone listings, and voter's lists. Within each study, there were differences in matching of controls to cases including: age (±5 years) and vital status in Iowa; frequency-matched 3:1 by race, age (±2 years) sex, and vital status in Nebraska; and cases and controls were stratified by age (±2 years) and province in Canada. Eligible study participants included

[66] Three population-based case-control studies included in the Presutti et al. (2016) analysis:
1. Brown, L. M., Burmeister, L. F., Everett, G. D., & Blair, A. (1993). Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes & Control*, *4*(2), 153-156.
2. Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A (1990) A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4D) in eastern Nebraska. Epidemiology 1(5):349–356
3. McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW (2001) NonHodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev 10(11):1155–1163

men ≥ 30 years of age (Iowa), white men and women ≥ 21 years of age (Nebraska), and men ≥ 19 years of age (Canada).[67] Pesticide exposure was assessed using questionnaires administered via telephone (Nebraska) and an in-person interview (Iowa) AND from a mailed questionnaire to all participants and a follow-up telephone interview for those who reported ≥10 hours per year of pesticide use in Canada. Participants in Canada and Nebraska received a list of chemicals and trade names for their questionnaires. Questionnaires also collected demographic, lifestyle, and occupational characteristics, and cancer risk factors including medical history. To validate pesticide use in Canada, the authors compared a subset of respondents self-reported pesticide use to pesticide suppliers' records of purchase and agreement in Canada was reported as excellent. In Nebraska and Iowa response rates for the study populations ranged from 78% - 91% (response rates for MM in Canada were not reported) and proxy respondents were used for those unable to complete questionnaires in Iowa, Nebraska, and Canada (Cases: 35%; Controls: 28%). Unconditional logistic regression was used to estimate ORs and 95% CIs for the association between malathion exposure and MM, adjusted for age, state or province of residence, ever diagnosed with any allergy, hay fever, or rheumatoid arthritis, and use of proxy respondent. Covariates were selected that showed a significant relationship with MM and those that created meaningful changes were kept within the final model. Duration of exposure for pesticide use including malathion in these statistical analyses was defined as either ever/never or years of use, and cumulative exposure information defined as lifetime days (years of pesticide usage multiplied by days per year of pesticide use) was only available in the Canadian cohort, where sufficient data was available. For study participants who were missing data related to pesticide duration of use, a condition imputation was performed. Median values for years of use and days per year were assigned based on the age and state/province-specific values to individuals who indicated exposure based on ever/never use, and imputed values were only provided when <35% of the data was missing for the cases, and when the missing data proportions varied by <20% for the cases and controls.

No evidence of a significant positive association was reported between malathion exposure and MM (OR = 1.19; 95% CI: 0.84 1.69; with n = 48 exposed cases and n = 226 exposed controls), based on ever use. And similarly, when proxy respondents were excluded from the analysis, no evidence of a significant positive association was reported (OR = 1.33; 95% CI: 0.91, 1.94; with n = 42 of 356 exposed cases, n = 194 of 1,945 exposed controls). When exposure was defined as years of pesticide usage, and malathion exposure was further stratified into the follow exposure categories: > 0 and ≤ 6 years, and > 6 years with the unexposed category as the referent, no evidence of a significant positive association was reported between malathion exposure and MM in either exposure category (> 0 and ≤ 6 years OR = 1.39; 95% CI: 0.86 2.25; with n = 25 of 515 exposed cases and n = 93 of 1,999 exposed controls; > 6 years OR = 1.12; 95% CI: 0.68, 1.84; with n = 22 exposed cases and n = 90 exposed controls). And similarly, when proxy respondents were excluded from the analysis, no evidence of a significant positive association was reported in either exposure category relative to the referent (> 0 and ≤ 6 years OR = 1.55; 95% CI: 0.93 2.58; with n = 22 of 340 exposed cases and n = 85 of 1,601 exposed controls, p-trend = 0.36; > 6 years OR = 1.20; 95% CI: 0.70, 2.07; with n = 19 of 340 exposed cases and n = 78 exposed of 1,601 controls, p-trend = 0.21). In the additional analysis that evaluated as lifetime days in the Canadian cohort only as sufficient data was available and malathion exposure was further stratified into the follow exposure categories: > 0 and ≤ 14.5 lifetime days and > 14.5 lifetime days, no evidence of a significant positive association was reported in either exposure category relative to the referent (> 0 and ≤ 14.5 lifetime days OR = 1.07; 95% CI: 0.58, 1.98; with n = 15 of 342 exposed cases and n = 63 of 1,349 exposed controls; > 14.5 lifetime days OR = 1.26; 95% CI: 0.71, 2.25; with n = 17 of 342 exposed cases and n = 61 of 1,349 exposed controls). And similarly, when proxy respondents were excluded from the analysis, no evidence of a significant

[67] Although women were eligible to participate in this study, women were excluded from the final analysis due to the small prevalence of women who reported pesticide usage.

positive association was reported in either exposure category relative to the referent (> 0 and ≤ 14.5 lifetime days OR = 1.31; 95% CI: 0.68, 2.52; with n = 13 of 239 exposed cases and n = 56 of 1,149 exposed controls; > 14.5 lifetime days OR = 1.46; 95% CI: 0.80, 2.66; with n = 16 of 239 exposed cases and n = 54 of 1,149 exposed controls).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the validation of MM diagnosis. Recall bias was a potential study limitation, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls and this may have contributed to exposure misclassification as well. Case and control selection methods differed between each study which likely led to selection bias, and different methods were used to collect pesticide use information (postal vs. telephone) potentially causing some misclassification of exposure. Certain participants who were prompted with a list of pesticide names may have remembered their pesticide exposures more accurately than those who were not prompted with pesticide names. Additionally, a large percentage of proxy respondents was reported by the study authors (~35% for cases, 28% for controls) which could have contributed to information bias and led to exposure misclassification.

- Leon et al. (2019) examined the association between pesticide exposure and cancer in agricultural workers, including malathion and MM, in a pooled analysis of data from three agricultural cohort studies, including AHS, as part of the AGRICOH as described in more detail above. Briefly, this study includes a pooled analysis of data from three cohorts to examine the association between exposure to pesticides, including malathion, and MM. The three prospective cohorts assessed all incident cases of NHL and subtypes self-reported during follow-up (the date of enrollment for AHS and AGRICAN participants and 1993 for CNAP, the earliest year of follow-up) and through periodic data linkages to cancer and mortality registries. Specifically, for the AHS, this meta-analysis includes data from the AHS private pesticide applicators (commercial applicators were excluded), who enrolled between 1993 – 1997, with registry linkages until December 31, 2010 (North Carolina) and December 30, 2011 (Iowa). Malathion exposure was assessed through self-report of ever exposure to pesticide active-ingredients (AHS) and self-report of crops cultivated combined with country-specific crop-exposure matrices (AGRICAN and CNAP); enrollment for the AGRICAN was 2005 – 2007 and for CNAP, owners and non-owners using a farm ("farm holders") and their families were included in at least one of five national agricultural and horticultural censuses performed during 1969, 1974, 1979, 1985, and 1989 by Statistics Norway. Cohort members were linked with appropriate cancer and mortality registries and the U.S National Death Index (AHS and CNAP only) to identify cases of NHL. Cox proportional hazard regression models were used to estimate the association between ever use of malathion and incident NHL for each cohort, with never exposure as the referent. The AHS cohort specific regression model was adjusted for sex, state of residence, livestock (animal production), and pesticides terbufos, lindane, DDT, permethrin, malathion, parathion, and carbaryl.[68] Resulting individual cohort estimates for malathion were then combined using random effects meta-analysis. Among the 316,270 agricultural workers included in the combined study population, 2,430 were cases of NHL (493 cases were participants of the AHS cohort). Authors considered MM a subtype of NHL. No evidence of a positive association was reported for malathion ever exposure and MM among all participants in the analysis (HR = 0.95; 95% CI: 0.67, 1.36, with n = 269 exposed cases).

[68] Each cohort Cox regression was adjusted for slightly different covariates: AGRICAN: sex, livestock, retirement status, number of selected types of crops for which pesticide treatment personally applied. CNAP: sex, livestock, dichlorvos, aldicarb, lindane, DDT, deltamethrin, mancozeb, linuron, glyphosate. AHS: sex, state, livestock, terbufos, lindane, DDT, permethrin, dicamba, parathion, carbaryl.

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. Strengths of the study included the combination of three, very large international prospective cohort studies which increased the ability to detect epidemiological associations. Study limitations included differing exposure measurement methods used within the three studies, and potential exposure misclassification since the analysis of the combined cohort did not consider re-entry tasks through which contact with previously applied pesticides may have occurred. For example, only one of the two cohorts, the AHS cohort, uses actual exposure information collected by individuals through self-administered questionnaires; the French AGRICAN study and the Norwegian CNAP study instead rely on information from a crop-exposure matrix (CEM) to derive estimates of ever-exposure to glyphosate (among other pesticides). No actual pesticide exposure measurements were made in the AGRICAN or CNAP studies nor were specific questions about specific pesticide applications or application practices asked; instead, a variety of very general and very generic assumptions were made which likely led to what might be a substantial degree of exposure misclassification. In addition, the study protocol was such that exposure misclassifications may have been exacerbated since analysis of the combined cohort did not consider re-entry tasks through which contact with previously applied pesticides may have occurred and which may equal or exceed pesticide exposure through application. An additional complication was that such re-entry work was not evenly distributed through the cohort. For example, 73% of the males and 56% of the females in AGRICAN reported performing re-entry work in vineyards which is a rarely reported crop in the US AHS (1%) -- and consisted itself of 97% male farmers. An additional limitation included the fact that the three cohorts differed in fundamental ways including the age of the participants (the AHS members tended to be younger at the start of follow-up) and there was a larger percentage of AGRICAN women participants. Further, different statistical adjustments were made depending on what covariates were measured in each of the individual cohorts: The AGRICAN study did not adjust for cigarette smoking, alcohol intake, or family history of cancer as the US AHS did but did adjust for animal production and for different pesticide active ingredients from those adjusted for and published in the US AHS study.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and multiple myeloma (MM). Four publications (Brown et al., 1993; Pahwa et al., 2012; Presutti et al., 2016; Leon et al., 2019;) assessed the association between exposure to malathion and MM, with none reporting evidence of a significant positive association. Brown et al. (1993) in a case-control study in Iowa reported no evidence of a significant positive association between malathion ever use through crop insecticides and MM, and no evidence of a positive association through animal insecticides and MM. The study quality was ranked moderate for regulatory purposes, with concerns related to the use of proxy respondents and recall bias which may have led to exposure misclassification and the use of two different subpopulations (farmers vs. nonfarmers) who have a different risk of disease. Additionally, reported risk estimates were among a very small number of cases (n<10), which severely restricts the ability to interpret with confidence the observed odds ratios. In the Cross-Canada Study of Pesticides and Health case-control study, Pahwa et al. (2012) reported no evidence of a positive association between exposure to malathion and MM, and no evidence of a significant positive associaton between exposure to malathion as a fumigant and MM. The study quality was moderate for regulatory purposes and study limitations included potential for selection bias and recall bias. Additionally, we note the number of exposed cases was very small (n<10). Presutti et al. (2016) reported no evidence of a significant positive association between malathion and MM based on ever use, years of pesticide use, and for lifetime days of pesticide use, using data from a pooled analysis that included three of the four case-control studies that make-up the North American Pooled Project (NAPP) in Nebraska, Iowa, and six Canadian provinces. This study was ranked moderate for regulatory

purposes and strengths included the validation of MM diagnosis. Study limitations included recall bias, selection bias, and some misclassification of exposure. Additionally, a large percentage of proxy respondents was reported by the study authors which could have contributed to information bias and led to exposure misclassification. Leon et al. (2019) examined the association between malathion exposure and MM among the three pooled agricultural cohort studies that make up the AGRICOH. One of the study populations included those of the AHS. No evidence of a positive association was reported for malathion ever exposure and MM among all participants in the analysis. The study was ranked low due to limitations with the pesticide exposure assessment and potential misclassification, methods used to measure covariates, and lack of adjustment for important potential confounders.

Melanoma

Two studies (Bonner et al., 2007; Lerro et al., 2015) assessed the association between exposure to malathion and melanoma.

- The association between melanoma and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. A Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, education, and carbaryl and parathion use[69]. Additionally, risk ratios were determined in a separate analysis for frequency (days of use per year), intensity (intensity score), and duration (years of use) of malathion exposure. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-days and intensity-weighted lifetime-days of exposure[70]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. The study authors conducted a sensitivity analysis to evaluate selection bias and concluded that selection bias had no effect on the reported risk estimates. In the melanoma analysis for IWLD with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile (T1 – T3: $0.47 \leq$ RRs ≤ 1.44; all CIs encompassed the null value of 1.0, with n = 7 – 15 exposed cases, with 14 cases in the non-exposed group)[71]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in IWLD exposure analysis (p-trend = 0.06). Additionally, no evidence

[69] In the melanoma analysis for malathion, the study authors further adjusted for carbaryl and parathion use since both have been associated with melanoma.

[70] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[71] Risk estimates for intensity-weighted lifetime days of exposure for melanoma reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.31 \leq$ RRs ≤ 1.16; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

of a significant positive association between melanoma for frequency, intensity, and duration[72] of malathion exposure was observed ($0.61 \leq RRs \leq 1.27$; all CIs encompassed the null value of 1.0, with n = 8 – 22 exposed cases, with 14 cases in the non-exposed group, p-trend > 0.05).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups. We also note the number of exposed cases was small among some exposure categories.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including melanoma, among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina, who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD – O – 3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history, and other covariates that may be potential confounders. The association between malathion exposure and melanoma was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 117 melanoma cases identified during the study period, 23 cases reported direct exposure to malathion. No evidence of a positive association was reported between malathion exposure and melanoma risk (RR = 0.90; 95% CI: 0.52, 1.53; with n = 23 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and melanoma (RR = 0.82; 95% CI: 0.42, 1.59; with n = 15 exposed cases).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

[72] In a separate analysis, frequency of use was defined as: <5 or ≥5 days of use per year, duration of use was defined as : ≤10 years of use or >10 years of use, and intensity was defined by tertiles; however, the tertiles were not specified in the table by the study authors.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and melanoma. This determination was based on two available studies (Bonner et al., 2007; Lerro et al., 2015) that investigated the association between malathion exposure and melanoma in the AHS prospective cohort. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and melanoma among pesticide applicators enrolled in the AHS prospective cohort, based on lifetime days and intensity-weighted lifetime days of use. Additionally, no evidence of a significant positive association for frequency, intensity, and duration of malathion exposure was observed. The study quality was ranked high for regulatory purposes and several strengths were noted including the prospective cohort study design as part of the AHS, the ascertainment of cancer using established cancer registries, and the strengths of the AHS exposure assessment approach. We note the small number of exposed cases in some of the exposure categories which limits the ability to interpret with confidence the observed rate ratios. Lerro et al. (2015) reported no evidence of a positive association between malathion exposure and melanoma risk among female spouses, and no evidence of a positive association was reported for malathion exposure and melanoma in additional analyses that investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment. The overall quality of the study was ranked moderate for regulatory purposes. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate statistical methods used. Additional details regarding the duration of time for pesticide usage (e.g., days, months, years) was not provided but would have been helpful.

Ovarian Cancer

One study (Lerro et al., 2015) examined the association between malathion exposure and ovarian cancer.

Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints including ovarian cancer among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina and who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n = 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD – O – 3 codes from date of enrollment through whichever event was earlier - data of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. malathion lifetime ever exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires were used to collect information on demographics, reproductive history and other covariates that may be potential confounders. The association between malathion exposure and ovarian cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Models were additionally adjusted for number of live births, menopause status at enrollment, and oral contraceptive use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (220 males were excluded, 907 women with cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure all were excluded) and 5,704 reported lifetime ever use of malathion. Of the 85 ovarian cancer cases identified during the study period, 16 cases reported direct exposure to malathion. No evidence of a positive association was reported between malathion exposure and ovarian

cancer risk among AHS spouses (RR = 0.89; 95% CI: 0.48, 1.67). When ovarian cancer was stratified by menopausal status at enrollment, no evidence of a significant positive association was reported between malathion exposure and ovarian cancer for pre-menopausal participants (*Premenopausal* - RR = 2.14; 95% CI: 0.78, 5.93 with n = 8 exposed cases) and no evidence of a positive association was reported between malathion exposure and ovarian cancer for postmenopausal participants (*Postmenopausal* - RR = 0.57; 95% CI: 0.24, 1.33 with n = 8 exposed cases). In additional sensitivity analyses that investigated associations between ever/never malathion exposure and ovarian cancer diagnosed five or more years after study enrollment among AHS spouses, no evidence of a significant positive association was reported between malathion ever exposure and ovarian cancer (RR = 1.28; 95% CI: 0.64, 2.56 with n = 15 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment. We note the number of exposed cases was small (10 < exposed cases < 19).

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and ovarian cancer. One study (Lerro et al. 2015), investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints including ovarian cancer among participants in the prospective AHS cohort. The study reported no evidence of a positive association between malathion exposure and ovarian cancer risk among AHS spouses When ovarian cancer was stratified by menopausal status at enrollment, no evidence of a significant positive association was reported between malathion exposure and ovarian cancer for pre-menopausal participants and no evidence of a positive association was reported between malathion exposure and ovarian cancer for postmenopausal participants. In additional sensitivity analyses that investigated associations between ever/never malathion exposure and ovarian cancer diagnosed five or more years after study enrollment among AHS spouses, no evidence of a significant positive association was reported between malathion ever exposure and ovarian cancer. The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate statistical methods used.

Pancreatic Cancer

The association between malathion and pancreatic cancer was evaluated in two AHS studies (Andreotti et al., 2009; Lerro et al., 2015)

- Andreotti et al. (2009) conducted a case-control analysis of the AHS cohort to evaluate the association between pesticides, including malathion, and pancreatic cancer incidence. The study population consisted of licensed private and commercial pesticide applicators and their spouses enrolled in the AHS. Incident pancreatic cancer cases diagnosed from enrollment (1993-1997) through 2004 were identified through state cancer registry files in Iowa and North Carolina. Participants with any cancer reported at enrollment were excluded from the analysis. Pesticide exposure (ever/never) was assessed via a self-administered questionnaire completed at enrollment and again shortly thereafter. Unconditional logistic regression was used to calculate ORs and 95% CIs for

the association between ever/never exposure to malathion among spouses and pesticide applicators, adjusting for age, smoking, diabetes, and applicator type. Further analyses stratified intensity-weighted lifetime days (IWLD) of malathion use among applicators (for spouses, only ever/never pesticide use was available), and two categories (low- and high-use) were created based on median level among controls. ORs and 95% CIs were reported for each category with the non-exposed group (never use) as the referent, adjusting for diabetes, age, and smoking status (never, past, current). Among the study population (n = 82,596), there were 93 incident pancreatic cancer cases (64 applicators, 29 spouses), and of those cases with information on malathion use, 15 reported ever exposure to malathion and 41 reported no malathion exposure. Of the 82,503 pancreatic cancer-free controls, 19,357 reported ever exposure to malathion and 31,260 reported no malathion exposure. No evidence of a positive association was reported between malathion exposure and pancreatic cancer among pesticide applicators and spouses (OR = 0.40; 95% CI: 0.20, 0.90, with n = 15 exposed cases) based on ever/never use, with the data and significant odds ratio of less than 1 even suggesting that increased exposure is *protective* of pancreatic cancer risk.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the prospective design, and ability to identify cancer cases through linkage to cancer registries. Although the exposure assessment approach examined cumulative lifetime exposure to malathion which may be considered a study strength, the study included indirect exposure of spouses, making the reported results less reliable. We note the number of exposed cases was small (10 < exposed cases < 19).

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including pancreatic cancer among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina, who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD-O-3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires were used to collected information on demographics, reproductive history and other covariates that may be potential confounders. The association between malathion exposure and pancreatic cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 47 pancreatic cancer cases identified during the study period, 14 cases reported direct exposure to malathion. No evidence of a significant positive association was reported between malathion exposure and pancreatic risk (RR = 1.50; 95% CI: 0.69, 3.26; with n = 14 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and – similarly --no evidence of a significant positive association was reported for malathion exposure and pancreatic cancer (RR = 1.46; 95% CI: 0.62, 3.44; with n = 12 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment. We note the number of exposed cases was small (10 < exposed cases < 19).

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and pancreatic cancer. Two studies (Andreotti et al., 2009; Lerro et al., 2015) assessed the association between malathion exposure and pancreatic cancer among the AHS prospective cohort. Andreotti et al. (2009) reported no evidence of a positive association between malathion and pancreatic cancer among pesticide applicators based on ever use. The overall quality of the study was ranked moderate for regulatory purposes. Study strengths included the prospective design, and ability to identify cancer cases through linkage to cancer registries. Although the exposure assessment approach examined cumulative lifetime exposure to malathion which may be considered a study strength, the study assessed indirect exposure of spouses, making the reported results less reliable. Lerro et al. (2015) reported no evidence of a significant positive association between malathion exposure and pancreatic risk among female spouses of the pesticide applicators. Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a significant positive association was reported for malathion exposure and pancreatic cancer. We note the exposed number of cases for both analyses was small which limits the ability to interpret with confidence the observed risk ratios. The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework.

Prostate Cancer

Eight studies (Bonner et al., 2007; Band et al., 2011; Barry et al., 2011; Barry et al., 2012; Koutros et al., 2011; Koutros et al., 2013a; Koutros et al., 2013b; Christensen et al., 2016) – all except Band et al. (2011) from part of the AHS prospective cohort -- examined the relationship between malathion exposure and prostate cancer.

- The association between prostate cancer and specific pesticides including malathion was evaluated by Bonner et al. (2007). The study population consisted of pesticide applicators living in Iowa and North Carolina, enrolled in the AHS prospective cohort. Exposure was assessed through a self-administered enrollment questionnaire, and investigators used this questionnaire data to calculate lifetime exposure-days for pesticides including malathion. Exposure values were further modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days (IWLD) of exposure. Cases were identified using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. Incident cases were determined beginning at study enrollment (1993-1997) through December 31, 2002. Poisson regression was used to calculate RRs and 95% CIs for individual pesticide exposures, and was adjusted for age, family history of cancer, sex, state of residence, alcohol, smoking, year of enrollment, and education. Among the study population (n = 19,717), 12,290 reported exposure to malathion and 7,427 reported no exposure to malathion. Tertiles were constructed for each of the two exposure metrics used in this study (lifetime exposure-

days and intensity-weighted lifetime-days of exposure[73]), and two reference groups (the non-exposed and the low-malathion exposed applicators) were used. In the prostate cancer analysis for IWLD of exposure with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile (T1 – T3: 0.98 ≤ RRs ≤ 1.20; all CIs encompassed the null value of 1.0, with n = 88 – 94 exposed cases, with 135 cases in the non-exposed group)[74]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.98). A sensitivity analysis conducted to determine if selection bias was present indicated that minimal bias was present and the study authors concluded this bias had no effect on the reported risk estimates.

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure and intensity-weighted lifetime days of exposure, in addition to two referent groups.

- Band et al. (2011) evaluated the potential association between pesticide exposure including malathion, and prostate cancer among male pesticide applicators in a population-based case-control study in British Columbia, Canada. The study population included a subset of male cancer patients who previously enrolled in a case-control study. All participants were ascertained via the British Columbia Cancer Registry and all diagnoses were histologically confirmed. Prostate cancer cases were diagnosed between 1983 and 1985. Controls included men who were diagnosed with cancers other than prostate, lung, or unknown primary site from 1983 through 1990 and were age-matched to the cases. Exposure was assessed using self-reported questionnaires provided at study enrollment that were completed at home and returned within 6-weeks and a Job Exposure Matrix was used to estimate lifetime cumulative exposure level by aggregating exposure over all jobs. Next-of-kin served as proxy respondents for deceased subjects (18.4% of cases and 17.2% of controls). Conditional logistic regression was used to calculated ORs and 95% CIs for the association between malathion and prostate cancer, adjusting for smoking years, alcohol consumption, pipe years, education level, and proxy respondent. Among the 1,153 cases and 3,999 controls eligible for this analysis, 82 cases and 210 controls reported exposure to malathion. Evidence of a borderline positive association was reported between malathion and prostate cancer (OR = 1.34; 95% CI: 1.01, 1.78; with n = 82 exposed cases, p<0.05) based on ever/never use. In an exposure-response analysis, where low and high categories of lifetime exposure were created by dividing the exposed controls into two equal halves, evidence of a borderline positive association was reported between malathion exposure and prostate cancer in the high exposure category (OR = 1.49; 95% CI: 1.02, 2.18; with n = 46 exposed cases, p < 0.05) with the no exposure as the referent and no evidence of a significant positive association was reported for the low exposure category (OR = 1.18; 95% CI: 0.78, 1.78; with n = 36 exposed cases, p > 0.05). A significant exposure-response trend was reported (p = 0.03).

  The overall quality of the study was moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the ascertainment of cases and controls from cancer registries, histological confirmation of diagnoses, and reasoned selection of potential confounders and

[73] Tertiles for lifetime days included the following: >0-9 (T1), 10-39 (T2), >39 (T3); tertiles for IWLD included the following: >0-58 (T1), >59-245 (T2), >245 (T3).

[74] Risk estimates for intensity-weighted lifetime days of exposure for prostate cancer reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.81 ≤ RRs ≤ 1.20; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

covariates. Use of cancer patients in both case and control groups may have decreased differential recall bias but may have increased risk of selection bias. Additional limitations included the potential for recall-bias due to inaccurate recall by proxy respondents (18.4% of the cases, 17.2% of the controls).

- Barry et al. (2011) and Barry et al. (2012) investigated the association between pesticide exposures including malathion and prostate cancer, and genetic variation among Base Excision Repair (BER) and the nucleotide excision repair (NER) pathway genes using a nested case-control study within the AHS. The study population included white male pesticide applicators living in Iowa or North Carolina who were diagnosed with prostate cancer between enrollment (1993 – 1997) and 2004. Cases were ascertained through state cancer registries. Controls included white male applicators with no previous cancer history (except non-melanoma skin cancer), who were frequency-matched to cases (2:1) by birth date (±1 year). Pesticide exposure, including malathion, was assessed through two self-administered questionnaires at study enrollment and shortly thereafter (1993 - 1997), and exposure was classified into intensity-weighted lifetime days of use and categorized into non-exposed, low, and high exposure groups. Unconditional logistic regression was used to investigate the association between malathion and prostate cancer risk, adjusting for state and age and estimated associations between BER gene variant alleles and prostate cancer. We only report on findings between malathion exposure and risk of prostate cancer here as that is the main focus of this document. Among the total cases (n = 776) and controls (n = 1,444), 314 cases and 657 controls reported malathion exposure and 225 cases and 399 controls reported no malathion exposure, respectively. No evidence of a positive association was reported between malathion exposure and prostate cancer among white male pesticide applicators in either the low or high exposure categories, with the non-exposed group as the referent (*Low* – OR = 0.88; 95% CI: 0.69, 1.13; with n = 162 exposed cases and n = 329 exposed controls; *High* – OR = 0.80; 95% CI: 0.62, 1.04; with n = 152 cases and n = 328 controls; p-trend = 0.13).

  The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure to malathion.

- Koutros et al. (2011) evaluated the association between specific pesticides, including malathion, and prostate cancer among male licensed pesticide applicators in a nested case-control analysis within the AHS prospective cohort. The study population (n=2,500) included male pesticide applicators living in Iowa and North Carolina enrolled in the AHS prospective cohort. Incident cases were determined beginning at study enrollment (1993-1997) through 2004 using cancer registry files from Iowa and North Carolina, and vital status was confirmed through the state death registries and the National Death Index. At follow-up, men were also asked to submit a DNA sample from buccal cells. Controls (n=1,444) included pesticide applicators (males only) who had not been previously diagnosed with prostate cancer, were not deceased at the time of follow-up, and had provided a DNA sample of buccal cells. The controls were frequency-matched to the cases (2:1) via birthdate (+/- 1 year). Exposure was assessed using data from two self-administered questionnaires completed at enrollment to determine malathion usage, and to further classify malathion usage by lifetime exposure days. Among the 776 cases and 1,444 controls, 315 cases and 661 controls reported malathion exposure. Lifetime exposure days were categorized as non-exposed, low, or high exposure, based on the median cut-point determined from the distribution of lifetime exposure days of both the controls and cases. Unconditional logistic regression was used to calculate ORs and 95% CIs for the association between malathion exposures and prostate cancer, adjusted for state, age, and family history of prostate cancer. No evidence of a positive association was reported between malathion exposure and

prostate cancer in either the low or high exposure categories (*Low* – OR=0.85; 95% CI: 0.67, 1.09; with n=173 exposed cases and n=351 exposed controls; *High* – OR=0.80; 95% CI: 0.62, 1.04; with n=142 exposed cases and n=360 exposed controls; p-trend=0.149).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure to malathion.

- Koutros et al. (2013a) investigated the potential association between prostate cancer and specific pesticides including malathion, adding cases through 2007.[75] The study population (n = 54,412) included male pesticide applicators participating in the AHS. Pesticide exposure was assessed by information obtained via self-administered questionnaires, and this information was used to calculate lifetime pesticide usage for 50 pesticides. Exposure values were modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days of exposure metric. Incident prostate cancer cases were identified through cancer registry files in Iowa and North Carolina, and cases diagnosed between study enrollment (1993-1997) until December 31, 2007 were included in this analysis. Incident cancer cases were then subdivided into prostate cancer or aggressive prostate cancer based on the Gleason score tumor ranking scale provided by a medical pathologist.[76,77] A Poisson regression was used to calculate RRs, adjusting for state, age, race, family history of prostate cancer, smoking, fruit servings, and leisure-time physical activity in the winter. Four quartiles were constructed (n = 184-189 cases/quartile) for prostate cancer and (n = 93-95 cases/quartile) for aggressive prostate cancer based on exposure, and RRs were reported for each quartile. Evidence of a positive association was reported for Q4 of the aggressive prostate cancer exposure category (RR for Q4 vs. nonexposed = 1.43, 95% CI: 1.08, 1.88; n=93). No evidence of a significant positive association was observed for prostate cancer or aggressive prostate cancer in any other quartile ($1.03 \leq RRs \leq 1.28$; all CIs encompassed the null value of 1.00). Evidence of a linear (monotonic) trend across all categories was observed for aggressive prostate cancer (p-trend = 0.04), but no evidence of a linear (monotonic) trend across categories was observed for total prostate cancer (p-trend = 0.62).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure to malathion. Study limitations included missing data among the cases (~30% of the cases), the fact that the Gleason scores used in the study were not standardized prior by the centralized pathologic review, and the potential for exposure misclassification.

- Koutros et al. (2013b) investigated SNP-environment interactions between confirmed prostate cancer susceptibility loci and various pesticides, including malathion, and prostate cancer risk among participants in the prospective AHS cohort. The study population consisted of male licensed pesticide

[75] Koutros et al. (2013a) is a follow-up to the following study: Alavanja, M. C., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C. F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Sandler, D.P., Blair, A., & Coble, J. (2003). Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *American journal of epidemiology*, *157*(9), 800-814.

[76] Johnson CH, ed. SEER Program Coding and Staging Manual 2004, Revision 1. Bethesda, MD: National Cancer Institute, 2004. (NIH publication no. 04-5581).

[77] Aggresive prostate cancer included cases with tumor(s) defined as one of the following: distant stage or poorly differentiated (Gleason score 7-10), deadly prostate cancer, or Gleason ≥ 7.

applicators (N=55,747) in Iowa and North Carolina, who completed the enrollment questionnaire (between 1993-1997). The study used newly genotyped data in 32 prostate genomic-wide association studies that have identified single-nucleotide polymorphisms (GWAS SNPs) to continue to explore possible SNP-pesticide interactions and risk of prostate cancer in AHS subjects included in a nested case-control study. Genotyping was performed using Applied Biosystems TaqManH SNP Genotyping Assays. The association SNPs and prostate cancer and the interaction between SNPs and malathion use with prostate cancer risk were estimated using unconditional logistic regression to determine odd ratios (ORs) and 95% confidence intervals (CIs) adjusting for age and state. Among the 55,747 male pesticide applicators who completed the enrollment questionnaires, 2,220 were included in the final analysis. Among the *EHBP1* SNP region and the TT genotype 52 prostate cancer cases were identified that reported direct exposure to malathion and among the *rs2710647* SNP region for the CT+CC genotype 190 prostate cancer cases were identified with direct exposure to malathion. The authors reported a significant interaction between SNP regions and malathion exposure (i.e., the effect of malathion on prostate cancer were significantly different between the genotypes, p-interaction = 0.003). Thus, high and low exposures were investigated separately: for subjects with *EHBP1* SNP region (i.e., TT genotype), evidence of a strong association was reported between the high exposure category for malathion and prostate cancer (OR: 3.43; 95% CI: 1.44, 8.15, with n=28 exposed cases), and no evidence of a significant positive association was observed at the low exposure category for malathion and prostate cancer (OR: 2.17; 95% CI: 0.91, 5.14 with n = 24 exposed cases). Note that the lower bound 0.91 of the estimated OR = 2.17 of the low exposure category was relatively close to the reference value of 1 in the determination of the statistical significance of an estimate. For the subjects with *rs2710647* SNP region (i.e., CT+CC genotype), no evidence of a positive association was reported for all exposure categories for malathion and prostate cancer (0.80 ≤OR≤ 0.96; all 95% CI encompass the null value of 1.0, with n=91-99 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate use of statistical methods (e.g., genotyping and quality control). Additional details regarding the duration of time for pesticide usage (e.g., days, months, years) were not provided but would have been helpful.

- Christensen et al. (2016) evaluated the potential association between pesticide exposures, including malathion and prostate cancer and modifications of risk by single-nucleotide polymorphisms on sex hormones using a nested case-control study within the AHS prospective cohort. The study population included white male pesticide applicators living in Iowa or North Carolina. Cases included white male AHS study participants who were cancer-free at enrollment, had physician-diagnosed prostate cancer between enrollment (1993 – 1997) and 2004, and provided a buccal cell sample later used for DNA testing. Cases (including tumor characteristics such as stage and Gleason score for aggressive prostate cancer) were ascertained through state cancer registries. Controls included white male applicators and were frequency-matched to the cases (2:1) by birth date (±1 year). Pesticide exposure was assessed through two self-administered questionnaires at study enrollment and again shortly thereafter, and exposure was classified via intensity-weighted lifetime exposure days and categorized into non-exposed, low, and high exposure groups using the median as the cut point. Unconditional logistic regression was used to investigate the association between malathion and prostate cancer, adjusting for state, age, and race. Among the total cases (n = 776) and controls (n = 1,444), 173 cases and 351 controls reported low malathion exposure, 142 cases and 310 controls reported high malathion exposure, and 225 cases and 399 controls respectively, reported no malathion exposure. No evidence of a positive association was reported between malathion exposure and prostate cancer among white male pesticide applicators in either the low or high exposure categories, with the non-

exposed group as the referent (Low – OR = 0.85; 95% CI: 0.67, 1.09, with n = 173 exposed cases; High – OR = 0.80; 95% CI: 0.62, 1.04, with n = 142 exposed cases; p-trend = 0.149).

The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and exposure assessment approach which examined cumulative lifetime exposure to pesticides.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and prostate cancer. Eight studies (Bonner et al., 2007; Band et al., 2011; Barry et al., 2011; Barry et al., 2012; Koutros et al., 2011; Koutros et al., 2013a; Koutros et al., 2013b; Christensen et al., 2016) examined the relationship between malathion exposure and prostate cancer in two study populations including pesticide applicators in the AHS, and among male pesticide applicators in a population-based case-control study in British Columbia, Canada. Seven of these publications examined this association among the AHS prospective cohort population. Bonner et al. (2007) first examined the association between malathion and prostate cancer from study enrollment in 1993-1997 through 2002. This study reported no evidence of a significant positive association based on lifetime days and intensity-weighted lifetime days of use. Follow-up studies of the AHS cohort further evaluated the relationship between malathion and considered additional factors, including potential genetic risk factors (Barry et al., 2011; Barry et al., 2012; Christensen et al., 2016; Koutros et al., 2013b), family history of prostate cancer (Koutros et al., 2011), and prostate cancer type (Koutros et al., 2013a). These follow-up studies reported the same general findings as first reported by Bonner et al. (2007). Specifically, these studies reported no evidence of a significant positive association between malathion exposure and prostate cancer overall within the AHS cohort. The study quality for these AHS studies was either high or moderate for regulatory purposes and all benefited from the general strengths of the AHS including the prospective study design and linkage to cancer registries to ascertain cases. Study limitations were noted, namely potential for exposure misclassification and missing data among cases (~30% of the cases). In a sub-analysis of aggressive prostate cancer, Koutros et al. (2013a) reported evidence of a positive association for malathion exposure and aggressive prostate cancer at the highest exposure quartile, along with evidence of a linear (monotonic) trend across quartiles for aggressive prostate cancer. Similarly, Koutros et al. (2013b) investigated SNP-environment interactions between confirmed prostate cancer susceptibility loci and various pesticides, including malathion. Based on this sub-analysis, the authors reported evidence of a strong association between the high exposure category for malathion and prostate cancer. In contrast, Christensen et al. (2016) examined separate genetic risk factors and reported no evidence of a positive association in either low or high malathion exposure categories. Both Koutros studies (Koutros et al., 2013a, 2013b) were ranked moderate for regulatory purposes, and study limitations included missing data among the cases (~30% of the cases) and the Gleason scores used in the study were not standardized prior by the centralized pathologic review. Christensen et al. (2016) was ranked high quality for regulatory purposes.

Outside of the AHS cohort, Band et al. (2011), evaluated the association between malathion and prostate cancer in a population-based case-control study among farm workers in British Columbia, Canada. Evidence of a borderline positive association was observed between prostate cancer and malathion in the high exposure category of the exposure-response analysis, along with a significant exposure-response trend. No evidence of a significant positive association was reported in the low exposure category for malathion, and no evidence of a significant positive association was reported between malathion and prostate cancer based on ever/never use. The study was moderate quality for regulatory purposes and limitations included selection bias, and recall bias due to proxy respondents inaccurate recall of exposure.

While some studies reported positive findings, the overall evidence was considered insufficient because the body of evidence was limited to two study populations with mixed results. In particular, several studies of the AHS cohort reported no evidence of a significant positive association, whereas the remaining case-control study by Band et al. (2011) reported evidence of a borderline positive association. A further sub-analysis of prostate cancer type and genetic factors in the AHS cohort also provided evidence an association between malathion exposure and aggressive prostate cancer and SNP regions, respectively. However, these sub-analyses were exploratory and require further replication in other study populations.

Soft Tissue Sarcoma

One study evaluated the association between malathion and soft tissue sarcoma (Pahwa et al., 2011)

Pahwa et al. (2011) investigated the potential association between malathion exposure and soft tissue sarcoma (STS) among men in the Cross-Canada Study of Pesticide and Health (CCSPH), a matched population-based case-control study. The study population included males ≥19 years old who lived in one of six Canadian provinces and completed a postal questionnaire. Deceased participants were excluded from this analysis. Cases of STS included those adult males diagnosed between September 1991 to December 1994 and were ascertained via provincial cancer registries or hospital ascertainment (Quebec only). Cases were validated by a pathologist who reviewed pathology slides. Controls were randomly selected males from either health insurance records, telephone directories (Ontario) or voters lists (British Columbia), who resided in the same Canadian provinces as cases and were matched to cases via age (± 2 years). A postal questionnaire was mailed to cases and controls to assess pesticide exposure, and follow-up telephone interviews regarding detailed pesticide use were conducted for each subject who reported more than 10 hours per year of pesticide use. The response rates for cases and controls were 67.1% and 48.0%, respectively.[78] Among the total STS cases (n=357) and population controls (n=1,506), 38 (10.6%) cases and 127 (8.4%) controls reported exposure to malathion. Conditional logistic regression was used to calculate ORs and 95% CIs for the association between individual pesticide exposures including malathion and STS, matching on age and province of residence (~4-5 controls/case). Models were additionally adjusted for relevant medical history variables[79] including family history of cancer (1st degree relative). No evidence of a significant positive association was reported between malathion ever use and STS among men in the CCSPH (OR=1.19; 95% CI: 0.80, 1.78; with n=38 exposed cases). When further adjusted for statistically significant medical history variables, no evidence was similarly reported of a significant positive association between malathion ever use and STS among men in the CCSPH (OR=1.23; 95% CI: 0.81, 1.85; with n=38 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included age- and province- matching of the cases to the controls, case ascertainment, and exposure assessment. A limitation of the study was the response rate to the mailed questionnaires. Only 67.1% of the contacted cases and 48% of the contacted controls responded to the questionnaire and were included in the analysis. There may have been differences between those who responded and those who did not respond. Potential recall bias was also considered a limitation, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls.

[78] McDuffie, H. H., Pahwa, P., Robson, D., Dosman, J. A., Fincham, S., Spinelli, J. J., & McLaughlin, J. R. (2005). Insect repellents, phenoxyherbicide exposure, and non-Hodgkin's lymphoma. J Occup Environ Med, 47(8), 806-816. https://doi.org/10.1097/01.jom.0000167260.80687.78

[79] Medical history variables included: mononucleosis, whooping cough, history of measles, rheumatoid arthritis, and a positive family history of cancer in a first-degree relative.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and STS. This determination is based on a very limited body of evidence that consisted of one case-control study (Pahwa et al., 2011) that investigated the potential association between exposure to malathion and soft tissue sarcoma (STS) among participants of the CCPHS. No evidence of significant positive associations were reported between malathion exposure and STS. The study was moderate quality for regulatory purposes based on the study quality criteria provided in the OPP Framework. Study strengths included age matched cases to the controls, case ascertainment, and exposure assessment. A limitation of the study was the response rate to the mailed questionnaires. Only 67.1% of the contacted cases and 48% of the contacted controls responded to the questionnaire and were included in the analysis. There may have been differences between those who responded and those who did not respond. Potential recall bias was also considered a limitation, as the cases living with the outcome may have remembered certain past exposures more accurately than the controls.

Thyroid Cancer

Two studies (Lerro et al., 2015; Lerro et al., 2021) examined the association between malathion exposure and thyroid cancer.

- Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints, including thyroid cancer, among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina, who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n= 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD-O-3 codes from date of enrollment through whichever event was earlier: date of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever-exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history, and other covariates that may be potential confounders. The association between malathion exposure and thyroid cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (exclusions were: 220 males, 907 women with a cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure), and 5,704 reported lifetime ever use of malathion. Among the 91 thyroid cancer cases identified during the study period, 22 cases reported direct exposure to malathion. Evidence of a moderately strong positive association was reported between malathion exposure and thyroid cancer risk (RR = 2.04; 95% CI: 1.14, 3.63; with n = 22 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and evidence of a moderately strong positive association was reported for malathion exposure and thyroid cancer (RR = 2.22; 95% CI 1.18, 4.17; with n = 19 exposed cases).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the

prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

- Lerro et al. (2021) investigated the association between exposure to malathion and other pesticides and thyroid cancer using data from the AHS prospective cohort. The study population (n=53,096) consisted of male pesticide applicators living in Iowa and North Carolina who were enrolled in the AHS cohort and who did not have a history of cancer at enrollment (1,096 participants reporting cancer at enrollment, 341 not living in either Iowa or North Carolina, and 1,531 female pesticide applicators were excluded). Incident cancer cases were identified using Iowa and North Carolina state cancer registry files from enrollment (1993-1997) through December 2014 in North Carolina and December 2015 in Iowa. International Classification of Diseases for Oncology ICD – O – 3 codes were used to classify cancer sites. Malathion exposure was assessed through the enrollment questionnaire (1993 – 1997) and the first follow-up interview five years after enrollment (1999 – 2005). Among the 85 thyroid cancer cases, 59 (69.4%) reported ever malathion exposure at study enrollment, and of these, 46 were cases of papillary thyroid cancer. Multiple imputation was used to estimate pesticide exposures after enrollment for individuals who did not complete the interview (37%, n = 20,986). Cox proportional hazard regression models were used to estimate HRs and 95% CIs for ever use at enrollment and intensity-weighted days of use of malathion, and other pesticides, compared with no use, adjusting for state, applicator type (commercial, private), cigarette smoking history at enrollment (never, former, current smoker, missing), body mass index (<25, 25–30, >30 kg/m2, missing), and correlated pesticides (Spearman $\rho > 0.4$; specifically imazethapyr for malathion analyses). Authors excluded applicators missing information for a pesticide of interest from the analysis of that pesticide. No evidence of a positive association was reported for malathion ever use at enrollment and either thyroid cancer (HR= 1.07; 95% CI: 0.66, 1.74; with n=59 exposed cases) or the subset papillary thyroid cancer (HR=1.09; 95% CI: 0.63, 1.90; with n = 46 exposed cases). For the analysis of cumulative exposure, categories of intensity-weighted days of exposure were divided into quartiles for each pesticide with 20+ exposed cases or into two groups divided at the median for pesticides with 10 -19 exposed cases. Specifically for malathion, four quartiles of exposure were created. No evidence of a significant positive association was reported for any exposure category of intensity-weighted lifetime days of malathion use and thyroid cancer ($0.43 < HR < 1.28$; all 95% CIs encompassed the null value 1.0; with n = 7-8 exposed cases per category; p-trend=0.12).

  The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The general strengths of the publication included the underlying prospective design of the AHS, the exposure assessment, and the availability of a U.S. cancer registry to comprehensively identify cancer cases.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and thyroid cancer. Two studies (Lerro et al., 2015; Lerro et al., 2021) examined the association between malathion exposure and thyroid cancer. Lerro et al. (2015) reported evidence of a moderately strong positive association between malathion exposure and thyroid risk among spouses of farm applicators. Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and evidence of a moderately strong positive association was reported for malathion exposure and thyroid cancer. The overall quality of study was ranked moderate. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate statistical methods used. Additional details regarding the

duration of time for pesticide usage (e.g., days, months, years) was not provided but would have been helpful. Lerro et al. (2021) reported no evidence of a significant positive association between malathion intensity-weighted lifetime days of malathion use and thyroid cancer or the subset papillary thyroid cancer among pesticide applicators in the AHS prospective cohort. The study was deemed high quality for regulatory purposes.

Uterine Cancer

One study (Lerro et al., 2015) examined the association between malathion exposure and uterine cancer.

Lerro et al. (2015) investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints including uterine cancer among participants in the prospective AHS cohort. The study population consisted of female spouses of pesticide applicators enrolled in the AHS between 1993 and 1997, living in Iowa and North Carolina and who completed the enrollment questionnaire (N = 32,345) and had complete data for the analysis (n = 30,003). Incident cases were identified through linkage with Iowa and North Carolina state cancer registries and cancer site classified according to ICD-O-3 codes from date of enrollment through whichever event was earlier - data of death, movement out of state, or December 31, 2011 for Iowa residents or December 31, 2010 for North Carolina residents. Malathion lifetime ever exposure (direct exposure) was assessed using data obtained from self-administered questionnaires completed by spouses at enrollment. Questionnaires also collected demographic information, reproductive history, and other covariates that may be potential confounders. The association between malathion exposure and uterine cancer was estimated using Poisson regression analysis to determine RRs and 95% CIs, adjusting for age, state of residence, smoking (pack-years smoked), race, alcohol use, educational attainment, body mass index (BMI), family cancer history, lawn/garden pesticide application and correlated/associated pesticide use. Models for cancers of the ovaries, breast, uterus, and all sites combined (all cancers) were additionally adjusted for number of live births, menopause status at enrollment, and oral contraceptive use. Among the 32,345 spouses of private applicators who completed the enrollment questionnaires, 30,003 female spouses were included in the final analysis (220 males were excluded, 907 women with cancer diagnosis prior to enrollment, 110 missing follow-up data, and 1,105 who were missing information on pesticide exposure all were excluded) and 5,704 reported lifetime ever use of malathion. Of the 231 uterine cancer cases identified during the study period, 58 cases reported direct exposure to malathion. No evidence of a significant positive association was reported between malathion exposure and uterine cancer risk among AHS female spouses (RR = 1.28; 95% CI: 0.90, 1.83). When uterine cancer was stratified by menopausal status at enrollment, no evidence of a significant positive association was similarly reported between malathion exposure and uterine cancer for pre-menopausal participants (*Premenopausal* - RR = 1.47; 95% CI: 0.86, 2.49 with n = 27 exposed cases) and no evidence of a positive association was reported between malathion exposure and uterine cancer for postmenopausal participants (*Postmenopausal* - RR = 0.98; 95% CI: 0.58, 1.67 with n = 26 exposed cases). In an additional sensitivity analyses that investigated associations between ever/never malathion exposure and uterine cancer diagnosed five or more years after study enrollment among AHS spouses, no evidence of a significant positive association was reported between malathion ever exposure and uterine cancer (RR = 1.09; 95% CI: 0.71, 1.67 with n = 37 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. This determination was based on the general strengths of the AHS, including the prospective design and use of cancer registries to identify cancer cases. The exposure assessment was limited to ever/never exposure and additional details regarding the duration of time for pesticide usage (e.g., days, months, years) would have strengthened the exposure assessment.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and uterine cancer. One study (Lerro et al., 2015) examined the association between malathion exposure and uterine cancer among AHS spouses, and reported no evidence of a significant positive association. The overall quality of the study was ranked moderate for regulatory purposes based on the study quality criteria provided in the OPP Framework. The investigators assessed indirect exposure based on self-reported pesticide use from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses.

3.6.2 Noncarcinogenic Health Outcomes

For noncarcinogenic health outcomes, EPA conducted a review of 67 publications which investigated the relationship between malathion exposure and non-carcinogenic adverse health effects including: autism spectrum disorder; amyotrophic lateral sclerosis; autoimmune disease (antinuclear antibodies); rheumatoid arthritis; birth defects; birth effects; cerebral palsy; depression; diabetes; dream enacting behaviors; end stage renal disease; endometriosis; eye disorders; fatal injury; gestational hypertension; hearing loss; kidney function; monoclonal gammopathy of undetermined significance; myocardial infarction; nervous system function (children and adults); neurodevelopmental/neurobehavorial effects in children; olfactory impairment, Parkinson's disease; respiratory effects (including asthma, chronic bronchitis, rhinitis, wheeze); recurrent pregnancy loss; sleep apnea; stroke; suicide; testosterone level changes; thyroid disease (including hyperthyroid, hypothyroid, and other thyroid disease); and weight gain in adults. The 67 studies for these health outcomes are described below.

Autism Spectrum Disorder

Two publications (Sagiv et al., 2018; von Ehrenstein et al., 2019) examined the relationship between malathion exposure and Autism Spectrum Disorder (ASD) among the CHAMACOS cohort in Salinas Valley, California and residents of the Central Valley, California.

- Sagiv et al. (2018) conducted a prospective cohort study to investigate the associations between prenatal residential proximity to agricultural use of organophosphate pesticides, including malathion, and ASD in children enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort. The study population (n = 235-354 depending on ASD outcome test) consisted of children born to mothers recruited and enrolled between October 1999 and October 2000 from health clinics serving low-income residents of California's Salinas Valley. Inclusion criteria for mothers included the following: ≥ 18 years of age, < 20 weeks of gestation, were eligible for low-income health insurance, spoke English or Spanish, and were planning to deliver at the public hospital. Of the 601 mothers enrolled, 536 live-born infants (including twins) were delivered, and 235-354 were included in the final analysis depending on ASD outcome test. Children were included in the final analysis of prenatal residential proximity to malathion only if their prenatal residential location was known during pregnancy for at least 75 days of each trimester. Women were interviewed during pregnancy and at several time points until their children were 14 years old. Children and mothers were administered several instruments to assess parent and teacher reports of ASD related behaviors. Social Responsiveness Scale, Version 2 (SRS-2) at age 14 y (parent), Behavioral Assessment Scale for Children, Version 2 (BASC-2) at 7, 10½, and 14 y, (parent, teacher at 7y). Study staff tested children's ability to recognize mental state of others through facial expressions using the Evaluación Neuropsicológica Infantil (ENI) Facial Expression Recognition Test at 9y, and the NEPSY-II Affect Recognition subtest at age 12y. Tests were performed by bilingual

psychometricians. Pesticide exposure was determined using global positioning system (GPS), GIS, and Department of Pesticide Regulation Pesticide Use Reports (PUR) data to estimate potential exposure from pesticide use within 1 km of maternal residences during pregnancy. The amount of malathion 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. Average pesticide use during the entire pregnancy was determined by summing the trimester-specific estimates of malathion applied and dividing by the number of trimesters included in the assessment for each residence. Children whose residential address was known for ≥75 days per trimester during at least two trimesters were included in the analysis. Authors stated that buffer distance of 1 km was selected because it was the distance that most strongly correlated with measured agricultural pesticide concentrations in house-dust samples.[80] Malathion use among participants with a 14-year SRS-2 score (n=236) was 4.1 kg (geometric mean, 95% CI: 3.4-5.1) within a 1-km radius of maternal residence. Linear regression was used to assess the association between log_{10}-tranformed prenatal malathion exposure within 1 km of maternal residence and ASD. Covariate inclusion in the multivariable analysis was determined using a directed acyclic graph (DAG) with all models adjusted for maternal education at delivery (<6th grade, 7-12th grade), maternal age at delivery (categorical: age 18 to 24, 25 to 29, 30 to 34, 35 to 45 y), marital status at delivery (not married to/living with child's father, married to/living with child's father), years in the US at delivery (≤1, 2–5, 6–10, and 11+years), depression (Center for Epidemiologic Studies Depression Scale (CES-D)≥ 16, CES-D<16) at the 9-year mark, parity (nulliparous, 1+), country of birth (United States, Mexico, or other), and quality of the home environment at the 10 ½-year visit using the Home Observation for the Measurement of the Environment-Short Form (HOME-SF). Models additionally included child's age at assessment (continuous), sex, and language in which questionnaire administered to the mother (Spanish, English). Results were presented as change in outcome score for a 10-fold increase in prenatal OP pesticide use, along with corresponding 95% CIs and p-values. No evidence of a significant association was reported between a 10-fold increase in prenatal malathion exposure use within 1-km of residence during pregnancy and the 14 years of age SRS-2 Total T-score (β = 0.50; 95% CI: –0.70, 1.80; with n = 235), the SRS-2 DSM-V compatible Social Communication and Interaction (SCI) T-score (β = 0.40; 95% CI: -0.90, 1.60; with n = 235), and the SRS-2 DSM-V compatible Restricted and Repetitive Behaviors (RRB) T-score (β = 0.90; 95% CI: –0.30, 2.10; with n = 235). For reference, the T-score standardized mean=50 (SD= 10) and *higher* SRS score indicates more ASD-related traits. Similar results were reported in a sensitivity analysis using 10-fold increase of malathion use within 3-km of residence during pregnancy. No evidence of a significant association was reported between a 10-fold increase in prenatal malathion exposure use within 1 km of residence during pregnancy and BASC-2 Social Skills T-score Teacher report at 7-y (β = -1.70; 95% CI: –5.40, 2.00; with n = 270), BASC-2 Social Skills T-sore- Parent Report at 7-y, 10 ½, and 14 y[81] (β = -0.70; 95% CI: –1.90, 0.50; with n = 354), and Affect Recognition ENI at 9-y (β = -0.10; 95% CI: –0.20, 0.10; with n = 310), and Affect Recognition NEPSY-II at 12-y (β = 0.10; 95% CI: -0.50, 0.60; with n = 307). For reference, *lower* scores are consistent with more ASD-related traits and BASC-2 T-score standardized mean=50 (SD= 10); ENI mean=6.6 (SD=1.2);NEPSY-II mean=26.6 (SD=3.6).

The overall quality of the study was ranked low. Strengths of the study included the cohort study design, and the assessments used to determine ASD in children. Limitations include the use of

[80] This determination was made based from Harnly ME, Bradman A, Nishioka M, McKone TE, Smith D, McLaughlin R, et al. 2009. Pesticides in dust from homes in an agricultural area. Environ Sci Technol 43(23):8767–8774, PMID: 19943644, https://doi.org/10.1021/es9020958 and Gunier RB, Ward MH, Airola M, Bell EM, Colt J, Nishioka M, et al. 2011. Determinants of agricultural pesticide concentrations in carpet dust. Environ Health Perspect 119(7):970–976, https://doi.org/10.1289/ehp.1002532.

[81] Repeated measures analysis (Generalized Estimating Equations) of parent report at age 7, 10½, and 14 years.

California PUR data to measure potential pesticide use, including malathion, 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 coefficients between malathion use and other organophosphates were 0.26-0.61. As such, the approach may lack the specificity to assess malathion 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. Specifically, 601 mothers enrolled in the study, but only 537 were followed to delivery, and only 353 were followed through to age 7, 337 through age 9 year and 333 through 14 years and had information on malathion exposure. Additionally, test scores were available from 235-354 individuals, depending on the test. 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.

- von Ehrenstein et al. (2019) examined the association between prenatal and infant exposure to ambient pesticides, including malathion, and ASD among children in a population-based case-control study in CA. The authors used 1998-2010 birth data from the Office of Vital Statistics to create a statewide sample population (n=33,921 cases, n=339,210 controls) and matched records from the California Department of Developmental Services via probabilistic linkage based on parent and child identifiers to select cases diagnosed with ASD (Diagnostic and Statistical Manual of Mental Disorders, fourth edition, revised) up to 31 December 2013. Controls were matched to cases by birth year and sex at 10 controls to one case (10:1). Participants from the statewide sample population (n=33,921 cases, n=339,210 controls) were excluded due to missing or implausible information on gestational age or birth weights (n=3,401 cases, n = 42,519 controls, non-singleton births, and controls who died before age 6 (n=1,296). Additionally, the sample was further limited to participants who lived in eight agricultural counties[82] at birth and time of diagnosis (38,331 participants, n=2,961 cases, n = 35,370 controls). Pesticide exposure was assessed using CA DPR PUR data, GIS, and GPS geolocated residential addresses on the birth certificate to determine pounds of pesticide applied per acre per month in a 2,000 m radius buffer around the residential birth address reported on the participant's birth certificate. Authors also tested a 2,500 m buffer in a sensitivity analysis. Developmental period specific averages were calculated for the three months before gestation, each month during gestation, and the first year of life. Control periods were truncated to match length of periods for cases. Among the final study population (38,311), 642 (22%) of the 2,961 cases and 7,277 (21%) of the 35,370 controls were exposed to malathion. Sociodemographic and pregnancy information were obtained from birth records. Unconditional logistic regression was used to assess the association between proximity of residential address to malathion and ASD, adjusted for sex, birth year, nitrogen oxides (traffic related air pollution), maternal age, maternal education, and maternal race/ethnicity. Confounders were selected based on previous knowledge and were identified from birth records and the CA Line Source (CALINE4) emissions records. In the analysis between malathion ever exposure at 2,000 m buffer around the residential address at birth and all ASD cases, adjusted simultaneously for all three exposure timepoints, *3 months before pregnancy*, *pregnancy*, and *first year of life*, no evidence of a significant positive association was reported between malathion exposure during *pregnancy* or the *first year of life* relative to ASD (*Pregnancy*- OR=1.08; 95% CI:

[82] Sample restricted to eight major agricultural counties in California - San Joaquin, Stanislaus, Merced, Madera, Fresno, Kings, Tulare, and Kern.

0.97, 1.20; *First year of life*-OR=1.09; 95%CI: 0.98, 1.20). No evidence of a positive association was reported for malathion exposure at *3 months before pregnancy* and ASD (*3 months before pregnancy* – OR=0.94; 95% CI: 0.82,1.08). In the same analysis where cases were limited to those with ASD and the comorbidity of intellectual disability, adjusted simultaneously for all three exposure timepoints, no evidence of a significant positive association was reported between malathion ever exposure during *pregnancy* or the *first year of life* (*Pregnancy*- OR=1.05; 95% CI: 0.81, 1.37; *first year of life*- OR=1.29; 95% CI: 1.00, 1.65). No evidence of a positive association was reported for malathion ever exposure *3 months before pregnancy* and ASD with intellectual disability comorbidity (OR=0.72; 95% CI: 0.50, 1.03).

In an additional analysis, where malathion exposure at the three exposure timepoints were considered separately among all ASD cases, no evidence of a significant positive association was reported between ever malathion exposure and ASD for the first timepoint (*3 months before pregnancy* – OR=1.01; 95% CI: 0.89, 1.15) and evidence of a borderline slight positive association was observed at the two additional time points (*Pregnancy*-OR=1.11; 95% CI: 1.01, 1.22; with n=642 exposed cases, 7,277 exposed controls; *First year of life*- OR=1.11; 95% CI: 1.01, 1.21; with n = 784 exposed cases, 8,911 exposed controls). When further adjusted for all other pesticides considered in the model, no evidence of a significant positive association was reported between malathion ever exposure at any exposure timepoint and ASD (*3 months before pregnancy* – OR=0.97 95% CI: 0.84, 1.11; *Pregnancy*-OR=1.05; 95% CI: 0.95, 1.16; with n=642 exposed cases, 7,277 exposed controls; *First year of life*-OR=1.07; 95% CI: 0.97, 1.18; with n = 784 exposed cases, 8,911 exposed controls).

In an additional analysis, where malathion exposure during the three exposure timepoints were considered separately among ASD cases with intellectual disability comorbidity, no evidence of a significant positive association was reported between ever malathion exposure and ASD for the *3 months before pregnancy, Pregnancy* and *First year of life* timepoints (*3 months before pregnancy*-OR=0.83; 95% CI: 0.59, 1.17; *Pregnancy*-OR=1.12; 95% CI: 0.89, 1.41; with n=99 exposed cases, 7,277 exposed controls; *First year of life*- OR=1.23; 95% CI: 0.99, 1.52; with n = 125 exposed cases, 8,911 exposed controls). When further adjusted for all other pesticides considered in the model, no evidence of a significant positive association was reported between malathion ever exposure during *3 months before pregnancy, Pregnancy* or the *First year of life* timepoints and ASD (*3 months before pregnancy*- OR=0.73; 95% CI: 0.51, 1.05; *Pregnancy*-OR=0.94; 95% CI: 0.74, 1.21; with n = 99 exposed cases, 7,277 exposed controls; *First year of life*- OR=1.02; 95% CI: 0.80, 1.29; with n = 125 exposed cases, 8,911 exposed controls).

The overall quality of the study was ranked low. Strengths of the study included the use of the California Department of Developmental Services records to determine ASD and intellectual disability in children and case to control matching via Office of Vital Statistics birth records based on parent and child identifiers, random selection of cases from birth records as well. The registry-based design limited recall bias and participation bias, common limitations of case-control studies that rely on self-reported past exposures and self-selection, respectively. Limitations included the use of California PUR data to measure potential pesticide use, including malathion, within a 2000 m proximity to the residential address listed on the birth certificate. 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 pesticides evaluated, during the pregnancy time period. Specifically, the correlation coefficients between malathion use and other organophosphates were 0.21-0.65. As such, the approach may lack the specificity to assess malathion and be unable to distinguish between factors associated with geographic proximity to agricultural land and pesticide use more generally. And finally, the study relied on the residential address found on the birth

certificate. Residential address on the birth certificate may not be the same address during the 3 months before pregnancy, during the pregnancy, or the first year of life exposure periods.[83]

EPA Conclusion

Overall, there *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and Autism Spectrum Disorder (ASD) among children. This determination was based on two studies (Sagiv et al., 2018; von Ehrenstein et al., 2019) that examined the relationship between malathion exposure and ASD among the CHAMACOS cohort in Salinas Valley, California and residents of the Central Valley, California. Sagiv et al. (2018) reported no evidence of a significant association between a 10-fold increase in prenatal malathion exposure use within 1-km of residence during pregnancy and the several outcomes testing ASD in children. In a second study, von Ehrenstein et al. (2019), reported either no evidence of a significant positive association or no evidence of a positive association between malathion ever exposure and ASD *3 months before pregnancy*, *pregnancy*, and during the *first year of life* in an analysis that estimated at 2-km buffer around the maternal residence during pregnancy. Both studies were ranked low for regulatory purposes. Both used geospatial methods and CA PUR data to estimate malathion exposure based on proximity of prenatal residence to malathion agricultural use. The approach may lack the specificity to assess malathion and be unable to distinguish between factors associated with geographic proximity to agricultural land and pesticide use more generally. And finally, the publications relied on the residential address found on the birth certificate. Residential address on the birth certificate may not be the same address the three months before pregnancy, during the pregnancy, or the first year of life exposure periods.

Amyotrophic Lateral Sclerosis

The association between malathion exposure and Amyotrophic Lateral Sclerosis (ALS) was evaluated in one AHS study (Kamel et al., 2012) described below.

Kamel et al. (2012) investigated the association between pesticide exposure, including malathion, and ALS among private pesticide applicators and their spouses in the AHS prospective cohort. Cases of ALS were identified using vital statistics data in Iowa and North Carolina and the National Death Index from enrollment through February 7, 2010 and were defined as having ALS listed as an underlying or contributing cause of death on the death certificate. Pesticide exposure (ever use and days of use) was self-reported via questionnaire completed at study enrollment (1993 – 1997) and shortly thereafter. Authors compared the 41 cases of ALS to the rest of the AHS cohort (84,689) and unconditional logistic regression was used to calculate ORs and 95% CIs for the association between malathion exposure and ALS, adjusting for age and gender.[84] Among the 41 cases and 84,689 controls, 14 (39%) cases and 39,200 (50%) controls reported malathion exposure. No evidence of a positive association was reported between malathion exposure and ALS among a small number of cases (OR = 0.60; 95% CI: 0.30, 1.30; with n = 14 exposed cases).

The quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. As part of the AHS, this study benefited from the strengths of the AHS study cohort

[83] Bell ML, Belanger K. Review of research on residential mobility during pregnancy: consequences for assessment of prenatal environmental exposures. J Expo Sci Environ Epidemiol 2012;22:429-38. https://doi.org/10.1038/jes.2012.42

[84] ALS incidence is greater in men and risk of ALS increases with increased age.

including the prospective cohort study design, case ascertainment, and the exposure assessment. However, we note the small number of malathion exposed cases.

EPA Conclusion

Overall, there *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and ALS. One study (Kamel et al., 2012) examined the association between malathion exposure among AHS participants and ALS and reported no evidence of a positive association. The quality of the study was ranked high for regulatory purposes based on strengths included the prospective cohort study design, case ascertainment, and exposure assessment. We note, however, that the number of exposed cases was small which restricts the ability to interpret with confidence the observed ORs.

Autoimmune Disease

Four studies (De Roos et al., 2005; Parks et al., 2016; Meyer et al., 2017; Parks et al., 2019) examined the effects of malathion exposure and autoimmune disease including antinuclear antibodies (markers of autoimmune disease) and rheumatoid arthritis.

Antinuclear Antibodies – markers of autoimmune disease

One study, Parks et al. (2019), examined the association between malathion exposure and the risk of developing systemic autoimmunity (autoimmune disease).

Parks et al. (2019) investigated the association between pesticide exposure, including malathion, and autoimmune disease among pesticide applicators enrolled in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study within the AHS, a large prospective cohort of farmers from Iowa and North Carolina. The study population included male private pesticide applicators living in Iowa or North Carolina who were enrolled in the AHS. Additionally, eligible participants of the BEEA were $\geq$ 50 years of age, completed the AHS enrollment questionnaire and the two follow-up interviews (1999-2003 and 2005-2010), had never been diagnosed with cancer other than non-melanoma skin cancer, and did not report a doctor diagnosis of systemic autoimmune disease at AHS enrollment. Among the 699 male private pesticide applicators enrolled in the BEEA study between June 2010 and September 2013, 668 were included in this analysis and of those, 110 reported exposure to malathion.

Markers of autoimmune disease, including Anti-nuclear antibodies (ANA), extractable nuclear antibodies (ENA) and anti-dsDNA antibodies, were detected in serum extracted from non-fasting blood specimens of study participants. Samples were collected in participant's home and were shipped cold via overnight delivery before processing and storage at -80^{o} C. ANA was measured using immunofluorescence assay using a standardized protocol in a rheumatology laboratory experienced in high-throughput testing. Samples positive for ANA were subsequently tested for ENA and anti-dsDNA antibodies. ANA positivity was based on highest reading observed and was divided into three exclusive categories of positivity to indicate an increasing threshold for ANA positivity: "Any ANA" ($\geq$ 1:80 dilution at 2+ intensity reading), "Moderate-higher" ($\geq$ 1:80 dilution at 3/4+ intensity reading), and "Higher" ($\geq$ 1:160 dilution at 3/4+ intensity reading). Pesticide exposure was assessed from pesticide use data reported on enrollment questionnaires (1993 – 1997), during the two follow-up interviews (1999 – 2003 and 2005 – 2010), and at BEEA enrollment to determine lifetime use of malathion. Among the 665 study participants, 529 reported exposure to malathion and of these, 211 had positive ANA level and 318 with malathion use had a negative result. The association between lifetime use of malathion reported at enrollment and ANA positivity level (Any ANA, Moderate-higher, Higher) compared to those with no detectable ANA was

assessed using three separate multivariable logistic regression models to determine ORs and 95% CIs adjusted for covariates measured at BEEA interview including: age, BMI, state, ever smoked, spring or summer season of blood draw, and use of agricultural pesticides in the past 12 months. No evidence of a positive association was reported for lifetime use of malathion and any of the three ANA categories (*Any ANA* – OR = 0.62; 95% CI: 0.42, 0.91; with n = 99 exposed cases; *Moderate-higher ANA* – OR = 0.87; 95% CI: 0.53, 1.45; with n = 66 exposed cases; *Higher ANA* – OR = 0.67; 95% CI: 0.34, 1.30; with n=46 exposed cases), with the no detectable ANA group as the referent.

In an additional analysis, the authors examined the association between malathion exposure and the presence of ENA or anti-dsDNA autoantibodies compared to those with no ANA level detected, adjusted for age. Nine (60%) of the 15 cases with ENA/anti-dsDNA detected and 318 (82%) of the 386 with no ANA level detected reported malathion ever exposure. No evidence of a positive association was reported for the association between malathion ever exposure and detection of ENA/anti-dsDNA autoantibodies among participants (OR = 0.31; 95% CI: 0.10, 0.95; with n = 9 malathion exposed cases of ENA/anti-dsDNA out of 15, and n = 318 malathion exposed participants with no detectable ANA out of 386; p-value 0.041).

The quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The study benefited from the general strengths of the AHS, including the prospective cohort study design and the exposure assessment approach which examined cumulative lifetime exposure to malathion. Additionally, the outcome of autoimmune disease markers ANA and ENA/anti-dsDNA were detected using laboratory methods rather than via self-report by the participant. A noted limitation of the study is the ambiguity around the temporality of the exposure and the outcome. It is unclear if ANA developed after exposure to pesticides or before or whether ANA appeared in the past but was no longer present at time of blood sample collection.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and autoimmune disease. One study (Parks et al., 2019) examined the association between malathion exposure among agricultural workers and risk of autoimmune disease among a subset of the AHS prospective cohort population; those enrolled in the Biomarkers of Exposure and Effect in Agriculture sub cohort. Parks et al. (2019) reported no evidence of a positive association between malathion exposure and biomarkers for autoimmune disease. The quality of the study was ranked moderate and benefited from the general strengths of the AHS, including the prospective cohort study design and the exposure assessment approach which examined cumulative lifetime exposure to malathion. A noted limitation of the study is the ambiguity around the temporality of the exposure and the outcome. It is unclear if ANA developed after exposure to pesticides or before or whether ANA appeared in the past but was no longer present at time of blood sample collection.

Rheumatoid Arthritis

The association between malathion exposure and rheumatoid arthritis (RA) was evaluated in three publications (De Roos et al., 2005, Parks et al., 2016, Meyer et al., 2017) described below.

- De Roos et al. (2005) investigated the association between pesticide exposure, including malathion, and RA among spouses of pesticide applicators participating in the AHS, using a nested case-control study design. The study population included female spouses of pesticide applicators enrolled in the AHS who completed both the enrollment (1993-1997) and 5-year follow-up (1999-2003) questionnaires. Cases included female spouses who reported a physician diagnosis of RA as an adult

either at enrollment or on the follow-up interview and whose RA diagnosis was also physician confirmed. Controls were also selected from women in the AHS population who completed the follow-up questionnaire. Additionally, controls did not report any history of systemic autoimmune disease. Each case (n = 135) was matched to five controls (n = 675) based on birth year. Pesticide exposure was assessed using data collected on the enrollment questionnaires. The association between malathion exposure and RA was assessed using unconditional logistic regression to estimate ORs and 95% CIs, adjusting for birth date and state of residence. Of the 135 physician-confirmed prevalent and incident RA cases and 675 controls, 36 cases (28.4%) and 150 controls (23.6%) reported exposure to malathion. No evidence of a significant positive association was reported between malathion exposure and RA in female spouses (OR = 1.30; 95% CI: 0.80, 2.00; with n = 36 exposed cases and n = 150 exposed controls). When the association between malathion exposure and RA was stratified by state of residence, no evidence of a significant positive association was reported among women living in Iowa (OR = 1.20; 95% CI: 0.70, 2.00; with n = 23 exposed cases and n = 114 exposed controls) or in North Carolina (OR = 1.60; 95% CI: 0.80, 3.50; with n = 13 exposed cases and n = 6 exposed controls).

The quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The cross-sectional design (inclusion of prevalent cases of RA) was a main limitation since temporality for exposure in relation to the outcome for these cases could not be determined, and the study was thus ranked low quality for this reason. We note also the number of cases that reported malathion exposure for RA (in North Carolina only) was small (10 < exposed cases $\leq$ 19).

- Parks et al. (2016) investigated the association of malathion and other pesticide exposures and RA among wives of pesticide applicators in the AHS. Using a cohort study design, women (n = 24,293) self-reported physician-diagnosed RA and pesticide use through questionnaires completed at enrollment (Phase 1: 1993 and 1997) for prevalent RA cases and follow up (Phase 2, 1998–2003; Phase 3, 2005–2010) for incident RA cases. Cases of self-reported RA were classified as confirmed if the self-reported RA was supported by physician data or probable if participants self-reported taking of medications specific to RA on a screening questionnaire.[85] Logistic regression was used to estimate ORs and CIs, adjusting for age, state, and pack-years smoking. Of the 271 total cases of RA among study participants, 58 (22%) reported exposure to malathion, and of the 129 incident cases of RA, 23 (19%) reported malathion exposure. Of the 23,570 non-cases with complete data, 4,671 (20%) reported malathion exposure. No evidence of a significant positive association between malathion exposure and all (incident and prevalent) RA cases was observed (OR = 1.10; 95% CI: 0.80, 1.40) and no evidence of a positive association between malathion and incident RA cases only was observed (OR = 0.86; 95% CI: 0.55, 1.40).

  The quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its cohort design. Study limitations included the use of proxy respondents (~22 filled out screening questionnaires) and the self-reported outcomes among several of the study participants. Although study authors indicated that some RA cases were physician-confirmed during later phases of the study, some RA cases were self-reported earlier on in the study and were contacted at a later date to provide additional data via questionnaire to validate their RA case status. This self-validating method is not the same as the cases which were ascertained by a physician, and likely led to bias and exposure misclassification.

[85] The study authors reported that identifying the probable cases (those who self-reported taking of medications specific to RA) provided "more power to focus on incident cases, which may minimize the influence of recall bias or healthy worker effect." (Parks et al. 2016)

- Meyer et al. (2017) investigated the potential association between exposure to pesticides including malathion and RA in male pesticide applicators in the AHS. The study population included male pesticide applicators enrolled in the AHS between 1993 – 1997, who completed at least one follow-up questionnaire (Phase II: 1999 – 2003, Phase III: 2005 – 2010, Phase IV: 2013 – 2015). Incident RA cases were identified either through self-reported use of disease-modifying antirheumatic drugs, use of steroids for RA, or self-reported RA diagnosis by a rheumatologist on the follow-up questionnaires. Eligible cases who reported RA on the follow-up interview were screened by telephone to confirm their diagnosis and to confirm use of disease-modifying antirheumatic drugs. Non-cases included pesticide applicators who did not report RA and had complete covariate data. Pesticide exposure was self-reported on the enrollment questionnaires and used to determine ever use and cumulative lifetime days of use for specific pesticides including malathion. Among the total probable incident RA cases (n=220) and non-cases (n=26,134), 87 (67%) cases and 8,983 (66%) non-cases reported exposure to malathion, based on ever/never use. The association between malathion exposure (ever use, lifetime use, and intensity-weighted lifetime days of use) vs. no use and RA was estimated using logistic regression models adjusted for age, pack-years smoking, education, and state of enrollment. It was decided *a priori* that exposure-response analysis would only be conducted for those pesticides with >20 exposed cases and an OR >1.20 for ever use. Covariates were selected based on hypothesized or observed associations with RA and pesticide use overall, and covariates included in the final model were confirmed using selection by stepwise regression. No evidence of a significant positive association was reported between malathion exposure and incident RA cases among male pesticide applicators (OR=1.05; 95% CI: 0.73, 1.53; with n=87 exposed cases). Exposure-response analysis was not conducted for malathion because the ever use OR was not >1.20.

  The quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The study benefited from the general strengths of the AHS, including the prospective study design and cumulative pesticide exposure assessment. Cases of incident RA were self-reported and while authors attempted to reduce over-reporting using a screening tool among those reporting RA on the Phase III questionnaire to gather more information about the disease and medications prescribed for RA, these reports were not confirmed via medical record. As such, the outcome assessment was considered a limitation of the study. Additionally, the stepwise selection procedures were considered a limitation as these are generally appropriate only for studies conducting exploratory analyses for purposes of hypothesis generation; purported statistical significance arising from studies that use this technique are not valid and cannot be relied upon. However, since the study mentioned that "covariates were selected based on hypothesized or observed associations with rheumatoid arthritis" this infers that the stepwise procedure was not automated and instead relied on the thoughtful selection of covariates, which is further reinforced since P(enter) and P(leave) were not indicated by the authors.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and RA. There were three available publications (De Roos et al., 2005, Parks et al., 2016, Meyer et al., 2017) that examined the association between malathion exposure and RA among participants in the AHS prospective cohort. De Roos et al. (2005) and Parks et al. (2016) reported no evidence of a significant positive association among wives of pesticide applicators in the AHS. The third publication, Meyer et al. (2017), examined the association between malathion exposure and RA among male pesticide applicators in the AHS and reported no evidence of a positive association between malathion exposure and incident RA cases among male pesticide applicators. All three studies were ranked moderate quality for regulatory purposes and while they benefited from exposure assessment approach used by the AHS, the outcome was self-reported and if

clinically confirmed via medical records would have made the assessment stronger. Lastly, we note the small number of exposed cases reported for RA (North Carolina only) in De Roos et al. (2005).

Birth Defects

Three studies (Grether et al., 1987; Thomas et al., 1992; Haraux et al., 2018) examined the association between malathion exposure and birth defects in newborn babies including congenital abnormalities, spontaneous abortions, still births, intrauterine growth retardation, and hypospadias in male offspring.

- Grether et al. (1987) evaluated the potential association between maternal exposures to malathion following aerial applications and congenital abnormalities in their offspring, using data from a semi-ecological study. Children born with congenital abnormalities between 1981 – 1982 were identified using hospital discharge records, and the zip code the of the mother's residence was linked to zip codes that had been treated prior with pesticides including malathion via aerial application. Mothers who resided in these following zip codes receiving aerial applications were included in this study: Santa Clara, San Mateo, and Alameda counties. Aerial application information was obtained from the California Department of Food and Agriculture and was used to calculate exposure scores on a monthly basis for all zip codes which received applications. Aerial applications made by the state between July 1, 1981 and August 1982 within a 13,000 square mile range were assessed in this study. No additional exposure details were provided by the study authors. For children born with congenital abnormalities, the first trimester of gestation was used in calculating the exposure score. The date of birth indicated from hospital discharge records (along with the maternal residence zip code) was used, and for full-term children, the first trimester was determined by counting back from nine months. A different month was calculated for children born early – at either six or eight months of gestation – as indicated by hospital documentation as 'extreme immaturity' or 'other preterm'. No statistical methods were mentioned by the study authors, so it is unclear which statistical model was used to determine the association between congenital abnormalities in children following maternal exposure to pesticides including malathion, in addition covariate selection for the model. Although the study does report relative risk estimates for select congenital abnormalities[86] without reasoning, it is also unclear if these results are part of a potentially larger data set containing additional diagnoses in children from 1981 – 1982. Odds ratios for the following congenital abnormalities in children were reported in this study: anomalies of the ear, bowed legs, varus deformities, clubfoot, and tracheoesophageal fistula. Among the total number of potentially exposed children (n = 22,465 births) for 1982, 152 reported congenital abnormalities. A total of 17,050 unexposed births in 1982 were reported, and a total of 37,854 unexposed births were reported in 1981. Evidence of strong association was observed for anomalies of the ear in children (OR: 4.49; 95% CI: 1.19, 16.92, with n = 11) and evidence of a moderately strong association was observed for bowed legs in children (OR: 2.99; 95% CI: 1.32, 6.75, with n = 25) following maternal exposure to aerial application of malathion relative to the 1981 unexposed group. For children born with varus deformities and clubfoot, evidence of a positive association was observed relative to maternal malathion exposure, in comparison to the 1981 unexposed group (OR varus deformities: 1.72; 95% CI: 1.16, 2.55, with n = 99; OR clubfoot: 1.47; 95% CI: 1.09, 1.96, with n = 174). It should be noted that when the metatarsus varus diagnosis was excluded from the varus deformities abnormality group, as well as from the clubfoot abnormality group of children, no evidence of a significant positive association was reported (OR varus deformities: 1.03; 95% CI: 0.58, 1.82, with n = 50; OR clubfoot: 1.12; 95% CI: 0.79, 1.61, with n = 125). Additionally, when the 'unspecific' diagnosis was excluded from the bowed legs abnormality, although elevated, no evidence of a significant positive association was

reported (OR: 3.37; 95% CI: 0.31, 37.16, with n = 3). For anomalies of the ear, when the 'other' diagnosis was excluded, an odds ratio was not reported due to the very small number of cases reported (n = 1). No evidence of a significant positive association was observed between maternal malathion exposure following aerial applications and tracheoesophageal fistula in children relative to 1982 unexposed group (OR: 2.66; 95% CI: 0.55, 12.78, with n = 9). The study authors concluded that no associations were reported between maternal malathion exposure following aerial applications and any congenital abnormalities in children; however, Table 1 of their same study reports statistically significant and elevated odds ratios for a number of congenital abnormalities.

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The semi-ecological study design was considered a study limitation due to an inability to extrapolate observed associations from the group level to the individual level, along with several additional limitations mentioned in the paragraph above. Lastly, we note a very small number of cases (n <10) and/or the very wide confidence intervals reported for a number of the reported congenital abnormalities. The study was ranked low quality for regulatory purposes.

- Thomas et al. (1992) evaluated the potential association between maternal exposures to malathion following aerial applications and reproductive abnormalities in their offspring, using data from a nested case-control study. The study population included pregnant women (n = 7,450) with a confirmed pregnancy during September 1, 1981 – June 30, 1982 at one of three Kaiser Permanente medical centers located in Redwood City, Hayward, or Santa Clara, California. Women who were pregnant with more than one child, whose pregnancy was terminated by an induced abortion, or pregnant women < 18 years of age, were excluded from this study. Cases included pregnant women from the study population who reported one the following reproductive abnormalities instead of a normal live birth: spontaneous abortion (loss of pregnancy at < 28 weeks gestation), reportable congenital abnormalities, intrauterine growth retardation (live birth with birth weight < 2 standard deviations lower than the mean of resulting gestational age), or stillbirth (loss of pregnancy at > 27 weeks gestation). Controls were randomly selected from the study population as well and were randomly selected from the subgroup of women who had live births. All outcomes of pregnancies were ascertained using Kaiser-Permanente medical files and/or using state live and fetal death records. A mailed questionnaire was completed by the cases and controls to obtain additional details regarding past pregnancies, residential histories, past employers, and potential confounding factors. Study participants who did not complete the mailed questionnaire were contacted by telephone. The response rate for the cases ranged from 70% - 88%. Medical records and birth certificate data were also used to ascertain participant details. The residential addresses during pregnancy were converted to geospatial coordinates, with these coordinates then linked to the completed survey data, and then further linked to 'spray corridors' which provided the location of aerial applications of malathion within the area during the same time frame. Information regarding exposure frequency within each spray corridor was also available. Once this data was combined, the following three indicators were used to determine maternal exposure by each week of gestation: the number of applications within a spray corridor containing the participants residence on a given week, the distance for the closest corridor active on a given week, and the total amount of area of covered by the active spray corridors within 1 km, weighted by the amount of applications and inversely by the distance from the maternal residence. These exposure classifications were further defined broadly as either direct exposure (exposure occurring with an active corridor) indirect exposure (exposure occurring with a 1 km distance of an active corridor), or no exposure (exposure occurring in > 1 km distance of an active corridor). Logistic regression was used to determine the association between maternal malathion exposure following aerial applications and intrauterine growth retardation, and for reportable abnormalities. The study authors do not specifically define the covariates considered and adjusted for in the statistical model within the method section of the study, but mention within the results section

that intrauterine growth retardation was adjusted for gestational age, race, indoor insecticide use, cigarette smoking during the first trimester, education, and nulliparity. Similarly for reportable abnormalities, there was mention within the results section by the study authors about adjustment for Asian race and outdoor insecticide use. For stillbirths and spontaneous abortions, a case-cohort analysis was conducted to determine the association following maternal malathion exposures. This analysis consisted of randomly selecting controls (n = 1,000) from the initial study population, and then randomly selecting cases from the initial case group using the same sampling fraction (16.7%) that was used to compose the control group. Each case could then be compared to a group of controls who were at risk at the same gestational age, as when the case died. Exposure in this analysis was defined as either 1.) any exposure or 2.) distance-weighted cumulative number of exposures and each exposure was further evaluated at four different time periods (i.e., gestational ages); however, the study only reported results for two of these four time periods – gestational age of the case when the outcome occurs (GA), and gestational age of the case one month prior to the outcome (GA – 4). Similar to the other two reported outcomes, the study authors do not specifically define the covariates considered and adjusted for in the statistical model for stillbirths and spontaneous abortions within the method section of the study. Within the results section they mention that spontaneous abortions were adjusted for age, tap water consumption, alcohol consumption, prior miscarriages, nausea (protective), and stillbirths were adjusted for facility. Among the total of 856 cases and 1,128 controls reported in this study, 559 of the cases resulted in spontaneous abortion, 37 of the cases resulted in stillbirth, 97 of the cases resulted intrauterine growth retardation, and 163 of the cases resulted in reportable congenital abnormalities. For reportable congenital abnormalities and intrauterine growth retardation, exposure was reported as either direct malathion exposure during the first trimester or direct malathion exposure during the second trimester. Evidence of a strong association but with borderline significance was reported between direct maternal exposure during the second trimester to malathion following aerial applications and gastrointestinal abnormalities (RR: 4.14, 95% CI: 1.01, 16.60). We note the very wide confidence interval and the lower bound of the confidence interval being very close to 1.0, that would indicate no association. No evidence of a significant positive association was observed, although elevated relative risk was reported, for any other abnormalities pertaining to the limb, orofacial, and all but chromosomal relative to direct exposure during the first trimester ($1.20 \leq RRs \leq 3.35$; all CIs encompassed the null value of 1.0). For intrauterine growth retardation, no evidence of a positive association was observed relative to direct maternal exposure during the first trimester to malathion following aerial applications (RR: 0.90; 95% CI: 0.54; 1.49). For spontaneous abortions and stillbirths, the following four exposure variables were included: direct exposure at gestational age (GA), direct exposure at GA – 4, indirect exposure at GA, and indirect exposure at GA – 4. No evidence of a significant positive association was reported in any of these exposure categories for spontaneous abortions $0.91 \leq$ adjusted RRs ≤ 1.20; all CIs encompassed the null value of 1.0) and for stillbirths ($0.99 \leq$ adjusted RRs ≤ 1.95; all CIs encompassed the null value of 1.0). The corresponding number of cases for each of the reported reproductive abnormalities within the study table was not provided.

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. Although the nested case-control study design and ascertainment of the cases were considered study strengths, the study had several limitations. A primary limitation of this study were the statistical methods used, as they were lacking details regarding the statistical test(s) performed and did not specifically define the adjusted confounders used in each test, and instead only briefly mentioned the confounders afterwards within the text of the results section of the paper. Furthermore, within the reported results tables, the corresponding number of cases for each reproductive abnormality was not reported. Additionally, exposure misclassification was likely due to errors including those that may have occurred during the aerial applications, due to unexpected malathion

drift outside the range of a defined spray corridor, as well as differences in exposures of the residences and differences in exposure among the pregnant women in the study.

- Haraux et al. (2018) evaluated the potential association between prenatal pesticide exposure including malathion, and isolated hypospadias (male birth defect in which the opening of the urethra is located on the underside of the penis instead of the tip) in newborn babies. Using data from a case-control study conducted in Picardy, France, suspect newborn hypospadias cases were initially identified at one of eleven maternity wards involved within the study, and were ascertained by a pediatric endocrinologist and urologist from March 2011 – March 2014. At the time of hypospadias diagnosis, the location of the urethral meatus (proximal, middle, or distal) was noted in addition to any other genitalia abnormalities that may have been associated. Controls were then matched to the cases based on gestational age, same birth month, and same maternity unit with three controls to every one case. Newborn babies delivered within the same month at a similar gestational age (< or > 35 weeks absent menstruation) and at the same maternity ward as the cases, were included within the control group. Both the cases and the controls were clinically examined and newborn babies born with hypospadias who further met the following criteria were included within the study: a normal hormonal profile, no additional genital abnormalities, no congenital syndromes, no family history of hypospadias. Fetal exposure to pesticides was measured through the meconium (the initial stools secreted following birth) of the newborns within the study, and meconium samples were collected and tested to detect pesticide concentrations, including malathion, daily by nurses until the first stools were observed. Meconium samples were stored at 4°C during transport and then at -80°C prior to analysis, and samples were assessed using liquid chromatography paired with tandem mass spectrometry. The limit of detection (LOD) for malathion was reported as 0.05 ng/g and the limit of quantification was 0.30 ng/g, and the median malathion concentration was 9.1 ng/g (IQR: 4.40, 11.60). The overall % of cases and controls with >LOD for malathion was 74.7%, with 80.0% for cases and 72.4% for controls. Parental data was also obtained using medical records and through a questionnaire for the following information: educational level, age, height, smoking status, weight, employment status, medication, and folate intakes during gestation, as well as family history of hypospadias, undescended testis, testicular cancer, as well as gynecological, obstetrics, and endocrine histories. A total of 25 cases and 58 controls were reported, with the majority of hypospadias cases of the distal form (n = 22), two of the middle form, and one of the proximal form. A conditional logistic regression was conducted to calculate adjusted ORs and 95% CIs for individual pesticides, including malathion, adjusted for primiparity and low birth weight. An elevated but not significant positive association was observed between hypospadias and malathion concentrations at the low exposure level (low level adjusted OR: 3.08; 95% CI: 0.64, 14.89 with n = 13 cases, 23 controls, and no evidence of a significant positive association was observed at the high exposure level (adjusted high level adjusted OR: 1.64; 95% CI: 0.37, 7.20 with n = 8 cases, 18 controls). Lastly, a very small number of exposed cases were observed.

  The overall quality of the study was ranked moderate based on the study quality criteria outlined in the OPP Framework. Haraux et al. (2018) used a case-control design to assess the relationship between hypospadias and pesticide exposure. Cases were ascertained by physicians, and three controls were matched to each case in this study. Limitations included no mention of laboratory QA/QC procedures by the study authors or use of field or laboratory blanks, for example. Additionally, the study authors mentioned that the diapers holding the meconium may be contaminated with trace amounts of other pesticides unaccounted for. This was the first study to use meconium sampling to determine fetal exposure, as meconium biomarker testing was a relatively new approach. As a result, it is unclear how meconium sampling would compare to other biomonitoring mediums used in the past such as cord blood and maternal urine and blood sampling, to detect prenatal exposure of pesticide metabolites. An initial validation study was completed by Whyatt and

Barr et al., 2001[87], which the study authors indicated reported correlations between the number of pesticides detected in maternal urine and meconium samples and the number of pesticides detected in maternal and cord blood in pregnancy; however, further studies are needed to validate these results. Lastly, a very small number of exposed cases were observed (n = 8 – 13 cases).

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and birth defects among newborn infants. Three publications (Grether et al., 1987; Thomas et al., 1992; Haraux et al., 2018) examined the association between malathion exposure and birth defects in newborn babies including congenital abnormalities, spontaneous abortions, still births, intrauterine growth retardation, and hypospadias in male offspring. Grether et al. (1987) evaluated the potential association between maternal exposures to malathion following aerial applications and congenital abnormalities in their offspring including anomalies of the ear, bowed legs, varus deformities, clubfoot, and tracheoesophageal fistula. Evidence of a strong association was observed for anomalies of the ear in children and evidence of a moderately strong association was observed for bowed legs in children, following maternal exposure to aerial application of malathion relative to the 1981 unexposed group. For children born with varus deformities and clubfoot, evidence of a positive association was observed relative to maternal malathion exposure, in comparison to the 1981 unexposed group. No evidence of a significant positive association was observed between maternal malathion exposure following aerial applications and tracheoesophageal fistula in children relative to 1982 unexposed group. The study was ranked low quality for regulatory purposes. The semi-ecological study design was considered a study limitation due to the inability to extrapolate observed associations from the group level to the individual level, along with several additional limitations. We also note a very small number of cases (n <10) and/or the very wide corresponding confidence intervals reported for a number of the reported congenital abnormalities, which severely restricts the ability to interpret with confidence the observed ORs. The second study, Thomas et al. (1992), evaluated the potential association between maternal exposures to malathion following aerial applications and reproductive abnormalities in their offspring including spontaneous abortion, reportable congenital abnormalities, intrauterine growth retardation, or stillbirth. Reportable congenital abnormalities were defined as pertaining to the: limb, orofacial, gastrointestinal, and all but chromosomal. Evidence of a strong association but of borderline significance was reported between direct maternal exposure during the second trimester to malathion following aerial applications and gastrointestinal abnormalities was observed. Although elevated, no evidence of a significant positive association was observed for any other abnormalities pertaining to the limb, orofacial, and all but chromosomal relative to direct exposure during the first trimester. For intrauterine growth retardation, no evidence of a positive association was observed relative to direct maternal exposure during the first trimester to malathion following aerial applications. For spontaneous abortions and stillbirths, no evidence of a significant positive association was reported in any of the direct or indirect exposure categories. The study quality was ranked low for regulatory purposes. A primary limitation of this study was the statistical methods used, as they were lacking details regarding the statistical test(s) performed and did not specifically define the adjusted confounders used in each test, and instead only briefly mentioned the confounders afterwards within the text of the results section of the paper. Furthermore, within the reported results tables, the corresponding number of cases for each reproductive abnormality was not reported. Additionally, exposure misclassification was likely due to errors including those that may have occurred during the aerial applications, due to unexpected malathion drift outside the range of a defined spray corridor, as well as differences in exposures of the residences and differences in exposure among the pregnant women in the study. A third publication, Haraux et al. (2018), evaluated the association between malathion and

[87] Whyatt, R. M., & Barr, D. B. (2001). Measurement of organophosphate metabolites in postpartum meconium as a potential biomarker of prenatal exposure: a validation study. *Environmental health perspectives*, *109*(4), 417-420.

the birth defect, hypospadias, in newborn babies and reported no evidence of a significant positive association. The study was ranked moderate quality for regulatory purposes. Study limitations included no mention of laboratory QA/QC procedures by the study authors, and potential cross-contamination of other pesticides found during meconium sampling. We also note that the number of exposed cases was small ($10 < \text{exposed cases} \leq 19$) which limits the ability to interpret with confidence the observed odds ratios.

Birth Effects

Four studies (Eskenazi et al., 2004; Wolff et al., 2007; Sathyanarayana et al., 2010; Ling et al., 2018) evaluated the association between malathion exposure and birth effects including head circumference, birth weight, length of gestation, crown-heel length, preterm birth, and ponderal index among children in New York City, in the AHS in Iowa and North Carolina, and in California.

- Eskenazi et al. (2004) investigated the potential association between maternal exposure to pesticides, including malathion, and subsequent fetal growth changes among their offspring. Using data from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), an ongoing prospective cohort study, the study population included pregnant women residing within Salinas Valley, California, < 20 weeks of gestation, aged ≥ 18 years old, Medi-Cal eligible, whose primary language was English or Spanish, and sought prenatal care at either Natividad Medical Center hospital or one of its five medical centers within the area and planned to deliver at the hospital. The eligibility period spanned one year (October 1999 – October 2000) and 601 (53.2%) of the 1,130 eligible women in this study participated in this study. Exclusion criteria for this study included: women with hypertension (n = 15), twin or still births (n = 8), a history of diabetes or gestational diabetes (n = 26), and for women whose birthweight data was not within range (< 500g). The study authors did include newborns (n = 11) born with congenital abnormalities, as their exclusion would not materially change the results. Pesticide exposure for certain pesticides, including malathion, were measured using pesticide-specific metabolites. The metabolite-specific metabolite for malathion measured in this study was malathion dicarboxylic acid (MDA). Two spot urine samples were collected during pregnancy to measure the metabolites, once at 13 weeks (mean time; ranged from 4 – 29 weeks) and another at 26 weeks (mean time; ranged from 18 – 39 weeks) of gestation. Urine samples were then stored at -80°C prior to shipping and analysis for metabolite levels at the Centers for Disease Control (CDC), using liquid or gas chromatography with tandem mass spectrometry. The LOD for MDA was 0.29 µg/L, with 30.1% of the MDA urine samples (at least one of the two urine samples obtained during pregnancy) being above the LOD; the median level[88] of MDA metabolites was 0.2 µg/L (0.2 – 28.90 µg/L). Due to this small percentage of samples being above the LOD, the exposure for MDA was assessed as a categorial variable, and exposure categories were defined as: referent group (no detectable levels), detectable levels lower than the median, and detectable levels at or above the median level. Of the 482 women who provided samples to be tested for specific urinary metabolites including MDA, a total of 382 urine samples were measured for MDA. The study authors reported MDA levels measured within ~100 urine samples were ineligible due to technical issues. Of the total 382 MDA urine samples, 74 samples were detectable less than the median MDA level, 75 were detectable at or above the median MDA level, and 233 were not detectable. Metabolite levels measured below the limit of detection (LOD) were calculated as the LOD/√2, and missing values were imputed using a regression analysis to predict the value based on the metabolite values of women for other metabolites at that same time point. Creatinine concentrations of the urine samples were also measured and adjusted and unadjusted associations for creatinine concentrations were

[88] The median level of the measured MDA metabolites was the mean of the two samples collected during pregnancy and was not adjusted for creatinine.

performed relative to the pesticide exposures. Study authors indicated that laboratory QA/QC procedures were carried out in this study. Detailed interviews were also conducted at two points during pregnancy and once immediately after delivery by a bilingual interviewer (English/Spanish) to obtain information regarding demographics, health histories, past pregnancies, and agricultural exposure. All health and prenatal histories were confirmed via medical records. The outcome was broadly defined as fetal growth changes at delivery and was further defined and measured as birth weight (grams), crown-heel length (distance from the crown of the head to the heel of the newborn) (centimeters), head circumference (centimeters), and ponderal index (grams/centimeters^3). An additional outcome -- length of gestation (weeks) -- was also assessed in a separate analysis. A linear regression model was used to estimate fetal growth changes (further defined above) at delivery from several pesticide exposures including MDA, the malathion-specific metabolite, adjusting for maternal BMI, poverty level, gestational age, gestational age^2, timing of urine collection, maternal age, infant sex, parity, country of birth, weight gain, and timing of entry into prenatal care. Additionally, a separate linear regression model was performed to determine the change in length of gestation relative to pesticide exposures including MDA, adjusting for maternal age, parity, poverty level, country of birth, timing of entry into prenatal care, and timing of urine collection. Covariates were selected from associations observed in the literature and were included within the models when the coefficient of exposure change by ≥ 10%. Both models reported results that were unadjusted for urinary creatinine concentrations. Results for the analysis of the association between malathion exposure and birth weight, birth length, ponderal index, head circumference, and length of gestation are reported below:

- For birth weight, no evidence of a significant association was reported between urinary metabolite levels of MDA in pregnant women and birth weight in newborns (< MDA median β: -45 grams; 95% CI: -154, 63, p-value = 0.41; ≥ MDA median β: 56 grams; 95% CI: -49, 161, p-value = 0.29).

- For crown-heel length, no evidence of a significant association was reported between urinary metabolite levels of MDA in pregnant women and crown-heel length in newborns (< MDA median β: -0.53 centimeters; 95% CI: -1.18, 0.11, p-value = 0.11; ≥ MDA median β: 0.14 centimeters; 95% CI: -0.48, 0.76, p-value = 0.66).

- For head circumference, no evidence of a significant association was reported between urinary metabolite levels of MDA in pregnant women and head circumference in newborns (< MDA median β: -0.16 centimeters; 95% CI: -0.52, 0.19, p-value = 0.37; ≥ MDA median β: 0.11 centimeters; 95% CI: -0.24, 0.46, p-value = 0.53).

- For ponderal index, no evidence of a significant association was reported between urinary metabolite levels of MDA in pregnant women and ponderal index in newborns (< MDA median β: 0.05 g/cm^3; 95% CI: -0.05, 0.14, p-value = 0.33; ≥ MDA median β: 0.02 g/cm^3; 95% CI: -0.07, 0.12, p-value = 0.60).

- For length of gestation, no evidence of a significant association was reported between urinary metabolite levels of MDA in pregnant women and length of gestation in newborns (< MDA median β: -0.13 weeks; 95% CI: -0.55, 0.30, p-value = 0.55; ≥ MDA median β: -0.21 weeks; 95% CI: -0.62, 0.20, p-value = 0.32).

The overall quality of the study was ranked moderate based on the study quality criteria outlined in the OPP Framework. Eskenazi et al. (2004) used a prospective cohort design to assess the relationship between birth effects including fetal growth changes in newborns and pesticide exposure measured through urinary metabolites. Study strengths included the study design, the use of medical

record data, the quantitative procedures used to measure the exposure, and the use of laboratory QA/QC methods. A primary limitation of the study included the potential for exposure misclassification due the transient and variable nature of exposures to pesticides. Furthermore, pesticides metabolites usually stay within the body for a short amount of time, making it challenging to accurately measure longer-term exposures. 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. An additional limitation included the potential for selection bias since the study population was focused within a specific area of California, making it less likely that it reflected the general population. The study was ranked moderate quality for regulatory purposes.

- Wolff et al. (2007) investigated the potential association between maternal exposure to pesticides including malathion and fetal growth changes among their offspring. Using data from the Children's Environmental Health Study[89], an ongoing prospective cohort study, the study population included pregnant women who resided in New York City and sought prenatal care at either the prenatal clinic at Mount Sinai hospital in New York City or at one of the two private practices within the hospital. Exclusion criteria for this study included women who: were multiparas; had multiple gestations; prior pregnancy complications; had their first prenatal visit at >26 weeks of gestation; had a history of diabetes, thyroid disease, or hypertension; consumed more than two alcoholic beverages per day; or used illegal drugs. Additionally, mother-child pairs were excluded if the child was born with a congenital abnormality or were severely premature (defined as < 1500 grams or <32 weeks of gestation). The eligibility period spanned from March 1998 – March 2002 and 404 mother-child pairs of the 479 eligible women participated in this study. Pesticide exposure for certain pesticides including malathion was measured using pesticide-specific metabolites. The metabolite-specific metabolite for malathion measured in this study was malathion dicarboxylic acid (MDA). Urine and blood samples were collected during the third trimester of pregnancy to measure the metabolites between 26 and 28 weeks of gestation, and cord blood samples were obtained at birth. Urine samples were analyzed for metabolite levels at the Centers for Disease Control (CDC) prior to January 2001. Study authors indicated that laboratory QA/QC procedures were carried out in this study. Detailed interviews were also conducted during the third trimester of pregnancy by a bilingual interviewer (English/Spanish) to obtain information regarding demographics, health histories, past pregnancies, and pesticide exposures. The outcome was broadly defined as fetal growth changes at delivery, and was further defined and measured as birth weight (grams), length (centimeters), head circumference (centimeters), and ponderal index (grams/centimeters^3), and gestational age (weeks), and confirmed using the hospital database. A linear regression model was used to estimate fetal growth changes (further defined above) at delivery from several pesticide metabolites including MDA, the malathion-specific metabolite, adjusting for, maternal age, gestational age (except for gestational age outcome), maternal BMI * infant weight gain (median quantiles) and race/ethnicity. Reported results below are creatinine adjusted.

- For <u>birth weight</u>, no evidence of a significant association was reported between prenatal exposure to MDA and birth weight among newborns (birth weight β±SE: 59 ± 53 grams, p-value = 0.27).

[89] Additional study details were found in: Berkowitz et al., 2003, 2004.

- For crown-heel length, no evidence of a significant association was reported between prenatal exposure to MDA and crown-heel length among newborns (length β±SE: -0.032 ± 0.30 centimeters, p-value = 0.91).

- For ponderal index, no evidence of a significant association was reported between prenatal exposure to MDA and ponderal index among newborns (ponderal index β±SE: 0.035 ± 0.036 g/cm^3, p-value = 0.33).

- For head circumference, no evidence of a significant association was reported between prenatal exposure to MDA and head circumference (head circumference β±SE: 0.23 ± 0.20 centimeters, p-value = 0.25).

- For gestational age, no evidence of a significant association was reported between prenatal exposure to MDA and gestational age (gestational age β±SE: -0.30 ± 0.22 weeks, p-value = 0.16).

  The overall quality of the study was ranked moderate based on the study quality criteria outlined in the OPP Framework. Wolff et al. (2007) used a prospective cohort design to assess the relationship between birth effects including fetal growth changes in newborns and malathion exposure measured through the MDA urinary metabolite. Study strengths included the study design, the use of hospital data to confirm the outcome, and the use of laboratory QA/QC methods. Study limitations included the single urinary sample taken during once pregnancy to assess malathion exposure and the potential for exposure misclassification due the transient and variable nature of exposures to pesticides. Furthermore, pesticides metabolites usually stay within the body for a short amount of time, making it challenging to measure exposure precisely. 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. Additionally, selection bias was possible since the people who participated in the study may have been more likely to frequent hospitals and medical centers which may not be reflective of the general population. The study was ranked moderate quality for regulatory purposes.

- Sathyanarayana et al. (2010) investigated in a cross-sectional analysis of the AHS prospective cohort, the potential association between maternal exposure to pesticides including malathion and subsequent birth weight among their offspring. The study population consisted of female spouses enrolled in the AHS who had given singleton[90] birth within five years of study enrollment and had complete information on all covariates (n = 2,246). Spouses completed self-administered questionnaires within one month of husband's enrollment. The spouse questionnaire was used to assess pesticide exposure and the family health questionnaire was used to obtain detailed information regarding the most recent pregnancy and pregnancy outcome and activities during early pregnancy including pesticides. Using this response data, overall pesticide exposure was first categorized based on pesticide-related tasks as one of the following: no exposure, indirect exposure, residential exposure, or agricultural exposure[91] during the first trimester of pregnancy for each study participant. Individual pesticide exposures were then assessed based on ever/never use. Data on temporal specificity of individual pesticide exposures

[90] Singleton birth defined as a birth event that resulted in a single, live born child.

[91] Exposure categories: No exposure = women who answered negatively to all exposure questions; indirect exposure = pruning, picking, harvesting, or weeding; residential exposure = applying pesticides within the home or garden; agricultural exposure = applying or mixing pesticides to crops or fixing pesticide application equipment.

(including malathion) during the most recent pregnancy were not available and exposure during the first trimester of pregnancy could not be further characterized. The outcome was defined as birth weight, a continuous variable, in grams for their most recent birth. Linear regression was used to estimate the association between pesticide exposure and change in birth weight,[92] adjusted for maternal BMI at enrollment (considered both as BMI and BMI squared), height, parity, smoking (ever smoked during pregnancy), state of residence, and preterm status (<37 weeks, 37 weeks or more). Of the 2,246 females who reported live birth pregnancies, 1,162 (52%) indicated no exposure to pesticides, and 764 (34%), 278 (12%), and 42 (2%) reported indirect, residential, and agricultural exposures during their first trimester of pregnancy, respectively. No evidence of a significant association was determined in birthweight at each of the four categories of exposure (-72 grams ≤ β ≤ 9 grams; all CIs encompassed the null value of 0; n = 42 – 764 women). No evidence of a significant reduction was reported between mother's ever use of malathion and offspring's birth weight (β = -59 grams; 95% CI: -118, 0.50 grams; with n = 307 exposed participants).

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The cross-sectional study design was the main limitation since temporality for exposure in relation to the outcome could not be determined, thus the study was ranked low quality for regulatory purposes.

- Ling et al. (2018) investigated the association between malathion and other pesticides with preterm birth among infants born in households within 2 km of agricultural fields in the state of California. The study population consisted of children born in California's agricultural regions between 1998 and 2010, that were randomly selected from California birth records. Pesticide exposure was assessed by combining California's Pesticide Use Reports (PUR), land use maps, and geocoded birth addresses. Of the 24,693 cases of preterm births and 220,297 non-cases of preterm births included in the analysis, 23.6% (n=5,696) of cases and 23.3% (n=51,530) of non-cases were exposed to malathion in the first trimester of pregnancy. Of the 24,693 cases of preterm births and 220,297 non-cases of preterm births included in the analysis, 23.6% (n=5,715) of cases and 23.3% (n=51,429) of controls were exposed to malathion in the second trimester of pregnancy. Logistic regression was used to calculate ORs and 95% CIs to estimate the exposure between malathion never/ever exposure and pre-term birth rate for first and second trimester, adjusting for year of birth, sex of infant, maternal age, maternal education, maternal race/ethnicity, parity, prenatal care in first trimester, payment type of prenatal care, maternal birthplace, and neighborhood SES. No evidence of a significant positive association was reported between malathion exposure in the first trimester of pregnancy to pre-term birth in the analysis adjusted for infant's year of birth and sex only (OR:1.01; 95%CI: 0.98, 1.04, n=5,696 exposed cases) and no evidence of a positive association in the analysis adjusted for year of birth, infant sex, maternal age, maternal education, maternal race/ethnicity, parity, prenatal care in first trimester, payment type of prenatal care, maternal birthplace, and neighborhood SES (OR:0.99; 95%CI: 0.96, 1.03, n=5,696 exposed cases). Similarly, the investigators examined the second trimester of pregnancy and reported no evidence of a significant positive association between malathion exposure in the second trimester of pregnancy and pre-term birth in an analysis adjusted for infant's year of birth and sex only (OR:1.02; 95%CI: 0.99, 1.05, n=5,715 exposed cases) and no evidence of a positive association in the analysis adjusted for year of birth, infant sex, maternal age, maternal education, maternal race/ethnicity, parity, prenatal care in first trimester, payment type of prenatal care, maternal birthplace, and neighborhood SES (OR: 1.00; 95% CI: 0.97, 1.03; n=5,715 exposed cases).

[92] Change in birth weight was reported as a multiple regression estimate coefficient with an associated 95% CI in grams.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The primary strength of the study was that it was able to systematically identify cases using the California birth defect registry and randomly select controls for all healthy births registered in California. The exposure assessment approach used geospatial information on California pesticide use records. This approach helped to minimize recall bias that would be associated with using a questionnaire. However, the investigators provided no information to demonstrate that this approach can reliably estimate the individual-level exposure of mothers.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between maternal malathion exposure and several birth effects in children. Four studies (Eskenazi et al., 2004; Wolff et al., 2007; Sathyanarayana et al., 2010; Ling et al., 2018) evaluated the association between malathion exposure and various birth effects including head circumference, birth weight, length of gestation, crown-heel length, preterm birth, and ponderal index among children in New York City, in the AHS in Iowa and North Carolina, and in California. Two of these studies (Eskenazi et al., 2004; Wolff et al., 2007) evaluated the association between prenatal exposure to malathion and birth effects among newborns in California and in New York City including head circumference, birth weight, length of gestation, crown-heel length, and ponderal index. Prenatal exposure was measured through detectable levels of the urinary malathion metabolite, MDA. In both studies, no evidence of a significant association was reported between MDA and length of gestation, crown-heel length, ponderal index, head circumference, and birth weight. Both studies were ranked moderate quality for regulatory purposes. Study limitations included recall bias, potential exposure misclassification due the transient and variable nature of exposures to pesticides, and the single urinary sample taken once during pregnancy to assess malathion exposure (Wolff et al., 2007 only). The third study, Sathyanarayana et al. (2010), examined the association between maternal malathion exposure and birth weight in their children among the AHS population and reported no evidence of a significant association between mother's ever use of malathion and offspring's reduced birth weight. The study quality was ranked low for regulatory purposes due to the cross-sectional study design since temporality for exposure in relation to the outcome could not be determined. And finally, Ling et al. (2018), investigated the association between malathion and other pesticides with preterm birth rates in agricultural regions in California, and reported no evidence of a significant positive association between malathion exposure in the first and second trimesters of pregnancy to pre-term birth rates when the reported risk estimates were adjusted for infant's year of birth and sex only. The study was ranked moderate for regulatory purposes due to the study's use of PUR data at the county-level to determine exposure; the investigators provided no information to demonstrate that this approach can reliably estimate the individual-level exposure of mothers.

Cerebral Palsy

One study (Liew et al., 2020) examined the association between malathion exposure during pregnancy and cerebral palsy among young children.

Liew et al. (2020) evaluated the association between prenatal exposure to pesticides, including malathion, and cerebral palsy in children using data from a case-control study design. Cases included children born between 1998 – 2010 who were ≤ 3 years of age, living in the state of California, diagnosed with cerebral palsy (CP) according to the California Department of Developmental Services (DDS), and living at one of

the DDS regional facilities maintained by the state.[93] Cases were ascertained using records held by the California DDS, and were identified through the linkage of birth records and DDS data maintained by the state. A random subset of controls, who were selected from birth record data for the state of California, were included in this study. A 1:10 ratio of cases to controls was used in addition to the cases being matched to the controls by gender and birth year. The controls selected for this study were part of a larger group of controls that were initially used for an autism study, preventing the controls from having an autism or CP diagnosis according to the DDS records by 2013. Maternal exposure to pesticides including malathion was determined based on pesticide applications that were made within a residential proximity of 2 km as reported by the California Department of Pesticide Use Reporting (PUR). Specifically, a geographic information system-based residential ambient pesticide estimate system combined California's PUR data, land use maps maintained by the California Department of Water Resources (CDWR), and the geocoded maternal residential addresses obtained from birth records to produce an estimate of pesticide exposures on a monthly-basis during pregnancy.[94] The monthly estimates for each pesticide were determined by taking the total amount of pesticide applied (measured in pounds) within a 2 km buffer zone of the maternal residence, and weighting that amount by the proportion of area treated with pesticides within the 2 km buffer area. Exposure estimates for each pesticide were calculated during each trimester of pregnancy defined as the following: 1st trimester (0 – 12 weeks of gestation), 2nd trimester (13 – 25 weeks of gestation), and 3rd trimester (26 – 37 weeks of gestation). Unconditional logistic regression was used to assess the relationship between prenatal exposure to select pesticides, including malathion, and CP in children and odds ratios (ORs) were calculated with corresponding 95% confidence intervals. Variables were selected based on literature review. Adjusted ORs were adjusted for birth year, mother's race, maternal education, maternal age, DDS regional center, and maternal birthplace. Adjusted ORs were also co-adjusted within the same model for prenatal exposure to other pesticides during the first trimester of pregnancy. Among the 1,661 *female* total cases and 16,924 *female* total controls, 443 of the cases and 4,013 of the controls reported exposure to malathion. Among the 2,244 *male* total cases and 22,453 *male* total controls, 529 of the cases and 5,361 of the controls reported exposure to malathion. No evidence of a significant positive association was reported between prenatal exposure to malathion and CP among *female* children in either the adjusted or co-adjusted models (adjusted OR: 1.10; 0.97, 1.24; co-adjusted OR: 1.13; 95% CI: 0.99, 1.30). Similarly, among *male* children, no evidence of a significant positive association was observed between prenatal exposure to malathion and CP in either the adjusted or co-adjusted models (adjusted OR: 0.95; 0.86, 1.06; co-adjusted OR: 1.04; 95% CI: 0.92, 1.17).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the strengths of the case-control study design, case ascertainment using state records and birth certificate data, the case-control matching, and the objective measure of exposure thus removing potential for recall bias. The primary limitation of the study was that it relied on a geospatial approach to assess pesticide exposure based on residential address, PUR data, and land use data on malathion. While this approach helps minimize recall bias, the method relied on a 2 km buffer to assign exposure based on distance to agricultural land where malathion was reported to have been applied instead of relying on study participants to provide exposure data. This approach has not been

[93] The DDS defines cerebral palsy as "(1) a non-progressive lesion or disorder in the brain occurring during intrauterine life or the perinatal period and characterized by paralysis, spasticity, or the abnormal control of movement or posture that is manifest before 2 or 3 years of age, and (2) other significant motor dysfunction appearing before the age of 18 years." (Liew et al. 2020)

[94] Ling, C., Liew, Z., Von Ehrenstein, O. S., Heck, J. E., Park, A. S., Cui, X., ... & Ritz, B. (2018). Prenatal exposure to ambient pesticides and preterm birth and term low birthweight in agricultural regions of California. *Toxics*, *6*(3), 41.
Roberts, E. M., English, P. B., Grether, J. K., Windham, G. C., Somberg, L., & Wolff, C. (2007). Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. *Environmental health perspectives*, *115*(10), 1482-1489.

validated so it is unclear if residence within 2 km of agriculture land can provide a reliable estimate of maternal exposure during pregnancy. Additionally, the study did not account for possible residential mobility[95] of mothers between pregnancy and childbirth with residency geocoded 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, possibly causing exposure misclassification. Additional limitations included live birth bias since it was possible fetuses exposed to higher levels of pesticides may not have survived to full-term gestation so certain effects were not observed, and the lack of information regarding parental employment prevented the study authors from being informed about potential parental occupational pesticide exposures. As a result, this could have underestimated total exposures among the study participants

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between prenatal exposure to malathion and CP among children. One study, Liew et al. (2020), examined this association and reported no evidence of a significant positive association for pregnant women who resided in a 2 km buffer zone of malathion applications and CP in both female and male children. Liew et al. (2020) was ranked moderate quality for regulatory purposes due the study's reliance on a geospatial approach to assess pesticide exposure based on residential address, PUR data, and land use data on malathion, as well as the inability to account for residential mobility of mothers, and the potential underestimation of total pesticide exposures among the study participants.

Depression

Two studies (Beard et al., 2013; Beard et al., 2014) examined the association between malathion exposure and depression.

- Beard et al. (2013) investigated the potential association between pesticide exposure, including malathion, and incident depression among wives of farmers enrolled in the AHS prospective cohort. The study population consisted of female spouses (n = 16,893) enrolled in the AHS living in Iowa and North Carolina with no history of physician-diagnosed depression at enrollment, and with complete data on depression at enrollment and complete covariate data. Cases included farmers' wives who self-reported incident depression between the time of study enrollment (1993-1997) through study follow-up (2005-2010), and cases were ascertained through responses to questions during the telephone follow-up interview. The non-cases included farmer's wives who did not report incident depression. Exposure was assessed during study enrollment for 50 different pesticides including malathion using self-administered questionnaires. Of the 1,054 cases, 203 (20%) reported exposure to malathion. The association between malathion ever use, and indirect exposure through farmer's use of malathion and incident depression among farmers' wives was estimated using log-binomial regression to determine RRs and 95% CIs. Inverse probability weights were applied to adjust for education level, age at enrollment, ever diagnoses with diabetes, state of residence, and drop out, as well as to account for the substantial number of women (n = 10,639) within the study population who did not complete a follow-up interview (1,342 due to death). No evidence of a positive association was reported for wives' malathion ever use and self-reported incident depression (RR = 0.96; 95% CI: 0.82, 1.12; with n = 203 exposed cases,) and no evidence of a positive

[95] Past studies have indicated that around 11 – 32 % of pregnant women move their residence at least one time throughout pregnancy, and the median move distances were between 4.2 – 10 km (Lupo et al., 2010; Strickland et al., 2017; Pereira et al., 2016)

association was reported for husband's ever use of malathion and self-reported incident depression among wives' who never used malathion (RR = 1.00; 95% CI: 0.81, 1.23; with 286 (71%) cases with indirect exposure).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design and exposure assessment approach which examined lifetime exposure to malathion. However, the outcome was self-reported without medical record validation and pesticide exposure was self-reported which may have introduced exposure misclassification and was limited to ever use. Information on frequency and duration of use of pesticides was not available for wives.

- Beard et al. (2014) investigated potential association between pesticide exposure, including malathion, and self-reported depression among male pesticide applicators in the AHS prospective cohort. The study population included male pesticide applicators, enrolled in the AHS between 1993 – 1997, who also completed a follow up telephone interview between 2005 – 2010. Participants self-reported physician diagnoses of depression prior to enrollment only, at both enrollment and follow-up, or at follow-up only. Pesticide exposure was assessed via two self-administered questionnaires, completed during study enrollment and during the follow-up interview (2005-2010). Polytomous logistic regression was used to calculate ORs and 95% CIs for malathion. Inverse probability weighting adjusted for confounders including age, diabetes, education level, and state of residence, and accounted for subjects missing covariate data 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 a diagnosis of depression at enrollment but not follow-up, and 369 (80%) of those cases reported exposure to malathion. Of the 1,702 cases, 540 participants reported depression diagnosis at both enrollment and follow-up, and 410 (79%) of those cases reported exposure to malathion. Of the 1,702 cases, 688 participants reported depression diagnosis at follow-up only, and 503 (76%) of those cases reported exposure to malathion. There were 19,506 study participants who reported no physician diagnosis of depression, and 13,941 of those non-cases reported exposure to malathion. No evidence of a significant positive association was reported between malathion exposure and risk of depression for those who reported depression at enrollment only (OR = 1.30; 95% CI: 1.00, 1.70), risk of depression for those who reported depression at both enrollment and follow-up (OR = 1.20; 95% CI: 1.00, 1.60), or for those who reported depression at follow-up only (OR = 1.10; 95% CI: 1.00, 1.40).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design and exposure assessment approach which examined lifetime exposure to malathion. The study relied on the self-report of depression diagnosis. Confirmation of cases via medical records would have improved the reliability of the outcome classification 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 malathion exposure and depression. There were two available studies (Beard et al., 2013; Beard et al., 2014) of the AHS cohort that examined the association between malathion exposure and depression among male pesticide applicators and among wives of farmers. Beard et al. (2013) reported no evidence of a positive association for wives' malathion ever use and self-reported incident depression, and no evidence of a positive association based on husband's ever use of malathion as an exposure proxy. Similarly, Beard et al. (2014), reported no evidence of a significant positive association between malathion exposure and depression amongst those who reported depression

at enrollment only, at follow-up only, and at both enrollment and follow-up. Both studies were rated moderate quality and relied on self-reported physician diagnosis of depression rather than clinical or medical record confirmation.

Diabetes

Two studies (Montgomery et al., 2008; Starling et al., 2014) examined the association between malathion exposure and diabetes.

- Montgomery et al. (2008) investigated the association between pesticide exposure, including malathion, and incident diabetes among pesticide applicators in the AHS prospective cohort. The study population consisted of pesticide applicators enrolled in the AHS between 1993 and 1997 (n = 31,787), living in Iowa or North Carolina, who completed both the enrollment (1993-1997) and follow-up (1999-2003) questionnaires and did not report diabetes at enrollment and had complete information on diabetes and important covariates. Incident diabetes was identified via self-report at the 5-year follow-up interview. Pesticide exposure was assessed using self-reported data from the enrollment and follow-up questionnaires. Of the 52,393 applicators enrolled in the AHS, 33,457 (64%) provided updated information about their medical conditions on the follow-up survey. Of these, an additional 1,330 participants were excluded due to reported diabetes at enrollment, and 330 were excluded due to missing information on diabetes (n =238) or an important covariate (n = 102). Among the 1,176 diabetic cases, 434 (43%) reported ever use of malathion, and among the 30,611 non-cases with complete data, 14,639 (53%) reported ever use of malathion. The association between malathion exposure and diabetes was assessed using logistic regression to estimate ORs and 95% CIs, adjusted for age, state of residence, and body mass index (BMI). No evidence of a significant positive association was reported between malathion and diabetes (OR = 1.05; 95% CI: 0.91, 1.21; with n = 766 exposed cases and n = 20,397 exposed non-cases) based on ever use when adjusted for age only. Further adjusting the model for state of residence and BMI in addition to age, no evidence of a significant positive association was reported (OR = 1.10; 95% CI: 0.95, 1.27; with n = 766 exposed cases and n = 20,397 exposed non-cases).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The prospective cohort study design as part of the AHS and the detailed pesticide exposure assessment were strengths. 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. Also, due to a substantial proportion of applicators who did not complete in the follow-up questionnaire (36%), a potential of selection bias could be present if the follow-up status was associated with both diabetic incidence and malathion exposure (i.e., differential loss-to-follow-up).

- Starling et al. (2014) investigated the potential association between pesticide exposure, including malathion, and diabetes among wives of farmers in the AHS study. The study population included female spouses (n=13,637) of farmers who were part of the AHS, living in Iowa and North Carolina who reported ever mixing or applying pesticides prior to enrollment (1993 - 1997), completed at least one of the two follow-up interviews at 5-years or 10-years (2005 – 2010) after enrollment (N=15,034), self-reported a physician-diagnosis of diabetes after enrollment and who had complete information on BMI. Pesticide exposure was assessed using data gathered on enrollment questionnaires. Of the total 688 cases, 272 (41%) reported exposure to malathion, and of the 12,949 non- cases, 4,650 (36%) reported malathion exposure. Cox proportional hazard regression models were used to calculate HRs and 95% CIs to analyze the association between ever use of malathion

and incident diabetes among wives of farmers in the AHS, adjusting for BMI at enrollment and state of residence. No evidence of a significant positive association was reported between malathion ever use and incident diabetes in women (HR=1.05; 95% CI: 0.90, 1.23).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. 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.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and diabetes. Montgomery et al. (2008) reported no evidence of a significant positive association between ever use of malathion and diabetes among pesticide applicators and Starling et al. (2014) reported no evidence of a significant positive association among wives of pesticide applicators. Both studies were ranked moderate for regulatory purposes. Self-reported diagnosis of diabetes among the study participants and the inability to control for diet and exercise were considered study limitations in both studies and may have resulted in misclassification of some of the observed results and/or errors induced by confounding, respectively.

Dream Enacting Behaviors

One epidemiologic study (Shrestha et al., 2018a) was identified that assessed exposure to malathion and dream enacting behaviors (DEB) among farmers enrolled in the AHS.

Shrestha et al. (2018a) examined the association between pesticide exposure, including malathion, and DEB using data from the AHS cohort. The study population included male private pesticide applicators in the AHS living in Iowa and North Carolina who completed a follow-up interview (2013 – 2015) that screened for several Parkinson's disease prodromal symptoms including DEB. AHS participants self-reported information on DEB in response to "Have you ever been told, or suspected yourself, that you seem to 'act out dreams' while sleeping?" If they answered yes, they were prompted to answer additional questions on the frequency of symptoms. Participants self-reported physician-diagnosed Parkinson's disease during follow-up interviews and cases of DEB were validated using medical record data. Information on head injury was obtained from a more detailed take-home questionnaire completed shortly after enrollment (1993 – 1997) and the Phase II follow-up questionnaire completed five years after enrollment (1999-2003). Among the 20,591 male private applicators included in the analysis, 1,623 (7.9%) self-reported DEB during the follow-up interview and 1,001 of these also reported experiencing DEB symptoms three or more times. Pesticide exposure was reported through self-administered questionnaires completed at enrollment. Among the 1,623 cases, 1,042 DEB cases reported exposure to malathion. Multivariable logistic regression was used to assess the relationship between pesticide exposure and DEB, adjusting for age, smoking, alcohol consumption, marital status, education, state of residence, and head injury. To make inferences about all male farmers who enrolled in the study, authors used inverse probability weighting to account for the loss of participants and for missing information on covariates as only 23,478 (46%) of the 51,035 male private applicators in the AHS completed the follow-up survey (2013-2015). Cases were compared with cohort members who also completed the follow-up interview but did not report DEB (n=16,441). No evidence of a positive association was reported between malathion ever use and DEB among male pesticide applicators (OR=1.00; 95% CI: 0.90, 1.20; with n=1,042 exposed cases). Similarly, no evidence of a positive association was reported between malathion exposure and DEB among pesticide applicators who reported three or more episodes of DEB (n=17,321), (OR=1.00; 95% CI: 0.90, 1.20, with n=495 exposed cases). And finally, no evidence of a positive

association was reported between malathion ever use and DEB when PD patients were excluded (OR=1.00; 95% CI: 0.90, 1.20, with n=740 exposed cases).

The overall study quality was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the cohort study design and the reliability of the AHS questionnaire to ascertain pesticide exposure. While the study had several strengths, it was determined to be of moderate quality because of limitations in the ascertainment of the outcome and the potential risk of selection bias due to loss to follow-up. Ascertainment of the outcome relied on self-report by survey participants and may have introduced misclassification if participants cannot reliably report that their symptoms are consistent with typical prodromal PD symptoms. Given that the study was prospective, this source of outcome misclassification is likely to be non-differential because study subjects provided information on pesticide use before reporting DEB during Phase IV follow-up in 2013-2015. Loss to follow-up is another important limitation because only 46% of the study subjects originally enrolled completed the Phase IV survey in 2013-2015. This may introduce selection bias if study subject's status of participation in the follow-up phases is related to both their disease status for DEB and their malathion exposure.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and DEB among farmers enrolled in the AHS. One available study (Shrestha et al., 2018a) assessed the association between malathion and DEB among farmers in the AHS and reported no evidence of a positive association among pesticide applicators. The overall quality of the study was ranked moderate. Study limitations included the self-reported outcome and the potential risk of selection bias if study subject's status of participation in the follow-up phases was related to both their disease status for DEB and malathion exposure.

End Stage Renal Disease

Two studies (Lebov et al., 2015; Lebov et al., 2016) examined the association between malathion exposure and end stage renal disease (ESRD).

- Lebov et al. (2015) evaluated the association between pesticide exposure, including malathion, and ESRD. The study population consisted of female spouses of pesticide applicators enrolled in the AHS. ESRD cases were identified through linkage with the US Renal Data System and first renal replacement therapy (i.e., dialysis initiation or renal transplantation) date was used to identify ESRD cases occurring between study enrollment (1993-1997) and end of follow-up (December 31, 2011). Direct and indirect pesticide (husbands' exposure among wives with no exposure) exposure was assessed using information obtained via self-administered questionnaires completed at enrollment; results for direct exposure to malathion (wives' personal use) were not reported, and the number of cases and non-cases with direct malathion exposure was not reported. Among the 64 ESRD cases and the 13,653 non-cases with indirect exposure to pesticides who reported no prior use, 36 (64.3%) cases and 8,793 (70.2%) non-cases reported indirect exposure to malathion. Cox proportional hazards models were used to calculate HRs and 95% CIs for the association between malathion and ESRD among female spouse of pesticide applicators adjusting for state and age. No evidence of a positive association was reported between indirect exposure to malathion and ESRD among these female spouse of pesticide applicators (HR= 0.71; 95% CI: 0.41, 1.22; with n=36 exposed cases). In an additional analysis that considered the association between husbands' cumulative lifetime use of malathion and ESRD among wives who reported no direct pesticide exposure, husband's malathion lifetime exposure was divided at the median at the following cut points: 1.0 – 13.5 days of use and >13.5-217.0 days of use. No evidence of a significant positive association was reported for female

spouses' indirect malathion exposure at either lower exposure level (*1.0 – 13.5 days of use* – HR=1.18; 95% CI: 0.47, 2.93; with n=8 exposed cases), higher exposure level (*>13.5-217.0 days of use* – HR=0.87; 95% CI: 0.35, 2.16; with n=8 exposed cases), or an exposure-response trend (p-trend >0.05), with the non-exposed group as the referent.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The general strengths of the study were the underlying prospective design of AHS and availability of a U.S. registry to comprehensively identify ESRD cases. Study limitations included the indirect assessment of pesticide exposure for applicator wives using husband use information as a surrogate. This approach has not been validated and may not be a reliable proxy for direct exposure by female spouses. Lastly, we note the very small numbers of exposed cases being used in some of the estimated HRs, which limits the ability to interpret with confidence the observed hazard ratios.

- Lebov et al. (2016) evaluated the association between pesticide exposure, including malathion, and ESRD among male pesticide applicators enrolled in the AHS prospective cohort. The study population included male pesticide applicators, enrolled in the AHS (1993 – 1997) living in Iowa and North Carolina who were >18 years old. ESRD cases were identified from enrollment through follow-up (December 31, 2011) by linking the AHS cohort data with the United States Renal Data System. Pesticide exposure was assessed via self-administered questionnaires administered at enrollment and shortly thereafter at home. Among the 24,429 with this exposure information, 136 ESRD cases were identified and of these, 83 cases reported malathion exposure. Lifetime pesticide exposure was modified by an intensity factor to account for the variation in pesticide application practices to produce an estimate of intensity-weighted lifetime days of exposure for including malathion. An investigation of the association between intensity-weighted lifetime days of use of malathion and ESRD among applicators, was conducted with the following tertiles used as cut-points: <644 days of use, ≥644 – <1792 days of use, and >1792 days of use, with the no exposure group as the referent. Cox proportional hazards models were used to calculate HRs and 95% CIs for the association between malathion and ESRD among male pesticide applicators, adjusting for state and age. No evidence of a significant positive association was reported between malathion exposure and ESRD among male pesticide applicators at any exposure category and no evidence of a significant exposure-response trend, with the no exposure group as the referent (0.87 <HR <1.44; all 95%CIs encompassed the null value of 1.0; n=27 - 28 exposed cases and n=3,626– 6,577 exposed non-cases; p-trend= 0.874).

  The overall quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The general strengths of the study were the underlying prospective design of AHS, the exposure assessment, and the availability of a U.S. registry to comprehensively identify ESRD cases.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and ESRD. Two studies investigated the association between malathion and ESRD among the AHS study population. Lebov et al. (2015) reported no evidence of a significant positive association between indirect malathion exposure (through husband's exposure) and end-stage renal disease (ESRD) among the female wives of pesticide applicators enrolled in the AHS. The overall quality of the study was ranked moderate with the primary limitation being the indirect assessment of pesticide exposure for applicator wives using husbands' use information as a surrogate. This approach has not been validated and may not be a reliable proxy for direct pesticide

exposure. We also note that the number of exposed cases was very small which severely restricts the ability to interpret with confidence some of the observed HRs as well as the ability to assess the exposure-response relationship. Lebov et al. (2016) reported no evidence of a significant positive association between malathion exposure and ESRD among male pesticide applicators, based on intensity-weighted lifetime days of exposure with the no exposure group as the referent. The overall quality of the study was ranked high. The general strengths of the study were the underlying prospective design of AHS, the exposure assessment, and the availability of a U.S. registry to comprehensively identify ESRD cases.

Endometriosis

One study (Li et al., 2020) assessed the association between malathion exposure and endometriosis among women.

Li et al. (2020) examined the association between pesticide exposures, including malathion, determined via specific urinary biomarkers and endometriosis among women using a prospective cohort design of the Endometriosis Natural History, Diagnosis, and Outcomes (ENDO) Study. The cohort was a pool of 626 women from an *operative* cohort and a *population* cohort (as called by the authors); and women were excluded from the cohort if they met one of the following: breastfeeding less than 6 months; use of an injectable hormone treatment within the past two years; cancer history besides nonmelanoma skin cancer; a confirmed history of laparoscopic endometriosis; or primary language was not English or Spanish. Additional study details described below were previously included in Buck Louis et al. (2011).[96] The *operative* cohort included 495 menstruating women living in Salt Lake City, Utah or San Francisco, California, aged 18 – 44 years, who were scheduled for a laparoscopy or laparotomy at one of 14 participating surgical centers in Salt Lake City, Utah, or San Francisco, California (five locations in Utah, nine locations in California) between 2007 and 2009; and the *population* cohort included 131 women, who were scheduled to undergo a standardized pelvic magnetic resonance imaging (MRI) for the assessment of endometriosis, matched to the women in the operative cohort by age and residence (within 50 miles of the participating surgical centers). Of the 495 women in the *operative* cohort, 3 women had non-detectable/limited urine volume and 21other women did not undergo laparoscopy or laparotomy. Among 471 women who underwent laparoscopy or laparotomy, 188 (40%) were diagnosed with endometriosis. Of the 131 women in the *population* cohort, 4 women had non-detectable/limited urine volume and 4 other women did not undergo pelvis MRI. Among 123 women who underwent pelvis MRI, 14 (11%) were diagnosed with endometriosis. Therefore, among the total of 594 women who underwent laparoscopy, laparotomy, or MRI, there were 202 (34%) women diagnosed with endometriosis.
Study participants in both cohorts provided demographic information via a telephone or in-person interview about 2 months before the laparotomy, laparoscopy, or MRI procedures. Blood and urine samples were obtained along with two self-administered screening instruments. Individual urine samples obtained at enrollment were used to assess pesticide exposure, including malathion, via detection and quantification of pesticide metabolites in the collected urine samples using an ultrahigh performance liquid chromatography paired with tandem mass spectrometry. The malathion-specific metabolite measured by the study authors in this study was malathion dicarboxylic acid (MDA). Urine samples were kept frozen (-20°C) until analyzed, and the study authors mentioned residual urine samples analyzed from a past study[97] were used in this study to measure pesticide metabolite concentrations. The limit of detection for MDA metabolites was 0.004 ng/mL. The specific urinary metabolite of malathion, MDA, had a detection frequency of 97.6% in this study, and a median concentration of 0.22 ng/mL (<LOD-23

[96] Louis, G. M. B., Hediger, M. L., Peterson, C. M., Croughan, M., Sundaram, R., Stanford, J., ... & ENDO Study Working Group. (2011). Incidence of endometriosis by study population and diagnostic method: the ENDO study. *Fertility and sterility*, *96*(2), 360-365.

[97] Mumford, S. L., Weck, J., Kannan, K., & Buck Louis, G. M. (2017). Urinary phytoestrogen concentrations are not associated with incident endometriosis in premenopausal women. *The Journal of nutrition*, *147*(2), 227-234.

ng/mL). A total of 619 urine samples were collected with 520 of the samples collected from Utah and 99 of the samples from California sites, and MDA concentrations were significant higher in Utah than in California ($p < 0.05$). Laboratory QA/QC procedures were carried out and included the use of procedural blanks as well as testing the accuracy and precision of analysis of urine samples. The operating surgeons performing such procedures completed an operative report immediately following surgery to provide gynecologic and pelvic pathology, and the Revised American Society for Reproductive Medicine classification was used to determine endometriosis staging (stage 1 (minimal) to stage 4 (severe).[98] Surgeons were also asked to obtain additional biospecimens during surgery for histology purposes. A random sample (n = 96) selected *a priori* of women from the operative cohort and all women from the population cohort, also underwent a pelvic magnetic resonance imaging (MRI) to determine endometriosis and any other pathological findings within the pelvic area. All randomized MRIs were double-read by trained radiologists, and women were required to follow-up when their MRI pathology screenings indicated further clinical attention. In this study, approximately 11% of women from the operative cohort and 14% of women from the population cohort reported follow-up. Log-transformed concentrations were categorized into four quartiles and unconditional logistic regression was used to calculate ORs and 95% CIs (the 1st quartile was used as the reference) for the association between pesticide exposure and endometriosis, adjusted for race, parity, race/ethnicity, household income, drinking, smoking, surgical site, age, and urinary creatinine (µg/g). The four quartiles of MDA metabolite urinary creatine-adjusted concentrations were: <0.004 – 0.12 µg/g creatinine, 0.13 - 0.24 µg/g creatinine, 0.25 – 0.49 µg/g creatinine and 0.50 – 24.30 µg/g creatinine. No evidence of a significant positive association was observed between any exposure quartile of malathion exposure (urinary concentration ng/mL) and endometriosis among all participants ($0.80 \leq$ adjusted ORs ≤ 1.11; all CIs encompassed the null value of 1.0) and a significant exposure-response trend was reported (p-trend <0.001); however, it is unclear the direction of the trend, given the reported ORs of the quartiles in article. Similar results were reported for the analyses where each cohort was considered separately. When cases were restricted to more severe endometriosis (stages III and IV, n = 333) in the operative cohort, no evidence of a significant positive association was reported between malathion exposure and endometriosis in any quartile of malathion exposure ($0.61 <$ adjusted ORs < 1.35; all 95% CIs encompassed the null value 1.0; p-trend =0.002) and a significant exposure-response trend was reported. Finally, when cases were restricted to those by inclusion of parity conditional on gravidity (n=469), no evidence of a significant positive association was reported between malathion exposure and endometriosis, in any exposure quartile of malathion ($0.71 <$ adjusted ORs < 1.09; all 95% CIs encompassed the null value 1.0; p-trend <0.001) and a significant but inverse exposure-response trend was reported.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The overall strengths of the study included the prospective cohort design, the laboratory QA/QC procedures to determine exposure, and the extensive measures used to determine the outcome. Study limitations included the use of a single urine sample to determine past pesticide exposure(s). Also, the study authors mentioned the urine samples used in this study had been used for analysis in a previous study causing potential concern for urine sample contamination and inaccurate metabolite measurements in this study.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and endometriosis including AMD. One available study Li et al. (2020) examined the association between pesticide exposures, including malathion,

[98] Canis, M., Donnez, J. G., Guzick, D. S., Halme, J. K., Rock, J. A., Schenken, R. S., & Vernon, M. W. (1997). Revised american society for reproductive medicine classification of endometriosis: 1996. *Fertility and Sterility*, *67*(5), 817-821.

determined via specific urinary biomarkers and endometriosis among women using a prospective cohort design of the Endometriosis Natural History, Diagnosis, and Outcomes (ENDO) Study. No evidence of a significant positive association was observed between malathion exposure for MDA urinary creatinine-adjusted concentrations and for MDA unadjusted urinary creatinine-adjusted concentrations. A statistically significant ($p < 0.05$) dose-response trend was observed in the operative cohort and in both cohorts combined; however, the direction of the trend is not clearly stated in the study. This study quality was ranked moderate for regulatory purposes. Strengths of the study included the prospective cohort design, the laboratory QA/QC procedures to determine exposure, and the extensive measures used to determine the outcome. Study limitations included the use of a single urine sample to determine past pesticide exposure(s) and potential concern for urine sample contamination and inaccurate metabolite measurements in this study.

Eye Disorders

Two studies (Kirrane et al., 2005; Montgomery et al., 2017) assessed the association between malathion exposure and eye disorders among wives of pesticide applicators.

- Kirrane et al. (2005) investigated the association between pesticide exposures, including malathion, and retinal degeneration and other eye disorders among wives of AHS pesticide applicators using a cross-sectional analysis of the AHS prospective cohort. The study population included wives of pesticide applicators living on a farm in Iowa and North Carolina who completed the spouse's questionnaire. Physician-diagnosis of retinal degeneration was self-reported on the spouse's questionnaire as was pesticide exposure. A total of 31,173 women self-reported both exposure (wives ever use of pesticides) and outcome (eye disorders) on questionnaires completed at enrollment (1993 – 1997). Logistic and hierarchical logistic regression modeling were used to evaluate potential associations between malathion exposure and eye disorders, adjusting for age and state of residence. The authors reported 23.8% (~ 66 – 67) of the 281 cases of eye disorders and 19.5%[99] of non-cases were exposed to malathion. No evidence of a positive association was reported between malathion exposure and eye disorders, based on ever use (OR = 1.00; 95% CI: 0.60, 1.40).

  The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS including the prospective design, and the exposure assessment approach which examined cumulative lifetime exposure to malathion. However, because of the cross-sectional study design, it was impossible to determine temporality and the study was thus ranked low quality for this reason.

- Montgomery et al. (2017) conducted a nested case-control study among the AHS study population as a follow-up study to Kirrane et al., (2005) to determine if incident cases of age-related macular degeneration (AMD) were associated with previous pesticide exposure including malathion. The study population included pesticide applicators and their spouses, enrolled in the AHS prospective cohort, who completed both enrollment (1993 - 1997) and follow-up telephone interviews (1999 - 2003 and 2005 – 2010) and were $\geq$ 50 years old on September 1, 2007 (AMD is rare before that age). Cases included AHS study participants (men and women) who self-reported either a physician-diagnosis of AMD during 1994 to 2007 or early signs of AMD. AMD diagnosis was confirmed by review of medical records, and supporting pathology or retinal photographs were reviewed by the study optometrist and ophthalmologist, respectively. Controls were selected from the cohort participants who did not have confirmed or possible AMD. Lifetime days of pesticide exposure was

[99] The total number of noncases was not reported by the study authors (only various ranges) due to missing data. Thus, we are unable to calculate an exact number of noncases exposed to malathion.

assessed via self-report on questionnaires administered at enrollment. The association between malathion exposure and AMD was assessed using logistic regression to determine ORs and 95% CIs, adjusted for age, gender, and smoking. Among the total 161 cases and 39,108 controls, 103 (68%) cases and 19,889 (53%) controls reported exposure to malathion. Evidence of a moderately strong association was reported between malathion and AMD based on ever/never exposure (OR = 2.20 95% CI: 1.50, 3.30 n = 103 exposed cases and n = 19,889 exposed controls). When the data were further stratified by gender, evidence of a moderately strong association was reported between malathion exposure and AMD among men (OR = 2.00; 95% CI: 1.10, 3.70, with n = 76 exposed male cases and n = 15,902 exposed male controls) and evidence of a moderately strong association was reported among females based on ever/never exposure (OR = 2.40; 95% CI: 1.40, 3.90, with n = 27 exposed female cases and n = 3,987 exposed female controls). When incident AMD cases were stratified by early and late AMD and adjusted for age, gender and smoking status, evidence of a moderately strong association was reported for malathion exposure and for *early AMD* and for *late AMD* when compared to controls (Early AMD – OR = 2.80; 95% CI: 1.40 5.40, with n = 40 exposed cases and n = 19,889 exposed controls; Late AMD – OR = 2.00; 95% CI: 1.10, 3.60, with n = 45 exposed cases and n = 19,889 exposed controls). And, when late AMD was compared to early AMD (reference group), no evidence of a positive association was reported (OR: 0.70; 95% CI: 0.30, 1.70, with n = 45 exposed late AMD cases and n = 40 exposed early AMD cases).

In an additional analysis of the cumulative days of malathion exposure among male pesticide applicators where exposure was divided into three exposure categories, >0 - 10 days, >10 – 100, and >100 days of cumulative exposure, with the no exposure category as the referent, evidence of a moderately strong association was reported for the high exposure category (OR = 2.00; 95% CI: 1.10, 3.90; with n = 17 exposed cases, n = 1,352 exposed controls; p-trend 0.093), with the no exposure group as the referent. No evidence of a positive association was reported for any other exposure category ($0.80 \leq OR \leq 0.90$; all 95% CIs encompassed the null value of 1.0; with n = 12 – 15 cases per exposure category).

The quality of the study was ranked high based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including its prospective design, ability to confirm AMD cases through review of medical records, pathology, and retinal photographs, and an exposure assessment approach which examined cumulative lifetime exposure to malathion.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and eye disorders including AMD. There were two available studies that examined eye disorders (Kirrane et al., 2005; Montgomery et al., 2017). Kirrane et al. (2005) reported no evidence of a positive association between malathion exposure and retinal degeneration among wives of farmers in a cross-sectional analysis of the AHS population and was ranked low quality. In an update to Kirrane et al. (2005) that included longer follow-up time and analysis of both pesticide applicators and pesticide applicators wives together and then separately, Montgomery et al. (2017) reported evidence of a moderately strong association between malathion and AMD based on ever/never exposure. For cumulative days of malathion use, evidence of a moderately strong association was seen at the high exposure category only and no evidence of a significant exposure-response relationship was reported among male pesticide applicators in the AHS. Additionally, moderately strong associations were reported between ever use of malathion and AMD among men and AMD among women in the AHS, and among early AMD cases and among late AMD cases in subsequent subanalyses. The study quality was ranked high for regulatory purposes.

Fatal Injury

One study (Waggoner et al., 2013) examined the association between malathion exposure and fatal injury.

Waggoner et al. (2013) investigated the association between specific pesticides, including malathion, and fatal injury among male private pesticide applicators enrolled in the AHS prospective cohort. The study population consisted of AHS male private pesticide applicators (n=51,035) living in Iowa and North Carolina who completed both enrollment questionnaires (1993 – 1997). Fatalities were identified through state death registries and the National Death Index. Cases were defined as any mortality that occurred in an occupational setting, including motor vehicle accidents, from enrollment (1993 – 1997) until the end of follow-up (December 31, 2008) or date of death (whichever was earlier). The non-case group included private pesticide applicators who did not suffer from a deadly injury during the study, regardless of vital status. Pesticide exposure was self-reported on the enrollment questionnaires. Among the total study population (n=51,035), 22,952 (50%) private pesticide applicators reported exposure to malathion. And of the 281 fatal injuries, 210 (71%) reported exposure to malathion. Cox proportional hazards models were used to calculate HRs and 95% CIs for fatal injuries and individual pesticides based on ever/never exposure, adjusted for age and state. No evidence of a positive association was reported between malathion exposure and fatal injury among male private pesticide applicators in the AHS, based on ever/never use (HR: 0.96; 95% CI: 0.75, 1.24; with n=210 exposed cases).

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP framework. While Waggoner et al. (2013) leveraged the AHS's prospective design and mortality data available through the National Death Index, it has important methodological limitations. The original aim of AHS was to examine the association between chronic pesticide exposure and cancer outcomes. In contrast to cancer, fatal injury is an acute event, so it is unclear if self-reported pesticide use at enrollment is a valid measure of exposure during the time interval that preceded fatal injury. The investigators also mention that frequency of pesticide use may be an "indicator" of other activities that could increase the risk of fatal injury. For example, individuals who use more pesticides may also use more complex farm equipment more frequently, increasing the chance of an occupational accident that could lead to death. As such, more definitive information is needed on cause of fatal injury and the contributing events that lead to accidents before any conclusions can be drawn from the AHS study population.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and fatal injury. There was one available study, Waggoner et al. (2013), that reported no evidence of a positive association between malathion exposure and fatal injury among male pesticide applicators in the AHS. The study quality was ranked low. While the prospective study design and collection of mortality data available through the National Death Index were study strengths; however, it is not clear if pesticide use at enrollment is a valid measure of exposure during the time interval that preceded fatal injury, as pesticide use could be more of an indicator of use of complex farm equipment which would increase risk of fatal injury.

Gestational Hypertension

One study (Ledda et al., 2015) evaluated the association between malathion exposure and gestational hypertension.

Ledda et al. (2015) investigated the potential association between pesticide exposure, including malathion, and hypertension among pregnant women using data from a cross-sectional study. The study

population included pregnant women who were ~ 22 weeks gestation, living in Sicily, Italy, and were recruited by primary care or occupational physicians between 2007 and 2013. Women with the following conditions were restricted from the study: hypertension prior to pregnancy; diabetes; anemia; kidney or heart disease; urinary tract infection; toxemia of pregnancy; metabolic disorders; multiple pregnancies; antiphospholipid antibody syndrome; a BMI <19 or > 35 kg/m^2; and women who reported prior complications with pregnancies and/or deliveries. During enrollment, study participants completed a questionnaire administered by trained personnel to obtain accurate details regarding medical history, demographics, and pesticide exposures. Maternal exposure during the first trimester (~ 22 weeks gestation) was sub-categorized into quartiles and defined as the following: no exposure, indirect exposure (exposure via pruning, planting, etc.,), domestic exposure (exposure via gardening or within the home), or occupational exposure. The outcome, hypertension, was measured through blood pressure measurements taken at the time of the study using an oscillometric sphygmomanometer that was validated and automated. Three separate blood pressure measurements were taken during a 20-minute interval by a medical doctor and the mean of these three recordings was used. Gestational hypertension in this study was defined as having a systolic blood pressure measurement of ≥ 140 mm Hg and/or diastolic blood pressure measurement of ≥ 90 mm Hg. A conditional logistic regression model was used to calculate ORs and 95% CIs for pesticide exposure including malathion and gestational hypertension, adjusting for smoking, age, BMI, and alcohol drinking habits. Among the 2,203 study participants included in the analysis, 582 (26%) reported no exposure, 534 (24%) reported indirect exposure, 613 (28%) reported domestic exposure, and 474 (22%) reported occupational exposure. Evidence of a slight positive association was reported between malathion exposure and gestational hypertension (adjusted OR: 1.14; 95% CI: 1.08, 1.19 with n = 48 women).

The overall quality of the study was ranked low based on the study quality criteria outlined in the OPP Framework. Ledda et al. (2015) relied on a cross-sectional study design that assessed the relationship between gestational hypertension and pesticide exposure, including malathion. As such, the study was unable to assess the temporal association between malathion exposure and gestational hypertension. Another limitation is the use of conditional logistic regression instead of another statistical analysis method because in this study the cases were not matched to the controls.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and gestational hypertension. One study, Ledda et al. (2015) evaluated this potential association and reported evidence of a slight positive association for malathion exposure. This study was ranked low quality for regulatory purposes as it relied on a cross-sectional design that was unable to assess the temporality of the relationship between malathion exposure and gestational hypertension. Another limitation is the use of conditional logistic regression instead of another statistical analysis method, because in this study the cases were not matched to the controls.

Hearing Loss

One study (Crawford et al., 2008) examined the association between malathion exposure and hearing loss.

Hearing loss among AHS study participants was reviewed by Crawford et al. (2008) to investigate its potential association with malathion and other pesticides in a prospective cohort study. The study population consisted of white male pesticide applicators living in Iowa and North Carolina who completed both questionnaires at enrollment and the five-year follow-up interview. Cases were subjects who indicated experiencing hearing loss during a follow-up interview, and controls were subjects who

answered "no" to the question. 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 adjusting for age, state, solvent exposure, metal exposures, and noise exposure. Among the study population (n = 14,229), 4,926 hearing loss cases and 9,303 controls were identified. Investigators reported 341 cases and 657 controls were missing data. Categories of exposure were constructed based on cumulative lifetime days of exposure (i.e., referent, low exposure, medium exposure, and high exposure[100]), and ORs were reported for each group. Evidence of a positive and evidence of a slight positive association between hearing loss and cumulative lifetime days of malathion exposure was reported in the medium and high dose exposure groups, respectively (OR medium exposure = 1.32; 95% CI: 1.18, 1.46 with n = 1,116 cases (24%), 1,825 controls (20%); OR high exposure = 1.20; 95% CI: 1.08, 1.34 with n = 1,128 cases (24%), 1,851 controls (21%)), and no evidence of a significant positive association was observed in the low dose exposure group (OR low exposure = 1.09; 95% CI: 0.98, 1.21 with n = 1,027 cases (22%), 1,992 (22%) controls; 95% CI encompassed the null value of 1.0). Additionally, no evidence of a significant p-trend was reported (p-trend = 0.09).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the prospective design of AHS and exposure assessment approach. The self-reported outcome diagnosis was a limitation and may have been underreported in the study due to societal stigmas associated with hearing loss, along with missing data among the cases and the controls.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and hearing loss among AHS study participants. There was one available study, Crawford et al. (2008), that reported evidence of a positive and slight positive association between hearing loss and malathion use in the medium and high exposure groups, respectively, and no evidence of a significant positive association in the low exposure group. Additionally, no evidence of a significant p-trend was reported. The study quality was ranked moderate for regulatory purposes. The prospective study design was a study strength, and the missing data and the potential underreported outcome due to societal stigmas were considered study limitations.

Kidney Function

One AHS study (Shearer et al., 2021) examined the association between malathion exposure and chronic kidney function.

Shearer et al. (2021) evaluated the association between pesticide exposure, including malathion, and chronic kidney function. The study population consisted of male pesticide applicators in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study, a sub-cohort in the Agricultural Health Study (AHS). Eligible participants included male applicators of the BEEA study, who were enrolled during 2010 – 2017, were ≥ 50 years old, resided in either Iowa or North Carolina, and had completed the enrollment AHS questionnaire (1993–1997) in addition to two follow-up interviews (1999–2003 and 2005–2010). Kidney function was measured using creatinine serum concentration collection from 1,545 BEEA participants who did not have lipemia or hemolysis; the estimated glomerular filtration rate

[100] The low, medium, and high exposure categories for malathion were defined as the following within the study: 0.88 – 57.75 (low exposure), 58 – 212 (medium exposure), and >212 cumulative lifetime days of use (high exposure).

(eGFR) was calculated with the chronic kidney disease (CKD) epidemiology collaboration (CKD-EPI) equation[101] that uses a combination of measured serum creatinine levels in addition to race, gender, and age for each study participant. Among the 1,545 BEEA participants, 155 of the 204 CKD cases and 1,100 of 1,341 non-cases reported exposure to malathion. Multivariable logistic regression models were used to calculate odds ratios (ORs) and 95% CIs for the association between malathion and CKD status among male pesticide applicators adjusting for age, state residence, BMI, history of diabetes, history of hypertension, alcohol consumption, smoking status, and correlated pesticides (carbaryl and diazinon were moderately correlated with malathion based on exposure days, with Pearson correlation $\rho = 0.27$ for carbaryl and $\rho = 0.27$ for diazinon). No evidence of a positive association was reported between CKD and malathion exposure among pesticide applicators based on ever/never use (OR: 0.70; 95% CI: 0.50-1.00 with n=155 exposed cases). Additional analyses were done for the total population (i.e., 1,545 BEEA participants) and for only the active farmers (i.e., a subgroup of the total population) to evaluate the association between applicators' cumulative intensity-weighted lifetime days of use of malathion and CKD, where cumulative intensity-weighted lifetime days was divided at the following cut points: 20-385 days of use, >385-1,080 days of use, >1,080-2,940 days of use, and >2,940-117,600 days of use. No evidence of a positive association was reported between malathion exposure and CKD among male pesticide applicators in the total population for any exposure category ($0.40 \leq$ ORs ≤ 0.80; all 95% CIs encompassed or well less than the null value;) with n=17-24 exposed cases. No evidence of a positive association was reported for male pesticide applicators in the active farmers population for any exposure category ($0.30 \leq$ OR ≤ 0.80; the 95% CIs encompassed the null value 1.0 for the 20-385, >385-1080, and >1080-2940 days of use exposure categories but the >2940-117600 days of use has a 95% CI:0.20-0.70; with n=12-25 exposed cases). For both the total population and active farmers population, a statistically significant negative trend (i.e., increasing exposure would reduce the risk of CKD) was observed (total population p-trend = 0.005; active farmers population p-trend = 0.01).

The quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The study benefited from the general strengths of the AHS, including the prospective cohort study design and the exposure assessment approach which examined cumulative lifetime exposure. Additionally, the outcome of kidney function markers, estimated glomerular filtration rate (eGFR) and serum concentration were detected using laboratory methods. A noted limitation of the study was the single measurement used to determine CKD status. Authors reported typically, CKD diagnosis is determined by several measurements of eGFR (<60 mL/min/1.73 m^2) sustained over three months. Another limitation of the study was the ambiguity around the temporality of the exposure and the outcome. It is unclear if CKD developed after exposure to pesticides or before or whether CKD appeared in the past but was no longer present at time of serum sample collection.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and chronic kidney disease. One publication (Shearer et

[101] CKD-EPI Formula from Levey et al. 2019: Black Female: ≤62 (≤0.7) GFR =166 X $(Scr/0.7)^{-0.329}$ X $(0.993)^{Age}$
>62 (>0.7) GFR =166 X $(Scr/0.7)^{-1.209}$ X $(0.993)^{Age}$; Black Male: ≤ 80 (≤0.9) GFR =163 X $(Scr/0.9)^{-0.411}$ X $(0.993)^{Age}$
>80 (>0.9) GFR= 163 X $(Scr/0.9)^{-1.209}$ X $(0.993)^{Age}$;White or other Female: ≤62 (≤0.7) GFR= 144 X $(Scr/0.7)^{-0.329}$ X $(0.993)^{Age}$
>62 (>0.7) GFR =144 X $(Scr/0.7)^{-1.209}$ X $(0.993)^{Age}$; White or other Male:> 80 (>0.9) GFR =141 X $(Scr/0.9)^{-0.411}$ X $(0.993)^{Age}$
>80 (>0.9) GFR =141 $(Scr/0.9)^{-1.209}$ X $(0.993)^{Age}$

al., 2021) examined the association between malathion exposure and chronic kidney function and reported no evidence of a positive association between CKD and malathion exposure. The quality of the study was ranked moderate for regulatory purposes. The study benefited from the general strengths of the AHS, including the prospective cohort study design and the exposure assessment approach which examined cumulative lifetime exposure to malathion. A noted limitation of the study was the ambiguity around the temporality of the exposure and the outcome. It is unclear if CKD developed after exposure to pesticides or before or whether CKD appeared in the past but was no longer present at time of serum sample collection. Another noted limitation of the study was the single measurement used to determine CKD status. Authors reported typically, CKD diagnosis is determined by several measurements of eGFR (<60 mL/min/1.73 m^2) sustained over three months.

Monoclonal Gammopathy of Undetermined Significance

The association between malathion exposure and monoclonal gammopathy of undetermined significance (MGUS), a pre-cursor to multiple myeloma, was evaluated in one AHS study (Landgren et al., 2009).

Landgren et al. (2009) investigated the potential association between pesticide exposure, including malathion and MGUS among the AHS prospective cohort. MGUS is a pre-malignant disorder of the plasma cells that usually precedes multiple myeloma. The study population (n = 678) included a stratified random sample (based on lifetime of organophosphate use) of male pesticide applicators in the AHS cohort living in Iowa or North Carolina who completed all three follow-up phases of the AHS and were enrolled in a neurobehavioral study nested within the AHS cohort. Applicators who reported a history of lymphoproliferative malignancy were excluded. Cases and non-cases of MGUS were determined from participant blood serum samples collected and that were reviewed by three study personnel between 2006 - 2007 for participants living in Iowa and collected in 2008 for participants living in North Carolina, and samples were then analyzed by three study personnel. All study participants reported pesticide exposure through a self-administered questionnaire completed at enrollment (1993 – 1997) and occupational exposures, medical histories, and lifestyle factors at follow-up interviews conducted five years after enrollment. Logistic regression models were used to calculate ORs and 95% CIs for malathion use at enrollment and risk of MGUS, adjusting for age and education level. Among the 677 male applicators included in the analysis, 27 of the 38 MGUS cases and 489 of the 639 non-cases reported exposure to malathion. No evidence of a positive association was reported between ever exposure to malathion and MGUS among (OR = 0.70; 95% CI: 0.30, 1.50, with n = 27 exposed cases).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. The ranking was based on the general strengths of the AHS, including the prospective design; additionally, the determination of MGUS cases through serum samples that were reviewed by three study personnel. The exposure assessment approach only included ever/never use, and an exposure-response assessment of cumulative lifetime exposure to malathion would have been helpful.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and MGUS. One study, Landgren et al. (2009), reported no evidence of a positive association between malathion exposure and MGUS among male pesticide applicators in a subset of the AHS population, and was ranked for regulatory purposes to be of moderate quality.

Myocardial Infarction

Two AHS studies (Mills et al., 2009; Dayton et al., 2010) examined the association between malathion exposure and myocardial infarction (MI).

- Mills et al. (2009) evaluated the association between pesticide usage including malathion, and MI among male pesticide applicators in the AHS prospective cohort. The study population (n = 54,609) included male pesticide applicators living in Iowa and North Carolina enrolled in the AHS. Cases of MI resulting in death among AHS participants that occurred after enrollment (1993 -1997) through December 31, 2006 were identified through linkage to state and national death records. Cases of incident non-fatal MI included those AHS participants who reported a physician diagnosis of MI since enrollment on the 5-year follow-up questionnaire (1999 – 2003). Fatal and non-fatal cases of MI were analyzed separately due to different follow-up times. Pesticide exposure was assessed using self-reported pesticide exposure on questionnaires completed at study enrollment and at the 5-year follow-up. Cox proportional hazards regression was used to calculate HRs and 95% CIs for fatal and non-fatal MI risk for individual pesticides, adjusted for age, smoking, and state of residence for the fatal MI analysis, and age, smoking, state of residence and BMI for the non-fatal MI analysis. Among the 476 fatal MI cases, 68% (n ~ 323 - 324 reported exposure to malathion, and of the 839 non-fatal MI cases, 74% (n ~ 620 - 621) reported malathion exposure. No evidence of a positive association was reported for self-reported ever use of malathion and fatal MI (HR= 0.81; 95% CI: 0.66, 1.00; with n ~ 323 - 324 exposed cases) and no evidence of a significant positive association was reported for malathion exposure and non-fatal MI (HR= 1.02; 95% CI: 0.86, 1.21; with n ~ 620 – 621 exposed cases).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths include the prospective design of AHS and exposure assessment approach. With respect to limitations, fatal and nonfatal MI incidence were ascertained using state/national death registries and self-report, respectively. The use of registry data on mortality allowed the investigators to evaluate fatal MI in the entire AHS cohort, where non-fatal MI could only be evaluated in 32,024 of the total 54,609 participants enrolled in AHS (58%). The follow-up period for non-fatal MI was only a median time of 5 years, whereas the median follow-up time for fatal MI was 11.8 years. An additional limitation in the evaluation of non-fatal MI is that ascertainment relied on self-report and has not been validated. The investigators acknowledged these limitations and suggest that this approach may result in misclassification, most likely non-differential, because studies in other populations suggest that only 60-68% of self-reported MI cases could be validated based on medical chart review.

- Dayton et al. (2010) investigated the association between pesticide use, including malathion, and non-fatal incident MI among female participants (i.e., female applicators and female spouses of applicators) in the AHS prospective cohort. A total of 22,425 women who completed both the enrollment questionnaire (1993 – 1997) and follow-up phone interview, self-reported physician-diagnosed MI after enrollment and pesticide use including malathion. Of the 168 incident MI cases, 31 (21%) reported exposure to malathion; of the 22,257 non-cases, 4,639 (22%) reported exposure. Logistic regression was used to calculate ORs and 95% CIs, controlling for age, BMI, smoking status, and state of residence. Based on this approach, the investigators reported no evidence of a positive association between ever use of malathion and non-fatal MI among farm women (OR=0.90, 95% CI 0.60, 1.30, n = 31 malathion exposed cases).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths include the prospective design of AHS and exposure assessment

approach. As with Mills et al. (2009), a limitation of the investigators' evaluation of non-fatal MI is that the outcome ascertainment relied on self-report and has not been validated. The investigators acknowledge this in the discussion of their findings and suggest that this approach may result in misclassification because studies in other populations suggest that only 60-68% of self-reported MI cases could be validated based on medical chart review.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and MI. There were two studies of the AHS cohort that examined that association between malathion exposure and MI. Mills et al. (2009) reported no evidence of a positive association for fatal MI and no evidence of a significant positive association for non-fatal MI, based on ever/never use of malathion amongst male pesticide applicators in the AHS. Dayton et al. (2010) further examined the relationship between malathion exposure and non-fatal MI among female participants of the AHS. The study reported no evidence of a positive association. Both studies were moderate quality and a limitation of both studies was the self-report of the outcome which could have resulted in misclassification.

Nervous System Function

Three studies (Engel et al., 2007; Starks et al., 2012a; Starks et al., 2012b) examined the association between malathion exposure and nervous system function including neurobehavioral (central nervous system) function in children and adults and peripheral nervous system function in adults.

Neonatal Central Nervous System Function

Engel et al. (2007) investigated the potential association between maternal exposure to pesticides including malathion and neonatal neurobehavioral effects among their offspring. Using data from the Children's Environmental Health Study[102], an ongoing, prospective cohort study, the study population included pregnant women who resided in New York City and sought prenatal care at either the prenatal clinic at Mount Sinai hospital in New York City or at one of the two private practices within the hospital. The eligibility period spanned from May 1998 – July 2001 and 404 mother-child pairs of the 479 eligible women were willing to participate in this study. Pesticide exposures for certain pesticides including malathion were measured using pesticide specific metabolites. The metabolite-specific metabolite for malathion measured in this study was malathion dicarboxylic acid (MDA). Urine and blood samples were collected during the third trimester of pregnancy to measure the metabolites at a mean of 31.2 weeks of gestation. Urine samples were analyzed for metabolite levels at the Centers for Disease Control (CDC). Study authors indicated that laboratory QA/QC procedures were carried out in this study. Detailed interviews were also conducted during the third trimester of pregnancy to obtain information regarding demographics, health histories, past pregnancies, and pesticide exposures. Following the child's birth, the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) was administered by trained interviewers prior to being discharged from the hospital. Women were ineligible to participate in this assessment if any of the following occurred: the child was admitted to the neonatal intensive care unit following birth, the parent was unwilling to participate, the child was born and the parents were discharged from the hospital over the weekend, if the trained staff were not available, or if the child was not able to be tested. The BNBAS consists of 28 neonatal behavioral and 18 primitive indices, and neonatal behavior was group by the following seven domains: habituation; orientation; motor performance; regulation of state; range of state; levels of stimulation; autonomic stability; and amount and

[102] Additional study details were found in studies Berkowitz et al., 2003, 2004.

type of abnormal primitive reflexes. A generalized linear regression model[103] was used to evaluate the association between each of the six neonatal behavior domains mentioned above (except for abnormal reflexes) and several pesticide metabolites including MDA, the malathion-specific metabolite, adjusting for several covariates.[104] A Poisson regression was used to assess the association between abnormal reflexes due to the nature of the data relative to urinary MDA by calculating risk ratios (RRs) and corresponding 95% confidence intervals (CIs), adjusting for anesthesia during labor, examiner, PON1 enzyme tertiles, overdispersion, and urinary creatinine. Backward elimination was used to determine the final adjusted covariates with a 20% change in the beta coefficient of the metabolite in the model as the criterion of inclusion/exclusion of a variable. A total of 311 mother-child pairs completed the study. The limit of detection (LOD) for MDA was 0.30 µg/L (n = 283) with a 21.6% detection rate. Due to the low detection rate, MDA metabolite concentrations were treated as a categorial variable, and dichotomized within the analysis as either above or below the limit of detection. For the Poisson regression, evidence of a moderately strong association was observed between prenatal exposure to malathion and the number of abnormal reflexes in neonatal babies (RR: 2.24; 95% CI: 1.55, 3.24 n =242). For the linear models, no evidence of a statistically significant change was observed for habituation, orientation, motor performance; regulation of state, range of state, levels of stimulation, and autonomic stability in newborns following prenatal exposure to malathion (habituation β: 0.440, 95% CI: -0.145, 1.025 with n = 148; orientation β: -0.100, 95% CI: -0.597, 0.405 with n = 240; motor β: -0.050, 95% CI: -0.233, 0.156 with n = 257; range of state β: -0.040, 95% CI: -0.281, 0.199 with n = 256; regulation of state β: -0.090, 95% CI: -0.480, 0.303 with n = 256; autonomic stability β: 0.090, 95% CI: -0.274, 0.463 with n = 256).

The overall quality of the study was ranked moderate based on the study quality criteria outlined in the OPP Framework. Engel et al. (2007) used a prospective cohort design to assess the relationship between neurobehavioral function in neonates following prenatal exposure to malathion measured through the MDA urinary metabolite. Study strengths included the study design, the use of hospital data to confirm the outcome, and the use of laboratory QA/QC methods. Study limitations included the single urinary sample taken during once pregnancy to assess malathion exposure, the potential for exposure misclassification due the transient and variable nature of exposures to pesticides, the lack of adjustment for the multiple tests performed, and the use of automated backward elimination as a statistical method to determine which confounders/covariates remained. Furthermore, pesticides metabolites usually stay within the body for a short amount of time, making it challenging to measure exposure. 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. The study was ranked moderate quality for regulatory purposes.

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[103] The authors did not mention about the link function or what kind of link function was used in the data analysis using generalized linear model. However, it could be that the authors mis-called the statistical terminology "generalized linear model" instead of "linear regression model" which is typically used to analyze continuous or reasonably assumed to be continuous data.

[104] For the habitation outcome, the following covariates were adjusted for in the model: drug use during gestation, urinary creatinine, examiner, and paraoxonase 1 (PON1) enzyme tertiles; for the orientation outcome the following covariates were adjusted for in the model: BMI prior to pregnancy, jaundice in neonates, examiner, PON1 enzyme tertiles, and urinary creatinine ; for the motor outcome, the following covariates were adjusted for in the model: infant age at examination, caffeine consumption during gestation, examiner, drug use during gestation, PON1 enzyme tertiles, and urinary creatinine by using a generalized linear model; for range of state, the following covariates were adjusted for in the model: infant age at examination, PON1 enzyme tertiles, examiner, and urinary creatinine; for the regulation of state, the following covariates were adjusted for in the model: maternal education, PON1 enzyme tertiles, examiner, and urinary creatinine; for autonomic stability, the following covariates were adjusted for in the model: infant age at examination, smoking during gestation, examiner, PON1 enzyme tertiles, and urinary creatinine.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and neonatal central nervous system function. There was one available study that examined central nervous system function (Engel et al., 2007) that reported evidence of a moderately strong association between prenatal exposure to malathion and the number of abnormal reflexes in neonatal babies, though no evidence of a statistically significant change was observed for habituation, orientation, motor performance, regulation of state, range of state, levels of stimulation, and autonomic stability in newborns following prenatal exposure to malathion. The study was ranked moderate quality for regulatory purposes, and study strengths included the study design, the use of hospital data to confirm the outcome, and the use of laboratory QA/QC methods. Study limitations included the single urinary sample taken during once pregnancy to assess malathion exposure and the potential for exposure misclassification due to the transient and variable nature of exposures to pesticides. We note, too that no adjustments for multiple testing were performed and the authors used automated backward elimination as a statistical method to determine which confounders/covariates remained, a technique perhaps appropriate only for exploratory analysis.

Central Nervous System Function (Adult)

The association between malathion and adult central nervous system function was evaluated in one AHS study (Starks et al., 2012a) described below.

Starks et al. (2012a) investigated the association between long-term pesticide usage, including malathion, and neurobehavioral function of the central nervous system. The study participants consisted of male pesticide applicators living in Iowa and North Carolina who completed all AHS interviews, were free of medical conditions that could influence central or peripheral nervous system testing results, reported drinking <42 alcoholic beverages per week,[105] and reported no history of pesticide poisoning at enrollment pre-screening or during the ten-year follow-up interview (Phase III). A total of 1,807 male AHS participants were originally eligible to participate in the study, with 701 (39%) agreeing to participate.[106] Of the 701 participants, 128 (18%) reported ever use of malathion. Authors reported participants' pesticide use and age were similar to non-participants (data not provided). Neurobehavioral function for each of the study participants was determined through a series of nine tests to assess central nervous system function, conducted during a single visit to a testing center in Iowa (Dubuque and Iowa City) or North Carolina (Greenville and Wilmington) between 2006 to 2008. Tests assessed memory, motor speed, fine motor coordination, sustained attention, verbal learning, and visual scanning and

[105] Participants were eligible to participate in the study even if they had consumed up to 41 alcoholic drinks per week on average in the past year (though not if they had consumed alcohol on the day of testing or if they had a past diagnosis of alcoholism). Alcohol intoxication and excessive alcohol use could have influenced the outcomes measured in this study including reaction time, balance, and motor skills. The Centers for Disease Control and Prevention defines heaving drinking as consuming 15 drinks or more per week for men and eight drinks or more per week for women and states that alcohol intoxication can cause impaired brain function resulting in reduced reaction time and loss of balance and motor skills. https://www.cdc.gov/alcohol/faqs htm#heavyDrinking

[106] The potential for selection bias was high: those pesticide applicators who participated (39%) may have been more likely to have experienced neurobehavioral symptoms and therefore could be more inclined to go through the effort to travel to the testing centers than asymptomatic pesticide applicators, overestimating the true association.

processing[107] and were administered by trained technicians blinded to pesticide exposure status. An additional questionnaire given at the time of neurobehavioral testing provided information on the participant's smoking status, alcohol use, head injury, antidepressant use, caffeine use, exposure to other potentially neurotoxic substances (organic solvents, soldering, welding fumes), and tests of potential reading ability, affect, and visual acuity. Pesticide exposure (ever use and lifetime-days of use) for malathion was assessed using self-reported pesticide use data from questionnaires and telephone interviews conducted at enrollment and every five years thereafter between 1993 and 2007. The additional questionnaire, given at the time of neurobehavioral testing, provided further data on participant's pesticide use in the last 12 months. To enrich the sample for applicators with higher lifetime use of organophosphate pesticides (OP), authors oversampled the high end of the OP lifetime use distribution based on the lifetime days of use of 10 OPs assessed in detail in Phase I of the AHS study. To allow for an enriched sample for OP use, authors selected a stratified sample among eligible participants, based on equal sampling from the upper and lower portion of the OP lifetime day's distribution (Iowa cutpoint ~75%, North Carolina cutpoint ~66%). Even though the cutpoint was shifted for selection, all analyses were based on lifetime use of pesticides - which included data from all AHS interviews and the neurobehavioral appointment. The sampling frame allowed for an enriched sample for OP use but was not used as an analytical variable. The authors multiplied the parameter estimates for the timed tests (Continuous Performance Test, Digit-Symbol, Grooved Pegboard, Sequence A and Sequence B) by -1 so that lower scores indicated poorer test performance for all neurobehavioral outcomes assessed. Cumulative lifetime days of pesticide use variables were log-transformed to normalize the distribution. Separate linear regression models were created with backwards elimination[108] for each neurobehavioral outcome measure and pesticide usage including malathion, adjusting for age and reading score as well as for outcome-specific covariates that included positive affect score, negative affect score, visual acuity, caffeine consumption, state of residence, and level of education. Final models included only covariates with p-values < 0.20. Alcohol consumption was selected as a potential confounder but did not remain in the final regression models ($p > 0.20$). No evidence of a statistically significant association was reported for malathion exposure and any neurobehavioral tests for malathion ever use ($-6.13 \leq$ all $\beta \leq 0.58$, all $p \geq 0.05$ for the associated model of malathion regression coefficients).[109] For the log transformed cumulative lifetime days of use, a statistically significant decrease was determined for digit-symbol ($\beta = -1.75$, $p < 0.05$); no evidence of a statistically significant association was reported for any other neurobehavioral tests ($-0.90 \leq$ all $\beta \leq 0.16$, all $p \geq 0.05$ for the associated model of malathion regression coefficients). Additionally, a statistically significant interaction between state of residence (IA or NC) and malathion use was reported for Grooved Pegboard. Specifically, a statistically significant increase in Grooved Pegboard was observed among AHS men exposed to malathion in Iowa, based on ever-use and lifetime days of use ($\beta = 5.85$, $p < 0.05$ for ever-use, $\beta = 2.46$, $p < 0.05$ for lifetime days). No significant interactions were determined among AHS men exposed to malathion in North Carolina and no significant

[107] Eight computerized tests from the Neurobehavioral Evaluation System, Version 3 and the Manual Grooved Pegboard test were administered by trained technicians blinded to pesticide exposure status and included the following: the continuous performance test to assess sustained attention, digit-symbol test for visual scanning and processing, finger tapping (dominant hand) test for motor speed, grooved pegboard (dominant hand) for fine motor coordination, auditory verbal learning test (AVLT)-total recall for verbal learning and memory, AVLT-delayed recall and AVLT recognition for memory, and sequences A and B latency tests for motor speed and scanning.

[108] With respect to automatic stepwise selection procedures, these are 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. For problems connected to use of these procedures, 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.

interactions were observed for any additional neurobehavioral outcomes mentioned above relative to malathion use.

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. Study strengths included the prospective cohort study design, the administration of neurobehavioral testing by trained technicians, and the pesticide exposure assessment. Several study limitations were noted including the potential for selection bias, the backwards selection method used in the statistical analysis, and eligibility criteria. The potential for selection bias was high: those pesticide applicators who participated (39%) may have been more likely to have experienced neurobehavioral symptoms and therefore could be more inclined to go through the effort to travel to the testing centers than asymptomatic pesticide applicators, overestimating the true association. With respect to stepwise selection procedures, these are 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. Participants were eligible to participate in the study even if they had consumed up to 41 alcoholic drinks per week on average in the past year (though not if they had consumed alcohol on the day of testing or if they had a past diagnosis of alcoholism). Alcohol intoxication and excessive alcohol use could have influenced the outcomes measured in this study including reaction time, balance, and motor skills.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and central nervous system function by conducting neurobehavioral tests in adults among pesticide applicators in the AHS cohort. There was one available study that examined neurobehavioral function (Starks et al., 2012a) that reported evidence of a significant decrease between lifetime days of malathion use and the digit symbol neurobehavioral test, and no evidence of significant association for any other neurobehavioral function outcome measures for both ever-use and lifetime days of malathion use. The study was ranked low quality. Several study limitations were noted including the potential for selection bias, use of backwards selection in the statistical analysis which is appropriate for a hypothesis generating study, and the fact that participants who consumed up to 41 alcoholic drinks per week were eligible to participate even though that quantity of alcohol might certainly have an effect on neurobehavioral function.

Peripheral Nervous System Function (Adults)

The association between malathion and adult peripheral nervous system function was evaluated in one AHS study (Starks et al., 2012b) described below.

Starks et al. (2012b) investigated the association between long-term pesticide usage, including malathion, and function of the peripheral nervous system (PNS). The study population (n = 678) consisted of male pesticide applicators participating in the AHS, living in Iowa and North Carolina, who completed all AHS questionnaires (the self-administered questionnaires at enrollment into the AHS between 1993 and 1997 and two 5-year follow-up telephone interviews), neurological testing, and a questionnaire at the time of the neurological test visit. Details on the study are presented above in Starks et al. (2012a). PNS function for each of the study participants was determined through a series of tests that were conducted during a single visit to one of four testing centers in Iowa (Dubuque and Iowa City) and North Carolina (Greenville and Wilmington) between 2006 and 2008 and included a neurological **physical exam** (6 tests), **electrophysiological measures** (4 tests), and corresponding **quantitative functional tests** (4 tests)

that were administered by a single physician who was blinded to pesticide exposure status.[110] Authors multiplied the parameter estimates for peroneal nerve distal motor latency and short F-wave latency, sway speed, and vibrotactile threshold by -1 so that lower scores indicated poorer test performance for all PNS function outcomes. Separate linear and logistic regression models were created for each PNS function outcome measure and malathion using backwards elimination,[111] to determine if an association existed between each PNS function outcome measure and malathion, adjusting for age as well as for outcome-specific covariates that included body mass index (BMI), height, state, foot temperature, and previous pesticide poisoning. Final models included only covariates with p-values < 0.20. Alcohol consumption was selected as a potential confounder but did not remain in the final regression models ($p > 0.20$). Cumulative lifetime days of pesticide use variables were log-transformed to normalize the distribution. For exposure-response analysis, log-transformed lifetime days of use for malathion, were split at the median among the pesticide users to create two exposure categories (low: ≤ median, and high: > median), with never use as the referent category. Of the 678 male pesticide applicators in this study, 525 (77.4%) reported ever use of malathion and the median log-transformed lifetime days of use was 37.0 days for malathion.

Test results are reported below:

- In the analysis of malathion exposure and **neurological physical examination outcomes**, the study reported no evidence of a significant positive associations between malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for either ever use of lifetime days of malathion use ($0.73 \leq ORs \leq 1.37$, all 95% CIs encompassed the null value of 1.0). Similarly, results from the dose-response model indicated no evidence of significant positive association between log-transformed lifetime days of use of malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for the low exposure (≤ 37.0 days) and the high exposure (> 37.0 days) groups, relative to the controls ($0.71 \leq ORs \leq 1.53$, all 95% CIs encompassed the null value of 1.0, and all p-trends ≥ 0.05).

- For the analysis of malathion exposure and the **electrophysiological tests**, no evidence of a significant association was reported for malathion and distal motor amplitude, distal motor latency, nerve conduction velocity, short F-wave latency for either ever use or lifetime days of use of malathion ($-0.70 < \beta < 0.28$, all 95% CIs encompassed the null value 0).

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[110] The PNS function tests included neurological physical exam, electrophysiological tests, and quantitative functional tests. Neurological physical examinations assessed ankle reflex, postural tremor, Romberg test for balance, tandem gait, toe proprioception, and toe vibration; electrophysiological tests of the dominant peroneal motor nerve assessed distal motor amplitude (mV), distal motor latency (msec), nerve conduction velocity (m/sec), and short F-wave latency (msec); and Quantitative functional PNS tests assessed hand strength dominant and non-dominant hand, sway speed with eyes open (mm/s), sway speed with eyes closed (mm/s), and vibrotactile threshold dominant and non-dominant toe (log μ).

[111] With respect to automatic stepwise selection procedures, these are 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. For problems connected to use of these procedures, 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

- For the analysis of malathion exposure and the **quantitative functional PNS tests**, no evidence of a significant association was reported for ever use of malathion and for log-transformed lifetime days of malathion and sway speed with eyes opened and closed, hand strength and vibrotactile threshold ($-0.24 < \beta < 0.53$, all 95% CIs encompassed the null value 0).

  The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. Study strengths included the prospective cohort study design, the detailed exposure assessment, and the administration of neurobehavioral testing by trained technicians. Several study limitations were noted including the potential for selection bias, the automated backwards selection method used in the statistical analysis, the lack of correction for multiple comparisons, and the eligibility criteria. The potential for selection bias was high: those pesticide applicators who participated (39%) may have been more likely to have experienced neurobehavioral symptoms and therefore could be more inclined to go through the effort to travel to the testing centers than asymptomatic pesticide applicators, overestimating the true association. With respect to automatic stepwise selection procedures, these are 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. Although several outcomes were considered, the study did not correct for multiple comparisons using statistical methods such as the Benjamini-Hochberg test. Participants were eligible to participate in the study even if they had consumed up to 41 alcoholic drinks per week on average in the past year (though not if they had consumed alcohol on the day of testing or if they had a past diagnosis of alcoholism). Alcohol intoxication and excessive alcohol use could have influenced the outcomes measured in this study including reaction time, balance, and motor skills. Lastly, although the exposure assessment was considered a study strength, as it was able to collect data on ever/ never and cumulative pesticide use, it should be noted the study authors indicated that different methods were used to collect pesticide use information throughout the study phases (checklist vs. open-ended questions vs. in-person interviews vs. take-home questionnaires). Certain participants who were prompted with a list of pesticide names may have remembered their pesticide exposures more accurately than those who were not prompted with pesticide names.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and adult peripheral nervous system function. There was one available study that examined the association between malathion exposure and peripheral nervous system function in adults (Starks et al., 2012b). In the analysis of malathion exposure and **neurological physical examination outcomes**, the study reported no evidence of a significant positive associations between malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for either ever use of lifetime days of malathion use. Similarly, results from the dose-response model indicated no evidence of significant positive association between log-transformed lifetime days of use of malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for the low exposure and the high exposure groups, relative to the controls. For the analysis of malathion exposure and the **electrophysiological tests**, no evidence of a significant association was reported for malathion and distal motor amplitude, distal motor latency, nerve conduction velocity, short F-wave latency for either ever use or lifetime days of use of malathion. For the analysis of malathion exposure and the **quantitative functional PNS tests**, no evidence of a significant association was reported for ever use of malathion and for log-transformed lifetime days of malathion and sway speed with eyes opened and closed, hand strength and vibrotactile threshold. The study was ranked low quality. While the study benefited from the prospective cohort study design and case identification using trained neurobehavior technicians, several study limitations were noted including the potential for

selection bias, use of automated backwards selection in the statistical analysis which is generally only appropriate for a hypothesis-generating study, and the fact that participants who consumed up to 41 alcoholic drinks per week were eligible to participate even though that quantity of alcohol might certainly have an effect on neurobehavioral function. Additionally, although several outcomes were considered, the study did not correct for multiple comparisons using statistical methods such as the Benjamini-Hochberg test.

Neurodevelopmental/Neurobehavorial Effects

One study (Eskenazi et al., 2007) evaluated the association between maternal exposure to pesticides including malathion and neurodevelopmental/neurobehavorial effects in children.

Eskenazi et al. (2007) investigated the potential association between maternal exposure to pesticides including malathion as determine by urine measurements and subsequent neurodevelopmental/ neurobehavorial effects among their offspring. Using data from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), an ongoing, prospective cohort study, the study population included pregnant women residing within Salinas Valley, California, < 20 weeks of gestation, aged ≥ 18 years old, Medi-Cal eligible, and whose primary language was English or Spanish and sought prenatal care at either Natividad Medical Center hospital or one of its five medical centers within the area and planned to deliver at the hospital. The eligibility period spanned one year (October 1999 – October 2000). Exclusion criteria for this study included: women and children with no prenatal and concurrent measured DAP metabolites (n = 3); children without a neurodevelopmental assessment (n = 71); twins (n = 8); children with Bayley scores that were not high enough for standardization (n = 5); children for whom the psychometrician performed too few tests necessary for statistical adjustment for the assessment (n = 4); and children born with a medical condition that could affect the study assessment (n = 3, conditions included: deafness, Down syndrome, and hydrocephalus), The study authors did include newborns (n = 11) born with congenital abnormalities, as their exclusion would not materially change the results. Pesticide exposure for certain pesticides including malathion was measured using urinary pesticide specific metabolites, and the specific metabolite for malathion was malathion dicarboxylic acid (MDA). Interviews were conducted to determine demographic details of the study participants and spot urine samples were collected at the same time to measure urinary metabolites, both at baseline and twice at follow-up, including once at 13 weeks gestation (mean time + SD: 13.4 ± 5.2 weeks) and another at 26 weeks (mean time + SD: 25.9 ± 2.6 weeks) of gestation, and after delivery (mean time + SD: 8.8 ± 17.9 days). Urine samples were stored at -80°C prior to being shipped and analyzed for metabolite levels at the Centers for Disease Control (CDC) using liquid or gas chromatography with tandem mass spectrometry. Metabolite levels measured below the limit of detection (LOD) were calculated as the LOD/√2, and missing values were imputed by randomly predicting the value based on the metabolite values of women for other metabolites at that same time point. The study authors reported MDA levels measured within 91 urine samples were missing due to analytical issues. Creatinine concentrations of the urine samples were also measured, and adjusted and unadjusted associations for creatinine concentrations were performed relative to the pesticide exposures including for malathion. Study authors indicated that laboratory QA/QC procedures were carried out in this study but few study details were provided including the use of blanks and spikes and detection limits. Detailed interviews were also conducted at two points during pregnancy and once immediately after delivery by a bilingual interviewer to obtain information regarding demographics, health histories, past pregnancies, and agricultural exposure. All health and prenatal medical records were abstracted via a registered nurse. The Peabody Picture Vocabulary Test and the Center for Epidemiologic Studies Depression Scale were administered at the 6-month and 12-month after delivery visits to determine maternal scholastic abilities and mental health. Additionally, when the children were 2-years of age, the mothers completed the Child Behavior Checklist (CBCL), to assess their child's emotional/behavioral welfare. For the CBCL, the study authors chose

three scales upon which to focus: the Attention Problems syndrome scale, the DSM-oriented Attention Deficit/Hyperactivity scale, and the DSM-oriented Pervasive Developmental Disorder scale – based on screening past animal data. Children were interviewed at 6, 12, and 24 months of age to assess neurodevelopment using the Bayley Scales of Infant Development test. Two indices – the Bayley Mental Development Index (MDI) and the Psychomotor Development Index (PDI) – make up this test in an effort to determine cognitive abilities and large muscle and fine motor coordination among children. Both the MDI and PDI indices were administered by trained, bilingual psychometricians who were blinded to pesticide exposure and were overseen by a clinical neuropsychologist. The Infant Toddler Home Observation for Measurements of Environment (HOME) was also administered at the 6-, 12-, and 24-month visits. A linear regression model was used to determine the association between maternal urinary pesticide metabolites and change in the MDI and PDI indices used in the Bayley Scales of Infant Development Test, adjusting for location, exact age at assessment, breast-feeding duration, sex, HOME score, maternal PPVT, parity, household income above poverty threshold, and identity of psychometrician. Covariates were selected: i.) from associations observed in the literature; ii.) when related to the conditions used for testing and were included within the models when the association was $p < 0.10$, and iii.) when related to neurodevelopment within the literature but not found within this study's data. The model reported results that were unadjusted for urinary creatinine concentrations. Of the 1,130 eligible women who participated in this study, 396 mother-infant pairs, 395 mother-infant pairs, and 372 mother-infant pairs were assessed at the 6-, 12-, and 24-months visits, using the Bayley Scales of Infant Development. At the 2-year visit, 356 women reported on their children's behavior using the Child Behavior Checklist. The median metabolite level for MDA was 0.82 µg/L, with 39% of the MDA maternal urine samples (at least one of the two urine samples obtained during pregnancy) being above the LOD. The reported LOD for MDA was 0.2 ng/mL[112]. Due to this small percentage of samples being above the LOD, the exposure for MDA was assessed as a categorial variable, and exposure categories were defined as: <LOD (no detectable levels), < median level detected, and ≥ median level detected. No evidence of a significant association was observed between prenatal urinary MDA metabolites in either exposure category (< median detected, ≥ median detected) and children's MDI scores at their 6-month, 12-month and 24-months visits, relative to the referent ($-1.09 \leq \beta s \leq 2.40$; all CIs encompassed the null value of 0). Similarly, for children's PDI scores at their 6-month, 12- month, and 24-month visits, no evidence of a significant association was observed for prenatal urinary MDA metabolites in either exposure category (< median detected, ≥ median detected), relative to the referent category ($-1.45 \leq \beta s \leq 0.75$; all CIs encompassed the null value of 0).

The overall quality of the study was ranked moderate based on the study quality criteria outlined in the OPP Framework. Eskenazi et al. (2007) used a prospective cohort design to assess the relationship between neurodevelopmental/neurobehavorial effects in children following maternal exposure to pesticides, measured through urinary metabolites. Study strengths included the study design, the use of medical record data, the use of trained psychometricians who were blinded to exposure, and the neurodevelopmental tests used to measure the outcome. A primary limitation of the study included the potential for exposure misclassification due to the transient and variable nature of exposures to pesticides. Further, pesticide metabolites from malathion usually stay within the body for a short amount of time, making it challenging to estimate longer-term exposure from a single measurement. 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

[112] The study authors mentioned the LOD for MDA was measured and reported in a separate study, Olsson et al., (2003); Olsson, A. O., Nguyen, J. V., Sadowski, M. A., & Barr, D. B. (2003). A liquid chromatography/electrospray ionization–tandem mass spectrometry method for quantification of specific organophosphorus pesticide biomarkers in human urine. *Analytical and bioanalytical chemistry*, *376*(6), 808-815.

differences in biomarkers of exposure among individuals, and possibly between comparison groups. The study was ranked moderate quality for regulatory purposes.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between maternal malathion exposure and neurodevelopmental/neurobehavorial effects in children. One study (Eskenazi et al., 2007) examined the association between maternal pesticide exposures by measuring urinary metabolites including MDA, and neurodevelopmental /neurobehavorial effects in children using the Bayley Scales of Infant Development test. No evidence of a significant association was observed between prenatal urinary MDA metabolites in any exposure category and children's MDI scores at their 6-month, 12-month and 24-months visits, relative to the referent. Similarly, for children's PDI scores at their 6-month, 12- month, and 24-month visits, no evidence of a significant association was observed for prenatal urinary MDA metabolites in any exposure category, relative to the referent ($-1.45 \leq \beta s \leq 0.75$; all CIs encompassed the null value of 0).

The study quality was ranked moderate for regulatory purposes due to potential exposure misclassification due to the transient and variable nature of exposures to pesticides. Furthermore, pesticides metabolites usually stay within the body for a short amount of time, making it challenging to measure exposure precisely.

Olfactory Impairment

Two AHS studies (Shrestha et al., 2019a; Shrestha et al., 2020a) examined the association between malathion exposure and olfactory impairment.

- Shrestha et al. (2019a) evaluated the association between high pesticide exposure events (HPEE) for specific pesticides including malathion and olfactory impairment among private pesticide applicators. The study population consisted of private pesticide applicators enrolled in the AHS, an ongoing, prospective cohort study. HPEE was reported through take-home self-administered questionnaires at enrollment (1993 – 1997) and olfactory impairment (OI) was ascertained through self-report during the latest follow-up period of the AHS (2013 – 2015). Logistic regression was used to calculate adjusted ORs for individual pesticides including malathion involved in the highest exposure HPEE at enrollment and OI among private pesticide applicators relative to OI with no HPEE history at enrollment, adjusting for smoking, education, state, age at enrollment, marital status, alcohol drinking, history of head injury, and sex. Among the total number of private pesticide applicators who reported HPEE (n = 1,845), 7 reported OI and 44 reported no OI where the highest exposure HPEE involved malathion. No evidence of a significant positive association was reported between malathion involved in the highest exposure HPEE and OI among private pesticide applicators among a very small number of cases (OR: 1.44; 95% CI: 0.64, 3.22 n = 7).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the Framework. Study strengths included the AHS prospective cohort, and the exposure assessment including high pesticide exposure events (HPEEs). The outcome was self-reported, and the outcome assessment would have been strengthened by clinical confirmation of self-reported outcome. We also note the very small sample size that included only 7 cases reporting exposure to malathion.

- Shrestha et al. (2020a) evaluated the association between exposure to specific pesticides, including malathion, and olfactory impairment among pesticide applicators in the AHS. The study population

consisted of private pesticide applicators (mainly farmers) enrolled in the AHS prospective cohort. Olfactory impairment[113] was self-reported during the Phase IV follow-up interview (2013- 2016). Pesticide exposure was self-reported on the questionnaires administered at enrollment (Phase I1: 1993- 2007) and shortly after and on follow-up interviews (Phase II: 1999-2003, Phase III: 2005-2010, and Phase IV: 2013-2016). Among the 52,394 applicators enrolled in the AHS, 24,145 (46.1%) completed the Phase IV follow-up survey and 20,409 of these participants had complete data on olfaction and baseline covariates and were included in the final analysis. Among these 20,409 participants who reported data on olfaction on the Phase IV questionnaire, 1,579 (79.1%) of 2,069 cases of olfactory impairment and 12,742 (72.8%) of the 18,340 non-cases reported ever exposure to malathion. Logistic regression was used to estimate ORs and 95% CIs for the association between individual pesticides including malathion use reported at enrollment and olfactory impairment among private pesticide applicators based on ever use and intensity-weighted lifetime days of use. Models were adjusted for age at enrollment, sex, smoking status, education, state of residence, history of performing other farming tasks that may result in airborne irritants at least once per year[114], and correlated pesticides (ever use with Spearman correlation >0.40)[115]. Evidence of a slight positive association was reported between the association of malathion ever use and olfactory impairment (OR=1.29; 95% CI: 1.15, 1.45 n = 1,579 exposed cases, 12,742 exposed controls). In the exposure-response analysis, intensity-weighted lifetime days (IWLD) of malathion (product of years of use and days per year weighted by exposure intensity) were divided into four exposure categories including never exposure (referent) and tertiles of days use for malathion (>0–360, >360–1,344 and >1,344 days). Evidence of a slight positive association was reported for the >0–360 exposure category of IWLD of malathion use and olfactory impairment (>0–360 OR: 1.24; 95% CI:1.05,1.47; with n=281) and evidence of a positive association was reported for the >1,344 days exposure category of IWLD of malathion use and olfactory impairment (>1,344 days OR: 1.33; 95% CI:1.13,1.58; with n=311). No evidence of a significant positive association was reported for the >360–1,344 exposure category of IWLD of malathion use and olfactory impairment among pesticide applicators in the AHS (>360–1,344 OR: 1.17; 95% CI:0.98,1.38; with n=280).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the Framework. Study strengths included the AHS prospective cohort, and the exposure assessment including ever use and cumulative use exposure response analysis. The outcome was self-reported, and the outcome assessment would have been strengthened by clinical confirmation of self-reported outcome.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and olfactory impairment. Two publications (Shrestha et al., 2019a; Shrestha et al., 2020a) examined the association between malathion exposure and olfactory impairment among the AHS prospective cohort population. Shrestha et al., 2019a reported no evidence of a significant positive association between malathion and OI among private pesticide applicators. Shrestha et al., 2020a, reported evidence of a slight positive association between of

[113] Participants were asked to respond to two questions pertaining to olfactory impairment on the Phase IV follow-up questionnaire and included "do you suffer from a loss of sense of smell or significantly decreased sense of smell?" and "When did you start losing your sense of smell" which had four possible response choices: < 1, 1-5, and >10 years prior to the Phase IV follow-up.

[114] Farming tasks that may result in airborne irritants (e.g., fumes, solvents, metals, and dusts) included repairing engines, handling stored grain, replacing asbestos brakes, welding, painting, and working in swine confinement areas.

[115] Correlated pesticides were not specified by authors.

malathion ever use and olfactory impairment. Additionally, in the dose-response analysis, evidence of a slight positive association in the lowest exposure category of IWLD of malathion use, and evidence of a positive association in the highest exposure category of IWLD of malathion use were reported relative to olfactory impairment. No evidence of a significant positive association was reported for the middle category of IWLD malathion use and olfactory impairment among pesticide applicators in the AHS. The quality of both studies was moderate. Study strengths included the prospective cohort design of the AHS and the exposure assessment. We noted limitations including the self-reported outcome assessment which could have been strengthened through clinical confirmation of olfactory impairment and the potential over adjustment of several covariates in the analysis. Additionally, authors adjusted for correlated pesticides; however, they did not specify which pesticides were correlated with each other and this would have been helpful in the assessment. Lastly, in Shrestha et al, 2019a, we also note that the number of exposed cases was very small which severely restricts the ability to interpret with confidence the observed OR.

Parkinson's Disease

Five studies (Firestone et al., 2005; Kamel et al., 2007; Firestone et al., 2010; Wang et al., 2014; Shrestha et al., 2020b) assessed the association between malathion exposure and Parkinson's disease (PD).

- Firestone et al. (2005) and Firestone et al. (2010) investigated the potential association between malathion exposure and PD in a population-based case-control study in Western Washington State. The study population included 404 incident PD cases and 526 age- and sex-matched control participants from the Group Health Cooperative (GHC) and the University of Washington. Newly diagnosed PD patients were identified between 1992 and 2000 via provider referrals and databases. A panel of neurologists confirmed case status. Exposure to pesticides, including malathion, was self-reported along with exposure to other industrial toxicants during a structured interview. Interviewers were blinded to disease status; however, authors reported that visible outward manifestations of PD made it impossible to completely blind interviewers to case-control status. Among the 397 participants included in the occupational exposure analysis in Firestone et al. (2005), 8 (5%) of the 156 cases and 10 (4%) of the 241 controls reported malathion exposure. Among the 526 participants included in the pesticide exposure analysis in Firestone et al. (2010), 10 (4%) of the 252 cases and 12 (4%) of the 326 controls reported malathion exposure. Unconditional logistic regression models were used in both studies, adjusting for age, smoking status, sex (only included in the 2005 data analysis; the 2010 data analysis only included males), and ethnicity (only included in the 2010 data analysis). Firestone et al. (2005) reported no evidence of a positive association between malathion exposure and PD in men (OR = 1.01, 95% CI: 0.37, 2.72; n = 8 exposed cases and 10 exposed controls), and Firestone et al. (2010) reported no evidence of a positive association between malathion exposure and PD among men. (OR = 1.00, 95% CI: 0.39-2.30).

  The overall quality of both studies was ranked low based on the study quality criteria provided in the OPP Framework. The case-control study design and outcome confirmation by a trained neurologist were strengths; however, several limitations were noted including potential for recall bias, interviewer bias, and self-reported exposures. Authors reported interviewers were blinded from case-control status of participants but the outward manifestations of PD made complete blinding impossible. Additionally, the nature of the case-control study imparts potential for recall-bias, and it is possible that those with PD may have remembered their exposures to pesticides more (or possibly less) clearly than those without a diagnosis of PD. Authors reported that subjects were blinded to the study hypothesis to minimize recall bias.

- Kamel et al. (2007) investigated the association between pesticide exposure, including malathion, and PD in the AHS prospective cohort at enrollment and Phase 1 follow-up. The study population (n = 52,393) consisted of male pesticide applicators and their spouses enrolled in the AHS living in Iowa and North Carolina who completed both the enrollment (1993 – 1997) and follow-up (1999-2003) questionnaires. Cases of PD included AHS study participants who self-reported a physician diagnosis of PD at enrollment (prevalent PD – n = 83 cases), and at the 5-year follow-up (incident PD – n = 78 cases) through 2003. Non-cases included AHS study participants who did not indicate PD at enrollment (n = 79,557) or at follow-up (n = 55,931). Pesticide exposure was assessed for 50 different pesticides including malathion using self-administered questionnaires at study enrollment (1993 - 1997). Odds ratios and 95% CIs were calculated for the association between individual pesticides and PD using a hierarchical regression model, adjusted for state, age, and type of participant (applicator or spouse). Among the 78 incident cases of PD, 41 (55%) incident cases and 49 (67%) prevalent cases reported exposure to malathion based on ever use. No evidence of a significant positive association was reported for malathion exposure and incident PD (OR = 1.20; 95% CI: 0.60, 2.10; with n = 49 exposed cases) and for prevalent PD (OR = 1.10; 95% CI: 0.60, 2.00; with n = 41 exposed cases), based on ever use of malathion.

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Although study strengths included the AHS study cohort, several study limitations were noted, including the lack of clinical confirmation of self-reported PD cases and the potential for recall bias. Recall bias is particularly important because the study included prevalent PD cases that may recall previous exposures differently than study subjects without PD. Study authors also indicated that for the prevalent cases of PD, the diagnosis date was unknown in addition to the duration of use for pesticides, making it difficult to assess temporality (i.e., whether the disease preceded the outcome); this information was known for incident cases in this study.

- Wang et al. (2014) investigated the association between ambient exposure to malathion, and other organophosphates, and PD using a population-based case-control study design. Study participants (357 incident PD Cases, 752 population controls) included residents of CA living in the Central Valley, CA (Kern, Tulare, and Fresno counties) who enrolled between 2001-2007 (cases) and 2002-2011 (controls). Eligible cases were diagnosed with PD within the past 3 years, lived in CA for at least 5 years (current resident in Kern, Tulare, or Fresno county), confirmed by movement disorder specialists at University of California, Los Angeles (UCLA) to have clinically probable or possible PD and had no other diagnosed neurological or psychiatric condition, and were not in the last stages of terminal illness. Patients were recruited from large medical groups, neurologists, and public service announcements. Of the 1,167 PD patients initially recruited to participate, 357 incident idiopathic PD cases were ultimately included in the final analyses (604 did not meet eligibility criteria, 90 could not be examined by movement disorder specialists, 107 did not meet criteria for idiopathic PD on examination by movement disorder specialists, six withdrew from the study, and three were excluded due to PD diagnosis outside of the study period). Controls were recruited from Medicare lists (2001) and residential tax assessor records thereafter, using random selection and enrollment of residential parcels via phone and mail and clustered random selection of five houses with enrollment via in-person home visits. Of the 1,212 potential controls recruited via random selection and the 4,756 individuals identified through the clustered random selection strategy, 752 controls with complete data were included in the final analyses. Demographic information including residential and workplace addresses were collected from cases and controls via telephone interviews. Pesticide exposure between 1974 and 1999 was assessed using a Geographic Information System (GIS)-based method that merged California Department of Pesticide Regulations agricultural pesticide use reports (PUR) data with geocoded address information (residential and workplace) and California Department of Water Resources (CDWR) land-use maps to estimate ambient exposure to malathion

and other pesticides. Participant exposure to individual pesticides, including malathion, was determined by summing the annual pounds of malathion applied in each 500-meter circular buffer surrounding the workplace or the residence, weighted by the proportion of acreage treated within the buffer, to obtain 26 annual exposure values for each pesticide separately for workplace and residential addresses. Authors chose the 500-meter buffer distance based on review of previous literature and it was the intermediate distance among studies reviewed (buffer distances ranged from 200 m - 1200 m). Then, the annual exposure estimates for each participant were averaged across the 26-year study period (1974-1999). Participants were considered to be exposed to a particular pesticide if their 26-year average ambient exposure was greater than or equal to the corresponding residential or workplace medians observed in the controls. Participants were considered unexposed to malathion if they were never exposed to at least the median value of the control group for any organophosphate pesticide or if they did not live or work within 500 m of a pesticide application in the study area during the study time period (1974 - 1999). Among the 357 incident PD Cases and 752 population controls included in the final analysis, 142 (39.8%) cases and 244 (32.4%) controls reported exposure to malathion. Logistic regression was used to assess the association between ambient malathion exposure and PD based on residential address exposure alone, workplace address exposure alone, and residential and workplace address exposures combined. All models were adjusted for gender, race (white vs non-white), age at diagnosis (cases), age at interview (controls $\leq$ 60 or > 60 years), education (<12, 12, >12 years), smoking (current, former, never), having a first-degree family member with PD (yes, no), and exposure to other non-organophosphate pesticides (*e.g.,* organochlorines, dithiocarbamates, and paraquat). Evidence of a moderately strong association was reported between ambient malathion exposure at the *residence and workplace* and incident PD (OR = 2.16; 95% CI: 1.36, 3.43; with n = 52 exposed cases and n = 81 exposed controls). Evidence of a strong association was reported between ambient malathion exposure at the *workplace* and incident PD (OR = 3.16; 95% CI: 1.88, 5.32; with n = 44 exposed cases and n = 43 controls). And evidence of a moderately strong association was reported between ambient malathion exposure at the *residence only* and incident PD (OR=2.69; 95% CI: 1.45, 5.01; with n = 25 exposed cases and n = 31 controls).

The overall study quality was ranked moderate based on the study quality criteria in the OPP Framework. Study strengths included the case-control study design and clinical confirmation of reported PD diagnosis. Study limitations included control selection, the GIS approach to exposure assessment, and the possible under-reporting of PUR data among farmers. Controls were recruited separately using a population-based approach that relied on Medicare enrollee lists and residential tax-collector records. This approach may have introduced selection bias if cases and controls represent populations with different demographics, lifestyle factors, potential for exposure, and willingness to participate in the study and was considered a limitation. We note that authors state that there was no reason to suspect cases and controls would choose to differentially participate in the study based on whether they lived or worked near agricultural plots during the exposure period. For the exposure estimation, the study relied on residential and occupational proximity to malathion agricultural use as determined from the California PUR data mapped to residential and occupational addresses rather than measuring direct exposure. Although farmers were mandated to report their restricted pesticide use as of 1970, some under-reporting may have occurred two years later in 1972, which was the start of the exposure assessment period for this study. The reported results suggest that the GIS approach used to assess exposure had limited ability to investigate exposure to malathion specifically, rather than general residential/workplace proximity to agricultural land in the three counties of interest. Given that this GIS approach has not been validated, it is unclear if being present at addresses within 500 m of agricultural land can provide a reliable estimate of true exposure. Another limitation of the study included the participant's ability to recall their home and workplace addresses from years in the past (up to 26 years using a telephone interview). We note that cases

tended to be older and less educated, more likely to be male, and more likely to have never smoked or to have stopped smoking than controls.

- In a more recent publication of the AHS cohort, Shrestha et al. (2020b) further investigated the association between pesticide use, including malathion, and incident PD among pesticide applicators and their spouses in the AHS. The study population for this analysis included male pesticide applicators (n=38,274) and their spouses (n=27,836) living in Iowa and North Carolina, who completed the enrollment questionnaire (1993 – 1999) and at least one follow-up survey (Phase II - 1999 – 2003, Phase III- 2005 – 2010, Phase IV- 2013 – 2016) or the PD validation screening questionnaire (2012-2017). Cases of incident PD (n=491) included AHS study participants who self-reported a physician diagnosis of PD on one of the follow-up questionnaires or on the PD validation survey, physical evaluation, medical records, or via linkage to state death registries and the National Death Index. The investigators then excluded prevalent cases of PD identified at enrollment and participants with inconsistent or insufficient information across follow-up surveys. Pesticide exposure ever use and intensity-weighted lifetime days of use was assessed using responses to the enrollment questionnaires and the Phase II questionnaire. Among the 66,110 participants (applicators and spouses) included in the analysis, 28,496 (48.7%) non-cases and 253 (62.6%) cases reported malathion exposure. Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% CIs for the potential association between pesticide exposure and PD, adjusting for sex, state of residence, education, smoking status, and ever use of correlated pesticides (Spearman correlation >0.40). No evidence of a significant positive association was reported for the association between ever use of malathion and PD among pesticide applicators and spouses in the AHS (HR=1.01; 95% CI: 0.78, 1.30; with n=253 exposed cases). For the intensity-weighted lifetime days of use analysis, with the following tertiles of exposure for malathion: >0–≤384, >384–≤1344, and >1344 days of exposure, no evidence of a significant positive association was reported between intensity-weighted lifetime days malathion use and incident PD in any exposure of intensity-weighted lifetime days of malathion use (0.83 <HR <1.26; all 95% CIs encompassed the null value 1.0; with n=47 - 68 cases per exposure category; p-trend=0.12)[116].

  The overall quality of the study was ranked moderate based on the study quality criteria outlined in the OPP framework. Study strengths included the general design of the AHS, including its prospective design and ability to assess pesticide use in well-characterized agricultural study population in Iowa and North Carolina. The study was also able to follow-up on the AHS cohort through 2016 and identified 372 incident PD cases, whereas the previous study by Kamel et al. (2007) was limited to Phase II follow-up through 2005 and included only 72 incident PD cases. While the study had several strengths, the study also had several limitations related to Phase III and Phase IV follow-up of the AHS cohort. Most importantly, selection bias may be present in the study because only 24,145 of the 52,394 applicators (46%) enrolled in the AHS cohort in 1993-1997 completed the Phase IV follow-up survey. This degree of loss-to-follow up could introduce selection bias and makes it difficult to assess the association between pesticide use and PD without additional information to evaluate the potential direction and magnitude of bias based on characteristics of study participants that were lost to follow-up. An additional limitation is that no additional information on pesticide use was collected during Phase III and Phase IV of the AHS cohort. Follow-up during these phases covers a 13-year period of potential pesticide use, so this may have introduced exposure misclassification if subjects changed their pesticide use practices during that period. Finally, as with Kamel et al. (2007), the investigators ascertained incident PD cases based on self-report by study participants or through death records. This may introduce misclassification if there is disagreement between self-report of diagnosis and clinical exam by neurological specialists. The AHS investigators suggest that self-

[116] The authors did not specify correlated pesticides

report of PD is reliable, based on previous work that showed 84% agreement between self-report of medical diagnosis and clinical confirmation (Tanner et al., 2011), but it is unclear how potential misclassification may impact the results reported by Shrestha et al. (2020b).

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and PD. There were five studies available to assess this potential relationship (Firestone et al., 2005; Kamel et al., 2007; Firestone et al., 2010; Wang et al., 2014; Shrestha et al., 2020b). Both Firestone et al. (2005) and Firestone et al. (2010) reported no evidence of a positive association between malathion exposure and PD in men. The overall quality of both studies was ranked low for regulatory purposes and several study limitations were noted including interviewer bias and recall bias. Authors reported interviewers were blinded from case-control status of participants, but the outward manifestations of PD made complete blinding impossible. Additionally, the nature of the case-control study imparts potential for recall-bias, and it is possible that those with PD may have remembered their exposures to pesticides more clearly than those without a diagnosis of PD. A third study, Kamel et al. (2007), reported no evidence of a significant positive association and was ranked moderate quality for regulatory purposes. Although study strengths included the AHS study cohort, several study limitations were noted, including the lack of clinical confirmation of self-reported PD cases and the potential for recall bias. Wang et al. (2014), investigated the association between ambient exposure to malathion, and PD among residents living in the Central Valley area of California, using a GIS based exposure assessment and PUR data. The study reported evidence of a moderately strong to strong association between ambient malathion exposure and PD based on residential address exposure, workplace address exposure, and residential and workplace address exposures combined. The study was ranked moderate quality for regulatory purposes, and study limitations included control selection, the GIS approach to exposure assessment, and the possible under-reporting of PUR data among farmers. Additionally, the study relied on the participant's ability to recall their home and workplace addresses from years in the past (up to 26 years using a telephone interview). A fifth study, Shrestha et al. (2020b), was particularly notable because the study provided more recent, prospective follow-up of the AHS cohort through 2016 and included 372 incident PD cases. The study first examined ever-never use of malathion at enrollment and incident PD in the entire AHS cohort and reported no evidence of a significant positive association between ever use of malathion and incident PD, and no evidence of a significant positive association between ever use of malathion and prevalent PD. Shrestha et al. (2020b) further assessed cumulative, lifetime malathion use among AHS applicators and reported no evidence of a significant positive association between malathion use and incident PD in any exposure category of IWLD of malathion use and no evidence of a significant exposure-response relationship. This study was ranked moderate for regulatory purposes.

Respiratory Effects

Eleven studies (Hoppin et al., 2002; Hoppin et al., 2006a; Hoppin et al., 2006b; Hoppin et al., 2007; Hoppin et al., 2008; Hoppin et al., 2009; Hoppin et al., 2017; Valcin et al., 2007; Slager et al., 2010; Henneberger et al., 2014; Rinsky et al., 2019) examined the association between malathion exposure and respiratory effects including asthma, chronic bronchitis, rhinitis, and wheeze.

Asthma

Three studies (Hoppin et al., 2008; Hoppin et al., 2009; Henneberger et al., 2014) examined the association between malathion exposure and asthma.

- The association between pesticide exposure, including malathion, and adult-onset asthma was investigated by Hoppin et al. (2008) in a cross-sectional analysis of female participants of the AHS prospective cohort. The study population consisted of female participants enrolled in the AHS (n = 25,814) who completed study enrollment questionnaires (1993 – 1997) that included questions on pesticide usage and physician's diagnosis of asthma. Cases of self-reported physician-diagnosed asthma as an adult (> 19 years old), were subdivided into atopic or nonatopic asthma based on self-reported eczema and/or hay fever. Pesticide use information collected from the enrollment questionnaires was used to determine lifetime total years of pesticide use and frequency of pesticide application. Polytomous logistic regression was used to calculate ORs and 95% CIs for the association between specific pesticides and asthma, adjusting for age, state, smoking status, BMI, and whether the participant grew up on a farm. Among the 25,814 females included in the analysis, 702 reported adult-onset asthma (282 atopic asthma, 420 nonatopic asthma) and 25,112 participants reported that they did not have asthma. Among the 282 atopic and 420 nonatopic asthma cases, 76 and 100 reported ever use of malathion, respectively. And, among the 25,112 control subjects, 5,004 reported ever use of malathion. Evidence of a positive association was reported for malathion exposure and atopic asthma among farm women, based on ever use (OR = 1.60: 95% CI: 1.22, 2.10; with n = 76 exposed cases) and no evidence of a significant positive association was reported for nonatopic asthma, based on malathion ever use (OR = 1.18; 95% CI: 0.94, 1.49; with n = 100 exposed cases).

  The overall quality of the study was ranked low for regulatory purposes based on the study quality criteria provided in the OPP Framework. The cross-sectional study design was a main limitation since temporality of exposure in relation to the outcome could not be determined. Additionally, the study relied on self-report of the outcome.

- In a separate study on male farmers, Hoppin et al. (2009) investigated the association between pesticide exposure including malathion, and adult-onset asthma among male private pesticide applicators using a cross-sectional analysis of data from the AHS prospective cohort. Cases included male private pesticide applicators in the AHS, aged ≥ 20 years who self-reported physician-diagnosed asthma with onset after 19 years old on the self-administered questionnaires completed at enrollment and shortly thereafter (1993-1997). Cases were subdivided into atopic asthma (those reporting history of physician-diagnosed hay fever or eczema) and nonatopic asthma (no history of physician-diagnosed hay fever or eczema). Pesticide exposure (ever use and IWLD of use) was assessed using data collected on the enrollment questionnaires. Polytomous logistic regression was used to calculate ORs and 95% CIs to evaluate the association between pesticide exposure and adult-onset asthma, adjusting for age, state of residence, smoking, BMI, and high pesticide exposure events (pesticide poisoning). Among the 19,704 private pesticide applicators included in this analysis, 441 reported asthma (n = 127 atopic asthma cases and n = 314 nonatopic asthma cases) and 19,263 reported no history of asthma. 87 (69%) of the 127 atopic asthma cases and 229 (74%) of the 314 nonatopic asthma cases reported malathion exposure. Among those who reported no history of asthma (n = 19,263), 12,150 (64%) reported exposure to malathion. Evidence of a positive association was reported for nonatopic asthma, based on ever/never malathion use (OR = 1.35; 95% CI: 1.04, 1.75; with n = 87 exposed cases). No evidence of a significant positive association was reported for atopic asthma based on ever/never malathion use (OR = 1.08; 95% CI: 0.74, 1.59; with n = 87 exposed cases). In an exposure-response analysis using the median as the cut-point of malathion intensity-adjusted exposure to create two exposure categories (1 – 110 days and > 110 days), evidence of a borderline positive association was reported in both exposure categories for non-atopic asthma (1 – 110 days OR = 1.36; 95% CI: 1.01, 1.83; with n = 109 exposed cases; > 110 days OR = 1.36; 95% CI: 1.02, 1.83 with n = 114 exposed cases), with no evidence of an exposure-response trend (p-trend = 0.90). No evidence of a significant positive association was reported for either exposure category of

atopic asthma (0.79 < OR < 1.41; all 95% CIs encompassed the null value of 1.0; with n = 30 – 55 cases per exposure category), with evidence of a significant exposure-response trend (p-trend = 0.01). In an additional analysis, controls with allergy (atopy) were excluded from the comparison group to determine if the difference in the reported results for atopic and non-atopic asthma was due to atopy alone. Evidence of a positive association was reported for malathion exposure and non-atopic asthma, and no evidence of a significant positive association for atopic asthma (*nonatopic asthma* – OR = 1.38; 95% CI: 1.06, 1.79; with n = 229 exposed cases*; atopic asthma* – OR = 1.10; 95% CI: 0.75, 1.62; with n = 87 exposed cases) when allergic individuals were removed from the control group. And, evidence of a significant positive association was reported for atopy alone (OR = 1.30; 95% CI: 1.17, 1.45; with n = 1,276 exposed cases). Finally, to determine if the results were due to another co-morbid respiratory disease or asthma, those with chronic bronchitis and farmer's lung were excluded from the analysis. No evidence of a significant positive association was reported for *atopic* asthma (OR = 1.15; 95% CI: 0.71, 1.85; with n = 56 exposed cases) and for *nonatopic* asthma (OR = 1.33; 95% CI: 0.97, 1.83; with n = 146 exposed cases).

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The cross-sectional study design was a main limitation since temporality of exposure in relation to the outcome could not be determined. Additionally, the study relied on self-report of the outcome.

- Henneberger et al. (2014) investigated pesticide usage, including malathion, and asthma exacerbation among asthmatic pesticide applicators (commercial and private) enrolled in the AHS. The study population consisted of pesticide applicators living in Iowa and North Carolina who completed both enrollment questionnaires of the AHS study (1993 – 1997) and self-reported physician-diagnosed active asthma.[117] Cases included those participants with active asthma who also reported exacerbation of asthma on the enrollment questionnaire.[118] Current (pesticide used in the 12 months before enrollment) and former (pesticide used in the past but not in the 12 months before enrollment) pesticide exposure was assessed for malathion using data from the self-administered questionnaires completed at enrollment. Among the 926 pesticide applicators with active asthma, 202 reported asthma exacerbation and 724 reported no asthma exacerbation following pesticide exposure. Among the 202 participants with asthma exacerbation, 87 reported malathion exposure. And, among the 724 participants without exacerbation, 359 reported current malathion exposure.[119] Logistic regression was used to calculate ORs and 95% CIs for the association between malathion and asthma exacerbation, adjusting for age (years), state of residence, ever smoked, allergic status, and adult onset of asthma, in addition to separate indicator variables for current and past exposure. No evidence of a positive association was reported between exposure and asthma exacerbation (OR=0.80; 95% CI: 0.40, 1.30; with n=87 exposed cases).

  The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The cross-sectional study design was a main limitation since temporality for exposure in relation to the outcome could not be determined. Additionally, the study relied on self-report of the outcome.

[117] Active asthma was defined as "at least one episode of wheezing or whistling in the past 12 months" and "having breathing problems in the same time period." (Henneberger et al., 2014)

[118] Exacerbation of asthma was defined as a "self-reported visit to a hospital emergency room or doctor for an episode of wheezing or whistling during the past 12 months." (Henneberger et al., 2014)

[119] The study authors mentioned the total number of cases reported with and without exacerbated asthma included the total number of never and current users of pesticides, and does not include former users. As a result, the corresponding total reported may be smaller than the actual total number of users.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and asthma. Three available AHS studies (Hoppin et al., 2008; Hoppin et al., 2009; Henneberger et al., 2014) examined the association between malathion exposure and asthma. Hoppin et al. (2008) reported evidence of a positive association for malathion exposure and atopic asthma among farm women and no evidence of a significant positive association for nonatopic asthma, based on malathion ever use. The subsequent, Hoppin et al. (2009), reported the reverse: evidence of a positive association between ever use of malathion and adult-onset non-atopic asthma among male farmers in the AHS, and no evidence of a significant positive association for atopic asthma. Furthermore, in an exposure-response analysis using the median as the cut-point of malathion intensity-adjusted exposure to create two exposure categories, evidence of a borderline positive association was reported in both exposure categories for non-atopic asthma and no evidence of a significant positive association was reported for either exposure category of atopic asthma. Evidence of a significant exposure-response trend was reported for atopic asthma, and no evidence of an exposure-response trend was reported for non-atopic asthma. The third study, Henneberger et al. (2014), evaluated asthma exacerbation among asthmatic pesticide applicators in the AHS and reported no evidence of a positive association between exacerbated asthma and current malathion exposure. The quality of each of the three AHS studies was ranked low for regulatory purposes due to the cross-sectional study design as temporality for exposure in relation to the outcome could not be determined. Additionally, the studies relied on self-report of the outcome.

Chronic bronchitis

Three publications (Hoppin et al., 2007; Valcin et al., 2007; Rinsky et al., 2019) examined the association between malathion exposure and chronic bronchitis.

- Hoppin et al. (2007) evaluated the potential association between exposure to pesticides including malathion and chronic bronchitis among pesticide applicators in a cross-sectional analysis of the AHS prospective cohort. The study population (n = 20,908) included male pesticide applicators enrolled in the AHS cohort living in Iowa or North Carolina who completed both the enrollment questionnaire and the mailed questionnaire shortly after enrollment (1993 – 1997). Prevalent cases included private pesticide applicators (males only) who self-reported a physician diagnosis of chronic bronchitis at > 19 years of age on the mailed questionnaire completed shortly after enrollment. Pesticide exposure was assessed using responses collected on the enrollment questionnaire. Base logistic regression was used to calculate ORs and 95% CIs to estimate the association between malathion ever/never exposure and chronic bronchitis, adjusting for state of residence, age, gender, and pack years smoking. Among the 654 cases and 20,254 non-cases included in the analysis, 75% of cases (n ~ 490 - 491) and 64% of non-cases (n ~ 12,962 – 12,963) reported exposure to malathion. Evidence of a positive association was reported between malathion exposure and chronic bronchitis among male pesticide applicators (OR = 1.66; 95% CI: 1.38, 1.99). When additionally adjusted for correlated pesticides however,[120] the odds ratio remained significant but was reduced (OR = 1.44; 95% CI: 1.19, 1.76). In an additional analysis where lifetime days of exposure of malathion was divided into categories based on prevalence of pesticide use (five categories for highly used chemicals (prevalence >50%); four categories for pesticides with prevalence between 30% and 50%; and three categories for prevalence less than 30%, with the no exposure group as the referent), lifetime use was divided into the following categories for malathion: 1 – 14, 15 – 55, 56 – 170, 171 – 235, and 236+ lifetime days

[120] Spearman correlation coefficients were calculated for the following correlated pesticides: carbaryl (0.26), chlordane (0.24), and diazinon (0.21).

of use. Evidence of a positive association was reported between malathion exposure and chronic bronchitis in the low, medium, and high exposure categories with the exception of the second highest exposure category, relative to the no exposure group as the referent (1 – 14 lifetime days OR = 1.47; 95% CI: 1.17, 1.85; with n ~ 183 - 184 exposed cases, n ~ 5,266 unexposed cases; 15 – 55 lifetime days OR = 1.46; 95% CI: 1.15, 1.86; with n ~ 156 – 157 exposed cases, n ~ 4,050 – 4,051 unexposed cases; 56 – 170 lifetime days OR = 1.35; 95% CI: 1.01, 1.80; with n ~ 85 exposed cases, n ~ 2,227 – 2,228 unexposed cases; 236+ lifetime days OR = 1.70; 95% CI: 1.11, 2.59; with n ~ 32 – 33 exposed cases, n ~ 607 – 608 unexposed cases). No evidence of a significant positive association was reported for the second highest exposure category (171 – 235 lifetime days OR = 1.48; 95% CI: 0.96, 2.29; with n ~ 26 - 27 exposed cases, n ~ 607 – 608 unexposed cases), and no evidence of an exposure-response trend (p-trend = 0.105) was reported.

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The cross-sectional study design was a main limitation since temporality for exposure in relation to the outcome could not be determined. Additionally, the study relied on self-report of the outcome.

- In a separate study, Valcin et al. (2007) investigated occupational risk factors for chronic bronchitis, including exposure to malathion and other pesticides, among women by conducting a cross-sectional analysis of the AHS prospective cohort. The 21,541 study participants included non-smoking female spouses of pesticide applicators enrolled in the AHS who completed the spouse questionnaire shortly after enrollment (1993 - 2000). Cases of physician diagnosed chronic bronchitis when ≥20 years old were self-reported by participants on the spouse questionnaire completed shortly after enrollment. In addition to health outcome information, the self-administered spouse questionnaire also included detailed information on pesticide exposures and potential confounders. Logistic regression was used to calculate ORs and 95% CIs for lifetime days of exposure to specific pesticides, including malathion, adjusting for age and state of residence and further adjusted for additional variables within each chemical class. Of the 583 cases, 24% (n ~ 139 - 140) reported malathion exposure, while 19% (n ~ 3,982) of the 20,958 controls reported exposure. Evidence of a positive association was reported between malathion and chronic bronchitis (OR = 1.34; 95% CI: 1.10, 1.63; with n ~ 139 - 140 exposed cases). When the model was further adjusted to account for exposure to other herbicides[121] in addition to age and state of residence, no evidence of a significant positive association was reported (OR = 1.21; 95% CI: 0.95, 1.53; with n ~ 139 - 140 exposed cases).

  The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The cross-sectional study design was a main limitation since temporality for exposure in relation to the outcome could not be determined. Additionally, the study relied on self-report of the outcome.

- Rinsky et al. (2019) examined the association between exposure to insecticides potentially used in animal production, including malathion, and self-reported prevalent chronic bronchitis and COPD diagnosis among farmers enrolled in the prospective cohort AHS. The study population included private pesticide applicators from Iowa and North Carolina, enrolled in the AHS who completed both

[121] The authors did not explicitly state which pesticides but said, "After adjusting for the all pesticides in their respective groups, some associations were attenuated."

the enrollment questionnaire (1993 - 1997) and the follow-up interview (2005-2010), had complete data on COPD, and had complete covariate data (n = 22,491 of the 53,394 private pesticide applicators enrolled in the AHS). Of the 22,491 farmers included in the analysis, 922 (4%) reported COPD diagnosis only; 254 (1%) reported both a COPD diagnosis and chronic bronchitis symptoms; 962 (4%) reported chronic bronchitis symptoms only; and 20,353 reported no COPD. Among the participants reported malathion exposure, 673 (73%) reported COPD diagnosis only; 207 (81%) reported COPD diagnosis and chronic bronchitis symptoms; 749 (78%) reported chronic bronchitis symptoms only; and, 15,135 reported no COPD. Covariate data including demographic information, lifestyle, medical history, and farming activities, were collected on the enrollment questionnaire. Exposure was assessed using self-reported use of insecticides registered for use on or around animals, including malathion ever use and lifetime days of use. Lifetime days of use were categorized as never use, ≤ median days of use, > median days of use, based on distribution of the study population. Cases of COPD were divided into four categories to capture the potential variety of manifestations and severity of COPD and included the following categories: COPD diagnosis only, COPD-related diagnosis and chronic bronchitis symptoms, chronic bronchitis symptoms only, and no COPD.[122] The association between COPD and malathion exposure was assessed using polytomous logistic regression models to calculate ORs and 95% CIs for each of the outcome disease categories chronic bronchitis and malathion based on ever/never exposure and lifetime days of exposure, adjusted for age at follow-up interview, state, gender, smoking status (never, former, current), and education (< high school degree, high school graduate/GED, some college, ≥ college graduate). Evidence of a slight positive association was reported for malathion ever use and chronic bronchitis symptoms alone (OR = 1.22; 95% CI: 1.05, 1.43, with 749 exposed cases). Evidence of a positive association was reported for malathion ever use and COPD diagnosis and chronic bronchitis symptoms (OR = 1.85; 95% CI: 1.32, 2.60, with 207 exposed cases). No evidence of a significant positive association was reported for malathion ever exposure and COPD diagnosis alone (*COPD diagnosis alone* – OR = 1.03; 95% CI: 0.88, 1.20, with 673 exposed cases). Similar results were reported for malathion ever use when adjusting for type of animal produced on the farmer's property. Evidence of a slight positive association was reported for malathion ever use and the chronic bronchitis symptoms only outcome when adjusted for type of animal produced (OR = 1.21; 95% CI: 1.03, 1.41, with 749 exposed cases). Evidence of a positive association was reported for malathion ever use adjusted for animal produced and the COPD diagnosis and chronic bronchitis symptoms outcome (OR = 1.84; 95% CI: 1.31, 2.60, with 207 exposed cases). No evidence of a significant positive association was reported between malathion ever use adjusted for animal produced and the COPD only outcome (OR = 1.04; 95% CI: 0.90, 1.22, with 673 exposed cases).

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. Study strengths included the exposure assessment approach which examined cumulative lifetime exposure to malathion and a prospective study design, and the exploration of effect modification by smoking. A major study limitation was that temporal ordering of exposure and outcomes was not possible because prevalent cases were not able to be excluded from the analysis (diagnosis symptoms were not collected from all farmers at study enrollment).

[122] Four disease categories: 1) COPD diagnosis only- physician diagnosis of COPD, chronic bronchitis, or emphysema with no report of classic chronic bronchitis symptoms meeting the classical case definition (cough and phlegm for ≥ 3 months during two consecutive years); 2) COPD-related diagnosis and chronic bronchitis symptoms- physician diagnosis of COPD, chronic bronchitis, or emphysema, and report of symptoms consistent with classical case definition of chronic bronchitis; 3) Chronic bronchitis symptoms only- symptoms consistent with chronic bronchitis classical case definition but no report of physician diagnosis of COPD, chronic bronchitis, or emphysema; and, 4) No COPD – Pesticide applicators who did not report a diagnosis or symptoms consistent with chronic bronchitis.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and chronic bronchitis. Three publications (Hoppin et al., 2007; Valcin et al., 2007; Rinsky et al., 2019) examined the association between malathion exposure and chronic bronchitis among agricultural populations. Hoppin et al. (2007) reported evidence of a positive association between malathion exposure and chronic bronchitis among male pesticide applicators in the AHS based on ever use, and when further adjusted for cumulative lifetime days of exposure, in every exposure category besides the second highest exposure category; however, the exposure-response trend was not statistically significant. Valcin et al. (2007) reported no evidence of a significant positive association between chronic bronchitis and malathion in their analysis of female spouses of AHS pesticide applicators. Rinsky et al. (2019) reported evidence of a positive association for malathion ever use and COPD diagnosis and chronic bronchitis symptoms and reported for malathion ever use adjusted for animal produced and the COPD diagnosis and chronic bronchitis symptoms outcome. Both Hoppin et al. (2007) and Valcin et al. (2007) used cross-sectional study designs. As such, the studies were unable to assess temporality between malathion exposure and chronic bronchitis and were judged to be of low quality for regulatory purposes. Rinsky et al. (2019) was also ranked low quality because the temporal ordering of exposure and outcomes was not possible since prevalent cases were not able to be excluded from the analysis.

Rhinitis

Two AHS studies (Slager et al., 2009; Slager et al., 2010) examined the association between malathion exposure and rhinitis.

- Slager et al. (2009) investigated the association between exposure to pesticides, including malathion, and current rhinitis through a cross-sectional analysis of the commercial pesticide applicators in the AHS prospective cohort. A total of 2,245 commercial pesticide applicators from Iowa completed the self-administered questionnaire at enrollment (1993 – 1997) and 46% of those completed the self-administered mail-in questionnaire shortly after enrollment. The outcome of current rhinitis (a stuffy, runny, or itchy nose in the past 12 months) along with additional medical history was reported on the questionnaire administered shortly after enrollment. Pesticide exposure, ever use and lifetime exposure, was assessed using responses from both the enrollment questionnaire and the mail-in questionnaire completed shortly after. Logistic regression models were used to calculate ORs and 95% CIs to analyze the association between ever use of malathion and current rhinitis, adjusting for age, education, and growing up on a farm. Of the 1,664 cases of rhinitis reported in the study group, 288 (17%) reported exposure to malathion; and, among the 581 respondents who reported no current rhinitis, 78 (14%) reported exposure to malathion. No evidence of a significant positive association was reported between exposure to malathion and current rhinitis based on use within the past year (OR = 1.28; 95% CI: 0.96, 1.69). The study authors noted that the comparison group influenced the risk estimate for malathion, and the odds ratio for malathion reached statistical significance when the referent group was based on never use only (instead of former use as well) (OR = 1.49; 95% CI: 1.10, 2.00).

  The overall quality of the study was ranked low based on the OPP Framework. While the study benefited from the strength of the AHS exposure assessment, the cross-sectional study design was a main limitation since temporality for exposure in relation to the outcome could not be determined. Additionally, the study relied on self-report of the outcome.

- In a separate AHS study, Slager et al. (2010) investigated the association between exposure to malathion and other pesticides and current rhinitis among private pesticide applicators through a cross-sectional analysis of the AHS cohort. A total of 21,958 private pesticide applicators from Iowa and North Carolina completed questionnaires that assessed both exposure to pesticides and the outcome of current rhinitis. The outcome, current rhinitis, was defined by the number of rhinitis episodes[123] reported by the study participant within the past year. Backward selection was used to determine the final models[124]. Logistic and polytomous regression models were used to calculate ORs and 95% CIs to analyze the association between malathion and current rhinitis, adjusting for several covariates[125]. In the polytomous model, the outcome (current rhinitis) was further stratified into categories based on the number of rhinitis episodes within a given year, and ORs were calculated for each of the following categories of rhinitis episodes within a year: 1 episode, 2 episodes, 3 – 6 episodes, 7 – 12 episodes, and 13+ episodes, respectively. Of the 14,629 cases of rhinitis reported in the study group, 2,529 (17%) reported exposure to malathion; and, among the 7,329 respondents who reported no current rhinitis, 995 (14%) reported exposure to malathion. In the polytomous model, evidence of a slight positive association was reported between malathion exposure and those who reported 3 – 6 episodes of rhinitis within a year (OR = 1.10; 95% CI: 1.04, 1.12), along with a significant p-trend ($p = 0.011$). No evidence of a significant positive association was reported for the 1, 2, 7 – 12, and 13 + episodes of rhinitis within a year ($0.97 \leq OR \leq 1.08$; all CIs encompassed the null value of 1.0). For the dichotomous logistic regression model (ever/never exposure), evidence of a borderline slight positive association between malathion and current rhinitis was reported (OR = 1.06; 95% CI: 1.01, 1.11).

  The overall quality of the study was ranked low based on the OPP Framework. The cross-sectional study design was a main limitation since temporality for exposure in relation to the outcome could not be determined. Additionally, the study relied on self-reported outcome, backwards selection was used, and a very large number of covariates were included in the final models. Lastly, the number of study participants exposed to malathion relative to the outcome (rhinitis) was not provided by the study authors (only the total number of participants who experienced the outcome) for both the polytomous and logistic models.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and rhinitis. Two available studies (Slager et al., 2009; Slager et al., 2010) examined rhinitis among private pesticide applicators in the AHS. Slager et al. (2009) reported no evidence of a significant positive association among commercial pesticide applicators based on use within the past year. Slager et al. (2010) examined rhinitis among commercial

[123] A rhinitis episode was defined as a runny, itchy, or stuffy nose, and was self-reported by the study participant.

[124] With respect to backward selection (automatic stepwise) procedures, these are 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. For problems connected to use of these procedures, 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.

[125] "Odds ratios were adjusted for: age, race, education, state of residence, body mass index, currently working on a farm, years mixing pesticides, repairing engines, repairing pesticide equipment, welding, painting, handling stored grain, handling stored hay, working in swine areas, working with hogs, other farm animals, butchering animals, growing cabbage, Christmas trees, field corn, sweet corn and hay relative to the reference category of no rhinitis in the past year." (Slager et al., 2010)

pesticide applicators in the AHS, and reported evidence of a slight positive association between malathion and current rhinitis based on ever/never exposure as well as for one of the rhinitis categories only (3 – 6 episodes/year) in the polytomous model. No evidence of a significant positive association was reported in any of the four other rhinitis categories. The overall study quality of both studies was ranked low for regulatory purposes. The cross-sectional study design was the main limitation and additionally the statistical methods used to select the regression model covariates.

Wheeze

Four AHS studies (Hoppin et al., 2002; Hoppin et al., 2006a; Hoppin et al., 2006b; Hoppin et al., 2017) examined the association between malathion exposure and wheeze.

- Hoppin et al. (2002) evaluated the association between exposure to pesticides, including malathion, and the prevalence of wheeze among pesticide applicators in a cross-sectional analysis of the AHS prospective cohort. The study population consisted of 20,468 pesticide applicators living in Iowa and North Carolina enrolled in the AHS, who completed both the enrollment questionnaires (1993 - 1997). Wheeze in the past year and pesticide exposure were self-reported on the self-administered questionnaires completed at enrollment and shortly following enrollment. Logistic regression was used to estimate the association between malathion ever use and wheeze in the past year, adjusting for age, state, past smoking, current smoking, and asthma/atopy. Of the 20,468 participants included in the analysis (3,838 reported wheeze and 16,630 reported no wheeze) 34.7% (n = ~1,331 – 1,332) of those with wheeze reported exposure to malathion and 30.7% (n ~ 5,105) of those who reported no wheeze also reported exposure to malathion. Evidence of a slight positive association between malathion exposure and wheeze among pesticide applicators (OR = 1.14; 95% CI: 1.02, 1.28) based on ever use in the past year. Further, the authors reported evidence of a linear (monotonic) trend across categories based on five ordinal categories of exposure (p-trend = 0.01).

  The overall quality of the study was ranked low based on the study quality criteria outlined in the OPP framework. Hoppin et al. (2002) relied on a cross-sectional design that assessed the relationship between prevalent cases of wheeze and pesticide exposure. As such, the study was unable to assess temporal association between malathion exposure and wheeze.

- In a separate AHS study, Hoppin et al. (2006a) investigated the association between pesticides including malathion, and the prevalence of wheeze using a cross-sectional analysis of the AHS prospective cohort. Study participants included private pesticide applicators (n=17,920) and commercial pesticide applicators (n=2,255) enrolled in the AHS between 1993 – 1997. Cases of wheeze were defined as participants who reported episodes of wheezing or whistling in the chest in the year before study enrollment and were self-reported on the enrollment questionnaire. Pesticide exposure (current use and past use) was also reported on the enrollment questionnaire. To evaluate lifetime pesticide use, authors created three variables: never use, former use (use but not in the past year) and current use (used in the past year). Among the total study participants, 19% of the 17,920 private applicators and 22% of the 2,255 commercial applicator study participants reported wheeze in the past year. Among private applicators, 35%, 48%, and 17% reported never, former, or current use of malathion; among commercial applicators, 44%, 40%, and 16% reported never, former, or current use of malathion, respectively. Logistic regression was used to estimate ORs and 95% CIs for the association between malathion and wheeze, adjusted for age, BMI, smoking, asthma/atopy, and previous use of pesticides. State of residence was also included as a potential confounder in the analyses for farmer applicators only; commercial applicator participants resided only in Iowa. Chlorimuron-ethyl adjustment was included in models for commercial applicators. No evidence of a significant positive association was reported between current malathion use and wheeze among

private pesticide applicators (OR=1.13; 95% CI: 1.00, 1.27) and no evidence of a positive association was reported among commercial applicators (OR=0.95; 95% CI: 0.69, 1.31). Additional results for commercial applicators were described in a separate publication (Hoppin et al., 2006b).

The overall quality of the study was ranked low based on the study quality criteria outlined in the OPP Framework. Hoppin et al. (2006a) relied on a cross-sectional design that assessed the relationship between prevalent cases of wheeze and pesticide exposure. As such, the study was unable to assess the temporal association between malathion exposure and wheeze.

- The results for commercial applicators are described in more detail in an additional AHS study, Hoppin et al. (2006b). Among the 486 commercial applicators that reported wheeze in the past year, 211 (44%) reported never use, 186 (39%) former use, and 85 (18%) reported current use of malathion. No evidence of a significant positive association was reported for current use of malathion and wheeze in the past year (OR=1.06; 95% CI: 0.78, 1.45; with n=85 exposed cases and n=282 exposed non-cases).

  The overall study quality was determined to be of low based on the study quality criteria outlined in the OPP Framework. Hoppin et al. (2006b) relied on a cross-sectional design that assessed the relationship between prevalent cases of wheeze and pesticide exposure. As such, the study was unable to assess the temporal association between malathion exposure and wheeze.

- Hoppin et al. (2017) investigated the association between pesticide exposure including malathion, and allergic and non-allergic wheeze among male private pesticide applicators through a cross-sectional analysis of the AHS prospective cohort. The study population (N=22,134) consisted of male private pesticide applicators who completed the AHS enrollment (1993 - 1997) and follow-up questionnaires (2005 – 2010) and reported symptoms of wheeze. Wheeze was defined as at least one episode of wheeze or whistling in the chest i*n the past year* with a physician-diagnosis of hay fever for allergic wheeze, or as at least one episode of wheeze or whistling in the chest *in the past year* without a diagnosis of hay fever for non-allergic wheeze. Non-cases were participants without wheeze but could have had allergy as authors reported they were interested in allergy as a modifier of wheeze not as an outcome. Pesticide exposure data reported at enrollment and follow-up was used to create three definitions for exposure current use (use since the last AHS interview), past use (not used since the last AHS interview); and never use of malathion. Polytomous logistic regression was used to determine the association between wheeze and malathion exposure, with allergic and non-allergic wheeze investigated separately. Models were adjusted for age, BMI, state, smoking, and current asthma, as well as for days spent applying pesticides and days driving diesel tractors. Among the 1,310 allergic wheeze cases, 12% reported current use of malathion, and among the 3,939 non-allergic wheeze cases, 13% reported current use of malathion within the past year. Of the 16,885 control subjects, 11% reported current use of malathion within the past year. Evidence of a positive association was reported between current malathion use and allergic wheeze in the past year (OR = 1.48; 95% CI: 1.19, 1.86; with n ~157 exposed cases) and a slight positive association for non-allergic wheeze in the past year (OR = 1.29; 95% CI: 1.13, 1.46; with n ~512 exposed cases).

  A further analysis considered the association between cumulative days of use of malathion and allergic and non-allergic wheeze among male private applicators. Authors divided the distribution of users of malathion into the following exposure categories based on frequency of use: 1 day, 2 days, 3-4 days, 5-7 days, 8-100 days of malathion *in the past year*. Past use (not used since the last AHS interview) and never use were also included in the model and never use served as the referent category for the analysis. In the exposure-response analysis for allergic wheeze, evidence of a positive association was reported for wheeze in the past year and the following exposure categories of

malathion: 2 days, 3 – 4 days, and 5 – 7 days (OR past use = 1.39; 95% CI: 1.20, 1.61 with n = 871 exposed cases; OR 2 days use = 1.93; 95% CI: 1.36, 2.75 with n = 46 cases; OR 3 – 4 days: 2.00; 95% CI: 1.30, 3.08 with n = 29 cases; OR 5 – 7 days use = 1.85; 95% CI: 1.12, 3.04 with n = 21 cases). No evidence of a significant positive association was reported for any other exposure category of malathion and allergic wheeze in the past year ($0.99 < OR < 1.08$; all 95% CIs encompassed the null value of 1.0; with n = ~14 - 42 cases per exposure category). No evidence of a significant positive association was reported in the highest exposure category of 8-100 days of use of malathion in the last year (OR = 1.08; 95% CI: 0.60, 1.95; with n = 14 exposed cases). For the analysis with the non-allergic wheeze, using the same exposure categories, evidence of a positive association was reported in the following exposure categories: 2 days and 8-100 days (OR 2 days = 1.32; 95% CI: 1.06, 1.64 with n = 126 cases; 8 – 100 days = 1.57; 95% CI: 1.16, 2.12 with n = 63 cases), and evidence of a borderline positive association at 3-4 days (OR 3 – 4 days = 1.34; 95% CI: 1.01, 1.77 with n = 73 cases). No evidence of a significant positive association was reported for non-allergic wheeze and the following exposure categories: past use, 1 day, and 5 – 7 days of malathion use ($1.09 \leq OR \leq 1.19$; all 95% CIs encompassed the null value of 1.0; with n = 49 – 206 cases per exposure category). The authors did not report a p-trend statistic for the exposure-response analysis for either allergic or non-allergic wheeze and malathion; however, inspection of the ORs associated with each category suggests an exposure-response trend may not exist for either allergic or non-allergic wheeze.

The overall quality of the study was ranked low based on the study quality criteria outlined in the OPP framework. Hoppin et al. (2017) relied on a cross-sectional design that assessed the relationship between current wheeze and pesticide exposure. As such, the study was unable to assess the temporal association between malathion exposure and wheeze.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and wheeze. Four AHS studies (Hoppin et al., 2002; Hoppin et al., 2006a; Hoppin et al., 2006b; and Hoppin et al., 2017) examined the association between malathion exposure and wheeze in the AHS prospective cohort study population. Hoppin et al. (2002) reported evidence of a slight positive association between malathion exposure and wheeze among pesticide applicators, based on ever use. In subsequent follow-on studies, Hoppin et al. (2006a) reported no evidence of a significant positive association between current malathion use and wheeze for farmer (private) applicators based on ever use within the year before enrollment, and Hoppin et al. (2006b) reported no evidence of a significant positive association between current malathion use and wheeze among commercial applicators, based on ever use. In a fourth study on the AHS that included a cross-sectional analysis of malathion exposure in the past year and wheeze in the past year using the responses from the 2005-2010 follow-up survey rather than from enrollment, Hoppin et al. (2017) reported evidence of a positive association between malathion exposure in the past year for allergic wheeze based on ever use. Evidence of a positive association was reported in three of the five exposure categories of allergic wheeze in the exposure-response analysis. No evidence of a significant positive association was reported in the highest exposure category. For non-allergic wheeze, evidence of a slight positive association was reported between malathion and wheeze based on ever use and evidence of a positive association was reported in the highest exposure category of malathion use in the past year in the exposure-response analysis, along with two other exposure categories. The authors did not report a p-trend statistic for the exposure-response analysis for either allergic or non-allergic wheeze and malathion; however, inspection of the ORs associated with each category suggests an exposure-response trend may not exist for either allergic or non-allergic wheeze. All four studies were ranked low quality, as they relied on a cross-sectional design that was unable to assess the temporality of the relationship between cases of pesticide exposure and wheeze. Additionally, health outcomes were self-reported.

Recurrent Pregnancy Loss

The association between malathion exposure and recurrent pregnancy loss among women was investigated in one publication (Pandey et al., 2020).

Pandey et al. (2020) evaluated the association between pesticide exposure including malathion and recurrent pregnancy loss among women in a case-control study conducted in India. Cases included women (n = 70) who were patients that had suffered two or more spontaneous miscarriages before the 20th week of gestation. Controls consisted of healthy women (n = 70) who had successfully delivered at least one child during the same time frame. Women in both groups were identified and enrolled into the study between January 2012 and January 2015 from King George's Medical University located in Lucknow, Utter Pradesh, India, and were excluded from the study if they met any of the following criteria: tuberculosis, hypertension, diabetes, any endocrine disorder, breast cancer, genital cancer, colon cancer, immunocompromised symptoms, or any other malignancies. Both the cases and controls were interviewed to obtain information on family history, smoking/tobacco chewing use, age, and alcohol consumption, in addition to collecting 5 mL blood samples. Blood samples were collected prior to the 20th week of gestation for the cases, and on Day 21 following the end of the menstrual cycle for women in the control group. Serum was abstracted from the blood samples after 10 minutes, and stored until analyzed at -20°C. Pesticide residues including malathion were extracted and quantified from the blood, and analyzed using gas chromatography. A Mann-Whitney test was used to determine whether a significant difference existed for malathion concentrations among the cases and control groups. No evidence of a statistically significant difference ($p = 0.22$) was reported for malathion exposure among the cases and control groups (mean±SD exposure level for cases: 2556 ±1027; mean±SD exposure level for controls: 1253.7±1421).

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. Although the study design was considered a strength, several study limitations were noted. No ascertainment of the cases was mentioned, laboratory QA/QC procedures were not carried out, and the statistical methods used in the study were considered rudimentary. Additionally, the interview conducted by the study authors to obtain supplemental details of the study participants did not include questions pertaining to their occupational/agricultural exposures, as well as detailed medical histories of both the cases and controls. These study details could have strengthened both the exposure and outcome assessments. Furthermore, additional details regarding how the cases and controls were recruited in this study could have provided clarity to rule out selection bias as a concern. The quality was ranked low for regulatory purposes.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and recurrent pregnancy loss among females. One study (Pandey et al., 2020) examined this potential association and reported no evidence of a statistically significant difference for malathion exposure among the cases and control groups ($p = 0.22$). The quality of the study was ranked low for regulatory purposes. The case-control study design was considered a strength; however, several study limitations were noted. No ascertainment of the cases was mentioned, laboratory QA/QC procedures were not carried out, and the statistical methods used in the study were considered rudimentary. Additionally, the interview conducted by the study authors to obtain supplemental details of the study participants did not include questions pertaining to their occupational/agricultural exposures, as well as detailed medical histories of both the cases and controls. These study details could have strengthened both the exposure and outcome assessments. Furthermore, additional details regarding how the cases and controls were recruited in this study could have provided clarity to rule out selection bias as a concern. The quality was ranked low for regulatory purposes.

Sleep Apnea

The association between malathion exposure and sleep apnea was evaluated in one publication (Baumert et al., 2018).

Baumert et al. (2018) evaluated the association between malathion exposure and sleep apnea in male pesticide applicators using data from the Agricultural Lung Health Study (ALHS), a case-control study of current asthma nested within the AHS cohort. ALHS participants were identified via an AHS follow-up telephone interview (2005 – 2010) and enrolled into the ALHS between 2009 and 2013. Cases of sleep apnea included male pesticide applicators who self-reported physician-diagnosed sleep apnea with treatment on the ALHS computer-assisted telephone survey. The controls were randomly selected from the AHS cohort and included study participants who did not self-report physician-diagnosed sleep apnea. AHS exposure questionnaires completed at enrollment (1993 – 1997) and at 5-year and 10-year follow-up time points (1999 – 2003, 2005 – 2010) were used to assess ever use of malathion. Among the 1,569 male pesticide applicators participating in the study, 162 (69.2%) of the 234 sleep apnea cases and 979 (73.3%) of the 1,335 cases reported exposure to malathion. Logistic regression was used to evaluate the association between malathion ever use and sleep apnea, adjusting for state of residence, age, BMI, history of diabetes, asthma, hypertension, and cardiovascular disease. No evidence of a positive association was reported between malathion exposure and sleep apnea based on ever/never use (OR=0.94; 95% CI: 0.67, 1.32).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Authors reported that the response rate for enrollment into the ALHS nested case-control study was 50% and this possibly could have introduced selection bias if there were differences between those that responded and those that did not. This information was not available as sleep apnea was not asked about on the earlier questionnaire. Additionally, cases of sleep apnea with treatment were self-reported allowing the potential for misclassification of the outcome. The outcome assessment would have been strengthened with medical record confirmation.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and sleep apnea. One study (Baumert et al., 2018) examined this association among male pesticide applicators enrolled in the AHS and reported no evidence of a positive association, based on ever use of malathion. The quality of the study was ranked moderate for regulatory purposes. The quality of the study was ranked moderate for regulatory purposes and limitations included the potential for selection bias due to the 50% response rate to enroll in the ALHS and self-reported outcome.

Stroke

The association between malathion exposure and stroke was evaluated in one publication (Rinsky et al., 2013) described below.

Rinsky et al. (2013) examined the association between pesticide exposure, including malathion, and the risk of stroke mortality among AHS prospective cohort participants. The study population consisted of male pesticide applicators (n=51,603) enrolled in the AHS living in Iowa and North Carolina. Cases of stroke mortality included AHS study participants who died from a stroke between study enrollment (1993 – 1997) through December 31, 2008, and vital status of each case was ascertained using state death certificates. The non-cases included AHS study participants who did not suffer from stroke mortality.

Pesticide exposure was assessed for 50 different pesticides, including malathion, using self-administered questionnaires completed at study enrollment. Of the 308 cases of fatal stroke and of the 51,295 non-cases, 206 (73%) cases and 34,127 (69%) non-cases reported malathion exposure. HRs and 95% CIs were calculated using Cox proportional hazards analysis, adjusting for smoking status, alcohol intake, and state of residence. No evidence of a positive association was reported between malathion exposure and stroke mortality (HR=0.99; 95% CI: 0.76, 1.29; with n=206 exposed cases) based on ever/never use.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. As part of the AHS, this study benefited from the strengths of the AHS study cohort including the prospective cohort study design and case ascertainment. Although the study investigated stroke mortality, details regarding stroke morbidity were not provided. This was a potential study limitation as the measure here did not capture the number of total incident stroke cases (including both fatal and non-fatal).

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and stroke. Rinsky et al. (2013) examined the association between malathion exposure and stroke among male pesticide applicators enrolled in the AHS and reported no evidence of a positive association between malathion exposure and stroke mortality. The quality of the study was ranked moderate for regulatory purposes. As part of the AHS, this study benefited from the strengths of the AHS study cohort including the prospective cohort study design and case ascertainment. Although the study investigated stroke mortality, details regarding stroke morbidity were not provided. This was a potential study limitation as the number of incident stroke cases may have been underreported.

Suicide

One study (Beard et al., 2011) evaluated the potential relationship between malathion exposure and suicide.

Beard et al. (2011) evaluated the potential association between pesticide exposure including malathion and suicide among pesticide applicators and their spouses in the AHS prospective cohort. Cases of suicide that occurred after enrollment (1993-1997) through May 2009 were identified by linking the AHS cohort to state mortality files and the National Death Index. Pesticide exposure was assessed via a self-administered questionnaire at enrollment. Cox proportional hazards models were used to analyze the association between malathion ever exposure and suicide risk to calculate HRs and 95% CIs, adjusting for age at enrollment, sex, number of children, frequency of alcohol consumption within the past year, and smoking. Among the study population (n = 81,998), 40,702 reported exposure to malathion. Among the 110 cases of suicide that occurred between enrollment (from 1993 to 1997) and May 2009, 62 cases reported ever exposure to malathion. No evidence of a positive association was reported between malathion exposure and suicide (HR = 0.93; 95% CI: 0.61, 1.42) based on ever/never use.

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths include the prospective design of AHS and the detailed 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.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and suicide. One AHS study (Beard et al., 2011) examined the association between malathion exposure and suicide and reported no evidence of a positive association among pesticide applicators. The quality of the study was ranked moderate for regulatory purposes based on the study quality criteria provided in the OPP Framework. Study strengths included the prospective design of the AHS and the AHS detailed 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.

Testosterone Level Changes

One study (Panuwet et al., 2018) investigated the association between malathion exposure and testosterone level changes among male farmers.

Panuwet et al. (2018) evaluated the association between malathion exposure and testosterone level changes in male farmers living in Thailand in a cross-sectional study. The study took place in 2006, and the study population (n = 136) included male farmers, who resided in either Inthakhin or Pong Yaeng, Thailand, and were 20 – 65 years of age. Study participants were required to be occupationally exposed to pesticides, physically fit, and be willing to provide urine and blood samples in addition to completing a survey (n = 133). Pesticide exposures including malathion was measured via urinary metabolites, and the specific metabolite for malathion was malathion dicarboxylic acid (MDA). Self-collected urine samples were obtained from the first morning void, and samples were then brought to a follow-up appointment where blood samples were also drawn from the study participants. Urine samples were then stored at -20°C prior to being shipped and after delivery, and were analyzed a year later (in 2007) for pesticide metabolites including MDA, using liquid chromatography with tandem mass spectrometry. The collected urine samples were creatinine-adjusted as a way to normalize the amount of metabolites detected due to the dilution of the urine. Serum was extracted from the collected blood samples within 24 hours after the follow-up appointment, prior to being stored at -70°C until analyzed six and eight years later (in 2012 and 2014) for testosterone. Testosterone levels (both total and free) were measured and assessed in the triplicate using the serum samples. Total testosterone is the total amount of testosterone found within the blood of an individual, which consists of both free and bound testosterone. Linear regression was used to evaluate the association between the urinary malathion metabolite (MDA) and changes in serum testosterone levels (both total and free) among male farmers, adjusting for smoking status, age, BMI, crop type, years of use, and documentation of previous pesticide use before sample collection. The MDA limit of detection (LOD) was 0.30 µg/L. Due to the low detection frequency, MDA exposure was treated as categorical variable (detected vs not detected) in the statistical analysis, and the outcome was modeled separately for total testosterone and free testosterone. The statistical models were further stratified by location (either Inthakhin or Pong Yaeng, Thailand), and adjusted for the same covariates. Among the total number of male farmers (n = 136) participating in the study, 67 study participants resided in the Pong Yaeng and 69 study participants resided in Inthakhin, Thailand. The limit of detection ranged for Pong Yaeng ranged from <LOD – 24.3 µg/g creatinine with a detection frequency of 10.4%, and in Inthakhin the limit of detection ranged from <LOD – 939 µg/g creatinine with a detection frequency of 26.1%. No evidence of a significant association was reported between the urinary metabolite MDA and changes for either total testosterone levels and for free testosterone levels among male farmers living in Inthakhin, Thailand (total testosterone β = -0.086; 95% CI: -0.219, 0.048, p-value = 0.216; free testosterone β = -0.026; 95% CI: -0.125, 0.074, p-value = 0.617). For farmers residing in Pong Yaeng, Thailand, the association between the urinary metabolite MDA and changes in total and free testosterone

levels among farmers could not be estimated as mentioned by the study authors, due to the low metabolite detection frequency (<25%).

The overall quality of the study was ranked low based on the study quality criteria provided in the OPP Framework. The cross-sectional study design was the main limitation since temporality for exposure in relation to the outcome could not be determined, thus the study was ranked low quality for regulatory purposes.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and testosterone levels changes in male farmers. One study (Panuwet et al., 2018) evaluated the association between malathion exposure via the urinary metabolite, MDA, and testosterone level changes for both total and free testosterone in male farmers living in either Pong Yaeng or Inthakhin, Thailand. No evidence of a significant association was reported between MDA and changes for either total testosterone levels and for free testosterone levels among male famers living in Inthakhin, Thailand. For farmers residing in Pong Yaeng, Thailand, the association between MDA and changes in total and free testosterone levels among farmers could not be estimated as mentioned by the study authors, due to the low metabolite detection frequency (<25%).

Thyroid disease

Six studies (Goldner et al., 2010; Goldner et al., 2013; Shrestha et al., 2018b; Shrestha et al., 2018c; Shrestha et al., 2019b; Lerro et al., 2018) investigated the association of malathion exposure and thyroid disease including hyperthyroid disease, hypothyroid disease, and other thyroid disease.

Hyperthyroid disease

Three studies (Goldner et al., 2010; Shrestha et al., 2018b; Shrestha et al., 2019b) investigated the association between malathion exposure and hyperthyroid disease among female spouses and pesticide applicators in the AHS.

- Goldner et al. (2010) evaluated the association between prevalent thyroid disease and malathion and other pesticides among female spouses of male private applicators in a cross-sectional analysis of data from the AHS prospective cohort. The study population included all female spouses of male private applicators who completed both the enrollment questionnaire on pesticide exposure (1993 – 1997) and the follow-up telephone interview collecting information on history of thyroid disease (1999 – 2003) and had complete data on all covariates. Cases of physician-diagnosed thyroid disease were ascertained through self-report during follow-up interviews (1999 – 2003) and were further classified into three subgroups: hypothyroidism, hyperthyroidism, and other thyroid disease. Pesticide exposure among female spouses of male private applicators was reported through the Spouse Enrollment Questionnaire given at enrollment (1993 – 1997) and included direct pesticide exposure (ever use of malathion), but not indirect pesticide exposure of the spouse (husband's use of the pesticide). Polytomous logistic regression was used to analyze the association between ever use of malathion and the occurrence of thyroid disease, adjusting for BMI, age at enrollment, smoking status, hormone replacement therapy (ever/never), and education. Among the 2,043 total cases of thyroid disease reported among female spouses, there were 64 (17.3%) hyperthyroid cases, 220 (19.7%) hypothyroid cases, and 98 (17.50%) 'other' thyroid cases reported ever use of malathion. No evidence of a positive association was reported for the association between malathion exposure (ever use) and

hyperthyroid disease among female spouses of pesticide applicators (OR = 0.97; 95% CI: 0.73, 1.30; with n = 64 exposed cases).

The overall study quality was ranked low based on the study quality criteria provided in the OPP Framework. Authors were not able to analyze incident cases separately from prevalent cases due to the way the data were collected. The cross-sectional study design was a limitation since temporality for exposure in relation to the outcome could not be determined. Reliance on self-report of a physician's diagnosis on the outcome without clinical confirmation was another limitation. Additionally, the investigators were only able to assess ever/never exposure and did not have more detailed exposure information to assess cumulative malathion exposure.

- Shrestha et al. (2018b) evaluated the association between exposures to pesticides including malathion and incident thyroid disease. The study population consisted of female spouses of pesticide applicators enrolled in the AHS, an ongoing prospective cohort study. For this analysis, the study population included 24,092 AHS female spouses. Hyperthyroid and hypothyroid disease status was ascertained through self-report during follow-up interviews during Phase II (1999 – 2003), Phase III (2005 – 2010) and Phase IV (2013 – 2016) of the study. Validation of self-reported cases of hyperthyroid and hypothyroid disease was carried out using medical record data; however, study authors reported that for hyperthyroid disease, only 32% of the study participants who self-reported hyperthyroid disease confirmed their diagnosis using medical record confirmation and thyroid disease. Pesticide exposure was reported through self-administered questionnaires at enrollment (1993 – 1997). The Cox proportional hazards models were used to calculate separate HRs and 95% CIs for hypothyroid and hyperthyroid disease, adjusted for smoking, education, and state and then HRs and 95% CIs adjusted for smoking, education, state, and correlated pesticides. The authors used multiple imputation with a fully conditional specification method to impute missing covariates for 1,273 spouses missing information on smoking status and 3,106 on education. The authors created five imputed datasets, performed regression analysis in each dataset, and obtained the pooled parameter estimates. For hyperthyroid disease, no evidence of a positive association was reported for malathion exposure (HR: 1.01; 95% CI: 0.82, 1.26 with n=107 exposed cases, 410 unexposed cases) based on ever use. When further adjusted for correlated pesticides, no evidence of a significant positive association was similarly reported (HR: 1.08; 95% CI: 0.85, 1.39 with n=107 exposed cases, 410 unexposed cases). An additional analysis that only included thyroid cases as defined by *receipt of treatment* in AHS spouses also reported no evidence of a significant positive association between malathion exposure and hyperthyroid disease (HR: 1.06; 95% CI: 0.84, 1.35 with n=86 exposed cases, 313 unexposed cases). And finally, an additional analysis that only included thyroid cases as defined by those confirmed by medical records or validation questionnaire, reported no evidence of a significant positive association between malathion exposure and hyperthyroid disease among female spouses of pesticide applicators (HR: 1.21; 95% CI: 0.86, 1.70 with n=45 exposed cases, 146 unexposed cases).

  The overall study quality was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the prospective cohort design, and the extensive methods used to obtain exposure information for several pesticides including malathion. Study limitations included self-reported outcome by the study participants and potential selection bias. Since thyroid disease was self-reported in this study (only 32% of the self-reported cases were ascertained via medical records), it was likely some cases misclassified their thyroid disease status and subtype. Potential selection bias was likely if study subject participation in the follow-up phases was related to their disease status for hyperthyroidism. The pesticide use information was limited to use prior to enrollment and did not account or pesticide use that occurred after enrollment and may have led to

exposure misclassification. Additional study details regarding frequency and duration of pesticide use for malathion would have been useful but were not provided.

- Shrestha et al. (2019b) evaluated the association between incident hyperthyroid disease and exposures to pesticides including malathion. The study population consisted of private pesticide applicators enrolled in the AHS, an ongoing, prospective cohort study. Pesticide exposure was reported through self-administered questionnaires at enrollment (1993 – 1997), and hyperthyroid disease status was ascertained through self-report during follow-up interviews during Phase II (1999 – 2003), Phase III (2005 – 2010) and Phase IV (2013 – 2016) of the study. Cases of hyperthyroid disease were validated using medical record data or two validation questionnaires. Validation by medical records was accomplished among only 32% of self-reported cases. The Cox proportional hazards model was used to calculate HRs for hyperthyroid disease, adjusting for smoking, education, state, and sex. Authors restricted their analysis to exposures with at least 10 thyroid disease cases in the overall analysis, but for the stricter case analysis[126] at least 5 exposed cases were required due to the limited sample size. No evidence of a positive association between malathion and hyperthyroidism among private applicators was reported in the overall analysis and the stricter case definition analysis (n=35,150) (Overall HR: 0.68; 95% CI: 0.52,0.88), with n=158 exposed cases; Stricter Case Definition HR: 0.56; 95% CI: 0.33,0.94). An additional sub-analysis that investigated the association between malathion exposure (based on ever/never use) and hyperthyroid risk among private applicators when females were excluded (n=34,375) found no evidence of a positive association (HR: 0.66; 95% CI: 0.50, 0.87, with n=149 exposed cases).

  This overall quality of the study was ranked moderate based on the study quality criteria in the OPP Framework. Strengths of the AHS as noted above, including the prospective study design and pesticide-use information. The study was limited by the reliance on self-report of hyperthyroidism diagnosis even though they attempted to validate cases via medical records (32% confirmed by medical personnel), and the pesticide use information was limited to use prior to enrollment and did not account or pesticide use that occurred after enrollment and may 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 malathion exposure and hyperthyroid disease. Three publications (Goldner et al., 2010. Shrestha et al., 2018b; Shrestha et al., 2019b) examined the relationship between malathion exposure and hyperthyroid disease among AHS study participants. Goldner et al. (2010) reported no evidence of a positive association between malathion ever use and hyperthyroid disease among female spouses of pesticide applicators enrolled in the AHS. The publication was ranked low due to a cross-sectional study design since temporality for exposure in relation to the outcome could not be determined and was limited by self-reported outcome. Shrestha et al. (2018b) reported no evidence of a positive association among female spouses of pesticide applicators in the AHS, based on malathion ever use and longer follow-up time and was ranked moderate quality. A third publication (Shrestha et al., 2019) reported no evidence of a positive association among private pesticide applicators in the AHS based on malathion ever use and was ranked moderate quality. Both Shrestha et al. (2018b) and Shrestha et al. (2019b) attempted to validate the self-reported hyperthyroidism diagnosis via medical record confirmation, however only 32% of attempted cases were ultimately clinically confirmed. Potential selection bias was also likely if study subject participation in the follow-up phases was related to

[126] In this study, the stricter case definition was inclusive of a.) cases confirmed via medical records or validation questionnaire; or, b.) cases who reported having hyperthyroidism ≥ 2 times on validation surveys.

their disease status for hyperthyroidism. An additional limitation of all three publications was that only ever use of pesticides prior to enrollment was captured rather than pesticide use that occurred after enrollment and this may have led to exposure misclassification.

Hypothyroid disease

Five studies (Goldner et al., 2010; Goldner et al., 2013; Shrestha et al., 2018b; Shrestha et al., 2018c; Lerro et al., 2018) investigated the association of malathion exposure hypothyroid disease.

- Goldner et al. (2010) evaluated the association between prevalent thyroid disease and malathion and other pesticides among female spouses of male private pesticide applicators in a cross-sectional analysis of data from the AHS prospective cohort. The study population included all female spouses of male private applicators who completed both the enrollment questionnaire on pesticide exposure (1993 – 1997) and the follow-up telephone interview collecting information on history of thyroid disease (1999 – 2003) and had complete data on all covariates. Cases of physician-diagnosed thyroid disease were ascertained through self-report during follow-up interviews (1999 – 2003) and were further classified into three subgroups: hypothyroidism, hyperthyroidism, and other thyroid disease. Pesticide exposure among female spouses of male private applicators was reported through the Spouse Enrollment Questionnaire given at enrollment (1993 – 1997) and included direct pesticide exposure (ever use of malathion), but not indirect pesticide exposure of the spouse (husband's use of the pesticide). Polytomous logistic regression was used to analyze the association between ever use of malathion and the occurrence of thyroid disease, adjusting for BMI, age at enrollment, smoking status, hormone replacement therapy (ever/never), and education. Among the 2,043 total cases of thyroid disease reported among female spouses, there were 64 (17.3%) hyperthyroid cases, 220 (19.7%) hypothyroid cases, and 98 (17.50%) 'other' thyroid cases reported ever use of malathion. No evidence of a significant positive association was reported for the association between malathion exposure and hypothyroid disease (OR = 1.10; 95% CI: 0.92, 1.30; with n = 220 exposed cases).

  The overall study quality was ranked low based on the study quality criteria provided in the OPP Framework. Authors were not able to analyze incident cases separately from prevalent cases due to the way the data were collected. The cross-sectional study design was a limitation since temporality for exposure in relation to the outcome could not be determined. Reliance on self-report of the outcome without clinical confirmation was another limitation. Additionally, the investigators were only able to assess ever/never exposure and did not have more detailed exposure information to assess cumulative malathion exposure.

- Shrestha et al. (2018b) evaluated the association between thyroid disease and exposures to pesticides including malathion. The study population consisted of female spouses of pesticide applicators enrolled in the AHS, an ongoing, prospective cohort study. Pesticide exposure was reported through self-administered questionnaires at enrollment (1993 – 1997), and thyroid disease, both hyperthyroid and hypothyroid disease status, was ascertained through self-report during follow-up interviews during Phase II (1999 – 2003), Phase III (2005 – 2010) and Phase IV (2013 – 2016) of the study. Validation of self-reported cases of hyperthyroid and hypothyroid disease was carried out using medical record data. The Cox proportional hazards model was used to calculate separate HRs for hypothyroid and hyperthyroid disease, controlling for smoking, education, and state. Authors restricted their analysis to exposures with at least 10 thyroid disease cases in each exposure category for all but the stricter case analysis for which at least 5 exposed cases in each exposure category were required. For this analysis, the study population included 24,092 AHS female spouses. Authors used multiple imputation with fully conditional specification method to impute missing covariates for

1,273 spouses missing information on smoking status and 3,106 on education. Authors created five imputed datasets, performed regression analysis in each dataset, and obtained the pooled parameter estimates. For hypothyroid disease, no evidence of a significant positive association was reported for malathion exposure (HR: 1.10; 95% CI: 0.97, 1.24; with n = 362 exposed cases, 1,205 unexposed cases). No evidence of a significant positive association was reported between malathion exposure and hypothyroid disease when further adjusted for correlated pesticides (HR: 1.08; 95% CI: 0.94, 1.24; with n = 362 exposed cases, 1,205 unexposed cases). An additional analysis that only included thyroid cases as defined by receipt of treatment in AHS spouses, reported no evidence of a significant positive association between malathion exposure and hypothyroid disease (HR: 1.11; 95% CI: 0.98, 1.26 with n = 330 exposed cases, 1,073 unexposed cases), and a further analysis that only included thyroid cases which were validated according to the *stricter case definition standards* (ascertained via medical record data; confirmed via validation questionnaire; reported thyroid disease at least twice in follow-up surveys). Evidence of a slight positive association for malathion exposure and hypothyroid disease for female spouses of pesticide applicators under 60 (HR: 1.24; 95% CI: 1.02, 1.49; with n = 144 exposed cases, 462 unexposed cases) and evidence of a positive association for malathion exposure and hypothyroid disease for female spouses of pesticide applicators over 60 (HR: 1.72; 95% CI: 1.30, 2.28; with n = 77 exposed cases, 132 unexposed cases).

The overall study quality was ranked moderate based on the study quality criteria provided in the OPP Framework. Study strengths included the prospective cohort design, and the extensive methods used to obtain exposure information for several pesticides including malathion. Study limitations included potential selection bias and self-reported hypothyroid disease. Selection bias was likely if study subject participation in the follow-up phases was related to their disease status for hypothyroidism and since thyroid disease was self-reported in this study, it was likely some cases misclassified their thyroid disease status and subtype. Additional study details regarding frequency and duration of pesticide use for malathion would have been useful but were not provided.

- In a separate study, Goldner et al. (2013) evaluated the potential association between hypothyroid disease and malathion and other pesticides using data from the AHS prospective cohort. The study population included male commercial and private pesticide applicators enrolled in the AHS, living in North Carolina and Iowa. Thyroid disease status was self-reported during follow-up interviews during Phase II (1999 – 2003) and Phase III (2005 – 2010) of the study. While the study investigated three subgroups of thyroid disease (hypothyroidism, hyperthyroidism, and 'other' thyroid disease), results for malathion exposure were only reported for hypothyroidism. Pesticide exposure was reported through two self-administered questionnaires at enrollment (1993 – 1997) and captured pesticide exposures that occurred prior to enrollment. Among the 22,246 AHS study participants, 461 hypothyroid cases were reported, and of these, 362 reported ever use of malathion. Of the 21,327 non-cases (no thyroid disease) with complete data, 15,261 reported ever use of malathion. Logistic regression was used to analyze the association between ever use of malathion and the occurrence of thyroid disease, adjusting for BMI, age at enrollment, and education. Evidence of a slight positive association was reported between exposure to malathion and hypothyroid disease among male commercial and private pesticide applicators, based on ever/never use (OR = 1.29; 95% CI: 1.03, 1.62; with n = 362 exposed cases and n = 15,261 exposed non-cases).

  The overall study quality was ranked moderate based on the study quality criteria in the OPP Framework. The prospective cohort study design and the detailed pesticide exposure information were considered study strengths. Limitations included self-reported diagnosis of thyroid disease rather than clinical confirmation which may have led to some cases misclassifying their thyroid disease subtype. Authors were unable to analyze incident cases separately from prevalent cases due to

the manner in which data were collected. And as such, it is difficult to discern the temporal ordering of exposure and outcomes.

- Shrestha et al. (2018c) evaluated the association between incident hypothyroid disease and exposures to pesticides including malathion. The study population consisted of private pesticide applicators enrolled in the AHS, an ongoing prospective cohort study. Hypothyroid disease status was ascertained through self-report during follow-up interviews during Phase II (1999 – 2003), Phase III (2005 – 2010) and Phase IV (2013 – 2016) of the study. Among the total number of study participants (n=34,879), 829 hypothyroid cases and 34,050 non-cases were reported among private pesticide applicators. Validation of self-reported cases of hypothyroid disease was carried out using medical record data or two validation questionnaires. Pesticide exposure was reported through self-administered questionnaires at enrollment (1993 – 1997). Among the hypothyroid cases (n=829), 629 reported exposure to malathion, based on ever/never use. The Cox proportional hazards model was used to calculate HRs for hypothyroid disease, adjusting for smoking, education, state, and sex. Covariates were selected *a priori* based on potential for causal relationship identified in prior literature. Evidence of a slight positive association was reported between malathion exposure and hypothyroid disease among private applicator based on ever/never use (HR: 1.23; 95% CI: 1.04, 1.46, p-value=0.02). A further analysis investigating the association between intensity-weighted lifetime days of use of malathion and hypothyroid disease among private applicators, was conducted with the following tertiles intensity-weighted lifetime days of use for malathion used: T1: >0 - ≤ 360 days of use,T2: >360 - ≤ 1,395 days of use, and T3: >1,395 days of use. Evidence of a positive association was reported for the middle (T2) and no evidence of a significant positive association was reported for the high exposure (T3) and low exposure (T1) categories (T2: *>360 - ≤ 1,395 days* – HR: 1.48; 95% CI: 1.16, 1.88; with n=103 exposed cases, p-value=0.00); T1: >0 - ≤ 360 days - HR: 1.16; 95% CI: 0.89, 1.50; with n=103 exposed cases, p-value=0.27; T3: >1,395 days - HR: 1.16; 95% CI: 0.89, 1.50; with n=106 exposed cases, p-value=0.27), and no evidence of a significant exposure-response trend (p-trend=0.68).

  Additional sub-analyses investigated the association between malathion exposure and hypothyroidism (based on ever/never use) while placing various restrictions on the case definition and no evidence of a significant positive association was reported for hypothyroid cases that were further restricted to those taking thyroid-related medications only (n=35,073) (HR=1.19; 95% CI: 1.00, 1.42; with n=567 exposed cases, p-value=0.05). And, evidence of a slight positive association was reported for the analysis in each of the following analyses: 1) hypothyroid cases when female applicators were excluded (n=34,375) (HR=1.25; 95% CI: 1.05, 1.49; with n=600 exposed cases, p-value=0.01); 2) ever use of malathion and hypothyroidism risk using inverse probability of censoring weights (HR: 1.23; 95% CI: 1.04, 1.46 n=621, p-value=0.02); 3) hypothyroid cases were restricted to those confirmed by a validation questionnaire or medical records or who reported having hypothyroid disease >2 times or more in surveys (n=34,464) (HR=1.44; 95% CI: 1.10, 1.88; with n=273 exposed cases; p-value=0.01).

  The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Strengths including the prospective cohort study design and the extensive methods used to assess cumulative pesticide exposure. Study limitations included the potential risk of bias due to loss to follow-up, and the possibility of selection bias if study subject participation in the follow-up phases was related to their disease status for hypothyroidism.

- Lerro et al. (2018) investigated the association between pesticide exposure including malathion and hypothyroidism using data from the Biomarkers of Exposure and Effect in Agriculture (BEEA) study, a subset 679 men from the AHS prospective cohort. The BEEA study was conducted from June 2010

to September 2013 and participants included male pesticide applicators who were part of the AHS, lived in North Carolina or Iowa, and were ≥ 50 years of age at enrollment for BEEA with no previous diagnosis of cancer (besides skin cancer). Eligible BEEA participants completed the AHS questionnaires at enrollment (1993 – 1997) and follow-up (1999 – 2003, 2005 – 2010), had no history of cancer, and no history of self-reported thyroid disease or thyroid medication use. Blood samples were collected by a trained phlebotomist and serum samples were measured to confirm subclinical hypothyroidism in each case, which the study reported as thyroid-stimulating hormone (TSH) levels >4.5 mIU/L.71 Pesticide exposure was assessed using data from the study questionnaires completed at enrollment and exposure data including frequency (average days/year) and duration (years) of use for individual pesticides including malathion was obtained. Intensity-weighted lifetime days of use were calculated for each pesticide by multiplying lifetime exposure days by an intensity-weighted factor. A logistic regression was performed to determine ORs and 95% CIs for the association between malathion and hypothyroidism, adjusting for age, smoking, state, BMI, and correlated pesticides. The following intensity-weighted lifetime days of use exposure categories for malathion were used: 20 – 276 days, >276 – 780 days, >780 – 2,250 days, and >2,250 – 117,600 days. No evidence of a significant positive association was reported between any exposure category of malathion intensity-weighted lifetime days of use and laboratory confirmed subclinical hypothyroidism (0.95 < OR <1.50; all 95% CIs encompassed the null value of 1.0; with 12 – 18 cases per exposure category; with a p-trend=0.30).

The overall quality of the study was ranked moderate based on the study quality criteria provided in the OPP Framework. Strengths including the prospective study design, laboratory confirmation of subclinical hypothyroidism, and the cumulative pesticide exposure assessment. The analysis was limited to cumulative pesticide use prior to enrollment and may have led to exposure misclassification. We note the number of exposed cases was small (exposed cases ≤ 19).

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and hypothyroid disease. Five publications (Goldner et al., 2010; Goldner et al., 2013; Shrestha et al., 2018b; Shrestha et al., 2018c; Lerro et al., 2018) examined the relationship between malathion exposure and hypothyroid disease among AHS study participants and the evidence is mixed. For female spouses of male pesticide applicators enrolled in the AHS, Goldner et al. (2010) reported no evidence of a significant positive association between malathion ever use and hypothyroid disease. The publication was ranked low due to a cross-sectional study design since temporality for exposure in relation to the outcome could not be determined and was limited by self-reported outcome. Shrestha et al. (2018b) reported no evidence of a significant positive association among female spouses of pesticide applicators in the AHS, based on malathion ever use and longer follow-up time and was ranked moderate quality. Study limitations included self-reported diagnosis of thyroid disease and selection bias if study subject participation was related to their outcome. Among male pesticide applicators in the AHS, Goldner et al. (2013) reported evidence of a slight positive association between exposure to malathion and hypothyroid disease based on ever use and was ranked moderate quality due to the self-reported diagnosis of thyroid disease. A fourth publication (Shrestha et al., 2018c) reported evidence of a slight positive association between malathion exposure (based on ever/never use) and hypothyroid disease among private pesticide applicators enrolled in the AHS. Additionally, for intensity-weighted lifetime days of exposure, evidence of a positive association for the mid- exposure category only was reported, with no evidence of a significant positive association observed for the low and high exposure categories, and for the exposure-response trend. The study quality was ranked moderate and study limitations were noted including the self-reported diagnosis of thyroid disease and the possibility of selection bias if study subject participation in the follow-up phases was related to their

disease status for hypothyroidism. Finally, a fifth publication, (Lerro et al. 2018), reported no evidence of a significant positive association at any exposure level relative to the non-exposed group for subclinical hypothyroidism among male participants in the AHS. The quality of the study was ranked moderate for regulatory purposes. We also note that the number of exposed cases was small which limits the ability to interpret with confidence the observed odds ratios.

Other Thyroid disease

One study (Goldner et al., 2010) evaluated the potential relationship between malathion exposure and other thyroid disease in women.

Goldner et al. (2010) evaluated the association between prevalent thyroid disease and malathion and other pesticides among female spouses of male private applicators in a cross-sectional analysis of data from the AHS prospective cohort. The study population included all female spouses of male private applicators who completed both the enrollment questionnaire on pesticide exposure (1993 – 1997) and the follow-up telephone interview collecting information on history of thyroid disease (1999 – 2003) and had complete data on all covariates. Cases of physician-diagnosed thyroid disease were ascertained through self-report during follow-up interviews (1999 – 2003) and were further classified into three subgroups: hypothyroidism, hyperthyroidism, and other thyroid disease. Pesticide exposure among female spouses of male private applicators was reported through the Spouse Enrollment Questionnaire given at enrollment (1993 – 1997) and included direct pesticide exposure (ever use of malathion), but not indirect pesticide exposure of the spouse (husband's use of the pesticide). Polytomous logistic regression was used to analyze the association between ever use of malathion and the occurrence of thyroid disease, adjusting for BMI, age at enrollment, smoking status, hormone replacement therapy (ever/never), and education. Among the 2,043 total cases of thyroid disease reported among female spouses, there were 64 (17.3%) hyperthyroid cases, 220 (19.7%) hypothyroid cases, and 98 (17.50%) 'other' thyroid cases reported ever use of malathion. No evidence of a positive association was reported for the association between malathion exposure and other thyroid disease (OR = 0.97; 95% CI: 0.77, 1.20; with n = 98 exposed cases).

The study quality was ranked low based on the study quality criteria provided in the OPP Framework. Authors were not able to analyze incident cases separately from prevalent cases due to the way the data were collected. The cross-sectional study design was a limitation since temporality for exposure in relation to the outcome could not be determined. Reliance on self-report of the outcome without clinical confirmation was another limitation. Additionally, the investigators were only able to assess ever/never exposure and did not have more detailed exposure information to assess cumulative malathion exposure.

EPA Conclusion

Overall, there is *no epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and other thyroid disease. One publication (Goldner et al., 2010) examined the relationship between malathion exposure and other thyroid disease among female spouses of private pesticide applicators enrolled in the AHS. Goldner et al. (2010) reported no evidence of a positive association between malathion ever use and other thyroid disease among female spouses of pesticide applicators enrolled in the AHS. The publication was ranked low due to a cross-sectional study design since temporality for exposure in relation to the outcome could not be determined and the study was also limited by self-reported outcome.

Weight Gain

One study (LaVerda et al., 2015) evaluated the potential relationship between malathion exposure and weight gain in pesticide applicators.

LaVerda et al. (2015) investigated the association between exposure to malathion and other pesticides and weight gain using the AHS prospective cohort study. The study population (n = 8,365) included male pesticide applicators residing in Iowa or North Carolina, aged 20 years or older. Exposure information, including ever use of specific pesticides as well as duration and frequency of exposure, was assessed by self-administered questionnaires at study enrollment (1993 – 1997). During study enrollment, participants also self-reported body mass index (BMI) at age 20 and at study enrollment. At follow-up telephone interviews conducted 5 years after study enrollment, participants reported BMI and updated pesticide exposure. Also at follow-up, participants reported diet history through a self-administered questionnaire. Exposure was assessed by combining follow-up exposure data with enrollment exposure data to estimate lifetime exposure metrics. Of the 8,365 study participants, 5,703 reported ever exposure to malathion, while 2,424 reported never exposure (238 subjects had missing malathion exposure data). Analyses considered cumulated pesticide exposure days from age 20 to age at follow-up. The mean cumulated malathion exposure days from age 20 to follow-up was 44.2 days (SD = 154.8). Multiple linear regression was used to assess the association between malathion exposure as a continuous variable and unit change in BMI ($kg/m^2/d$; BMI associated with 100 cumulative exposure days between age 20 and age and follow-up). Results for malathion indicated evidence of a positive association between malathion exposure days and adjusted BMI at age 20, for age, smoking, daily kilocalories consumed, and daily hours of heavy lifting ($\beta = 0.07$, $p = 0.01$). To investigate the potential effect modification of weight-related health conditions diagnosed in 2,586 participants (cancer excluding nonmelanoma skin cancer, diabetes, heart disease, lupus, and/or amyotrophic lateral sclerosis (ALS)), these participants were excluded, and results from the medical exclusions analysis were similar to the overall analysis ($\beta = 0.08$, $p = 0.03$). To investigate the potential effect modification of the state variable, a stratified analysis was conducted, and results indicated no positive association between cumulative malathion exposure days and increased BMI in Iowa (adjusted analysis $\beta = 0.03$, $p = 0.449$), but a positive association in North Carolina (adjusted analysis $\beta = 0.09$, $p = 0.018$; significance based on Bonferroni-adjusted p value = 0.003).

The overall study quality was ranked low based on the study quality criteria provided in the OPP Framework. Strengths of LaVerda et al. (2015) included the prospective cohort study design, questionnaires and interviews that assessed specific pesticide exposure including duration and frequency of exposure, the adjustment for other pesticides in the models, and the use of the Bonferroni adjustment to minimize chance effects due to multiple comparisons and the chance of type I error. The study was limited by the self-reported outcome (BMI) including a retrospective report of BMI at age 20 collected during study enrollment (the mean age at follow-up was 56.4 years, indicating the mean age at enrollment was approximately 51 years old). This introduced the potential for outcome misclassification. The inclusion of variables for daily kilocalories consumed and daily hours of heavy lifting in the adjusted models attempted to control for the influence of physical activity and diet on BMI; however, the crude approximation for physical activity (defined as “heavy lifting” and based on participant responses to questions about hours of heavy lifting at time of interview and, retrospectively, 10 years prior to study enrollment) and the use of a diet history questionnaire may not have appropriately captured these critical influences on BMI. The use of questionnaires to assess exposure and confounder information may have introduced the potential for recall bias and exposure misclassification. However, the AHS participant cohort has demonstrated high reliability for self-reported information for pesticide use, demographic, and

lifestyle factors[127]. Finally, the medical exclusions list included diseases that may also be associated with exposure to pesticides investigated in this study; however, authors presented stratified results (with and without medical exclusions) to partially account for this effect modifier and the potential effect modification of state of residence, and found similar results for malathion analyses across these stratifications.

EPA Conclusion

Overall, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and other thyroid disease. One publication (LaVerda et al., 2015) examined the relationship between malathion exposure and weight gain and reported no evidence of a positive association between malathion exposure and increased BMI. The publication was ranked moderate. Strengths of the study included the prospective cohort study design, questionnaires and interviews that assessed specific pesticide exposure including duration and frequency of exposure, the adjustment for other pesticides in the models, and the use of the Bonferroni adjustment to minimize chance effects due to multiple comparisons and the chance of type I error. The study was limited by the self-reported outcome (BMI) including a retrospective report of BMI at age 20 collected during study enrollment (the mean age at follow-up was 56.4 years, indicating the mean age at enrollment was approximately 51 years old). This introduced the potential for outcome misclassification.

3.7 Epidemiology Conclusion

OPP performed a systematic review of the epidemiologic literature on malathion exposure and identified 109 peer-reviewed publications that investigated malathion exposure and a range of adverse health outcomes, including 42 studies on carcinogenic health outcomes and 67 on the non-carcinogenic health outcomes affecting several organs, as well as autoimmune disease, Parkinson's disease, myocardial infarction, respiratory effects and birth effects and birthweight in children. OPP's conclusions on the available evidence for these outcomes are summarized below.

3.7.1 Carcinogenic Health Outcomes

Twenty-four cancer outcomes were examined in 42 epidemiologic studies, with most cancer outcomes investigated in one or two studies.

OPP concluded there was *no epidemiological evidence* of a clear associative or causal relationship between malathion exposure and three cancer outcomes: colon cancer, esophageal cancer, and rectal cancer. This conclusion was based on evidence that was limited to studies on each cancer outcome that reported no evidence of a positive association between malathion exposure and the cancer outcome (e.g., all reported OR effect estimates were ≤ 1.0).

OPP concluded there was *insufficient epidemiological evidence* of a clear associative or causal relationship between malathion exposure and eighteen cancer outcomes: all cancers, bladder cancer, brain and spinal cancer (glioma), breast cancer, colorectal cancer, childhood cancer, gastric cancer, kidney cancer, lung cancer, leukemia, lymphatic-hematopoietic cancer, Hodgkin lymphoma, melanoma, multiple myeloma, non-Hodgkin lymphoma (NHL), ovarian cancer, pancreatic cancer, prostate cancer, thyroid cancer, soft tissue carcinoma, and uterine cancer. The majority of these cancer outcomes were also only investigated in a single study population, with breast cancer investigated in five studies, prostate cancer

[127] Blair, A., Tarone, R., Sandler, D., Lynch, C. F., Rowland, A., Wintersteen, W., . . . Alavanja, M. C. (2002). Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. Epidemiology, 13(1), 94-99.

examined in eight studies, and NHL in eleven studies. Given the limited number of studies available for each outcome other than prostate cancer, there was minimal confidence in the available evidence so additional epidemiological evidence could substantively affect the overall magnitude or direction of any observed associations. Further information on the evidence for each health endpoint is summarized below.

- For all cancers, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and cancer (all sites) among pesticide applicators. This determination was based on a limited body of evidence that consisted of two cohort studies (Bonner et al., 2007; Lerro et al., 2015) that reported no evidence of a significant positive association between malathion exposure on adults and cancer (all sites). The overall quality of Bonner et al. (2007) was ranked high and Lerro et al. (2015) was ranked moderate for regulatory purposes.

- For bladder cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and bladder cancer. This determination was based on two studies (Bonner et al., 2007, Koutros et al., 2016), that investigated the potential association between malathion exposure and bladder cancer among the AHS prospective cohort each with increasing follow-up time and number of cases. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and bladder cancer among pesticide applicators enrolled in the AHS prospective cohort, based on lifetime days and intensity-weighted lifetime days of use, or for frequency, intensity, and duration of malathion exposure. The study quality was ranked high quality for regulatory purposes. The second publication, Koutros et al. (2016), reported no evidence of a significant positive association between malathion exposure and bladder cancer among AHS pesticide applicators based on ever/never use, or for intensity-weighted lifetime days of malathion use and intensity-weighted lifetime days of malathion use stratified by smoking status (never, former, and current smoker strata). The quality of the study was ranked high for regulatory purposes.

- For brain and spinal cancer (glioma), there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and brain and spinal cancer. This determination was based on three studies (Lee et al., 2005; Yiin et al., 2012; Lerro et al., 2015) that investigated the relationship between malathion exposure and brain and spinal cancers among separate populations in the United States. Lee et al. (2005) reported no overall evidence of a positive association between malathion exposure and glioma in a case-control study in Nebraska, but when the data was further stratified by type of respondent (self or proxy), evidence of a strong association was reported among cases who completed the interview themselves (self), among a small number of exposed cases and corresponding wide confidence interval. We note the number of exposed cases was small ($10 <$ exposed cases < 19) and no evidence of a positive association was reported between malathion ever use and glioma among those cases who had a proxy respondent. The study was ranked low quality for regulatory purposes due to the large proportion of proxy respondents, reference group to nonfarmers, ever use assessment rather than exposure-response, and self-report of exposure. Yinn et al. (2012) in a case-control analysis among participants of the Upper Midwest Health Study reported no evidence of a positive association between malathion ever use and glioma, when either self+proxy or self-only respondents were considered. The quality of the study was ranked moderate for regulatory purposes due to the large proportion of proxy respondents, self-report of exposure, selection and recall bias. The third publication, Lerro et al. (2015), reported no evidence of a significant positive association between malathion exposure and brain cancer among female spouses in the AHS cohort. The overall quality of the study was ranked moderate for regulatory purposes. The investigators assessed indirect exposure based on self-reported pesticide use

from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses. We note the number of exposed cases was small (10 < exposed cases < 19).

- For breast cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and breast cancer. This determination was based on five publications (Engel et al., 2005; Mills and Yang, 2005; Lerro et al., 2015; Engel et al., 2017; Golmohammadzadeh et al., 2019) that examined the association between malathion exposure and breast cancer among women in the AHS, women living in California, and women living in Iran. Engel et al. (2005), Engel et al. (2017), and Lerro et al. (2015) reported no evidence of a significant positive association between malathion use and breast cancer among women in the AHS, and all three studies were ranked moderate quality. Mills and Yang (2005) reported evidence of a moderately strong association between malathion use (at the mid exposure level only) and breast cancer among Hispanic women diagnosed from 1988 to 1994 among a small number of cases, using pesticide use data and geospatial analysis in a case-control study. No evidence of a significant positive association was reported at the low or high exposure levels, and no evidence of a positive association was reported between any exposure level of malathion use and breast cancer that was diagnosed from 1995 to 2001. The study was ranked low quality for regulatory purposes, as the exposure assessment approach relied on county-level pesticide use record information as a surrogate measure of exposure to estimate individual-level exposure. We note the number of exposed cases was small (10 < exposed cases < 19). Golmohammadzadeh et al. (2019) reported no evidence of a statistically significant difference in mean serum malathion levels and breast cancer in women living in Iran. The study was ranked low quality for regulatory purposes and limitations included the statistical analysis methods and reporting.

- For childhood cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and cancer in children. This determination was based on a limited body of evidence that consisted of two case-control studies in children. The first study, Flower et al. (2004), reported no evidence of a significant positive association between prenatal malathion exposure and childhood cancer in the AHS prospective cohort. We note the number of exposed cases was small. The overall quality of the study was moderate for regulatory purposes as the study relied on ever/never exposure assessment. The second study, Park et al. (2020), evaluated the association between prenatal malathion exposure and childhood cancer, specifically childhood acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), using a record-based study in California. While evidence of a positive association for ALL was seen in children who resided in a 4,000m buffer zone of malathion applications using a single pesticide model, no evidence of a significant positive association was observed for ALL relative to malathion when further adjusted using the hierarchical statistical model. For AML in children, no evidence of a positive association was observed in children who resided in a 4,000m buffer zone of malathion applications. Park et al. (2020) was ranked moderate quality for regulatory purposes due to the study's reliance on a geospatial approach to assess pesticide exposure based on residential address and land use data for malathion, as well as not accounting for residential mobility of mothers.

- For colorectal cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a causal or clear associative relationship between malathion exposure and colorectal cancer. This determination was based on two studies (Bonner et al., 2007; Lee et al., 2007) that examined the relationship between malathion exposure and colorectal cancer. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and colorectal cancer among pesticide applicators enrolled in the AHS prospective cohort, based on lifetime days and

intensity-weighted lifetime days of use or for frequency, intensity, and duration of malathion exposure. The study quality was ranked high for regulatory purposes. A second study, Lee et al. (2007), similarly reported no evidence of a positive association between malathion exposure and colorectal cancer based on ever use. The study quality was ranked high for regulatory purposes.

- For gastric cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and gastric cancer. This determination was based on two publications (Lee et al. 2004; Mills and Yang, 2007) that examined the association between malathion exposure and stomach cancer. Lee et al. (2004) reported no evidence of a positive association between malathion exposure and gastric cancer in a case-control study of residents in Nebraska and was low quality for regulatory purposes. Several limitations were noted including selection bias, recall bias, comparison of farmers to non-farmers, and a large number of proxy respondents compared to self-respondents. Both groups could have different levels of pesticide use knowledge, memory of use, and different motives for responding. Mills and Yang (2007) used a geospatial method to estimate malathion exposure based on residential proximity to agricultural malathion use and risk of gastric cancer among Hispanic farm workers in a nested case-control study. No evidence of a significant positive association was reported for malathion ever vs. never use. For the exposure-response analysis, no evidence of a significant positive association was reported in any exposure group when the no exposure quartile was the referent. With the low exposure quartile as the referent in the multivariable-adjusted analysis, evidence of a moderately strong association was observed in the highest exposure quartile with the low exposure quartile as the referent, and no evidence of a significant positive association was reported in the mid-exposure. Additionally, no evidence of a significant positive association was reported between malathion and gastric cancer in any quartile in the age-adjusted analysis. The publication was ranked low quality for regulatory purposes due to the exposure assessment approach.

- For kidney cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a causal or clear associative relationship between malathion exposure and kidney cancer. This determination was based on two publications (Bonner et al., 2007; Andreotti et al., 2020) that evaluated the association between malathion exposure and kidney cancer and renal cell carcinoma, a type of kidney cancer, among pesticide applicators in the AHS. The first study, Bonner et al. (2007), reported no evidence of a significant positive association between malathion exposure and kidney cancer among pesticide applicators, based on lifetime days and intensity-weighted lifetime days of use. The study quality was ranked high for regulatory purposes. A second study, Andreotti et al. (2020), reported no evidence of a significant positive association for any exposure quartile of malathion and renal cell carcinoma, with the no exposure group as the referent, and no evidence of a significant exposure-response trend. Similar results were reported for lifetime days of malathion exposure and renal cell carcinoma. The study was determined to be high quality for regulatory purposes.

- For lung cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and lung cancer. This determination was based on four available publications (Pesatori et al., 1994; Bonner et al., 2007; Bonner et al., 2017; Lerro et al., 2015) that were used to examine the association between malathion exposure and lung cancer among male pesticide applicators and their female spouses. Pesatori et al. (1994) reported no evidence of a significant positive association between malathion ever exposure and lung cancer. The study was low quality for regulatory purposes and had substantive limitations in its exposure assessment. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and lung cancer among pesticide applicators, based on lifetime days and intensity-weighted lifetime days of use. The third study, Lerro et al. (2015),

reported no evidence of a positive association between malathion exposure and lung cancer risk for female spouses in the AHS cohort. The fourth study, Bonner et al. (2017), reported no evidence of a significant positive association between malathion exposure and lung cancer among pesticide applicators for intensity-weighted days of exposure. Bonner et al. (2007) was ranked high quality, and Lerro et al. (2015) and Bonner et al. (2017) were determined to be of moderate quality for regulatory purposes.

- For leukemia, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and leukemia in adults. This determination was based on four publications (Brown et al., 1990; Mills et al., 2005; Bonner et al., 2007; Lerro et al., 2015) that assessed the association between malathion exposure and leukemia among study populations in Minnesota and Iowa, California, and the AHS cohort. Brown et al. (1990) reported no evidence of a positive association between malathion exposure and leukemia among adult males in Minnesota and Iowa, and similarly, when the data was further stratified based on pesticide use at least 20 years prior to the interview, no evidence of a significant positive association was reported.[128] The study was ranked moderate quality for regulatory purposes and limitations included the use of proxy respondents and recall bias which likely led to exposure misclassification and the comparison of two different subpopulations (farmers vs. nonfarmers) who have a different risk of disease. Mills et al. (2005) reported no evidence of a significant positive association between high malathion use and total leukemia among farmers living in California. When further stratified by type of leukemia, although elevated, the investigators reported no evidence of a significant positive association between high exposure (compared to low exposure) to malathion and lymphocytic leukemia in the total study population, and no evidence of a significant positive association between high exposure to malathion and granulocytic leukemia in the total study population. The analysis of total leukemia was also stratified by gender and evidence of a strong association was reported between high exposure (compared to low exposure) to malathion and total leukemia among females, but not males. The study was ranked low quality for regulatory purposes due to a number of study limitations including the ecologic exposure assessment approach that potentially led to exposure misclassification. The two additional studies, Bonner et al. (2007) and Lerro et al. (2015), were both part of the AHS cohort. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and leukemia among pesticide applicators based on lifetime days and intensity-weighted lifetime days of use, and the study quality was ranked high for regulatory purposes. Lerro et al. (2015) reported no evidence of a positive association between malathion exposure and leukemia risk among AHS spouses based on ever/never use. The overall quality of the study was ranked moderate for regulatory purposes. The indirect exposure based on self-reported pesticide use from spouses' husbands was considered a study limitation, as this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses.

- For lymphatic-hematopoietic cancers (all), there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and lymphatic-hematopoietic cancers (all). This determination was based on two studies, (Bonner et al., 2007; Alavanja et al., 2014), that investigated the relationship between malathion exposure and lymphatic-hematopoietic cancers (all) among pesticide applicators enrolled in the AHS prospective cohort. Both studies reported no evidence of a significant positive association, based on lifetime days and intensity-weighted lifetime days of use. Both studies were ranked high quality for regulatory purposes.

[128] See Footnote 37.

- For Hodgkin Lymphoma (HL), there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and HL. This determination was based on two studies (Karunanayake et al., 2012, Latifovic et al., 2020) that assessed the association between malathion exposure and HL among residents in Canada and the United States. Both publications reported no evidence of a significant positive association between malathion exposure and HL among males and both were moderate quality for regulatory purposes. Limitations included potential for selection bias, recall bias, low response rate, and a large number of proxy respondents.

- For non-Hodgkin's Lymphoma (NHL), there is *insufficient* epidemiological *evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and NHL among several study populations in the United States and Canada, including the AHS cohort. While eleven epidemiologic studies (Cantor et al., 1992; McDuffie et al., 2001; Waddell et al., 2001; De Roos et al., 2003; Mills et al., 2005; Bonner et al., 2007; Hohenadel et al., 2011; Alavanja et al., 2014; Lerro et al., 2015; Koutros et al. 2019; Leon et al., 2019) were identified that assessed exposure to malathion and NHL, EPA notes that among these many different investigations, there are essentially only four study populations in which the studies are based: an NCI pooled dataset of case-control studies in the U.S. Midwest (distinct from the AHS study population), the AHS population, the cross-Canada case-control study series (Cross-Canada Study in Pesticide and Health, or CCSPH), and the international pooled study performed by the AGRICOH consortium. The results of the studies were mixed. The two high quality studies from the AHS (Bonner et al. (2007) and Alavanja et al. (2014) were prospective cohort studies and did not find any evidence of an association between exposure to malathion and NHL using a variety of metrics, nor were any trends seen with increasing exposure. Similar findings were present in the moderate quality rated AHS Lerro et al. (2015) study conducted with AHS spouses as well as the most recent study, that of Leon et al. (2019); this latter study, however, was rated as low quality for regulatory purposes. The study by Koutros et al. (2019) was a pooled analysis of four population-based case-control studies conducted in Kansas, Iowa, Minnesota and Nebraska in the 1980s by NCI and in the various Canadian locations (Ontario, Quebec, Manitoba, Saskatchewan, British Columbia, and Alberta) by the CCSPH between 1991 and 1994. The authors reported several moderately strong ORs resulting from a number of different analyses. While the overall quality of the pooled CCSPH study was ranked moderate, there were a number of limitations. Recall bias was a potential study limitation as was the fact that case and control selection methods differed between each study and may have led to selection bias. Further, different methods were used to collect pesticide use information (postal vs. telephone vs. in-person interviews) potentially causing some misclassification of exposure. Additionally, proxy respondents accounted for a large percentage of respondents. The last publication, Mill et al., 2005, reported no evidence of a significant positive association for total NHL among farm workers in California, and evidence of a strong association between high exposure to malathion and NHL-extranodal and no evidence of a significant positive association for NHL-nodal, in a stratified analysis. However, the study was ranked low quality for regulatory purposes since the exposure assessment relied on county-level pesticide use record information as a surrogate measure of exposure for each study participant. Additionally, the investigators reported that the statistical power of the study was low. Given the two high quality prospective cohort studies from the AHS that showed no association between malathion and NHL and the limitations cited regarding the several moderate quality studies that appeared to show an association, we conclude that the evidence is mixed and that there is insufficient epidemiological evidence of a clear associative or causal relationship between malathion exposure and NHL.

- For NHL subtypes, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and NHL subtypes. This determination was based on three publications (Waddell et al., 2001; Alavanja et al., 2014; Koutros et

al., 2019), and the results were mixed. Waddell et al. (2001) reported no evidence of a significant positive association between malathion and follicular, diffuse, small lymphocytic, and 'other' subtypes of NHL, and when the analysis was further adjusted for fonofos and diazinon, among farmers relative to non-farmers in an analysis of several pooled studies from Iowa, Minnesota, Kansas, and Nebraska. The second study, Koutros et al. (2019), reported evidence of a positive association for the follicular lymphoma and diffuse large B cell lymphoma subtypes, and no evidence of a significant positive association for the small lymphocytic lymphoma subtype and 'other' subtype, relative to malathion exposure. Both studies were ranked moderate quality for regulatory purposes, and noted study limitations are mentioned above (see NHL conclusion). The third publication, Alavanja et al. (2014), reported no evidence of a significant positive association between malathion and any NHL subtypes among the AHS cohort, based on ever exposure and lifetime days of malathion exposure. The study quality was ranked high for regulatory purposes.

- For multiple myeloma (MM), there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and MM. This determination was based on four publications (Brown et al., 1993; Pahwa et al., 2012; Presutti et al., 2016; Leon et al., 2019). Brown et al. (1993) reported no evidence of a significant positive association between malathion ever use through crop insecticides and MM, and no evidence of a positive association through animal insecticides and MM, in a case-control study in Iowa. The study quality was ranked moderate for regulatory purposes and limitations included the use of proxy respondents and recall bias which likely led to exposure misclassification and compared two different subpopulations (farmers vs. nonfarmers) who have a different risk of disease. In the Cross-Canada Study of Pesticides and Health case-control study, Pahwa et al. (2012) reported no evidence of a positive association was observed between exposure to malathion as a chemical class and MM, and no evidence of a significant positive associaton between exposure to malathion as a fumigant and MM. The study quality was moderate for regulatory purposes and study limitations included potential for selection bias and recall bias. Presutti et al. (2016) reported no evidence of a significant positive association between malathion and MM based on ever use, years of pesticide use, and for lifetime days of pesticide use, using data from a pooled analysis that included three of the four case-control studies that make-up the North American Pooled Project (NAPP) in Nebraska, Iowa, and six Canadian provinces. This study was ranked moderate for regulatory purposes, and study limitations included recall bias, selection, and some misclassification of exposure. Additionally, a large percentage of proxy respondents was reported by the study authors which could have contributed to information bias and led to exposure misclassification. Leon et al. (2019) examined the association between malathion exposure and MM among the three pooled agricultural cohort studies that make up the AGRICOH. One of the study populations included those of the AHS. No evidence of a positive association was reported for malathion ever exposure and MM among all participants in the analysis. And the study was ranked low for regulatory purposes due to limitations with the pesticide exposure assessment and potential misclassification, methods used to measure covariates and incomplete adjustment for important potential confounders.

- For melanoma, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and melanoma. This determination was based on two available studies (Bonner et al., 2007; Lerro et al., 2015) that investigated the association between malathion exposure and melanoma in the AHS prospective cohort. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and melanoma among pesticide applicators enrolled in the AHS prospective cohort, based on lifetime days and intensity-weighted lifetime days of use. Additionally, no evidence of a significant positive association for frequency, intensity, and duration of malathion exposure was observed. The study quality was ranked high for regulatory purposes. Lerro et al. (2015) reported no

evidence of a positive association between malathion exposure and melanoma risk among female spouses of pesticide applicators. Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and melanoma. The study was ranked moderate for regulatory purposes. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate statistical methods used. However, the investigators assessed indirect exposure based on self-reported pesticide use from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses.

- For ovarian cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and ovarian cancer. One study (Lerro et al. 2015), investigated the association between organophosphate (OP) pesticides, including malathion, and cancer of various endpoints including ovarian cancer among participants in the prospective AHS cohort. The study reported no evidence of a positive association was reported between malathion exposure and ovarian cancer risk among AHS spouses. The overall quality of the study was ranked moderate regulatory purposes. The ranking was based on the general strengths of the AHS, including its prospective design, ability to identify cancer cases through linkage to cancer registries, and the adequate statistical methods used. However, the investigators assessed exposure based on self-reported pesticide use from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses.

- For pancreatic cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and pancreatic cancer. This determination was based on two AHS studies (Andreotti et al., 2009; Lerro et al., 2015) that examined the association between malathion exposure and pancreatic cancer. Andreotti et al. (2009) reported no evidence of a positive association between malathion and pancreatic cancer among pesticide applicators based on ever use. The second study, Lerro et al. (2015), reported no evidence of a significant positive association between malathion exposure and pancreatic cancer risk among female spouses of pesticide applicators. Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a significant positive association was reported for malathion exposure and pancreatic cancer. Both studies were ranked moderate quality for regulatory purposes. The investigators assessed indirect exposure based on self-reported pesticide use from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses.

- For prostate cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and prostate cancer. This determination was based on eight studies (Bonner et al., 2007; Band et al., 2011; Barry et al., 2011; Barry et al., 2012; Koutros et al., 2011; Koutros et al., 2013a; Koutros et al., 2013b; Christensen et al., 2016) that examined the association among the AHS prospective cohort and in a population-based case-control study among farm workers in British Columbia, Canada. Bonner et al. (2007) reported no evidence of a significant positive association between malathion exposure and prostate cancer among pesticide applicators enrolled in the AHS prospective cohort, based on lifetime days and intensity-weighted lifetime days of use. The study quality was ranked high for regulatory purposes. A second study, Band et al. (2011), evaluated the association between malathion and prostate cancer in a population-based case-control study among farm workers in British Columbia, Canada. Evidence of a borderline positive association was observed between prostate cancer and malathion in the high exposure category of the exposure-response analysis, along with a significant exposure-response trend. No evidence of a significant positive association was reported in the low exposure category for

malathion, and no evidence of a significant positive association was reported between malathion and prostate cancer based on ever/never use. The study was moderate quality for regulatory purposes and limitations included selection bias and recall bias due to proxy respondents inaccurate recall of exposure. Koutros et al. (2011) reported no evidence of a significant positive association for prostate cancer or aggressive prostate cancer relative to malathion exposure for any of the stratified exposure categories, and no evidence of a linear (monotonic) trend across categories for total prostate cancer. There was evidence of a linear (monotonic) trend across all categories for aggressive prostate cancer. The study quality was ranked moderate due to the general strengths of the AHS including the prospective study design (three were nested-case control) and linkage to cancer registries to ascertain cases. Study limitations included missing data among the cases (~30% of the cases), the Gleason scores used in the study were not standardized prior by the centralized pathologic review, and the potential for exposure misclassification. Barry et al. (2011) and Barry et al. (2012) reported no evidence of a positive association between malathion exposure and prostate cancer among white male pesticide applicators in either the low or high exposure categories, with the non-exposed group as the referent. The study was ranked high quality for regulatory purposes. Koutros et al. (2013a) reported evidence of a positive association for malathion exposure and aggressive prostate cancer at the highest exposure quartile only, along with evidence of a linear (monotonic) trend across quartiles for aggressive prostate cancer; no evidence of a significant positive in any of the lower quartiles was observed. Additionally, no evidence of a significant positive association was observed for malathion exposure and total prostate cancer in any of the exposure quartiles, with no evidence of a linear (monotonic) trend across quartiles for total prostate cancer. Koutros et al. (2013b) reported evidence of a strong association between the high exposure category for malathion and prostate cancer for the *EHBP1* SNP region for the TT genotype. No evidence of a significant positive association was observed at the low dose exposure category to malathion and prostate cancer for the TT genotype. Among the *rs2710647* SNP region for the CT+CC genotype, no evidence of a positive association was reported for all exposure categories for malathion and prostate cancer. Christensen et al. (2016) reported no evidence of a positive association between malathion exposure and prostate cancer, and the study quality was high for regulatory purposes.

- For soft tissue sarcoma, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and STS. This determination is based on a very limited body of evidence that consisted of one case-control study (Pahwa et al., 2011) that investigated the potential association between exposure to malathion and soft tissue sarcoma (STS) among participants of the Cross-Canada Study of Pesticides and Health Study. No evidence of significant positive associations were reported between malathion exposure and STS. The study was moderate quality for regulatory purposes.

- For thyroid cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and thyroid cancer. Two studies (Lerro et al., 2015; Lerro et al., 2021) examined the association between malathion exposure and thyroid cancer. Lerro et al. (2015) reported evidence of a moderately strong positive association between malathion exposure and thyroid risk. Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and evidence of a moderately strong positive association was reported for malathion exposure and thyroid cancer. We note the number of exposed cases was small. The study quality was ranked moderate for regulatory purposes. The investigators assessed indirect exposure based on self-reported pesticide use from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses. Lerro et al. (2021) reported no evidence of a significant positive association between malathion intensity-weighted lifetime days of malathion use and thyroid

cancer or the subset papillary thyroid cancer among pesticide applicators in the AHS prospective cohort. The study was ranked high quality for regulatory purposes.

- For uterine cancer, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and uterine cancer. One study (Lerro et al., 2015) examined the association between malathion exposure and uterine cancer among AHS spouses, and reported no evidence of a significant positive association. The study was ranked moderate for regulatory purposes. The investigators assessed indirect exposure based on self-reported pesticide use from spouses' husbands, and this approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses.

3.7.2 Noncarcinogenic Health Outcomes

Thirty-four non-carcinogenic health outcomes were examined in 67 epidemiologic studies. OPP concluded there was *no epidemiological evidence* of a clear associative or causal relationship between malathion exposure and the following outcomes: amyotrophic lateral sclerosis (ALS), autoimmune disease (antinuclear antibodies), dream enacting behavior, fatal injury, kidney function, monoclonal gammopathy of undetermined significance, neurodevelopmental/neurobehavorial effects in children, recurrent pregnancy loss, sleep apnea, stroke, suicide, testosterone level effects, and other thyroid disease. This conclusion was based on evidence that was limited to a one or two studies on each health outcome that reported no evidence of a positive association between malathion exposure and the health outcome of interest (e.g., reported OR effect estimates were ≤ 1.0).

OPP concluded there was *insufficient epidemiological evidence* of a clear associative or causal relationship between malathion exposure and the remaining health effects: autism spectrum disorder, birth defects, birth effects, birthweight, cerebral palsy, depression, diabetes, end stage renal disease, endometriosis, eye disorders, gestational hypertension, hearing loss, myocardial infarction (MI), nervous system function (neonatal, central, and peripheral nervous system in adults), olfactory impairment, Parkinson's disease (PD), respiratory effects (asthma, chronic bronchitis, rhinitis, wheeze), rheumatoid arthritis, hyperthyroid disease, hypothyroid disease, and weight gain in adults. The majority of these effects were also only investigated in a single study population, and frequently reported no evidence of a significant positive association (e.g., OR > 1.00 but not significant). Given the limited number of studies available for each outcome, there was generally minimal confidence in the available evidence, so additional epidemiological evidence could substantively affect the overall magnitude or direction of any observed associations. Further information on the evidence for each health endpoint is summarized below.

- For autism spectrum disorder, there *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and Autism Spectrum Disorder (ASD) among children. This determination was based on two studies (Sagiv et al., 2018; von Ehrenstein et al., 2019) that examined the relationship between malathion exposure and Autism Spectrum Disorder (ASD) among the CHAMACOS cohort in Salinas Valley, California and residents of the Central Valley, California. Sagiv et al. (2018) reported no evidence of a significant association between a 10-fold increase in prenatal malathion exposure use within 1-km of residence during pregnancy and the several outcomes testing ASD in children. In a second study, von Ehrenstein et al. (2019), reported either no evidence of a significant positive association or no evidence of a positive association between malathion ever exposure and ASD *3 months before pregnancy*, *pregnancy*, and during the *first year of life* in an analysis that estimated at 2-km buffer around the maternal residence during pregnancy. Both studies were ranked low for regulatory purposes. Both used geospatial methods and CA PUR data to estimate malathion exposure based on proximity of prenatal residence

to malathion agricultural use. The approach may lack the specificity to assess malathion and be unable to distinguish between factors associated with geographic proximity to agricultural land and pesticide use more generally. And finally, the publications relied on the residential address found on the birth certificate. The residential address on the birth certificate may not be the same residential address during the three months before pregnancy, during the pregnancy, or the first year of life exposure periods.

- For birth defects, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and birth defects among newborn babies. Three publications (Grether et al., 1987; Thomas et al., 1992; Haraux et al., 2018) examined the association between malathion exposure and birth defects in newborn babies including congenital abnormalities, spontaneous abortions, still births, intrauterine growth retardation, and hypospadias in male offspring and the results were mixed. Grether et al. (1987) reported evidence of a strong association for anomalies of the ear in children and evidence of a moderately strong association for bowed legs in children, following maternal exposure to aerial application of malathion relative to the 1981 unexposed group, among a small number of exposed cases. For children born with varus deformities and clubfoot, evidence of a positive association was observed relative to maternal malathion exposure, in comparison to the 1981 unexposed group. The semi-ecological study design was considered a main limitation due to an inability to extrapolate observed associations from the group level to the individual level. The study was ranked low quality for regulatory purposes, and we note a very small number of cases (n <10). The second study, Thomas et al. (1992), reported evidence of a borderline strong association between direct maternal exposure during the second trimester to malathion following aerial applications and gastrointestinal abnormalities. We note the very wide confidence interval and the lower bound of the confidence interval being very close to 1.0, that would indicate no association. The quality of the study was ranked low for regulatory purposes, and study limitations included the statistical methods used, potential exposure misclassification from errors that may have occurred during the aerial applications due to unexpected malathion drift outside the range of a defined spray corridor, and exposure differences in exposures among residences and among pregnant women in the study. And the third publication, Haraux et al. (2018), evaluated the association between malathion and the birth defect, hypospadias, in newborn babies and reported an elevated but not significant positive association at the low exposure level of malathion and no evidence of a significant positive association at the high exposure level, among a very small number of exposed cases. The study was ranked moderate quality for regulatory purposes and study limitations included no mention of laboratory QA/QC procedures, and potential contamination of trace amounts of other pesticides of the meconium samples.

- For birth effects, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and birth effects. This determination was based on four publications (Eskenazi et al., 2004; Wolff et al., 2007; Sathyanarayana et al., 2010; Ling et al., 2018) that examined the association between prenatal malathion exposure and various birth effects including head circumference, birth weight, crown-heel length, ponderal index, length of gestation, and preterm birth among children in New York City, Iowa, North Carolina, and California. Eskenazi et al. (2004), Wolff et al. (2007), and Ling et al. (2018) were moderate quality for regulatory purposes and exposure assessments were limited by either geospatial exposure assessment or a single measurement of malathion during pregnancy. Sathyanarayana et al. (2010) was low quality for regulatory purposes due to the cross-sectional study design since temporality for exposure in relation to the outcome could not be determined. The available evidence for each birth effect is reported below.

- For birth weight, Eskenazi et al. (2004), Wolff et al. (2007), and Sathyanarayana et al. (2010) reported no evidence of a significant positive association between prenatal malathion exposure and birth weight among mother/infant dyads in a New York City cohort, in a California cohort, and among the AHS cohort in a cross-sectional analysis, respectively.

- For length of gestation, Eskenazi et al. (2004) and Wolff et al. (2007) reported no evidence of a significant association between prenatal malathion exposure and length of gestation among mother/infant dyads in a New York City cohort and in a California cohort.

- For head circumference, Eskenazi et al. (2004) and Wolff et al. (2007) reported no evidence of a significant association between prenatal malathion exposure and head circumference among mother/infant dyads in a New York City cohort and in a California cohort.

- For ponderal index, Eskenazi et al. (2004) and Wolff et al. (2007) reported no evidence of a significant association between prenatal malathion exposure and ponderal index among mother/infant dyads in a New York City cohort and in a California cohort.

- For crown-heel length, Eskenazi et al. (2004) and Wolff et al. (2007) reported no evidence of a significant association between prenatal malathion exposure and crown-heel length among mother/infant dyads in a New York City cohort and in a California cohort.

- For preterm birth, Ling et al. (2018) reported no evidence of a significant positive association between malathion exposure in the first and second trimesters of pregnancy to pre-term birth rates in agricultural regions in California.

- For cerebral palsy, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between prenatal exposure to malathion and CP among children. This determination was based on one available study (Liew et al., 2020) that reported no evidence of a significant positive association for pregnant women who resided in a 2 km buffer zone of malathion applications and CP in both female and male children. Liew et al. (2020) was ranked moderate quality for regulatory purposes due the study's reliance on a geospatial approach to assess pesticide exposure based on residential address, PUR data, and land use data on malathion, not accounting for residential mobility of mothers, and the potential underestimation of total pesticide exposures among the study participants.

- For depression, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and depression. There were two available studies (Beard et al., 2013; Beard et al., 2014) of the AHS cohort that examined the association between malathion exposure and depression among male pesticide applicators and among wives of farmers. Beard et al. (2013) reported no evidence of a positive association for wives' malathion ever use and self-reported incident depression, and no evidence of a positive association based on husband's ever use of malathion as an exposure proxy. Similarly, Beard et al. (2014), reported no evidence of a significant positive association between malathion exposure and depression amongst those who reported depression at enrollment only, at follow-up only, and at both enrollment and follow-up. Both studies were rated moderate quality and relied on self-reported physician diagnosis of depression rather than clinical or medical record confirmation.

- For diabetes, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and diabetes. This determination was based on two available studies (Montgomery et al., 2008; Starling et al., 2014). Montgomery et al.

(2008) reported no evidence of a positive association between ever use of malathion and diabetes among AHS pesticide applicators and Starling et al. (2014) reported no evidence of a significant positive association among wives of pesticide applicators. Both studies were ranked moderate quality and study limitations included self-reported diagnosis of diabetes, the inability to control for diet and exercise, and possible selection bias in Montgomery et al. (2008) since a large number of participants who did not complete a follow-up questionnaire might have been diabetic at study enrollment.

- For end-stage renal disease (ESRD), there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and ESRD. This determination was based on two available publications (Lebov et al., 2015; Lebov et al., 2016). Lebov et al. (2015) evaluated the association between malathion exposure and ESRD among the wives of pesticide applicators enrolled in the AHS. No evidence of a significant positive association was reported between indirect malathion exposure and ESRD based on ever/never use, and no evidence of an exposure-response trend was observed. The overall quality of the study was ranked moderate. Study limitations included the indirect assessment of pesticide exposure for applicator wives using husband's use information as a surrogate. This approach has not been validated and may not be a reliable proxy for direct malathion exposure by female spouses. Lebov et al. (2016) directly assessed malathion exposure and ESRD among male pesticide applicators and reported no evidence of a significant positive association based on intensity-weighted lifetime days of exposure, with the no exposure group as the referent. The overall quality of the study was ranked high. b

- For endometriosis, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and endometriosis. This determination was based on one available study, Li et al. (2020), that examined the association between pesticide exposures, including malathion, determined via specific urinary biomarkers and endometriosis among women using a prospective cohort design of the Endometriosis Natural History, Diagnosis, and Outcomes (ENDO) Study. No evidence of a significant positive association was observed between malathion exposure for MDA urinary creatinine-adjusted concentrations and for MDA unadjusted urinary creatinine-adjusted concentrations. A statistically significant ($p < 0.05$) dose-response trend was observed in the operative cohort and in both cohorts combined; however, the direction of the trend is not clearly stated in the study. This study quality was ranked moderate for regulatory purposes. Strengths of the study included the prospective cohort design, the laboratory QA/QC procedures to determine exposure, and the extensive measures used to determine the outcome. Study limitations included the use of a single urine sample to determine past pesticide exposure(s) and potential concern for urine sample contamination and inaccurate metabolite measurements in this study.

- For eye disorders, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and eye disorders including AMD. There were two available studies that examined eye disorders and the results were mixed (Kirrane et al., 2005; Montgomery et al., 2017). Kirrane et al. (2005) reported no evidence of a positive association between malathion exposure and retinal degeneration among wives of farmers in a cross-sectional analysis of the AHS population and was ranked low quality. In an update to Kirrane et al. (2005) that included longer follow-up time and analysis of both pesticide applicators and pesticide applicators wives together and then separately, Montgomery et al. (2017) reported evidence of a moderately strong association between malathion and AMD based on ever/never exposure. For cumulative days of malathion use, evidence of a moderately strong association was seen at the high exposure category only and no evidence of a significant exposure-response relationship was reported among male pesticide applicators in the AHS. Additionally, moderately strong associations were reported

between ever use of malathion and AMD among men and AMD among women in the AHS, and among early AMD cases and among late AMD cases in subsequent subanalyses. The study quality was ranked high for regulatory purposes.

- For gestational hypertension, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and gestational hypertension. One study, Ledda et al. (2015), evaluated the potential association between pesticide exposure, including malathion, and hypertension among pregnant women and reported evidence of a slight positive association for malathion exposure. This study was ranked low quality for regulatory purposes, as it relied on a cross-sectional design that was unable to assess the temporality of the relationship between malathion exposure and gestational hypertension.

- For hearing loss, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and hearing loss among AHS study participants. This determination was based on one available study, Crawford et al. (2008), that reported evidence of a positive and slight positive association between hearing loss and malathion use in the medium and high exposure groups, and no evidence of a significant positive association in the low exposure group. Additionally, no evidence of a significant p-trend was reported. The study quality was ranked moderate for regulatory purposes. The prospective study design was a study strength, and the missing data and the potential underreported outcome due to societal stigmas were considered study limitations.

- For myocardial infarction, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and MI. This determination was based on two studies of the AHS cohort that examined that association between malathion exposure and MI. Mills et al. (2009) reported no evidence of a positive association for fatal MI and no evidence of a significant positive association for non-fatal MI, based on ever/never use of malathion amongst male pesticide applicators in the AHS. Dayton et al. (2010) further examined the relationship between malathion exposure and non-fatal MI among female participants of the AHS. The study reported no evidence of a positive association. Both studies were moderate quality and a limitation of both studies was the self-report of the outcome which could have resulted in misclassification.

- For neonatal central nervous system function, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and neonatal central nervous system function. This determination was based on one available study that examined central nervous system function (Engel et al., 2007) in neonates. Evidence of a moderately strong association was reported between prenatal exposure to malathion and the number of abnormal reflexes in neonatal babies, and no evidence of a statistically significant change was observed for habituation, orientation, motor performance; regulation of state, range of state, levels of stimulation, and autonomic stability in newborns following prenatal exposure to malathion. The study was ranked moderate quality for regulatory purposes. Study strengths included the study design, the use of hospital data to confirm the outcome, and the use of laboratory QA/QC methods. Study limitations included the single urinary sample taken during once pregnancy to assess malathion exposure and the potential for exposure misclassification due the transient and variable nature of exposures to pesticides.

- For central nervous system function in adults, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and central nervous system function by conducting neurobehavorial tests in adults. This determination was based

on one available study that examined central nervous system function (Starks et al., 2012a) that reported evidence of a significant decrease between lifetime days of malathion use and the digit symbol neurobehavorial test, and no evidence of significant association for any other neurobehavioral function outcome measures for both ever-use and lifetime days of malathion use, among pesticide applications in the AHS population. The study was ranked low quality for regulatory purposes. While the study benefited from the prospective cohort study design, case identification using trained neurobehavior technicians, and the AHS exposure assessment approach, several study limitations were noted including the potential for selection bias, use of automated backwards selection in the statistical analysis which is appropriate for a hypothesis generating study, and the fact that participants who consumed up to 41 alcoholic drinks per week were eligible to participate even though that quantity of alcohol might certainly have an effect on neurobehavioral function.

- For peripheral nervous system function in adults, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion and adult peripheral nervous system function. This determination was based on one study that examined the association between malathion exposure and peripheral nervous system function in adults (Starks et al., 2012b). In the analysis of malathion exposure and **neurological physical examination outcomes**, the study reported no evidence of a significant positive associations between malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for either ever use of lifetime days of malathion use. Similarly, results from the dose-response model indicated no evidence of significant positive association between log-transformed lifetime days of use of malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for the low exposure and the high exposure groups, relative to the controls. For the analysis of malathion exposure and the **electrophysiological tests**, no evidence of a significant association was reported for malathion and distal motor amplitude, distal motor latency, nerve conduction velocity, short F-wave latency for either ever use or lifetime days of use of malathion. For the analysis of malathion exposure and the **quantitative functional PNS tests**, no evidence of a significant association was reported for ever use of malathion and for log-transformed lifetime days of malathion and sway speed with eyes opened and closed, hand strength and vibrotactile threshold. The study was ranked low quality for regulatory purposes. While the study benefited from the prospective cohort study design and case identification using trained neurobehavior technicians, several study limitations were noted including the potential for selection bias, use of automated backwards selection in the statistical analysis which is appropriate for a hypothesis-generating study, and the fact that participants who consumed up to 41 alcoholic drinks per week were eligible to participate even though that quantity of alcohol might certainly have an effect on neurobehavioral function. Additionally, although several outcomes were considered, the study did not correct for multiple comparisons using statistical methods such as the Benjamini-Hochberg test.

- For olfactory impairment, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and olfactory impairment. This determination was based on two available studies, Shrestha et al. (2019a) and Shrestha et al. (2020a). Shrestha et al. (2019a) reported no evidence of a significant positive association between malathion and OI among private pesticide applicators. Shrestha et al., 2020a, reported no evidence of a significant positive association between ever use and the >360–1,344 exposure category of cumulative IWLD of use of malathion among the pesticide applicators in the AHS and olfactory impairment. Evidence of a slight positive association was reported between the association of malathion and olfactory impairment based on ever use and was reported for the >0–360 exposure category of intensity-weighted lifetime days of malathion use and olfactory impairment. Evidence of a positive association was reported for the >1,344 days exposure category of intensity-

weighted lifetime days of malathion use and olfactory impairment. The quality of both studies were moderate, and the self-reported outcome was a limitation.

- For Parkinson's disease (PD), there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and PD. This determination was based on five studies (Firestone et al., 2005; Kamel et al., 2007; Firestone et al., 2010; Wang et al., 2014; Shrestha et al., 2020b) that reported mixed results. Firestone et al. (2005) and Firestone et al. (2010) investigated the potential association between malathion exposure and PD in a population-based case-control study in Western Washington State, and both studies reported no evidence of a positive association between malathion exposure and PD in men. The quality of the study was ranked low for regulatory purposes for both studies. Several limitations were noted including potential for recall bias, interviewer bias, and self-reported exposures. Authors reported interviewers were blinded from case-control status of participants but the outward manifestations of PD made complete blinding impossible. A third study, Kamel et al. (2007), reported no evidence of a significant positive association and was ranked moderate quality for regulatory purposes. Wang et al. (2014) investigated the association between ambient exposure to malathion and PD among residents living in the Central Valley area of California, using a GIS based exposure assessment and PUR data. The study reported evidence of a moderately strong to strong association between ambient malathion exposure and PD based on residential address exposure, workplace address exposure, and residential and workplace address exposures combined. The study was ranked moderate quality for regulatory purposes, and study limitations included control selection, the GIS approach to exposure assessment, and the possible under-reporting of PUR data among farmers. Additionally, the study relied on the participant's ability to recall their home and workplace addresses from years in the past (up to 26 years using a telephone interview). A fifth study, Shrestha et al. (2020b), was particularly notable because the study provides more recent, prospective follow-up of the AHS cohort through 2016. The study first examined ever-never use of malathion at enrollment and incident PD in the entire AHS cohort and reported no evidence of a significant positive association between ever use of malathion and incident PD, and no evidence of a significant positive association between ever use of malathion and prevalent PD. Shrestha et al. (2020b) further assessed cumulative, lifetime malathion use among AHS applicators and reported no evidence of a significant positive association between malathion use and incident PD in any exposure category of IWLD of malathion use and no evidence of a significant exposure-response relationship. This study was ranked moderate for regulatory purposes.

- For respiratory effects (asthma, chronic bronchitis, rhinitis, and wheeze), there were eleven studies of the AHS cohort (Hoppin et al., 2002; Hoppin et al., 2006a; Hoppin et al., 2006b; Hoppin et al., 2007; Hoppin et al., 2008; Hoppin et al., 2009; Hoppin et al., 2017; Valcin et al., 2007; Slager et al., 2010; Henneberger et al., 2014; Rinsky et al., 2019) that examined the association between malathion exposure and respiratory effects including asthma, chronic bronchitis, rhinitis, and wheeze. Most of these studies were limited in quality because they all relied on cross-sectional study designs and were unable to assess the temporal relationship between malathion exposure and respiratory effects.

    - For the respiratory effect of asthma, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and asthma. This determination was based on three AHS studies (Hoppin et al., 2008; Hoppin et al., 2009; Henneberger et al., 2014) that examined the association between malathion exposure and asthma. The reported results among the three studies were mixed. Hoppin et al. (2008) reported evidence of a positive association for malathion exposure and atopic asthma among farm women and no evidence of a significant positive association for nonatopic asthma, based on malathion ever use. The subsequent Hoppin et al. (2009) study reported evidence of a positive association between ever use of malathion and adult-onset non-atopic asthma among male farmers in the

AHS, and no evidence of a significant positive association for atopic asthma. Furthermore, in an exposure-response analysis using the median as the cut-point of malathion intensity-adjusted exposure to create two exposure categories, evidence of a borderline positive association was reported in both exposure categories for non-atopic asthma, and no evidence of a significant positive association was reported for either exposure category of atopic asthma. Evidence of a significant exposure-response trend was reported for atopic asthma, and no evidence of an exposure-response trend was reported for non-atopic asthma. The third study, Henneberger et al. (2014), evaluated asthma exacerbation among asthmatic pesticide applicators in the AHS and reported no evidence of a positive association between exacerbated asthma and current malathion exposure. The quality of each of the three AHS studies was ranked low for regulatory purposes due to the cross-sectional study design as temporality for exposure in relation to the outcome could not be determined. Additionally, the studies relied on self-report of the outcome.

- For the respiratory effect of chronic bronchitis, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and chronic bronchitis. This determination was based on three publications (Hoppin et al., 2007; Valcin et al., 2007; Rinsky et al., 2019) that examined the association between malathion exposure and chronic bronchitis among agricultural populations. The reported results among the three studies were mixed. Hoppin et al. (2007) reported evidence of a positive association between malathion exposure and chronic bronchitis among male pesticide applicators in the AHS based on ever use, and when further adjusted for cumulative lifetime days of exposure, in every exposure category besides the second highest exposure category; however, the exposure-response trend was not statistically significant. Valcin et al. (2007) reported no evidence of a significant positive association between chronic bronchitis and malathion in their analysis of female spouses of AHS pesticide applicators. Rinsky et al. (2019) reported evidence of a positive association for malathion ever use and COPD diagnosis and chronic bronchitis symptoms and reported for malathion ever use adjusted for animal produced and the COPD diagnosis and chronic bronchitis symptoms outcome. Both Hoppin et al. (2007) and Valcin et al. (2007) used cross-sectional study designs. As such, the studies were unable to assess temporality between malathion exposure and chronic bronchitis and were judged to be of low quality for regulatory purposes. Rinsky et al. (2019) was also ranked low quality because the temporal ordering of exposure and outcomes was not possible since prevalent cases were not able to be excluded from the analysis.

- For the respiratory effect of rhinitis, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and rhinitis. This determination was based on two available studies (Slager et al., 2009; Slager et al., 2010) that examined rhinitis among private pesticide applicators in the AHS. Slager et al. (2009) reported no evidence of a significant positive association among commercial pesticide applicators based on use within the past year. Slager et al. (2010) examined rhinitis among commercial pesticide applicators in the AHS, and reported evidence of a slight positive association between malathion and current rhinitis based on ever/never exposure as well as for one of the rhinitis categories only (3 – 6 episodes/year) in the polytomous model. No evidence of a significant positive association was reported in any of the four other rhinitis categories. The overall study quality of both studies was ranked low for regulatory purposes. The cross-sectional study design was the main limitation and additionally the statistical methods used to select the regression model covariates.

- For the respiratory effect of wheeze, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and wheeze. This determination was based on four AHS studies (Hoppin et al., 2002; Hoppin et al.,

2006a; Hoppin et al., 2006b; and Hoppin et al., 2017) that examined the association between malathion exposure and wheeze in the AHS prospective cohort study population. The reported results among the four studies were mixed. Hoppin et al. (2002), reported evidence of a slight positive association between malathion exposure and wheeze among pesticide applicators, based on ever use. In subsequent follow-on studies, Hoppin et al. (2006a) reported no evidence of a significant positive association between malathion use among private pesticide applicators and no evidence of a positive association between malathion use among commercial pesticide applicators. Hoppin et al. (2006b) reported no evidence of a significant positive association between current malathion use and wheeze among commercial applicators, based on ever use. Both studies were ranked as low quality because the studies relied on a cross-sectional design that assessed the relationship between prevalent cases of wheeze and pesticide exposure. As such, the study was unable to assess the temporal association between malathion exposure and wheeze. In a fourth study on the AHS that included a cross-sectional analysis of malathion exposure in the past year and wheeze in the past year using the responses from the 2005-2010 follow-up survey rather than from enrollment, Hoppin et al. (2017) reported evidence of a positive association between malathion exposure in the past year for allergic wheeze based on ever use. Evidence of a positive association was reported in three of the five exposure categories of allergic wheeze in the exposure-response analysis. No evidence of a significant positive association was reported in the highest exposure category. For non-allergic wheeze, evidence of a slight positive association was reported between malathion and wheeze based on ever use and evidence of a positive association was reported in the highest exposure category of malathion use in the past year in the exposure-response analysis, along with two other exposure categories. The authors did not report a p-trend statistic for the exposure-response analysis for either allergic or non-allergic wheeze and malathion; however, inspection of the ORs associated with each category suggests an exposure-response trend may not exist for either allergic or non-allergic wheeze. All four studies were ranked low quality, as they relied on a cross-sectional design that was unable to assess the temporality of the relationship between cases of pesticide exposure and wheeze. Additionally, health outcomes were self-reported.

- For rheumatoid arthritis, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and rheumatoid arthritis. This determination was based on three publications (De Ross et al., 2005; Parks et al., 2016; Meyer et al., 2017) that examined the association between malathion exposure and RA among participants of the AHS prospective cohort. De Roos et al. (2005) and Parks et al. (2016) reported no evidence of a significant positive association among wives of pesticide applicators in the AHS. The third publication, Meyer et al. (2017), examined the association between malathion exposure and RA among male pesticide applicators in the AHS and reported no evidence of a positive association between malathion exposure and incident RA cases among male pesticide applicators. All three studies were ranked moderate quality for regulatory purposes and while they benefited from exposure assessment approach used by the AHS, the outcome was self-reported and if clinically confirmed via medical records would have made the assessment stronger.

- For hyperthyroid disease, there is there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and hyperthyroid disease. Three publications (Goldner et al., 2010. Shrestha et al., 2018b; Shrestha et al., 2019b) examined the relationship between malathion exposure and hyperthyroid disease among AHS study participants. Goldner et al. (2010) reported no evidence of a positive association between malathion ever use and hyperthyroid disease among female spouses of pesticide applicators enrolled in the AHS and was low quality for regulatory purposes. The main limitation was the cross-sectional study design since temporality for exposure in relation to the outcome could not be determined.

Shrestha et al. (2018b) with longer follow-up time and additional cases, similarly reported no evidence of a positive association among female spouses of pesticide applicators in the AHS. A third publication (Shrestha et al., 2019) also reported no evidence of a positive association among private pesticide applicators in the AHS based on malathion ever use. Both Shrestha et al. (2018b) and Shrestha et al. (2019b) were ranked moderate quality for regulatory purposes. Both attempted to validate the self-reported hyperthyroidism diagnosis via medical record confirmation, however only 32% of attempted cases were ultimately clinically confirmed. Potential selection bias was also likely if study subject participation in the follow-up phases was related to their disease status for hyperthyroidism. An additional limitation of all three publications was that only ever use of pesticides prior to enrollment was captured rather than pesticide use that occurred after enrollment and this may have led to exposure misclassification.

- For hypothyroid disease, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and hypothyroid disease. This determination was based on five publications (Goldner et al., 2010; Goldner et al., 2013; Shrestha et al., 2018b; Shrestha et al., 2018c; Lerro et al., 2018) that examined the relationship between malathion exposure and hypothyroid disease among AHS study participants and the evidence is mixed. For female spouses of male pesticide applicators enrolled in the AHS, Goldner et al. (2010) reported no evidence of a significant positive association between malathion ever use and hypothyroid disease. The publication was ranked low due to a cross-sectional study design since temporality for exposure in relation to the outcome could not be determined and was limited by self-reported outcome. Shrestha et al. (2018b) reported no evidence of a significant positive association among female spouses of pesticide applicators in the AHS, based on malathion ever use and longer follow-up time and was ranked moderate quality. Study limitations included self-reported diagnosis of thyroid disease and selection bias if study subject participation was related to their outcome. Among male pesticide applicators in the AHS, Goldner et al. (2013) reported evidence of a slight positive association between exposure to malathion and hypothyroid disease based on ever use and was ranked moderate quality due to the self-reported diagnosis of thyroid disease. A fourth publication (Shrestha et al., 2018c) reported evidence of a slight positive association between malathion exposure and hypothyroid disease among private pesticide applicators enrolled in the AHS. For intensity-weighted lifetime days of exposure, evidence of a positive association for the mid-exposure category only was reported, with no evidence of a significant positive association observed for the low and high exposure categories, or for the exposure-response trend. The study quality was ranked moderate and study limitations were noted including the self-reported diagnosis of thyroid disease and the possibility of selection bias if study subject participation in the follow-up phases was related to their disease status for hypothyroidism. Finally, a fifth publication, (Lerro et al. 2018), reported no evidence of a significant positive association at any exposure level relative to the non-exposed group for subclinical hypothyroidism among male participants in the AHS. The quality of the study was ranked moderate for regulatory purposes.

- For weight gain, there is *insufficient epidemiological evidence* at this time to conclude that there is a clear associative or causal relationship between malathion exposure and other thyroid disease. This determination was based on one publication (LaVerda et al., 2015) that examined the relationship between malathion exposure and weight gain and reported no evidence of a positive association between malathion exposure and increased BMI. The study was ranked moderate quality for regulatory purposes. Strengths of the study included the prospective cohort study design, questionnaires and interviews that assessed specific pesticide exposure including duration and frequency of exposure, the adjustment for other pesticides in the models, and the use of the Bonferroni adjustment to minimize chance effects due to multiple comparisons and the chance of type I error. The study was limited by the self-reported outcome (BMI) including a retrospective report of

BMI at age 20 collected during study enrollment (the mean age at follow-up was 56.4 years, indicating the mean age at enrollment was approximately 51 years old). This introduced the potential for outcome misclassification.

4 OVERALL CONCLUSION

For this Malathion Tier II Incident and Epidemiology Report, HED found that overall, the majority of malathion incidents were low in severity (78% in IDS, 73% in SENSOR-Pesticides, NPIC 79%). In both IDS and SENSOR, malathion incidents appear to be decreasing over time. In Main IDS, most individuals reported being exposed to malathion during application, and indoor exposure. NPIC found that most malathion cases were related to spills, primarily indoors. SENSOR-Pesticides (from 2010-2017) found the main contributing factor in malathion case reports involved pesticide user spills or splashes (both for occupational and residential users). Of the occupational malathion cases reported in SENSOR-Pesticides, nearly 75% involved agricultural workers exposed to pesticide residues while working in treated fields. The California PISP (from 2012-2017) found that most malathion incidents involved fieldworkers exposed to either pesticide residue or from off-site movement of the pesticide. Reported symptoms continue to include mostly neurological, gastrointestinal and respiratory effects. In addition, HED did not identify any aberrant effects outside of those anticipated and documented as a result of general OP toxicity.

HED conducted a systematic review of the epidemiologic literature on malathion in order to assess the epidemiologic evidence on the potential adverse effects of malathion exposure and identified 109 publications that investigated a range of health outcomes, including 42 publications on carcinogenic health outcomes and 67 on the non-carcinogenic outcomes. There were individual studies that identified positive associations between malathion and some adverse health effects; however, the overall evidence was based on a small body of studies (i.e., typically only one or two studies per health outcome) that often had substantive limitations with respect to their study design, exposure assessment approach, or outcome assessment. As such, HED concluded that overall, there was insufficient epidemiologic evidence to suggest a clear associative or causal relationship exists between malathion exposure and the adverse health effects examined in the available epidemiologic literature. The Agency will continue to monitor the epidemiology data and – if a concern is triggered – additional analysis will be conducted.

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6 APPENDIX A: SUMMARY OF INCIDENTS REPORTED

Table A-1. Single AI Malathion Incidents Reported to Main IDS from 2014-February 25, 2021						
Incident Package Report	**Incident Date**	**Location**	**Reg Number**	**Product Name**	**Exposure Severity**	**Incident Description**
028917 - 00002	4/30/2016	DALLAS, TX	000769-00620-053883	MARTIN'S 57% MALATHION	Major	A 79-year-old female unintentionally ingested 1/2 cup of product.
029049 - 00012	6/05/2016	CA	Unknown	Unknown	Moderate	An adult male experienced a cough and nasal discharge 12 hours following application of the product to his backyard by his landlord. He also experienced his chest feeling raw and a warm head. Four days later, his cough was persisting but the other symptoms were diminished.
029049 - 00035	6/14/2016	TX	000239-00739	ORTHO MALATHION PLUS INSECT SPRAY CONCENTRATE	Moderate	An adult female used the diluted product and developed a redness, swelling and itching on her arms.
029141 - 00012	6/18/2016	CA	Unknown	SPRECTRACIDE MALATHION	Moderate	A 42-year-old male intentionally ingested the product along with gasoline and THC+ and alcohol.
028934 - 00001	6/27/2016	CA	Unknown	Unknown	Moderate	A 69-year-old male mixed 3 cc of malathion with his insulin shot and injected it. He was transported to the hospital for observation and discharged the next day.
029141 - 00016	7/10/2016	CA	Unknown	MALATHION	Moderate	A 53-year-old male was admitted to the hospital after presenting symptoms of nausea, vomiting, diarrhea, diaphoresis, ataxia and drowsiness after an alleged application of the product to his residence by his neighbor.
029273 - 00010	8/14/2016	VIAN, OK	046515-00019-008845	SPECTRACIDE MALATHION INSECT SPRAY CONCENTRATE	Moderate	A 52-year-old male put 2 tsp of the product in his body wash to treat chiggers. He experienced red bloody stools.
029358 - 00005	7/08/2016	AUSTIN, KY	046515-00019-008845	SPECTRACIDE MALATHION INSECT SPRAY CONCENTRATE	Moderate	Adult female used the outdoor product inside her home, in her kitchen cabinets. She was diagnosed with asthma after the exposure.
029386 - 00014	8/11/2016	CA		UNKNOWN	Moderate	A 57-year-old female post office worker was exposed to a malathion application at the post office by the janitorial staff. She was transported to the ER. Symptoms not reported.

Table A-1. Single AI Malathion Incidents Reported to Main IDS from 2014-February 25, 2021						
Incident Package Report	**Incident Date**	**Location**	**Reg Number**	**Product Name**	**Exposure Severity**	**Incident Description**
029452 - 00125	10/08/2016	CA	000239-00739	ORTHO MAX MALATHION INSECT SPRAY CONCENTRATE	Moderate	An 83-year-old female used the diluted product with gloves on. She developed a rash, redness, and blisters on both arms.
029015 - 00022	5/16/2016	NC	000239-00739	ORTHO MALATHION 50 SPRAY	Moderate	A senior female (>65-year-old) used the product. 48 hours later, she experienced a nosebleed which were intermittent to chronic. She saw a doctor who thought the nosebleed was from hypertension.
029626 - 00005	1/12/2017	FL	046515-00019	SPECTRACIDE MALATHION INSECT SPRAY CONCENTRATE	Moderate	An adult male was mixing the product. It was windy and the product blew into his home. He and his wife inhaled it. It was irritation and they experienced coughing and left the home.
029574 - 00079	4/26/2016	TX		MALATHION	Moderate	An adult male applied the product and immediately developed chest pain.
029993 - 00086	4/10/2017	AR	000239-00739	ORTHO MAX MALATHION INSECT SPRAY CONCENTRATE	Moderate	An adult female was exposed to the product on her hand and washed it after about 20 minutes. Two hours later she developed itchiness and welts on her arms that spread across her chest that got worse throughout the day. She went to the MD who gave her a steroid shot and prescribed prednisone.
030208 - 00004	6/27/2017	POCAHONTAS, AR		MALATHION (NON-SPECIFIC)	Moderate	Several individuals were exposed when a mosquito control truck sprayed the city. They reported various symptoms including nausea, vomiting, abnormal mentation, confusion, dizziness, drowsiness, and shortness of breath.
030664 - 00001	1/02/2018	WEST BABYLON, NY		UNKNOWN	No or Unknown Symptoms	A homeowner reported a glass bottle of malathion broke and spilled on the concrete floor of his attached garage last night. He, his wife, daughter, and houseguest reported headaches and "discomfort" after the odor entered the home from the garage.
030765 - 00001	10/11/2017	CA		UNKNOWN	Moderate	A 39-year old male applied malathion in his own backyard and forgot to wash his hands after the application and before eating. Two hours later, he experienced symptoms of severe pain in his stomach and was hospitalized for two days.
030838 - 00001	3/13/2018	TOA ALTA, PR		UNKNOWN	Minor	Teachers at an elementary school reported exposure to malathion vapors after the had been applied by a neighbor. The school was evacuated to protect the student.

Table A-1. Single AI Malathion Incidents Reported to Main IDS from 2014-February 25, 2021						
Incident Package Report	**Incident Date**	**Location**	**Reg Number**	**Product Name**	**Exposure Severity**	**Incident Description**
031130 - 00005	5/05/2018	OH		UNKNOWN PRODUCT	Moderate	One-year old female ate potentially treated potting soil. She experienced vomiting. The symptom was not related to the product however, it was diagnosed as a stomach bug because everyone else in the house became ill.
031130 - 00012	5/23/2018	LAKE WOOD, FL	000239-00739	MALATHION 50 PLUS INSECT SPRAY	Moderate	An adult female sprayed her rose bushes with the product and found a crack in the bottle. The product ran down her arm and smelled really strong. After the application, she showered and went to bed. She woke up around 4am experiencing shaking, numbness and nausea.
031341 - 00052	7/04/2018	IA	000239-00739	ORTHO MAX MALATHION INSECT SPRAY CONCENTRATE	Moderate	An adult male was exposed the airborne product. The wind blew it into his face, arms and into his mouth. Approximately 20-30 minutes later, he developed respiratory distress and was taken to the ER via ambulance after blacking out. He was discharged after seven hours. Three days later, he was still experiencing labored breathing, headaches and dizziness. Doctors are unsure if this was heat-related or product related.
031453 - 00001	8/12/2018	PEARLAND, TX	067760-00040-053883	MARTIN'S MALATHION 57%	Moderate	A 55-year-old male used the product. He mixed it more concentrated than the label directed. The wind blew back a mist onto his arms, face and legs, in his eyes and he breathed it in. He did not bathe for 3 days after. He developed fatigue, headache, malaise, muscle pain and throat irritation.
031736 - 00007	12/18/2018	SAINT JOSEPH, MO	046515-00019-008845	SPECTRACIDE MALATHION INSECT SPRAY CONCENTRATE	Moderate	A 64-year-old female used the product in her home. She went to the ER and had elevated CPK. No other information provided regarding symptoms.
032332 - 00001	7/24/2019	ATLANTA, GA		UNKNOWN	No or Unknown Symptoms	An unlicensed applicator made her own pesticide mix which included malathion and applied it to mattresses for bedbugs in approximately 15 homes.
032700 - 00130	10/25/2019	CA	000239-00739	MALATHION INSECT SPRAY (CONCENTRATE)	Moderate	An adult male had put the product in a milk jug and accidentally drank it. He experienced confusion and intermittent vision loss. All symptoms resolved by the next day.
033415 - 00009	6/17/2020	SEMMES, AL	046515-00019-008845	SPECTRACIDE MALATHION INSECT SPRAY CONCENTRATE	Moderate	A 67-year-old male sprayed the product inside his house to get rid of bugs. He made the product double strength. A couple days later he started symptoms (shortness of breath, nausea, dizziness). He has preexisting condition of blood clots.
033386 - 00001	6/02/2018	MS	000239-00739	MALATHION INSECT SPRAY CONCENTRATE	Major	A senior female (>65-year-old) spilled the product in the trunk of her car two years ago. Since then she has been experiencing bronchitis and sinus infections.

Table A-1. Single AI Malathion Incidents Reported to Main IDS from 2014-February 25, 2021						
Incident Package Report	**Incident Date**	**Location**	**Reg Number**	**Product Name**	**Exposure Severity**	**Incident Description**
033386 - 00005	6/04/2020	CA	000239-00739	MALATHION PLUS INSECT SPRAY CONCENTRATE	Moderate	An adult female applied malathion to plants in a greenhouse. The next day she developed migraine, body aches, vomiting, anorexia.

Table A-2. Malathion Incident Reports to PISP involving Case Hospitalization, 2012-2017				
Year	**Days Hospitalized**	**Ag/Non-Ag**	**Medical Description**	**Narrative Description**
2017	2	Non-Ag	He experienced nausea, vomiting, and severe abdominal pain. The doctor noted ongoing mild bradycardia and EKG changes, his lungs remained clear, no miosis present, and his oxygen stats were good. He was hospitalized for 2 days.	08-la-18. A man diluted malathion in a sprayer & got some on his hands while closing the lid. He did not wear gloves. He treated several plants & trees in his yard. Afterwards, he ate some food without washing his hands. He then developed symptoms.
2016	1	Non-Ag	Shortness of breath, throat irritation, chest tightness, mouth and nose numbness. Slight tachycardia (103) and hypertension (153/103) noted at the ER.	58-pla-16. A janitor threw a malathion container into the dumpster outside the backdoor of a post office. Three workers complained about the odor that entered through the open back door. This worker developed symptoms.
2016	1	Non-Ag	Nausea, vomiting, diarrhea, diaphoresis, ataxia, pinpoint pupils, drowsiness, high blood pressure. In ward: oxygen saturation was 94% on ra. He stated his only symptoms were lightheadedness & inability to focus.	47-sha-16. A woman sprayed malathion in the backyard of her home. Her boyfriend developed symptoms and sought care 2 days later. He stated his symptoms were due to not taking his blood pressure medication. He did not say how he may have been exposed.
2016	Indeterminate	Non-Ag	Vomiting, shaking, sinus tachycardia, lacrimation, diaphoresis, moderate yellowish bronchial secretion, dyspnea, fever, metabolic acidosis, nystagmus, pneumonitis, hypoxia with o2 sat 80s. Given atropine & 2-pam. Admitted for at least 6 days.	43-ker-16. A man drank alcohol and an unknown amount of malathion in a suicide attempt. He is an alcoholic and admitted to being depressed. Marijuana may also have been used. His vomit smelled like gasoline, an odor suspected to be from the malathion.
2015	Indeterminate	Non-Ag	Paramedics reported man was not arousable and had a lot of unspecified secretions prior to transport. Doctor reported pupils at 3 mm and decreased level of consciousness.	53-mrn-15. A 69-year old diabetic man injected 2-3 ccs of malathion mixed with his insulin. He was taken to a hospital by paramedics where he was hospitalized.

Table A-2. Malathion Incident Reports to PISP involving Case Hospitalization, 2012-2017				
Year	**Days Hospitalized**	**Ag/Non-Ag**	**Medical Description**	**Narrative Description**
2014	Indeterminate	Non-Ag	Vomiting, uncontrolled defecation, seizures, tachypnea & tachycardia, twitching, salivary secretions, wet lung sounds. Responded to atropine and 2-pam, was intubated and sedated. Medical staff protected themselves from the off-gassing patient.	69-la-14. A 56-year old man ingested malathion in a self-harm attempt. He had cholinergic significant effects and was hospitalized at least four days. No investigation performed due to sensitive nature of case.
2014	1	Non-Ag	Nausea, diarrhea, vomiting, and occasional non-productive dry cough. Pupils 5-6mm. Symptoms resolved after receiving multiple doses of Ativan and 2-pam.	01-na-14. A homeless man ingested a mouthful of malathion in a self-harm gesture. He arrived at the emergency department ill and with a strong pesticide odor on his clothing. He had to be decontaminated outdoors due to the strong fumes.
2013	Indeterminate	Non-Ag	Vomiting, secretions, tearing, sweating, incontinence, salivation, acidosis, seizures. Given 2pam and atropine. After 9 days, he failed a breathing trial and received a tracheostomy. After 16 days he was minimally responsive; anticipated to be permanent.	47-riv-13. A 49-year old man reported ingesting one cup of malathion in a self-harm gesture. He was decontaminated upon arrival to ed. Hospital staff reported a strong odor. Due to the sensitive nature of this incident, no investigation was conducted.
2013	Indeterminate	Non-Ag	He had generalized weakness and felt "off". He has a history of COPD. He was hospitalized and given a breathing treatment, then spent time in a nursing home to regain strength. He was feeling much better at the time of the interview, about a month later.	110-ora-14. An old container of malathion leaked in the garage of an elderly man's home and was absorbed into the drywall, creating a strong odor. He went to stay with his daughter and hazmat contained the spill. A few days later he sought care.
2012	1	Non-Ag	He arrived somnolent and developed vomiting, diarrhea, diaphoresis, excessive salivation, hypotension, hypothermia and crackly lung sounds. He expired; summarized cause of death: cardiopulmonary arrest with acidosis due to ingestion of pesticide.	02-sac-13. A man ingested "3 cups" of one or more pesticides in a self-harm attempt. He had apparently also ingested the same product a week prior.

Table A-2. Malathion Incident Reports to PISP involving Case Hospitalization, 2012-2017				
Year	**Days Hospitalized**	**Ag/Non-Ag**	**Medical Description**	**Narrative Description**
2012	1	Non-Ag	He was awake on arrival. He developed bronchorrhea, vomiting, diarrhea, bradycardia and was given 2-pam and atropine. He also had miotic pupils, but it was unclear if symptom was due to heroin or organophosphate. He did well and was medically cleared.	76-sac-12. A man drank an unknown amount of malathion in a self-harm gesture. He also had a history of heroin use. Ems and er staff noted that he smelled strongly of the product.
2012	7	Non-Ag	Vomiting, diarrhea, coarse lung sounds, pinpoint pupils, "feels rigid," excessive secretions, tachycardia.	51-sbd-14. A man intentionally ingested malathion & was admitted to the ICU. 2 days later, his family brought in the bottle. Two hospital housekeepers were exposed to its contents when the discarded bottle broke in the trash room. See 2012-1099 & 1100.
2012	1	Non-Ag	Vomited twice at home & again once en route to the hospital. Ed noted he was 'a bit lethargic' on arrival, but had no further symptoms, but a murmur was discovered. He was admitted to the pediatric ward overnight for observation & released the next day.	23-ker-12. As he & his young son were in the yard, a father glanced over and saw him with a container of malathion used for spraying fruit trees. Dad saw the liquid around his mouth & shirt. He was taken for care.
2012	1	Non-Ag	Nose and throat irritation, burning throat, some cough, shortness of breath, and bronchial irritation. O2 sat 96%, smoker w/COPD. Ed administered 2-pam. Exam found coarse breath sounds. He washed at home before seeking care.	06-but-12. A man intended to spray malathion insecticide as an herbicide at home. As he cleaned a tank that had some leftover in it, the mix splashed up onto him and he inhaled some fumes. He washed, felt ill & was hospitalized overnight for observation.

7 APPENDIX B: SUMMARY OF EPIDEMIOLOGIC STUDIES AND STUDY QUALITY ASSESSMENT

7.1 Table B-1: Summary of Epidemiologic Studies on Cancer

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
All Cancers							
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	The authors reported no evidence of a positive association for malathion ever exposure and overall NHL (i.e., all subtypes considered together) (OR = 0.98; 95% CI: 0.82, 1.16, with n = 1,208 exposed cases), and no evidence of a significant positive association for malathion and any of the NHL subtypes in the meta-analysis (0.84 <HR <1.18; all 95% CIs encompassed the null value of 1.0; with n= 114 – 1208 exposed cases per category, p-trend >0.05).	Moderate
Bladder Cancer							
Bonner et al. (2007)	Enrollment (1993-1997) through December 31, 2002	AHS	Prospective Cohort n=57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	In the bladder cancer analysis for IWLD with the non-exposed group as the referent, no evidence of a positive association was observed in any tertile (T1 – T3: 0.85 ≤ RRs ≤ 0.91; all CIs encompassed the null value of 1.0, with n = 8	High

[129] For additional reported results including risk measures, number of cases/non-cases, and p-values, refer to the individual study summaries written above.

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						– 9 exposed cases, with 14 cases in the non-exposed group)[130]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.91).	
Koutros et al. 2016	1993-1997 (Enrollment) to 2010/2011	AHS	Prospective Cohort n=54,344 male pesticide applicators	AHS Survey Instrument – Ever/Never Use and Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	The study results suggested no evidence of a significant positive association between malathion exposure and risk of bladder cancer (RR = 1.01; 95% CI: 0.65, 1.58) based on ever/never use. Further analyses considered intensity-weighted lifetime days of malathion use. Low and high exposure categories were created, split at the median exposure value based on cumulative intensity-weighted days, and RRs were reported for each category. Adjusting for the aforementioned factors, the researchers observed no evidence of a significant positive association between	Moderate

[130] Risk estimates for intensity-weighted lifetime days of exposure for bladder cancer reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.71 \leq RRs \leq 1.40$; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						malathion exposure and bladder cancer in the first three categories (RR = 1.00; 95% CI: 0.62, 1.59 for the 1st quartile median category, RR = 1.15; 95% CI: 0.71, 1.86 for the 2nd quartile median category, RR = 1.14; 95% CI: 0.71, 1.83 for the 3rd quartile median category, with n = 27-29 exposed cases in all categories and n = 49 unexposed cases), no evidence of a positive association between malathion exposure and bladder cancer in the last exposure category (RR = 0.95; 95% CI: 0.60, 1.52 for the 4th quartile median category, with n=29), and there was no evidence of increasing risk of bladder cancer with increased use of malathion (p-trend = 0.73).	
Brain Cancer							
Lee et al. 2005	July 1st, 1988 to June 30, 1993	Nebraska Health Study II, adults living in eastern Nebraska	Case-control study 251 cases, 498 controls	Telephone interview Ever use	Nebraska Cancer Registry and participating hospitals in Lincoln and Omaha	Evidence of a strong association was reported among cases who completed the interview themselves. We note the small number of exposed cases and corresponding wide confidence interval (OR	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						= 3.40; 95% CI: 1.20, 9.30; with n = 11 exposed cases and n = 17 exposed controls). No evidence of a positive association was reported between malathion ever use and glioma among those cases who had a proxy respondent (OR= 0.80; 95% CI: 0.20, 2.80; with n = 5 exposed cases and n = 12 exposed controls).	
Yiin et al. (2012)	Glioma	1995 - January 1997	Upper Midwest Health Study (UMHS) in Iowa, Michigan, Minnesota and Wisconsin	Case-control (n=798 cases, n=1,175 population-based controls)	Phone interview - participants were asked to report lifetime pesticide use on the farm, at other non-farm jobs, and in the house and garden through 1992, not limited to pesticides on the supplied list	Reported pesticide use in non-farm jobs No evidence of a positive association between malathion and glioma when proxy respondents were included, OR = 0.69 (95% CI 0.30, 1.56) No evidence of a significant positive association between malathion and glioma when proxy respondents were excluded, OR = 1.04 (95% CI 0.45, 2.40) Reported house and garden pesticide use No evidence of a positive association between malathion and glioma when proxy respondents were	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						included, OR = 0.82 (95% CI 0.56, 1.20) No evidence of a positive association between malathion and glioma when proxy respondents were excluded, OR = 0.72 (95% CI 0.44, 1.18)	
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	Among the 38 brain cancer cases identified during the study period, 11 cases reported direct exposure to malathion. No evidence of a significant positive association was reported between malathion exposure and brain cancer (RR = 1.57; 95% CI: 0.65, 3.78; with n = 11 exposed cases).	Moderate
Breast Cancer							
Engel et al. (2005)	1993-1997 (Enrollment) to 2000	AHS	Prospective Cohort n = 30,145 (309 breast cancer cases)	AHS Survey Instrument – Ever/Never Malathion Use (Indirect Exposure, based on Husband Self-Report)	Cancer registries in Iowa and North Carolina, coded via ICD-O-2	The authors reported no evidence of a positive association between ever use of malathion and breast cancer incidence among all wives in the cohort (RR = 0.90; 95% CI: 0.70, 1.20; with 63 exposed cases). A subset analysis conducted for wives who reported no prior pesticide use (n = 13,449) considered husbands' malathion use and no evidence of a significant positive association was reported	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						between husband's malathion use and wife's risk of breast cancer (RR = 1.40; 95% CI: 1.00, 2.00; with 101 cases indirectly exposed).	
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a significant positive association between malathion exposure and breast cancer risk among AHS spouses (RR = 1.05; 95% CI: 0.88, 1.26). When breast cancer was analyzed based on estrogen receptor (ER) or progesterone receptor (PR) status, no evidence of a positive association was identified for *ER+PR+* (*ER+PR+* - RR = 1.00; 95% CI: 0.79, 1.26; with n = 124 exposed cases out of n = 595 total cases) and no evidence of a significant positive association was identified for *ER-PR-* (*ER-PR-* - RR = 1.17; 95% CI: 0.77, 1.78; with n = 40 exposed cases) based on ever use. When breast cancer was stratified by menopausal status at enrollment, no evidence of a significant positive association was reported between malathion	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						exposure and breast cancer for either pre- or postmenopausal participants (*Premenopausal* - RR = 1.04; 95% CI: 0.78, 1.38 with n = 80 exposed cases; *Postmenopausal* - RR = 1.03; 95% CI: 0.81, 1.30 with n = 132 exposed cases). In an additional sensitivity analyses that investigated associations between ever/never malathion exposure and breast cancer diagnosed five or more years after study enrollment among AHS spouses, no evidence of a significant positive association was reported between malathion ever exposure and breast cancer (RR = 1.10; 95% CI: 0.89, 1.35 with n = 156 exposed cases) and between malathion ever exposure and breast cancer diagnosed five or more years after enrollment, based on ER/PR status (*ER+PR+* - RR = 1.11 95% CI: 0.85, 1.45; with n = 95 exposed cases; *ER-PR-* - RR= 1.04 95% CI: 0.62 to 1.74).	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
Mills and Yang, 2005	Breast cancer diagnosis 1988-2001	Cohort of Hispanic farm worker women who were members of the United Farm Workers of America (UFW) at any time between 1973 and 2001.	Nested case-control, n=128 breast cancer cases and n=640 controls	Records to verify occupational history, grower's contracts to establish the crop/commodity the member was exposed to, and the pesticide use records (PUR) from the California Department of Pesticide Regulation to determine maneb use	State cancer registry files	Evidence of a moderately strong association was reported for the medium exposure level only of malathion use and breast cancer diagnosed from 1988 to 1994 (OR: 2.95; 95%CI: 1.07, 8.11 with n = 16 exposed cases) among a small number of cases. No evidence of a significant positive association was reported at the low or high exposure levels (1.68 < all ORs < 1.89; all 95% CIs encompassed 1.0; n=9-14 malathion exposed cases per exposure category). No evidence of a positive association was reported between any exposure level of malathion use and breast cancer that was diagnosed from 1995 to 2001 (0.50 < all ORs < 0.79; all 95% CIs encompassed 1.0; n=14-18 malathion exposed cases per exposure category).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
Engel et al. (2017)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 22,271 wives of pesticide applicators	AHS Survey Instrument – Ever/Never Malathion Use (Direct Exposure and Indirect Exposure, based on Husband Self-Report)	Cancer registries in Iowa and North Carolina	For the association between wives' reported ever use of malathion at enrollment and incident breast cancer, no evidence of a positive association was reported (HR = 1.00; 95% CI: 0.90, 1.20; with n = 226 exposed cases and 5,561 exposed non-cases) and similar results were reported when further adjusted for other pesticides associated with breast cancer in the current analysis (HR = 1.00; 95% CI: 0.80, 1.20; with n = 226 exposed cases and 5,561 exposed non-cases). In table 4 of the study, associations between the husbands' use of individual insecticides and risk of breast cancer among farmers' wives who never used pesticides in the AHS were reported. Both adjustments, without and with pesticides associated with breast cancer, encompassed the null value (0.60 < HR < 1.40; all 95% CIs encompassed the null value of 1.0; with 216 exposed cases and 100 exposed non-cases).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
Golmohammadzadeh et al. 2019	March - May 2018	Breast cancer patients and hospital-based controls (healthy women) at the Cancer Research Center at Imam Hospital in Sari, Iran	Case-control (n=123; n=72 cases, n=51 controls)	Serum levels	Breast cancer diagnosis and stages of breast cancer (I-IV)	No evidence of a positive association between mean serum malathion level and risk of breast cancer, OR=1.0 (95% CI 0.99-1.01).	March - May 2018
Cancers of the Large Intestine							
Bonner et al. (2007)	Enrollment (1993-1997) through December 31, 2002	AHS	Prospective Cohort n=57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.81). Additionally, no evidence of a significant positive association between colorectal cancer for frequency, intensity, and duration of malathion exposure was observed ($0.83 \leq RRs \leq 1.27$; all CIs encompassed the null value of 1.0, with n = 18 – 45 exposed cases, with 40 cases in the non-exposed group, p-trends > 0.05).	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
Lee et al. (2007)	1993-1997 (Enrollment) to 2002	AHS	Prospective Cohort n = 56,813 pesticide applicators	AHS Survey Instrument – Ever/Never Malathion Use	Cancer registries in Iowa and North Carolina, coded via ICD-O-2	No evidence of a positive association was reported between exposure to malathion and colorectal cancer based on ever use (OR = 0.80; 95% CI: 0.60, 1.10; with n = 169 exposed cases and n = 80 unexposed cases).	High
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and colon risk (RR = 0.89; 95% CI: 0.58, 1.37; with n = 38 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and colon cancer (RR = 0.95; 95% CI: 0.58, 1.57; with n = 29 exposed cases).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS-Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a significant positive association was reported between malathion exposure and rectal cancer (RR = 0.92; 95% CI: 0.43, 1.97; with n = 12 exposed cases)	Moderate
Lee et al. (2007)	1993-1997 (Enrollment) to 2003	AHS	Prospective Cohort n = 56,813 pesticide applicators	AHS Survey Instrument – Ever/Never Malathion Use	Cancer registries in Iowa and North Carolina, coded via ICD-O-3	No evidence of a positive association was reported between exposure to malathion and rectal cancer based on ever use (OR = 1.00; 95% CI: 0.60, 1.70; with n = 57 exposed cases and n = 21 unexposed cases).	High
Childhood Cancer							
Flower et al. (2004)	1993-1997 (Enrollment)	AHS	Prospective Cohort for parents and child cases were identified retrospectively and prospectively following parental enrollment N = 17,280 children	AHS Survey Instrument – Ever/Never Malathion Use completed by parents	Birth certificates and cancer registries in Iowa and North Carolina	No evidence of a significant positive association was observed between parental malathion exposure and childhood cancer among a small (n = 11) number of cases (OR = 1.12; 95% CI: 0.57, 2.20).	Moderate
Park et al. (2020)	Children born between 1998 and 2011 and cancer diagnosis until 2012	Cancer cases less than 6 years of age and cancer-free controls were identified from birth certificates.	Case-control (n=162 cases of childhood leukemia [only 132 included in the analysis], n=9,805 controls)	Residential addresses as listed on the birth certificate were mapped using GIS and linked to the data from the PUR and land-use surveys from California's	Leukemia cases (diagnosed between 1986 and 2012) were identified from the California Cancer	Acute lymphoblastic leukemia (ALL): Evidence of a positive association between malathion and ALL in the single pesticide model, adjusted OR=1.54 (95% CI 1.06, 2.22).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
		Both cases and controls were born between 1998 and 2011 and living in rural areas in California.		Public Land Survey System (PLSS)	Registry: ALL (International Classification of Childhood Cancer, Third Edition [ICCC] code 011] and AML (ICCC code 012)	No evidence of a significant positive association between malathion and ALL in the multipesticide model (HLM), adjusted OR=1.16 (95% CI 0.73, 1.85) Acute myeloid leukemia (AML): No evidence of a positive association between malathion and AML in the single pesticide model, adjusted OR=0.99 (95% CI 0.46, 2.12) No evidence of a positive association between malathion and AML in the multipesticide model (HLM), adjusted OR=0.79 (95% 0.42, 1.48)	
Esophageal Cancer							
Lee at al. (2004)	1988 - 1990	Nebraska Health Study II, adults living in eastern Nebraska	Case-control study 251 cases, 498 controls	Telephone interview Ever use prior to 1985	Nebraska Cancer Registry and medical records from participating hospitals	No evidence of a positive association was reported for malathion ever use and esophageal cancer among farmers in Nebraska, among a small number of cases (OR = 0.70; 95% CI: 0.40, 1.50; with n = 12 exposed cases).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
Gastric Cancer							
Lee at al. (2004)	1988 - 1990	Nebraska Health Study II, adults living in eastern Nebraska	Case-control study 251 cases, 498 controls	Telephone interview Ever use prior to 1985	Nebraska Cancer Registry and medical records from participating hospitals	No evidence of a positive association was reported between malathion ever use and gastric cancer (OR = 0.80; 95% CI: 0.40, 1.60; with n=14 exposed cases).	Low
Mills and Yang (2007)	1988-2003	United Farm Workers of America (UFW)	Nested case-control n=139,000 for the whole cohort; n=100 gastric cancer cases (78 males, 22 females) and n=210 controls Controls were matched on age, sex, and Hispanic ethnicity.	Records to verify occupational history, grower's contracts to establish the crop/commodity the member was exposed to, and the pesticide use records (PUR) from the California Department of Pesticide Regulation to determine maneb use	State cancer registry files	No evidence of a significant positive association was reported between malathion ever use and gastric cancer (OR = 1.43; 95% CI: 0.84, 2.44; n = 69 exposed cases) based on ever/never use. Malathion exposure was further stratified into quartiles, based on county-level pounds of use, and the following quartiles were reported for malathion: 0 lbs, 1-11 lbs, 12-42 lbs, and 43-8,164 lbs. Evidence of a moderately strong association was observed in the highest exposure quartile in the multivariable-adjusted analysis with the low exposure quartile (1-11 lbs) as the referent (OR: 2.61; 95% CI:1.18; 5.76 with n = 30 exposed cases). No evidence of a significant positive association was reported	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						in the 12-42 lbs exposure quartile with the low exposure quartile as the referent (OR: 1.96; 95% CI: 0.88, 4.38), and no evidence of a significant positive association was reported in any exposure quality with the no exposure quartile as the referent (0.72 < OR < 1.49, all 95% CIs encompassed the null value of 1.00; with n = 14-30 cases per exposure category). Additionally, no evidence of a significant positive association was reported between malathion and gastric cancer (in any quartile) in the age-adjusted analysis (0.50 < OR < 1.28, all 95% CIs encompassed the null value of 1.00; with n = 14-30 cases per exposure category).	
Kidney Cancer							
Bonner et al. (2007)	Enrollment (1993-1997) through December 31, 2002	AHS	Prospective Cohort n=57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	In the kidney cancer analysis for IWLD with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile of exposure (T1 – T3: 0.78 ≤ RRs ≤ 1.53; all CIs encompassed the	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						null value of 1.0, with n = 4 – 10 exposed cases, with 8 cases in the non-exposed group)[131]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.68).	
Andreotti et al. (2020)	1993-1997 (Enrollment) to 2014/2015	AHS pesticide applicators	Prospective Cohort n= 55,873 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	No evidence of a significant positive association was reported for any exposure category (0.90 < rate ratio < 1.09; all 95%s encompassed the null value of 1.0; with n=33-34 exposed cases per category; p-trend =0.56)	High
Lung Cancer							
Pesatori et al. (1994)	1965 - 1982	Pesticide applicators	Nested case-control study	Telephone interview, questionnaire	Death certificate records	No evidence of a significant positive association was reported between malathion ever exposure and lung cancer among pest control operators when compared to dead controls (OR = 1.6; 95% CI: 0.5, 4.6; with n = 11 cases, n = 13 deceased controls) and no evidence of a positive association was	Low

[131] Risk estimates for intensity-weighted lifetime days of exposure for kidney reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure ($0.92 \leq RRs \leq 2.00$; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						observed when compared to living controls (OR = 1.0; 95% CI: 0.4, 2.6; with n = 11 cases, n = 29 living controls).	
Bonner et al. (2007)	Enrollment (1993-1997) through December 31, 2002	AHS	Prospective Cohort n=57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	In the lung cancer analysis for IWLD of exposure with the non-exposed group as the referent, no evidence of a positive association was observed in any tertile (T1 – T3: 0.78 ≤ RRs ≤ 1.00; all CIs encompassed the null value of 1.0, with n = 14 – 21 exposed cases, with 31 cases in the non-exposed group)[132]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.42 for IWLD).	High
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and lung cancer risk (RR = 1.00; 95% CI: 0.60, 1.65; with n = 30 exposed cases). Additional analyses	Moderate

[132] Risk estimates for intensity-weighted lifetime days of exposure for lung cancer reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.75 ≤ RRs ≤ 1.46; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a significant positive association was reported for malathion exposure and lung cancer (RR = 1.11; 95% CI: 0.63, 1.93; with n = 26 exposed cases).	
Bonner et al. (2017)	1993-1997 (Enrollment) to 2010 (North Carolina) & 2011 (Iowa)	AHS	Prospective Cohort n = 57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Malathion Use	Cancer registries in Iowa and North Carolina	Evidence of a moderately strong association was observed for lung cancer in the lowest tertile of exposure only (HR: 2.18; 95% CI: 1.03, 4.59; with n =7 exposed cases, 423 unexposed cases), relative to the unexposed group. No evidence of a positive association was observed in the mid- and highest exposure tertile ($0.63 \leq HR \leq 0.89$; all CIs encompassed the null value of 1.0; with n = 10 - 15 exposed cases, p-trend = 0.855), relative to the unexposed group. For intensity-weighted lifetime days, no evidence of a significant	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						positive association was observed in the low, mid, or highest exposure tertile ($0.56 \leq HR \leq 1.39$; all CIs encompassed the null value of 1.0; with n = 10 - 11 exposed cases, n = 423 unexposed cases, p-trend = 0.599), relative to the unexposed group, and no evidence of a statistically significant p-trend was observed	
Lymphohematopoietic cancers							
Hodgkin Lymphoma							
Karunanayake et al. 2012	September 1991 to December 1994	Cross-Canada Study of Pesticides and Health Study (CCSPH)	Population based Case-Control Study (Cases=316 cases, Controls=1,506)	Postal questionnaire, follow-up telephone interview	Provincial cancer registries, hospital medical records	No evidence of a significant positive association was reported between malathion exposure and HL, when stratified by age group and province of residence (OR=1.07; 95% CI: 0.65, 1.74; with n=27 exposed cases and n=127 exposed controls). In an additional analysis that further adjusted for medical variables that were statistically significant in bivariable analysis ($p < 0.20$) including history of measles, acne, hay-fever, shingles, and a positive family history (1st degree relative) of	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						cancer, no evidence of a positive association was reported (OR=0.97; 95% CI: 0.58, 1.63).	
Latifovic et al. 2020	1976 – 1994 (enrollment)	North American Pooled Project (NAPP) Male farmers living in Nebraska, Kansas, and Canada	Pooled analysis of three case-control studies (Cases of HL=507, Controls=3,886	Postal questionnaire, follow-up telephone interview	State and Provincial cancer registries, hospital medical records	No evidence of a positive association was reported between indirect exposure to malathion and ESRD among these female spouse of pesticide applicators (HR= 0.71; 95% CI: 0.41, 1.22; with n=36 exposed cases). In an additional analysis that considered the association between husbands' cumulative lifetime use of malathion and ESRD among wives who reported no direct pesticide exposure, husband's malathion lifetime exposure was divided at the median at the following cut points: 1.0 – 13.5 days of use and >13.5-217.0 days of use. No evidence of a significant positive association was reported for female spouses' indirect malathion exposure at the lower exposure level and no evidence of an exposure-response trend (1.0 – 13.5 days of use – HR=1.18; 95% CI: 0.47, 2.93; with n=8 exposed	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						cases). No evidence of a positive association was reported for female spouses' indirect malathion exposure at the higher exposure level (>13.5-217.0 days of use – HR=0.87; 95% CI: 0.35, 2.16; with n=8 exposed cases; p-trend >0.05), with the non-exposed group as the referent.	
Leukemia							
Brown et al. (1990)	1980 up to 1987	Males (both farmers and nonfarmers) living in Iowa and Minnesota	Population-based case-control interview study	Self-reported Malathion use collected during in-person interviews	Tumor registry database or a special surveillance network including hospital and pathology records in Iowa and Minnesota	Risk of leukemia for mixing, handling, or applying malathion: No evidence of a positive association between malathion and leukemia, OR=0.9 (95% 0.4, 1.9) No evidence of a significant positive association between ever/never use of malathion and leukemia, OR=1.2 (95% CI 0.8, 2.0) No evidence of a significant positive association between malathion use at least 20 years before the study and leukemia, OR=1.5 (0.8, 2.9) Risk of leukemia based on number of days per year malathion used on crops:	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						No evidence of a significant positive association between malathion use 1-4 days/year and leukemia, OR=1.2 (95% 0.3, 3.9) No evidence of a positive association between malathion use 5-9 days/year and leukemia, OR=0.8 (95% 0.2, 4.4) No cases for >10 days/year malathion use Risk of leukemia based on number of days per year malathion used on animals: No evidence of a positive association between malathion use 1-4 days/year and leukemia, OR=0.5 (95% 0.1, 1.3) No cases for malathion use 5-9 days/year Evidence of a moderately strong association between malathion > 10 days/year and leukemia, OR=3.2 (95% 1.0, 10.0)	
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and leukemia risk (RR = 0.89; 95% CI: 0.58, 1.37; with n = 38 exposed cases).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and leukemia (RR = 0.95; 95% CI: 0.58, 1.57; with n = 29 exposed cases).	
Mills et al. (2005)	1973 - 2001	United Farm Workers of America (UFW) California	Nested case-control	Three separate records/databases: UFW records to verify occupational history, grower's contracts to establish the crop/commodity the member was exposed to, and the California Department of Pesticide Regulation to determine specific pesticide usage	Cancer registries in California	No evidence of a significant positive association between high malathion use and total leukemia (OR = 1.83; 95% CI: 0.91, 3.67; n = 51 total cases). When further stratified by type of leukemia, although elevated, the investigators reported no evidence of a significant positive association between high exposure to malathion and lymphocytic leukemia in the total study population (OR=2.88; 95% CI 0.94, 8.80; n = 23 cases) and no evidence of a significant positive association between high exposure to malathion and granulocytic leukemia	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						in the total study population (OR=1.79; 95% CI: 0.63, 5.08; n = 20 cases). The analysis of total leukemia was also stratified by gender and it was reported that there was evidence of a strong association between high exposure to malathion and total leukemia among females (OR=4.91; 95% CI: 1.21-19.89; n = 16 female cases), and no evidence of a significant positive association between high exposure to malathion and total leukemia among males (OR=1.19; 95% CI: 0.51, 2.76; n = 35 male cases).	
Lymphatic-hematopoietic cancers (all)							
Alavanja et al. 2014	1993-1997 (Enrollment) to 2001	AHS	n=57,284 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries and National Death Index in Iowa and North Carolina	No evidence of a positive association was reported between ever malathion exposure and overall risk of NHL (RR = 0.90; 95% CI: 0.80, 1.10; with n = 332 exposed cases).	High
Bonner et al. (2007)	Enrollment (1993-1997) through	AHS	Prospective Cohort n=57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and	In the lymphatic-hematopoietic cancers (all) analysis for IWLD with the non-exposed	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
	December 31, 2002				North Carolina	group as the referent, no evidence of a significant positive association was observed in any tertile (T1 – T3: 0.81 ≤ RRs ≤ 1.25; all CIs encompassed the null value of 1.0, with n = 16 – 24 exposed cases, with 34 cases in the non-exposed group)[133]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.25).	
Non-Hodgkin's Lymphoma							
Cantor et al. (1992)	1980 up to 1984	Males (both farmers and nonfarmers) living in Iowa and Minnesota	2 population-based case-control interview studies	Self-reported Malathion use collected during in-person interviews	Pathology reviews were conducted to confirm NHL and subtypes	For malathion, when the data was stratified by pesticide applications (to crops or to animals), no evidence of a significant positive association was reported between malathion exposure and NHL among farmers based on ever/never use from animal applications or from crop applications (animal applications OR: 1.30; 95% CI: 0.90,	Moderate

[133] Risk estimates for intensity-weighted lifetime days of exposure for lymphatic-hematopoietic cancers (all) reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.87 ≤ RRs ≤ 1.49; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						2.10 with n = 43 cases, 67 controls; crop applications OR: 1.50; 95% CI: 0.80, 2.70 with n = 21 cases, 30 controls).	
De Roos et al. (2003)	1979 – 1983	Four Midwestern states within the United States- Iowa, Nebraska, Kansas, and Minnesota	Pooled analysis of three case-control studies N = 870 cases, 2,569 controls	Self-reported Malathion use through questionnaires administered by interviewers to study participants or proxy respondents using a series of exposure-related questions asked in various ways (e.g., directly vs. open-ended questions)	Nebraska Lymphoma Study Group and local hospitals (Nebraska); state cancer registry (Kansas & Iowa); surveillance program in hospitals and pathology laboratories (Minnesota)	No evidence of a significant positive association was reported between malathion exposure and NHL for both the logistic and hierarchical regressions (OR = 1.10; 95% CI: 0.60, 1.80; OR = 1.10; 95% CI: 0.70, 1.70), respectively.	Moderate
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never Malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and total cancer risk (RR = 0.64; 95% CI: 0.41, 0.99; with n = 34 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						and NHL (RR = 0.60; 95% CI: 0.37, 0.98; with n = 27 exposed cases).	
Leon et al. (2019)	1969, 1974, 1979, 1985, & 1989 (census data from Norwegian CNAP study) 1993 – 1997 (US AHS) through 2010 (North Carolina) & 2011 (Iowa) 2005-2007 through 2009 (French AGRICAN study)	AHS, Norwegian CNAP study, & French AGRICAN study	Prospective Cohort N = 316,270 agricultural workers included in the combined study population	Self-reported Ever/Never Malathion Use	Cancer and mortality registries and the U.S National Death Index (AHS and CNAP only) & self-report	The authors reported no evidence of a positive association for malathion ever exposure and overall NHL (i.e., all subtypes considered together) (OR = 0.98; 95% CI: 0.82, 1.16, with n = 1,208 exposed cases), and no evidence of a significant positive association for malathion and any of the NHL subtypes in the meta-analysis (0.84 <HR <1.18; all 95% CIs encompassed the null value of 1.0; with n= 114 – 1208 exposed cases per category, p-trend >0.05).	Low
Koutros et al. (2019)	1980s (US) 1991 – 1994 (Canada)	Men in the US and Canada	Pooled analysis using four population-based case-control studies N = 1,690 cases, 5,131 controls	Interview-led questionnaires in-person or via the telephone or mail	Pathology reviews were conducted in each study, and NHL and subtypes were coded via ICD – O – 1 using original histology codes	Evidence of a positive association between malathion and NHL, based on ever/never use (OR: 1.63; 95% CI: 1.33, 2.0). When the data was mutually adjusted further, evidence of a positive association was reported between malathion and NHL among the study participants (OR: 1.43; 95% CI: 1.14, 1.81).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
McDuffie et al. (2001)	September 1991 to December 1994	Cross-Canada Study of Pesticides and Health Study (CCSPH)	Population based Case-Control Study (Cases=517 cases, Controls=1,506)	Postal questionnaire, follow-up telephone interview	Provincial cancer registries, hospital medical records	Evidence of a positive association was reported for any malathion exposure when the model was adjusted for age and province of residence (OR = 1.77; 95% CI: 1.28, 2.46; with n = 72 exposed cases and n = 127 exposed controls), and when the model was further adjusted for additional medical variables (OR = 1.83; 95% CI: 1.31, 2.55; with n = 72 exposed cases and n = 127 exposed controls). 1.25, 2.68; with n = 50 exposed cases and n = 88 exposed controls; ≤ 2 days per year OR = 1.75; 95% CI: 1.02, 3.03; with n = 22 exposed cases and n = 39 exposed controls), with the no exposure group as the referent.	Moderate
Waddell et al. (2001)	1981-1986 (enrollment)	National Cancer Institute - Kansas, Iowa, Minnesota, Nebraska;	Pooled analysis using three population-based case-control studies	Interview-led questionnaires in-person or via the telephone or mail	Pathology reviews and classification by Working Formula (National Cancer Institute)	Evidence of a positive association was reported between malathion ever use and NHL among farmers relative to non-farmers (OR = 1.60; 95% CI 1.20, 2.20; with n = 91 exposed cases and n = 147 exposed controls). No evidence of a significant positive association was reported	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						between malathion use and NHL among direct respondent farmers (proxy respondents excluded), relative to non-farmers (OR=1.20; 95% CI: 0.90, 1.80; with n = 68 exposed cases, 121 exposed controls). Additional analyses were conducted for malathion exposure and NHL among direct respondent farmers (proxy respondents excluded) relative to non-farmers when stratified by several categories: 1) state of residence – Iowa, Kansas, Minnesota, Nebraska; 2) Age at first use of malathion (<20 years ago, >20 years ago); 3) Years of malathion use - <10 years, 10-19 years, >20 years; 4) days per year of malathion use - < 5 days, >5days; 5) protective gear – used, not used. For the analysis by age at first use of malathion, evidence of a positive association was reported between malathion use and NHL among direct respondents farmers who were ≥ 20 years of	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						age, relative to non-farmers (≥ 20 years of age– OR = 1.70; 95% CI: 1.10, 2.90; with n=35 exposed cased). No evidence of a positive association was reported between malathion use and NHL among direct respondents < 20 years of age, relative to non-farmers (OR: 0.90; 95% CI: 0.50, 1.60 with n = 22 exposed cases). No evidence of a significant positive association was reported for state of residence, years of malathion use, days per year of malathion use, or use of protective gear (1.00< ORs < 2.70; 95% CIs encompassed the null value 1.0; with n = 3 - 43 exposed cases per category).	
Hohenadel et al. (2011)	September 1991 to December 1994	Cross-Canada Study of Pesticides and Health Study (CCSPH)	Population based Case-Control Study (Cases=517 cases, Controls=1,506)	Postal questionnaire, follow-up telephone interview	Provincial cancer registries, hospital medical records	For individual pesticide effects, in the malathion and DDT exposure group, evidence of a moderately strong association was reported between malathion exposure and NHL (OR = 2.03; 95% CI: 1.41, 2.94 with 52 cases and 95 controls). For individual pesticide effects, in the malathion and carbaryl exposure	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						group evidence of a positive association between malathion exposure and NHL was reported (OR = 1.75; 95% CI: 1.22, 2.52 with 52 cases and 106 controls). For individual pesticide effects in the malathion and glyphosate exposure group evidence of a positive association between malathion exposure and NHL was reported (OR = 1.95; 95% CI: 1.29, 2.93 with 41 cases and 72 controls). For individual pesticide effects in the malathion and mecoprop exposure group, evidence of a positive association between malathion exposure and NHL was reported (OR = 1.76; 95% CI: 1.20, 2.60 with 44 cases and 92 controls). For individual pesticide effects, no evidence of a significant positive association between malathion exposure and NHL was reported among men in the malathion and 2,4-D exposure group (OR = 1.73; 95% CI: 0.81, 3.66 with 11 cases and	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						21 controls) among a small number of cases (n ≥10≤20).	
Melanoma							
Bonner et al. (2007)	Enrollment (1993-1997) through December 31, 2002	AHS	Prospective Cohort n=57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	No evidence of a significant positive association was observed in any tertile (T1 – T3: 0.47 ≤ RRs ≤ 1.44; all CIs encompassed the null value of 1.0, with n = 7 – 15 exposed cases, with 14 cases in the non-exposed group)[134]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in IWLD exposure analysis (p-trend = 0.06). Additionally, no evidence of a significant positive association between melanoma for frequency, intensity, and duration[135] of malathion exposure was observed (0.61 ≤ RRs ≤ 1.27; all CIs encompassed the null value of 1.0, with n = 8 – 22 exposed cases,	High

[134] Risk estimates for intensity-weighted lifetime days of exposure for melanoma reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.31 ≤ RRs ≤ 1.16; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

[135] In a separate analysis, frequency of use was defined as: <5 or ≥5 days of use per year, duration of use was defined as : ≤10 years of use or >10 years of use, and intensity was defined by tertiles; however, the tertiles were not specified in the table by the study authors.

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						with 14 cases in the non-exposed group, p-trends > 0.05).	
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and melanoma risk (RR = 0.90; 95% CI: 0.52, 1.53; with n = 23 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a positive association was reported for malathion exposure and melanoma (RR = 0.82; 95% CI: 0.42, 1.59; with n = 15 exposed cases).	Moderate
Multiple Myeloma							
Brown et al. (1993)	1981 - 1984 (Enrollment)	Males (both farmers and nonfarmers) living in Iowa	Population-based case-control study (578 cases, 1,245 controls)	Self-reported use collected during in-person interviews	Tumor registry database/ hospital and pathology records	No evidence of a significant positive association was reported between malathion ever use through crop insecticides and MM (OR = 1.90; 95% CI: 0.80, 4.60), and no evidence of a positive association was reported for malathion ever use through animal	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						insecticides and MM (OR = 0.80; 95% CI: 0.30, 1.90). Both reported risk estimates were among a very small number of cases (n<10), with nonfarmers as the referent.	
Pahwa et al. (2012)	1991-1994	Men from six Canadian provinces	Population based Case-Control Study (n= 316 cases, 1,506 controls)	Self-administered questionnaire, telephone follow-up interview	Provincial cancer registries, hospital medical records	No evidence of a positive association was observed between exposure to malathion as a chemical class and MM (OR = 0.97; 95% CI: 0.62, 1.53; with n = 32 exposed cases and n = 127 exposed controls). No evidence of a significant positive association was observed between exposure to malathion as a fumigant and MM (OR = 1.16; 95% CI: 0.44, 3.11; with n = 6 exposed cases and n = 23 exposed controls).	Moderate
Presutti et al. (2016)	North American Pooled Project (NAPP) in the 1980s, Cross Canada Study of Pesticides and Health (CCSPH) in the early 1990s	Subset of two NAPP studies (Iowa, Nebraska) and CCSPH	Case-control (n=547 MM cases, n=2700 controls)	Self-reported information on pesticide use, farming activities and demographic characteristics was obtained through standardized interviews with participants. Information on duration of pesticide use	Incident MM cases were identified using state and provincial cancer registry records, with the exception of Nebraska and Quebec, where cases were recruited	Ever/never use of malathion: Model 1: No evidence of a significant positive association between self-reported ever/never use of malathion and MM, OR=1.19 (95% CI 0.84, 1.69) Model 2: No evidence of a significant positive association between self-reported ever/never use of malathion and	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
				(years) was collected in all studies, and it was evaluated for malathion using years of self-reported use. Cumulative exposure was investigated using a composite lifetime days (LD) metric, defined as: LD=years of pesticide use x days/year of pesticide yes. Analyses of cumulative exposure were restricted to the Canadian subset.	from hospitals.	MM when proxy respondents were excluded, OR=1.33 (95% CI 0.91, 1.94) Median years of self-reported exposure: Model 1: No evidence of a significant positive association between MM and >0 and ≤ 6 years of malathion use, OR=1.39 (95% CI 0.86, 2.25); > 6 years of malathion use, OR=1.39 (95% CI 0.86, 2.25), p-trend=0.36 Model 2: No evidence of a significant positive association between MM and >0 and ≤ 6 years of malathion use, OR=1.55 (95% CI 0.93, 2.58); > 6 years of malathion, OR=1.20 (95% CI 0.70, 2.07) when proxy respondents were excluded, p-trend=0.21	
Leon et al. (2019)	1969, 1974, 1979, 1985, & 1989 (census data from Norwegian CNAP study)	AHS, Norwegian CNAP study, & French AGRICAN study	Prospective Cohort N = 316,270 agricultural workers included in the combined study population	Self-reported Ever/Never Malathion Use	Cancer and mortality registries and the U.S National Death Index (AHS and CNAP only) & self-report	No evidence of a positive association was reported for malathion ever exposure and MM among all participants in the analysis (HR = 0.95; 95% CI: 0.67,	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
	1993 – 1997 (US AHS) through 2010 (North Carolina) & 2011 (Iowa) 2005-2007 through 2009 (French AGRICAN study)					1.36, with n = 269 exposed cases).	
Ovarian Cancer							
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS	Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS	When ovarian cancer was stratified by menopausal status at enrollment, no evidence of a significant positive association was reported between malathion exposure and ovarian cancer for pre-menopausal participants (Premenopausal - RR = 2.14; 95% CI: 0.78, 5.93 with n = 8 exposed cases) and no evidence of a positive association was reported between malathion exposure and ovarian cancer for postmenopausal participants (Postmenopausal - RR = 0.57; 95% CI: 0.24, 1.33 with n = 8 exposed cases). In an additional sensitivity analyses that investigated associations between ever/never malathion exposure and ovarian	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						cancer diagnosed five or more years after study enrollment among AHS spouses, no evidence of a significant positive association was reported between malathion ever exposure and ovarian cancer (RR = 1.28; 95% CI: 0.64, 2.56 with n = 15 exposed cases).	
Pancreatic Cancer							
Andreotti et al. (2009)	1993-1997 (Enrollment) – 2004	AHS pesticide applicators and spouses	Case-Control Study (Cases= 93, Controls=82,503)	AHS Survey Instrument – Ever/Never Use and Cumulative Lifetime Days	Cancer registries in Iowa and North Carolina	No evidence of a positive association between malathion exposure and pancreatic cancer among pesticide applicators and spouses (OR=0.90; 95% CI: 0.60, 1.60, with n=23 exposed cases) based on ever/never use. No evidence of a significant positive association among pesticide applicators in either exposure category with the never exposure group as the referent and no evidence of an exposure-response trend (<176 – OR=1.40; 95% CI: 0.80, 2.70, with n=16 exposed cases; >177 – OR=0.50; 95% CI: 0.20, 1.30, with n=5 exposed cases, p-trend=0.32).	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS	Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS	No evidence of a significant positive association was reported between malathion exposure and pancreatic risk (RR = 1.50; 95% CI: 0.69, 3.26; with n = 14 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and no evidence of a significant positive association was reported for malathion exposure and pancreatic cancer (RR = 1.46; 95% CI: 0.62, 3.44; with n = 12 exposed cases).	Moderate
Prostate Cancer							
Barry et al. (2011), Barry et al. (2012)	1993-1997 (Enrollment) to 2004	AHS	Nested case-control n = 776 cases, n = 1,444 controls	AHS Survey Instrument – Ever/Never Malathion Use and Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and prostate cancer among white male pesticide applicators in either the low or high exposure categories, with the non-exposed group as the referent (*Low* – OR = 0.88; 95% CI: 0.69, 1.13; with n = 162 exposed cases and n = 329 exposed controls; *High* – OR = 0.80; 95% CI: 0.62, 1.04; with n =	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						152 cases and n = 328 controls; p-trend = 0.13).	
Bonner et al. (2007)	Enrollment (1993-1997) through December 31, 2002	AHS	Prospective Cohort n=57,310 pesticide applicators	AHS Survey Instrument – Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	In the prostate cancer analysis for IWLD of exposure with the non-exposed group as the referent, no evidence of a significant positive association was observed in any tertile (T1 – T3: 0.98 ≤ RRs ≤ 1.20; all CIs encompassed the null value of 1.0, with n = 88 – 94 exposed cases, with 135 cases in the non-exposed group)[136]. There was no evidence of a statistically significant trend of increasing risk with increased exposure in the IWLD exposure analysis (p-trend = 0.98).	High
Band et al. (2011)	1983-1985 Cases 1983-1990 Controls	British Columbia, Canada	Case-control study (Cases=1,153, Controls=3,999)	Questionnaire, Job Exposure Matrix – Ever/Never Use and Cumulative Lifetime Use	British Columbia Cancer Registry	No evidence of a significant positive association was reported between malathion exposure and prostate cancer in the high exposure category (OR = 1.49; 95% CI: 1.02, 2.18; with n = 46	Moderate

[136] Risk estimates for intensity-weighted lifetime days of exposure for prostate cancer reported in Bonner et al. (2007) are provided here with the non-exposure group as the referent group. Additional non-statistically significant risk estimates reported for IWLD with the low-exposure referent group and for lifetime days of exposure (0.81 ≤ RRs ≤ 1.20; all CIs encompassing the null value of 1.0; p-trends ≥ 0.05) can be found in Tables 2 & 4 in Bonner et al. (2007).

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						exposed cases, $p < 0.01$) with the no exposure as the referent and no evidence of a significant positive association was reported for the low exposure category (OR = 1.18; 95% CI: 0.78, 1.78; with n = 36 exposed cases, $p < 0.05$). A significant exposure-response trend was reported ($p = 0.03$).	
Christensen et al. (2016)	Enrollment (1993 – 1997) and 2004	AHS	Nested case-control n = 776 cases, n = 1,444 controls	AHS Survey Instrument – Ever/Never Malathion Use and Cumulative Lifetime Use	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and prostate cancer among white male pesticide applicators in either the low or high exposure categories, with the non-exposed group as the referent (Low – OR = 0.85; 95% CI: 0.67, 1.09, with n = 173 exposed cases; High – OR = 0.80; 95% CI: 0.62, 1.04, with n = 142 exposed cases; p-trend = 0.149).	High
Koutros et al. (2011)	Enrollment (1993-1997) through 2004	AHS	Study population (n=2,500)	AHS Survey Instrument	Cancer registries in Iowa and North Carolina	No evidence of a positive association was reported between malathion exposure and prostate cancer in either the low or high exposure categories (*Low* – OR=0.85; 95% CI: 0.67, 1.09; with	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						n=173 exposed cases and n=351 exposed controls; *High* – OR=0.80; 95% CI: 0.62, 1.04; with n=142 exposed cases and n=360 exposed controls; p-trend=0.149).	
Koutros et al. (2013a)	1993-1997 (Enrollment) to 2007	AHS	Prospective Cohort n = 54,412 pesticide applicators	AHS Survey Instrument – Cumulative Malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a significant positive association was observed for prostate cancer or aggressive prostate cancer relative to malathion exposure for any of the stratified exposure categories ($1.03 \leq RRs \leq 1.43$; all CIs encompassed the null value of 1.00), and there was no evidence of a linear (monotonic) trend across categories for total prostate cancer (p-trend = 0.62). There was evidence of a linear (monotonic) trend across all categories for aggressive prostate cancer (p-trend= 0.04).	Moderate
Koutros et al. (2013b)	1993-1997 (Enrollment)	AHS Male licensed pesticide applicators	Nested Case Control Study (N=55,747)	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina Genotyping was performed using Applied	For the EHBP1 SNP region for the TT genotype, evidence of a strong association was reported between the high exposure category for malathion and prostate cancer (OR: 3.43; 95% CI: 1.44,	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
					Biosystems TaqManH SNP Genotyping Assays	8.15, with n=28 exposed cases). No evidence of a significant positive association was observed at the low dose exposure category to malathion and prostate cancer (OR: 2.17; 95% CI: 0.91, 5.14 with n = 24 exposed cases). Among the rs2710647 SNP region for the CT+CC genotype, no evidence of a positive association was reported for all exposure categories for malathion and prostate cancer (0.80 ≤OR≤ 0.96; all 95% CI encompass the null value of 1.0, with n=91-99 exposed cases).	
Soft Tissue Sarcoma							
Pahwa et al. (2011)	September 1991 to December 1994	Cross-Canada Study of Pesticides and Health Study (CCSPH)	Population based Case-Control Study (n= 357 cases, 1,506 controls)	Self-administered questionnaire, telephone follow-up interview	Provincial cancer registries, hospital medical records	No evidence of a significant positive association was reported between malathion ever use and STS among men in the CCSPH (OR=1.19; 95% CI: 0.80, 1.78; with n=38 exposed cases), among a small number of cases (n >10-<20). When further adjusted for statistically significant medical history variables, no evidence of a significant positive association was reported	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						between malathion ever use and STS among men in the CCSPH (OR=1.23; 95% CI: 0.81, 1.85; with n=38 exposed cases), among a small number of cases (n >10-<20).	
Thyroid Cancer							
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	Evidence of a moderately strong positive association was reported between malathion exposure and thyroid risk (RR = 2.04; 95% CI: 1.14, 3.63; with n = 22 exposed cases). Additional analyses investigated associations between ever/never malathion exposure and cancer diagnosed five or more years after study enrollment, and evidence of a moderately strong positive association was reported for malathion exposure and thyroid cancer (RR = 2.22; 95% CI 1.18, 4.17; with n = 19 exposed cases).	Moderate
Lerro et al. (2021)	Enrollment questionnaire (1993 – 1997) and the first follow-up interview five years	AHS	prospective cohort study population (n=53,096)	AHS Questionnaire	International Classification of Diseases for Oncology (ICD-O-3) codes were used to	No evidence of a positive association was reported for malathion ever use at enrollment and either thyroid cancer (HR= 1.07; 95% CI: 0.66, 1.74; with n=59 exposed cases) or	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
	after enrollment (1999 – 2005)				classify cancer sites.	the subset papillary thyroid cancer (HR=1.09; 95% CI: 0.63, 1.90; with n = 46 exposed cases). For the analysis with of cumulative exposure, categories of intensity-weighted days of exposure were divided into quartiles for each pesticide with 20+ exposed cases or into two groups divided at the median for pesticides with 10 -19 exposed cases. Specifically, for malathion, four quartiles of exposure were created. No evidence of a significant positive association was reported for any exposure category of intensity-weighted lifetime days of malathion use and thyroid cancer (0.43 < HR < 1.28; all 95% CIs encompassed the null value 1.0; with n = 7-8 exposed cases per category; p-trend=0.12).	
Uterine Cancer							
Lerro et al. (2015)	1993-1997 (Enrollment) to 2010/2011	AHS Wives of pesticide applicators	Prospective Cohort n = 30,003 wives of pesticide applicators	AHS Survey Instrument – Ever/Never malathion Use	Cancer registries in Iowa and North Carolina	No evidence of a significant positive association was reported between malathion exposure and uterine cancer risk among AHS	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						spouses (RR = 1.28; 95% CI: 0.90, 1.83). When uterine cancer was stratified by menopausal status at enrollment, no evidence of a significant positive association was reported between malathion exposure and uterine cancer for pre-menopausal participants (Premenopausal - RR = 1.47; 95% CI: 0.86, 2.49 with n = 27 exposed cases) and no evidence of a positive association was reported between malathion exposure and uterine cancer for postmenopausal participants (Postmenopausal - RR = 0.98; 95% CI: 0.58, 1.67 with n = 26 exposed cases). In an additional sensitivity analyses that investigated associations between ever/never malathion exposure and uterine cancer diagnosed five or more years after study enrollment among AHS spouses, no evidence of a significant positive association was reported between malathion ever exposure and uterine	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results[129]	Study Quality
						cancer (RR = 1.09; 95% CI: 0.71, 1.67 with n = 37 exposed cases).	

7.2 Table B-2: Summary of Epidemiologic Studies on Other Health Outcomes

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Amyotrophic Lateral Sclerosis (ALS)							
Kamel et al. (2012)	Enrollment 1993 - 1997; mortality data was collected through February 2010	Agricultural Health Study (AHS) - private pesticide applicators and spouses	Cohort (n=84,739)	Questionnaires collected information on lifetime pesticide use as well as demographics, lifestyle, and medical history at enrollment and some applicators filled out supplemental questionnaires. Cohort members provided information on ever use and on years and days per year of use of any pesticide.	Death certificate	No evidence of a positive association between ever use of malathion and ALS, OR=0.6 (95% CI 0.3, 1.3)	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Autism Spectrum Disorder							
Sagiv et al. (2018)	Enrolled between October 1999 and October 2000	Children enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort	Prospective cohort	Self-reported information on pesticide use	Several instruments used to assess parent and teacher reports of ASD related behaviors. Social Responsiveness Scale, Version 2 (SRS-2) at age 14 y (parent), Behavioral Assessment Scale for Children, Version 2 (BASC-2) at 7, 10½, and 14 y, (parent, teacher at 7y). Study staff tested children's ability to recognize mental state of others through facial expressions using the Evaluación Neuropsicológica Infantil (ENI) Facial Expression Recognition Test at 9y, and the NEPSY-II Affect Recognition subtest at age 12 y.	No evidence of a significant association was reported between a 10-fold increase in prenatal malathion exposure use within 1-km of residence during pregnancy and the 14years of age SRS-2 Total T-score (β = 0.50; 95% CI: –0.70, 1.80; with n = 235), the SRS-2 DSM-V compatible Social Communication and Interaction (SCI) T-score (β = 0.40; 95% CI: -0.90, 1.60; with n = 235), and the SRS-2 DSM-V compatible Restricted and Repetitive Behaviors (RRB) T-score (β = 0.90; 95% CI: –0.30, 2.10; with n = 235).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
von Ehrenstein et al. (2019)	Children born between 1998 and 2010 and diagnosed with autism spectrum disorder until December 31, 2013	Children born in California between 1998 and 2010. Cases were identified from California Department of Developmental Services (DDS); controls were selected from birth records.	Matched case-control (n=2,961 ASD cases and n=35,370 controls matched on birth year and sex). Out of the 2,961 ASD cases, 445 had ASD with intellectual disability comorbidity.	Data from California state mandated Pesticide Use Reporting (PUR) were integrated into a geographic information system (GIS) tool to estimate prenatal and infant exposures to diazinon (measured as pounds of pesticides applied per acre/month within 2000 m of the maternal residence)	Children with a primary diagnosis of autistic disorder (code 299.00) were identified from the California Department of Developmental Services (DDS) client development evaluation report, which implements criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, revised (DSM-IV-R)	Pesticide exposure defined as ever versus never malathion exposure in specific developmental period (Model 1): Evidence of a slight positive association between ever/never exposure to malathion during pregnancy and ASD, adjusted OR=1.11 (95% CI 1.01, 1.22) Evidence of a significant positive association between ever/never exposure to malathion during the first year of life and ASD, adjusted OR=1.11 (95% CI 1.02, 1.21)	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Autoimmune Disease							
Parks et al. (2019)	1993-1997 (enrollment) 1999 – 2003 2005–2010 (5-Year and 10-Year Follow-Up)	Biomarkers of Exposure and Effect in Agriculture (BEEA) study within the AHS	Prospective Cohort N = 699 male private pesticide applicators	Self-reported cumulative use of Malathion	Blood samples collected and measured for antibodies via laboratory testing Presence of the following antibodies in blood serum: Anti-nuclear antibodies (ANA), extractable nuclear antibodies (ENA) and anti-dsDNA antibodies	No evidence of a positive association was reported for lifetime use of malathion and any of the three ANA categories (Any ANA – OR = 0.62; 95% CI: 0.42, 0.91; with n = 99 exposed cases; Moderate-higher ANA – OR = 0.87; 95% CI: 0.53, 1.45; with n = 66 exposed cases; Higher ANA – OR = 0.67; 95% CI: 0.34, 1.30; with n=46 exposed cases), with the no detectable ANA group as the referent.	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Birth Effects							
Eskenazi et al. (2004)	Enrollment October 1999 - October 2000; pregnancy outcome measured 2000-2001	CHAMACOS longitudinal birth cohort, Salinas Valley, CA	Cohort (n=1,130 eligible women; n=488 included in the study and n=382 have MDA levels available)	Malathion dicarboxylic acid (MDA) was measured from spot urine samples from the pregnant women at the time of the two pregnancy interviews (mean 13 weeks gestation and mean 26 weeks gestation). For analysis, the two pregnancy measurements of each urinary MDA were averaged for each woman. A large proportion of women had non-detectable levels of MDA. MDA was categorized into three groups < LOD for both pregnancy measurements, and for those with at least one detectable level, subdivided below and above the median of the average pregnancy level.	Infant birth weight, crown-heel length, and head circumference were obtained from hospital delivery logs and medical records. Infant ponderal index, a measure of proportionality of growth, was calculated as (birth weight in grams × 100)/(length in centimeters). Gestational age was obtained from medical records and was based on ultrasound procedures for 25% of women.	No evidence of a positive association between maternal MDA levels and length of gestation (weeks): detectable levels of MDA < median: adjusted β= -0.13 (95% CI -0.55, 0.30), p-value=0.55 detectable levels of MDA ≥ median: adjusted β= -0.21 (95% CI -0.62, 0.20), p-value=0.32 No evidence of a (significant) positive association between maternal MDA levels and birth weight(g): detectable levels of MDA < median: adjusted β= -45 (95% CI -154, 63), p-value=0.41 detectable levels of MDA ≥ median: adjusted β= 56 (95% CI -49, 161), p-value= 0.29	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Wolff et al. (2007)	March 1998 - March 2002	Children's Environmental Health Study, New York	Cohort (n=404 mother-infant pairs)	Maternal urine and blood samples were taken in the third trimester (generally between 26 and 28 weeks)	Information on delivery characteristics and birth outcomes, including birth weight, length, head circumference, gestational age, and infant gender were obtained from a computerized perinatal database within the Department of Obstetrics, Gynecology, and Reproductive Science at Mount Sinai Hospital. Measurements of weight, length, and head circumference at birth were based on standardized clinical techniques.	**No creatinine adjustment (β± SE, p-value)** No association between maternal MDA levels and birth weight: β = 39 ± 52, p-value=0.46 (Model 1) No association between maternal MDA levels and birth length: β = -0.16 ± 0.28, p-value=0.56 (Model 1) No association between maternal MDA levels and ponderal index: β = 0.039 ± 0.035, p-value=0.27 (Model 1) No association between maternal MDA levels and head circumference: β = 0.15 ± 0.19, p-value=0.44 (Model 1) No association between maternal MDA levels and gestational age: β = -0.28 ± 0.21, p-value=0.18 (Model 2)	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Ling et al. (2018)	Between 1998 and 2010	Children born in California	24,693 cases of preterm births and 220,297 non-cases of preterm births	California's Pesticide Use Reports (PUR), land use maps, and geocoded birth addresses	California birth defect registry	No evidence of a significant positive association was reported between malathion exposure in the first trimester of pregnancy to pre-term birth in the analysis adjusted for infant's year of birth and sex only (OR:1.01; 95%CI: 0.98, 1.04, n=5,696 exposed cases) and no evidence of a positive association in the analysis adjusted for year of birth, infant sex, maternal age, maternal education, maternal race/ethnicity, parity, prenatal care in first trimester, payment type of prenatal care, maternal birthplace, and neighborhood SES (OR:0.99; 95%CI: 0.96, 1.03, n=5,696 exposed cases).	Moderate
Birth Defects							

Grether et al. (1987)	1981 - 1982	Live birth from mothers residing in treated zip codes of Alameda, San Mate, and Santa Clara counties	Cohort (n=22,456 exposed live births in 1982, n=17,050 unexposed live births in 1982 and n=37,854 unexposed live births in 1981)	The exposed group included mothers residing in malathion-treated zip codes from July 1981 through August 1982. Data regarding aerial treatment of malathion were provided by the California Department of Food and Agriculture and were used to determine monthly exposure "scores" for each treated zip code.	Birthweight information was extracted from birth certificate data files. Congenital anomalies were obtained from all 1981 and 1982 newborn hospital discharges.	**Compared to the 1981 unexposed group:** No evidence of an association between malathion and low birthweight, no estimates are provided. Evidence of a moderately strong association between malathion and ear anomalies: RR=4.49 (95% CI 1.19, 16.92) - based on only 10 diagnosed infants Evidence of a moderately strong association between malathion and bowed legs: RR=2.99 (95% CI 1.32, 6.75) - based on only 22 diagnosed infants Evidence of a positive association between malathion and varus deformities: RR=1.72 (95% 1.16, 2.55) No evidence of a significant positive association between malathion and varus deformities with metatarsus varus: RR= 1.03 (95% 0.58, 1.82) Evidence of a positive association between malathion and clubfoot grouping: RR=1.47 (95% 1.09, 1.96) No evidence of a significant positive association between malathion and clubfoot grouping with	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						metatarsus varus: RR=1.12 (95% 0.79, 1.61) **Compared to the 1982 unexposed group:** No evidence of a significant positive association between malathion and tracheoesophageal fistula: RR=2.66 (95% CI 0.55, 12.78) - based on only 9 diagnosed infants	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Thomas et al. (1992)	September 1, 1981 - June 30, 1982	Pregnant women from three Kaiser-Permanente facilities in the San Francisco Bay Area	Nested case-control and case-cohort (n=7,450 eligible pregnancies - full cohort; n=1,128 in study sample, n=1000 in analysis) Cases by outcome: n=474 spontaneous abortions n=26 stillbirths n=144 reportable anomalies n=78 intrauterine growth retardation	Residential information was ascertained via questionnaire. Residential data were converted to geographical coordinates and using UNIMATCH record linkage program, were linked with malathion spraying data from the U.S. Census Bureau DIME (Dual Independent Map Encoding).	Outcome for each pregnancy was extracted from Kaiser-Permanente records if the pregnancy terminated there or by search of state files of live births and fetal deaths.	**Spontaneous abortions:** No evidence of a significant positive association between direct exposure to malathion at the time of the outcome of the case and spontaneous abortion, adjusted HR=1.20 (95% CI 0.94, 1.52). No evidence of a positive association between direct exposure to malathion one month before the time of the outcome of the case and spontaneous abortion, adjusted HR=0.91 (95% CI 0.75, 1.12) No evidence of a significant positive association between distance weighted exposure to malathion at the time of the outcome of the case and spontaneous abortion, adjusted HR=1.12 (95% CI 0.82, 1.52)	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Haraux et al. (2018)	March 2011 - March 2014	Newborns from the Picardy region of northern France	Matched case-control (n=25 cases, n=58 controls)	Exposure to pesticides was evaluated directly by assaying the meconium for pesticides. The six pesticides most commonly used in the Picardy region (and concentrations of their metabolites, for some compounds) were analyzed using an ultra-high-pressure liquid chromatography-tandem mass spectrometry method. Exposure information was classified into three categories ("low" when < LOD, "moderate" when between the LOD and the median, and "high" when > median).	All newborns underwent a comprehensive clinical examination. Newborns with hypospadias and normal hormonal profile but no other genital malformations, congenital syndromes or known family history of hypospadias were included as cases.	No evidence of a significant positive association between low level of malathion and hypospadias, adjusted OR=3.08 (95% CI 0.64, 14.89), p-value=0.16 No evidence of a significant positive association between high level of malathion and hypospadias, adjusted OR=1.64 (95% CI 0.37, 7.20), p-value=0.51	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Birthweight							
Sathyanarayana et al. (2010)	1993-1997 (enrollment)	AHS	Cross-Sectional n = 2,246 female spouses who had a singleton birth within 5 years of AHS enrollment	AHS Survey Instrument – Ever/Never Malathion Use	Study subjects reported the weight in pounds and ounces for each most recent birth	No evidence of a significant association was determined in birthweight at each of the four categories of exposure (-72 grams ≤ β ≤ 9 grams; all CIs encompassed the null value of 0; n = 42 – 764 women). No evidence of a significant reduction was reported between mother's ever use of malathion and offspring's birth weight (β = -59 grams; 95% CI: -118, 0.50 grams; with n = 307 exposed participants).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Cerebral Palsy							
Liew et al. (2020)	1998 - 2010	Children born in California to mothers who lived in proximity (within 2 km) to any agricultural pesticide application recorded in the California Pesticide Use Reporting (PUR) system	Case-control (n=3,905 CP cases, n=39,377 controls)	The study geocoded maternal residential addresses listed on the birth certificate using an automated approach, and estimated residential ambient pesticide exposure using a geographic information system (GIS)-based Residential Ambient Pesticide Estimation System. The model integrates California's PUR, land use maps, and geocoded birth addresses to generate estimates of pesticide exposure during each month of pregnancy.	CP cases were identified through diagnostic records maintained at the California Department of Developmental Services (DDS) and linked these to California birth certificate records with a linkage success rate of 86.3%.	**Female offspring:** No evidence of a significant positive association between malathion and CP, Model 1: OR=1.10 (95% CI 0.97, 1.24); Model 2: OR=1.13 (95% CI 0.99, 1.30) **Male offspring:** No evidence of a positive association between malathion and CP, Model 1: OR=0.95 (0.86, 1.06) No evidence of a significant positive association between malathion and CP, Model 2: OR=1.04 (95% CI 0.92, 1.17)	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Depression							
Beard et al. (2013)	1993-1997 (enrollment) to 2005-2010 (follow-up)	AHS	Prospective Cohort N = 16,893 female spouses	AHS Survey Instrument – Ever/Never Malathion Use Self-reported	Self-reported incident depression between the time of study enrollment (1993-1997) to study follow-up (2005-2010)	No evidence of a positive association was reported for wives' malathion ever use and self-reported incident depression (RR = 0.96; 95% CI: 0.82, 1.12; with n = 203 exposed cases,) and no evidence of a positive association was reported for husband's ever use of malathion and self-reported incident depression among wives' who never used malathion (RR = 1.00; 95% CI: 0.81, 1.23; with 286 (71%) cases with indirect exposure).	Moderate
Beard et al. (2014)	1993-1997 (enrollment) to 2005-2010 (follow-up)	AHS	Prospective Cohort N = 21,208 male applicators	AHS Survey Instrument – Ever/Never Malathion Use Self-reported	Self-reported incident depression between the time of study enrollment (1993-1997) to study follow-up (2005-2010)	No evidence of a significant positive association was reported between malathion exposure and risk of depression for those who reported depression at enrollment only (OR = 1.30; 95% CI: 1.00, 1.70), risk of depression for those who reported depression at both enrollment and follow-up (OR = 1.20; 95% CI: 1.00, 1.60), or for those who reported depression at follow-up only (OR = 1.10; 95% CI: 1.00, 1.40).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Diabetes							
Montgomery et al. (2008)	Enrollment (1993-1997) and follow-up (1999-2003)	AHS	Prospective Cohort N=33,457	AHS Survey Instrument	Self-Reported diabetes	No evidence of a significant positive association was reported between malathion and diabetes (OR = 1.05; 95% CI: 0.91, 1.21; with n = 766 exposed cases and n = 20,397 exposed non-cases) based on ever use when adjusted for age only. Further adjusting the model for state of residence and BMI in addition to age, no evidence of a significant positive association was reported (OR = 1.10; 95% CI: 0.95, 1.27; with n = 766 exposed cases and n = 20,397 exposed non-cases).	Moderate
Starling et al. (2014)	Enrollment (1993 - 1997), completed at least one of the two follow-up interviews at 5-years or 10-years (2005 – 2010)	AHS-wives of pesticide applicators	Prospective Cohort (N=15,034)	AHS Survey Instrument on Ever/Never Malathion Use	Self-Reported a Physician Diagnosis of diabetes after enrolment, who had complete information on BMI	No evidence of a significant positive association was reported between malathion ever use and incident diabetes in women (HR=1.05; 95% CI: 0.90, 1.23).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Dream Enacting Behavior							
Shrestha et al. (2018a)	1993-1997 (Enrollment) to 2013-2015 (Phase 5 Follow-up)	AHS	Prospective Cohort n = 23,478 subjects completing Phase 5 Questionnaire	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument – , "Have you ever been told, or suspected yourself, that you seem to 'act out dreams' while sleeping?" If they answered yes, they were prompted to answer additional questions on the frequency of symptoms.	No evidence of a positive association was reported between malathion ever use and DEB among male pesticide applicators (OR=1.00; 95% CI: 0.90, 1.20; with n=1,042 exposed cases). Similarly, no evidence of a positive association was reported between malathion exposure and DEB among pesticide applicators who reported three or more episodes of DEB (n=17,321), (OR=1.00; 95% CI: 0.90, 1.20, with n=495 exposed cases). And finally, no evidence of a positive association was reported between malathion ever use and DEB when PD patients were excluded (OR=1.00; 95% CI: 0.90, 1.20, with n=740 exposed cases).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
End State Renal Disease							
Lebov et al. (2015)	1993-2011 (Enrollment) to 2011	AHS	Prospective Cohort n = 31,142 wives of licensed applicators	Husband's Responses to AHS Survey Instrument Ever/Never Malathion Use and Cumulative Lifetime Use	Linkage with the United States Renal Data System and the National Death Index	No evidence of a significant positive association was reported for female spouses' indirect malathion exposure at the lower exposure level and no evidence of an exposure-response trend (*1.0 – 13.5 days of use* – HR=1.18; 95% CI: 0.47, 2.93; with n=8 exposed cases). No evidence of a positive association was reported for female spouses' indirect malathion exposure at the higher exposure level (*>13.5-217.0 days of use* – HR=0.87; 95% CI: 0.35, 2.16; with n=8 exposed cases; p-trend >0.05), with the non-exposed group as the referent.	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Lebov et al. (2016)	1993-1997 (Enrollment) to 2011	AHS	Prospective Cohort n = 55,580 Licensed male applicators	AHS Survey Instrument Ever/Never Malathion Use and Cumulative Lifetime Use	Linkage with the United States Renal Data System and the National Death Index	No evidence of a significant positive association was reported between malathion exposure and ESRD among male pesticide applicators at any exposure category and no evidence of a significant exposure-response trend, with the no exposure group as the referent (0.87 <HR <1.44; all 95%CIs encompassed the null value of 1.0; n=27 - 28 exposed cases and n=3,626– 6,577 exposed non-cases; p-trend= 0.874).	High
Endometriosis							

Li et al. (2020)	2007 - 2009	Endometriosis, Natural history, Diagnosis and Outcomes (ENDO) Study in reproductive-aged women from Utah and California scheduled for laparoscopy or laparotomy and women in the general population	Cross-sectional Operative group (n=188 women with and n=283 without endometriosis); Population group (n=14 women with and n=109 women without endometriosis)	Urinary metabolite of malathion (malathion dicarboxylic acid (MDA))	Endometriosis diagnosed by either laparoscopy/laparotomy or MRI	No evidence of a positive association between urinary concentration of MDA and endometriosis among the operative cohort: Adjusted OR for 2nd quartile=0.94 (95% CI 0.54, 1.63); adjusted OR for 3rd quartile=0.91 (95% CI 0.69, 2.06); adjusted OR for 4th quartile=1.19 (95% CI 0.69, 2.06); p-trend < 0.001 No evidence of a positive association between urinary concentration of MDA and endometriosis among the population cohort: Adjusted OR for 2nd quartile=0.86 (95% CI 0.53, 1.4); adjusted OR for 3rd quartile=1.62 (95% CI 0.29, 9.18); adjusted OR for 4th quartile=1.23 (95% CI 0.76, 1.97); p-trend=0.899 No evidence of a positive association between urinary concentration of MDA and endometriosis in the combined study population (operative and population cohort): Adjusted OR for 2nd quartile=1.18 (95% CI 0.72, 1.93); adjusted OR for 3rd	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						quartile=0.94 (95% CI 0.57, 1.54); adjusted OR for 4th quartile=0.76 (95% CI 0.46, 1.27); p-trend < 0.001)	
Eye disorders							
Kirrane et al. (2005)	1993-1997	AHS	Cross-sectional n = 31,173 wives of pesticide applicators	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument – Self-reported retinal or macular degeneration	No evidence of a positive association was reported between malathion exposure and eye disorders, based on ever use (OR = 1.00; 95% CI: 0.60, 1.40).	Low

Montgomery et al. (2017)	1993-1997	AHS	Nested Case-Control n = 161 cases, 39,108 controls	AHS Survey Instrument – Ever/Never Malathion Use	Cases were ascertained by physicians with supporting pathology or retinal photographs	Evidence of a moderately strong association was reported between malathion and AMD based on ever/never exposure (OR = 2.20 95% CI: 1.50, 3.30). When the data were further stratified by gender, evidence of a positive association was reported between malathion exposure and AMD among men (OR = 2.00; 95% CI: 1.10, 3.70, with n = 76 exposed male cases and n = 15,902 exposed male controls) and evidence of a moderately strong association was reported among females based on ever/never exposure (OR = 2.40; 95% CI: 1.40, 3.90, with n = 27 exposed female cases and n = 3,987 exposed female controls). When incident AMD cases were stratified by early and late AMD and adjusted for age, gender and smoking status, evidence of a moderately strong association was reported for malathion exposure and early AMD when compared to controls and evidence of a positive association for late	High

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						AMD when compared to controls (Early AMD – OR = 2.80; 95% CI: 1.40 5.40, with n = 40 exposed cases and n = 19,889 exposed controls; Late AMD – OR = 2.00; 95% CI: 1.10, 3.60, with n = 45 exposed cases and n = 19,889 exposed controls).	
Fatal Injury							
Waggoner et al. (2013)	1993-1997 (Enrollment) to 2008	AHS	Prospective Cohort n = 51,035 licensed male applicators	AHS Survey Instrument – Ever/Never Malathion Use	Annual linkage with death registries in NC and IA and the National Death Index. Injury deaths defined by ICD codes indicating a fatal injury.	No evidence of a positive association was reported between malathion exposure and fatal injury among male private pesticide applicators in the AHS, based on ever/never use (HR: 0.96; 95% CI: 0.75, 1.24; with n=210 exposed cases).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Gestational Diabetes							
Ledda et al (2015)	2007 - 2013	Women at approximately 22 weeks of gestation recruited by general and occupational physicians in Sicily, Italy	Cross-sectional (n=2203 pregnant women)	Structured questionnaire investigating environmental and occupational risk was administered by trained interviewers to gather accurate data on demographics, health habits, and pesticide and other chemical exposures. Exposure to pesticides in the first trimester of pregnancy was classified into four categories: no exposure (women reporting no exposure); indirect exposure (from planting, pruning, weeding, picking, or harvesting); domestic exposure (from pesticide use in the garden or in the house); occupational exposure (from work with pesticides).	Participants were considered to have GHY if they had systolic blood pressure (BP) ≥ 140 mm Hg and/or diastolic BP ≥ 90 mmHg. Women with hypertension before pregnancy, anemia, toxemia of pregnancy, kidney or heart disease, diabetes, urinary tract infection, metabolic disorders, antiphospholipid antibody syndrome, multiple pregnancies, a BMI < 19 or > 35 kg/m2, and those reporting complications during earlier pregnancies and/or deliveries were excluded.	Evidence of a slight positive association between malathion and gestational hypertension, adjusted OR=1.14 (95% CI 1.08, 1.19), p-value < 0.05	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Hearing Loss							
Crawford et al. (2008)	1993-1997 (Enrollment) to Five-Year Follow-Up	AHS	Prospective Cohort n = 14,229 licensed male applicators	AHS Survey Instrument – Ever/Never Malathion Use as well as frequency and duration of use	Self-reported hearing loss	Evidence of a positive and slight positive association between hearing loss and cumulative lifetime days of malathion exposure was reported in the medium and high dose exposure groups (OR medium exposure = 1.32; 95% CI: 1.18, 1.46 with n = 1,116 cases (24%), 1,825 controls (20%); OR high exposure = 1.20; 95% CI: 1.08, 1.34 with n = 1,128 cases (24%), 1,851 controls (21%)), and no evidence of a significant positive association was observed in the low dose exposure group (OR low exposure = 1.09; 95% CI: 0.98, 1.21 with n = 1,027 cases (22%), 1,992 (22%) controls; 95% CI encompassed the null value of 1.0). Additionally, no evidence of a significant p-trend was reported (p-trend = 0.09).	Moderate
Kidney Function							

Shearer et al. (2021)	The enrollment AHS questionnaire (1993–1997) in addition to two follow-up interviews (1999–2003 and 2005–2010).	Male pesticide applicators in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study, a subcohort in the Agricultural Health Study (AHS)	1,545 BEEA participants	AHS Questionnaire	Serum concentration collection, estimated glomerular filtration rate	No evidence of a positive association was reported between CKD and malathion exposure among pesticide applicators, based on ever/never use (OR: 0.70; 95% CI: 0.50-1.00 with n=155 exposed cases). No evidence of a positive association was reported between malathion exposure and CKD among male pesticide applicators in the total population for any exposure category ($0.40 \leq OR \leq 0.80$; the 95% CIs encompassed the null value 1.0 for the 20-385, >385-1080, and >1080-2940 days of use exposure categories but the >2940-117600 days of use has a 95% CI:0.20-0.70) with n=17-24 exposed cases. No evidence of a positive association was reported for male pesticide applicators in the active farmers population for any exposure category ($0.30 \leq OR \leq 0.80$; the 95% CIs encompassed the null value 1.0 for the 20-385, >385-1080, and >1080-2940 days of use exposure categories but the >2940-117600 days of use has a 95% CI:0.20-	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						0.70; with n=12-25 exposed cases).	
Monoclonal Gammopathy of Undetermined Significance (MGUS)							
Landgren et al. (2009)	Enrollment, Phase II-1999-2003, Phase III-2005-2010)	AHS	Enrollment, Phase II-1999-2003, Phase III-2005-2010	Self-administered questionnaire completed at enrollment (1993 – 1997) and occupational exposures, medical histories, and lifestyle factors at follow-up interviews conducted five years after enrollment	Participant serum samples collected from this subset of AHS participants between 2006 - 2007 for participants living in Iowa and in 2008 for participants living in North Carolina	No evidence of a positive association was reported between ever exposure to malathion and MGUS among (OR = 0.70; 95% CI: 0.30, 1.50, with n = 27 exposed cases).	Moderate
Myocardial Infarction							
Mills et al. (2009)	1993-1997 (enrollment) to 1999-2005	AHS	Prospective Cohort n = 54,609 non-fatal MI group, 32,024 non-fatal MI group after 5-year follow-up period	AHS Survey Instrument – Ever/Never Malathion Use	Fatal MI ascertained using state and national death databases Non-fatal MI ascertained AHS Survey Instrument	No evidence of a positive association was reported for self-reported ever use of malathion and fatal MI (HR= 0.81; 95% CI: 0.66, 1.00; with n ~ 323 - 324 exposed cases) and no evidence of a significant positive association was reported for malathion exposure and non-fatal MI (HR= 1.02; 95% CI: 0.86, 1.21; with n ~ 620 – 621 exposed cases).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Dayton et al. (2010)	1993-1997 (Enrollment) to 2005	AHS Female spouses of applicators and female applicators	Case-control analysis of the Prospective Cohort n=22,425 women in the AHS	AHS Survey Instrument – Ever/Never Use	State and National death databases, AHS Survey Instrument	No evidence of a positive association between ever use of malathion and non-fatal MI among farm women (OR=0.90, 95% CI 0.60, 1.30, n = 31 malathion exposed cases).	Moderate
Nervous System Function							
Engel et al. (2007)	May 1998 - July 2001	Inner city multiethnic cohort of mother-infant pairs from the Mount Sinai Children's Environmental Health Cohort, New York	Cohort (n=1,450 eligible mothers; n=311 in the final study)	Maternal blood was obtained during routine venipuncture at a mean gestational age of 31.2 (standard deviation, 3.7) weeks, and a urine sample was collected at the same time. Maternal urine samples were analyzed for malathion dicarboxylic acid (MDA) by the CDC in Atlanta, GA.	Abnormalities in neonatal behavior and/or primitive reflexes were measured and assessed by the Brazelton Neonatal Behavioral Assessment Scale (BNBAS)	No evidence of a significant positive association between third-trimester urinary MDA levels and habituation outcome: adjusted β= 0.444 (95% CI -0.145, 1.025) No evidence of a positive association between third-trimester urinary MDA levels and orientation outcome: adjusted β= -0.100 (95% CI -0.597, 0.405) adjusted RR = 2.24 (95% CI 1.55, 3.24)	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Starks et al. (2012a)	1993-1997 (Enrollment) 2007 (Phase II, 10-year Follow-Up)	AHS	Prospective Cohort n = 1,807 licensed male applicators (eligible), 701 (participated)	AHS Survey Instrument – Ever/Never Malathion Use; lifetime days of use	Neurobehavioral function was determined through nine tests to assess central nervous system (CNS) function, along with a questionnaire	No evidence of a statistically significant association was reported for malathion exposure and any neurobehavioral tests for malathion ever use ($-6.13 \leq$ all $\beta \leq 0.58$, all $p \geq 0.05$ for the associated model of malathion regression coefficients).[137] For the log transformed cumulative lifetime days of use, a statistically significant decrease was determined for digit-symbol ($\beta = -1.75$, $p < 0.05$)	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Starks et al. (2012b)	1993-1997 (Enrollment) Two 5-year follow-up telephone interviews (Phase 2 & 3)	AHS	Prospective Cohort n = 678 licensed male applicators	AHS Survey Instrument – Ever/Never Malathion Use; lifetime days of use was the sum of days of use calculated for each interview period	Neurological testing along with a questionnaire; the peripheral nervous system (PNS) function tests included a neurological physical exam, electrophysiological tests, and quantitative functional tests	No evidence of a significant positive associations between malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for either ever use of lifetime days of malathion use ($0.73 \leq ORs \leq 1.37$, all 95% CIs encompassed the null value of 1.0). no evidence of significant positive association between log-transformed lifetime days of use of malathion and ankle reflex, postural tremor, Romberg, tandem gait, toe proprioception, and toe vibration for the low exposure (≤ 37.0 days) and the high exposure (> 37.0 days) groups, relative to the controls ($0.71 \leq ORs \leq 1.53$, all 95% CIs encompassed the null value of 1.0, and all p-trends ≥ 0.05).	Low
Neuro-Developmental/ Neurobehavioral Effects							

Eskenazi et al. (2007)	Enrollment October 1999 - October 2000; outcomes measured 2000-2003	CHAMACOS longitudinal birth cohort, Salinas Valley, CA	Cohort (n=1,130 eligible women; n=356 mother-infant pair in analysis)	Malathion dicarboxylic acid (MDA) was measured from spot urine samples from the pregnant women at the time of the two pregnancy interviews (mean 13 weeks gestation and mean 26 weeks gestation). For analysis, the two pregnancy measurements of each urinary MDA were averaged for each woman. A large proportion of women had non-detectable levels of MDA. MDA was categorized into three groups < LOD for both pregnancy measurements, and for those with at least one detectable level, subdivided below and above the median of the average pregnancy level.	Neurodevelopmental and behavioral outcomes were assessed using the Bayley Scales of Infant Development, Second Edition (1993). Children were assessed on average (mean ± SD) at 6.6 ± 1.1 months, 12.8 ± 1.6 months, and 24.6 ± 1.1 months. Each scale is standardized by age to mean = 100 and SD = 15. Scores > 1 SD below the mean (i.e., < 85) indicate possible developmental delay.	**6 months:** No evidence of a (significant) positive association between maternal MDA levels and MDI of the Bayley Scales: detectable levels of MDA < median: adjusted β= 0.98 (95% CI -0.85, 2.81) detectable levels of MDA ≥ median: adjusted β= -0.25 (95% CI -2.10, 1.60) No evidence of a (significant) positive association between maternal MDA levels and PDI of the Bayley Scales: detectable levels of MDA < median: adjusted β= 0.42 (95% CI -2.34, 3.18) detectable levels of MDA ≥ median: adjusted β= -1.45 (95% CI -4.21, 1.32) **12 months:** No evidence of a significant positive association between maternal MDA levels and MDI of the Bayley Scales: detectable levels of MDA < median: adjusted β= 0.95 (95% CI -1.55, 3.46) detectable levels of MDA ≥ median: adjusted β= 2.40 (95% CI -0.13, 4.94) No evidence of a	Moderate

						(significant) positive association between maternal MDA levels and PDI of the Bayley Scales: detectable levels of MDA < median: adjusted β= -0.53 (95% CI -4.05, 3.00) detectable levels of MDA ≥ median: adjusted β= 0.75 (95% CI -2.81, 4.31) **24 months:** No evidence of a (significant) positive association between maternal MDA levels and MDI of the Bayley Scales: detectable levels of MDA < median: adjusted β= -1.09 (95% CI -4.51, 2.32) detectable levels of MDA ≥ median: adjusted β= 0.24(95% CI -3.03, 3.52) No evidence of a (significant) positive association between maternal MDA levels and PDI of the Bayley Scales: detectable levels of MDA < median: adjusted β= -0.73 (95% CI -3.87, 2.41) detectable levels of MDA ≥ median: adjusted β= 0.33 (95% CI -2.68, 3.35)	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Gunier et al. (2017)	Between October 1999 and October 2000	Children born to mothers who were recruited and enrolled from health clinics serving low-income residents of Salinas, California	Cohort study N= 337 children enrolled	Maternal Residence assessed	Neurodevelopment assessment. Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) and the Behavior Assessment System for Children 2 (BASC-2) to assess children's behavior at age seven	No evidence of a significant association was reported between malathion exposure at 1km from the maternal residence during pregnancy and any of the IQ scales used in the study at 7-years of age – Verbal Comprehension, Full-Scale IQ, Working Memory, Processing Speed, and Perceptual Reasoning) ($-1.30 \leq \beta \leq 0.80$; all CIs encompassed the null value of 0; all p-values > 0.05). No evidence of a significant association was reported between malathion exposure at 1km from the maternal residence during pregnancy and Full-Scale IQ at 7-years of age from multiple pesticide models ($\beta = 0.20$; 95% CI: -1.70, 2.1).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Neuropsychological Function							
Sullivan et al. (2018)	Deployment 1990 - 1991 Interviews 1997 - 1998	Gulf War-deployed preventative medicine personnel who had varying levels of pesticide exposures during their work as pesticide applicators or other preventative medicine roles. These veterans had a unique knowledge of pesticides and their usage during the war.	Cohort (n=159)	Data from interviews conducted in 1997-1998, current interview responses, and current exposure questionnaire responses were used to categorize exposure to pesticides and exposure to pesticide into high and low categories, resulting in four groups (group 1 = low pesticide, low pyridostigmine bromide (PB); group 2 = high pesticide, low PB; group 3 = low pesticide, high PB; group 4 = high pesticide, high PB).	A trained research assistant or neuropsychologist who was blind to the exposure status of the subject administered the neuropsychological tests. The battery assessed the functional domains of general intelligence, language, attention/executive abilities, psychomotor function, visuospatial skills, short-term memory, and mood.	Malathion-specific effects estimates are not significant but are not specifically reported for attention domain, motor domain, mood domain, language domain, and visuospatial	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Olfactory Impairment							
Shrestha et al. (2019a)	Enrollment (1993 – 1997)	AHS	Prospective Cohort	AHS Self-Administered Questionnaires	AHS Survey Instrument	No evidence of a significant positive association was reported between malathion involved in the highest exposure HPEE and OI among private pesticide applicators (OR: 1.44; 95% CI: 0.64, 3.22); however, we considered these study results for OI to be less reliable due to the small number of cases reported.	Moderate
Shrestha et al. (2020a)	1993-1997 (Enrollment) to 2016 (Phase IV)	AHS Male Pesticide Applicators	Prospective Cohort n= 52,394 licensed pesticide applicators	AHS Survey Instrument – Ever/Never Use and Cumulative Lifetime Use	AHS Survey Instrument	No evidence of a significant positive association was reported between malathion involved in the highest exposure HPEE and OI among private pesticide applicators (OR: 1.44; 95% CI: 0.64, 3.22); however, we considered these study results for OI to be less reliable due to the small number of cases reported.	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Parkinson's Disease							
Firestone et al. (2005, 2010)	1992-2002	Western Washington State population study of PD	Case-Control n = 250 cases, 388 controls	Face-to-face structured interview conducted by nurse practitioner: Demographics, medical and occupational history (job duration, 6 months), occupational and home-based pesticide use, drinking water source, residential history, and smoking history.	Subjects reported first and last year of use and frequency of exposure (number exposed days per year). Cumulative exposures categorized as ordinal Trained neurologist confirmed PD diagnoses by medical chart review, requiring at least 2 of 4 cardinal signs of PD (bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment), one of which had to be bradykinesia or resting tremor.	Firestone et al. (2005) reported no evidence of a significant positive association between malathion exposure and PD among men (OR = 1.01, 95% CI: 0.37, 2.72; n = 8 exposed cases and 10 exposed controls) among a very small number of exposed cases ($n \leq 10$). Firestone et al. (2010) reported no evidence of a positive association between malathion exposure and PD among men. (OR = 1.00, 95% CI: 0.39-2.30) among a very small number of exposed cases ($n < 12$).	Low
Kamel et al. (2007)	1993-1997 (Enrollment) 1999-2003 (Phase 2 Follow-up)	AHS	Cohort Cross-sectional n = 84,738 enrolled, 57,251 Phase 2 Follow-up	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument At enrollment and follow-up, ''Has a doctor ever told you that you had been diagnosed with Parkinson's disease?'	No evidence of a significant positive association was reported for malathion exposure and incident PD (OR = 1.20; 95% CI: 0.60, 2.10; with n = 49 exposed cases) and for prevalent PD (OR = 1.10; 95% CI: 0.60, 2.00; with n = 41 exposed cases), based on ever use of malathion.	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Wang et al. (2014)	January 1, 2001 - January 1, 2007	Persons with PD and population controls from Fresno, Tulare, and Kern counties (rural agricultural tri-county area in Central California)	Case-control (n=357 PD cases, n=752 population controls)	Employed a GIS-based system that combined PUR data, land use maps, and geocoded address information to produce estimates of pesticide exposure within a 500 m radius buffer around participants' occupational and residential addresses from 1974 to 1999. Annual ambient exposures were calculated for the individual pesticides (malathion, diazinon) for each participant by summing the pounds of pesticides applied in each buffer and weighting the total pound by the proportion of the acreage treated.	PD patients initially identified through neurologists, large medical groups, and public service announcements, and all potential cases were examined by study movement disorder specialists	Evidence of a positive association between exposure to malathion at residences only and PD, OR=2.16 (95% CI 1.36, 3.43) Evidence of a strong association between exposure to malathion at the workplace and PD, OR=3.16 (95% CI 1.88, 5.32) Evidence of a moderately strong association between exposure to malathion both at residences and workplaces, OR= 2.69 (95% CI 1.45, 5.01)	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Shrestha et al. (2020b)	1993-1997 (Enrollment) to 2017	AHS Pesticide Applicators and Spouses	Prospective Cohort n= 52,393 licensed pesticide applicators and spouses	AHS Survey Instrument – Ever/Never Use and Cumulative Lifetime Use	State and National death databases, AHS Survey Instrument, PD Screening Validation Questionnaire, physical evaluation, medical records	No evidence of a significant positive association was reported for the association between ever use of malathion and PD among pesticide applicators and spouses in the AHS (HR=1.01; 95% CI: 0.78, 1.30; with n=253 exposed cases). For the intensity-weighted lifetime days of use analysis, with the following tertiles of exposure for malathion: >0–≤384, >384–≤1344, and >1344 days of exposure, no evidence of a significant positive association was reported between intensity-weighted lifetime days malathion use and incident PD in any exposure of intensity-weighted lifetime days of malathion use (0.83 <HR <1.26; all 95% CIs encompassed the null value 1.0; with n=47 - 68 cases per exposure category; p-trend=0.12) .	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Rheumatoid Arthritis							
De Roos et al. (2005)	1993 – 1997 (Enrollment) 1999 – 2003 (5-Year Follow-Up)	AHS	Nested case-control study N = 135 female cases	AHS Survey Instrument – Ever/Never Malathion Use	Self-reported physician-diagnosed Rheumatoid arthritis Physician confirmed	No evidence of a significant positive association was reported between malathion exposure and RA in female spouses (OR = 1.30; 95% CI: 0.80, 2.00; with n = 36 exposed cases and n = 150 exposed controls). When the association between malathion exposure and RA was stratified by state of residence, no evidence of a significant positive association was reported among women living in Iowa (OR = 1.20; 95% CI: 0.70, 2.00; with n = 23 exposed cases and n = 114 exposed controls) or in North Carolina (OR = 1.60; 95% CI: 0.80, 3.50; with n = 13 exposed cases and n = 6 exposed controls).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Meyer et al. (2017)	1993 – 1997 (Enrollment) 1999 – 2003, 2005 – 2010, and/or 2013 – 2015 (Follow-Up)	AHS	Prospective cohort n = 26,134 male AHS study participants	AHS Survey Instrument – Ever/Never and cumulative Malathion use	Self-reported or physician diagnosed rheumatoid arthritis	No evidence of a significant positive association was reported between malathion exposure and incident RA cases among male pesticide applicators (OR=1.05; 95% CI: 0.73, 1.53; with n=87 exposed cases). Exposure-response analysis was not conducted for malathion because the ever use OR was not >1.20.	Moderate
Parks et al. (2016)	1993 – 1997 (Enrollment) 1999 – 2003 2005–2010 (Phases 2&3. 5-Year and 10-Year Follow-Up)	AHS	Nested case-control study N = 24,293 female cases	AHS Survey Instrument – Ever/Never Malathion Use	Self-reported physician-diagnosed Rheumatoid arthritis Physician confirmed	No evidence of a significant positive association between malathion exposure and all (incident and prevalent) RA cases was observed (OR = 1.10; 95% CI: 0.80, 1.40) and no evidence of a positive association between malathion and incident RA cases only was observed (OR = 0.86; 95% CI: 0.55, 1.40).	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Respiratory Effects							
Asthma							
Henneberger et al. (2014)	1993-1997 (Enrollment)	AHS	Cross-Sectional n = 926 commercial and private applicators	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Self-report of a doctor's diagnosis of asthma	No evidence of a positive association was reported between exposure and asthma exacerbation (OR=0.80; 95% CI: 0.40, 1.30; with n=87 exposed cases).	Low
Hoppin et al. (2008)	1993-1997 (Enrollment)	AHS	Cross-Sectional n = 25,814 farm women	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Self-report of a doctor's diagnosis of asthma after age 19 years and atopic status based on a self-reported history of doctor-diagnosed eczema or hay fever.	Evidence of a positive association was reported for malathion exposure and atopic asthma among farm women, based on ever use (OR = 1.60: 95% CI: 1.22, 2.10; with n = 76 exposed cases) and no evidence of a significant positive association was reported for nonatopic asthma, based on malathion ever use (OR = 1.18; 95% CI: 0.94, 1.49; with n = 100 exposed cases).	Low

Hoppin et al. (2009)	1993-1997 (Enrollment) and through second mailed questionnaire	AHS	Cross-Sectional n = 19,704 male applicators	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Self-report of a doctor's diagnosis of asthma	Evidence of a positive association was reported for nonatopic asthma, based on ever/never malathion use (OR = 1.35; 95% CI: 1.04, 1.75; with n = 87 exposed cases). No evidence of a significant positive association was reported for atopic asthma based on ever/never malathion use (OR = 1.08; 95% CI: 0.74, 1.59; with n = 87 exposed cases). evidence of a positive association was reported in both exposure categories for non-atopic asthma (1 – 110 days OR = 1.36; 95% CI: 1.01, 1.83; with n = 109 exposed cases; > 110 days OR = 1.36; 95% CI: 1.02, 1.83 with n = 114 exposed cases), with no evidence of an exposure-response trend (p-trend = 0.90). No evidence of a significant positive association was reported for either exposure category of atopic asthma (0.79 < OR < 1.41; all 95% CIs encompassed the null value of 1.0; with n = 30 – 55 cases per exposure category), with evidence of a significant exposure-	Low

						response trend (p-trend = 0.01). In an additional analysis, controls with allergy (atopy) were excluded from the comparison group to determine if the difference in the reported results for atopic and non-atopic asthma was due to atopy alone. Evidence of a positive association was reported for malathion exposure and non-atopic asthma, and no evidence of a significant positive association for atopic asthma (nonatopic asthma – OR = 1.38; 95% CI: 1.06, 1.79; with n = 229 exposed cases; atopic asthma – OR = 1.10; 95% CI: 0.75, 1.62; with n = 87 exposed cases) when allergic individuals were removed from the control group. And evidence of a significant positive association was reported for atopy alone (OR = 1.30; 95% CI: 1.17, 1.45; with n = 1,276 exposed cases). Finally, to determine if the results were due to another co-morbid respiratory disease or asthma, those with chronic bronchitis and farmer's lung were excluded from the	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						analysis. No evidence of a significant positive association was reported for atopic asthma (OR = 1.15; 95% CI: 0.71, 1.85; with n = 56 exposed cases) and for nonatopic asthma (OR = 1.33; 95% CI: 0.97, 1.83; with n = 146 exposed cases).	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Chronic Bronchitis							
Hoppin et al. (2007)	1993-1997 (Enrollment)	AHS	Cross-sectional n = 20,908 male applicators	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: "Has a DOCTOR ever told you that you had (been diagnosed with) chronic bronchitis? If yes, how old were you when a doctor first told you? < 20, 20–39, 40–59, 60+)"	Evidence of a positive association was reported between malathion exposure and chronic bronchitis among male pesticide applicators (OR = 1.66; 95% CI: 1.38, 1.99). When additionally adjusted for correlated pesticides = 1.70; 95% CI: 1.11, 2.59; with n ~ 32 – 33 exposed cases, n ~ 607 – 608 unexposed cases). No evidence of a significant positive association was reported for the second highest exposure category (171 – 235 lifetime days OR = 1.48; 95% CI: 0.96, 2.29; with n ~ 26 - 27 exposed cases, n ~ 607 – 608 unexposed cases), and no evidence of an exposure-response trend (p-trend = 0.105) was reported.	Low

Rinsky et al. (2019)	1993 – 1997 (Enrollment) 1999 – 2003 (Follow-Up Interview) 2005 – 2010	AHS	Prospective cohort n = 22,491 male AHS study participants	AHS Survey Instrument – Ever/Never Malathion Use	Follow-up interview, farmers were asked, "Have you ever been diagnosed with chronic bronchitis, emphysema, and COPD" in three separate questions	Evidence of a slight positive association was reported for malathion ever use and chronic bronchitis symptoms alone (OR = 1.22; 95% CI: 1.05, 1.43, with 749 exposed cases). Evidence of a positive association was reported for malathion ever use and COPD diagnosis and chronic bronchitis symptoms (OR = 1.85; 95% CI: 1.32, 2.60, with 207 exposed cases). No evidence of a significant positive association was reported for malathion ever exposure and COPD diagnosis alone (COPD diagnosis alone – OR = 1.03; 95% CI: 0.88, 1.20, with 673 exposed cases). Similar results were reported for malathion ever use when adjusting for type of animal produced on the farmer's property. Evidence of a slight positive association was reported for malathion ever use and the chronic bronchitis symptoms only outcome when adjusted for type of animal produced (OR = 1.21; 95% CI: 1.03, 1.41, with 749 exposed cases). Evidence of a positive association was reported for malathion	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						ever use adjusted for animal produced and the COPD diagnosis and chronic bronchitis symptoms outcome (OR = 1.84; 95% CI: 1.31, 2.60, with 207 exposed cases). No evidence of a significant positive association was reported between malathion ever use adjusted for animal produced and the COPD only outcome (OR = 1.04; 95% CI: 0.90, 1.22, with 673 exposed cases).	
Valcin et al. (2007)	1993 – 1997 (Enrollment)	AHS	Prospective cohort n = 21,541 female AHS study participants	AHS Survey Instrument – Cumulative Malathion Use	Self-reported doctor diagnosis from answering the following question, "Have you ever been diagnosed with chronic bronchitis, emphysema, and COPD"	Evidence of a positive association was reported between malathion and chronic bronchitis (OR = 1.34; 95% CI: 1.10, 1.63; with n ~ 139 - 140 exposed cases). When the model was further adjusted to account for exposure to other herbicides in addition to age and state of residence, no evidence of a significant positive association was reported (OR = 1.21; 95% CI: 0.95, 1.53; with n ~ 139 - 140 exposed cases).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Rhinitis							
Slager et al. (2009)	1993-1997 (Enrollment)	AHS Pesticide Applicators	Cross-Sectional n=21,958 private applicators	AHS Survey Instrument – Ever/Never Use and Cumulative Lifetime Use	AHS Survey Instrument	No evidence of a significant positive association was reported between exposure to malathion and current rhinitis based on use within the past year (OR = 1.28; 95% CI: 0.96, 1.69). The study authors noted that the comparison group influenced the risk estimate for malathion, and the odds ratio for malathion reached statistical significance when the referent group was based on never use only (instead of former use as well) (OR = 1.49; 95% CI: 1.10, 2.00).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Slager et al. (2010)	1993-1997	AHS	Cross-Sectional n = 21,958 private applicators	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Current rhinitis, self-report	In the polytomous model, evidence of a slight positive association was reported between malathion exposure and those who reported 3 – 6 episodes of rhinitis within a year (OR = 1.10; 95% CI: 1.04, 1.12), along with a significant p-trend (p = 0.011). No evidence of a significant positive association was reported for the 1, 2, 7 – 12, and 13 + episodes of rhinitis within a year ($0.97 \leq OR \leq 1.08$; all CIs encompassed the null value of 1.0). For the dichotomous logistic regression model (ever/never exposure), evidence of a slight positive association between malathion and current rhinitis was reported (OR = 1.06; 95% CI: 1.01, 1.11).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Wheeze							
Hoppin et al. (2002)	1994-1997 (Enrollment)	AHS	Cross-sectional n = 20,468	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Wheeze in past 12 months, self-report	Evidence of a slight positive association between malathion exposure and wheeze among pesticide applicators (OR = 1.14; 95% CI: 1.02, 1.28) based on ever use in the past year. Further, the authors reported evidence of a linear (monotonic) trend across categories based on five ordinal categories of exposure (p-trend = 0.01).	Low
Hoppin et al. (2006a)	1993-1997 (Enrollment)	AHS	Cross-sectional n = 20,175 (17,920 farmers and 2,255 commercial applicators)	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: "How many episodes of wheezing or whistling in your chest have you had in the past 12 months?"	No evidence of a significant positive association was reported between current malathion use and wheeze among private pesticide applicators (OR=1.13; 95% CI: 1.00, 1.27) and no evidence of a positive association was reported among commercial applicators (OR=0.95; 95% CI: 0.69, 1.31). Additional results for commercial applicators were described in a separate publication (Hoppin et al., 2006b).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Hoppin et al. (2006b)	1993-1997 (Enrollment)	AHS	Cross-sectional n = 2,255 commercial applicators from Iowa	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Wheeze in past 12 months, self-report	No evidence of a significant positive association was reported for current use of malathion and wheeze in the past year (OR=1.06; 95% CI: 0.78, 1.45; with n=85 exposed cases and n=282 exposed non-cases).	Low

Hoppin et al. (2017)	1993-1997 (Enrollment) – 2010	AHS Male Pesticide Applicators	Cross-sectional n=22,134	AHS Survey Instrument – Ever/Never Use AHS Survey Instrument	AHS Survey Instrument	Evidence of a positive association was reported between current malathion use and allergic wheeze in the past year (OR = 1.48; 95% CI: 1.19, 1.86; with n ~157 exposed cases) and a slight positive association for non-allergic wheeze in the past year (OR = 1.29; 95% CI: 1.13, 1.46; with n ~512 exposed cases). In the exposure-response analysis for allergic wheeze, evidence of a positive association was reported for wheeze in the past year and the following exposure categories of malathion: 2 days, 3 – 4 days, and 5 – 7 days (OR past use = 1.39; 95% CI: 1.20, 1.61 with n = 871 exposed cases; OR 2 days use = 1.93; 95% CI: 1.36, 2.75 with n = 46 cases; OR 3 – 4 days: 2.00; 95% CI: 1.30, 3.08 with n = 29 cases; OR 5 – 7 days use = 1.85; 95% CI: 1.12, 3.04 with n = 21 cases). evidence of a significant positive association was reported for non-allergic wheeze and the following exposure categories: past use, 1 day, and 5 – 7 days of	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						malathion use (1.09 ≤ OR ≤ 1.19; all 95% CIs encompassed the null value of 1.0; with n = 49 – 206 cases per exposure category).	
Recurrent Pregnancy Loss							
Pandey et al. (2020)	Between January 2012 and January 2015	Women (n = 70) who were patients that had suffered two or spontaneous miscarriages before the 20th week of gestation	Case control study (n=70)	Interviewed to obtain information on family history, smoking/tobacco chewing use, age, and alcohol consumption, in addition to collecting 5 mL blood samples	Interviewed to obtain information	No evidence of a statistically significant difference (p =0.22) was reported for malathion exposure among the cases and control groups (mean±SD exposure level for cases: 2556 ±1027; mean±SD exposure level for controls: 1253.7±1421).	Low
Sleep Apnea							
Baumert et al. (2018)	Enrollment (1993 – 1997) and at 5-year and 10-year follow-up time points (1999 – 2003, 2005 – 2010)	AHS/ALHS	A case-control study	AHS Survey Instrument – Ever/Never Use	AHS Survey Instrument, ALHS Survey Instrument	No evidence of a positive association was reported between malathion exposure and sleep apnea based on ever/never use (OR=0.94; 95% CI: 0.67, 1.32).	Moderate
Stroke							
Rinsky et al. (2013)	1993-1997 (Enrollment) Follow-up through 2008	AHS	Prospective cohort n = 51,603 AHS study participants	AHS Survey Instrument – Ever/Never Malathion Use	Vital status was ascertained at follow-up using death records	No evidence of a positive association was reported between malathion exposure and stroke mortality (HR=0.99; 95% CI: 0.76, 1.29; with n=206	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						exposed cases) based on ever/never use.	
Suicide							
Beard et al. (2011)	1993-1997 (Enrollment) Follow-up through May 2009	AHS	Prospective cohort n = 81,998 AHS study participants	AHS Survey Instrument – Ever/Never Malathion Use	Vital status was ascertained using death records	No evidence of a positive association was reported between malathion exposure and suicide (HR = 0.93; 95% CI: 0.61, 1.42) based on ever/never use.	Moderate
Testosterone Level Changes							
Panuwet et al. (2018)	2006	Farmers (20-65 years old) from Chiang Mai province in Thailand. Thais residing in Inthakhin and ethnic Hmong minorities residing in Pong Yaeng districts of Chiang Mai province.	Cross sectional (n=133)	Urine samples were analyzed for a variety of urinary biomarkers of pesticides using two different analytical methods. The common metabolites of OP insecticides (dialkylphosphate metabolites [DAPs]) were analyzed using gas chromatography-tandem mass spectrometry. Metabolites were analyzed using high performance liquid chromatography-atmospheric pressure ionization-tandem mass spectrometry	Serum samples were analyzed for levels of total and free testosterone as well as cotinine using enzyme-linked immunosorbent assay kits	No evidence of a positive association between MDA and total-testosterone, β= -0.086 (95% CI -0.033, 0.215), p-value=0.156 No evidence of a positive association between MDA and free-testosterone, β= -0.026 (95% CI -0.125, 0.074), p-value=0.617	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
				(HPLC-APCI-MS/MS)			
Thyroid Disease							
Hyperthyroid Disease							
Goldner et al. (2010)	1993-1997 (Enrollment)	AHS	Cross-sectional n = 16,529	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Self-reported history of physician diagnosed thyroid disease (hyperthyroid, hypothyroid, other)	No evidence of a positive association was reported for the association between malathion exposure (ever use) and hyperthyroid disease among female spouses of pesticide applicators (OR = 0.97; 95% CI: 0.73, 1.30; with n = 64 exposed cases).	Low
Shrestha et al. (2018b)	1993 – 1997 (Enrollment) 1999 – 2003 (Follow-Up Phase II) 2005 – 2010 (Follow-Up Phase III) 2013 – 2016 (Follow-Up Phase IV)	AHS	Prospective cohort n = 24,092 female AHS spouses	AHS Survey Instrument – Ever/Never Malathion Use	Self-reported diagnosis of thyroid disease using medical records for validation between time of study enrollment (1993-1997) to study follow-up (1999-2003, 2005 – 2010, 2013 - 2016)	For hyperthyroid disease, no evidence of a significant positive association was reported for malathion exposure (HR: 1.01; 95% CI: 0.82, 1.26 with n=107 exposed cases, 410 unexposed cases) based on ever use. When further adjusted for correlated pesticides, no evidence of a significant positive association was similarly reported (HR: 1.08; 95% CI: 0.85, 1.39 with n=107 exposed cases, 410 unexposed cases). An additional analysis that only included thyroid	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						cases as defined by receipt of treatment in AHS spouses also reported no evidence of a significant positive association between malathion exposure and hyperthyroid disease (HR: 1.06; 95% CI: 0.84, 1.35 with n=86 exposed cases, 313 unexposed cases). And finally, an additional analysis that only included thyroid cases as defined by those confirmed by medical records or validation questionnaire, reported no evidence of a significant positive association between malathion exposure and hyperthyroid disease female spouses of pesticide applicators (HR: 1.21; 95% CI: 0.86, 1.70 with n=45 exposed cases, 146 unexposed cases), among a small number of exposed cases.	
Shrestha et al. (2019)	1993 – 1997 (Enrollment) 1999 – 2003 (Follow-Up Phase II)	AHS	Prospective cohort n = 35,150 male AHS study participants	AHS Survey Instrument – Ever/Never Malathion Use	Self-reported diagnosis of thyroid disease using medical records or questionnaire data for validation between time of study enrollment (1993-1997) to study	No evidence of a positive association between malathion and hyperthyroidism among private applicators was reported in the overall analysis and the stricter	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
	2005 – 2010 (Follow-Up Phase III) 2013 – 2016 (Follow-Up Phase IV)				follow-up (1999-2003, 2005 – 2010, 2013 - 2016)	case definition analysis (n=35,150) (Overall HR: 0.68; 95% CI: 0.52,0.88), with n=158 exposed cases; Stricter Case Definition HR: 0.56; 95% CI: 0.33,0.94). An additional sub-analysis that investigated the association between malathion exposure (based on ever/never use) and hyperthyroid risk among private applicators when females were excluded (n=34,375) found no evidence of a positive association (HR: 0.66; 95% CI: 0.50, 0.87, with n=149 exposed cases).	
Hypothyroid Disease							
Goldner et al. (2010)	1993-1997 (Enrollment)	AHS	Cross-sectional n = 16,529	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Self-reported history of physician diagnosed thyroid disease (hyperthyroid, hypothyroid, other)	No evidence of a significant positive association was reported for the association between malathion exposure and hypothyroid disease (OR = 1.10; 95% CI: 0.92, 1.30; with n = 220 exposed cases).	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
Goldner et al. (2013)	1993 – 1997 (Enrollment) 1999 – 2003 (Follow-Up Phase II) 2005 – 2010 (Follow-Up Phase III)	AHS	Prospective cohort n = 22,246 male AHS study participants	AHS Survey Instrument – Ever/Never Malathion Use	Self-reported diagnosis of thyroid disease between time of study enrollment (1993-1997) to study follow-up (1999-2003, 2005 – 2010)	Evidence of a slight positive association was reported between exposure to malathion and hypothyroid disease male commercial and private pesticide applicators, based on ever/never use (OR = 1.29; 95% CI: 1.03, 1.62; with n = 362 exposed cases and n = 15,261 exposed non-cases).	Moderate
Shrestha et al. (2018b)	1993 – 1997 (Enrollment) 1999 – 2003 (Follow-Up Phase II) 2005 – 2010 (Follow-Up Phase III) 2013 – 2016 (Follow-Up Phase IV)	AHS	Prospective cohort n = 24,092 female AHS spouses	AHS Survey Instrument – Ever/Never Malathion Use	Self-reported diagnosis of thyroid disease using medical records for validation between time of study enrollment (1993-1997) to study follow-up (1999-2003, 2005 – 2010, 2013 - 2016)	For hypothyroid disease, no evidence of a significant positive association was reported for malathion exposure (HR: 1.10; 95% CI: 0.97, 1.24; with n = 362 exposed cases, 1,205 unexposed cases). No evidence of a significant positive association was reported between malathion exposure and hypothyroid disease when further adjusted for correlated pesticides (HR: 1.08; 95% CI: 0.94, 1.24; with n = 362 exposed cases, 1,205 unexposed cases). An additional analysis that only included thyroid cases as defined by receipt of treatment in AHS spouses, reported no evidence of a	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						significant positive association between malathion exposure and hypothyroid disease (HR: 1.11; 95% CI: 0.98, 1.26 with n = 330 exposed cases, 1,073 unexposed cases), and a further analysis that only included thyroid cases which were validated according to the *stricter case definition standards* (ascertained via medical record data; confirmed via validation questionnaire; reported thyroid disease at least twice in follow-up surveys). Evidence of a slight positive association for malathion exposure and hypothyroid disease for female spouses of pesticide applicators under 60 (HR: 1.24; 95% CI: 1.02, 1.49; with n = 144 exposed cases, 462 unexposed cases) and evidence of a positive association for malathion exposure and hypothyroid disease for female spouses of pesticide applicators over 60 (HR: 1.72; 95% CI: 1.30, 2.28; with n =	

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						77 exposed cases, 132 unexposed cases).	
Shrestha et al. (2018c)	1993 – 1997 (Enrollment) 1999 – 2003 (Follow-Up Phase II) 2005 – 2010 (Follow-Up Phase III) 2013 – 2016 (Follow-Up Phase IV)	AHS	Prospective cohort n = 34,879 male AHS study participants	AHS Survey Instrument – Ever/Never Malathion Use	Self-reported diagnosis of thyroid disease using medical records or questionnaire data for validation between time of study enrollment (1993-1997) to study follow-up (1999-2003, 2005 – 2010, 2013 - 2016)	Evidence of a slight positive association was reported between malathion exposure and hypothyroid disease among private applicators (HR: 1.23; 95% CI: 1.04, 1.46, p-value=0.02). A further analysis investigating the association between intensity-weighted lifetime days of use of malathion and hypothyroid disease among private applicators, was conducted with the following tertiles intensity-weighted lifetime days of use for malathion used: T1: >0 - ≤ 360 days of use,T2: >360 - ≤ 1,395 days of use, and T3: >1,395 days of use. Evidence of a positive association was reported for the middle (T2) and no evidence of a significant positive association was reported for the high exposure (T3) and low exposure (T1) categories (T2: >360 - ≤ 1,395 days – HR: 1.48; 95% CI: 1.16, 1.88;	Moderate

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						with n=103 exposed cases, p-value=0.00); T1: >0 - ≤ 360 days - HR: 1.16; 95% CI: 0.89, 1.50; with n=103 exposed cases, p-value=0.27; T3: >1,395 days - HR: 1.16; 95% CI: 0.89, 1.50; with n=106 exposed cases, p-value=0.27), and no evidence of a significant exposure-response trend (p-trend=0.68).	
Lerro et al. (2018)	1993 – 1997 (Enrollment) to 2013	AHS Biomarkers of Exposure and Effect in Agriculture (BEEA) study	Prospective cohort n=679 male AHS study participants in BEEA study	AHS Survey Instrument – Ever/Never Use	Blood samples and serum samples were measured to confirm subclinical hypothyroidism	No evidence of a significant positive association was reported between any exposure category of malathion intensity-weighted lifetime days of use and laboratory confirmed subclinical hypothyroidism (0.95 < OR <1.50; all 95% CIs encompassed the null value of 1.0; with 12 – 18 cases per exposure category; with a p-trend=0.30).	Moderate
Other Thyroid Disease							
Goldner et al. (2010)	1993-1997 (Enrollment)	AHS	Cross-sectional n = 16,529	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Self-reported history of physician diagnosed thyroid disease (hyperthyroid, hypothyroid, other)	No evidence of a positive association was reported for the association between malathion exposure and other thyroid disease (OR = 0.97; 95% CI:	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						0.77, 1.20; with n = 98 exposed cases).	
Weight Gain							
LaVerda et al. (2015)	Enrollment (1993 – 1997)	AHS	Prospective cohort study (n = 8,365)	AHS Survey Instrument – Ever/Never Malathion Use	AHS Survey Instrument: Self-reported BMI	Results for malathion indicated no evidence of a positive association between cumulated malathion exposure days and increased BMI, and results were similar for the unadjusted analysis (β = 0.007, p= 0.818) and evidence of a positive association between malathion exposure days and adjusted BMI at age 20, for age, smoking, daily kilocalories consumed, and daily hours of heavy lifting (β = 0.07, p =0.01). To investigate the potential effect modification of weight-related health conditions diagnosed in 2,586 participants (cancer excluding nonmelanoma skin cancer, diabetes, heart disease, lupus, and/or amyotrophic lateral sclerosis (ALS)), these participants were excluded, and results from the medical exclusions analysis were similar to the overall analysis (β = 0.08, p = 0.03). To	Low

First Author (Pub Year)	Study Period	Description of study population	Study Design	Exposure Measurement	Outcome Measurement	Primary Malathion Results	Study Quality
						investigate the potential effect modification of the state variable, a stratified analysis was conducted, and results indicated no positive association between cumulative malathion exposure days and increased BMI in Iowa (adjusted analysis β = 0.03, p = 0.449), but a positive association in North Carolina (adjusted analysis β = 0.09, p = 0.018; significance based on Bonferroni-adjusted p value = 0.003).	