UNIVERSITAT DE BARCELONA

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

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INTRODUCTION

1. INTRODUCTION

1.1 Acrylamide

Human acrylamide exposure is considered a public health concern. Acrylamide was first produced in 1893 by Moureu and its commercial production started in 1954 [1]. Acrylamide is also known either by its chemical name 2-propenamide or by its synonyms acrylic acid amide, acrylic amide, ethylenecarboxamide, propenamide, propeneamide, propenoic acid amide, and vinyl amide.

Acrylamide is an organic monomer compound which has low molecular weight and is water soluble. It is solid in its pure form (crystalline form), colorless and odorless at room temperature, and is mainly used for the preparation of polyacrylamide (**Figure 1**). Polyacrylamide compounds have been used in industries as a flocculant for the treatment of the public water supply, in paper and plum manufacture, textile, and mining; however, it has also been used as cosmetic additives, in biomedical research (gel electrophoresis), and in the formulation of grouting agents.

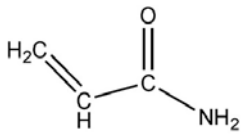


Figure 1. Chemical structure of acrylamide [2]

In 1985, the International Agency for Research on Cancer (IARC) evaluated for the first time the carcinogenic risk of acrylamide to humans, and although at that time no data on humans were available, there was *sufficient evidence* to catalog acrylamide as carcinogenic to experimental animals [1]. The second IARC monograph evaluation was published in 1994, and classified acrylamide as *probably carcinogenic to humans (Group 2A)* [3] due to the *sufficient evidence* for the carcinogenicity in experimental animals and the *inadequate evidence* in humans (only two cohort studies assessed the relation between acrylamide workers exposure and mortality, with inconsistent results [4, 5]). Nevertheless, already by the 1950s, several studies observed that acrylamide exposed workers (i.e. chemical industry) developed symptoms of neurotoxicity [6] .

Public health concern increased in 1997, during tunnel construction in Bjäre, Sweden. Tunnels workers had to use 1,400 tons of a sealant product (containing acrylamide) during the building period, but this fact resulted in the contamination of ground and surface water with acrylamide. The contamination caused neurotoxicity to workers, and death in cows and aquatic species [7]. One of the multiple studies that were carried to compare acrylamide blood levels between exposed workers and non-exposed controls (who were non-smokers) found elevated levels of acrylamide in blood in both groups, but higher in exposed workers. Due to these results, other hypotheses were proposed, such as endogenous formation and dietary exposure [8].

In spring 2002, acrylamide was discovered in some foods (bread, fried foods and coffee) treated at high temperatures, first by the Swedish National Food Administration and researchers from Stockholm University, and afterwards by the British Food Standard Agency [9], the Norwegian Food Control Authority [10], the Federal Office of Public Health (Switzerland) [11], the Bundesinstitut für Risikobewertung (Germany) [12], and the US Food and Drug Administration (FDA) [13].

1.1.1 Acrylamide formation in food

Acrylamide cannot be detected in natural foods [3]. It was during the autumn of 2002 that several researchers described the main mechanism by which acrylamide was formed in heated foods [14–17]. They observed that they could obtain acrylamide through a reaction between asparagine, an amino acid, and a reducing carbohydrate (i.e. glucose and fructose). The complex chemical reaction between an amino group of a protein and a carbonyl group of a reducing sugar is known as Maillard reaction, glycation, or non-enzymatic glycosylation [18, 19]. Part of the heat-treated food characteristics, such as taste, smell, texture, and appearance are due to the reaction discovered by Louis-Camille Maillard [18]. A large number of different substances besides acrylamide are formed during this procedure, called Maillard reaction products (MRPs) also known as advanced glycation end-products (AGEs). There are both positive and negative health effects on animals and humans with AGEs exposure [20]. In addition, other minor pathways that can synthesize acrylamide have been described [21].

Main pathway for acrylamide formation

Asparagine is the main amino acid precursor of acrylamide. After thermal treatment and in the presence of α-hydroxycarbonyl groups (i.e. reducing sugars), asparagine is converted to acrylamide through decarboxylation and deamination reactions; however, these reactions can also take place without sugars, but are much less efficient. In this route, the reaction between asparagine and the α-hydroxycarbonyl group results in the formation of a Schiff base in the Maillard reaction. This Schiff base can either be decarboxylated to form azomethine ylide, or transformed to Amadori compounds. The Amadori compounds can be decarboxylated to form acrylamide. The molecule azomethine ylide can follow three pathways: (a) directly form acrylamide; (b) hydrolyze to form 3-aminopropionamide (3-APA), and after a deamination reaction to form acrylamide; and (c) via a tautomerisation and yield to decarboxylated Amadori compound, which leads to acrylamide formation. The molecule 3-APA is considered the acrylamide precursor (**Figure 2**).

In presence of α, β, γ, δ-diunsaturated carbonyl or α-dicarbonyl (named as reactive carbonyls) compounds derived from the Maillard reaction, an α-amino group of an amino acid is degraded. A corresponding Schiff base is obtained, which leads to azomethine ylide to form acrylamide. This pathway is commonly known as the Strecker degradation route. This route can also form acetaldehyde during the thermal degradation of proteins (**Figure 2**).

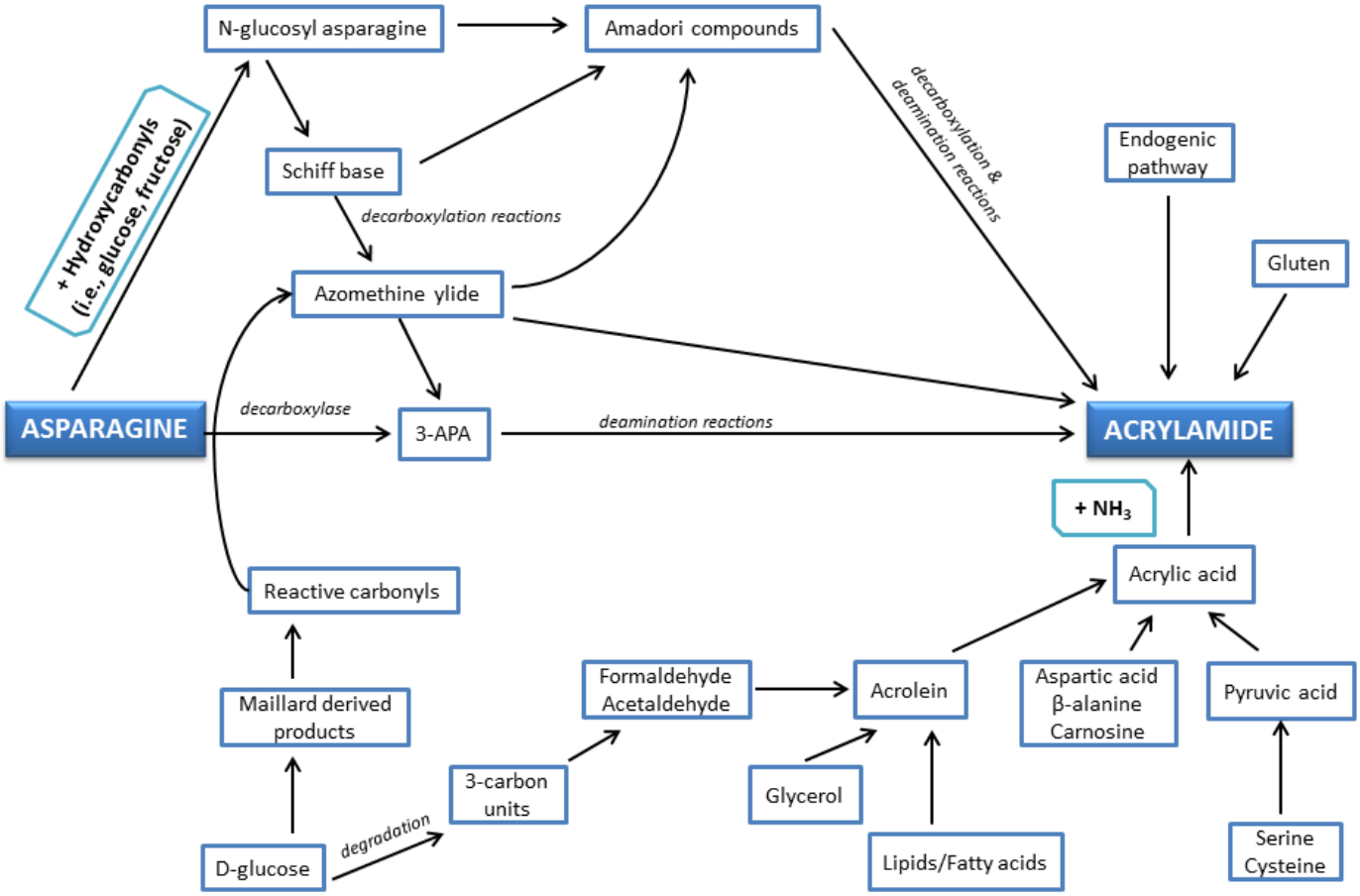


Figure 2. Pathways of acrylamide formation. Adapted from [21]

Minor pathways for acrylamide formation

Among the minor pathways, the acrolein pathway contributes most to acrylamide formation. It is well-known that deep-fried foods rich in asparagine (without the presence of reducing sugars) have a high concentration of acrylamide; thus, lipid metabolism plays a role in the synthesis of acrylamide [22]. The oxidation and degradation of lipids benefit the presence of free glycerol and 3-carbon units, which derives to acrolein. Acrolein can also be synthesized through formaldehyde and acetaldehyde. Then, acrolein is oxidated to form acrylic acid. The reaction between acrylic acid and ammonia (amino group of a degraded aminoacid) results in acrylamide formation. There are other substrates that can trigger formation of acrylic acid, such as aspartic acid, carnosine, or β-alanine. Serine and cysteine can be degradated to form pyruvic acid, which also leads to acrylamide formation. D-glucose can suffer several reactions that lead in the formation of 3-carbon compounds, which at the same time, forms formaldehyde and/or acetaldehyde, the precursors of acrolein. Other marginal pathways are through the effect of a *decarboxylase*, an enzyme that catalyzes the reaction asparagine to 3-APA, or by breaking down gluten or small peptides after thermal processing [23](**Figure 2**).

All of the pathways mentioned above are exogenous (are formed in food before it is eaten); however, it has been observed in *in vitro* studies that acrylamide can also be formed through

an endogenous pathway under specific physiological conditions; but it is thought to play a minimal role [24, 25].

Food sources of acrylamide

The total amount of acrylamide in foods varies depending on concentration and reactivity of the chemical components, temperature, pH, water content and duration of the thermal process.

- Concentration and reactivity of chemical components: As it has been pointed out, both free asparagine and reducing sugars are needed to form acrylamide; nonetheless, it has been observed that sugars with shorter chains are more likely to react and form acrylamide. For instance, α-hydroxycarbonyl groups are more likely to react than α-dicarbonyl groups. Likewise, the presence of fructose increases 2-fold the acrylamide formation compared to glucose, since fructose has lower fusion temperature than glucose (127 *versus* 157 ºC, respectively).
- Temperature: Acrylamide is not present in raw food products; it is usually formed at high temperatures during common cooking procedures such as frying, baking, roasting, grilling, and toasting. Generally, the increase of temperature and/or heating time, results in an increase in acrylamide formation (**Figure 3**). The water content of foods influences the temperature in which the synthesis of acrylamide starts, but usually involves temperatures above 120°C. In rare cases, acrylamide has also been found in foods (e.g. prunes and prune juice) cooked at temperatures lower than 100°C [26]. Interestingly, dark-roasted coffee has less acrylamide levels than medium-roasted coffee due to acrylamide evaporation/degradation (acrylamide boils at 193°C and roasting involves temperatures between 210 to 250°C) [27].

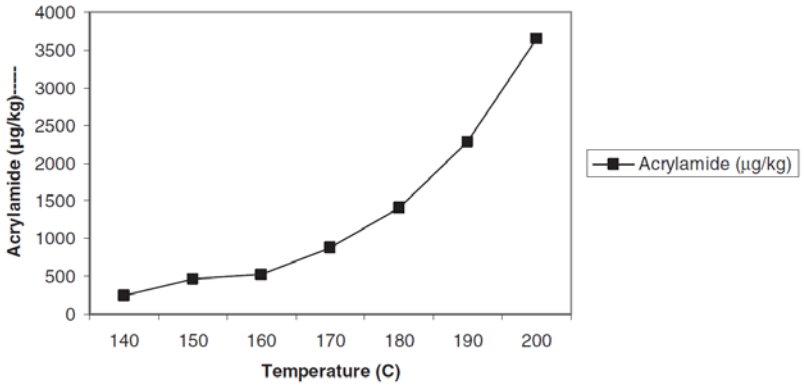


Figure 3. Acrylamide levels in potato crisps depending on temperature [28]

Exceptionally, glycidamide (metabolite of acrylamide) is also formed in some heat-treated foods (i.e. potato chips, French fries), but at very low concentrations [29]. Glycidamide formation might also be dependent on heating time [30].

- pH: It is widely established that pH levels influences acrylamide formation [31, 32]. A pH reduction (increase in acidity) has been shown to reduce the synthesis of

acrylamide. At lower pH there is an increment of proton concentration (H^+) which transforms the non-protonated α-amino groups of asparagine to protonated, thus, the first step of acrylamide formation is inhibited [33].

- Water content: Several studies have concluded that the total amount of water present in foods can increase or decrease acrylamide formation. It has been observed that acrylamide is formed in foods when water activity (a_w) is between 0.8 and 0.4. When a_w is <0.4, the formation of acrylamide is decreased. Water activity is a parameter linked to moisture content, therefore those foods with moisture content <5% will be more likely to follow the Maillard reaction and form acrylamide [34].

Acrylamide has been measured in a wide variety of foods and food products, mainly of which are consumed in a daily basis in many countries [30]; however, it is difficult to report acrylamide content in specific foods due to the great variation in acrylamide values within the same food items (**Table 1**). It is well known that food composition (i.e., nutrient contents) is affected, *inter alia*, by season, climate, soil, manufacturing procedures, and storage conditions. For instance, several studies have observed that these factors (such as year, storage temperature and time) have an impact on asparagine, glutamine, glucose, and fructose levels in potatoes, and consequently, affected acrylamide levels [35, 36].

Table 1. Differences in acrylamide levels within and between foods in Sweden

Food	Source	Acrylamide levels (ng/g)
Potato chips	Manufacturer 1	730
	Manufacturer 2	1,500
	Manufacturer 3	3,700
	Manufacturer 4	550
French fries	Vendor 1	610
	Vendor 2	1,300
	Vendor 3	620
	Vendor 4	1,900

Adapted from [26]

There is still limited information on acrylamide levels in home-cooked food; nevertheless, there exist general acrylamide monitoring databases of which, two of the most important are from Europe (EU), and the United States (US) [37, 38]. Although eating habits vary across and within regions (EU and US), the major food contributors to dietary acrylamide exposure are similar: French fries, potato chips, cereals, crispbread, bread, coffee, pies, and pastry [39].

Several epidemiologic studies have published major food determinants of dietary acrylamide exposure, and results are generally in agreement with the foods identified by the EU and US monitoring databases (**Table 2**). Freisling et al. assessed the principal food group determinants of acrylamide intake based in a 24-hour dietary recall (24hDR) in 13,486 men and 23,508 women from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, and identified bread, crisp bread, rusks, coffee, and potatoes [40]. In Finnish adults, the main dietary sources of acrylamide were coffee and casseroles rich in starch, and bread [41]. In a Polish population, they identified bread, French fries, potato crisps and roasted coffee as the main food contributors to acrylamide exposure [42]. The fifth Chinese Total Diet Study reported as major food sources vegetables, cereals, and potatoes [43]. In Sweden, the top

food group contributors were crispbread, coffee, other bread, fried potatoes, and buns and cakes [44].

Table 2. Reported amounts of acrylamide in foods by EFSA and the ACSP

Food	EFSA		ASBP	
	n[1]	Mean (max) μg/kg	n[1]	Mean (min-max) μg/kg
French fries	529	350 (2,668)	13	250 (0-1,260)
Potato crisps	242	675 (4,533)	21	736 (22-1,304)
Soft bread	150	30 (425)	6	30 (0-79)
Breakfast cereals	174	138 (1,290)	29	92 (0-348)
Coffee and coffee substitutes	151	527 (8,044)	65	263 (56-696)
Baby foods	55	69 (1,107)	54	17 (0-192)

n[1] Number of individual data analyzed for each food category
EFSA, European Food Safety Authority; ASBP, Agència de Salut Pública de Barcelona
Adapted from [30, 45]

1.1.2 Human acrylamide exposure

There are at least three routes by which humans can be exposed to acrylamide: oral, dermal, and inhalation; thus, 'human acrylamide exposure' reflects a combination from diet, smoking (and second-hand smoke exposure; SHS), drinking water, occupational sources, and personal care items.

Dietary acrylamide exposure

Epidemiological studies often use food frequency questionnaires (FFQ) to estimate dietary exposure, but this tool may not yield accurate estimates of dietary contaminants [46]. Despite the challenges in estimating dietary acrylamide exposure, several studies and organizations have reported means and/or medians of acrylamide consumption based on FFQs (**Table 3**).

Table 3. Dietary acrylamide estimates from several epidemiologic studies.

Study	Estimated mean acrylamide intake μg/kg bw per day	Source
EPIC	0.38	Obón-Santacana et al. [47]
FINRISK Study	0.44 (median, women) 0.41 (median, men)	Hirvonen et al. [41]
NHS-II	0.29	Wilson et al. [48]
NLCS	0.30	Hogervorst et al. [49]
TDS	0.21	Wong et al. [50]
Study in Poland	0.43	Mojska et al. [42]

EPIC; European Prospective Investigation into Cancer and Nutrition; FINRISK; Finnish population survey on risk factors on chronic, noncommunicable diseases; NHS-II, Nurses' Health Study II; NLCS, the Netherlands Cohort Study; and TDS, Hong Kong Total Diet Study

The Food and Agriculture Organization of the United Nations (FAO), and the World Health Organization (WHO) had a meeting in 2011 – Joint FAO/WHO Expert Committee on Food Additives (JECFA), and estimated a margin of exposure (MOE) or health-based guidance value using information from eight countries, representing all regions except Africa (no data were available). MOE is usually used for risk assessment when there is potentially no tolerable daily

intake for genotoxic compounds. The general population had a mean dietary acrylamide exposure ranging from 0.2-1.0 μg/kg body weight (bw) per day. Adults classified at percentiles 95th-97.5th had values of 0.6-1.8 μg/kg bw per day; however, adults highly exposed consumed 4 μg/kg bw per day. It is worth noting that children had 2-fold higher mean acrylamide levels (measured as μg/kg bw per day) compared to the general adult population. Their conclusion was that an average estimate of 1 μg/kg bw per day could be consumed for the general population and children, and for high acrylamide consumers 4 μg/kg bw per day. The MOE values calculated relative to the no-observed-adverse-effect levels (NOAEL), and the lower limit on the Benchmark dose for a 10% response ($BMDL_{10}$) indicated a potential human health concern [51].

In 2015, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) reported mean values of acrylamide exposure for the general population of 0.4-1.9 μg/kg bw per day. Adults classified at percentile 95th had values of 0.6-3.4 μg/kg bw per day. The EFSA panel also noted that infants were the most exposed group [30]. Similar results (mean intake of 0.4 μg/kg bw per day for age 2 and over) were published by the US FDA [52].

Drinking water also contains polyacrylamide/free acrylamide; however, levels in the EU and the US are very low (some samples measured were below the limit of detection) [53].

Occupational acrylamide exposure

In industry, acrylamide is used directly, as an intermediate, and as a monomer in the production of polyacrylamide. Direct uses of acrylamide include adhesives and grouts, polymer cross-linking, and photopolymerization systems. The primary use of acrylamide is in the production of polyacrylamides which are used in a number of industries including paper/pulp manufacture, oil, mining, wastewater treatment, soil conditioning, textile, and cosmetics. Polyacrylamide is also used for gel electrophoresis in biomedical research.

Occupational exposure usually occurs via inhalation or direct contact with skin or mucous membranes. Acrylamide has been evaluated as an industrial exposure in two retrospective occupational cohort studies of acrylamide workers from the US and the Netherlands. Both reported an excess of pancreatic cancer mortality [4, 5]; however, in subsequent follow-up evaluations, little evidence of an increase of pancreatic cancer death was reported [54, 55].

Furthermore, some personal care products (i.e. cosmetics) contain free acrylamide, which can be absorbed by dermal contact; however, dermal exposure is very difficult to estimate [56].

Tobacco acrylamide exposure

More than 8,000 compounds have been identified in tobacco, of which more than 60 are known to be carcinogenic. These carcinogenic compounds have been measured both in mainstream and side-stream smoke [57]. Acrylamide is also formed by the pyrolysis of tobacco, and tobacco use is considered an important source of acrylamide exposure. The US National Toxicology Program (NTP) estimated that smokers have a mean acrylamide exposure of 3.4 μg/kg bw per day.

Acrylamide exposure can also be measured using adducts of hemoglobin in red blood cells. Smokers have mean acrylamide hemoglobin adducts levels three to four times higher than

non-smokers (without occupational exposures) [30, 58, 59]. Second-hand smoke exposure also has been observed to influence levels of acrylamide hemoglobin adducts [60].

1.1.3 Acrylamide metabolism

Once acrylamide is consumed, it is absorbed by the gastrointestinal tract and, via the circulation, is distributed to peripheral tissues [51, 61]. Schabacker et al. observed that acrylamide monomers pass the monolayer of Caco-2 cells (human intestine model) through passive diffusion [62]. Despite the high bioavailability of dietary acrylamide, diet is complex, and interactions between acrylamide and food ingredients are possible due to the high reactivity of acrylamide with peptides (i.e. acrylamide uptake in humans has been hypothesized to be impaired by a diet rich in proteins) [62].

Several studies concluded that acrylamide does not accumulate in the body; however, it has been detected in several animal tissues (i.e. heart, thyroid, stomach, kidney, liver, and testis) [63]. Concern increased when a group from Germany found acrylamide levels in breast human milk, and also observed that acrylamide could pass the placenta and reach the developing fetus [64]. Acrylamide has a low molecular weight and is highly water soluble. These properties mean acrylamide is more likely to be biologically active in the body.

Acrylamide is metabolized in animals and in humans via at least two main pathways: conjugation with reduced glutathione for elimination, and conversion to a chemically reactive epoxide, glycidamide (**Figure 4**), in a reaction catalyzed by the cytochrome P450 enzyme complex Cyp2e1.

In animals and in humans, the conversion of acrylamide to glycidamide (2,3-epoxypropionamide) is known to primarily occur via the enzyme Cyp2e1; however, there could be other pathways that contribute to the formation of glycidamide, but these are likely to play only a minimal role. Both *in vitro* and *in vivo* studies confirmed that acrylamide is converted to glycidamide by Cyp2e1 in experimental animals and in humans; nevertheless, rats and mice have higher conversion rates than humans [65]. Studies in *CYP2E1*-knockout mice (absence of Cyp2e1) showed that mice did not excrete glycidamide or metabolites derived from glycidamide [66]. Furthermore, some authors observed that in presence of allyl and diallyl sulfide (inhibitors of cytochrome P450 2E1, extracted from garlic) the conversion of acrylamide to glycidamide was attenuated [67].

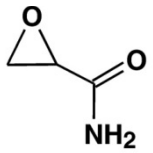


Figure 4. Chemical structure of glycidamide

Acrylamide reacts very slowly with DNA and has only been observed in *in* vitro studies; however, glycidamide clearly forms adducts with guanine and adenine [68]. In addition, acrylamide and glycidamide are alkylating agents that can react with plasma albumins, proteins, and the amino acid valine of hemoglobin to form *N-(2*-carbamoylethyl)valine (HbAA) and N-(2-carbamoyl-2-hydroxyethyl)valine (HbGA). These hemoglobin adducts (HbAA and HbGA) are considered valid biomarkers that reflect human internal exposure within the last

120 days (the average lifespan of erythrocytes) [17, 69]. Hemoglobin adduct measurements are reproducible with intraclass correlation in the range of 0.77 to 0.80 [70]. DNA adducts of acrylamide and glycidamide are not yet developed for use in human epidemiology studies.

Both acrylamide and glycidamide can be excreted from the body by conjugating with reduced glutathione-S-transferases (GST). This reaction results in the formation of glutathione conjugates which will be transformed to mercapturic acids (such as N-acetyl-S-(2-carbamoylethyl)-cysteine, or N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-cysteine), and eliminated in urine. Likewise, glycidamide can also be hydrolyzed by the microsomal epoxide hydrolase (mEH) to glyceramide, a less reactive metabolite that is also excreted in urine (**Figure 5**).

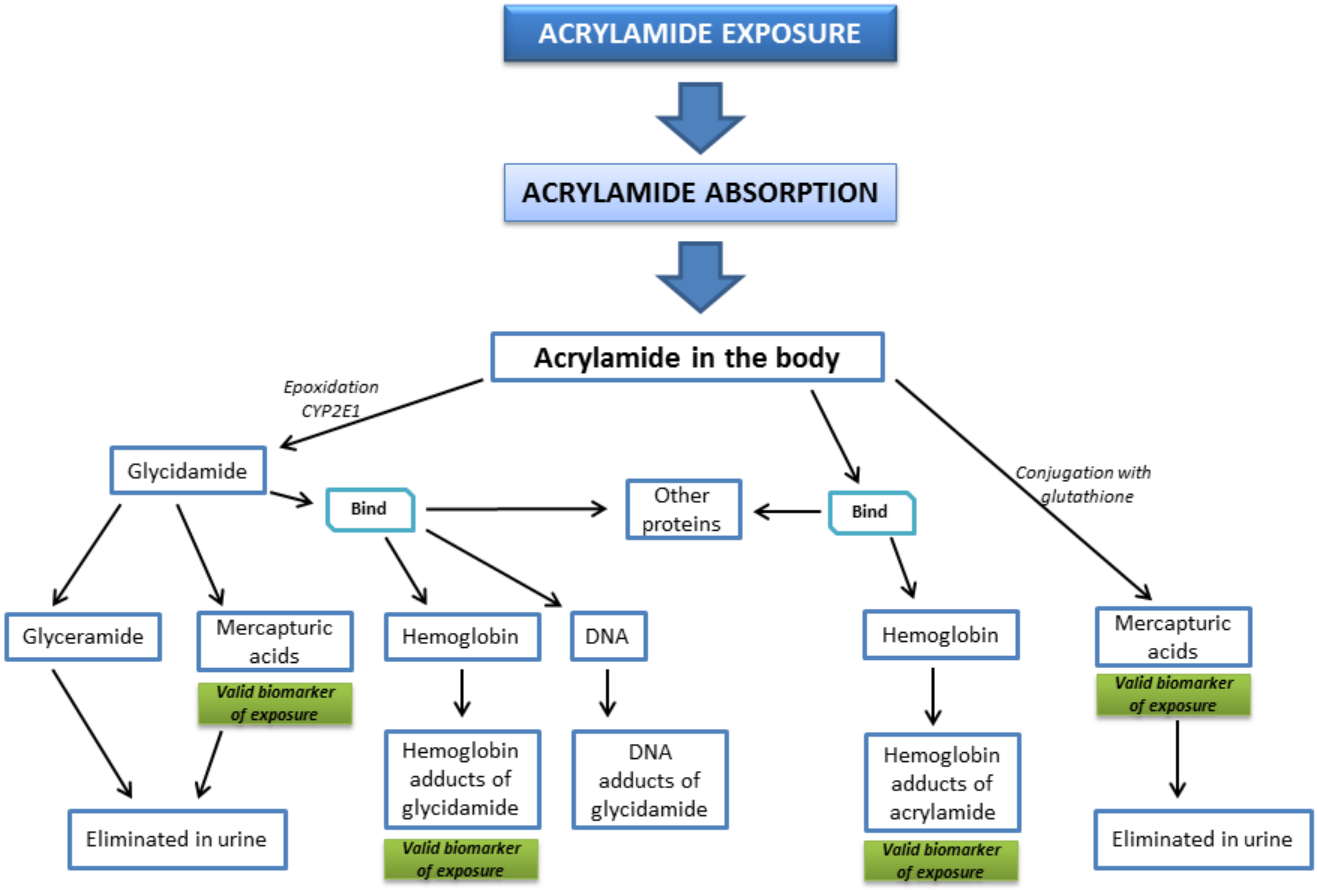


Figure 5. Pathways of acrylamide metabolism

Lately, it has been observed that acrylamide may also be catabolized by microorganisms, some of which are found in the human microbiome [21]. Mercapturic acids are also considered as valid biomarkers of acrylamide and glycidamide exposure; however, they reflect short-term exposure [71]. Fuhr et al. carried out a study where volunteers ingested 0.94 mg of acrylamide in a meal, and observed that the half-live for acrylamide was 2.4±0.4 hours, and for the mercapturic acids was between 17.4±3.9 and 25.1±6.4 hours [65].

It is important to highlight that individuals who consume the same amount of acrylamide could have different levels of glycidamide in the body (i.e., interindividual variation), and these

differences may be due to the microbiome, and to genetic variation in enzymes that are involved in acrylamide metabolism: Cyp2E1, GST, and mEH [72]. Additionally, Cyp2E1 is inducible by chronic heavy alcohol intake. In a cross-sectional study within EPIC, individuals with higher alcohol consumption had lower levels of HbGA. The authors concluded that alcohol may compete with acrylamide as a CYP2E1 substrate [73]. Likewise, HbAA and HbGA levels may vary by hemoglobin status, which can be influenced by gender, age, and lifestyle factors [74]. Further, as earlier mentioned, children are more exposed to dietary acrylamide than adults (measured as acrylamide exposure per unit of body weight), and tend to eat more acrylamide-containing foods; nonetheless, metabolic conversion rates also vary across age groups. Vesper et al. reported that the ratio HbGA to HbAA was higher in children than in non-smoking adults [60].

1.1.4 Acrylamide and disease

As already discussed above, acrylamide is rapidly distributed to all tissues and both acrylamide and glycidamide have the potential to bind sulfhydryl groups and aminoacids and/or proteins both in animals and in humans. This capacity allows acrylamide/glycidamide to have different effects in the body which can be differentiated into carcinogenic and non-carcinogenic effects.

Carcinogenic effects

The IARC classified acrylamide as *probably carcinogenic* (group 2A) to humans in 1994 [3], and the US Environmental Protection Agency (EPA), despite having a different system of classification, also classified acrylamide as a *probable human carcinogen* (Group B2) [75]. The European Chemicals Agency (ECHA) included acrylamide to the Candidate List of Substances of Very High Concern (SVHC) for Authorization, and classified acrylamide as *carcinogenic-category 2* (may cause cancer), *mutagenic-category 2* (may cause heritable genetic damage) [76].

There are two main hypotheses concerning the carcinogenic effect of acrylamide/glycidamide: One involving direct genotoxic action of the reactive epoxide metabolite (glycidamide), and the second one through non-genotoxic mechanisms. The genotoxicity hypothesis has more support based on the large body of evidence; however it is also possible that genotoxicity and the non-genotoxicity pathway in combination play a role in acrylamide related carcinogenesis [77–79].

- **Genotoxicity**:
  Several studies in rodents observed that after oral administration of acrylamide, tumor rates increased. The following tumor types have been identified in female rats: mammary gland, central nervous system, thyroid gland follicular epithelium, oral tissues, uterus, benign adenoma of the pituitary gland, and clitoral gland. Benign thyroid gland adenoma, oral tissue, epithelial hyperplasia, benign adrenal gland pheochromocytoma, and scrotal mesothelium have been reported for male rats. These studies evaluated chronic carcinogenicity in Fisher rats, using doses that ranged from 20 to 6,000 times the amount that humans in industrialized countries generally ingest through their diets [77].
  Mechanisms by which acrylamide/glycidamide induce tumor formation in animals still remain unclear. It has been mentioned before that both acrylamide and glycidamide

are alkylating agents. Alkylating agents are substances that can covalently bind to nucleophilic sites of DNA and proteins through transferring an alkyl group; however the cytotoxic effect is primarily caused by the reaction between alkylating agents and DNA, which may damage DNA replication and transcription [80]. Such damage can promote mutations and cell transformations leading to tumor initiation [81]. It has been observed that glycidamide preferently binds with guanine and adenine at position N7 and N1, respectively. Acrylamide also binds DNA (less efficient than glycidamide, and only observed in vitro) by a Michael-type process [82]. The carcinogenicity of acrylamide and glycidamide have been extensively analyzed in *in vitro, in vivo,* and in animal studies. DNA adducts of glycidamide have been associated with sister chromatid exchange frequency and induction of chromosomal aberrations in animal-cell assays [83, 84]. Moreover, an effect of acrylamide on kinesin proteins has been observed in *in vitro* studies [85, 86]. These proteins are involved in the cell division process, and are important for the proper separation of chromosomes. Additionally, it has also been described that acrylamide can bind to sulfhydryl groups of proteins that are implicated in DNA repair [87].

Despite both acrylamide and glycidamide being defined as mutagenic and clastogenic, it seems that the genotoxicity is driven by the clastogenic effect, and the epoxide metabolite glycidamide(alkylating agent) is playing the central role [77, 88].

- **Non-genotoxic effects**:

  Several authors have been postulated that acrylamide may also indirectly cause tumor development by non-genotoxic pathways. There are at least two hypotheses and are not necessary exclusive:

  a) The oxidative stress hypothesis proposes that acrylamide exposure decreases the levels of reduced-glutathione (as acrylamide conjugates with glutathione for elimination), and increases the levels of oxidized-glutathione. This fact results in a change of the redox status of the cell that may induce the carcinogenic process directly affecting gene expression, or indirectly, by dysregulation of transcription factor levels [77, 79].
  b) The hormonal hypothesis posits that acrylamide may influence hormone levels; nonetheless little is known in this area. Some gene expression studies after acrylamide/glycidamide exposure have been carried out, and observed that some genes involved in hormonal metabolism appeared to be dysregulated [89, 90]. Hogervorst et al. assessed the relation between dietary acrylamide intake and sex hormones levels in plasma from pre- and postmenopausal women, but they did not observe a clear association (perhaps because they did not evaluate hemoglobin adducts of acrylamide and glycidamide, but rather total intake based on FFQs)[91].

Non-carcinogenic effects

Regarding non-carcinogenic toxic effects, many studies with a number of animal species have shown that the nervous system is a principal site of acrylamide toxicity, and further investigations also reported that acrylamide is a neurotoxicant. The mechanisms by which acrylamide may cause neurotoxicity are by directly inhibiting neurotransmission, or

dysregulating neurotransmitter levels, or by the inhibition of kinesin-based fast axonal transport [92, 93].

Male rodents, after oral doses of acrylamide (> 7mg/kg body-weight per day), showed reduced fertility; but, in female rodents, no adverse effects on fertility or reproduction have been observed. With regards to developmental toxicity, acrylamide was shown to be fetotoxic in mice at maternal oral dose (45 mg/kg body-weight per day). Reproductive and developmental effects in humans have recently been studied in the European Prospective Mother–Child Study (NewGeneris) and the The Norwegian Mother and Child Cohort Study (MoBa), and concluded that prenatal exposure to dietary acrylamide may have a negative effect on fetal growth [94, 95].

1.2 Endometrial cancer epidemiology

1.2.1 Incidence and mortality

Cancer of the corpus uteri is the fifth and fourth most common incident cancer worldwide and in EU women, respectively (**Figure 6** and **Figure 7**); but is the principal gynecological tumor in developed countries.

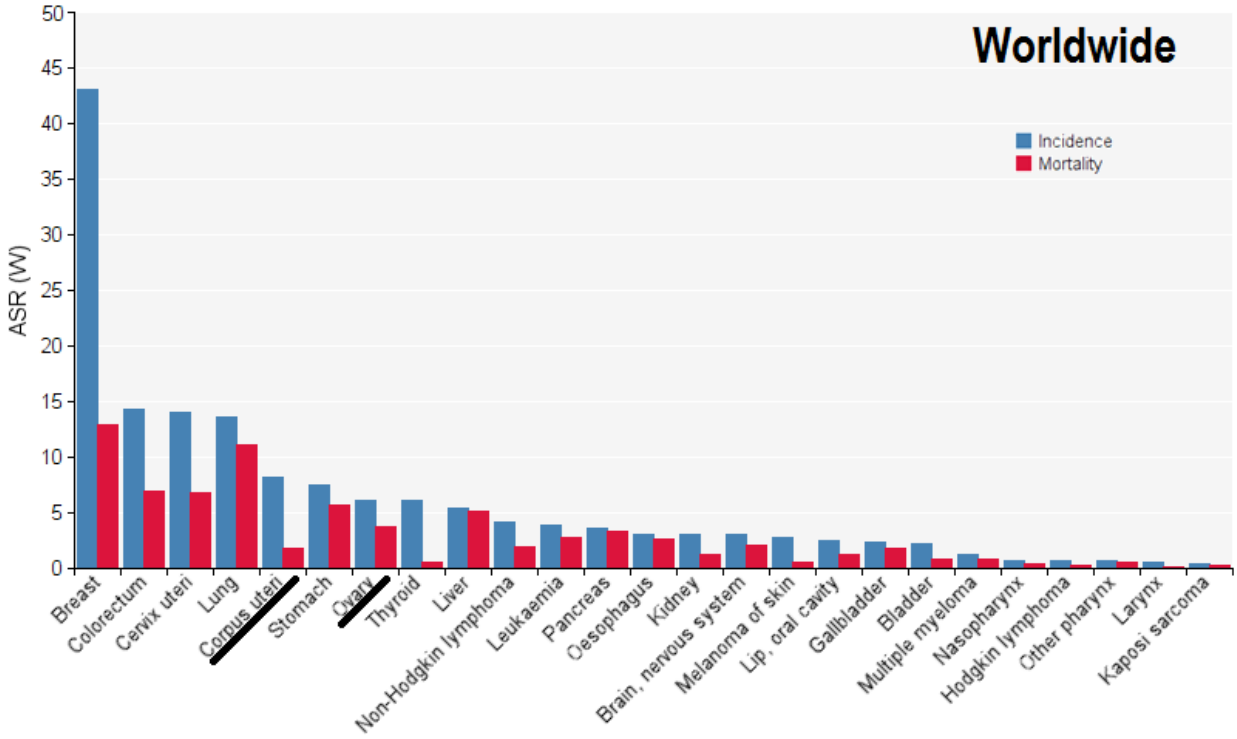


Figure 6. Estimated cancer age-standardized incidence and mortality rates (per 100,000 inhabitants) in worldwide women; GLOBOCAN-IARC 2012

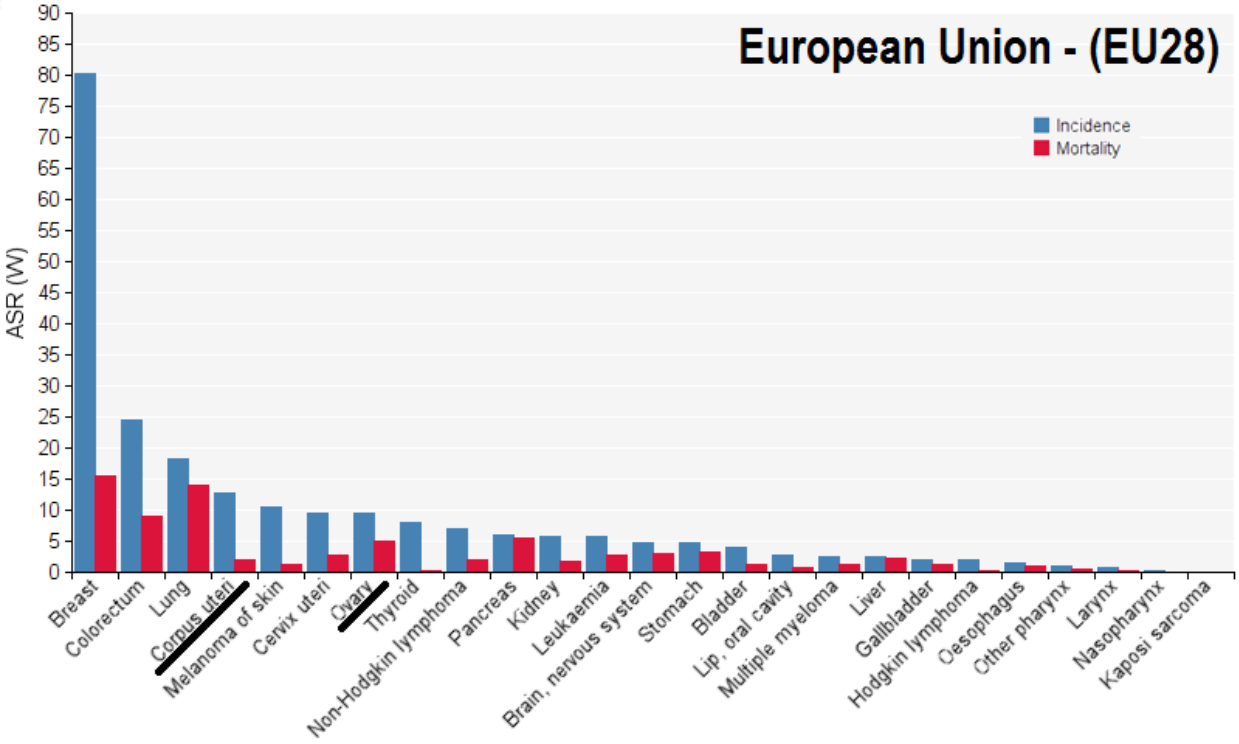


Figure 7. Estimated cancer age-standardized incidence and mortality rates (per 100,000 inhabitants) in European Union women; GLOBOCAN-IARC 2012

The most common type of corpus uteri cancer is endometrial carcinoma (EC) accounting for 95% of cases, the remaining 5% are sarcomas [96]. There is considerable international variation in incidence as well as mortality, and both rates increase dramatically with age. [97]. In fact, the mean age at diagnosis is 60 years; thus, EC is primarily a disease of postmenopausal women. EC prognosis is favorable, the mean age-standardized 5-year survival ranges from 74% to 91% [98]. Most of the EC cases are diagnosed at early stages due to clinical symptoms (abnormal uterine bleeding), thus, survival rates are high; however, 14% of EC cases are diagnosed in premenopausal women [98].

Interestingly, black women tend to have lower incidence of EC compared to white women; however, mortality is higher since black women are diagnosed with more aggressive EC (advanced stages). To date, it is still unclear if this is a biological or a sociodemographic effect [99].

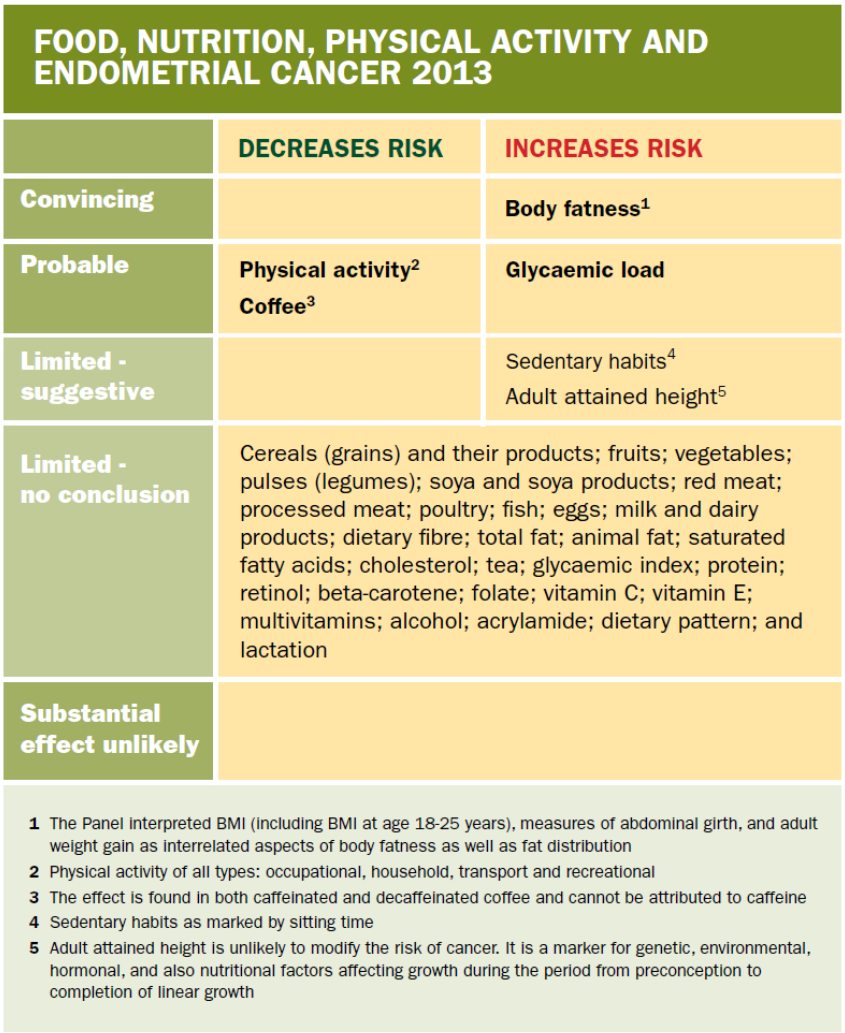
1.2.2 Risks factors

Established risk factors for EC are mainly related to estrogenic effects (endogenous and exogenous), such as greater lifetime exposure to estrogens (early menarche and late menopause), nulliparity, history of polycystic ovarian syndrome, obesity, and physical inactivity. Likewise, there is clear evidence that estrogen therapy unopposed by progesterone and Tamoxifen® use are a cause of EC [100]. Further, those women diagnosed with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome or those who have a first-degree relative with EC are at higher risk of developing EC than women without family history or HNPCC [101].

There is still no consensus about the role of diabetes in relation to EC, epidemiological studies report inconsistent results [102, 103].

On the other hand, the use of oral contraceptives (OCs, containing both estrogen and progesterone) is well stablished to lower the risk of EC, and this effect is maintained for years [98, 104, 105]. Postmenopausal women that use hormone-replacement therapy (HRT) with both hormones combined in the formula also have lower risk of EC. Likewise, cigarette smoking tends to lower the risk of developing EC, and this effect is thought to be more pronounced in recent smokers [106]; however, an EPIC study conducted in 249,986 women observed that smoking premenopausal women had higher risk of EC [107].

Although a diet high in fat has been observed to increase risk [101], and coffee consumption seems to decrease risk [108], few dietary components are associated with EC risk (**Figure 8**).



FOOD, NUTRITION, PHYSICAL ACTIVITY AND ENDOMETRIAL CANCER 2013

	DECREASES RISK	INCREASES RISK
Convincing		Body fatness[1]
Probable	Physical activity[2] Coffee[3]	Glycaemic load
Limited - suggestive		Sedentary habits[4] Adult attained height[5]
Limited - no conclusion	Cereals (grains) and their products; fruits; vegetables; pulses (legumes); soya and soya products; red meat; processed meat; poultry; fish; eggs; milk and dairy products; dietary fibre; total fat; animal fat; saturated fatty acids; cholesterol; tea; glycaemic index; protein; retinol; beta-carotene; folate; vitamin C; vitamin E; multivitamins; alcohol; acrylamide; dietary pattern; and lactation	
Substantial effect unlikely		

1 The Panel interpreted BMI (including BMI at age 18-25 years), measures of abdominal girth, and adult weight gain as interrelated aspects of body fatness as well as fat distribution
2 Physical activity of all types: occupational, household, transport and recreational
3 The effect is found in both caffeinated and decaffeinated coffee and cannot be attributed to caffeine
4 Sedentary habits as marked by sitting time
5 Adult attained height is unlikely to modify the risk of cancer. It is a marker for genetic, environmental, hormonal, and also nutritional factors affecting growth during the period from preconception to completion of linear growth

Figure 8. Summary of the evidence on food, nutrition, physical activity, and body fatness related to Endometrial Cancer; WCRF 2013[109]

1.2.3 Endometrial cancer subtypes

EC has long been classified into two types. Type-I tumors are mostly endometrioid adenocarcinomas, account about 80% of EC cases, and are characterized as estrogen-

dependent tumors. Type-II tumors are mainly serous carcinomas, which are believed to be estrogen independent and are usually diagnosed in elderly women [101]. Nonetheless, the main difference is that type-II generally has worse prognosis than type-I (**Table 4**).

Table 4. Differences between endometrial cancer types

	Type-I	Type-II
Grade	Low	High
Hormone receptor expression	Positive	Negative
Histology	Endometrioid	Non-endometrioid (serous, clear-cell carcinoma)
***TP53* mutation**	No	Yes
Prognosis	Good	Poor

Adapted from [98]

1.3 Ovarian cancer epidemiology

1.3.1 Incidence and mortality

Ovarian cancer is the seventh most common incident cancer worldwide and in EU women (**Figure 6** and **Figure 7**), and is the second leading gynecological tumor after cancer of the corpus uteri. Ovarian cancer rates are higher in developed countries, but rates are increasing in populations undergoing economic transition.

Several epidemiologic studies have suggested etiological differences between ovarian tumor subtypes. Almost 90% of the malignant ovarian tumors are epithelial ovarian cancer (EOC), which is the seventh most common cause of cancer death in women worldwide: the average 5-year survival rate ranges between 30% and 50% depending upon geographic region [110]. Usually women are diagnosed with later stages due to the lack of symptomatology, which results in high mortality. The stroma and the sex cord, and germ cell tumors account for about 5–6% and 2-3%, respectively. Most of EOC are diagnosed in postmenopausal women (median age of 63 years); whereas germ cell tumors are commonly diagnosed in younger women [111].

1.3.2 Risk factors

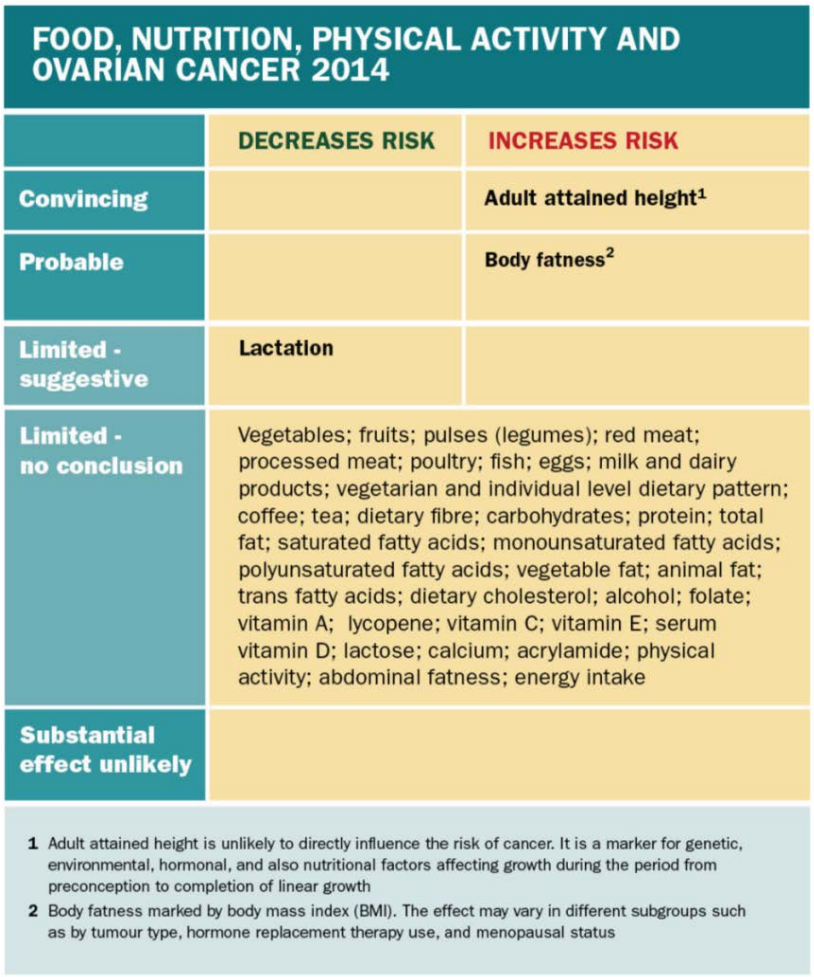
Despite the high incidence and mortality rates, few risk factors for EOC have been described. Epidemiological and genetic studies have observed that at least 7% of ovarian cancer cases are familial. First-degree relatives with ovarian cancer, families with HNPCC, or with mutations in the BRACA1 or BRACA2 genes have higher risk of developing ovarian cancer. For instance, women who carry BRACA1 or BRACA2 mutations have a 40 to 60% lifetime risk of EOC [112]; nonetheless, prophylactic bilateral oophorectomy reduces the risk of EOC by 90% in women with BRACA1 or BRACA2 mutations[113]. Other surgical procedures, such as hysterectomy or tubal ligation also reduce the risk of EOC.

In addition to genetic predisposition and age, higher number of ovulatory cycles (early menarche and late menopause), as well as adult attained height and body mass index (BMI), increase the risk of developing EOC. Infertility and the use of HRT in menopause have been associated with increased risk in several, but not all, epidemiologic studies. In the same line, there is evidence that sedentary behavior or low physical activity increases the risk of ovarian cancer. Alcohol and tobacco smoking are generally not associated with ovarian cancer, but

recent studies suggest that smoking could be associated with increased risk of the mucinous subtype [113–115].

Combined OCs are a strong and established protective factor for EOC, and the protection increases with OC duration and is thought to be maintained for at least three decades. In the same line, there is moderate evidence that exercise/physical activity reduces the risk of ovarian cancer. Parity and lactation have also been inversely related to ovarian cancer.[115]

With reference to diet, the epidemiological evidence is less consistent [110]. There is limited evidence for any association between foods or macro-/micronutrients and the risk of ovarian cancer (**Figure 9**).



FOOD, NUTRITION, PHYSICAL ACTIVITY AND OVARIAN CANCER 2014

	DECREASES RISK	INCREASES RISK
Convincing		Adult attained height[1]
Probable		Body fatness[2]
Limited - suggestive	Lactation	
Limited - no conclusion	Vegetables; fruits; pulses (legumes); red meat; processed meat; poultry; fish; eggs; milk and dairy products; vegetarian and individual level dietary pattern; coffee; tea; dietary fibre; carbohydrates; protein; total fat; saturated fatty acids; monounsaturated fatty acids; polyunsaturated fatty acids; vegetable fat; animal fat; trans fatty acids; dietary cholesterol; alcohol; folate; vitamin A; lycopene; vitamin C; vitamin E; serum vitamin D; lactose; calcium; acrylamide; physical activity; abdominal fatness; energy intake	
Substantial effect unlikely		

1 Adult attained height is unlikely to directly influence the risk of cancer. It is a marker for genetic, environmental, hormonal, and also nutritional factors affecting growth during the period from preconception to completion of linear growth

2 Body fatness marked by body mass index (BMI). The effect may vary in different subgroups such as by tumour type, hormone replacement therapy use, and menopausal status

Figure 9. Summary of the evidence on food, nutrition, physical activity, and body fatness related to Ovarian Cancer; WCRF 2014 [110]

1.3.3 Epithelial ovarian cancer subtypes

Several classifications have been proposed over the years; however, the most widely used is based on the histological types: serous, mucinous, endometrioid, clear cell, transitional cell tumors (Brenner tumors), squamous cell tumors, mixed epithelial tumors, and undifferentiated carcinoma [116].

In addition to the histological classification, ovarian tumors can be differentiated upon their behavior: benign, borderline, or malignant (or invasive). Borderline tumors are thought to be "tumors of low malignant potential"[112]. Two further classifications of ovarian cancer have been established according to tumor grade (ranging from low to high grade) and stage. One of the most commonly used classifications for both grade and stage is published by the International Federation of Gynecology and Obstetrics (FIGO) [117].

This thesis has included both borderline and invasive EOC; nevertheless, invasive EOCs are differentiated into six histologic subtypes (serous, mucinous, endometrioid, clear cell, not otherwise specified (NOS), and others) due to the clinical and risk factor differences between subtypes [114, 118].

Table 5. Differences between invasive epithelial ovarian cancer histologic subtypes

	Serous		Mucinous	Endometrioid		Clear cell	NOS
Grade	High	Low	-	High	Low	-	-
Mutations	*TP53* *BRACA* ½ *NF1*	BRAF KRAS	KRAS ERBB2 ampl	*TP53* *BRACA* ½ *PTEN*	*CTNNB1* *PTEN*	PTEN PI3KCA *ARID1A*	Unknown
Prognosis	Fair		Good	Good		Poor	Poor

Adapted from [112, 119]

1.4 Previous studies: Acrylamide and endometrial and epithelial ovarian cancer

The first prospective epidemiologic study to investigate dietary acrylamide intake and risk of endometrial and ovarian cancer was conducted in the Netherlands Cohort Study (NLCS) which included over 60,000 women and which began enrollment in 1986. Results showed that the highest quintile of acrylamide intake was associated with EC risk (especially in never-smokers: HR_{Q5vsQ1}: 1.99, 95% CI: 1.12-3.52; and EOC risk overall results: HR_{Q5vsQ1}:1.78, 95% CI: 1.10-2.88; and in never smokers: HR_{Q5vsQ1}: 2.22, 95% CI: 1.20-4.08) (**Table 6** and **Table 7**) [120] .

The second cohort was the Swedish Mammography Cohort (SMC) study of over 55,000 Swedish women who have been followed since the late 1980s. Neither EC nor EOC were associated with dietary acrylamide intake in the SMC; however, the range of total dietary intake of acrylamide was substantially lower in the Swedish cohort than the Dutch cohort [121, 122]. Likewise, an Italian hospital-based case-control study found no association between acrylamide intake (based on a limited number of food items) and EOC risk [123] (**Table 6** and **Table 7**).

Table 6. Summary of the published results on dietary intake of acrylamide and endometrial cancer risk

Study	Study design	Study size	Intake range (μg/day)	Overall results	Subgroup analyses: Never smokers	Publication year & Source
NLCS	Prospective cohort study	221 cases 62,573 participants	10-37 (Median Q1-Q5)	Q1: 1.00(ref) Q2: 0.95(0.59-1.54) Q3: 0.94(0.56-1.56) Q4: 1.21(0.74-1.98) Q5: 1.29(0.81-2.07) *p-trend:* 0.18	Q1: 1.00(ref) Q2: 1.16(0.63-2.15) Q3: 1.35(0.73-2.51) Q4: 1.30(0.69-2.46) Q5: 1.99(1.12-3.52) *p-trend:* 0.03	2007 – Hogervorst et al. [120]
SMC	Prospective cohort study	687 cases 61,226 participants	17-33 (Median Q1-Q4)	Q1: 1.00(ref) Q2: 1.10(0.89-1.36) Q3: 1.08(0.88-1.34) Q4: 0.96(0.59-1.78) *p-trend:* 0.72	Q1: 1.00(ref) Q2: 1.31(0.85-2.04) Q3: 1.30(0.83-2.02) Q4: 1.20(0.76-1.90) *p-trend:* 0.52	2009 – Larsson et al. [121]
NHS	Prospective cohort study	484 cases 88,671 participants	9-26 (Mean Q1-Q5)	Q1: 1.00(ref) Q2: 1.12(0.83-1.50) Q3: 1.31(0.97-1.77) Q4: 1.35(0.99-1.84) Q5: 1.41(1.01-1.97) *p-trend:* 0.03	Q1: 1.00(ref) Q2: 0.97(0.64-1.46) Q3: 1.35(0.90-2.02) Q4: 1.47(0.97-2.24) Q5: 1.43(0.90-2.28) *p-trend:* 0.04	2010 – Wilson et al. [124]

NLCS, The Netherlands Cohort Study; NHS, Nurses' Health Study; SMC, Swedish Mammography Cohort
Adapted from [30]

Table 7. Summary of the published results on dietary intake of acrylamide and epithelial ovarian cancer risk

Study	Study design	Study size	Intake range (μg/day)	Overall results	Subgroup analyses: Never smokers	Publication year & reference
Italian C-C	Case-control	1,031 cases 2,411 controls	10-32 (p20-p80)	Q1: 1.00(ref) Q2: 1.03(0.79-1.34) Q3: 1.09(0.83-1.44) Q4: 1.01(0.76-1.34) Q5: 0.97(0.73-1.31) *P-trend:* 0.80	*Not reported*	2006 – Pelucchi et al. [123]
NLCS	Prospective cohort study	195 cases 62,573 participants	10-37 (Median Q1-Q5)	Q1: 1.00(ref) Q2: 1.22(0.73-2.01) Q3: 1.12(0.65-1.92) Q4: 1.28(0.77-2.13) Q5: 1.78(1.10-2.88) *P-trend:* 0.02	Q1: 1.00(ref) Q2: 1.60(0.85-3.02) Q3: 1.64(0.84-3.19) Q4: 1.86(1.00-3.48) Q5: 2.22(1.20-4.08) *P-trend:* 0.01	2007 – Hogervorst et al. [120]
SMC	Prospective cohort study	368 cases 61,057 participants	17-33 (Median Q1-Q4)	Q1: 1.00(ref) Q2: 0.91 (0.68-1.21) Q3: 0.97(0.73-1.29) Q4: 0.86(0.63-1.16) *P-trend:* 0.39	*Not reported*	2009 – Larsson et al. [122]
NHS	Prospective cohort study	416 cases 88,672 participants	9-26 (Mean Q1-Q5)	Q1: 1.00(ref) Q2: 0.93(0.68-1.29) Q3: 1.29(0.94-1.76) Q4: 1.17(0.84-1.64) Q5: 1.25(0.88-1.77) *P-trend:* 0.12	Q1: 1.00(ref) Q2: 1.17(0.72-1.88) Q3: 1.04(0.63-1.74) Q4: 1.11(0.63-1.94) Q5: 1.19(0.66-2.15) *P-trend:* 0.04	2010 – Wilson et al. [124]

NLCS, The Netherlands Cohort Study; NHS, Nurses' Health Study; SMC, Swedish Mammography Cohort
Adapted from [30]

The most recent analysis of dietary acrylamide intake and EC and EOC risks was conducted in the Nurses' Health Study (NHS) of over 120,000 female US nurses. In the US cohort analysis, acrylamide intake data were based on multiple dietary assessments over twenty years of follow-up and over forty individual food items. Results showed a statistically significant positive association between the highest quintile of dietary acrylamide intake and EC risk (HR_{Q5vsQ1}: 1.41, 95%CI: 1.01-1.97). The results for ovarian cancer were suggestive, but not statistically significant (HR_{Q5vsQ1}: 1.25, 95%CI: 0.88-1.77). Associations were slightly stronger and significant in women with body mass index <25 kg/m^2 (**Table 6** and **Table 7**)[124].

Recently, a nested case-control study from the NHS cohort, based on HbAA and HbGA and EOC risk was published by Xie J et al., including 263 cases and 526 matched controls. No association between these adducts levels in blood and ovarian cancer risk was observed (**Table 8**), even when the analysis was restricted to non-smoking women. No effect measure modification was observed for HRT use, BMI, or menopausal status at blood donation [125].

Table 8. Summary of the published result on acrylamide and glycidamide hemoglobin adducts levels and epithelial ovarian cancer risk

Study	Study design	Study size	Hb-adducts range (pmol/g Hb)	Overall results	Subgroup analyses: Never smokers	Publication year & reference
NHS	Nested-case control	263 cases 526 matched controls	74-226 (p10-p90)	<u>Total adducts (HbAA+HbGA)</u> T1: 1.00(ref) T2: 0.83(0.56-1.24) T3: 0.79(0.50-1.24) *P-trend:* 0.08	<u>HbAA</u> T1: 1.00(ref) T2: 0.87(0.58-1.31) T3: 0.85(0.56-1.30) *P-trend:* 0.06 <u>HbGA</u> T1: 1.00(ref) T2: 1.14(0.77-1.71) T3: 0.80(0.52-1.23) *P-trend:* 0.36	2013 – Xie et al. [125]

NLCS, The Netherlands Cohort Study; NHS, Nurses' Health Study; SMC, Swedish Mammography Cohort; HbAA, hemoglobin adducts of acrylamide; HbGA, hemoglobin adducts of glycidamide
Adapted from [30]