

Pesticide exposure and liver cancer: a review

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Abstract

Purpose To review the epidemiologic literature examining pesticide exposure and liver cancer incidence.

Methods A search of the MEDLINE and Embase databases was conducted in October 2015. Eligibility criteria included examining hepatocellular carcinoma (HCC) or primary liver cancer, pesticides as an exposure of interest, and individual-level incidence. The review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Results Forty-eight papers were assessed for eligibility and 15 studies were included in the review. The majority of studies were conducted in China and Egypt ($n=8$), used a case–control design ($n=14$), and examined HCC ($n=14$). Most studies showed no association between self-reported and/or occupational exposure to pesticides and liver cancer risk. Six studies demonstrated statistically significant positive associations, including three biomarker-based studies (two using pre-diagnostic sera) that reported higher serum levels of dichlorodiphenyltrichloroethane (DDT) were associated with increased HCC risk. Studies indirectly

measuring pesticide exposure using self-reported exposure, occupation, job-exposure matrices, or geographic residence demonstrated inconsistent results. These studies were limited by exposure assessment methods, lack of confounder information, minimal case confirmation, selection bias, and/or over-adjustment.

Conclusions There is mixed evidence suggesting a possible association between specific pesticides and HCC risk, with the strongest evidence observed in biomarker-based studies. In particular, organochlorine pesticides, including DDT, may increase HCC risk. Future research should focus on improved pesticide exposure assessment methods, potentially incorporating multiple approaches including biomonitoring while considering the chemicals of interest, historical exposure to address latency periods, and examining specific chemicals and exposure pathways.

Keywords Liver cancer · Hepatocellular carcinoma · Pesticides · Epidemiology · Review

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Introduction

Primary liver cancer is the sixth most common cancer in the world and the second leading cause of cancer-related death [1]. Approximately 70–85% of primary liver cancer cases are hepatocellular carcinoma (HCC) [2]. The second most common histology is intrahepatic cholangiocarcinoma [3]. Over 80% of HCC cases occur in East Asia and sub-Saharan Africa [4]. Age-adjusted liver cancer incidence in China in 2012 was 33.7 per 100,000 among males and 10.9 per 100,000 among females [1]. In the U.S., HCC is the most commonly occurring type of primary liver cancer and is increasing in incidence [5]. Between 2000 and 2005 and 2006–2012, age-adjusted HCC incidence in the US increased from 7.5 to 10.0 per 100,000 among males (33% increase; $p < 0.00001$), and from 2.1 to 2.7 per 100,000 among females (27% increase; $p < 0.00001$) [6]. The majority of HCC risk factors contribute to carcinogenesis by promoting the formation and progression of cirrhosis [7]. In parts of Asia and sub-Saharan Africa, predominant risk factors include chronic hepatitis B virus (HBV) infection and exposure to aflatoxin, a mycotoxin produced by the *Aspergillus* fungus forming on foods in damp conditions [8]. In Japan and Egypt, the major risk factor is chronic hepatitis C virus (HCV) infection [3]. Major risk factors in the U.S. and Europe include chronic HCV infection, heavy alcohol consumption (≥ 3 more drinks per day), and metabolic syndrome [3, 9, 10]. Other risk factors include obesity, diabetes, non-alcoholic fatty liver disease (and non-alcoholic steatohepatitis), and cigarette smoking; coffee and tea consumption may be protective [11–16]. However, between 15 and 50% of HCC cases have no established risk factors [4].

Pesticides and liver cancer

Pesticides are chemicals used to destroy, mitigate, prevent, or repel pests such as insects, mice, weeds, fungi, and microorganisms. Pesticides can be delineated into functional groups (e.g., insecticides) according to the organisms they control, or chemical classes (e.g., organochlorines) according to similar chemical structures and biological mechanisms of action [17]. Humans are exposed to pesticides via dermal contact, ingestion, and inhalation [18]. Occupational exposure to pesticides occurs among individuals employed in agriculture, pesticide manufacturing, pesticide application, and forestry; family members may be exposed if pesticides are introduced into the home (e.g., on clothing). Non-occupational exposure can also occur via residential use or dietary ingestion from contaminated drinking water and food [17, 19]. Residential proximity to agricultural pesticide applications is an important source of ambient environmental exposure, where pesticides applied from the air and ground may drift from intended sites

[20, 21]. Pesticides are metabolized in the liver and are hypothesized to contribute to liver carcinogenesis through mechanisms of cell adhesion alterations, oxidative stress, genotoxicity, tumor promotion, immunotoxicity, and hormonal action [22–25]. Experimental studies have shown that exposure to dichlorodiphenyltrichloroethane (DDT), an organochlorine insecticide widely used in the mid-twentieth century, and its metabolite, dichlorodiphenyldichloroethylene (DDE), lead to the development of HCC and other liver tumors in rodents [26–28]. However, results from epidemiologic studies of pesticide exposure and liver cancer mortality in humans have been inconsistent [29–37].

Liver cancer is a significant and growing public health burden and a substantial number of cases are unexplained by known risk factors. A growing body of literature has examined pesticide exposure as a potential environmental factor related to liver cancer. However, to date, the literature has not been synthesized. Inconsistent results from epidemiologic studies may be due to methodological limitations such as selection bias. Importantly, it is difficult to reconcile results of studies that used different exposure assessment methods (e.g., biomarkers vs. self-report). The purpose of this review was to summarize the current epidemiologic literature examining the association between pesticide exposure and liver cancer and to interpret results in light of these challenges.

Methods

Search strategy

The review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [38]. The MEDLINE (January 1966 to October 2015) and Embase (1980 to October 2015) databases were searched for studies. In MEDLINE, the following terms were searched as exploded MeSH terms and in all fields (e.g., title and abstract): (liver neoplasms) and (agrochemicals OR environmental exposure OR rural population OR rural health) AND humans [MeSH] AND (risk OR epidemiologic studies OR incidence). In Embase, the following terms were searched as exploded Emtree terms: ‘liver cancer’ AND (‘environmental chemical’ OR ‘environmental exposure’ OR ‘rural area’ OR ‘rural population’) AND ‘human’ AND (‘risk’ OR ‘observational study’ OR ‘incidence’ OR ‘prospective study’ OR ‘controlled study’ OR ‘cohort analysis’). The Embase search was executed as broadly as possible (mapping to Emtree, searching free text in all fields, exploding using narrower Emtree terms). For both database searches, limits for humans, English language, and original research were applied.

Study selection

After combining studies from the MEDLINE and Embase searches, duplicates were removed and studies were screened by title and abstract for relevance. One review paper was removed during screening as it was not filtered during the database searches. Inclusion and exclusion criteria for this review were determined *a priori* by the authors. Full-text papers were evaluated according to the following eligibility criteria for inclusion into the review: the outcome of interest was HCC or primary liver cancer; an exposure of interest was pesticides (including proxies for exposure); and the study investigated individual-level risk of developing the outcome. Studies examining cancers other than liver were not considered due to internal validity concerns (i.e., inclusion of studies with adequate statistical power to detect an association and that ascertained liver cancer risk factors to assess potential confounding). All cited references in each evaluated paper were also examined for inclusion into the review. Among studies satisfying all eligibility criteria, the following information was extracted: study design, time period, sample size, source population, outcome, case confirmation, reference group, matching factors, exposure metric(s), measures of association, confounders, and effect modifiers. Potential sources of bias (e.g., selection) were evaluated for each study. Unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for five studies not reporting these results, but providing sufficient information for their calculation [39]. All exposure metrics used in each study are listed in Table 1. Results from each study included in the review are shown in Table 2. Among studies using multiple exposure metrics, one primary result is reported for each study in Table 2. Reporting of the primary result was performed according to the following order determined by quality and relevance of the exposure metric to pesticide exposure and/or scientific evidence supporting an association with liver cancer: DDT if measuring multiple organochlorine pesticides in biospecimens; self-reported pesticide exposure (overall); employment in agricultural occupation/job title (the occupation more relevant to pesticide exposure was reported, e.g., farmworker vs. farm manager); employment in agriculture industry. Results regarding specific pesticide chemical classes are reported in the text. Issues affecting internal validity (i.e., case confirmation, confounding, over-adjustment, and selection bias) as well as effect modification are reported.

Results

A total of 1,262 studies were screened (Fig. 1) and after exclusions, 48 full-text papers were assessed for eligibility.

After the exclusions described above, 15 studies were included in the review (Table 1) [40–54]. Most studies were conducted in China ($n=4$) and Egypt ($n=4$). The majority of studies used a case–control design ($n=14$ total; $n=12$ retrospective; $n=2$ prospective) and examined incident HCC as the outcome ($n=14$). Most studies confirmed diagnoses, including via histology, imaging (e.g., ultrasonography), elevated alpha-fetoprotein (AFP) levels, and clinical examination. Most studies evaluated more than one measure of pesticide exposure such as job title in addition to self-reported pesticide exposure; results among these studies were inconsistent. While a majority of studies showed no association between pesticide exposure and liver cancer ($n=9$; 60%), six studies (40%) reported statistically significant positive associations between pesticide exposure and liver cancer with ORs ranging between 2.19 and 4.07 (Table 2). These studies were conducted in China and Egypt. One study reported a statistically significant protective effect of employment in the agriculture industry, although there was no adjustment for major risk factors [44]. The strongest evidence for an association was observed among three biomarker-based studies (two of which were prospectively assessed) conducted in China directly measuring organochlorine pesticides in serum and demonstrating statistically significant linear trends with increasing DDT levels and increasing HCC risk [48, 49, 54].

DDT and other organochlorine pesticides

Biomonitoring

Three biomarker-based studies in China measured serum organochlorine pesticides and demonstrated statistically significant positive associations with HCC risk, with adjusted ORs ranging between 2.96 and 4.07 for DDT (Table 2), adjusting for variables including age, sex, hepatitis B surface antigen (HBsAg), and hepatitis C virus antibody (anti-HCV) [48, 49, 54]. Two of the three studies assessed exposure using sera preceding diagnosis [48, 49], while one study assessed exposure after diagnosis [54]. The time between blood draw and HCC diagnosis was between 0 and 17 years in McGlynn et al. [48], 1 and 7 years in Persson et al. [49], and not reported in Zhao et al. [54]. Zhao et al. [54] also showed statistically significant positive associations between both DDE and hexachlorocyclohexane (HCH), an organochlorine pesticide, and HCC. With the exception of Zhao et al. [54], all of these studies confirmed cases (e.g., via histology). Odds ratios for serum DDT and HCC risk increased between 39 and 65% after adjustment for confounders in two studies [48, 49]. Although Persson et al. [49] did not adjust for HCV, McGlynn et al. [48] showed that HCV was not a significant risk factor in a

Table 1 Studies included in review examining pesticide exposure and liver cancer

| Authors | Study area | Study design | Time period | Sex | Exposure | Outcome (n) | Reference population (n) | Matching factors | Case confirmation ^a |
|--------------------------|------------|----------------------------|-------------|-------------------|--|---|---|--|---|
| VoPham et al. [53] | U.S. | Retrospective case-control | 2000–2009 | Males and females | GIS: ZIP code combined with agricultural pesticide exposure database | 3,034 HCC cases | 14,991 controls | Age, sex, race, duration of California residence, year | Histology, cytology, microscopic confirmation NOS, laboratory test/marker study, direct visualization, radiology, other imaging (not based on clinical diagnosis only and not reported on autopsy/death certificate only) |
| Persson et al. [49] | China | Prospective case-control | 1992–2000 | Males and females | Serum DDT, DDE | 473 HCC cases | 492 controls | Age, sex, area of residence | Histology, imaging, AFP > 400 ng/mL, clinical diagnosis, death certificate |
| Schiefelbein et al. [51] | Egypt | Retrospective case-control | 2007–2009 | Males and females | Self-reported occupation | 148 HCC cases | 148 controls | Age, sex | Histopathology, US, AFP, clinical diagnosis, triphasicity |
| Zhao et al. [54] | China | Retrospective case-control | 2007–2009 | Males and females | Serum DDT, DDE, DDD, HCH | 345 HCC cases | 961 hospital-based controls | – | Not reported |
| Soliman et al. [52] | Egypt | Retrospective case-control | 2007–2009 | Males and females | Self-reported occupation; residential history (rural/urban) | 150 HCC cases | 150 controls, healthy cancer center visitors or non-relatives accompanying patients | Age, sex | Histology |
| Ferrand et al. [46] | France | Retrospective case-control | 2000–2003 | Males and females | Self-reported occupation and industry (ISCO and ISIC) | 125 HCC cases | 142 hospital-based controls | Age, date, hospital of admission | Histology, imaging, AFP > 400 ng/mL |
| Chang et al. [42] | China | Case-cohort | 1989–1998 | Females | Self-reported occupation; JEM | 360 liver cancer cases (histology not reported) | 3,186 controls, age-stratified sub-cohort | – | Imaging and microscopic confirmation in subsample |

Table 1 (continued)

| Authors | Study area | Study design | Time period | Sex | Exposure | Outcome (n) | Reference population (n) | Matching factors | Case confirmation ^a |
|-----------------------|---|----------------------------|--------------|-------------------|---|--|---|---|--|
| McGlynn et al. [48] | China | Prospective case-control | 1984–2001 | Males and females | Serum DDT, DDE | 168 HCC cases | 385 controls | Age, sex | Pathological review, biochemical assays, US, CT, clinical diagnosis |
| Ezzat et al. [45] | Egypt | Retrospective case-control | Not reported | Males and females | Self-reported pesticide exposure and occupation; JEM | 236 HCC cases | 236 hospital-based controls | Age, sex, rural/urban residence | Pathological or cytological confirmation, US, CT, AFP > 300 or 1,000 ng/mL, treatment |
| Porru et al. [50] | Italy | Retrospective case-control | 1997–1999 | Males | Self-reported occupation and industry (ISCO and ISIC) | 144 liver cancer cases (<i>n</i> = 94 HCC) | 283 hospital-based controls | Age, date of admission, hospital of admission | Histology, cytology, US, CT, AFP ≥ 400 ng/mL |
| Heinemann et al. [47] | France, Germany, Greece, Italy, Spain, United Kingdom | Retrospective case-control | 1990–1996 | Females | Self-reported occupation and occupational exposure to pesticides; JEM | 317 HCC cases (incident and prevalent) | 1,779 controls (<i>n</i> = 1,060 hospital-based) | Age | Histology, US, CT, MRI, AFP ≥ 500 ng/mL |
| Badawi et al. [41] | Egypt | Retrospective case-control | Not reported | Males and females | Self-reported pesticide exposure | 102 HCC cases | 96 hospital-based controls | – | None (AFP levels were higher in cases [mean 224 IU] vs. controls [mean 3.4 IU]; normal CEA levels in 96% cases [mean 3.3 ng/mL]) |
| Dossing et al. [44] | Denmark | Retrospective case-control | 1970–1984 | Males and females | Occupation and industry in Supplementary Pension Fund (ISIC) | 973 liver cancer cases (<i>n</i> = 599 HCC) | 15,348 controls | Birth year, sex | Histology |
| Cordier et al. [43] | Vietnam | Retrospective case-control | 1989–1992 | Males | Self-reported occupational pesticide exposure | 152 HCC cases | 241 hospital-based controls | Age, hospital, place of residence | Histology, US, AFP ≥ 500 ng/mL, clinical diagnosis |

Table 1 (continued)

| Authors | Study area | Study design | Time period | Sex | Exposure | Outcome (n) | Reference popu-lation (n) | Matching factors | Case confirmation ^a |
|--------------------|------------|----------------------------|--------------|-------------------|---|--------------|-----------------------------|------------------------------|--------------------------------|
| Austin et al. [40] | U.S. | Retrospective case-control | Not reported | Males and females | Self-reported pesticide exposure; occupation and industry on death certificates (U.S. Census Bureau Classified Index of Industries and Occupations) | 80 HCC cases | 146 hospital-based controls | Age, sex, race, study center | Histology, clinical diagnosis |

AFP alpha-fetoprotein, *CEA* carcinoembryonic antigen, *CT* computed tomography, *DDD* dichlorodiphenyldichloroethane, *DDE* dichlorodiphenyldichloroethylene, *DDT* dichlorodiphenyltrichloroethane, *GIS* geographic information system, *HBsAg* hepatitis B surface antigen, *HCC* hepatocellular carcinoma, *HCH* hexachlorocyclohexane, *ICD-O-3* International Classification of Diseases for Oncology Third Edition, *ISCO* International Standard Classification of Occupations, *ISIC* International Standard Industrial Classification of All Economic Activities, *JEM* job-exposure matrix, *MRI* magnetic resonance imaging, *NGS* not otherwise specified, *US*, ultrasonography; *U.S.* United States

^aCase identification using ICD codes: VoPham et al. [53] ICD-O-3 topography C22.0, ICD-O-3 histology 8170–8175; Chang et al. [42] ICD-9 155.0, 155.2

low-HCC rate region in China. McGlynn et al. [48] and Persson et al. [49] did not ascertain aflatoxin information, but cited previous research demonstrating low aflatoxin levels in the study area and no statistically significant association between corn consumption and HCC risk. Although two studies used population-based controls matched on major risk factors [48, 49], one study used hospital-based controls [54], representing a potential source of selection bias. A statistically significant interaction was observed between DDT and DDE in Zhao et al. [54] (interaction $p=0.001$) and McGlynn et al. [48] ($p=0.042$), where higher HCC risk was associated with increasing serum DDT and decreasing serum DDE. Zhao et al. [54] demonstrated higher serum DDT levels were associated with increased HCC risk among those with HBV ($p=0.001$), not heavily consuming alcohol ($p=0.001$), diabetes ($p=0.01$), higher serum aflatoxin levels ($p=0.005$), higher serum polyaromatic hydrocarbon levels ($p=0.0005$), and higher serum HCH levels (beta isomer; $p=0.0096$).

Self-reported pesticide exposure

Cordier et al. [43] interviewed participants from hospitals in Vietnam regarding occupational exposure to pesticides. Self-reported exposure to ≥ 30 L/year of organochlorine pesticides (adjusted OR 4.8, 95% CI 0.9, 25.1) compared to none is suggestive of an association with HCC among males in Vietnam, adjusting for age, hospital, place of residence, HBsAg, and alcohol consumption [43]. Over-adjustment may have occurred as Cordier et al. [43] matched on a variable correlated with pesticide exposure (place of residence), potentially biasing results towards the null. Usage of hospital-based controls represents a potential source of selection bias.

Residential history

VoPham et al. [53] assessed pesticide exposure by combining residential ZIP Codes with a pesticide exposure database in a geographic information system (GIS) in the U.S. ZIP Code-level organochlorine pesticide exposure ≥ 14.53 kg/km² compared to < 14.53 kg/km² (adjusted OR 1.87, 95% CI 1.17, 2.99) was associated with a statistically significant increase in HCC risk among individuals residing in agriculturally intensive areas in the U.S., adjusting for liver disease and diabetes and stratifying by the matching factors of age, sex, race, duration of California residence, and year [53]. VoPham et al. [53] did not have access to individual-level occupation, a potential confounder. There was a statistically significant interaction between ZIP Code-level organochlorine pesticide exposure and sex ($p=0.0075$), where pesticide exposure was associated with a statistically significant increase in HCC

Table 2 Selected primary results from studies examining pesticide exposure and liver cancer

| Study ^a | Exposure ^b | Unadjusted/minimally adjusted OR (95% CI) | Adjusted OR (95% CI) | Confounders adjusted for in multivariable analysis ^c |
|----------------------------------|--|--|---|---|
| VoPham et al. [53] | High ZIP Code pesticide exposure (≥ 1.85 kg/km ²) vs. low (≤ 0.07 kg/km ²) | 0.89 (0.81, 0.98) | 0.95 (0.82, 1.09) | Age, sex, race, duration of California residence, year, liver disease (HCV, HBV, unspecified hepatitis, alcoholic liver disease, non-specific cirrhosis), diabetes, rare genetic disorders ($\alpha 1$ antitrypsin deficiency, hemochromatosis, porphyria, tyrosinemia, Wilson disease), state buy-in (socioeconomic status) |
| Persson et al. [49] ^d | Highest DDT quintile (≥ 810 ng/g fat) vs. lowest (≤ 261 ng/g fat) | 1.93 (1.00, 3.74) ^e | 2.96 (1.19, 7.40) | Age, sex, area of residence, HBsAg, family history of HCC, history of acute hepatitis, smoking, alcohol consumption, occupation, DDE |
| Schieffelin et al. [51] | Ever employed in farming vs. never employed in farming | 1.9 (1.1, 3.2) | 2.8 (1.1, 7.2) | Age, sex, HBV (HBsAg or anti-HBc), anti-HCV, schistosomiasis, cirrhosis, blood transfusion |
| Zhao et al. [54] ^d | Highest DDT quartile (≥ 43.09 ng/mL fat) vs. lowest (<16.11 ng/mL fat) | 4.07 (2.76, 5.80) ^f | 4.07 (2.72, 6.10) | Age, sex, education, alcohol consumption, smoking, AFB1-alb, HBsAg, anti-HCV |
| Soliman et al. [52] | Ever employed in farming vs. never employed in farming | 1.68 (1.02, 2.76) | 1.58 (0.79, 3.16) | Age, sex, HBsAg and/or anti-HCV, schistosomiasis, water pipe use, smoking |
| Ferrand et al. [46] | General farmers vs. all other job titles | 0.9 (0.4, 2.4) ^g | 0.9 (0.3, 2.6) | Age, date, hospital of admission, alcohol consumption |
| Chang et al. [42] | Pesticide exposure ≥ 10 years vs. never exposed | 0.61 (1.14, 2.55) ^f | 0.54 (0.12, 2.32) | Age at baseline, smoking, alcohol consumption |
| McGlynn et al. [48] ^d | Highest DDT quintile (> 787 ng/g fat) vs. lowest (<265 ng/g fat) | 1.5 (0.9, 2.8) | 3.8 (1.7, 8.6) | Age, sex, HBsAg, residential commune, DDE |
| Ezzat et al. [45] | Agricultural pesticide exposure vs. never exposure | Rural males: 3.0 (1.8, 5.2) Rural females: 1.0 (0.1, 7.5) | Rural males: 2.5 (1.3, 5.0) Rural females: 1.3 (0.1, 12.6) | Age, HCV RNA, HBsAg |
| Porru et al. [50] | Field crop and vegetable farm workers vs. never employed in occupation | 2.57 (0.99, 6.65) ^f | 1.8 (0.6, 5.6) | Age, date of admission, hospital of admission, area of residence, education, HBsAg, anti-HCV, alcohol consumption |
| Heinemann et al. [47] | Pesticide exposure vs. no exposure | 1.75 (0.88, 3.47) ^f | 1.51 (0.57, 3.97) | Age, study center |
| Badawi et al. [41] | Pesticide exposure vs. no exposure | Not reported | 2.19 (1.41, 3.43) | Age, sex, occupation, smoking, family history of cancer, schistosomiasis, HBV (HBsAg, HBsAb, or HBcAb) |
| Dossing et al. [44] | Ever employed in agriculture vs. never employed in industry | Males: 0.52 (0.3, 0.9) Females: not reported | -- | Age |
| Cordier et al. [43] | Exposure to ≥ 1 pesticide type vs. no exposure | 1.55 (0.99, 2.42) ^f | 1.2 (0.6, 2.5) | Age, hospital, place of residence, HBsAg, alcohol consumption |
| Austin et al. [40] | Pesticide exposure vs. no exposure | 2.4 (CI not reported) | 2.1 (0.6, 6.9) | Age, sex, race, study center, herbicide exposure, fertilizer exposure |

AFB1-alb aflatoxin-albumin adducts, *anti-HBc* hepatitis B core antigen, *anti-HCV* hepatitis C virus antibody, *CI* confidence interval, *DDE* dichlorodiphenyldichloroethylene, *DDT* dichlorodiphenyltrichloroethane, *HBcAb* hepatitis B core antibody, *HBsAb* hepatitis B surface antibody, *HBsAg* hepatitis B surface antigen, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *HCV RNA* hepatitis C virus ribonucleic acid, *OR* odds ratio

^aVoPham et al. [53], Ezzat et al. [45], and Cordier et al. [43] reported results for specific pesticide chemical classes

^bSome studies reported results using multiple exposure metrics. One primary result is reported for each study according to methods section “Study selection”

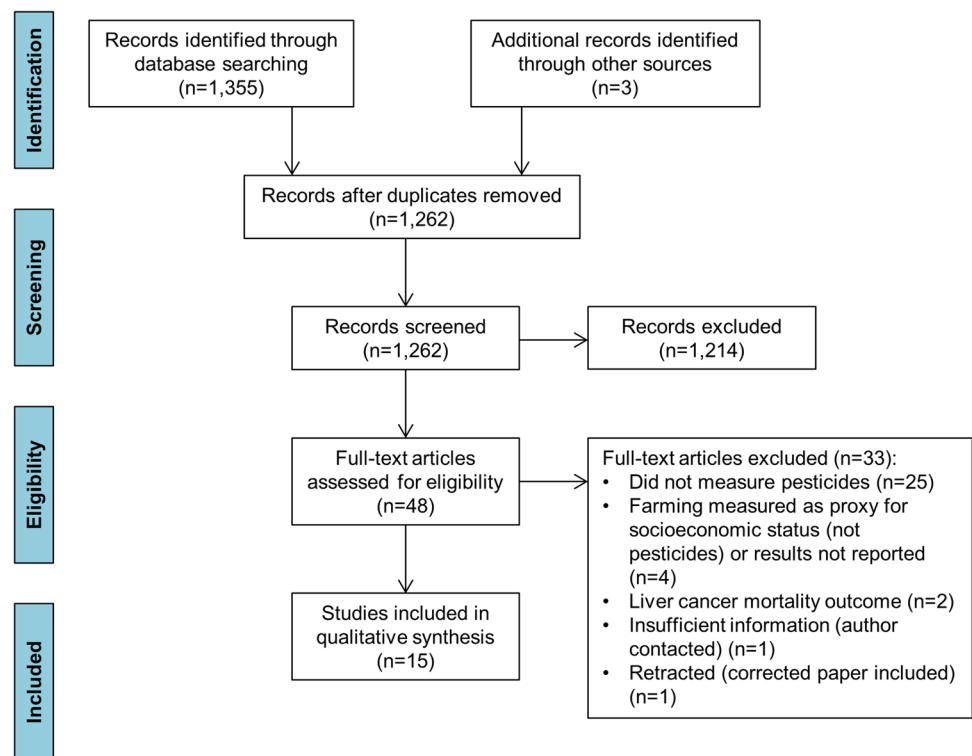
^cIncludes matching factors that were or were not included as adjustment or stratification variables in statistical models

^dp trend: Persson et al. [49] $p = 0.04$; Zhao et al. [54] $p < 0.0001$; McGlynn et al. [48] $p = 0.0024$

^eAdjusted for age, sex, area of residence, HBsAg, family history of HCC, history of acute hepatitis, smoking, alcohol consumption, and occupation

^fCalculated ORs and/or 95% CIs

^gAdjusted for hospital and age

Fig. 1 PRISMA study flow diagram

risk among males, but no association was observed among females [53].

Other pesticides

Self-reported pesticide exposure

Cordier et al. [43], a previously referenced study, found that self-reported exposure to ≥ 30 L/year of organophosphate pesticides (adjusted OR 4.7, 95% CI 1.1, 20.1) as well as other pesticides (adjusted OR 4.0, 95% CI 0.3, 47.0) compared to none suggested an association with HCC among males in Vietnam, although the sample size was small.

Job-exposure matrices

Ezzat et al. [45] estimated exposure using a job-exposure matrix (JEM), in addition to collecting self-reported information regarding occupational history, pesticide exposure, and agricultural activities in Egypt. Several selected pesticide chemical classes demonstrated statistically significant positive associations with HCC among males in rural Egypt, including carbamate pesticides (adjusted OR 2.9, 95% CI 1.4, 5.8) and organophosphate pesticides (adjusted OR 2.7, 95% CI 1.3, 5.3), adjusting for age, HCV RNA (ribonucleic acid), and HBsAg [45]. Regarding potential over-adjustment, Ezzat et al. [45] matched on rural/urban residence, although results were stratified by residence and

sex. Controls were recruited from a hospital orthopedic department. Effect modification by sex was reported, where occupational exposure to agricultural pesticides was associated with a statistically significant increased risk of HCC among males in rural Egypt, but was not associated with HCC among females [45].

Residential history

VoPham et al. [53] (referenced earlier) reported no association between ZIP Code-level organophosphate pesticides or carbamate pesticides and HCC in the U.S.

Mixed exposures and/or unspecified pesticides

Self-reported pesticide exposure

Studies ascertaining self-reported pesticide exposure mostly showed no association between pesticide exposure and liver cancer [40, 41, 43, 45, 47]. Exposure assessment methods included interviews and questionnaires. However, Badawi et al. [41] reported pesticide exposure vs. no exposure was associated with a statistically significant increased risk of HCC in Egypt (adjusted OR 2.19, 95% CI 1.41, 3.43), adjusting for age, sex, occupation, smoking, family history of cancer, schistosomiasis, and HBV (Table 2). Ezzat et al. [45] reported agricultural pesticide exposure vs. never exposure was associated with a statistically

significant increased risk of HCC among males in rural Egypt (adjusted OR 2.5, 95% CI 1.3, 5.0) (Table 2). Austin et al. [40] did not collect information regarding potential confounders including HCV. Badawi et al. [41] did not collect information regarding HCV, which is a major HCC risk factor in Egypt. Many studies used hospital-based controls, a potential source of selection bias [40, 41, 43, 45, 47].

Job-exposure matrices

Chang et al. [42] assessed exposure using a JEM, showing no association between liver cancer and ≥ 10 years of pesticide exposure compared to never exposure among females in China (adjusted OR 0.54, 95% CI 0.12, 2.32), adjusting for age at baseline, smoking, and alcohol consumption (Table 2). Metastatic liver cancer may have been included in the case group, as International Classification of Diseases, Ninth Revision (ICD-9) code 155.2 (malignant liver neoplasms not specified as primary or secondary) was used in the case definition [42]. Information on established liver cancer risk factors was not collected.

Occupation and industry

Several studies ascertained occupation and industry information from a national supplemental pension fund, interviews, and questionnaires [44, 46, 50–52]. Some studies coded occupational information using classification systems such as the International Standard Classification of Occupations and the International Standard Industrial Classification of All Economic Activities [44, 46, 50]. Job titles were typically defined as farmer, farm worker, and farm laborer, and industries as agriculture. Although farming occupation was generally not associated with liver cancer [46, 50, 52], Schiefelbein et al. [51] showed farming vs. never employed in farming was associated with a statistically significant increased risk of HCC in Egypt (adjusted OR 2.8, 95% CI 1.1, 7.2), adjusting for HBV, anti-HCV, schistosomiasis, cirrhosis, and blood transfusion (controls were matched to cases according to age and sex) (Table 2). Pesticide exposure defined based on working in the agriculture industry in Denmark was not associated with liver cancer (Table 2) [44].

Most of these studies confirmed diagnoses, although there is some potential evidence of inclusion of metastatic liver cancer in Dossing et al. [44]. Seven percent ($n=5$) of randomly sampled liver cancer patients were considered metastatic by pathologists, but not by the Denmark Cancer Registry [44]. Two studies did not collect or collected minimal confounder information [44, 46]. Porru et al. [50] may have over-adjusted by controlling for area of residence. Several studies used hospital-based or healthy cancer center visitors/non-relatives accompanying patients, a potential

source of selection bias [46, 50, 52]. Effect modification by HCV was reported in Soliman et al. [52], where farming occupation was associated with a statistically significant increased risk of HCC among individuals with HCV, but was not associated with HCC among those without HCV.

Residential history

VoPham et al. [53] (referenced earlier) reported that combined ZIP Code-level exposure to organochlorine, organophosphate, and carbamate pesticides was not associated with HCC risk in the U.S. after adjustment (Table 2).

Discussion

There is some evidence to suggest a positive association between exposure to particular pesticides and HCC in the published literature to date. To the best of our knowledge, this is the first review summarizing the epidemiologic literature on pesticide exposure and liver cancer. While most studies showed null results, several demonstrated statistically significant elevations in liver cancer risk associated with higher pesticide exposure ascertained directly via biomarkers or indirectly (e.g., self-report). Studies that indirectly measured pesticide exposure demonstrated inconsistent results, ranging from statistically significant positive associations to non-significant deficits in risk. The most convincing evidence was observed among three case-control studies directly measuring organochlorine pesticides such as DDT in serum (two of which were prospectively assessed). Aside from the possibility of chance, methodological issues likely contributed to inconsistent results, including pesticide exposure assessment, case confirmation, confounding, over-adjustment, and selection bias.

Pesticide usage in the U.S. has declined over the past 30 years [55], but remains a major issue in this country comprising 22% of the world pesticide market [56]. Worldwide pesticide production has continuously increased since 1940 [57]. China is the current global leader in usage [58] and developing countries, such as Vietnam, have experienced increasing use [59]. Specific pesticides demonstrated statistically significant associations with liver cancer among studies included in this review, including the organochlorine insecticide DDT. Broader pesticide chemical classes associated with increased risk of liver cancer include organochlorines, organophosphates, and carbamates, each comprised of pesticides showing carcinogenic potential [60–62]. Organochlorines are mostly insecticides, were widely used in the 1940s to 1960s, but have largely been banned in many countries due to adverse wildlife and human health effects and environmental persistence [18, 63]. DDT was banned in China in 1983, but use continues

for malarial control and dicofol insecticide production [48]. Organophosphates and carbamates, predominantly insecticides, began to increase in use following the ban of organochlorines. One-third of the insecticides used in China are organophosphates [64]. Both chemical classes were widely used in the U.S. during the 1980s and 1990s, but have since declined in usage replaced by more environmentally friendly chemicals [65]. Many of the pesticides from these chemical classes (e.g., DDT) are persistent organic pollutants, remaining in the environment for long periods of time and accumulating and passing from one species to another through the food chain [66]. DDT bioaccumulates in human adipose tissue. Pesticides outside of these chemical classes may be relevant to liver carcinogenesis, but their effects may not have been documented due to a lack of environmental persistence.

The high quality of the two biomonitoring case–control studies using pre-diagnostic sera, in terms of adjustment for established risk factors and exposure assessment, bolster confidence in their findings linking specific pesticides to HCC as direct measurement captures exposure from all sources [48, 49]. These studies were able to establish a temporal relationship as blood samples were collected prior to disease diagnosis. The use of biomonitoring to objectively quantify exposure also minimizes particular biases (e.g., recall bias from self-report). Both studies demonstrated statistically significant positive associations with organochlorine pesticides, including DDT. Humans are exposed to DDT and DDE through oral, inhalation, and dermal routes [67]. Humans can directly ingest DDE present in foods containing animal fat, especially as DDE is relatively more persistent than DDT [28]. Oral DDT and DDE exposure results in absorption by the intestinal lymphatic system and into portal blood. DDT and DDE are distributed in the lymph and blood to all body tissues and are subsequently stored in fat. DDT is initially metabolized in the liver to DDE and DDD (dichlorodiphenyldichloroethane). DDE and DDD are subsequently converted to DDA (2,2-bis(4-chlorophenyl)acetic acid), the primary urinary metabolite of DDT, in the liver and kidney. Conversion of DDT to DDE is predicted to occur slowly (<20% over 23 years). DDE metabolism is purported to occur at a slower rate compared to DDT. DDT is excreted as its metabolites through urine, feces, semen, and breast milk [67]. McGlynn et al. [48] and Persson et al. [49] measured serum DDT at study baseline, which occurred between 0 and 17 years preceding HCC diagnosis. These studies showed a statistically significant dose–response relationship between DDT and HCC risk. Zhao et al. [54] also showed a statistically significant positive association, but assessed serum measurements at study recruitment, which may be susceptible to reverse causation, especially as DDE was not associated with HCC in the prospective case–control studies [48, 49],

but was associated with a statistically significant increase in HCC risk in this retrospective case–control study [54]. Although these differing results may reflect variability in pesticide exposure across China, with more recent exposure in the Zhao et al. [54] study population, reverse causation cannot be ruled out as liver cancer is associated with weight loss [68], which has been linked with increases in DDT and DDE [69]. Since organochlorine compounds are stored in fat, loss of body fat may increase blood and organ concentrations [69]. It is not clear whether results from China, which has higher levels of pesticide exposure, would be generalizable to populations where pesticide exposure is relatively lower such as the U.S. For example, the highest serum DDT concentrations in the U.S. are lower than the 25th percentile in Chinese studies [3, 18, 49].

Studies demonstrating a statistically significant positive association between pesticide exposure and liver cancer risk were conducted in China and Egypt, while the majority of studies with null findings were conducted in Europe and the U.S. The major limitation of the evaluated studies was pesticide exposure assessment. Three studies used biomonitoring (two of which were prospectively assessed), considered the gold standard exposure assessment method that can assess long-term exposure to chemicals, particularly those with long biological half-lives whose concentrations are not affected by disease [17, 19]. Although informative, biomonitoring has limitations, including inability to determine the exact timing and amount at initial exposure (as levels change over time and may not reflect the magnitude of exposure), the source or route of exposure, a meaningful health benchmark, and difficulty in assessing exposure to chemicals with relatively shorter biological half-lives such as organophosphates and carbamates [70]. Most studies included in the literature review indirectly measured exposure. Proxy measures can be useful in capturing exposure to pesticides without known biomarkers or from residential use. Farmers and those involved in purchasing/using pesticides have been shown to provide reliable information [17]. GIS can integrate multiple exposure data sources to estimate ambient pesticide exposure based on location [53]. However, these measures are subject to exposure misclassification due to recall bias and uncertain geographic context. Most studies did not examine historical pesticide exposure, which would address a potential latency period of 20 years documented for some HCC risk factors [4]. Recent exposure may be irrelevant to hepatocarcinogenesis. Exposure misclassification was likely non-differential, particularly among the studies prospectively assessing exposure using biomonitoring [48, 49], using administrative data regarding geographic residence [53], occupational information from a pension fund [44], and studies blinding interviewers or

occupational physicians to case–control status [43, 46]. However, as many studies relied on self-report, differential exposure misclassification due to more accurate recall of past occupations, exposures, and residential history among cases cannot be ruled out, which may bias results towards or away from the null, contributing to the observed inconsistent results.

The majority of studies confirmed cases by histology, clinical examinations, imaging, elevated AFP levels, or a combination of these. Histological confirmation through biopsy or surgical resection is considered the gold standard [71], thus other confirmation methods may introduce misclassification of cases and controls. Misclassification from including metastatic liver cancer or non-HCC primary liver cancers in the case group could bias results (e.g., towards the null if pesticides are specifically associated with HCC and not other histologies or primary tumors). Prevalent HCC cases may differ from incident cases in their pesticide exposure experiences, where those with prevalent HCC may have survived due to relatively less pesticide exposure compared to incident cases [47].

Many studies did not adjust for established risk factors of HCC. The effect of confounding will vary according to geographic region, source population, and pesticide exposure assessment, reflecting differences in relationships with confounders in addition to exposure misclassification. When comparing effect estimates between studies adjusting for confounders compared to those not/minimally adjusting, several variables appear to exhibit strong confounding, particularly liver disease (HBV, HCV, and/or cirrhosis), alcohol consumption, age, and sex.

Over-adjustment, or controlling for variables highly correlated with the exposure of interest, may have affected results. Pesticide exposure is a largely rural phenomenon, as agricultural activities are more common in less densely populated areas [19]. Adjustment for variables that are inherently geographic, such as rural or urban residence, may produce comparable cases and controls, but may consequently impact results from measures designed/intended to capture the effect of pesticide exposure. For example, over-adjustment may have biased some results towards the null as pesticide use is expected to be more prevalent in rural areas, manifest in some studies matching on/adjusting for area of residence [43, 50]. However, several studies showed statistically significant results despite adjustment for/matching on geography-related variables [43, 45, 48, 49]. Badawi et al. [41] adjusted for farming occupation and pesticide exposure in the same multivariable model. These wide-ranging results highlight differential impacts of potential over-adjustment depending on pesticide exposure assessment and the study area. For example, rural–urban demarcations used in some studies may not accurately reflect pesticide exposure practices, obscuring variability

that may exist in pesticide use across particular study areas. The effect of over-adjustment should be considered in future research.

Many studies used hospital controls, representing a potential selection bias where conditions for admission and other cancers (e.g., bladder cancer [40]) may share pesticide exposure as a risk factor with HCC and bias results towards the null (assuming pesticides increase liver cancer risk). For example, Porru et al. [50] included hospital-based controls admitted for issues primarily related to the genitourinary, digestive, and circulatory systems. As bladder cancer, a genitourinary issue, has been linked with pesticide use [72], results may have been biased towards the null.

Future research should harness the advantages of multiple pesticide exposure assessment methods. Important considerations include historical exposure reconstruction to address latency periods, and evaluation of multiple and specific pesticides as well as multiple relevant sources and pathways (e.g., occupation, residential use, residential proximity to agricultural pesticide applications, and diet). Biomonitoring studies should consider pharmacokinetic variability in exposure assessment. Single biomarker measurements are subject to exposure misclassification, and including information regarding birth cohort, body mass index, and weight gain as surrogates for exposure onset and individual differences in absorption and excretion, respectively, in statistical analyses may reduce misclassification [73]. Additional information to consider includes diet, health conditions (e.g., thyroid disease), lactation, medications, metabolizing enzymes, occupation, and residence. Sequential longitudinal biomarkers can be obtained to determine secular trends affecting pharmacokinetic variability. Practical issues regarding biomonitoring should be considered, including invasiveness of collection, implementing quality assurance/control measures, and collection from sensitive populations such as children [74]. Given suggestive associations in some studies, future research should focus on specific pesticides, such as DDT, and pesticides from particular chemical classes including organochlorines, organophosphates, and carbamates, as well as others that may have similar biological mechanisms of action to any of these classes [89]. Focusing on individuals with an opportunity for exposure can allow for detecting health effects that would be rarer and more difficult to observe in the general population. For example, agricultural pesticide use is more common in rural areas. Closer examination of effect modification is warranted, including between pesticide exposure and HCC risk factors such as sex, alcohol consumption, diabetes, HCV, and HBV. Obesity and diabetes are continuing to increase in prevalence, particularly in developing countries, which has important implications especially if there is a true synergistic association

between diabetes and pesticide exposure [3, 54]. Results from studies utilizing multiple pesticide exposure metrics and/or examining multiple chemicals that were not reported in Table 2 should be examined [40, 41, 43–50, 52–54]. For example, Ezzat et al. [45] examined dithiocarbamate fungicides and bridged diphenyl acaricides. Anticipated National Institutes of Health-funded research includes two nested case–control studies conducted in the U.S. and Norway measuring 11 organochlorine pesticides using blood samples collected between the 1960s and 1970s [75]. Although this review only included published studies, the overall null findings suggest the absence of a publication bias in favor of statistically significant positive associations. Studies that examined multiple cancer outcomes were excluded ($n=11$). Excluding such studies may be viewed as a potential limitation as most were of prospective designs and thus less susceptible to certain biases compared to case–control studies (e.g., retrospective exposure assessment). However, as these studies were limited with respect to internal validity-related issues in statistical power (liver cancer is a rare outcome) and lack of adjustment for potential confounders critical to our association of interest, we a priori determined to exclude them from our review.

In summary, there is some evidence to suggest a positive association between particular pesticides and HCC. The most convincing evidence was observed in three studies directly measuring serum pesticide levels, although one of these studies used post-diagnostic sera and results may be subject to reverse causation. Specific pesticides, including the organochlorine DDT, demonstrated statistically significant positive associations with HCC. While many studies showed no association, these were largely limited by indirect pesticide exposure assessment methods likely resulting in exposure misclassification, minimal case confirmation, lack of adjustment for confounders, over-adjustment, and/or selection bias. Given the high prevalence of pesticide exposure in geographic areas with high HCC incidence and the high proportion of HCC cases in the U.S. that occur among those with no established risk factors, it is important to determine whether pesticides play a role in hepatocarcinogenesis. Future research should focus on improving pesticide exposure assessment, considering historical exposure, multiple pesticide exposures and exposure pathways, and the impact of specific organochlorine, organophosphate, and carbamate pesticides.

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