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## Research Article

# Pesticide Exposure and Risk of Cholangiocarcinoma: A Hospital-based Matched Case-control Study

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## Abstract

**Background:** Cholangiocarcinoma (CCA) caused by Opisthorchis viverrini is a well-known and significant public health issue in northeastern Thailand; however, pesticide exposure (PE) and CCA risk have not yet been established. Therefore, our research objective was to investigate the relationship between PE and CCA risk.

**Methods:** A hospital-based matched case-control study was carried out. All cases (in-patients) and controls (out-patients) were volunteers at a tertiary hospital in northeast Thailand. Between 2015 and 2019, 178 incident cases of pathologically-confirmed CCA and 356 controls were selected from the check-up clinic at Srinagarind Hospital outpatient database (two controls per case). The recruited controls were individually matched to the CCA cases based on sex, age (±5 years), and admission date (±3 months). During face-to-face interviews, a standardised pre-tested questionnaire was used to collect data. Multivariable conditional logistic regression was used to analyse the data.

**Results:** The respective frequency of PE between the 178 CCA cases and 356 controls was 77.0% vs. 87.6% for never, 14.6% vs. 5.3% for have but stopped, and 8.4% vs. 7.0% for current users. After adjusting for the highest educational attainment, smoking behaviour, alcohol use, and history of cancer in the family, PE was not significantly associated with CCA (p-value = 0.086). Using volunteers who have never used PE as the reference group, the respective odds of developing CCA for those who have ever used but have since stopped and currently using was 2.04 (adjusted OR = 2.04; 95 % CI: 1.03 – 4.04) vs. 0.83 (adjusted OR=0.83; 95% CI: 0.39 – 1.76) times more likely to develop CCA than those who had never used PE.

**Conclusion**: There is no association between PE and the risk of CCA. Future research should focus on enhancing PE assessment methods that consider complex chemical mixtures, chemicals of interest, historical exposure, and exposure pathways. In addition, it emphasised the need for more extensive and longer population-based cohort studies that include younger, non-occupationally exposed individuals during periods of developmental susceptibility.

**Keywords:** Cholangiocarcinoma; Pesticide; Epidemiology; Risk Factors

## Introduction

Cholangiocarcinoma (CCA) or biliary tract cancers include intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) [1, 2]. In many regions of the world, the incidence and mortality of CCA have increased. Mortality from ICC globally has trended to increase. In contrast, mortality from ECC has decreased in most nations—rates being around 1 to 2 per 100,000 person-years for ICC and below 1 per 100,000 person-years for ECC. Increased incidence is responsible for the rise in ICC mortality, while laparoscopic cholecystectomy is responsible for the decline in ECC mortality [3-5].

In Thailand and other parts of Southeast Asia, liver cancer and bile duct are considered one of the most common cancers in men and females (age-standardised rate, ASR=33.9, and 12.9 per 100,000, respectively) [1,6,7].The most common histological type is CCA, which comprises between 82.0% and 89.0 % of all primary liver cancers detected [8]. Thailand has one of the highest incidence rates of CCA globally [6]. In Thailand, the respective overall ASR for CCA between 1989 and 2018 for all ages in males vs. females was 36.1 per 100,000 person-years (95% CI; 35.3 to 36.8) vs. 14.4 per 100,000 person-years (95% CI; 13.9 to 14.8) [9]. The increased incidence is accompanied by low 5-year relative survival rates (5-30% between 2000 and 2014) because many cases go undetected for decades and are diagnosed late [10]. Between 1988 and 2015, the 5-year relative survival (RS) for CCA was 10.9% (95%CI: 10.3 to 11.6). Based on age at diagnosis, the respective highest and lowest 5-year RS rate was under 40 years at 25.4% (95%CI: 20.4 to 30.7) and between 51 and 61 at 9.4% (95%CI: 8.4 to 10.6). As for the decade of diagnosis, the respective highest and lowest 5-year RS rate was 1999–2003 at 14.5% (95%CI: 13.0 to 16.2) and 2009–2013 at 5% (95%CI: 4.1 to 6.1). Due to the high prevalence of liver fluke infection [12], the ASR of liver and bile duct cancer was between 53.4 and 94.8 per 100,000 for males and between 18.5 and 39.0 per 100,000 for females between 1988 and 2015 [11,12]. Several risk factors for CCA have been investigated in Thailand, but *Opisthorchis viverrini (O. viverrini)* is most often implicated in the genesis of CCA [13-17].

Cancer is a multifactorial disease in which genetic, environmental, and lifestyle factors all play a role. Pesticide exposure is recognized as a significant environmental risk factor linked to cancer development. The epidemiology of pesticide exposure and human cancer has been studied globally. Insecticides, herbicides, and fungicides are linked to cancers of the hematopoietic system and cancers of the prostate, pancreas, and liver [18]. However, the involvement of pesticides in CCA cancer is currently unknown. In developing countries, epidemiological research and evidence are insufficient to establish a link between pesticide exposure and the development of cancer. Consequently, the purpose of this narrative review was to summarize recent epidemiological research examining the links between pesticide exposure (PE) and the risk of CCA.

## Methods

### Study Design

We conducted a hospital-based matched case-control study of patients admitted to Srinagarind Hospital in Khon Kaen, Northeast Thailand. This facility is a regional tertiary referral centre and the primary teaching hospital for the Faculty of Medicine at Khon Kaen University (KKU).

A total of 178 cases were all incident cases with pathologically-confirmed CCA. The controls were 356 volunteers selected from the check-up clinic as the healthy section project database (two controls per case). We recruited CCA case and controls in the study between 2015 and 2019. The two controls were individually matched with each CCA case by sex, age (within 5 years), and date of admission (within 3 months). Age and sex were chosen as matching variables because previous studies have found that both factors were associated with CCA [19, 20].

Data were collected from the recruited in-patients through a face-to-face interview with a trained interviewer using a standardised pre-tested questionnaire. The questionnaire was developed by researchers and validated by specialists in the field of CCA, and a pilot test was carried out in 20 cases and controls. The questionnaire items were designed to obtain information about potential risk factors. They included questions on demographic variables, history of morbidity and cancer, exposure to pesticides and use of toxic chemicals, stool examination of *O. viverrini* and Praziquantel treatment, a history of eating local nitrite-containing foods, alcohol consumption, smoking behaviour, source of water consumption, and a family history of cancer.

#### *Eligibility Criteria and Operational Definitions*

The eligibility criteria for both cases and controls were as follows:

1. The volunteer had provided signed informed consent to participate in the study, and
2. The volunteer could speak and understand Thai, provide reliable information and was both well enough and sufficiently cognitively intact to respond to interview questions. The trained interviewer determined this criterion.

#### *Cases*

The following additional eligibility criteria were used to define an individual as a CCA case:

1. CCA was the primary diagnosis;
2. The CCA was histologically confirmed by pathologists from the Department of Pathology in the Faculty of Medicine, Khon Kaen University; and,

3. The volunteer was an in-patient who was an incident case of CCA and had been admitted and diagnosed between January 1, 2015, and December 31, 2019, at Srinagarind Hospital.

***Controls***

The following additional eligibility criteria were used to define an individual as a control:

1. The volunteer did not have CCA. They also had no history of hepatic disease, liver cancer, or other malignancy—as determined from medical records by a research assistant. The hospital departments from which the controls were recruited were the Departments of Otolaryngology, Ophthalmology, Rehabilitation, and Orthopaedics.
2. The volunteer was an in-patient admitted to Srinagarind Hospital between January 1, 2015, and December 31, 2019.
3. The volunteer was selected from the Srinagarind Hospital out-patient check-up clinic database.

The process for the recruitment and enrolment of cases and controls is summarised in Figure 1.

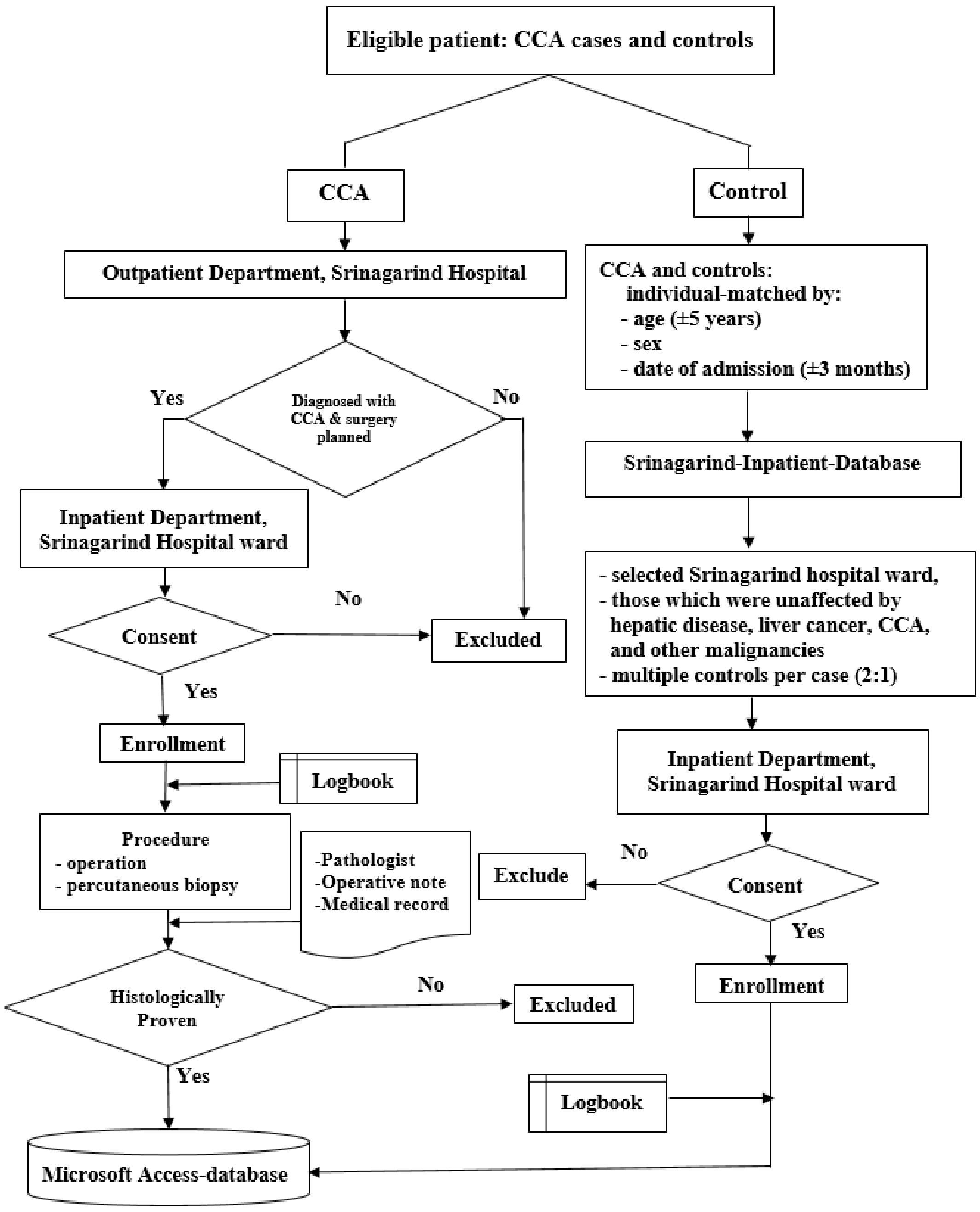


**Figure 1:** Patient enrollment, cases with CCA and controls (adapted from the association between praziquantel treatment and cholangiocarcinoma: a hospital-based matched case-control study) [38].

***Factor of interest***

The factor of interest was pesticide exposure (PE). The frequency of PE was categorised into three groups: never used (during lifetime), used but not currently (used, but not currently), and currently using (currently doing, never stopped) The trained interviewer showed a sample of PE to each participant when asking about the type of PE history.

***Potential Confounders***

Confounding factors were variables that were already known to be associated with CCA. These were various demographic variables, history of morbidity and cancer, pesticide exposure and use of toxic chemicals, stool examination of *O. viverrini* and Praziquantel treatment, a history of eating local nitrite-containing foods, alcohol consumption, smoking behaviour, source of water consumption, and a family history of cancer. A history of the use of toxic chemicals included the use of (1) herbicides, (2) rodenticides, (3) insecticides, and (4) fungicides.

Smokers (as opposed to those who 'never smoked') included ex-smokers (who smoked at least once a day in the past but quit more than one year ago), occasional smokers (those now smoking less than once per day), and current smokers (those smoking at least once per day).

Drinkers (as opposed to those who 'never drank') included ex-drinkers (consumed alcohol at least once a day in the past, but quit drinking more than a year previously), occasional drinkers (those presently using alcohol less than once a day), and current drinkers (those using alcohol at least once daily).

A history of eating local foods containing nitrites included eating (1) salted freshwater fish and salted meat, (2) fermented fish products such as *Pla-chao* and *Pla-chom*, (3) grilled or smoked meat, and (4) sausages (including Chinese sausages).

***Sample Size Calculation***

The estimated sample size required for the cases and controls was 178 and 356, respectively. This was based on estimating sample sizes in pair-matched case-control studies [21], adjusting the variance inflation factor [22], and the case-control ratio of 1:2.

***Data processing***

Data were entered on a computer using Microsoft Access [23]. Stata statistical software [24], was used for data verification, and data entry errors were corrected. When the questionnaires were incomplete, the researcher contacted the volunteers to obtain the missing information.

## Statistical Analyses

***Description of demographic characteristics of CCA and controls***

The characteristics of the subjects were summarised using descriptive statistics. Means and standard deviations, medians, and their ranges (minimum and maximum) were used for continuous variables, and frequency counts and percentages were used for categorical variables.

***Crude analysis***

A crude analysis was performed to determine the associations of PE and other factors with CCA without controlling for confounding variables. Bivariate conditional logistic regression computed the crude odds ratio (crude OR) and their 95 % confidence intervals (95 % CI).

***Multivariable analysis***

Multivariable conditional logistic regression [25, 26] was used to calculate the adjusted odds ratio (adjusted OR) and their 95 % confidence intervals (95 % CI) to investigate the effect of PE on CCA while controlling for the effects of confounding variables.

***Model fitting***

Candidate variables for the multivariable analysis were selected according to two criteria: firstly, variables in the crude analysis with a p-value of < 0.25, and secondly, variables from the literature shown to have an association with CCA. Next, the backwards stepwise elimination method was used as the model-fitting strategy. Finally, a likelihood ratio test was performed to assess the goodness-of-fit of the final model. All analyses were performed using Stata version 15.0 [24]. All test statistics were two-sided, and a p-value of < 0.05 was considered statistically significant.

***Ethical considerations***

The Human Research and Ethics Committee of Khon Kaen University reviewed and approved this project (registration: HE581141). Written informed consent was obtained from each of the patients in these studies. As for patient records in this study, all data were fully anonymised before use.

**Data Availability Statement:**

All relevant data are contained in the manuscript and its supporting information files.

## Results

***Matching Variables and Descriptive Epidemiology***

***Matching variables***

One hundred seventy-eight volunteers were recruited as CCA cases, and 356 were available for analysis as controls. None of the otherwise eligible patients declined to consent to participate. To summarise the outcome of the matching procedure. For all three matching variables, the percentages of CCA and control were the same or similar (Table 1).

| Variable | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Sex** | | | | |
| Male | 113 | 63.5 | 228 | 64.0 |
| Female | 65 | 36.5 | 128 | 36.0 |
| **Age at recruitment (years)** | | | | |
| Younger than 40 | 5 | 2.8 | 10 | 2.8 |
| 41 - 50 | 20 | 11.2 | 40 | 11.2 |
| 51 - 60 | 61 | 34.3 | 158 | 44.4 |
| Older than 60 | 92 | 51.7 | 148 | 41.6 |
| Mean (Standard deviation) | 59.7 (8.5) | | 58.9 (8.5) | |
| Median (Minimum: Maximum) | 61 (31:76) | | 59 (26:79) | |
| **Date of admission (Jan 2015-Dec 2019)** | | | | |
| 1st year recruitment | | | | |
| Jan 2015 – March 2015 | - | - | 1 | 0.6 |
| Apr 2015 – Jun 2015 | 4 | 2.3 | 3 | 1.7 |
| Jul 2015 – Sep 2015 | 3 | 1.7 | 7 | 3.7 |
| Oct 2015 – Dec 2015 | 4 | 2.3 | 3 | 1.7 |
| 2nd year recruitment | | | | |
| Jan 2016 – March 2016 | 8 | 4.5 | 4 | 2.2 |
| Apr 2016 – Jun 2016 | 8 | 4.5 | 6 | 3.3 |
| Jul 2016 – Sep 2016 | 6 | 3.4 | 12 | 6.6 |
| Oct 2016 – Dec 2016 | 10 | 5.6 | 6 | 3.3 |
| 3rd year recruitment | | | | |
| Jan 2017 – March 2017 | 8 | 4.5 | 6 | 3.3 |
| Apr 2017 – Jun 2017 | 9 | 5.1 | 8 | 4.4 |
| Jul 2017 – Sep 2017 | 9 | 5.1 | 20 | 11.0 |
| Oct 2017 – Dec 2017 | - | - | 3 | 1.7 |
| 4th year recruitment 2018 | | | | |
| Jan 2018 – March 2018 | 6 | 3.4 | 1 | 0.6 |
| Apr 2018 – Jun 2018 | 6 | 3.4 | 2 | 1.1 |
| Jul 2018 – Sep 2018 | 12 | 6.7 | 12 | 6.6 |
| Oct 2018 – Dec 2018 | 13 | 7.3 | 22 | 12.1 |

| | | | | |
|---|---|---|---|---|
| 5th year recruitment 2019 | | | | |
| Jan 2019 – March 2019 | 22 | 12.4 | 18 | 9.9 |
| Apr 2019 – Jun 2019 | 28 | 15.7 | 33 | 18.1 |
| Jul 2019 – Sep 2019 | 13 | 7.3 | 12 | 6.6 |
| Oct 2019 – Dec 2019 | 9 | 5.1 | 3 | 1.7 |

**Table 1:** Outcomes of CCA and controls matching procedure

***Descriptive epidemiology***

*Chemical exposure*

The occupations and occupational exposure of the 178 volunteers recruited as CCA cases follow. The respective current pesticide use was (99, 55.6%), (4, 2.2%), and (1, 0.6%) for rice farming, gardening, and TamRai. The proportional type currently used was pesticide (composite variables) (15, 8.4%), herbicide (12, 6.8%), insecticide (3, 1.7%), rodenticide (2, 1.1%), and fungicide (0, 0%). As for Hired for pesticide spraying, there were none (including, not ever, currently doing, or never stopped).

As for the 356 controls recruited, it included those who do rice farming, gardening, and cash crops (*Tam Rai*) for whom the respective current pesticide use was (135, 37.9%), (4, 1.1%), and (3, 0.8%). The respective type of pesticide (composite variables), herbicides, insecticides, rodenticides, and fungicide was (25, 7.0%), (23, 6.5%), (8, 2.2%), (2, 0.6%), and (1, 0.3%). Hired for spraying pesticides (i.e., current use) was 20.6%.

***General information***

As for CCA cases, they were frequently uneducated or had only finished primary school (168, 47.2%). Marital status was mostly married or common-law (161, 90.5%). Two-thirds worked in agriculture (119, 66.9%). Income averaged 8,747.8 baht (SD=12,898.7 baht) while the median was 3,000 baht (min=400 baht; max=62,000 baht)

As for the controls, most were uneducated or had only finished primary school (136, 76.4%). Marital status was mainly married or common-law (299, 84.0%). On the other hand, a sizeable minority worked in agriculture (147, 41.3%). Income averaged 21,3141.1 baht (SD=36,356.8 baht), while the median was 1,000 baht (min=600 baht; max=500,000 baht).

*History of Opisthorchis viverrini infection and use of praziquantel*

As for CCA cases, stool examination was available for most (123, 69.1%), and most took praziquantel to treat the *O. viverrini* infestation (132,74.2%). As for the controls, a stool examination was done for most (263, 73.9%), and just over half had taken praziquantel to treat an *O. viverrini* infestation (202, 56.7%).

*History of eating local food*

As for CCA cases, the majorit were eating grilled/smoked meat and grilled/smoked fish (172, 96.6%), followed by cooked fermented fish (*Pla-ra*) (166, 93.2%), salted freshwater fish and salted meat (118, 66.3%), fermented vegetables or fruit (116, 65.2%), Chinese or other sausages (110, 61.8%), partially cooked, raw meat (74, 41.6%), fermented products (69, 38.8%), raw fermented fish (*Pla-ra*) (63, 35.4%), raw, partially cooked of fermented fish (*Pla-ra*) (52, 29.2%), and raw, partially cooked, raw fish (35, 19.7%).

As for controls, grilled/smoked fish (346, 97.2%) were eaten by the majority, followed by cooked fermented fish (*Pla-ra*) (327, 91.9%), salted freshwater fish, salted meat (269, 75.6%), Chinese sausage/other sausages (256, 71.9%), fermented vegetables or fruit (227, 63.8%), raw, partially cooked, raw meat (132, 37.1%), fermented product (128, 36.0%), raw fermented fish (*Pla-ra*) (114, 32.0%), grilled/smoked meat, raw, partially cooked of fermented fish (*Pla-ra*) (80, 22.5%), and raw, partially cooked, raw fish (47, 13.2%).

*Smoking and Alcohol drinking history*

A sizable proportion of CCA cases were smokers (74, 41.6%) and drinkers (108, 60.7%). A comparable proportion of controls were smokers (126, 35.4%) and drinkers (204, 57.3%).

*History of cancer in family*

The respective proportion of CCA cases and controls with a history of cancer in family was 34.8% (n=62) and 27% (n=96) (Table 2).

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Agricultural occupation** | | | | |
| **Farm** | | | | |
| Never | 38 | 21.4 | 145 | 40.7 |
| Have but stopped | 41 | 23.0 | 76 | 21.4 |
| Current use | 99 | 55.6 | 135 | 37.9 |
| **Garden** | | | | |
| Never | 173 | 97.2 | 352 | 98.9 |
| Have but stopped | 1 | 0.6 | - | - |
| Current use | 4 | 2.2 | 4 | 1.1 |
| **Tam Rai** | | | | |
| Never | 176 | 98.9 | 353 | 99.2 |
| Have but stopped | 1 | 0.5 | - | - |
| Current use | 1 | 0.6 | 3 | 0.8 |

**Table 2A:** Baseline characteristics between CCA and controls: Chemical exposed

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Type of pesticide** | | | | |
| **Pesticide (Composite variables)** | | | | |
| Never | 137 | 77.0 | 312 | 87.6 |
| Have but stopped | 26 | 14.6 | 19 | 5.3 |
| Current use | 15 | 8.4 | 25 | 7.0 |
| **Herbicides** | | | | |
| Never used | 143 | 80.1 | 319 | 89.6 |
| Have but stopped | 23 | 13.1 | 14 | 3.9 |
| Current used | 12 | 6.8 | 23 | 6.5 |
| **Insecticide**s | | | | |
| Never used | 165 | 92.7 | 343 | 96.4 |
| Have but stopped | 10 | 5.6 | 5 | 1.4 |
| Current used | 3 | 1.7 | 8 | 2.2 |
| **Rodenticid**e | | | | |
| Never used | 171 | 96.1 | 347 | 97.5 |
| Have but stopped | 5 | 2.8 | 7 | 1.9 |
| Current used | 2 | 1.1 | 2 | 0.6 |
| **Fungicid**e | | | | |

| | | | | |
|---|---|---|---|---|
| Never used | 177 | 99.4 | 353 | 99.1 |
| Have but stopped | 1 | 0.6 | 2 | 0.6 |
| Current used | - | - | 1 | 0.3 |

**Table 2B:** Baseline characteristics between CCA and controls: Type of pesticides (Cont.)

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Sprayer, Hire for spraying pesticide** | | | | |
| Never used | 175 | 98.3 | 349 | 98.0 |
| Have but stopped | 3 | 1.7 | 5 | 1.4 |
| Current used | - | - | 2 | 0.6 |

**Table 2C:** Baseline characteristics between CCA and controls: Sprayer (Cont.)

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Highest education attainment** | | | | |
| Uneducated or only primary school | 168 | 47.2 | 136 | 76.4 |
| Hight school or Diploma | 53 | 14.9 | 22 | 12.4 |
| Bachelor's degree or higher | 135 | 37.9 | 20 | 11.2 |
| **Marital status** | | | | |
| Single | 4 | 2.2 | 19 | 5.3 |
| Married | 161 | 90.5 | 299 | 84.0 |
| Divorce | 3 | 1.7 | 10 | 2.8 |
| Spouse died | 10 | 5.6 | 28 | 7.9 |
| **Occupation** | | | | |
| Agriculture | 119 | 66.9 | 147 | 41.3 |
| Government service | 59 | 33.1 | 209 | 58.7 |
| **Income (Thai baht)** | | | | |
| lower than10,000 | 142 | 79.8 | 190 | 53.3 |
| 10,001 – 20,000 | 10 | 5.6 | 39 | 11.0 |
| 20,001 – 30,000 | 14 | 7.9 | 38 | 10.7 |
| 30,001 – 40,000 | 2 | 1.1 | 28 | 7.9 |
| 40,001 – 50,000 | 5 | 2.8 | 19 | 5.3 |
| More than 50,000 | 5 | 2.8 | 42 | 11.8 |
| Mean (Standard deviation) | 8,747.8 (12,898.7) | | 21,314.1 (36,356.8) | |
| Median (Minimum: Maximum) | 3,000 (400: 62,000) | | 10,000 (600: 500,000) | |

**Table 2D:** Baseline characteristics between CCA and controls: General information (Cont.)

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Fecal stool** | | | | |
| No | 55 | 30.9 | 93 | 26.1 |
| Yes | 123 | 69.1 | 263 | 73.9 |
| **Take PZQ for treat *O. viverrini* infestation** | | | | |
| No | 46 | 25.8 | 154 | 43.3 |
| Yes | 132 | 74.2 | 202 | 56.7 |

**Table 2E:** Baseline characteristics between CCA and controls: History of *O. viverrini* infection and use of Praziquantel (Cont.)

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Salted freshwater fish, salted meat** | | | | |
| Never | 37 | 20.8 | 76 | 21.9 |
| Have but stopped | 23 | 12.9 | 9 | 2.5 |
| Current used | 118 | 66.3 | 269 | 75.6 |
| **Raw, partially cooked raw fish** | | | | |
| Never | 87 | 48.9 | 277 | 77.8 |
| Have but stopped | 56 | 31.4 | 32 | 9.0 |
| Current used | 35 | 19.7 | 47 | 13.2 |
| **Raw, partially cooked raw meat** | | | | |
| Never | 59 | 33.1 | 205 | 57.6 |
| Have but stopped | 45 | 25.3 | 19 | 5.3 |
| Current used | 74 | 41.6 | 132 | 37.1 |
| **Fermented product** | | | | |
| Never | 83 | 46.6 | 213 | 59.8 |
| Have but stopped | 26 | 14.6 | 15 | 4.2 |
| Current used | 69 | 38.8 | 128 | 36.0 |
| **Grilled/smoked meat, Grilled/smoked fish** | | | | |
| Never | 1 | 0.6 | 7 | 2.0 |
| Have but stopped | 5 | 2.8 | 3 | 0.8 |
| Current used | 172 | 96.6 | 346 | 97.2 |
| **Chinese sausage/other sausages** | | | | |
| Never | 54 | 30.3 | 90 | 25.3 |
| Have but stopped | 14 | 7.9 | 10 | 2.8 |
| Current used | 110 | 61.8 | 256 | 71.9 |
| **Fermented vegetables or fruit** | | | | |
| Never | 44 | 24.7 | 124 | 34.8 |

| | | | | |
|---|---|---|---|---|
| Have but stopped | 18 | 10.1 | 5 | 1.4 |
| Current used | 116 | 65.2 | 227 | 63.8 |
| **Cooked Fermented fish (*Pla-ra Suk*)** | | | | |
| Never | 3 | 1.7 | 26 | 7.3 |
| Have but stopped | 9 | 5.1 | 3 | 0.8 |
| Current used | 166 | 93.2 | 327 | 91.9 |
| **Raw, partially cooked of fermented fish (*Pla-ra Su*k Dip)** | | | | |
| Never | 108 | 60.7 | 271 | 76.1 |
| Have but stopped | 18 | 10.1 | 5 | 1.4 |
| Current used | 52 | 29.2 | 80 | 22.5 |
| **Raw fermented fish (*Pla-r*a Dip)** | | | | |
| Never | 95 | 53.4 | 237 | 66.6 |
| Have but stopped | 20 | 11.2 | 5 | 1.4 |
| Current used | 63 | 35.4 | 114 | 32.0 |

**Table 2F:** Baseline characteristics between CCA and controls: History of eating local food (Cont.)

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **Smoking behavior** | | | | |
| Never smoked | 104 | 58.4 | 230 | 64.6 |
| Smoker | 74 | 41.6 | 126 | 35.4 |
| **Alcohol use** | | | | |
| Never used | 70 | 39.3 | 152 | 42.7 |
| Drinkers | 108 | 60.7 | 204 | 57.3 |

**Table 2G:** Baseline characteristics between CCA and controls: History of smoking and alcohol drinking (Cont.)

| Characteristic | CCA | | Controls | |
|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) |
| **History of cancer in family** | | | | |
| No | 116 | 65.2 | 260 | 73.0 |
| Yes | 62 | 34.8 | 96 | 27.0 |

**Table 2H:** Baseline characteristics between CCA and controls, History of cancer in family (Cont.)

*Crude analysis*

The results of the crude analysis are presented in Table 3. Factors found to be significantly associated with CCA (p-value 0.05) included herbicide use, insecticide use, fungicide use, highest educational attainment, occupation, income, Praziquantel treatment, and eating salted freshwater fish, salted meat, raw or partially cooked fish, raw or partially cooked meat, fermented products, Chinese sausage or other sausages, fermented vegetables or fruit, and cooked fermented fish (Pla-ra). Meanwhile, the following factors were not significantly associated with CCA (p-value > 0.05): herbicide, insecticide and fungicide use, smoking behavior, and the consumption of salted freshwater fish, salted meat, and grilled or smoked meat (Table 3).

| Characteristics | Cholangiocarcinoma | | Controls | | Crude OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | |
| **Composite variables** | | | | | | | |
| **1. Pesticide** | | | | | | | **0.001** |
| Never | 137 | 77.0 | 312 | 87.6 | 1 | - | |
| Ever but have stopped | 26 | 14.6 | 19 | 5.3 | 3.09 | 1.64 - 5.81 | |
| Current use | 15 | 8.4 | 25 | 7.0 | 1.40 | 0.69 - 2.81 | |
| **2. Herbicides** | | | | | | | **0.001** |
| Never | 141 | 80.1 | 318 | 89.6 | 1 | - | |
| Ever but have stopped | 23 | 13.1 | 14 | 3.9 | 3.60 | 1.78 - 7.28 | |
| Current use | 12 | 6.8 | 24 | 6.5 | 1.18 | 0.56 - 2.49 | |
| **3. Insecticides** | | | | | | | **0.029** |
| Never | 165 | 92.7 | 343 | 96.4 | 1 | - | |
| Ever but have stopped | 10 | 5.6 | 5 | 1.4 | 3.97 | 1.36 - 11.62 | |
| Current use | 3 | 1.7 | 8 | 2.3 | 0.78 | 0.19 - 3.17 | |
| **4. Rodenticide** | | | | | | | **0.648** |
| Never | 171 | 96.1 | 347 | 97.5 | 1 | - | |
| Ever but have stopped | 5 | 2.8 | 7 | 2.0 | 1.48 | 0.44 - 4.95 | |
| Current use | 2 | 1.1 | 2 | 0.6 | 2.00 | 0.28 - 14.20 | |
| **5. Fungicide** | | | | | | | **0.667** |
| Never | 177 | 99.4 | 353 | 99.2 | 1 | - | |
| Ever but have stopped | 1 | 0.6 | 2 | 0.6 | 0.10 | 0.09 - 11.03 | |
| Current use | - | - | 1 | 0.3 | NA | NA | |
| | | | | | | | |

**Table 3A:** Crude odds ratio for CCA associations with PE and various risk factors, Type of pesticides.

| Characteristics | Cholangiocarcinoma | | Controls | | Crude OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | |
| **6. Sprayer, Hire for spraying pesticide** | | | | | | | **0.429** |
| Never | 175 | 98.3 | 349 | 98.0 | 1 | - | |
| Ever | 3 | 1.7 | 5 | 1.4 | 1.23 | 0.26 - 5.75 | |
| Yes | - | - | 2 | 0.6 | NA | NA | |

**Table 3B:** Crude odds ratio for CCA associations with PE and various risk factors, Sprayer (Cont.)

| Characteristics | Cholangiocarcinoma | | Controls | | Crude OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | |
| **7. Highest education attainment** | | | | | | | **< 0.001** |
| Uneducated and primary school | 168 | 47.2 | 136 | 76.4 | 1 | - | |
| Hight school or Diploma | 53 | 14.9 | 22 | 12.4 | 0 .47 | 0.26 - 0.83 | |
| Bachelor's degree and higher | 135 | 37.9 | 20 | 11.2 | 0 .18 | 0.10 - 0.31 | |
| **8. Marital status** | | | | | | | **0.163** |
| Single | 4 | 2.3 | 19 | 5.3 | 1 | - | |
| Married | 161 | 90.5 | 299 | 83.9 | 2.55 | 0.86 - 7.53 | |
| Divorce | 3 | 1.7 | 10 | 2.8 | 1.36 | 0.26 - 6.97 | |
| Spouse as dead | 10 | 5.6 | 28 | 7.9 | 1.67 | 0.46 - 6.03 | |
| **9. Occupation** | | | | | | | **< 0.001** |
| Agriculture | 119 | 66.9 | 147 | 41.3 | 1 | - | |
| Government service | 59 | 33.2 | 209 | 58.7 | 0.37 | 0.25 - 0.54 | |
| **10. Income (Thai baht)** | | | | | | | **< 0.001** |
| lower than10,000 | 142 | 79.8 | 190 | 53.4 | 1 | - | |
| 10,001 – 20,000 | 10 | 5.6 | 39 | 10.9 | 0.33 | 0.15 - 0.71 | |
| 20,001 – 30,000 | 14 | 7.8 | 38 | 10.7 | 0.52 | 0.26 - 1.03 | |
| 30,001 – 40,000 | 2 | 1.1 | 28 | 7.9 | 0.11 | 0.02 - 0.45 | |
| 40,001 – 50,000 | 5 | 2.8 | 19 | 5.3 | 0.36 | 0.13 - 0.10 | |
| More than 50,000 | 5 | 2.8 | 42 | 11.8 | 0.18 | 0.07 - 0.48 | |

**Table 3C:** Crude odds ratio for CCA associations with PE and various risk factors, General information (Cont.)

| Characteristics | Cholangiocarcinoma | | Controls | | Crude OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | |
| **11. Fecal stool** | | | | | | | **0.260** |
| No | 55 | 30.9 | 93 | 26.1 | 1 | - | |
| Yes | 123 | 69.1 | 263 | 73.9 | 0.80 | 0.54 - 1.18 | |
| **12. Take PZQ for treat *O. viverrini* infestation** | | | | | | | **< 0.001** |
| No | 46 | 25.8 | 154 | 43.3 | 1 | - | |
| Yes | 132 | 74.2 | 202 | 56.7 | 2.61 | 1.67 - 4.09 | |

**Table 3D:** Crude odds ratio for CCA associations with PE and various risk factors, History of *Opisthorchis viverrini* infection and use of Praziquantel (Cont.)

| Characteristics | Cholangiocarcinoma | | Controls | | Crude OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | |
| **13. Salted freshwater fish, salted meat** | | | | | | | **< 0.001** |
| Never | 37 | 20.8 | 76 | 21.9 | | | |
| Ever but have stopped | 23 | 12.9 | 9 | 2.5 | 35.3 | 4.53 - 275.49 | |
| Current used | 118 | 66.3 | 269 | 75.6 | 1.01 | 0.63 - 1.60 | |
| **14. Raw, partially cooked raw fish** | | | | | | | **< 0.001** |
| Never | 87 | 48.9 | 277 | 77.8 | | | |
| Ever but have stopped | 56 | 31.5 | 32 | 8.9 | 7.85 | 4.21 - 14.62 | |
| Current used | 35 | 19.7 | 47 | 13.2 | 2.64 | 1.51 - 4.63 | |
| **15. Raw, partially cooked raw meat** | | | | | | | **< 0.001** |
| Never | 59 | 33.2 | 205 | 57.6 | 12.15 | 5.82 - 25.37 | |
| Ever but have stopped | 45 | 25.3 | 19 | 5.3 | 2.42 | 5.82 - 25.37 | |
| Current used | 74 | 41.6 | 132 | 37.1 | | 1.54 - 3.79 | |
| **16. Fermented product** | | | | | | | **< 0.001** |
| Never | 83 | 46.6 | 213 | 59.8 | | | |
| Ever but have stopped | 26 | 14.6 | 15 | 4.2 | 6.78 | 2.85 - 16.11 | |
| Current used | 69 | 38.7 | 128 | 35.9 | 1.35 | 0.91 - 1.99 | |
| **17. Grilled/smoked meat, Grilled/smoked fish** | | | | | | | **0.074** |
| Never | 1 | 0.6 | 7 | 1.9 | | | |
| Ever but have stopped | 5 | 2.8 | 3 | 0.8 | 15.15 | 1.04 - 219.76 | |
| Current used | 172 | 96.6 | 346 | 97.2 | 3.51 | 0.43 - 28.45 | |
| **18. Chinese sausage/other sausages** | | | | | | | **< 0.001** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Never | 54 | 30.3 | 90 | 25.3 | | | |
| Ever but have stopped | 14 | 7.9 | 10 | 2.8 | 6.77 | 1.44 - 31.85 | |
| Current used | 110 | 61.8 | 256 | 71.9 | 0.72 | 0.48 - 1.09 | |
| **19. Fermented vegetables or fruit** | | | | | | | **< 0.001** |
| Never | 44 | 24.7 | 124 | 34.8 | | | |
| Ever but have stopped | 18 | 10.1 | 5 | 1.4 | 10.88 | 3.53 - 33.52 | |
| Current used | 116 | 65.2 | 227 | 63.7 | 1.41 | 0.94 - 2.14 | |
| **20. Cooked Fermented fish (Pla-ra Suk)** | | | | | | | **< 0.001** |
| Never | 3 | 1.7 | 26 | 7.3 | | | |
| Ever but have stopped | 9 | 5.1 | 3 | 0.8 | 23.91 | 4.10 - 139.40 | |
| Current used | 166 | 93.3 | 327 | 91.9 | 4.28 | 1.28 -14.37 | |
| **21. Raw, partially cooked of fermented fish (Pla-ra Suk Dip)** | | | | | | | **< 0.001** |
| Never | 108 | 60.7 | 271 | 76.1 | | | |
| Ever but have stopped | 18 | 10.1 | 5 | 1.4 | 20.24 | 4.58 - 89.41 | |
| Current used | 52 | 29.2 | 80 | 22.5 | 1.75 | 1.15 - 2.67 | |
| **22. Raw fermented fish (Pla-ra Dip)** | | | | | | | **< 0.001** |
| Never | 95 | 53.4 | 237 | 66.6 | | | |
| Ever but have stopped | 20 | 11.2 | 5 | 1.4 | 20.19 | 4.64 - 87.80 | |
| Current used | 63 | 35.4 | 114 | 32.0 | 1.42 | 0.93 - 2.14 | |

**Table 3E:** Crude odds ratio for CCA associations with PE and various risk factors, History of eating local food. (Cont.).

| Characteristics | Cholangiocarcinoma | | Controls | | Crude OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | |
| **23. Smoking behavior** | | | | | | | **0.063** |
| Never smoked | 104 | 58.4 | 230 | 64.6 | 1 | - | |
| Smoker | 74 | 41.6 | 126 | 35.4 | 1.61 | 0.97 - 2.67 | |
| **24. Alcohol use** | | | | | | | **0.210** |
| Never used | 70 | 39.3 | 152 | 42.7 | 1 | - | |
| Drinkers | 108 | 60.7 | 204 | 57.3 | 1.49 | 0.79 - 2.80 | |

**Table 3F:** Crude odds ratio for CCA associations with PE and various risk factors, History of smoking and alcohol drinking (Cont.)

| Characteristics | Cholangiocarcinoma | | Controls | | Crude OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | |
| **25. History of cancer in family** | | | | | | | **0.053** |
| No | 116 | 65.2 | 260 | 73.1 | 1 | - | |
| Yes | 62 | 34.8 | 96 | 26.9 | 1.49 | 0.99 - 2.23 | |

**Table 3G:** Crude odds ratio for CCA associations with PE and various risk factors, History of cancer in family (Cont.).

*Association between CCA, chemical exposures and nitrite-containing food: combining variables*

We combined four chemical variables (herbicide, rodenticide, insecticide, and fungicide use) into one variable, 'Never', 'Used but have stopped using', and 'Currently using', depending on whether the volunteers reported ever having used one or more of the chemicals on at least one occasion. The decision to combine a variable was made because of concern that volunteers may have risk accumulation [27]. The outcome of combining these variables in a univariate analysis is presented in Table 3.

*Multivariable analysis*

Table 4 shows the outcome of the multivariable analysis of the factors associated with CCA. After adjusting for highest educational attainment, smoking behaviour, alcohol consumption, and history of cancer among relatives, pesticide exposure (PE) was not significantly associated with CCA (p-value = 0.086). Using subjects who never used PE as the reference group, the respective odds of developing CCA for those who have used but stopped using and currently using was 2.04 (95 % CI: 1.03 – 4.06) and 0.83 (95 CI: 0.39 – 1.76) times more likely to develop CCA than those who had never used the PE (Table 4).

| Characteristic | CCA | | Controls | | Crude OR | Adjusted OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | | |
| **Composite variables** | | | | | | | | |
| **1. Pesticide** | | | | | | | | 0.086 |
| Never | 137 | 77.0 | 312 | 87.6 | 1 | 1 | - | |
| Have but stopped | 26 | 14.6 | 19 | 5.3 | 3.09 | 2.04 | 1.03 - 4.06 | |
| Current use | 15 | 8.4 | 25 | 7.0 | 1.40 | 0.83 | 0.39 - 1.76 | |
| **2. Highest education attainment** | | | | | | | | < 0.001 |
| Uneducated or primary school | 168 | 47.2 | 136 | 76.4 | 1 | 1 | - | |
| Hight school or Diploma | 53 | 14.9 | 22 | 12.4 | 0 .47 | 0.49 | 0.27 - 0.90 | |
| Bachelor's degree or higher | 135 | 37.9 | 20 | 11.2 | 0 .18 | 0.18 | 0.10 - 0.32 | |
| **3. Smoking behavior** | | | | | | | | 0.681 |
| Never smoked | 104 | 58.4 | 230 | 64.6 | 1 | 1 | - | |
| Smoker | 74 | 41.6 | 126 | 35.4 | 1.61 | 1.13 | 0.64 - 2.0 | |
| **4. Alcohol use** | | | | | | | | 0.206 |
| Never used | 70 | 39.3 | 152 | 42.7 | 1 | 1 | - | |
| Drinker | 108 | 60.7 | 204 | 57.3 | 1.49 | 1.60 | 0.76 - 3.36 | |

| Characteristic | CCA | | Controls | | Crude OR | Adjusted OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|---|
| | Number (n = 178) | Percent (%) | Number (n = 356) | Percent (%) | | | | |
| **Composite variables** | | | | | | | | |
| **5. History of cancer in family** | | | | | | | | 0.022 |
| No | 116 | 65.2 | 260 | 73.1 | 1 | 1 | - | |
| Yes | 62 | 34.8 | 96 | 26.9 | 1.49 | 1.69 | 1.07 - 2.65 | |

**Table 4:** Adjusted odds ratios for CCA associations with pesticide exposure and various risk factors.

## Discussion

The current study investigated the associations between PE and the risk of developing CCA. The results showed that after adjusting for the highest educational attainment, smoking behaviour, alcohol use, and history of cancer in the family, PE was not significantly associated with CCA (p-value = 0.086). Compared to those who had never used PE, those who had used but stopped, and those who were still using had 2.04 (95 %CI: 1.03 – 4.06) and 0.83 (95% CI: 0.39 – 1.76) times the odds of developing CCA, respectively. (Table 4)

*Environmental exposures, including pesticides and liver cancer*

Twenty-eight liver cancer studies have documented a positive association between developing cancer and exposure to aflatoxin, air pollution, polycyclic aromatic hydrocarbons, asbestos, chimney sweeping occupation, and paints. By comparison, there was an inverse association between ultraviolet radiation and a null/inconsistent result for organic solvents, pesticides, perfluorooctanoic acid, nuclear radiation, iron foundry occupation, and brick kiln pollution [28]. There were five studies on liver cancer and nonalcoholic fatty liver disease (NAFLD). The NAFLD studies found a positive association with cancer and exposure to heavy metals, methyl tertiary-butyl ether, and selenium and no association with trihalomethanes [28]. Even though recent evidence suggests causal relationships between pesticide exposure and cancer, the most robust evidence exists for acute myeloid leukemia (AML) and colorectal cancer (CRC) [29].

*Exposures to pesticides and risk of cancer: empirical findings*

*Pesticides*

Pesticides are chemicals that are used to control pests like insects and are thought to influence human hepatocarcinogenesis through oxidative stress, genotoxicity, and immunotoxicity [30]. Several studies revealed that dichlorodiphenyltrichloroethane (DDT), an organochlorine insecticide, was associated with increased HCC risk (IARC Group 2A) [30, 31]. A prospective cohort study conducted by the Korean Veterans Health Study discovered that Agent Orange exposure (a TCDD-contaminated herbicide used during the Vietnam War) was associated with an increased risk of liver cancer (adjusted HR=1.16, 95% CI 1.01–1.34) [32]. The association was stronger among those who served in the Vietnam War for more than six months and those who served in a military unit with greater exposure risk [32]. A cross-sectional study in the same population found similar results, even though data collection for liver cancer was based on self-report [33].

Relatedly, a US-based Agricultural Health Study prospective cohort reported that higher intensity-weighted lifetime days of occupational metolachlor (a herbicide) exposure was associated with an increased risk of liver cancer (adjusted RR=3.18; 95% CI: 1.10–9.22)[34]. In a retrospective case-control study in China, self-reported pesticide exposure was associated with an increased HCC risk (adjusted OR=1.99; 95% CI: 1.10–3.60) [36]. By contrast, a retrospective case-control study in the US found that geospatial-based residential exposure to pesticides (from organochlorines, organophosphates, and carbamates) was not associated with HCC risk [35]. While the latter found a possible positive association with organochlorine pesticides, the study was limited to participants living in agriculturally intensive areas. The retrospective Canadian Census Health and Environment Cohort study reported an inverse association with liver cancer risk among male agricultural workers (adjusted HR=0.51; 95% CI; 0.38–0.68). There was no association among female agricultural workers compared to all other employed individuals. Problematically, the healthy worker effect and/or residual confounding from smoking and alcohol consumption could have influenced the findings [37].

In summary, mixed evidence suggests a possible link between specific pesticides and HCC risk, with biomarker-based studies providing the most substantial evidence. In particular, organochlorine pesticides (such as DDT) may increase the risk of HCC. However, previous research did not demonstrate a link between pesticide exposure and CCA cancer risk.

*CCA and O. viverrini*

CCAs arise primarily from the epithelial lining of the bile duct (intra- and extrahepatic bile duct) and exclude gallbladder and ampulla of Vater malignancies [5, 7]. CCAs are uncommon in most countries, except in Thailand [4].

In Thailand and other parts of Southeast Asia, CCA ranks as one of the most common cancers in males and females (age-standardised rate, ASR 33.9 and 12.9 per 100,000, respectively) [5]. This is likely due to the high incidence of liver fluke infection [6]. The high ASRs of CCA in northern and northeastern Thailand are linked to opisthorchiasis, an endemic parasitic disease caused by *O. viverrini* infections. *O. viverrini* is now classified as a Group 1 carcinogenic agent by the International Agency for Research on Cancer (IARC) [39]. The most significant risk factor for CCA is *O. viverrini* infection [13-16]. Human infection with *O. viverrini* is primarily caused by eating raw or undercooked cyprinoid fish, the intermediate host containing *O. viverrini* larvae [40-41].

Though high-quality studies on organophosphate insecticides have been published since the IARC monograph in 2017, there are still gaps in the literature for human carcinogenic evidence, including many potentially carcinogenic pesticides that were not included in the IARC assessment. For AML and CRC, there is consistent and high-quality evidence vis-à-vis the carcinogenicity of pesticides. However, for liver cancer, such as hepatocellular carcinoma, most studies found no association between self-reported and/or occupational pesticide exposure and liver cancer risk. Moreover, no association between pesticide exposure and CCA cancer was found in prior research.

*CCA, according to location and cell type*

Since CCA can be divided into papillary and tubular CCA based on cell type and intrahepatic and extrahepatic based on location, several risk factors have distinct associations based on CCA cell type and location. Therefore, we further analysed the association between PE and CCA according to cell type and location and found no statistically significant associations with PE (p-value > 0.05).

**Conclusions**

This study demonstrated no association between PE and CCA risk. Future research should focus on improved PE assessment methods that consider complex chemical mixtures, the chemicals of interest, historical exposure, and exposure pathways. To justify regulatory action that would reduce human exposure to pesticides in the future, more large-scale and longer population-based cohort studies should include younger, non-occupationally exposed individuals during developmental periods of susceptibility.

**Advantages and disadvantages of exposure and risk of CCA**

*Strengths of the study*

This is the first study to examine environmental exposures, including pesticides, the risk of CCA cancer, and all CCA cases confirmed by histology. CCA is an ICD-O-3 diagnosis, and only the cases with coding C22.1, C24.0, C24.8, and C24.9 (excluding C24.1, Ampulla of Vater) were included. Histological confirmation via biopsy or surgical resection is the gold standard; consequently, other confirmation methods may have misclassified CCA and non-CCA cases.

*Limitations of the study*

The current research found no link between PE and the risk of CCA. This was perhaps impacted by indirect pesticide exposure assessment methods, which most likely resulted in exposure misclassification. We propose that new techniques and methods be used to improve the sensitivity and precision of exposure assessment.

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