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**Association between dietary intake of acrylamide and increased risk of mortality in women:
evidence from the E3N prospective cohort**

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Abstract

Acrylamide is an organic compound classified as probably carcinogenic to humans because of sufficient evidence in animals but not in humans. Other health risks associated with acrylamide intake are still not fully elucidated. We aimed to study the relationship between acrylamide dietary intake and mortality in the E3N (Etude Epidémiologique auprès de femmes de l'Education Nationale) French cohort.

We studied 72 585 women of the E3N prospective cohort, which completed a food frequency questionnaire in 1993. The E3N food consumption database and the food contamination database obtained from the second French total diet study were used to estimate participants' average daily acrylamide dietary intake. We estimated the associations between acrylamide dietary intake and all-cause or cause-specific mortality using Cox proportional hazard models.

During follow-up (1993-2014), we identified 6 441 deaths. The mean acrylamide dietary intake was 32.6 µg/day, with coffee consumption as principal contributor (48.6%). In the fully adjusted model, we found a non-linear association between acrylamide dietary intake and all-cause mortality and a linear positive association with cardiovascular disease (HR per one STD increment [95%CI]: 1.11 [1.02; 1.21]), all-cancer (HR [95%CI]: 1.05 [1.01; 1.10]) and lung cancer (HR [95%CI]: 1.22 [1.09; 1.38]) mortality, while we observed no association with breast (HR [95%CI]: 0.94 [0.86; 1.03]) and colorectal (HR [95%CI]: 1.12 [0.97; 1.29]) cancer mortality. We highlighted an interaction between acrylamide dietary intake and smoking status in the models for all-cause and all-cancer mortality: when stratifying on smoking status, statistically significant positive associations were observed only in current smokers.

This study on a large prospective cohort following more than 70 000 women for over 20 years suggests that higher acrylamide dietary intakes are associated with an increased risk of mortality. Therefore, it is essential to keep reducing acrylamide contamination and prevent dietary intake of acrylamide, especially among smokers.

Keywords: acrylamide; diet; mortality; woman; cohort

1. Introduction

Acrylamide is an organic and soluble compound. Occupational exposure was first studied, as acrylamide is mostly used as polyacrylamide in various industries, such as water treatment, soil stabiliser, paper, cosmetics, textile, food packaging, or tobacco (EFSA, 2015; Koszucka et al., 2020). Acrylamide is also found in cigarette smoke. In 2002, acrylamide was discovered in food and described as a heat-induced contaminant that appears at low moisture levels and during high-temperature (>120°C) cooking methods, such as baking, roasting or frying (EFSA, 2015). It is mostly formed in plant-based foods during the Maillard reaction between the asparagine amino acid and reducing sugars (Mottram et al., 2002), but several other processes can induce acrylamide formation (Keramat et al., 2011). In the general non-smoking population, diet is the main source of exposure to acrylamide with the highest levels of acrylamide frequently found in potato-fried products, bread, biscuits, and coffee or coffee substitutes. In smokers, cigarette smoke may represent a more important source of acrylamide than diet (EFSA, 2015).

In 1994, with sufficient evidence from animal studies, but limited in human studies, acrylamide was classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer (IARC) (IARC, 1994). Although acrylamide's carcinogenicity in humans remains uncertain, the European Food Safety Authority (EFSA) reported margins of exposure (MOEs) that suggest a risk of neoplastic effects based on animal evidence (EFSA, 2015). Moreover, despite inconsistent epidemiological results on whether or not exposure to acrylamide through diet increases the risk of human cancers, the American Food and Drug Administration (FDA) recommended that industries and consumers both reduce acrylamide formation in food (FDA, 2022). In 2019, acrylamide was identified as a high-priority agent recommended for a re-evaluation of its classification because of new evidence on its mechanisms and effects in human cancers (Marques et al., 2019). Acrylamide is also known to be neurotoxic in animals and humans (especially through occupational exposure) (Erkekoglu & Baydar, 2014), reprotoxic, and toxic for development (EFSA, 2015).

A Danish study estimated that dietary exposure to acrylamide is annually responsible for the loss of 1.8 years of healthy life per 100 000 inhabitants (Jakobsen et al., 2016). Only one epidemiological study investigated the dietary intake of acrylamide in relation to cancer mortality in elderly Chinese and identified an increased risk of all-cancer, digestive and respiratory cancer mortality (Liu et al., 2017). To the best of our knowledge, no previous study investigated the association between acrylamide dietary intake and all-cause or cardiovascular disease (CVD) mortality.

32 The main goal of the present study was to investigate the association between acrylamide dietary
33 intake and the risk of all-cause and cause-specific mortality in the E3N (Etude Epidémiologique
34 auprès de femmes de l'Education Nationale) French prospective cohort.

35 2. Material and methods

36 2.1. E3N cohort

37 The E3N cohort is an ongoing prospective cohort established in 1990 that includes 98 995 women
38 born between 1925 and 1950 who were members of the MGEN, a health insurance for workers of
39 the French national education system. Anthropometric, lifestyle, and health characteristics were
40 collected every 2 to 3 years through self-administered questionnaires. Only 3% of the E3N women
41 are lost to follow-up (Clavel-Chapelon et al., 1997; Clavel-Chapelon & E3N Study Group, 2015). All
42 participants gave consent to this study, that was approved by the CNIL (the French National
43 Commission for Data Protection and Privacy).

44 2.2. Dietary questionnaire

45 The third questionnaire (Q3), sent in 1993, was a semi-quantitative food frequency questionnaire
46 (FFQ) containing 208 food items and divided in two parts. The first part focused on the frequency and
47 amount of food and drinks consumed during the previous year, and was organised in eight meal
48 occasions according to French dietary habits. The second part asked more detailed questions about
49 the food items belonging to the food groups included in the first part. The validity and reproducibility
50 of this dietary questionnaire were previously confirmed (van Liere et al., 1997).

51 We estimated the daily food intakes by multiplying the quantity of food consumed by the frequency
52 of eating occasions. Daily nutrient intakes were derived from the French food composition table of
53 the CIQUAL, the French Information Centre on Food Quality (ANSES, 2013).

54 2.3. Ascertainment of acrylamide dietary intake

55 Food contamination levels were derived from the second French total diet study (TDS2) performed
56 by the Anses, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES,
57 2011). Briefly, between 2007 and 2009, 20 280 food items were purchased in 8 French regions in
58 order to analyse the concentrations of more than 400 contaminants in 1 352 composite samples
59 prepared as consumed (i.e., peeling, frying, etc.), according to French cooking habits (Sirot et al.,
60 2009). In particular, acrylamide was analysed in 192 samples prepared from 2 280 common French
61 products known to contribute to acrylamide dietary intake, such as bread, breakfast cereals,
62 croissant-like pastries, biscuits, chocolate-based products, potato crisps, French fries, or coffee (Sirot
63 et al., 2012).

64 The E3N food consumption database and the TDS2 food contamination database were then merged
65 to estimate for each participant the acrylamide dietary intake (in μg of acrylamide/day), by summing,
66 over all food items, the product of the quantity consumed of the food item (in g of food/day) and the
67 concentration of acrylamide in the food item (in μg of acrylamide/g of food) (Mancini et al., 2020).

68 We estimated acrylamide dietary intake according to the lower bound (LB) scenario, in which the
69 concentration values below the limit of detection (LOD, 4 $\mu\text{g}/\text{kg}$) were replaced by 0, and the
70 concentration values below the limit of quantification (LOQ, 10 $\mu\text{g}/\text{kg}$) were replaced by the LOD
71 (Sirot et al., 2012). As there were few values that were not detected (ND) or not quantified (NQ)
72 (respectively 11% and 15% of 192 analysed samples), estimates of acrylamide dietary intakes
73 according to the LB scenario were similar to those derived from the middle-bound (MB) scenario
74 where ND and NQ values are replaced by $\frac{1}{2}$ LOD and $\frac{1}{2}$ LOQ, respectively.

75 2.4. Ascertainment of mortality

76 We identified deaths that occurred during the follow-up period, i.e., from the baseline of the study
77 considered as the date of completion of the dietary questionnaire sent in 1993 to the most recent
78 update of the mortality database linked to the cohort (i.e., November 2014). The vital status of the
79 participants was obtained from the MGEN, postal services, municipal registries, physicians, or next of
80 kin. Causes of death were coded by the French centre of epidemiology on medical causes of death
81 (Inserm-CépiDc) according to the 9th (before 2000) or the 10th (after 2000) international classification
82 of diseases (ICD). The ICD9 140-208 and ICD10 C00-C97 codes were used for all-cancer mortality,
83 ICD9 390-459 and ICD10 I00-I99 for CVD mortality, ICD9 174 and ICD10 C50 for breast cancer
84 mortality, ICD9 162 and ICD10 C33-C34 for lung cancer mortality and ICD9 153, 154.0-154.1 and
85 ICD10 C18-C20 for colorectal cancer mortality.

86 2.5. Study population

87 We included all the women who answered the dietary questionnaire sent in 1993 (N=74 522). We
88 then excluded 1 491 women with extreme values of energy intakes, i.e., below the 1st or above the
89 99th percentiles of the ratio of energy intake on energy requirements (corresponding to the basal
90 metabolic rate based on sex, age and weight multiplied by the physical activity level (Schofield,
91 1985)), and 446 women lost to follow-up. Finally, for all-cause mortality analyses, our study
92 population included 72 585 women. For all-cancer or CVD mortality analyses, the study population
93 was composed of 72 416 women as we additionally excluded 169 participants with unknown cause of
94 death. Finally, for specific-cancers mortality analyses, 178 women were further excluded because of
95 unknown primary location of cancer leading to death, resulting in a study population of 72 238
96 participants. The flow chart is presented in **Supplementary figure 1**.

97 2.6. Statistical analyses

98 2.6.1. Descriptive analyses

99 We described the characteristics (mean and standard deviation (STD) for continuous variables,
100 number and proportion for categorical variables) of the overall population, and according to vital
101 status and quartiles of acrylamide dietary intake.

102 We analysed the distribution of acrylamide dietary intake and identified its main contributing food
103 groups whose correlations with acrylamide dietary intake were estimated using Spearman rank
104 correlation coefficients.

105 2.6.2. Main analyses

106 Cox proportional hazard models with age (continuous, years) as the time scale were used to estimate
107 hazard ratios (HR) and their 95% confidence intervals (CI) for the association between acrylamide
108 dietary intake and the risk of all-cause, CVD, all-cancer, and specific-cancers (breast, lung or
109 colorectal) mortality. Each participant was followed-up from the age of dietary assessment to the age
110 at death, age at the last completed E3N questionnaire, or the end of the follow-up period (i.e., the
111 last update of the mortality database, in November 2014), whichever occurred first. For cause-
112 specific mortality analyses, we censored all other causes of death at the age at death.

113 Acrylamide dietary intake, the main exposure variable, was divided by its STD to estimate HR for one
114 STD increment.

115 Adjustment covariates were derived from the literature and we built a directed acyclic graph (DAG,
116 <http://www.dagitty.net>, **Supplementary figure 2**) that shows the hypothetical causal structure of the
117 variables. Confounders were defined using the 2nd questionnaire (Q2) sent in 1992 in order to respect
118 temporality, given that the dietary questionnaire (Q3) sent in 1993 assessed the last 12 months'
119 dietary consumptions, except for physical activity that was defined at Q3 (as it was not collected at
120 Q2).

121 We started by fitting an unadjusted model (model 1) and then adjusted our main analyses (model 2)
122 on BMI at Q2 (continuous, kg/m²), birth cohort (\leq 1930, (1930 – 1935], (1935 – 1940], (1940 – 1945],
123 >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status at Q2 (never or former
124 smoker, current smoker), physical activity at Q3 (continuous, MET-hours/week), menopausal status
125 combined with recent (i.e., during the last year) use of menopausal hormone therapy (MHT) at Q2
126 (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal
127 and no information on whether and when MHT was used), and dietary characteristics all measured at

128 Q3: total energy intake excluding energy from alcohol and lipids (continuous, kcal/day), alcohol
129 intake (continuous, g of ethanol/day), and lipids intake (continuous, g/day).

130 In order to examine dose-effect relations and departures from linearity, we used restricted cubic
131 splines functions for all continuous variables and examined whether they improved the main model's
132 fit compared to a model including a single linear term; continuous variables were modelled using
133 restricted cubic splines if the p-value of the non-linearity test was below 0.1, and using a linear
134 variable otherwise. For the main exposure variable, we compared several models with an increasing
135 number of knots: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th
136 percentiles or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles; we selected the model (and
137 the related number of knots) with the smallest Akaike information criterion (AIC) value. For all other
138 continuous covariates (i.e. BMI, physical activity, total energy intake excluding energy from alcohol
139 and lipids, alcohol intake, lipids intake, and coffee consumption), we used 4 knots by default as
140 suggested by Harrell (Harrell, 2001), at the 5th, 35th, 65th and 95th percentiles. Moreover, we also
141 performed all main analyses with the main exposure variable in quartile groups to facilitate
142 comparisons between models and to estimate the association for the highest exposure category
143 compared to the lowest (as reference). Linear trends across categories were examined by modelling
144 the median value of each category as a continuous variable.

145 As cigarette smoke was also a source of exposure to acrylamide, we tested the multiplicative
146 interaction between acrylamide dietary intake and smoking status, and we performed stratified
147 analyses on smoking status if the p-value for the interaction test was <0.1.

148 Finally, as coffee is the main contributing food group to acrylamide dietary intake and as its
149 consumption may also be associated with mortality, we repeated our analyses for non-coffee
150 acrylamide dietary intake (i.e., acrylamide coming from all food groups except coffee), with an
151 additional adjustment on coffee consumption (continuous, mL/day; model 3). We also tested the
152 multiplicative interaction between non-coffee acrylamide dietary intake and smoking status. When
153 the p-value for the interaction test was <0.1, we stratified analyses on smoking status.

154 We imputed covariates with less than 5% of missing values with the median for continuous variables
155 and with the modal category for categorical variables. We created an "unknown value" category
156 when there were more than 5% of missing values (menopausal status and recent MHT use).

157 We considered a p-value strictly lower than 0.05 as statistically significant. We used the Statistical
158 Analysis System software package version 9.4 to build the database (SAS Institute, Cary, North
159 California), and R version 4.1.2 to perform the statistical analyses.

2.6.3. Sensitivity analyses

We tested models 2 and 3 with the dietary intake of acrylamide or non-coffee acrylamide, respectively, modelled with restricted cubic spline functions irrespectively of the non-linearity test, to visually assess non-linear trends beyond statistical significance and make comparisons between all the studied mortality outcomes. Moreover, participants with prevalent chronic disease at baseline may have adopted a healthier diet after diagnosis. Thus we performed a sensitivity analysis in which we additionally adjusted the main model (model 2) on diagnosis of diabetes before inclusion (yes, no; for all causes of death), diagnosis of cancer before inclusion (yes, no; for all-cause and cancer mortality), diagnosis of high cholesterol before inclusion (yes, no; for all-cause and CVD mortality) and diagnosis of hypertension before inclusion (yes, no; for all-cause and CVD mortality). Furthermore, we performed two other sensitivity analyses (on model 2 for all-cause mortality): first, we used the energy adjustment residual method by substituting the main exposure variable with the residual of the regression of acrylamide dietary intake on energy (Willett et al., 1997); second, we explored the potential influence of reverse causation by excluding participants who died or were censored during the first five years of follow-up.

3. Results

3.1. General characteristics

During an average of 19 years of follow-up (STD 4.1), we identified 6 441 deaths, including 3 473 from cancer, 896 from CVD, 953 from breast cancer, 364 from lung cancer, and 317 from colorectal cancer.

The mean acrylamide dietary intake in our study population was 32.6 µg/day (STD 19.4) (**Table 1**). Its median was 29.5 µg/day (IQR 18.9-42.2). The graph of acrylamide dietary intake distribution is presented in **Supplementary figure 3**.

Characteristics of the study population overall and according to quartiles of acrylamide dietary intake are shown in **Table 1**, and according to vital status in **Supplementary table 1**. Compared to participants in the 1st quartile of acrylamide dietary intake, participants in the 4th quartile were younger (51.4 vs 54.4 years) and more frequently overweight or obese (20.5% vs 16.3%), current smokers (18.1% vs 8.2%), and premenopausal (60.3% vs 43.2%). They also had a higher intake of energy (1 453.2 vs 1 210.7 kcal/day), alcohol (14.1 vs 8.9 g ethanol/day), lipids (101.2 vs 77.8 g/day), and coffee (642.2 vs 89.1 mL/day).

The food group that contributed the most to acrylamide dietary intake was coffee (48.6%), which was highly correlated with acrylamide intake (spearman rank correlation coefficient 0.79)

192 **(Supplementary table 2)**. Other contributing food groups were bread and salty cereal products
193 (17.9%) and starch food (16.5%, most of the acrylamide coming from French fries) **(Supplementary**
194 **figure 4)**.

195 3.2. Acrylamide dietary intake and mortality

196 The associations between acrylamide dietary intake and all-cause or cause-specific mortality (models
197 1 and 2) are presented in **Table 2** and in **Figure 1**. We observed a non-linear association between
198 acrylamide dietary intake and all-cause mortality, which was inverse up to the median after which
199 the association became positive (p-value for the overall association=0.003). Setting the minimum
200 value of acrylamide dietary intake as reference, we observed a statistically significant increased risk
201 of all-cause mortality above a threshold of 4.8 STD of acrylamide dietary intake. For all other
202 outcomes, we observed linear associations. We highlighted a positive and statistically significant
203 association between acrylamide dietary intake and all-cancer mortality (HR [95%CI] per one STD:
204 1.05 [1.01; 1.10]), CVD mortality (HR [95%CI]: 1.11 [1.02; 1.21]) and lung cancer mortality (HR
205 [95%CI]: 1.22 [1.09; 1.38]). We observed no statistically significant association with breast cancer
206 mortality (HR [95%CI]: 0.94 [0.86; 1.03]) and colorectal cancer mortality (HR [95%CI]: 1.12 [0.97;
207 1.29]).

208 We observed a statistically significant interaction between acrylamide dietary intake and smoking
209 status for analyses on all-cause ($p_{\text{interaction}}=0.017$) and all-cancer ($p_{\text{interaction}}=0.008$) mortality. In
210 analyses stratified by smoking status, the association between acrylamide dietary intake and all-
211 cause mortality was not statistically significant in never or former smokers (p-value for the overall
212 association=0.282). On the contrary, we observed a non-linear association in current smokers which
213 showed a statistically significant increase in risk above a threshold of 5.6 STD of acrylamide dietary
214 intake when using the minimum value of this intake as reference (p-value for the overall association
215 <0.001). We observed similar results for all-cancer mortality: HR [95%CI]: 1.01 [0.96; 1.06] in never or
216 former smokers, and HR [95%CI]: 1.24 [1.13; 1.37] in current smokers (**Table 3** and **Figure 2**).
217 Therefore, the association between acrylamide dietary intake and all-cause or all-cancer mortality
218 risk was modified by smoking status in such a way that only current smokers experienced an
219 increased risk.

220 Finally, in analyses based on non-coffee acrylamide dietary intake (model 3), the associations with
221 mortality were not statistically significant, except for a non-linear association borderline with
222 statistical significance with all-cause mortality (p-value for the overall association=0.066) and a
223 suggestive linear and positive association with colorectal cancer mortality (HR [95%CI]: 1.14 [1.00;
224 1.30]) (**Table 4** and **Figure 3**). We highlighted a statistically significant interaction between non-coffee

225 acrylamide dietary intake and smoking status only for analyses on all-cause mortality
226 ($p_{\text{interaction}}=0.031$). When stratifying the analyses on smoking status, the association between non-
227 coffee acrylamide dietary intake and all-cause mortality was not statistically significant in never or
228 former smokers (p -value for the overall association=0.555), while we observed a non-linear
229 association in current smokers, which showed a statistically significant increased risk above a
230 threshold of 6.4 STD of non-coffee acrylamide dietary intake when using the minimum value of this
231 intake as reference (p -value for the overall association 0.005) (**Table 5** and **Figure 4**). Consequently,
232 the association between non-coffee acrylamide dietary intake and the risk of all-cause mortality was
233 modified by smoking status such that only current smokers presented an increased risk.

234 3.3. Sensitivity analyses

235 When representing all the associations between acrylamide dietary intake and mortality using
236 restricted cubic splines, irrespective of the statistical significance of the non-linearity test, we
237 observed similar associations compared to the main analyses. When comparing spline graphs of
238 model 2 (total acrylamide dietary intake as main exposure variable) with the spline graphs of model 3
239 (non-coffee acrylamide dietary intake as main exposure variable), we observed different shapes of
240 associations, especially for CVD, breast cancer, lung cancer and colorectal cancer mortality risk.
241 Indeed, we observed higher HRs in model 3 compared to those in model 2 for CVD, breast and
242 colorectal cancer mortality, while on the contrary, HRs are lower in model 3 for lung cancer mortality
243 compared to those observed in model 2 (**Supplementary table 3** and **Supplementary figure 5**).

244 When additionally adjusting model 2 on diagnosis of diabetes, cancer, high cholesterol or
245 hypertension before inclusion, results didn't change compared to those of model 2 (**Supplementary**
246 **table 4** and **Supplementary figure 6**).

247 Finally, we obtained similar results for the relation between acrylamide dietary intake and all-cause
248 mortality after adjusting model 2 for energy with the residual method (**Supplementary table 5** and
249 **Supplementary figure 7**) and after excluding participants who died or were censored during the first
250 five years of follow-up (**Supplementary table 6** and **Supplementary figure 8**).

251 4. Discussion

252 In this study, we observed a non-linear association between acrylamide dietary intake and all-cause
253 mortality. We also highlighted positive linear associations between acrylamide dietary intake and
254 CVD, all-cancer and lung cancer mortality, and no associations with breast and colorectal cancer
255 mortality. Moreover, we found no association between non-coffee acrylamide dietary intake and
256 mortality, except for a non-linear association with all-cause mortality and a linear and positive

257 association with colorectal cancer mortality, both borderline with statistical significance. We
258 highlighted an interaction between acrylamide dietary intake and smoking status for all-cause and
259 all-cancer mortality analyses, and an interaction between non-coffee acrylamide dietary intake and
260 smoking status for all-cause mortality analysis only, whereby the association between high
261 acrylamide intake and mortality was restricted to current smokers.

262 In our study population, the estimated mean acrylamide dietary intake (32.6 µg/day) was in line with
263 a review of 101 studies including 68 studies from 26 countries (Timmermann et al., 2021). In another
264 French study based on a different population of women, the estimated mean dietary intake of
265 acrylamide (30.1 µg/day) was very similar to our estimates (Bellicha et al., 2022). Finally, our
266 estimate of mean acrylamide dietary exposure (0.55 µg/kg bw/day) was consistent with Anses'
267 estimation, of 0.43 µg/kg bw/day in French adults (ANSES, 2011), and of the same magnitude as
268 EFSA's estimation for the European population (0.5 µg/kg bw/day) (EFSA, 2015).

269 Acrylamide is easily absorbed and distributed in the human organism and highly metabolised, mostly
270 in the liver. Acrylamide is then transformed by the cytochrome P4502E1 (CYP2E1) in glycidamide, an
271 epoxide known for its mutagenic properties. Glycidamide can bind to DNA to form DNA adducts,
272 leading to genotoxicity and carcinogenicity (EFSA, 2015). At least 6% of an ingested acrylamide dose
273 is converted to the glycidamide epoxide, which is known to be more genotoxic than acrylamide
274 (Koszucka et al., 2020). Acrylamide can also be harmful through other pathways, such as disruption
275 of the endocrine system or the production of reactive oxygen species (ROS) leading to oxidative
276 stress (Adani et al., 2020; Benisi-Kohansal et al., 2021; EFSA, 2015; Koszucka et al., 2020; Liu et al.,
277 2017). Finally, acrylamide and glycidamide can form covalent adducts with haemoglobin. In addition,
278 they can be detoxified by conjugation with glutathione (GSH), leading to mercapturic acids, which are
279 then excreted in the urine.

280 Our results highlighted a non-linear association between acrylamide dietary intake and all-cause
281 mortality, with an increased mortality risk at the highest acrylamide dietary intakes. No previous
282 study investigated the association between acrylamide dietary intake and all-cause mortality. Only
283 one article studied the associations between several haemoglobin biomarkers of acrylamide and all-
284 cause mortality (Huang et al., 2018). The authors reported that, in non-smokers, an acrylamide
285 haemoglobin biomarker (HbAA) was associated with an increased risk of all-cause mortality, while a
286 glycidamide biomarker (HbGA) was inversely associated with all-cause mortality. In contrast, no
287 association was found in smokers. However, this study was based on an internal exposure
288 assessment that encompasses all sources of acrylamide exposure, and therefore the results were not
289 directly comparable with our findings. Finally, we can hypothesise that the non-linear association

290 found between acrylamide dietary intake and all-cause mortality was due to potential compensation
291 mechanisms of the organism until a threshold above which harmful effects of acrylamide can be
292 observed.

293 Our findings also suggested a positive association between acrylamide dietary intake and CVD
294 mortality. We found no study on acrylamide dietary intake and CVD mortality and only few studies
295 investigated the association between acrylamide exposure estimated through blood or urinary
296 biomarkers and CVD or CVD mortality (Huang et al., 2018; Wang et al., 2022; Zhang et al., 2018).
297 Nevertheless, the results of these studies are inconsistent. Moreover, they are not directly
298 comparable with ours. However, the authors of the most recent study concluded that acrylamide
299 exposure (estimated from urinary biomarkers) may affect CVD partly from oxidative stress,
300 inflammation, and TGF- β 1 mechanisms, which may explain our results (Wang et al., 2022).

301 Concerning cancer mortality, we observed a positive association with all-cancer mortality, which was
302 consistent with another study on Chinese elderly (Liu et al., 2017). This study reported a positive
303 association between dietary intake of acrylamide and respiratory cancer mortality, which is in line
304 with the positive association we observed with lung cancer mortality. However, Liu et al. also found a
305 positive association with digestive cancer mortality while we did not see a significant association with
306 colorectal cancer mortality in our study. This difference may be explained by the fact that the studied
307 outcome is not exactly the same. Finally, we did not identify other studies investigating acrylamide
308 intake and specific-cancers mortality, such as breast cancer mortality.

309 In general, the lack of association with breast and colorectal cancer mortality we observed may be
310 explained in different ways. For breast cancer, even if acrylamide is known to be carcinogenic in the
311 mammary gland in rodents, the given doses of acrylamide in animal studies are frequently much
312 higher than the estimated doses in the general human population, and thus acrylamide dietary
313 intake may not have appreciable effects on breast cancer risk at the levels of exposure estimated in
314 the E3N cohort. In addition, non-significant results may be due to the fact that coffee (the main
315 contributor to acrylamide dietary intake) contains components with opposite effects on breast and
316 colorectal cancer (such as caffeine, phenolic acids, melanoidins, and the diterpenes kahweol and
317 cafestol), which could attenuate the potential positive association with acrylamide (Nehlig & Cunha,
318 2020). More generally, the beneficial health effects of coffee but also other components of the diet
319 (such as red berries, wasabi, garlic or other food items containing antioxidants) may mask or
320 attenuate the true association between acrylamide dietary intake and health (Koszucka et al., 2020;
321 Nehlig & Cunha, 2020).

322 Although coffee is an important confounder, we did not adjust our main analyses (model 2) on coffee
323 intake, because it was highly correlated with dietary intake of acrylamide (spearman rank correlation
324 coefficient 0.79). We addressed this issue by considering non-coffee acrylamide dietary intake as the
325 exposure, while adjusting on coffee intake (model 3). Our aim was to test the effect of acrylamide
326 independently of the effects of the other substances in coffee or in general with other factors related
327 to coffee consumption. For CVD mortality analyses, we observed a different shape of the spline
328 graph with higher HR in model 3 than in model 2. This change may be due to the fact that we
329 observed a more prominent residual protective effect of coffee in model 2 than in model 3. Indeed,
330 coffee is known to be inversely associated with CVD and CVD mortality (Hou et al., 2022; Kim et al.,
331 2019; Poole et al., 2017). For all studied outcomes, the association with non-coffee acrylamide was
332 not statistically significant, except for a non-linear association for all-cause mortality and a positive
333 association for colorectal cancer mortality, both of borderline statistical significance. Based on these
334 results, we can assume that non-coffee acrylamide intake has no effects on these outcomes per se,
335 and/or that non-coffee acrylamide intake was too low and homogenous among participants to allow
336 to observe any effect.

337 In addition, when stratifying the analyses on smoking status, associations were statistically significant
338 only in current smokers. This differential effect among smokers and non-smokers has been
339 inconsistently observed in previous studies. Indeed, a recent systematic review and meta-analysis on
340 cancer risk highlighted an interaction between acrylamide intake and smoking status only for lung
341 cancer with an increased risk limited to smokers, while no interaction between acrylamide and
342 smoking status was observed for other cancer sites (Filippini et al., 2022). Nevertheless, the authors
343 underline that these results have to be interpreted with caution due to the limited number of studies
344 and the high heterogeneity. On the contrary, other studies highlighted a significant and positive
345 association among non-smokers that was not significant among smokers (Bongers et al., 2012;
346 Hirvonen et al., 2010). As suggested by Filippini et al., this could be explained by the fact that tobacco
347 smoke may saturate the enzyme involved in the epoxidation of acrylamide to glycidamide, thus the
348 conversion of acrylamide to the genotoxic glycidamide could be more effective in non-smokers than
349 in smokers (Filippini et al., 2022; Schettgen et al., 2004). This hypothesis is not totally in contradiction
350 with our results since it may still partly explain the association that we observed in smokers. For low
351 acrylamide dietary intake, tobacco smoking could prevent the biotransformation of acrylamide to
352 glycidamide, but only up to a certain threshold beyond which the epoxidation of acrylamide restarts.
353 Another hypothesis to explain the difference between smokers and non-smokers when studying the
354 association between acrylamide dietary intake and all-cause or all-cancer mortality could be that as
355 smokers are exposed to acrylamide from both diet and cigarette smoking, they can reach more easily

356 a threshold above which acrylamide is harmful to human health. We can thus assume that in our
357 study population, the overall exposure to acrylamide in smokers is probably higher than in non-
358 smokers, leading to appreciable association with all-cause or all-cancer mortality. In addition, we
359 observed a significant interaction between acrylamide dietary intake and smoking status for all-
360 cancer mortality analyses, while it was not significant when studying breast, lung or colorectal cancer
361 mortality individually, probably due to limited statistical power. Moreover, since our analyses were
362 not adjusted for coffee and because coffee and tobacco are positively correlated, the observed
363 interactions between acrylamide dietary intake and smoking status may reflect an interaction
364 between acrylamide dietary intake and coffee. We tested the interaction between non-coffee
365 acrylamide dietary intake and smoking status, and observed a statistically significant interaction only
366 for all-cause mortality. In light of these results, the interaction between acrylamide and tobacco
367 remains to be clarified.

368 When interpreting our results, some limitations need to be taken into account. The E3N cohort
369 includes volunteer women who are not representative of middle-aged French women, as they are
370 leaner and they have a higher school education level (Clavel-Chapelon & E3N Study Group, 2015).
371 However, this does not prevent from comparing women with high and low levels of acrylamide
372 dietary intake. In addition, the dietary consumptions of the E3N participants and the estimation of
373 their acrylamide dietary intake may have been impacted by the time gap between the dietary
374 questionnaire (1993) and collection of food contamination data (2007-2009). However, we can
375 assume that the dietary consumptions of middle-aged women are stable over time, as suggested by
376 Thorpe et al. (Thorpe et al., 2019). Moreover, since its discovery in food in 2002, acrylamide levels in
377 foods have decreased especially in potato-fried products and coffee (ANSES, 2011). Therefore, in
378 1993, our study population was probably exposed at higher concentrations of acrylamide than what
379 we have estimated, and this potential exposure classification bias could have led to an attenuation of
380 the real association. Furthermore, acrylamide contamination levels are strongly related to cooking
381 processes (EFSA, 2015), but we do not have specific information on the individual cooking habits in
382 the E3N cohort. Indeed, for French fries intake, the level of acrylamide depends on the reducing
383 sugars level in the potato, the oil, the temperature and the duration of the frying (Sirot et al., 2012).
384 However, the TDS2 provided acrylamide levels measured in food prepared as consumed, according
385 to French cooking habits (Sirot et al., 2012). We can assume that for a given consumer, the
386 acrylamide level in French fries will vary from one time to another, and thus in a year, the TDS2
387 acrylamide level will be a good estimation of the mean level of acrylamide in all the French fries
388 consumed. Moreover, the E3N FFQ contained questions on the consumption of French fries
389 independently of the consumption of potatoes from other cooking methods. Finally, it would have

390 been interesting to better assess acrylamide exposure coming from tobacco smoking (for example
391 including quantitative information on the smoking habits such as type, amount and frequency of
392 tobacco consumed), or to measure levels of acrylamide's internal biomarkers of exposure, but this
393 type of information was not available in our study. In general, residual confounding cannot be
394 completely ruled out.

395 Our study also benefits from several strengths, including its prospective design. With more than
396 70 000 women followed over 19 years, it was possible to study the long-term health effects of a low
397 level and chronic acrylamide dietary intake. The large study population ensures a good statistical
398 power. The robustness of the results is comforted by the numerous sensitivity analyses. The
399 mortality database with causes of death was validated by the Inserm-CépiDc. As the dietary
400 questionnaire was previously validated (van Liere et al., 1997), and as food samples were prepared
401 "as consumed" to estimate their contamination level (Sirost et al., 2009), the measurement error of
402 the exposure is supposed to be limited. Finally, in the E3N study much information on lifestyle and
403 characteristics of participants was collected allowing us to take into account several potential
404 confounders.

405 5. Conclusion

406 We observed a non-linear association between acrylamide dietary intake and all-cause mortality.
407 This association was only significant in current smokers when stratifying on smoking status. We also
408 found that a higher acrylamide dietary intake was associated with an increased risk of CVD, all-cancer
409 and lung cancer mortality. For all-cancer mortality risk, this association was only significant among
410 current smokers. Moreover, we observed a non-linear association borderline with statistical
411 significance between non-coffee acrylamide dietary intake and all-cause mortality, that was limited
412 to current smokers when stratifying on smoking status. Our results also suggested that a higher non-
413 coffee acrylamide dietary intake increased the risk of colorectal cancer mortality. It is therefore
414 essential to keep reducing acrylamide contamination and to prevent dietary intake of acrylamide,
415 especially among smokers.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions Statement

All authors contributed to the study conceptualization and methodology. CM performed statistical analyses. CM wrote the first draft of the manuscript and all authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Table 1: Characteristics^a of the study population overall and among each quartile group of acrylamide dietary intake in the E3N cohort (N=72 585)

	Acrylamide dietary intake (µg/day)				
	All (N=72 585)	Q1 (N=18 146)	Q2 (N=18 146)	Q3 (N=18 147)	Q4 (N=18 146)
Acrylamide dietary intake (µg/day)	32.6 (19.4)	12.2 (4.5)	24.2 (3.0)	35.3 (3.6)	58.5 (17.2)
Coffee acrylamide dietary intake (µg/day)	17.7 (15.8)	4.0 (4.3)	11.7 (6.6)	19.4 (8.1)	35.6 (18.1)
Non-coffee acrylamide dietary intake (µg/day)	14.9 (10.3)	8.2 (4.3)	12.5 (6.3)	15.9 (7.8)	22.9 (13.7)
Age (years)	52.9 (6.7)	54.4 (7.0)	53.4 (6.8)	52.6 (6.5)	51.4 (6.1)
Follow-up duration (years)	19.0 (4.1)	18.9 (4.2)	19.0 (4.1)	19.1 (4.1)	19.0 (4.2)
Follow-up duration (person-years)	1 378 046	342 238	345 541	346 134	344 133
BMI (kg/m ²)	22.7 (3.1)	22.5 (3.1)	22.7 (3.1)	22.7 (3.1)	23.0 (3.2)
<18.5	2 645 (3.6)	825 (4.6)	672 (3.7)	616 (3.4)	532 (2.9)
[18.5 – 25)	56 794 (78.2)	14 373 (79.2)	14 301 (78.8)	14 221 (78.4)	13 899 (76.6)
≥25	13 146 (18.1)	2 948 (16.3)	3 173 (17.5)	3 310 (18.2)	3 715 (20.5)
Birth cohort					
≤1 930	7 295 (10.1)	2 635 (14.5)	2 032 (11.2)	1 561 (8.6)	1 067 (5.9)
(1 930 – 1 935]	9 996 (13.8)	3 082 (17.0)	2 752 (15.2)	2 415 (13.3)	1 747 (9.6)
(1 935 – 1 940]	14 710 (20.3)	3 864 (21.3)	3 840 (21.2)	3 642 (20.1)	3 364 (18.5)
(1 940 – 1 945]	17 811 (24.5)	4 152 (22.9)	4 178 (23.0)	4 658 (25.7)	4 823 (26.6)
>1 945	22 773 (31.4)	4 413 (24.3)	5 344 (29.5)	5 871 (32.4)	7 145 (39.4)
Education level					
<12 years	8 190 (11.3)	1 947 (10.7)	1 975 (10.9)	2 076 (11.4)	2 192 (12.1)
12 to 14 years	38 408 (52.9)	9 604 (52.9)	9 692 (53.4)	9 616 (53.0)	9 496 (52.3)
>14 years	25 987 (35.8)	6 595 (36.3)	6 479 (35.7)	6 455 (35.6)	6 458 (35.6)
Smoking status					
Never or former	63 425 (87.4)	16 666 (91.8)	16 139 (88.9)	15 753 (86.8)	14 867 (81.9)
Current	9 160 (12.6)	1 480 (8.2)	2 007 (11.1)	2 394 (13.2)	3 279 (18.1)
Physical activity (MET-hours/week)	46.4 (43.4)	46.6 (43.4)	46.5 (43.3)	46.3 (43.0)	46.2 (43.7)
Menopausal status and recent use of MHT					
Premenopausal	37 313 (51.4)	7 849 (43.2)	8 850 (48.8)	9 674 (53.3)	10 940 (60.3)
Menopausal and recent MHT use	10 047 (13.8)	2 692 (14.8)	2 752 (15.2)	2 474 (13.6)	2 129 (11.7)
Menopausal and no recent MHT use	21 671 (29.9)	6 569 (36.2)	5 635 (31.1)	5 138 (28.3)	4 329 (23.9)
Menopausal and no information on whether and when MHT was used	3 554 (4.9)	1 036 (5.7)	909 (5.0)	861 (4.7)	748 (4.1)
Energy intake (excluding energy from alcohol and lipids, kcal/day)	1 329.0 (356.6)	1 210.7 (325.4)	1 298.1 (333.8)	1 353.8 (347.0)	1 453.2 (374.0)
Alcohol consumption (g ethanol/day)	11.6 (13.9)	8.9 (12.4)	11.1 (13.3)	12.3 (13.7)	14.1 (15.7)
Lipid consumption (g/day)	88.9 (27.0)	77.8 (22.7)	85.6 (24.2)	91.1 (25.6)	101.2 (29.6)
Coffee intake (mL/day)	332.6 (282.2)	89.1 (103.2)	230.8 (137.3)	368.3 (161.7)	642.2 (310.2)

^a Mean (std) for continuous variables, N (%) for categorical variables

Table 2: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between acrylamide dietary intake and mortality risk in the E3N cohort (N=72 585)

		N deaths	Model 1 HR [95% CI]^a	Model 2 HR [95% CI]^a
All-cause mortality (N=72 585)	Acrylamide dietary intake (spline with 3 knots)	6 441		
	p-value for the overall association		<0.001	0.003
	p-value for the non-linearity test		<0.001	0.006
	Quartiles of acrylamide intake (min-max, µg/day)			
	Q1 (0.0-18.9)	1 857	Ref	Ref
	Q2 (18.9-29.5)	1 626	0.97 [0.90; 1.03]	0.97 [0.91; 1.04]
	Q3 (29.5-42.2)	1 560	1.01 [0.95; 1.08]	1.01 [0.95; 1.09]
Q4 (42.2-276.2)	1 398	1.07 [1.00; 1.15]	1.04 [0.96; 1.12]	
p-linear trend		0.028	0.204	
All-cancer mortality (N=72 416)	Acrylamide dietary intake (linear term)	3 473	1.07 [1.03; 1.12]	1.05 [1.01; 1.10]
	p-value		<0.001	0.024
	p-value for the non-linearity test		0.067	0.125
	Quartiles of acrylamide intake (min-max, µg/day)			
	Q1 (0.0-18.9)	916	Ref	Ref
	Q2 (18.9-29.5)	875	1.02 [0.93; 1.11]	1.01 [0.92; 1.11]
	Q3 (29.5-42.2)	871	1.07 [0.98; 1.18]	1.06 [0.96; 1.16]
Q4 (42.2-276.2)	811	1.12 [1.01; 1.23]	1.07 [0.96; 1.18]	
p-linear trend		0.013	0.166	
CVD mortality (N=72 416)	Acrylamide dietary intake (linear term)	896	1.11 [1.02; 1.20]	1.11 [1.02; 1.21]
	p-value		0.013	0.018
	p-value for the non-linearity test		0.293	0.261
	Quartiles of acrylamide intake (min-max, µg/day)			
	Q1 (0.0-18.9)	259	Ref	Ref
	Q2 (18.9-29.5)	226	1.01 [0.85; 1.21]	1.03 [0.86; 1.23]
	Q3 (29.5-42.2)	217	1.12 [0.93; 1.34]	1.15 [0.96; 1.39]
Q4 (42.2-276.2)	194	1.30 [1.08; 1.57]	1.33 [1.09; 1.63]	
p-linear trend		0.004	0.003	
Breast cancer mortality (N=72 238)	Acrylamide dietary intake (linear term)	953	0.95 [0.88; 1.03]	0.94 [0.86; 1.03]
	p-value		0.211	0.185
	p-value for the non-linearity test		0.100	0.141
	Quartiles of acrylamide intake (min-max, µg/day)			
	Q1 (0.0-18.9)	271	Ref	Ref
	Q2 (18.9-29.5)	246	0.94 [0.79; 1.12]	0.95 [0.80; 1.13]
	Q3 (29.5-42.2)	222	0.88 [0.73; 1.05]	0.88 [0.73; 1.06]
Q4 (42.2-276.2)	214	0.90 [0.75; 1.08]	0.90 [0.74; 1.09]	
p-linear trend		0.226	0.227	
Lung cancer mortality (N=72 238)	Acrylamide dietary intake (linear term)	364	1.28 [1.15; 1.42]	1.22 [1.09; 1.38]
	p-value		<0.001	0.001

	p-value for the non-linearity test		0.172	0.124
	Quartiles of acrylamide intake (min-max, µg/day)			
	Q1 (0.0-18.9)	84	Ref	Ref
	Q2 (18.9-29.5)	88	1.11 [0.82; 1.50]	1.07 [0.79; 1.44]
	Q3 (29.5-42.2)	90	1.21 [0.90; 1.62]	1.13 [0.84; 1.54]
	Q4 (42.2-276.2)	102	1.52 [1.14; 2.04]	1.34 [0.98; 1.83]
	p-linear trend		0.003	0.053
	Acrylamide dietary intake (linear term)	317	1.15 [1.01; 1.30]	1.12 [0.97; 1.29]
	p-value		0.035	0.113
	p-value for the non-linearity test		0.188	0.205
Colorectal cancer mortality (N=72 238)	Quartiles of acrylamide intake (min-max, µg/day)			
	Q1 (0.0-18.9)	73	Ref	Ref
	Q2 (18.9-29.5)	80	1.19 [0.86; 1.63]	1.16 [0.85; 1.60]
	Q3 (29.5-42.2)	84	1.34 [0.98; 1.84]	1.31 [0.95; 1.80]
	Q4 (42.2-276.2)	80	1.46 [1.06; 2.01]	1.39 [0.99; 1.96]
	p-linear trend		0.016	0.049

Model 1: Adjustment on age (years) as time-scale

Model 2: M1 + BMI (kg/m²), physical activity (MET-hours/week), birth cohort (≤ 1930 , (1930 – 1935], (1935 – 1940], (1940 – 1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day)

^a We present the results of linear associations between acrylamide dietary intake and mortality risk with HR [95% CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

Figure 1: Restricted cubic splines of acrylamide dietary intake in models 1 and 2 in association with all-cause mortality risk in the E3N cohort (N=72 585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

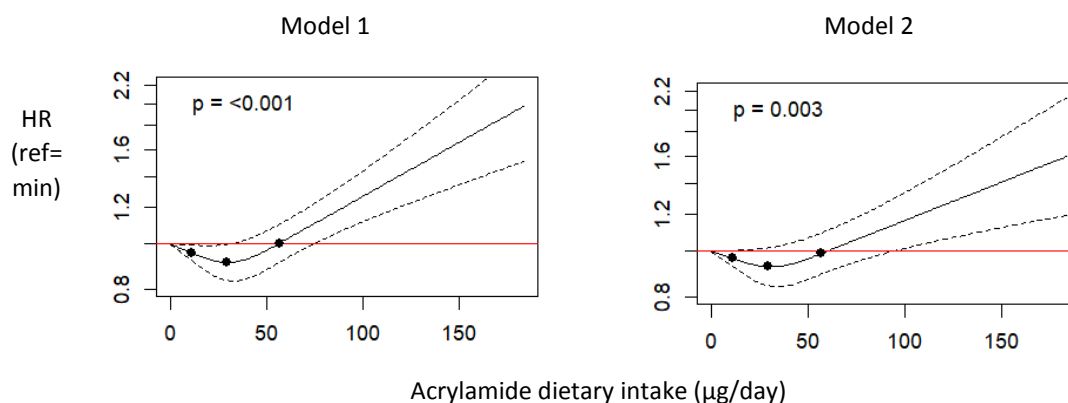


Table 3: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between acrylamide dietary intake and mortality risk, stratified on smoking status, in the E3N cohort (N=72 585)

		N deaths	Never or former smoker HR [95% CI] ^a		N deaths	Current smoker HR [95% CI] ^a
All-cause mortality (N=72 585)	Acrylamide dietary intake (spline with 3 knots)	5 493		Acrylamide dietary intake (spline with 5 knots)	948	
	p-value for the overall association		0.282	p-value for the overall association		<0.001
	p-value for the non- linearity test		0.112	p-value for the non- linearity test		0.024
	Quartiles of acrylamide intake (min-max, µg/day)			Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0-18.9)	1 682	Ref	Q1 (0.0-18.9)	175	Ref
	Q2 (18.9-29.5)	1 414	0.96 [0.90; 1.04]	Q2 (18.9-29.5)	212	0.99 [0.81; 1.21]
	Q3 (29.5-42.2)	1 319	1.00 [0.93; 1.08]	Q3 (29.5-42.2)	241	1.10 [0.90; 1.34]
Q4 (42.2-276.2)	1 078	1.00 [0.92; 1.09]	Q4 (42.2-204.4)	320	1.25 [1.03; 1.52]	
p-linear trend		0.829	p-linear trend		0.007	
All-cancer mortality (N=72 416)	Acrylamide dietary intake (linear term)	2 936	1.01 [0.96; 1.06]	Acrylamide dietary intake (linear term)	537	1.24 [1.13; 1.37]
	p-value		0.618	p-value		<0.001
	p-value for the non- linearity test		0.362	p-value for the non- linearity test		0.235
	Quartiles of acrylamide intake (min-max, µg/day)			Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0-18.9)	829	Ref	Q1 (0.0-18.9)	87	Ref
	Q2 (18.9-29.5)	758	1.00 [0.90; 1.10]	Q2 (18.9-29.5)	117	1.06 [0.80; 1.40]
	Q3 (29.5-42.2)	733	1.03 [0.93; 1.15]	Q3 (29.5-42.2)	138	1.16 [0.88; 1.53]
Q4 (42.2-276.2)	616	1.01 [0.90; 1.13]	Q4 (42.2-204.4)	195	1.36 [1.04; 1.78]	
p-linear trend		0.712	p-linear trend		0.009	

Model: Adjustment on age (years) as time-scale, BMI (kg/m²), physical activity (MET-hours/week), birth cohort (\leq 1930, (1930 – 1935), (1935 – 1940], (1940 – 1945], >1945), education level (<12 years, 12 to 14 years, >14 years), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day)

^a We present the results of linear associations between acrylamide dietary intake and mortality risk with HR [95% CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

Figure 2: Restricted cubic splines of acrylamide dietary intake in association with all-cause mortality risk, stratified on smoking status, in the E3N cohort (N=72 585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

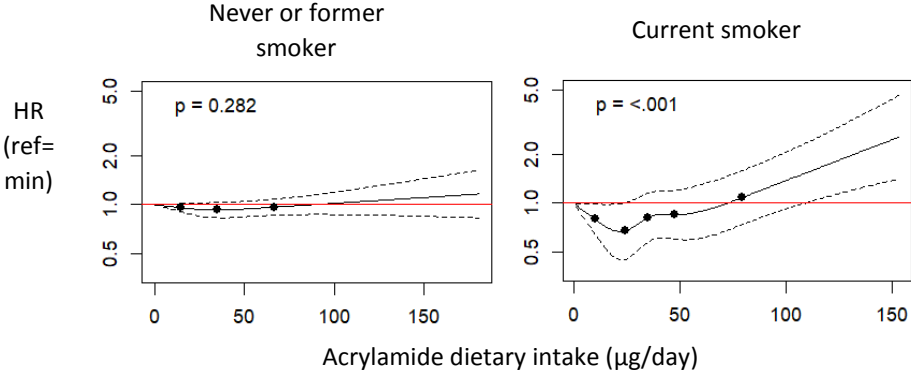


Table 4: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between non-coffee acrylamide dietary intake and mortality risk in the E3N cohort (N=72 585)

		N deaths	Model 3 HR [95% CI]^a
All-cause mortality (N=72 585)	Non-coffee acrylamide dietary intake (spline with 4 knots)	6 441	
	p-value for the overall association		0.066
	p-value for the non-linearity test		0.040
	Quartiles of non-coffee acrylamide intake (min-max, µg/day)		
	Q1 (0.0-7.8)	1 943	Ref
	Q2 (7.8-12.8)	1 715	1.03 [0.96; 1.10]
	Q3 (12.8-19.6)	1 468	0.97 [0.90; 1.04]
Q4 (19.6-209.3)	1 315	0.98 [0.90; 1.06]	
	p-linear trend		0.395
All-cancer mortality (N=72 416)	Non-coffee acrylamide dietary intake (linear term)	3 473	1.01 [0.97; 1.06]
	p-value		0.531
	p-value for the non-linearity test		0.344
	Quartiles of non-coffee acrylamide intake (min-max, µg/day)		
	Q1 (0.0-7.8)	982	Ref
	Q2 (7.8-12.8)	902	1.01 [0.92; 1.11]
	Q3 (12.8-19.6)	831	0.99 [0.90; 1.10]
Q4 (19.6-209.3)	758	0.98 [0.87; 1.09]	
	p-linear trend		0.586
CVD mortality (N=72 416)	Non-coffee acrylamide dietary intake (linear term)	896	1.07 [0.98; 1.17]
	p-value		0.129
	p-value for the non-linearity test		0.238
	Quartiles of non-coffee acrylamide intake (min-max, µg/day)		
	Q1 (0.0-7.8)	289	Ref
	Q2 (7.8-12.8)	255	1.11 [0.93; 1.32]
	Q3 (12.8-19.6)	175	0.89 [0.73; 1.09]
Q4 (19.6-209.3)	177	1.15 [0.92; 1.42]	
	p-linear trend		0.506
Breast cancer mortality (N=72 238)	Non-coffee acrylamide dietary intake (linear term)	953	1.02 [0.94; 1.12]
	p-value		0.588
	p-value for the non-linearity test		0.440
	Quartiles of non-coffee acrylamide intake (min-max, µg/day)		

	Q1 (0.0-7.8)	240	Ref
	Q2 (7.8-12.8)	268	1.20 [1.00; 1.43]
	Q3 (12.8-19.6)	229	1.07 [0.88; 1.30]
	Q4 (19.6-209.3)	216	1.07 [0.87; 1.33]
	p-linear trend		0.897
Lung cancer mortality (N=72 238)	Non-coffee acrylamide dietary intake (linear term)	364	1.03 [0.89; 1.18]
	p-value		0.714
	p-value for the non-linearity test		0.284
	Quartiles of non-coffee acrylamide intake (min-max, µg/day)		
	Q1 (0.0-7.8)	106	Ref
	Q2 (7.8-12.8)	91	0.97 [0.73; 1.30]
	Q3 (12.8-19.6)	92	1.04 [0.77; 1.40]
	Q4 (19.6-209.3)	75	0.93 [0.66; 1.31]
	p-linear trend		0.744
	Colorectal cancer mortality (N=72 238)	Non-coffee acrylamide dietary intake (linear term)	317
p-value			0.050
p-value for the non-linearity test			0.486
Quartiles of non-coffee acrylamide intake (min-max, µg/day)			
Q1 (0.0-7.8)		90	Ref
Q2 (7.8-12.8)		70	0.85 [0.62; 1.17]
Q3 (12.8-19.6)		75	0.98 [0.71; 1.36]
Q4 (19.6-209.3)		82	1.18 [0.83; 1.68]
p-linear trend			0.208

Model 3: Adjustment on age (years) as time-scale, BMI (kg/m²), physical activity (MET-hours/week), birth cohort (≤ 1930 , (1930 – 1935], (1935 – 1940], (1940 – 1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day), and coffee consumption (mL/day)

^a We present the results of linear associations between non-coffee acrylamide dietary intake and mortality risk with HR [95% CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

Figure 3: Restricted cubic splines of non-coffee acrylamide dietary intake in association with all-cause mortality risk in model 3 in the E3N cohort (N=72 585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

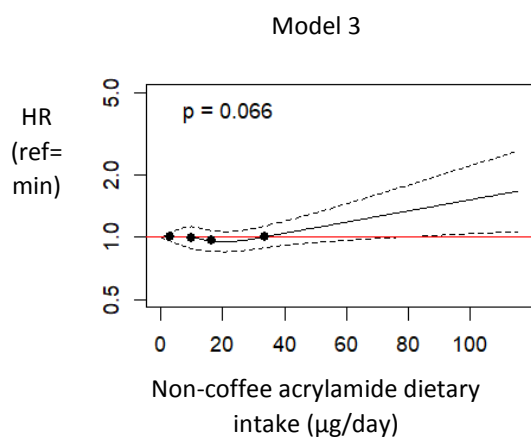


Table 5: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between non-coffee acrylamide dietary intake and all-cause mortality risk, stratified on smoking status, in the E3N cohort (N=72 585)

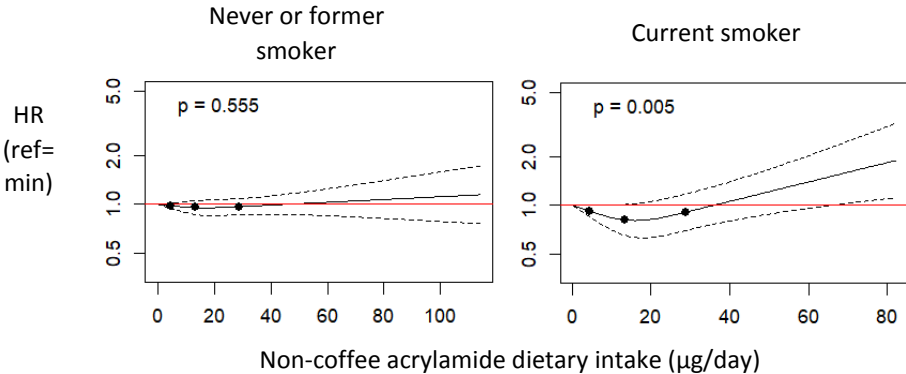
	N deaths	Never or former smoker HR [95% CI] ^a	N deaths	Current smoker HR [95% CI] ^a
Non-coffee acrylamide dietary intake (spline with 3 knots)	5 493		948	
p-value for the overall association		0.555		0.005
p-value for the non-linearity test		0.282		0.006
Quartiles of non-coffee acrylamide intake (min-max, µg/day)			Quartiles of non-coffee acrylamide intake (min-max, µg/day)	
Q1 (0.0-7.8)	1 640	Ref	Q1 (0.0-7.8)	Ref
Q2 (7.8-12.8)	1 485	1.03 [0.93; 1.11]	Q2 (7.8-12.8)	0.99 [0.83; 1.18]
Q3 (12.8-19.6)	1 275	0.99 [0.92; 1.07]	Q3 (12.8-19.6)	0.84 [0.69; 1.02]
Q4 (19.6-209.3)	1 093	0.97 [0.89; 1.06]	Q4 (19.6-124.0)	1.02 [0.84; 1.25]
p-linear trend		0.370	p-linear trend	0.994

Model: Adjustment on age (years) as time-scale, BMI (kg/m²), physical activity (MET-hours/week), birth cohort (≤1930, (1930 – 1935), (1935 – 1940), (1940 – 1945), >1945), education level (<12 years, 12 to 14 years, >14 years), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), alcohol consumption (g ethanol/day), and coffee consumption (mL/day)

^a We present the results of linear associations between non-coffee acrylamide dietary intake and mortality risk with HR [95% CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

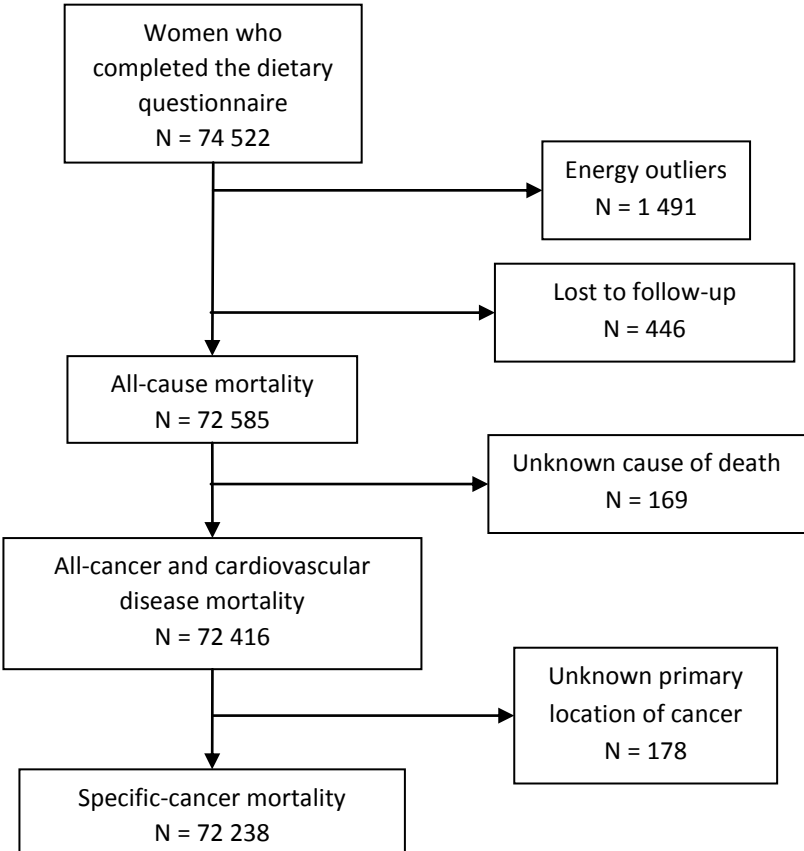
Figure 4: Restricted cubic splines of non-coffee acrylamide dietary intake in association with all-cause mortality risk, stratified on smoking status, in the E3N cohort (N=72 585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR,

and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

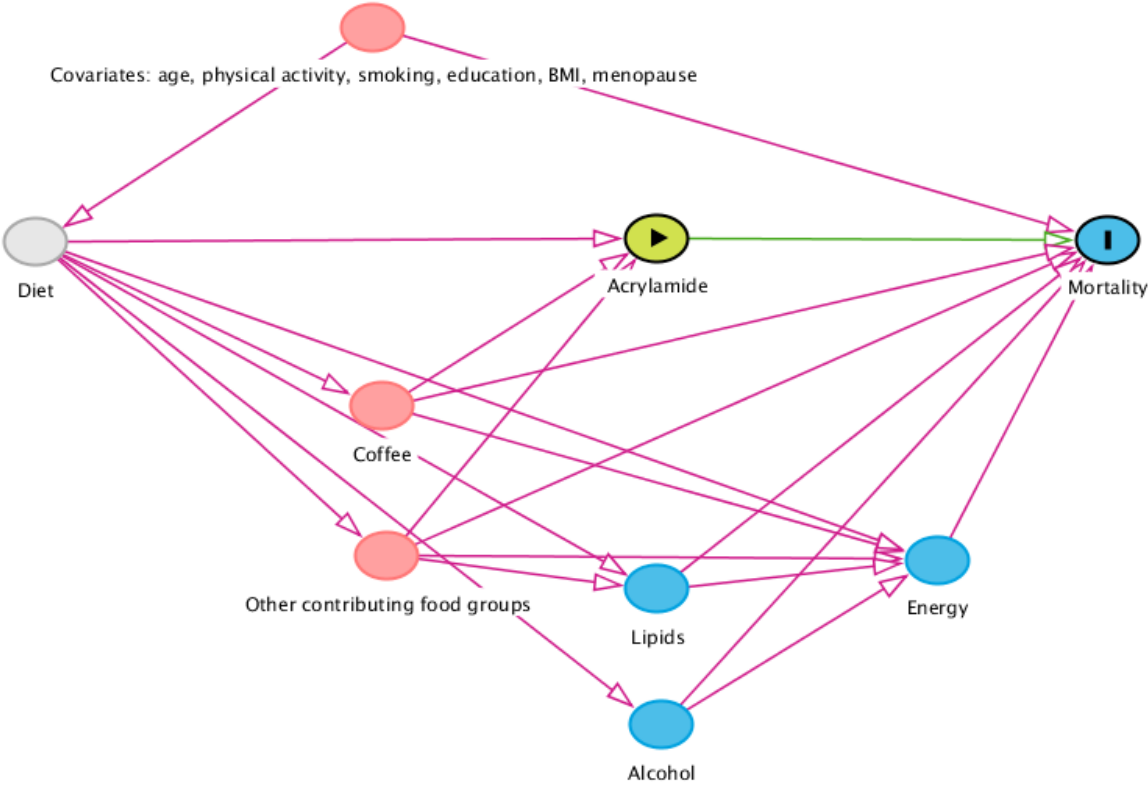


Appendix. Supplementary materials

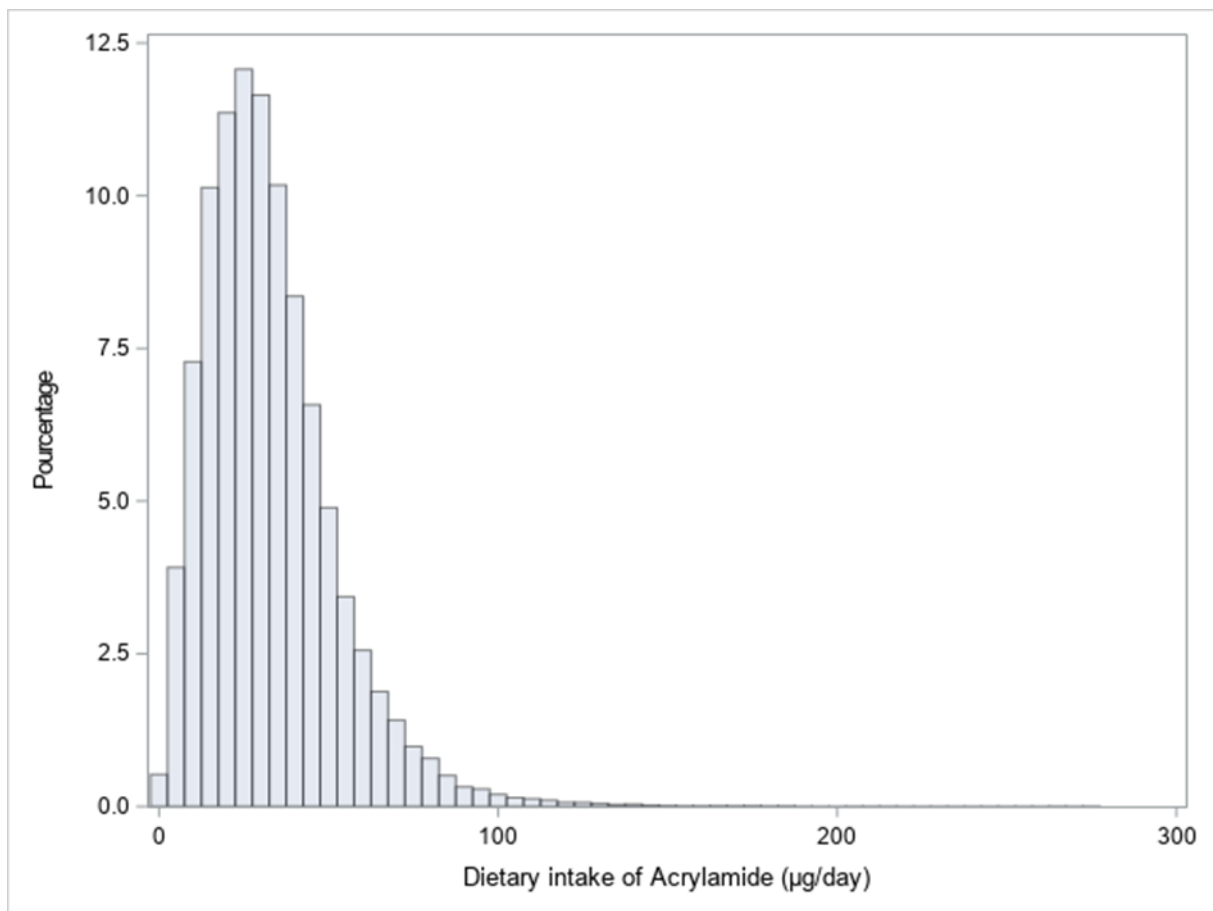
Supplementary figure 1: Flow chart



Supplementary figure 2: Directed acyclic graph



Supplementary figure 3: Distribution of acrylamide dietary intake in the E3N cohort (N=72 585)



Supplementary table 1: Characteristics^a of the study population overall and among vital status at the end of the follow-up in the E3N cohort (N=72 585)

	All (N=72 585)	Vital status	
		Non-deaths (N=66 144)	Deaths (N=6 441)
Acrylamide intake ($\mu\text{g}/\text{day}$)	32.6 (19.4)	32.8 (19.4)	30.9 (19.6)
Age (years)	52.9 (6.7)	52.5 (6.4)	57.7 (7.2)
Follow-up duration (years)	19.0 (4.1)	19.5 (3.5)	13.4 (5.6)
Follow-up duration (person-years)	1 378 046	1 291 705	86 341
BMI (kg/m^2)	22.7 (3.1)	22.7 (3.1)	23.4 (3.7)
<18.5	2 645 (3.6)	2 382 (3.6)	263 (4.1)
[18.5 – 25)	56 794 (78.2)	52 243 (79.0)	4 551 (70.7)
≥ 25	13 146 (18.1)	11 519 (17.4)	1 627 (25.3)
Birth cohort			
$\leq 1 930$	7 295 (10.1)	5 348 (8.1)	1 947 (30.2)
(1 930 – 1 935]	9 996 (13.8)	8 609 (13.0)	1 387 (21.5)
(1 935 – 1 940]	14 710 (20.3)	13 487 (20.4)	1 223 (19.0)
(1 940 – 1 945]	17 811 (24.5)	16 817 (25.4)	994 (15.4)
>1 945	22 773 (31.4)	21 883 (33.1)	890 (13.8)
Education level			
<12 years	8 190 (11.3)	7 261 (11.0)	929 (14.4)
12 to 14 years	38 408 (52.9)	34 930 (52.8)	3 478 (54.0)
>14 years	25 987 (35.8)	23 953 (36.2)	2 034 (31.6)
Smoking status			
Never or former	63 425 (87.4)	57 932 (87.6)	5 493 (85.3)
Current	9 160 (12.6)	8 212 (12.4)	948 (14.7)
Physical activity (MET-hours/week)	46.4 (43.4)	46.3 (43.1)	48.1 (45.7)
Menopausal status and recent use of MHT			
Premenopausal	37 313 (51.4)	35 681 (53.9)	1 632 (25.3)

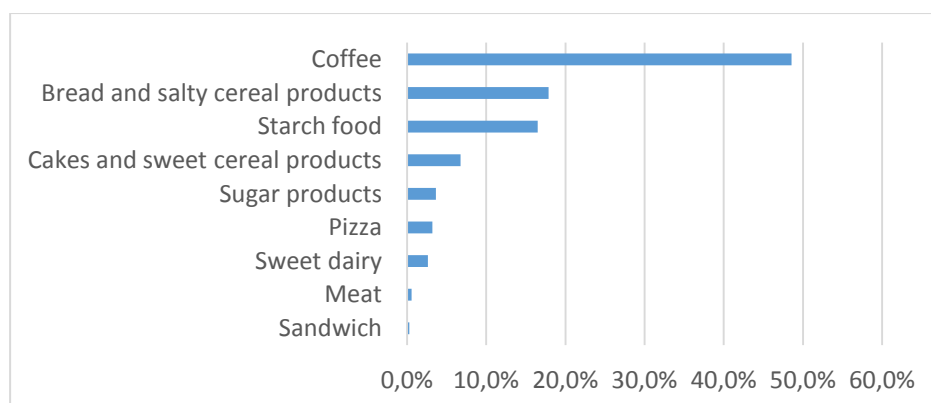
Menopausal and recent MHT use	10 047 (13.8)	9 133 (13.8)	914 (14.2)
Menopausal and no recent MHT use	21 671 (29.9)	18 198 (27.5)	3 473 (53.9)
Menopausal and no information on whether and when MHT was used	3 554 (4.9)	3 132 (4.7)	422 (6.6)
Energy intake (excluding energy from alcohol and lipids, kcal/day)	1 329.0 (356.6)	1 331.6 (356.4)	1 301.8 (357.4)
Alcohol consumption (g ethanol/day)	11.6 (13.9)	11.5 (13.8)	12.3 (15.3)
Lipid consumption (g/day)	88.9 (27.0)	89.3 (27.0)	85.6 (26.6)
Coffee intake (mL/day)	332.6 (282.2)	333.2 (282.4)	326.4 (280.8)

^a Mean (std) for continuous variables, N (%) for categorical variables

Supplementary table 2: Spearman rank correlation coefficients between acrylamide dietary intake and food groups consumption in the E3N cohort (N=72 585)

	Coffee intake (mL/day)	Bread and salty cereal products intake (g/day)	Starch food intake (g/day)
Acrylamide intake (µg/day)	0.79	0.10	0.26
p-value	<.0001	<.0001	<.0001

Supplementary figure 4: Food groups contribution to acrylamide dietary intake in the E3N cohort (N=72 585)



Supplementary table 3: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between acrylamide and non-coffee acrylamide dietary intake (modelled with restricted cubic spline functions) and mortality risk in the E3N cohort (N=72 585)

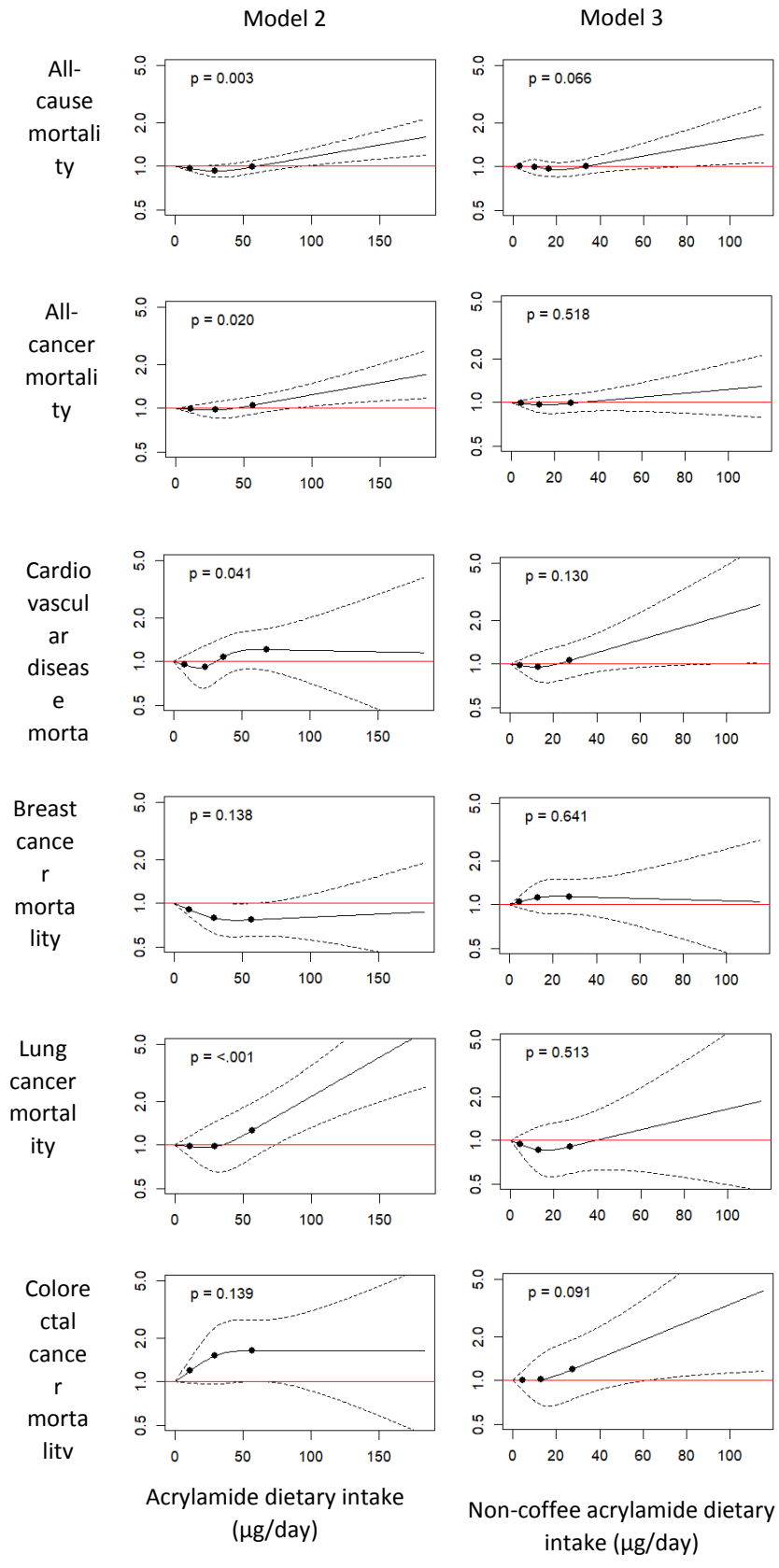
		N deaths	Acrylamide intake Model 2 HR [95% CI]	Non-coffee acrylamide intake Model 3 HR [95% CI]
All-cause mortality (N=72 585)	p-value for the overall association	6 441	0.003	0.066
	p-value for the non-linearity test		0.006	0.040
All-cancer mortality (N=72 416)	p-value for the overall association	3 473	0.020	0.518
	p-value for the non-linearity test		0.125	0.344
CVD mortality	p-value for the overall association	896	0.041	0.130

(N=72 416)	p-value for the non-linearity test		0.261	0.238
Breast cancer mortality (N=72 238)	p-value for the overall association	953	0.138	0.641
	p-value for the non-linearity test		0.141	0.440
Lung cancer mortality (N=72 238)	p-value for the overall association	364	<0.001	0.513
	p-value for the non-linearity test		0.124	0.284
Colorectal cancer mortality (N=72 238)	p-value for the overall association	317	0.139	0.091
	p-value for the non-linearity test		0.205	0.486

Model 2: Adjustment on age (years) as time-scale, BMI (kg/m²), physical activity (MET-hours/week), birth cohort (\leq 1930, (1930 – 1935], (1935 – 1940], (1940 – 1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day)

Model 3: M2 + coffee consumption (mL/day)

Supplementary figure 5: Restricted cubic splines of acrylamide and non-coffee acrylamide dietary intake in association with mortality risk in models 2 and 3, respectively, in the E3N cohort (N=72 585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.



Supplementary table 4: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between acrylamide dietary intake and mortality risk when adjusting for chronic diseases at baseline in the E3N cohort (N=72 585)

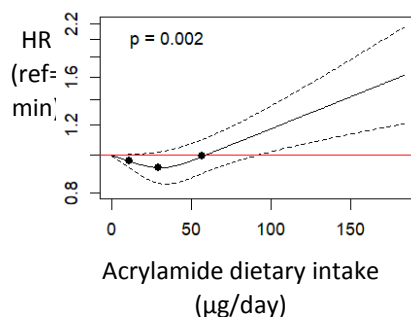
		N deaths	Model HR [95% CI]^a
All-cause mortality (N=72 585)	Acrylamide dietary intake (spline with 3 knots)	6 441	
	p-value for the overall association		0.002
	p-value for the non-linearity test		0.006
	Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0-18.9)	1 857	Ref
	Q2 (18.9-29.5)	1 626	0.97 [0.91; 1.04]
	Q3 (29.5-42.2)	1 560	1.03 [0.96; 1.10]
	Q4 (42.2-276.2)	1 398	1.04 [0.96; 1.12]
p-linear trend		0.166	
All-cancer mortality (N=72 416)	Acrylamide dietary intake (linear term)	3 473	1.06 [1.01; 1.10]
	p-value		0.015
	p-value for the non-linearity test		0.159
	Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0-18.9)	916	Ref
	Q2 (18.9-29.5)	875	1.01 [0.92; 1.11]
	Q3 (29.5-42.2)	871	1.08 [0.98; 1.19]
	Q4 (42.2-276.2)	811	1.07 [0.97; 1.19]
p-linear trend		0.108	
CVD mortality (N=72 416)	Acrylamide dietary intake (linear term)	896	1.11 [1.01; 1.21]
	p-value		0.023
	p-value for the non-linearity test		0.291
	Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0-18.9)	259	Ref
	Q2 (18.9-29.5)	226	1.03 [0.86; 1.23]
	Q3 (29.5-42.2)	217	1.14 [0.95; 1.38]
	Q4 (42.2-276.2)	194	1.32 [1.08; 1.62]
p-linear trend		0.004	
Breast cancer mortality (N=72 238)	Acrylamide dietary intake (linear term)	953	0.95 [0.87; 1.04]
	p-value		0.260
	p-value for the non-linearity test		0.178
	Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0-18.9)	271	Ref
	Q2 (18.9-29.5)	246	0.96 [0.80; 1.14]
	Q3 (29.5-42.2)	222	0.91 [0.76; 1.10]
	Q4 (42.2-276.2)	214	0.91 [0.75; 1.11]
p-linear trend		0.332	
Lung cancer mortality (N=72 238)	Acrylamide dietary intake (linear term)	364	1.22 [1.09; 1.38]
	p-value		0.001
	p-value for the non-linearity test		0.128
	Quartiles of acrylamide intake (min-max,		

	µg/day		
	Q1 (0.0-18.9)	84	Ref
	Q2 (18.9-29.5)	88	1.07 [0.79; 1.44]
	Q3 (29.5-42.2)	90	1.14 [0.84; 1.54]
	Q4 (42.2-276.2)	102	1.34 [0.98; 1.83]
	p-linear trend		0.053
	Acrylamide dietary intake (linear term)	317	1.12 [0.97; 1.29]
	p-value		0.111
	p-value for the non-linearity test		0.202
	Quartiles of acrylamide intake (min-max, µg/day)		
Colorectal cancer mortality (N=72 238)	Q1 (0.0-18.9)	73	Ref
	Q2 (18.9-29.5)	80	1.17 [0.85; 1.61]
	Q3 (29.5-42.2)	84	1.32 [0.95; 1.82]
	Q4 (42.2-276.2)	80	1.40 [0.99; 1.97]
	p-linear trend		0.048

Model: Adjustment on age (years) as time-scale, BMI (kg/m²), physical activity (MET-hours/week), birth cohort (≤ 1930 , (1930 – 1935], (1935 – 1940], (1940 – 1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), alcohol consumption (g ethanol/day), diabetes before inclusion (yes, no, for all causes of death), cancer before inclusion (yes, no, for all-cause and cancer mortality), high cholesterol before inclusion (yes, no, for all-cause and CVD mortality), and hypertension before inclusion (yes, no, for all-cause and CVD mortality)

^a We present the results of linear associations between acrylamide dietary intake and mortality risk with HR [95% CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

Supplementary figure 6: Restricted cubic splines of acrylamide dietary intake in association with all-cause mortality risk when adjusting for chronic diseases at baseline in the E3N cohort (N=72 585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.



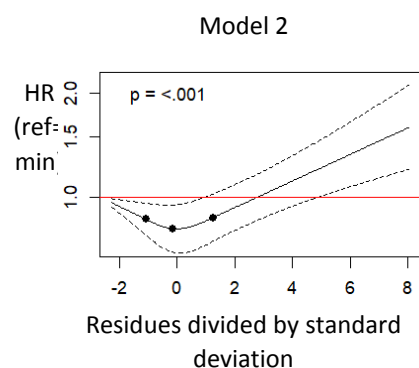
Supplementary table 5: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between acrylamide dietary intake and mortality risk with the energy adjustment residual method in the E3N cohort (N=72 585)

		N deaths	Model 2 HR [95% CI] ^a
All-cause mortality (N=72 585)	Acrylamide dietary intake (spline with 3 knots)	6 441	
	p-value for the overall association		<.001
	p-value for the non-linearity test		<.001
	Quartiles of acrylamide intake (min-max, µg/day)		
	Q1 (0.0-18.9)	1 857	Ref
	Q2 (18.9-29.5)	1 626	0.96 [0.90; 1.03]
	Q3 (29.5-42.2)	1 560	0.94 [0.88; 1.01]
Q4 (42.2-276.2)	1 398	1.05 [0.98; 1.13]	
	p-linear trend		0.171

Model 2: Adjustment on age (years) as time-scale, BMI (kg/m²), physical activity (MET-hours/week), birth cohort (≤1930, (1930 – 1935], (1935 – 1940], (1940 – 1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day)

^a We present the results of linear associations between acrylamide dietary intake and mortality risk with HR [95% CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

Supplementary figure 7: Restricted cubic splines of acrylamide dietary intake in association with all-cause mortality risk with the energy adjustment residual method in the E3N cohort (N=72 585). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.



Supplementary table 6: Hazard ratios (95% CI) estimated by Cox multivariable regression models for the association between acrylamide dietary intake and mortality risk when excluding participants who died or were censored during the first five years of follow-up in the E3N cohort (N=70 922)

	N deaths	Model 2 HR [95% CI] ^a
Acrylamide dietary intake (spline with 3 knots)	5 748	
p-value for the overall association		0.003
p-value for the non-linearity test		0.014
Quartiles of acrylamide intake (min-max, µg/day)		
All-cause mortality (N=70 922)		
Q1 (0.0-18.9)	1 659	Ref
Q2 (18.9-29.5)	1 461	0.98 [0.91; 1.05]
Q3 (29.5-42.2)	1 379	1.01 [0.94; 1.09]
Q4 (42.2-276.2)	1 249	1.06 [0.98; 1.15]
p-linear trend		0.090

Model 2: Adjustment on age (years) as time-scale, BMI (kg/m²), physical activity (MET-hours/week), birth cohort (≤1930, (1930 – 1935], (1935 – 1940], (1940 – 1945], >1945), education level (<12 years, 12 to 14 years, >14 years), smoking status (never or former smoker, current smoker), menopausal status and recent MHT use (premenopausal, menopausal and recent MHT use, menopausal and no recent MHT use, menopausal and no information on whether and when MHT was used), energy intake (excluding energy from alcohol and lipids, kcal/day), lipids consumption (g/day), and alcohol consumption (g ethanol/day)

^a We present the results of linear associations between acrylamide dietary intake and mortality risk with HR [95% CI] per one standard deviation, p-value and p-value for the non-linearity test, and of non-linear associations with p-values for the overall association and for the non-linearity test.

Supplementary figure 8: Restricted cubic splines of acrylamide dietary intake in association with all-cause mortality risk when excluding participants who died or were censored during the first five years of follow-up in the E3N cohort (N=70 922). The minimum value of the main exposure variable is taken as reference. The "p" represents the p-value for the overall association. Solid lines indicate HR, and dashed lines indicate 95% CI. The points represent percentiles as follows: 3 nodes at the 10th, 50th and 90th percentiles, 4 nodes at the 5th, 35th, 65th and 95th percentiles, or 5 nodes at the 5th, 27.5th, 50th, 72.5th and 95th percentiles.

