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Dietary intake and biomarkers of acrylamide exposure and risk of endometrial and ovarian cancer

A molecular epidemiologic study in the European Prospective Investigation into Cancer and Nutrition
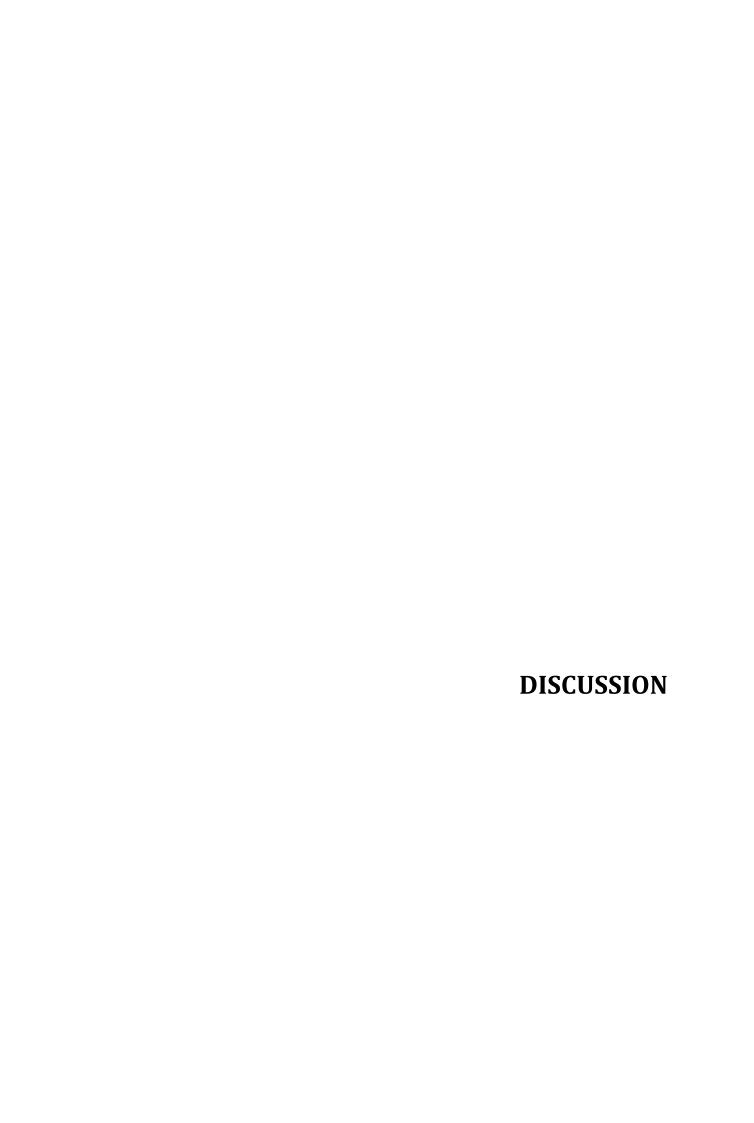
Mireia Obón Santacana

DISCUSSION

6. DISCUSSION

Each of the articles presented in this thesis included a discussion section. In the present chapter, some aspects, that that could not be more deeply discussed in the articles, are commented. Furthermore, current legislation, recommendations and future perspectives on this topic are provided.

6.1 General discussion

As described in this thesis, in the two prospective studies no overall association was observed between dietary intake of acrylamide and the risk of EC, type-I EC, EOC, or EOC subtypes; however, increased relative risks, as well as statistically significant monotonic dose-response trends, were observed for the association between dietary intake of acrylamide and type-I EC among women who both never smoked and never used OC. In addition, valid biomarkers of internal acrylamide exposure (measured as HbAA and HbGA) were used to further investigate the previous associations; but first, we wanted to evaluate if dietary items and lifestyle factors were determinants of HbAA and HbGA levels. In the cross-sectional study, we observed that dietary and lifestyle variables explained a moderate proportion of HbAA and HbGA biomarker levels. In the two nested case-controls studies, no evidence was observed to support our initial hypothesis that higher pre-diagnostic levels of HbAA and HbGA adducts would be associated with increased risk of developing EC or EOC in non-smoking post-menopausal women.

Results presented in this thesis indicate that dietary acrylamide intake was not a risk factor for overall EC or type-I EC risk in the EPIC cohort. Our results are in agreement with the Swedish prospective study, and the recently published Italian case-control study (in 2016), where no associations between acrylamide intake and EC risk were observed [121, 134]. In addition, two meta-analysis of epidemiological studies were published in 2014 and 2015 which included all four prospective studies (NLCS, SMC, NHS, and EPIC), and concluded that acrylamide intake was not related to EC risk; however, a modest association among women who never smoked and had the highest acrylamide intake was observed [135, 136]. The NHS was the only study that reported statistically significant increased relative risk for overall EC, but they did not report associations among never-smoking women [124]. By contrast, in the NLCS study, increased relative risks among never-smoking women were reported, but not for overall EC [120]. The EPIC study was the only one that reported increased relative risk for type-I EC with increasing acrylamide intake among women who both never smoked and were non-users of OC. All three prospective studies and the recent case-control study adjusted their statistical models by OC, but none showed results stratified by OC-use subgroups. Both smoking and OC use were considered confounding factors in our analysis, and one of the methods to control for confounding is by stratification.

The EPIC study also did not find an association between acrylamide intake and overall EOC or in any of the EOC subtypes (borderline or invasive). In comparing our results with previous epidemiological studies, null associations were also observed in the Italian case-control study and the SMC [122, 123]. Again, statistically significant positive associations and linear monotonic dose-response trends were observed in the NLCS (both in the entire cohort and

among never smoking women) [120]. The NHS reported some higher relative risks (for invasive and serous EOC), but none were statistically significant [124].

The first two studies of dietary intake presented in this thesis have some limitations that warrant further attention. (a) Dietary acrylamide exposure assessment using DQs (such as FFQs) that were not especially designed to capture acrylamide intake has been criticized for several reasons. Nutritional epidemiologists still have not found a perfect measure of dietary intake; however, it is widely known that the reproducibility and validity of FFQs in measuring nutrients intake (macro- and micronutrients) is high [132]. Acrylamide is a chemical compound that cannot be measured in raw foods, thus, obtaining reliable acrylamide intake estimates from FFQs is difficult. It is important to take into consideration that none of the epidemiological studies that assessed the relation between acrylamide intake and cancer risk (NLCS, NHS, SMC, Italian case-control, and EPIC) designed specific DQs for acrylamide intake, since their recruitment started between 1980 and 1992, before the discovery of acrylamide in foods in 2002. (b) Another challenge in estimating acrylamide content in foods is due to variation in preparation techniques (i.e., frying, baking, roasting). Some cooking methods that could have influenced total acrylamide intake were not assessed in all EPIC centers; however, information on relevant acrylamide food sources such as potatoes (except in Italy), bread, and breaded meats was available in all centers. (c) It is important to acknowledge that information bias may be present in our dietary acrylamide intake analyses. Information bias can be distinguished into non-differential or differential misclassification. Exposure misclassification is non-differential when it is unrelated to the occurrence of disease; nevertheless, when it is different between participants with and without disease, it is considered differential [137]. Non-differential exposure misclassification leads to attenuation of the possible true associations, and might have occurred in our study in a number of ways:

- Our acrylamide estimates might have been influenced by measurement error, such as reporting errors by participants due to the difficulty in remembering past exposures, database errors, incomplete food lists in FFQs, and cooking temperature and methods were not always accounted for.
- Different acrylamide databases were used to compile the EPIC acrylamide database, since some composition databases had incomplete data on acrylamide containing food; however, it has been observed in EPIC and in other studies that after energy intake adjustment, the validity of the intake estimates improved [138]. All statistical analyses presented in the first two articles of this thesis were stratified by country and age at recruitment, and adjusted for energy intake (using the residual method) and confounding variables. Due to the variation in dietary and cancer patterns across EPIC countries and centers, it is important to include the variable country and age at recruitment in the model, and one way to do so is including them as strata in a Cox proportional hazards model. The advantages of the strata inclusion are: fewer parameters to estimate in the model, and avoidance of the proportional hazard assumption for the country and age at recruitment effects.
- We cannot exclude the presence of residual confounding in our analyses due to misclassification of confounders such as BMI and reproductive history, or to

unmeasured variables. For example, we could not adjust by drinking water consumption and advanced glycation end-products (AGEs). It is widely known that polyacrylamide is used in water treatment; thus, residues of acrylamide may be found in potable water [3]. AGEs, have been linked to cardiovascular and diabetes mellitus diseases; moreover, several studies have pointed out that the expression of the receptor for AGEs (RAGE) could be associated with EC and EOC risk [139, 140].

As noted by Ferrari et al., the correlation coefficient between acrylamide intake based on a single 24-hDR and acrylamide intake derived from the food intake questionnaires in EPIC was low: 0.35 and 0.17 for crude and adjusted correlation coefficient, respectively [138]; nevertheless, an important limitation of a single 24-hDR is the high variability from day to day, thus a single assessment is likely not sufficient to accurately estimate average acrylamide intake. Several studies, including EPIC, have tested the validity of the method used to assess acrylamide intake estimates by using biochemical indicators of acrylamide intake. The correlation between acrylamide intake based on DQs and HbAA and HbGA was low in EPIC and in most of the studies (0.08 to 0.43) [138, 141–144]. This may be attributable to random or systematic errors in self-reported FFQs, acrylamide variability in foods, within-person variation (in diet or hemoglobin adducts circulation and metabolism), or other unknown factors (i.e., bioavailability). Interestingly, the NHS-II reported modest correlations (0.19 to 0.35), possibly because some commonly consumed foods in their cohort were additionally analyzed for acrylamide content (i.e., brands of breakfast cereals) and this information was included in their acrylamide database [48]. Finally, multiple subgroups were analyzed in both prospective studies, and some of the observed results might have been due to a combination of misclassification and chance.

A great advantage of the EPIC study for assessing the relation between acrylamide intake and cancer risk is the prospective cohort design, which is less prone to differential exposure misclassification (i.e., selection and recall biases), and participants were free of cancer at the start of the study; however, we also performed sensitivity analyses excluding the EC and EOC cases diagnosed during the first two years of follow-up to avoid reverse causation bias. Both prospective studies presented in this thesis had the largest sample size and had more cases than the previous published studies (**Table 11**). Consequently, we were able to investigate histological EC and EOC subtypes.

Table 11. Comparison of the total number of cases between studies

	Endometrial cancer cases	**Epithelial ovarian cancer cases**
NLCS	221	195
SMC	687	368
NHS	484	416
Italian case-control study	454	1,031
EPIC	1,382	1,191

EPIC; European Prospective Investigation into Cancer and Nutrition, NLCS, The Netherlands Cohort Study; NHS, Nurses' Health Study; SMC, Swedish Mammography Cohort

Despite the conflicting results between some of the studies, the limitations mentioned above, and taking into consideration that we were evaluating a probable carcinogen in humans, we additionally conducted two nested case-control studies to assess the relation between biomarkers of acrylamide exposure and EC and EOC risk in non-smoking postmenopausal women to assess internal exposure to acrylamide without the biases and problems associated with questionnaire-based assessment.

The EPIC cohort has designed several (nested) case-control studies, where cases and controls were matched by several factors depending upon the cancer site. Nested case-control studies are designed to study biologic precursors of disease (i.e., biomarkers of exposure, genetics), since compared to using the full cohort, study costs are reduced. As mentioned in the methodology section, the same matching variables were used to create the EPIC endometrial and ovarian (nested) case-control studies. Nevertheless, the two nested case-control studies presented in this thesis were based on non-smoking postmenopausal women (thus, individual matching was broken), which helped us to avoid confounding by smoking and hormonal fluctuations.

Before analyzing these two nested case-control studies, we first carried out a cross-sectional study with the purpose to identify which food groups and lifestyle variables were determinants of hemoglobin adduct concentrations of acrylamide and glycidamide in 801 non-smoking postmenopausal women. These women were controls from the endometrial and the ovarian nested case-control studies. In postmenopausal non-smoking women, the most important determinants of HbAA, HbGA, and HbAA+HbGA were 'salty biscuits, aperitif biscuits, and crackers', and 'dry cakes, and biscuits'. Alcohol intake and BMI were both identified as major determinants of the HbGA/HbAA ratio. Alcohol intake and BMI are factors that are thought to affect the activity of Cyp2e1 [40, 44], and the ratio of HbGA/HbAA was studied as it may reflect the metabolism of acrylamide to glycidamide. Three other studies evaluated food determinants of hemoglobin adducts, but different food groups were obtained [48, 141, 145]. The current study differs from the ones that were previously published in that both dietary and lifestyle variables were included in multiple linear regression analyses, and we observed that not only diet influences HbAA and HbGA levels. Thus, as earlier noted by Freisling et al., it is important to account for both alcohol intake and BMI by performing stratified analyses when acrylamide exposure is being evaluated [40].

After the evaluation of dietary and lifestyle determinants of hemoglobin adducts, a total of 768 non-smoking postmenopausal women (383 EC cases and 385 controls) were included in the first published nested case-control study of biomarkers of acrylamide intake and EC risk. We did not find any evidence for increased relative risk when women with the highest quintile of biomarker levels were compared to women with the lowest quintiles. Null associations were also found when we tried to replicate the results obtained in the prospective cohort analysis (increased relative risk were observed between acrylamide intake and type-I EC in women who were never smokers and non-users of OCs at baseline). The lack of association in this subgroup of women in the nested case-control study may be due to the small sample size, or, more

likely, the result observed in the prospective cohort analysis was due to misclassification and chance.

The second nested case-control study presented in this thesis (on hemoglobin adducts of acrylamide and EOC risk) was the first published study that included a European population; however, a nested case-control study conducted within the NHS and NHS-II (US) was previously published. In our study, although some of the ORs of the middle quintiles of HbGA and HbAA+HbGA showed statistically significant associations, the fourth and fifth quintiles of HbGA and HbAA+HbGA did not. No effect measure modification by BMI, alcohol intake, OC use, and HRT use were observed. The previously published nested case-control biomarker study also concluded that there were no associations between hemoglobin adducts and EOC or serous EOC risk [125].

One of the explanations for why we did not observe any associations between dietary acrylamide intake and EOC risk, but we did observe associations in the middle quintiles of HbGA and HbAA+HbGA, may be a result of limitations of the assessment of dietary acrylamide intake, as stated previously. However, the nested case-control studies also have weaknesses that should be acknowledged:

- Unlike the FFQs, that usually provide food consumption information in reference to the preceding year, HbAA and HbGA reflect exposure to acrylamide within the past 4 months (the average life-span of erythrocytes), and do not represent seasonal variation; however, biomarkers measured in red blood cells are known to be less sensitive to short-term fluctuations in diet, and thus, are thought to be good tools for determining long-term exposure [137].
- Unfortunately, in EPIC, only one blood sample was taken at baseline from each participant. Consequently, we were unable to assess intra-individual variation in biomarker levels. In the NHS-II, 45 non-smoking women provided two to three blood samples over a period of 1-3 years, and they observed no significant intra-individual variation, meaning that biomarkers of acrylamide exposure were reproducible over time in that population [48]. Vikström et al. observed high intra-individual variation, but only 13 participants were included in their study [146].
- There are many factors that can influence biomarkers levels (i.e., differences in absorption and metabolism) that could not be taken into account in our statistical models; however, one of the major disadvantages of these biomarkers is that they are expressed per gram of hemoglobin. Thus, all factors that contribute to hemoglobin variation may also influence HbAA and HbGA levels, such as gender, age, physical activity, smoking habits, and alcohol consumption. The EPIC nested case-control studies only included non-smoking postmenopausal women with similar ages at recruitment. Alcohol intake was included in all statistical models and was also evaluated in stratified analyses for effect measure modification. Physical activity was also evaluated, but was not included in final models since it was not a confounding variable. Consequently, hemoglobin variation was unlikely to affect the relative risks presented in this thesis.

- Although some of the participants had information on second-hand smoke (SHS) exposure, we were unable to perform exhaustive analyses; but other studies that could evaluate the effect of SHS on HbAA levels, reported null o very minor effects [59, 147].

- Despite having large sample size in both nested case-control studies, we could not evaluate in detail histological EC and EOC subtypes. Finally, as occurs in all epidemiological studies, and as was earlier mentioned, we cannot exclude the presence of residual confounding in our analyses; however, it is not expected to be strong since confounders had a weak impact in our results.

From a methodological point of view, one of the major strengths for both nested case-control studies is that they were designed to avoid confounding from smoking and hormonal fluctuations. Tobacco is one of the most important sources of acrylamide, and it is well established that cigarette smoke influences HbAA and HbGA adduct levels, and acrylamide has been postulated to influence hormone balance. In EPIC, information on the main risk factors of EC and EOC, and all blood samples were obtained at recruitment from controls and cases before they developed cancer. Moreover, the adduct levels were measured and analyzed in a randomized and blinded manner at the Centers for Disease Control and Prevention (CDC) Protein Biomarker Laboratory (Atlanta, US) under rigorous quality assurance/quality control laboratory protocols. Additionally, for each sample two independent measurements were performed. Further, to independently assess the laboratory reproducibility of the hemoglobin adducts measurements, 5% of the blood samples were sent in duplicate.

The overall impression is that dietary acrylamide intake appears unlikely to play a role in endometrial carcinogenesis in EPIC; nonetheless, it can be inferred from the evidence provided from this thesis that, if a true association exists between dietary acrylamide intake and EOC risk, the magnitude of effect is likely not a strong one. These results highlight the need for further evaluation of the association between acrylamide intake and EOC risk using biomarkers of acrylamide exposure, but with larger sample size in different populations (i.e., at least 500 cases and 1,000 controls), and in consortium studies.

The presence of acrylamide in commonly consumed foods represents a public health concern, since technically the entire population is exposed; however, there is currently no international or Spanish specific legislation on acrylamide exposure (there is no established maximum tolerance for acrylamide in food), but several recommendations by public organizations have been published [148, 149].

Since acrylamide and glycidamide are considered genotoxic and carcinogenic, the EFSA also could not establish a 'tolerable daily intake' (TDI) for dietary acrylamide exposure; however, the ESFA CONTAM Panel estimated a $BMDL_{10}$ of 0.17 and 0.43 mg/kg body weight per day to observe tumor and neurological effects, respectively [30]. Likewise, the JECFA has not established a 'toxicological reference value' (additional studies are needed) but MOE values have been used instead.

The official journal of the European Union published in 2013 several recommendations on the monitoring of acrylamide in food (2013/647/EU) [150]. Member States were invited to annually report to the Commission the acrylamide levels in foods that are known to major contribute to human dietary exposure. EFSA published 'acrylamide indicative values' for these foods, and the FoodDrinKEurope (represents the European food and drink industry) published the 'The FoodDrinKEurope Acrylamide Toolbox', which comprises several guidelines to help companies to voluntary reduce acrylamide levels in their food products (from the raw material selection to the finished product). To date, there are five published guidelines available in 23 European languages on 'biscuits, crackers & crispbreads', 'bread products', 'breakfast cereals', 'fried potato crisps', and 'fried potato products/French fries'. In addition, the Specialized Nutrition Europe (SNE) together with the FoodDrinKEurope also published a guideline in 'foods for infants and young children'[151].

There are also recommendations for the general population to reduce acrylamide levels in home cooking, such as the ones provided by the European Potato Processors' Association (EUPPA), or by the Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN)[149] (**Figure 12**).

PAN/TOSTADAS

• Si haces tostadas o pan precocinado, el color dorado es preferible al marrón oscuro.

Figure 12. Example extracted from the AECOSAN guideline to reduce acrylamide exposure

As it was mentioned in the introduction, dietary acrylamide intake from foods is not the only exposure route. The FDA established regulations for the use of polymers and copolymers of acrylamide in products in contact with food. Moreover, according to Regulation (EC n°. 1223/2009), acrylamide is listed as a banned component in cosmetic products (polyacrylamide use is allowed, but with maximum limits).
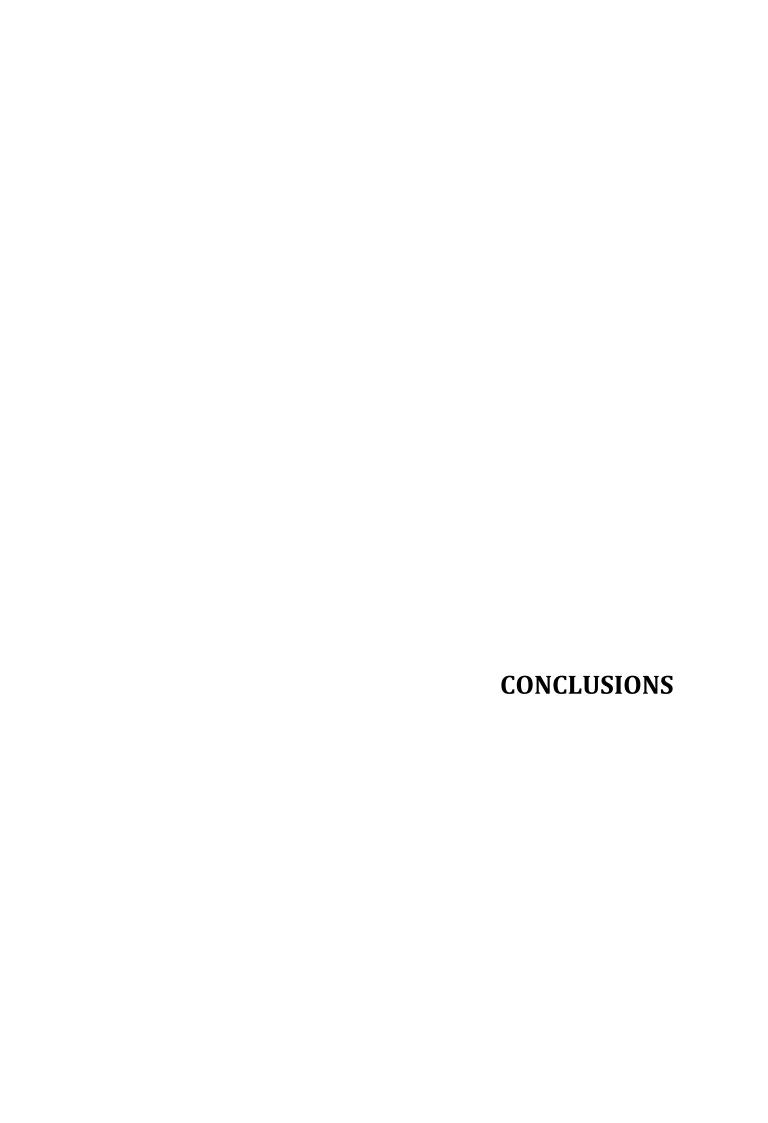
The last EFSA report concluded that the current levels of acrylamide intake '*are not a concern with respect to non-neoplastic effects*'; but they are for neoplastic effects [30]. Based on the arguments provided in this thesis, and despite the conflicting results, we believe that the precautionary approach should be adopted. People should follow the recommendations provided by their country specific 'food-based dietary guidelines' to pursue a balanced diet and healthy lifestyles to promote overall health and prevent chronic diseases.

In Spain, there are two official dietary guidelines promoted by the Spanish Ministry of Health, Social Services and Equality named '*Come sano y muévete: 12 decisiones saludables*' and '*Nutrición saludable de la infancia a la adolescencia. La alimentación de tus niños y niñas*'; however, there are other dietary and food guides developed at national and regional levels, such as the food pyramid of the Mediterranean Diet Foundation or the Iberoamerican Nutrition Foundation (FINUT) Healthy Lifestyles Guide.

6.2 Benefits of research, applicability and validity

The studies conducted in this thesis are the most comprehensive prospective investigations of dietary acrylamide, acrylamide and glycidamide biomarkers, and risk of endometrial and ovarian cancers to date, and represent a major step forward for nutritional epidemiology research.

The studies comprised in this thesis have directly addressed a lingering question about the potential role of acrylamide as a human carcinogen by investigating all dietary acrylamide exposure, measuring blood-based biomarkers of acrylamide and its major epoxide metabolite, glycidamide, and considering other risk factors such as age, education, geography, reproductive factors, smoking, BMI, coffee, fruit, vegetable, and meat intakes, and alcohol consumption. The results of this project have contributed to improve the knowledge about endometrial and ovarian cancer risk factors, and will also be evaluated by the WCRF Continuous Update Project. In addition, all the scientific publications that our group has provided to the scientific community (acrylamide exposure and pancreatic, esophageal, endometrial, and ovarian cancer risk [47, 152–154]) were included and evaluated in the latest EFSA Scientific Opinion on acrylamide in food. Moreover, WHO-IARC plans to re-evaluate the carcinogenicity of acrylamide in the near future, and these studies will contribute to the next expert evaluation and recommendations on the potential human carcinogenicity of acrylamide.

CONCLUSIONS

7. CONCLUSIONS

The main aim of this thesis was to evaluate whether dietary acrylamide exposure can be considered a risk factor for endometrial and epithelial ovarian cancers. There was very limited research from large prospective cohort studies on the association between dietary intake of acrylamide and the risk of these two cancers, and this thesis has contributed to increase the scientific epidemiological knowledge in this field.

The conclusions are presented as a response to each hypothesis specified at the beginning of this thesis:

Hypothesis 1: _High dietary acrylamide intake is associated with increased risk of endometrial cancer. Risk differs by tobacco smoking, alcohol consumption and oral contraceptive use._

- Dietary acrylamide intake does not increase the risk of overall endometrial cancer in EPIC.
- Statistically significant increased type-I I EC risks were observed among women who never smoked and never used oral contraceptives at baseline; however, we could not rule out the role of chance in this finding.
- No effect measure modification was observed by tobacco smoking, alcohol consumption, body mass index, and geographical region.

Hypothesis 2: _High dietary acrylamide intake is associated with increased risk of epithelial ovarian cancer. Risk differs by tobacco smoking, alcohol consumption and oral contraceptive use._

- Dietary acrylamide intake does not increase the risk of overall epithelial ovarian cancer and histological epithelial ovarian cancer subtypes in EPIC.
- No effect measure modification was observed by tobacco smoking, alcohol consumption, body mass index, oral contraceptive use, and geographical region.

Hypothesis 3: _Specific foods and lifestyle variables determine biomarker levels of acrylamide and its metabolite, glycidamide in non-smoking postmenopausal women. Biomarker levels are modified by heavy alcohol consumption._

- Dietary food group and lifestyle variables explain a moderate proportion of hemoglobin adducts of acrylamide and glycidamide variation in a subset of non-smoking postmenopausal women from the EPIC cohort.

- The main food group determinants of hemoglobin adducts of acrylamide and glycidamide, and its sum, were 'salty biscuits, aperitif biscuits, and crackers' and 'dry cakes and biscuits.'

- Alcohol intake and body mass index were identified as the main determinants for the ratio of hemoglobin adducts of glycidamide/hemoglobin adducts of acrylamide.

Hypothesis 4: _High biomarker levels of HbAA and HbGA are associated with increased endometrial cancer risk. Heavy alcohol consumption and oral contraceptive use modify effects._

- Hemoglobin adducts of acrylamide and glycidamide do not increase the risk of overall endometrial cancer or type-ı endometrial cancer in EPIC.

- No effect measure modification was observed by alcohol intake, body mass index, oral contraceptive use, hormonal replacement therapy use, and geographical region.

- The association observed under hypothesis 1 was not replicated using biomarkers of acrylamide exposure.

Hypothesis 5: _High biomarker levels of HbAA and HbGA are associated with increased epithelial ovarian cancer risk. Heavy alcohol consumption and oral contraceptive use modify effects._

– Hemoglobin adducts of acrylamide do not increase the risk of overall epithelial ovarian cancer or invasive serous epithelial ovarian cancer in EPIC.

– Associations with the third quintile of hemoglobin adducts of glycidamide, and the second and third quintiles of the sum of total adducts were observed for overall epithelial ovarian cancer.

– Participants classified in the fifth quintile of hemoglobin adducts of glycidamide or the sum of total adducts, showed statistically non-significant higher risks of overall epithelial ovarian cancer.

– No effect measure modification was observed by alcohol intake, body mass index, oral contraceptive use, hormonal replacement therapy use, and geographical region.

– To exclude the possibility of an association between acrylamide and ovarian cancer, additional nested case-control studies with larger sample size and pooled analyses of existing studies should be performed.