

REVIEW

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A Prospective Pooled Analysis of Meat Mutagens and Colorectal Adenoma and Cancer in the US and EPIC Studies: Findings with an Emphasis on Improving Exposure Measurements

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Abstract

Background: In animal studies, heterocyclic amines (HCAs) are recognized as having strong carcinogenicity, therefore we have hypothesized that HCAs might be associated with the risk of colorectal adenoma (CRA) and cancer (CRC). **Methods:** We used the Keywords of “Heterocyclic amines and colorectal cancer” to search, there were showing published articles (n=200). After reviews of titles, abstracts, and full articles, seven prospective cohort studies were included in the pooled analysis. Exposures to HCAs 2-amino-3,8-dimethylimidazo(4,5-j)quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), 2-amino-3,4,8-trimethylimidazo(4,5-f)quinoxaline (DiMeIQx), meat-derived mutagenicity (MDM), and the risk of CRA and CRC were examined. The estimated HCA intake as ng/day and ng/1,000 kcal/day by participants and by studies were examined. The ln(HR) and se(ln(HR)) were estimated from the multivariable-adjusted HR, 95%CI derived from seven published prospective studies in the US and EPIC. The random pooled multivariable-adjusted HR, 95%CI was analyzed using ln(HR) and se(ln(HR)) by STATA-10. **Results:** For CRC and HCA intake, the null association was observed for MDM, the random pooled multivariable-adjusted HR, (95%CI): 1.11, (1.00, 1.23); for PhIP: 1.00, (0.91, 1.09); and for DiMeIQx: 1.03, (0.87, 1.22). A significant positive association was seen for MeIQx, the random pooled multivariable-adjusted HR, (95%CI):1.12, (1.03, 1.22). For CRA and HCA intake, the null association was observed for MDM, randomly pooled multivariable-adjusted HR, (95%CI): 1.15, (0.99, 1.34), and for DiMeIQx: 1.09, (0.97, 1.23). A significant positive association was seen for PhIP, the random pooled multivariable-adjusted HR, (95%CI): 1.19, (1.02, 1.39), and for MeIQx: 1.17, (1.01, 1.35). The major instances of HCAs were contributed by chicken (54%-74%) for PhIP and by red meat (83%-92%) for MeIQx. However, the estimated PhIP intake (ng/1,000 kcal/day) was remarkably different between studies. **Conclusions:** We observed a positive association between exposures to MeIQx and the risk of both CRC and CRA which supports the hypothesis of the role of HCAs in developing CRA and CRC. Improving the quality of the estimated HCA intake would be highly concerned for further investigation.

Keywords: Meat mutagens- heterocyclic amines- colorectal adenoma and cancer- diet- cooking methods

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Introduction

In 2012, the estimated incidence of colorectal cancers (CRC) in the world was 1,360,602 cases and the disease has been predicted to increase by 60% to 2.2 million incident cases in 2030 (IARC, 2012). Red meat has been recognized as a major risk of colorectal cancer (WCRF, 2007; WCRF, 2011) and its underlying mechanisms were unclear. Epidemiological study on the association between meat mutagens of Heterocyclic amines, recognized as having strong carcinogenicity in rats and mice, and CRC has been intensely done, from 1997 (Augustsson et al., 1999; Destefani et al., 1997) to date (Le, 2018). However, previous prospective cohort studies have shown

inconsistent findings (Cross et al., 2010; Le et al., 2016; Ollberding et al., 2012). The recently published findings of a Meta-analysis of meat mutagens and colorectal adenoma and cancer suffer from numerous errors (Chiavarini et al., 2017) that have been highlighted and published elsewhere (Le, 2018). The association between Heterocyclic Amines (HCAs) intake and the risk of colorectal cancer (CRC) cannot be conclusively predicted as yet. Examination of existing prospective cohort studies on HCAs intake and CRC needs more in-depth analysis to come to a possible conclusion on any association with promoting primary cancer prevention and healthy diets that exclude CRC.

To date, 40 years have passed since HCAs were first detected in charred parts of broiled fish and meat in 1977

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(Sugimura, 1997). HCAs in food are mainly produced from sugar and amino acids in meat (red meats, poultry meats, and fish) upon heating (Sugimura, 2000); referred to as “Maillard Reaction” (Maillard, 1912). The work in 1912 entitled “Action of amino acids on sugars: formation of melanoidins in a methodical way”, and later work in 1953, “Dehydrated foods, the chemistry of browning reactions in model systems”, lead to the development of food technology worldwide to make food browned, tasty, and agreeable (Hodge, 1953; Maillard, 1912). HCAs are heated food-borne carcinogens/carcinogenicity and pluripotent in inducing malignancies in any part of the rats or mice including the liver, lung, stomach, urinary bladder, blood vessels, colon, skin, mammary gland, lymphoid tissue, oral cavity (Sugimura, 1997). HCAs include a group of more than 20 types (Sugimura, 2000) of chemical substances and in addition to a type of 2-amino-1,7-dimethylimidazo[4,5-g]quinoxaline (7-MeIQx) that was recognized and reported in 2007 (Turesky et al., 2007). Due to a limitation in technology, there are possible unrecognized types of HCAs that occur during heating meat at high temperatures and for an extended time. Exposure to HCAs, however, seems inevitable during the ordinary human life of eating heated red meat, poultry and fish either homemade or commercially supplied and fast foods, which may be a significant public health concern by the individual as well as all societies.

The American Cancer Research in 2007 and 2011 concluded that red meat (beef, pork, lamb, and goat from domesticated animals) increases the risk of CRC (WCRF (World Cancer Research Fund), 2007; WCRF (World Cancer Research Fund), 2011). Red meat was a major source of MeIQx intake in many previous studies (Byrne et al., 1998; Martinez et al., 2007; Wu et al., 2006). In 1993, the IARC (International Agency for Research on Cancer) concluded that HCAs of PhIP, MeIQx, DiMeIQx may be carcinogenic to humans (Group 2B) (IARC, 1993). In 2014, the National Institute of Health concluded that PhIP and MeIQx are “reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting genotoxicity data” (NTP (National Toxicology Program), 2014). Nearly 20 years ago, the Lancet published the null association between HCAs intake and the risk of CRC based on findings from a case-control study (Augustsson et al., 1999). Later, from 1996 to 2000, baseline or follow-up surveys, for determining prospective cohort participants included cooking methods (Cross et al., 2010; Ferrucci et al., 2012; Le et al., 2016; Ollberding et al., 2012). However, findings from these studies are inconsistent (Cross et al., 2010; Ferrucci et al., 2012; Le et al., 2016; Ollberding et al., 2012) as the individual prospective study is limited by specific population demographics with limited sample sizes. A systematic review and META analysis was performed. The aim was to address initial achievements and to learn the methods of assessment of HCA exposure for further better performing observational study on the association between HCA intake and the risk of CRA and CRC.

Materials and Methods

Data collection

We focused on pooled analysis by a systematic review and META analysis based on existing published prospective cohort studies. Using EndNote9 and the Keywords of “Heterocyclic amines and colorectal cancer” to search on 20 May 2022, there were showing published articles (n=200). Published articles were excluded based on title/abstract (n=192), there were remaining prospective cohort studies and systematic review and META analysis (n=8). One more prospective cohort study was added after the full-text article was reviewed. A systematic review and META analysis was excluded (n=2). There were eligible prospective cohort studies included in the pooled analysis of colorectal cancer (n=3) and colorectal adenoma (n=4), Figure 1.

Participated study populations

Study populations included Nurses’ Health Study (NHS, 65,875 female participants for CRC study) and Health Professionals Follow-up Study (HPFS, 29,615 male participants for CRC and 14,032 for CRA studies) operated by Channing Division of Network Medicine, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, and Harvard T.H. Chan School of Public Health (Le et al., 2016; Wu et al., 2006); The National Institute of Health, American Association of Retired Persons (NIH-AARP) Diet and Health Study (NIH-AARP, 300,948 male and female participants for CRC study) operated by National Cancer Institute (Cross et al., 2010); The Multiethnic Cohort Study (MEC, 131,763 male and female participants for CRC study) operated by University of Hawaii and the University of Southern California (Ollberding et al., 2012); The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO, 17, 072 male and female participants for CRA study) operated by National Cancer Institute (Ferrucci et al., 2012); The ursodeoxycholic acid (UDCA) trial (869 male and female participants for CRA study) operated by the University of Arizona (Martinez et al., 2007); and the European cohort (EPIC) study (21,452 participants for CRA study) operated by the Heidelberg Study, Table 1.

Estimation and validation of HCAs intake

Types of HCAs being examined

Exposures to HCAs 2-amino-3,8-dimethylimidazo(4,5-j)quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), 2-amino-3,4,8-trimethylimidazo(4,5-f)quinoxaline (DiMeIQx), meat-derived mutagenicity (MDM), and the risk of CRA and CRC were examined. The estimated amount of these four types of HCAs was presented in ng/1000 kcal/day or ng/day. For comparison, we converted from ng/day into ng/1000 kcal/day referred to as calories intake available in the published articles, Table 2.

Selection of food items contributed to HCAs intake

Based on a pilot study to predict a possible food item contributing high amounts of PhIP, MeIQx, and DiMeIQx, the results indicated that 18 food items of pan-fried

chicken (or turkey), broiled chicken (or turkey), grilled/BBQ chicken (or turkey), pan-fried fish, grilled/BBQ fish, broiled fish, pan-fried steak (NHS only), grilled/BBQ steak, broiled steak, pan-fried hamburger (HPFS only), broiled fish hamburger, grilled/BBQ hamburger, roast beef, homemade beef gravy, pan-fried bacon, broiled bacon, microwave bacon, and fried sausage predicted contributing a major of these three types of HCAs (Byrne et al., 1998). Food items contributing high amounts of PhIP were, from highest to lowest, grilled/BBQ chicken (or turkey), broiled chicken (or turkey), pan-fried chicken (or turkey), broiled fish, and grilled/BBQ steak (Five items: Chicken 3, red meat 1, and fish 1); MeIQx was pan-fried hamburger, pan-fried steak, homemade beef gravy, grilled/BBQ hamburger, grilled/BBQ steak, pan-fried chicken (or turkey), broiled steak, grilled/BBQ chicken (or turkey), and fried sausage (Nine items: Chicken 1, red meat 8, fish zero); DiMeIQx were pan-fried steak, grilled/BBQ chicken (or turkey), homemade beef gravy, pan-fried chicken (or turkey), broiled chicken (or turkey), pan-fried bacon, pan-fried hamburger, grilled/BBQ hamburger, and broiled steak (Nine items: chicken 3, red meat 6, fish zero) (Byrne et al., 1998). The above 18 food items plus two items of pan-fried pork chops/ham, and grilled pork chops/ham were included fully or partially in six prospective cohort studies in the US using the CHARRED Database to estimate intake amount by the study participants (Cross et al., 2010; Ferrucci et al., 2012; Le et al., 2016; Martinez et al., 2007; NCI (National Cancer Institute), 2006; Ollberding et al., 2012; Wu et al., 2006).

Follow-up and case ascertainment

The periods, from the baseline or follow-up surveys of cooking methods in estimating amount intake of HCAs to the last follow-up survey varied by study, ranging from shortest to longest, 31 months (Martinez et al., 2007), 3-5 years (Ferrucci et al., 2012), 7.2 years on average (Cross et al., 2010), 8.1 years in average (Ollberding et al., 2012), and 14 years (Le et al., 2016). The number of CRC was 418 (HPFS), 790 (NHS), 2,719 (NIH-AARP), and 1757 (MEC), giving a total of 5,684 CRC cases available from the present pooled analysis. For CRA, the number was 581 (HPFS), 379 (UDCA), 516 (EPIC), and 1,008 (PLCO), giving a total of 2,484 cases, Table 1.

Data analysis

Four prospective cohort studies on meat mutagens and CRC have shown 5 levels of exposure from quintile-1 to quintile-5 and multivariable-adjusted hazard ratios and 95% confident interval (HR, 95%CI) for PhIP, MeIQx, DiMeIQx. Of which only three studies of NHS, HPFS, and NIH-AARP analyzed for MDM. Analysis of pooled multivariable-adjusted hazard ratios and 95% confident interval (HR, 95%CI) was performed for quintile-5 versus quintile-1 for risk of CRC.

For CRA, exposure levels to HCAs have shown 5 quintiles (HPFS), 4 quintiles (EPIC and PLCO), and 3 quintiles (UDCA) for PhIP, MeIQx, DiMeIQx. Of these four studies, only three studies (HPFS, PLCO, and UDCA), were analyzed for MDM. Analysis of pooled

multivariable adjusted (HR, 95%CI) was performed for both quintile-3 versus quintile-1 and quintile-highest versus quintile-1 for risk of CRA due to the exposure to HCAs intake. The random pooled multivariable adjusted (HR, 95%CI) was the final finding for both CRA and CRC.

The $\ln(\text{HR})$ and $\text{se}(\ln(\text{HR}))$ were estimated from the multivariable-adjusted (HR, 95%CI) derived from seven published prospective studies, of which one study has shown results for both CRA and CRC (HPFS). The random pooled HR, 95%CI was analyzed using $\ln(\text{HR})$ and $\text{se}(\ln(\text{HR}))$ by STATA-10. The number of data pooled to estimate the risk of CRA and CRC was four for PhIP, MeIQx, DiMeIQx, and three for MDM.

Results

The number of CRC was 5,684 registered cases among 528,111 participants and for CRA were 2,484 registered cases among 53,425 participants. These four prospective cohort studies were conducted in the US and used the same CHARRED Database to calculate HCA intake, Table 1. Chicken contributed to the highest proportion of PhIP (54%-74%) while red meat contributed to the highest proportion of MeIQx (83%-92%) (Data not shown). The estimated HCA intake (ng/1,000 kcal/day) for quintiles-1 to quintile-5 was remarkably lower in the NIH-AARP for PhIP: 2.10, 10.90, 24.70, 49.40, and 123.50, respectively, when compared with that in the MEC: 35.34, 130.52, 256.05, 465.45, 1,027.30, respectively; for MeIQx: 0.50, 2.40, 5.30, 10.30, and 24.40, respectively in the NIH-AARP, when compared with that in the MEC: 3.09, 20.91, 49.02, 95.04, and 208.18, respectively in the MEC. In contrast, the estimated red meat intake (g/1000 kcal/day) for quintiles-1 to quintile-5 was lower in the NIH-AARP when compared with that in the MEC, as much as about 20% for all quintiles. The ratio of PhIP estimated in MEC per NIH-AARP, from quintile-1 to quintile-5, was 16.8, 12.0, 10.4, 9.4, and 8.2, respectively. The estimated HCA intake of PhIP and MeIQx in the HPFS (Quintile-5: 103.57 ng/1000 kcal/day) and PLCO (Quintile-4: 102.99 ng/1000 kcal/day) was similar to it in the NIH-AARP (Quintile-5: 123.60 ng/1000 kcal/day). The estimated PhIP intake was suggested to be lower in the EPIC (in Germany) than that in the UDCA (in the US), that is, 41.3 or higher ng/day (the highest exposure, quintile-4 in the EPIC) versus 90.0-1406.9 ng/day (the highest exposure, quintile-3 in the UDCA), Table 2.

For CRC, quintile-5 versus Quintile-1, the random multivariable-adjusted pooled HR, (95%CI) was 1.11, (1.00, 1.23), $P=0.052$, heterogeneity $P=0.685$ for MDM; HR, (95%CI): 1.00, (0.91, 1.09), $P=0.958$, heterogeneity $P=0.661$ for PhIP; HR, (95%CI): 1.12, (1.03, 1.22), $P=0.009$, heterogeneity $P=0.400$ for MeIQx; HR, (95%CI): 1.03, (0.87, 1.22), $P=0.763$, heterogeneity $P=0.013$ for DiMeIQx. Two methods, fixed and random, have shown similar results to pooled analysis for MDM, PhIP, and MeIQx, Table 3. The random weights of the pooled analysis, quintile-5 versus Quintile-1, were greatest by the NIH-AARP of 241.1, followed by MEC of 154.7, NHS of 67.5, and HPFS of 34.0 for PhIP. Similar random weights appeared for MeIQx and DiMeIQx, Figure 2.

Table 1. Characteristics of Prospective Cohort Studies on Meat Mutagens and Colorectal Adenoma and Cancer

| No | Lead author CRC | Journal | HCA | Quantile | Multivariable adjusted HR (95%CI) | Case | Participant | % | First baseline survey | Cooking methods survey time | Follow-up time | Population | Year | Exposure |
|---------------|------------------|----------|------------|----------|-----------------------------------|---------|-------------|------|-----------------------|-----------------------------|----------------|------------|------|----------|
| 1 | Cross (NH-AARP) | CR | MDM | 5 | 1.14 (1.01, 1.29) | 2,719 | 300,948 | 53.1 | 566,402 | 1995-1996 | 7 Years | US | 2010 | CHARRED |
| | | | PHIP | 5 | 0.99 (0.87, 1.12) | | | | | | | | | |
| | | | MeIQx | 5 | 1.19 (1.05, 1.34) | | | | | | | | | |
| | | | DiMeIQx | 5 | 1.17 (1.05, 1.29) | | | | | | | | | |
| 2 | Ollberding (MEC) | JIC | Total HCAs | 5 | 0.90 (0.76, 1.05) | 1,757 | 131,763 | 61.3 | 215,000 | 1999-2000 | 8.1 Years | US | 2012 | CHARRED |
| | | | PHIP | 5 | 0.95 (0.81, 1.11) | | | | | | | | | |
| | | | MeIQx | 5 | 1.01 (0.86, 1.19) | | | | | | | | | |
| | | | DiMeIQx | 5 | 0.88 (0.75, 1.03) | | | | | | | | | |
| 3 | Le (HPFS) | EHP | MDM | 5 | 1.02 (0.73, 1.41) | 418 | 29,615 | 57.5 | 51,529 | 1996 | 14 Years | US | 2016 | CHARRED |
| | | | PHIP | 5 | 1.01 (0.72, 1.41) | | | | | | | | | |
| | | | MeIQx | 5 | 1.22 (0.89, 1.68) | | | | | | | | | |
| | | | DiMeIQx | 5 | 0.88 (0.65, 1.19) | | | | | | | | | |
| | Le (NHS) | EHP | MDM | 5 | 1.04 (0.83, 1.30) | 790 | 65,785 | 54.1 | 121,700 | 1996 | 14 Years | US | 2016 | CHARRED |
| | | | PHIP | 5 | 1.14 (0.90, 1.45) | | | | | | | | | |
| | | | MeIQx | 5 | 1.07 (0.86, 1.34) | | | | | | | | | |
| | | | DiMeIQx | 5 | 1.15 (0.93, 1.43) | | | | | | | | | |
| Total CRC CRA | | | | | 5,684 | 528,111 | | | | | | | | |
| 4 | Wu (HPFS) | CEBP | MDM | 5 | 1.29 (0.97, 1.72) | 581 | 14,032 | 27.2 | 51,529 | 1996 | 7 Years | US | 2006 | CHARRED |
| | | | PHIP | 5 | 1.11 (0.85, 1.46) | | | | | | | | | |
| | | | MeIQx | 5 | 1.28 (0.95, 1.71) | | | | | | | | | |
| | | | DiMeIQx | 5 | 1.08 (0.86, 1.37) | | | | | | | | | |
| 5 | Martinez (JDCA) | Carcino. | MDM | 3 | 1.23 (0.86, 1.75) | 379 | 869 | 67.6 | 1,285 | 1996 | 31 months | US | 2007 | CHARRED |
| | | | PHIP | 3 | 0.97 (0.68, 1.39) | | | | | | | | | |
| | | | MeIQx | 3 | 1.32 (0.93, 1.88) | | | | | | | | | |
| | | | DiMeIQx | 3 | 1.37 (0.96, 1.94) | | | | | | | | | |
| 6 | Rohrman (EPIC) | AICN | MDM | 4 | 1.47 (1.13, 1.93) | 516 | 21,452 | 84 | 25,540 | 2002-2004 | 3-5 Years | Germany | 2009 | NA |
| | | | PHIP | 4 | 1.27 (0.97, 1.68) | | | | | | | | | |
| | | | MeIQx | 4 | 1.18 (0.92, 1.53) | | | | | | | | | |
| | | | DiMeIQx | 4 | 1.06 (0.86, 1.31) | | | | | | | | | |
| 7 | Ferreuci (PLCO) | BIC | MDM | 4 | 1.18 (0.96, 1.45) | 1,008 | 17,072 | 11 | 154,952 | 1993-2001 | 3-5 Years | US | 2012 | CHARRED |
| | | | PHIP | 4 | 0.99 (0.80, 1.23) | | | | | | | | | |
| | | | MeIQx | 4 | 0.99 (0.82, 1.20) | | | | | | | | | |
| | | | DiMeIQx | 4 | | | | | | | | | | |
| Total CRA | | | | | 2,484 | 53,425 | | | | | | | | |

HCAs, heterocyclic amines; PHIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; MDM: meat derived mutagenic activity

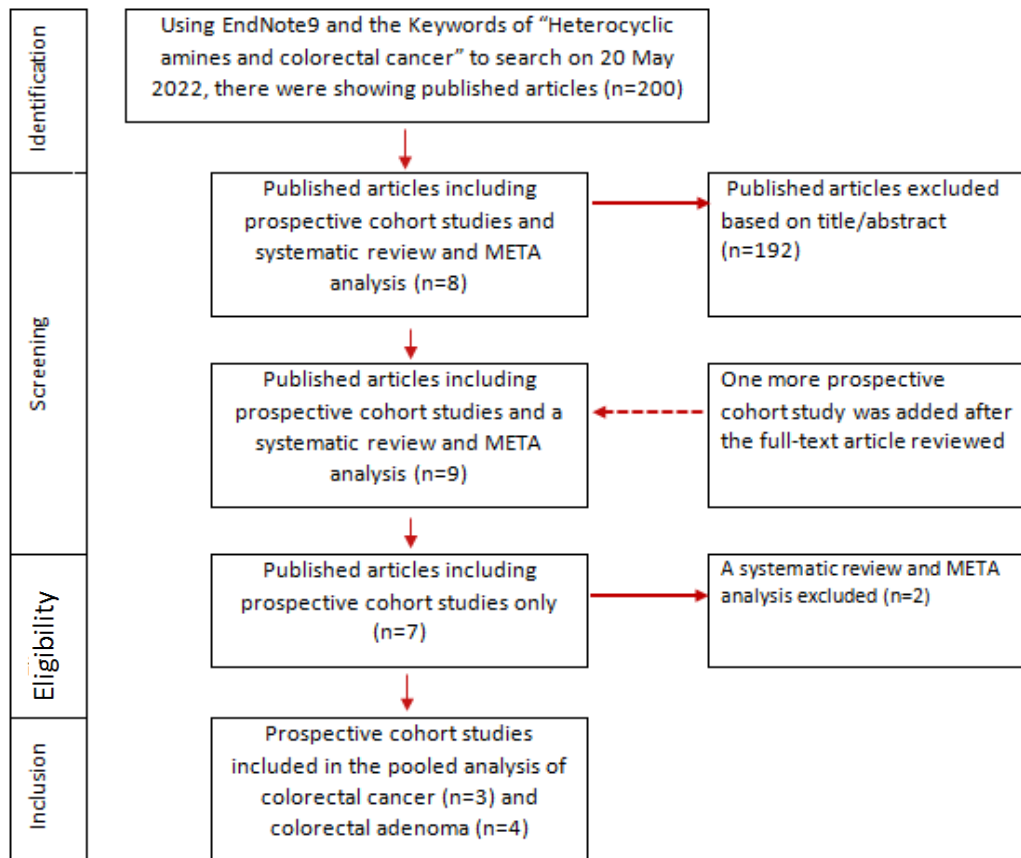


Figure 1. Flow Diagram of a Systematic Literature Search on Heterocyclic Amines Intake and the Risk of Colorectal Cancer and Adenoma based on Prospective Cohort Studies

For CRA, quintile-3 versus Quintile-1, the random pooled multivariable-adjusted HR, (95%CI) was 1.13, (0.92, 1.39), P=0.237, heterogeneity P=0.178 for MDM; HR, (95%CI): 1.11, (0.96, 1.29), P=0.169, heterogeneity

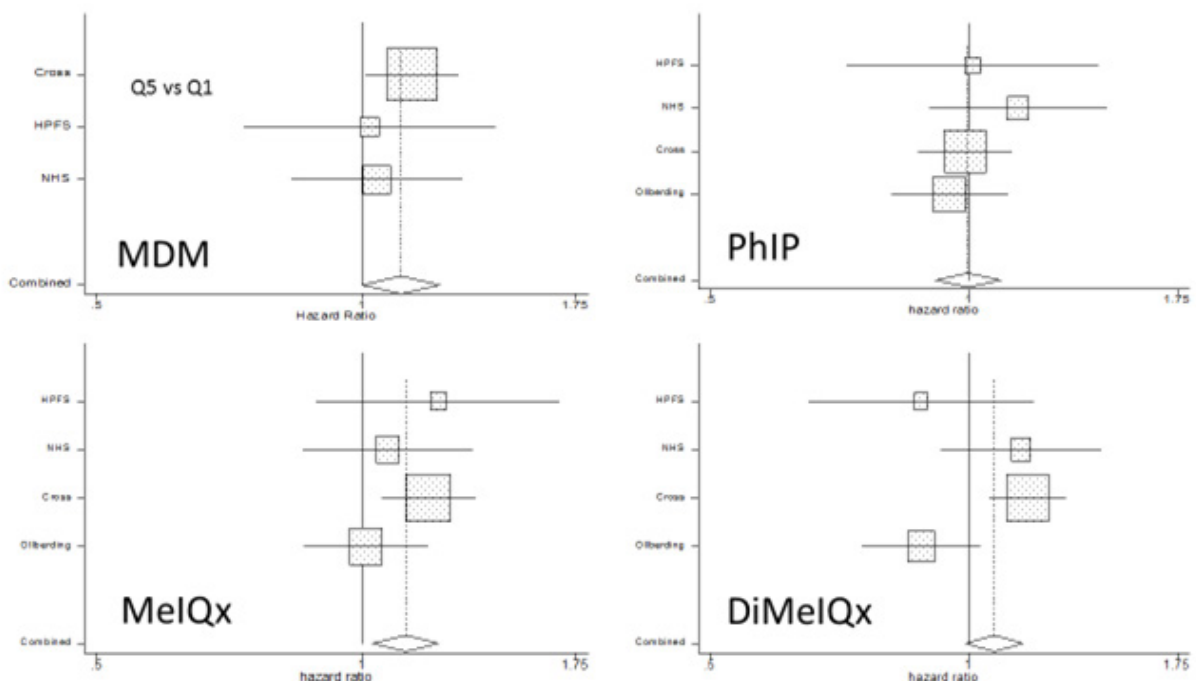


Figure 2. Pooled Multivariable-adjusted HR (95%CI) for Colorectal Cancer of Individual Study Populations and Overall Risk of Cancer based on Prospective Cohort Studies. Q5 vs Q1, Quintile-5 versus Quintile-1; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; MDM, meat derived mutagenic activity;

Table 2. Estimation of HCAIntake by Quintiles

| The lead author (study) | HCAs and some other indicators | Quintiles (Q1-Q5) | | | | |
|-----------------------------|--------------------------------|-------------------|-------------|---------------|----------|----------|
| | | Q1 | Q2 | Q3 | Q4 | Q5 |
| Colorectal Cancer | | | | | | |
| Cross (NIH-AARP) | MDM (ng/1,000 kcal/day) | 165 | 601 | 1,152.00 | 2,042.00 | 4,349.00 |
| | PhIP (ng/1,000 kcal/day) | 2.1 | 10.9 | 24.7 | 49.4 | 123.6 |
| | MeIQx (ng/1,000 kcal/day) | 0.5 | 2.4 | 5.3 | 10.3 | 24.4 |
| | DiMeIQx (ng/1,000 kcal/day) | 0 | 0.04 | 0.19 | 0.58 | 1.74 |
| | Red meat (g/1,000 kcal/day) | 9.5 | 20.9 | 30.7 | 42.1 | 61.6 |
| Oberding (MEC) | Total HCAs | 43.82 | 165.03 | 321.58 | 574.69 | 1,237.86 |
| | PhIP (ng/1,000 kcal/day) | 35.34 | 130.52 | 256.05 | 465.45 | 1,027.30 |
| | MeIQx (ng/1,000 kcal/day) | 3.09 | 20.91 | 49.02 | 95.04 | 208.18 |
| | DiMeIQx (ng/1,000 kcal/day) | 0.15 | 1.38 | 3.34 | 6.55 | 16.75 |
| | Red meat (g/1,000 kcal/day) | 7.41 | 16.56 | 24.55 | 33.37 | 47.99 |
| Colorectal Adenoma | | | | | | |
| Wu (HPFS) | MDM (ng/day) | 711 | 1,726.00 | 2,831.00 | 4,347.00 | 8,125.00 |
| | PhIP (ng/day) | 14.4 | 39.1 | 70.6 | 117.4 | 220.4 |
| | MeIQx (ng/day) | 1.5 | 5.3 | 9.9 | 17.3 | 35 |
| | DiMeIQx (ng/day) | 0 | 0.2 | 0.5 | 1.2 | 4 |
| | Calories (kcalo) by MDM | 1,827.00 | | | | 2,173.00 |
| | Calories (kcalo) by PhIP | 1,826.00 | | | | 2,128.00 |
| | Calories (kcalo) by MeIQx | 1,796.00 | | | | 2,265.00 |
| | Calories (kcalo) by DiMeIQx | 1,910.00 | | | | 2,108.00 |
| | MDM (ng/1,000 kcal/day) | 389.16 | | | | 3,739.07 |
| | PhIP (ng/1,000 kcal/day) | 7.89 | | | | 103.57 |
| | MeIQx (ng/1,000 kcal/day) | 0.84 | | | | 15.45 |
| | DiMeIQx (ng/1,000 kcal/day) | 0 | | | | 1.9 |
| | Ferrucci (PLCO) | MDM (ng/day) | 692 | 2,146.00 | 4,312.00 | 9,902.00 |
| PhIP (ng/day) | | 10.8 | 36.2 | 84.3 | 234.5 | |
| MeIQx (ng/day) | | 4.8 | 13.1 | 26.4 | 62.5 | |
| DiMeIQx (ng/day) | | 0 | 0.5 | 1.4 | 3.8 | |
| Calories (kcalo) | | 1,934.00 | 2,001.00 | 2,122.00 | 2,277.00 | |
| MDM (ng/1,000 kcal/day) | | 357.81 | 1,072.46 | 2,032.05 | 4,348.70 | |
| PhIP (ng/1,000 kcal/day) | | 5.58 | 18.09 | 39.73 | 102.99 | |
| MeIQx (ng/1,000 kcal/day) | | 2.48 | 6.55 | 12.44 | 27.45 | |
| DiMeIQx (ng/1,000 kcal/day) | | 0 | 0.25 | 0.66 | 1.67 | |
| Rohrmann (EPIC) | MDM (ng/day) | | | | | |
| | PhIP (ng/day) | <6.5 | 6.5-16.7 | 16.8-41.3 | 41.3+ | |
| | MeIQx (ng/day) | <3.8 | 3.8-9.2 | 9.3-19.8 | 19.9+ | |
| | DiMeIQx (ng/day) | <0.5 | 0.5-<1.5 | 1.5-<3.8 | 3.8+ | |
| Martinez (UDCA) | MDM (ng/day) | 0-1,710 | 1,724-4,389 | 4,390-136,556 | | |
| | PhIP (ng/day) | 0-27.9 | 28.3-89.5 | 90.0-1406.9 | | |
| | MeIQx (ng/day) | 0-10.7 | 10.8-30.6 | 30.7-403.9 | | |
| | DiMeIQx (ng/day) | 0-0.5 | 0.5-201 | 2.1-50.0 | | |

HCAs, heterocyclic amines; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx, 2-amino-3,8- dimethylimidazo[4,5-f]quinoxaline, DiMeIQx: 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; MDM, meat derived mutagenic activity

P=0.285 for PhIP; HR, (95%CI): 1.14, (1.00, 1.30), P=0.048, heterogeneity P=0.634 for MeIQx; HR, (95%CI): 1.00, (0.87, 1.15), P=0.999, heterogeneity P=0.296 for DiMeIQx. When we performed the pooled analysis, quintile-highest versus Quintile-1, the null

association was observed for MDM, the random pooled multivariable-adjusted HR, (95%CI): 1.15, 0.99, 1.34, P=0.069, heterogeneity P=0.514; and for DiMeIQx, the random pooled multivariable-adjusted HR, (95%CI): 1.09, 0.97, 1.23, P=0.142, heterogeneity P=0.394. We

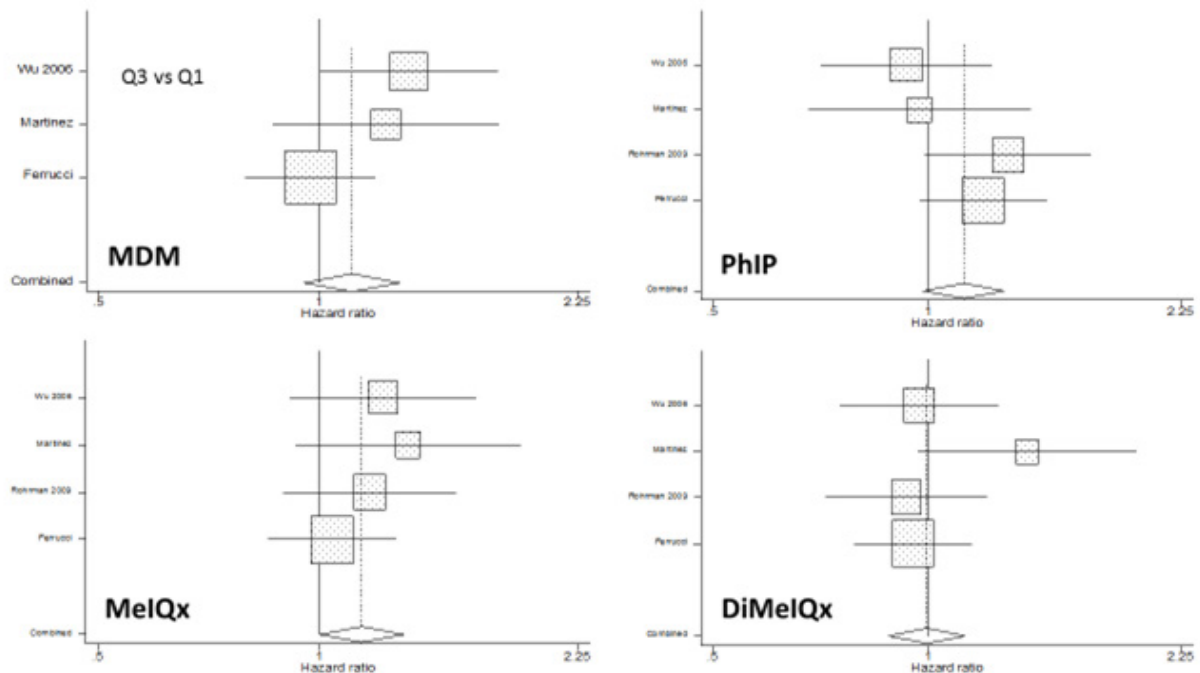


Figure 3. Pooled Multivariable-adjusted HR (95%CI) for Colorectal Adenoma of Individual Study Populations and Overall Risk of Adenoma (Q3 vs Q1) based on Prospective Cohort Studies. Q3 vs Q1, Quintile-3 versus Quintile-1; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; MDM, meat derived mutagenic activity

observed positive association for PhIP, the random pooled multivariable-adjusted HR, (95%CI): 1.19, 1.02, 1.39, $P=0.024$, heterogeneity $P=0.272$; and MeIQx, the random pooled multivariable-adjusted HR, (95%CI): 1.17, 1.01, 1.35, $P=0.040$, heterogeneity $P=0.327$, Table 4. The random weights of the pooled analysis, quintile-3 versus Quintile-1, were greatest by the PLCO of 63.4, followed by EPIC of 42.5, HPFS of 40.7, and UDCA of 26.2 for PhIP. We observed similar random weights for MeIQx and DiMeIQx, Figure 3, and noted similar observations in the pooled analysis, quintile-highest versus Quintile-1. The random weights appeared greatest by the PLCO of 59.9, followed by EPIC of 41.2, HPFS of 40.6, and UDCA of 25.7 for PhIP. We saw similar random weights for MeIQx

and DiMeIQx, Figure 4. To test for publication bias, the funnel plots appear symmetry, and there is evidence of bias-free using the Egger method for MDM, $P=0.189$; for PhIP, $P=0.480$; for DiMeIQx, $P=0.843$; and for DiMeIQx, $P=0.447$, Figure 5.

Discussion

The current pooled analysis of multivariable-adjusted hazard ratio and 95% confident interval was successfully performed for both CRA and CRC with a relatively large sample size of 2,484 CRA and 5,684 CRC, respectively. The findings were deemed consistent with the significant positive association between MeIQx intake and the

Table 3. Pooled Multivariable adjusted HR (95%CI) for Colorectal Cancer

| HCA | Method (Q-5 vs Q-1) | HR (95%CI) | p-value | Heterogeneity (p-value) | Data # |
|---------|---------------------|-------------------|---------|-------------------------|--------|
| MDM(a) | Fixed | 1.11 (1.00, 1.23) | 0.052 | 0.685 | 3 |
| | Random | 1.11 (1.00, 1.23) | 0.052 | | |
| MDM(b) | Fixed | 1.04 (0.96, 1.14) | 0.336 | 0.155 | 4 |
| | Random | 1.03 (0.91, 1.17) | 0.653 | | |
| PhIP | Fixed | 1.00 (0.91, 1.09) | 0.958 | 0.661 | 4 |
| | Random | 1.00 (0.91, 1.09) | 0.958 | | |
| MeIQx | Fixed | 1.12 (1.03, 1.22) | 0.009 | 0.400 | 4 |
| | Random | 1.12 (1.03, 1.22) | 0.009 | | |
| DiMeIQx | Fixed | 1.07 (0.99, 1.16) | 0.084 | 0.013 | 4 |
| | Random | 1.03 (0.87, 1.22) | 0.763 | | |

HCA, heterocyclic amines; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; MDM, meat derived mutagenic activity; Q-5 vs Q-1, Quintile-5 versus Quintile-1; Data #, Number of data used to calculate the risk; MDM(a), Not included total HCAs from the MEC study; MDM(b), Included total HCAs from the MEC study

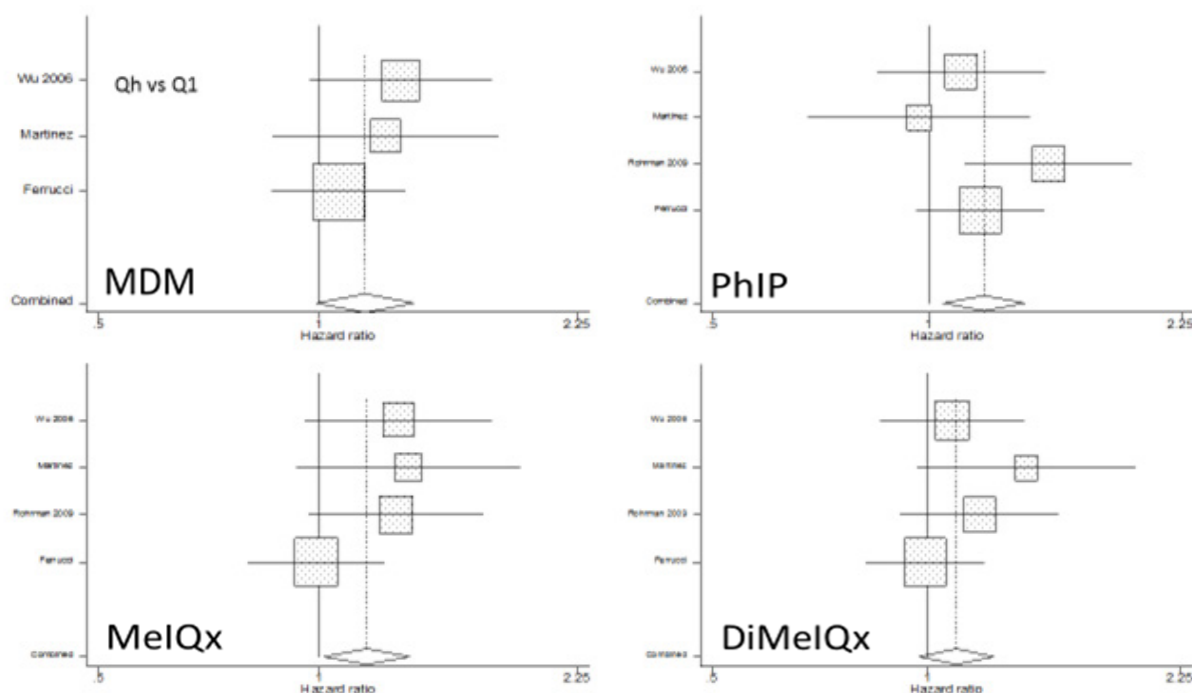


Figure 4. Pooled Multivariable-adjusted HR (95%CI) for Colorectal Adenoma of Individual Study Populations and Overall Risk of Adenoma (Qh vs Q1) based on Prospective Cohort Studies. Qh vs Q1, Quintile-highest versus Quintile-1; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; MDM, meat derived mutagenic activity

increased risk of both CRA and CRC. For CRA, we performed both pooled analyses for quintile-3 versus quintile-3 and quintile-highest versus quintile-1, because the origin exposure levels were 3 (one study) (Martinez et al., 2007), 4 (two studies) (Ferrucci et al., 2012; Rohrmann et al., 2009), and 5 (one study) (Wu et al., 2006). These

two approaches have shown consistent results of MeIQx and a positive association with the risk of CRA.

Because MeIQx was majority contributed by red meat, the present findings have to support the hypotheses of MeIQx acting as an underlying mechanism of red meat-induced malignancy in colorectal cancer. The present

Table 4. Pooled Multivariable adjusted HR (95%CI) for Colorectal Adenoma

| HCA | Method (Q-3 vs Q-1) | HR (95%CI) | p_value | Heterogeneity (p_value) | Data # |
|--------------|---------------------|-------------------|---------|-------------------------|--------|
| MDM(a) | Fixed | 1.11 (0.95, 1.28) | 0.190 | 0.178 | 3 |
| | Random | 1.13 (0.92, 1.39) | 0.237 | | |
| PhIP | Fixed | 1.12 (0.98, 1.27) | 0.096 | 0.285 | 4 |
| | Random | 1.11 (0.96, 1.29) | 0.169 | | |
| MeIQx | Fixed | 1.14 (1.00, 1.30) | 0.048 | 0.634 | 4 |
| | Random | 1.14 (1.00, 1.30) | 0.048 | | |
| DiMeIQx | Fixed | 0.99 (0.88, 1.12) | 0.918 | 0.296 | 4 |
| | Random | 1.00 (0.87, 1.15) | 0.999 | | |
| (Q-h vs Q-1) | | | | | |
| MDM (a) | Fixed | 1.15 (0.99, 1.34) | 0.069 | 0.514 | 3 |
| | Random | 1.15 (0.99, 1.34) | 0.069 | | |
| PhIP | Fixed | 1.19 (1.05, 1.36) | 0.008 | 0.272 | 4 |
| | Random | 1.19 (1.02, 1.39) | 0.024 | | |
| MeIQx | Fixed | 1.16 (1.01, 1.33) | 0.033 | 0.327 | 4 |
| | Random | 1.17 (1.01, 1.35) | 0.04 | | |
| DiMeIQx | Fixed | 1.09 (0.97, 1.23) | 0.142 | 0.394 | 4 |
| | Random | 1.09 (0.97, 1.23) | 0.142 | | |

HCA, heterocyclic amines; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeIQx, 2-amino-3,8- dimethylimidazo[4,5-f]quinoxaline; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; MDM, meat derived mutagenic activity; Q-3 vs Q-1, Quintile-3 versus Quintile-1; Q-h vs Q-1, Quintile-highest versus Quintile-1; Data #, Number of data used to calculate the risk; MDM(a), Missing in the EPIC study.

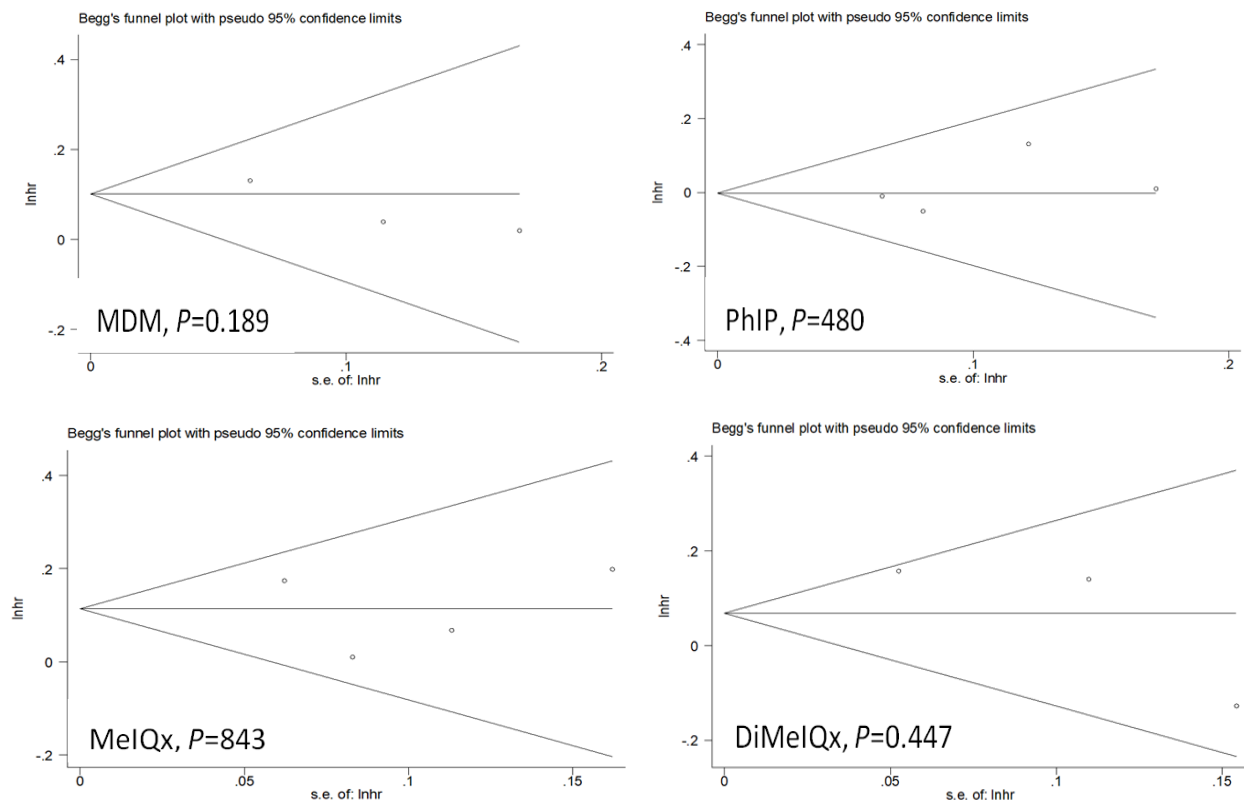


Figure 5. Funnel Plots of the Analyses of Meat Mutagens and the Risk of Colorectal Adenoma and Cancer based on Prospective Cohort Studies

observational findings add timely evidence to the role of MeIQx in developing colorectal cancer that supports the recent conclusions by the National Institute of Health that “MeIQx is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting genotoxicity data”(NTP (National Toxicology Program), 2014) and the conclusions of the American Cancer Research Fund in 2007 and 2011(WCRF (World Cancer Research Fund), 2007; WCRF (World Cancer Research Fund), 2011), and the International Agency for Research on Cancer in 1993 (IARC (International Agency for Research on Cancer), 1993). Because HCAs are created during cooking at over 100°C by frying, roasting, grilling, baking, oven broiling, barbecuing, microwaving, and smoking red meat, poultry, and fish for commercial and homemade foodstuffs worldwide, a label regarding the content and concentration of MeIQx and PhIP is urgently needed to help consumers understand the health risks of such foods and their consumptions.

The null association between MDM, PhIP, DiMeIQx, and CRC in the present study might be related to some issues in measuring and estimating the true amount of intake of these specific types of HCAs in the average lifestyle. Due to this limitation, the estimated amount of HCAs intake varied by study populations in the US. Some possible limitations might be that all seven prospective cohort studies on meat mutagens and CRA and CRC have not validated the designed questionnaire part of cooking methods to estimate HCAs intake by participants. An initial pilot survey estimated that individuals used about

18-20 food items of red meat, poultry, and fish, of which NHS used 8 items, HPFS used 8 items (one differed from NHS), and UDCA used 12 items (Byrne et al., 1998; Le et al., 2016; Martinez et al., 2007; Wu et al., 2006). Furthermore, the average cumulative intake of HCAs to enhance the estimate of long-term dietary intake was not available, because the previous studies simply completed only one baseline or follow-up survey only. Finally, the CHARRED Database only provided one set database for selected food items of red meat, poultry, and fish but was not detailed for specific real cooking methods by ethnic groups, such as White, African, Asian, and Latinos. Shortly, food tables of nutrients and substances should include indicators of the presence of chemical substances of PhIP, MeIQx, and DiMeIQx depending on cooking methods.

In conclusion, the present prospective pooled analysis has observed a significant positive association between MeIQx intake and the risk of both CRA and CRC that would support the hypothesis of the role of HCAs in developing CRC. Likewise, we recommend further improved research methodology for observational studies and the validation of the designed questionnaire of cooking methods and update of “CHARRED Database”.

Author Contribution Statement

Conceptualization: LCN, BTN, NTL. Data curation: NTL. Formal analysis: NTL. Funding acquisition: NTL. Methodology: BTN, NTL. Project administration: BTN, NTL. Visualization: LCN, BTN, NTL. Writing - original

draft: LCN, BTN, NTL. Writing - review & editing: LCN, BTN, NTL.

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Conflict of interest

There are no conflicts to disclose.

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