



Substance report

June 2022



Acrylamide



Table of contents

Authors and Acknowledgements.....	4
Glossary	5
1 Key messages	5
2 Introduction.....	6
2.1 How to use this document.....	6
2.2 Overview of acrylamide.....	6
3 Human exposure to acrylamide.....	7
3.1 Environmental exposure	8
3.2 Occupational exposure	9
3.3 Consumer exposure.....	9
4 Health impacts of acrylamide	9
4.1 Overview of key health impacts from acrylamide	9
4.2 Vulnerable target groups.....	11
4.3 Societal concerns	12
5 EU policies on acrylamide.....	12
6 Policy questions for acrylamide.....	13
6.1 Introduction.....	13
6.2 What is the current exposure of the EU population to Acrylamide?	13
6.3 Does the exposure to acrylamide differ significantly between countries and population groups? Are the main reasons for these differences related to different dietary habits or to other factors?	13
6.4 Which population groups are more at risk? Are there other sources of exposure of acrylamide that need to be discovered (e.g. smoking habits or other food sources)?	14
6.5 Is there an impact from the mitigation for the production in food processing and manufacturing and REACH restrictions on the distribution of acrylamide exposure? Do we need to implement other restrictions to decrease the level of exposure of acrylamide?	14
6.6 Are the exposure levels a concern for health? Is the exposure to acrylamide associated to cancer, neurological alterations and foetal growth in humans? Is the health risk dependent on long term or intermittent exposure to low quantity of acrylamide?.....	14
6.7 Are the health risks dependent on age and gender?.....	16
7 HBM4EU outputs to date	17
7.1 Categorisation.....	17
7.2 Key outputs.....	17
7.3 Key data gaps.....	20
8 Future recommendations	20
9 References	21
Appendix 1: Additional information on exposure routes.....	22

Appendix 2: Additional information on health effects	23
Figure 1.1 Overview of exposure routes and pathways for acrylamide.....	7
Figure 2.1 Overview of health effects associated with exposure to acrylamide	11
Table 1.1 Overview of CLP classifications for acrylamide	10
Table 2.1 Overview of EU policies relating to acrylamide	12
Table 3.1 HBM4EU categorisation for acrylamide	17

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The EEA has since updated this document to reflect the work developed before the conclusion of HBM4EU, with the support of the CGL and other colleagues.

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Glossary

Abbreviations	
C&L	Classification and Labelling
CLP	The 'Classification, Labelling, Packaging' Regulation Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
HBM	Human Biomonitoring
HBM4EU	European Human Biomonitoring Initiative
IARC	International Agency for Research on Cancer
OEL	Occupational Exposure Limits
REACH	The 'Registration, Evaluation, Authorisation and Restriction of Chemicals' Regulation Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
WP	Work package

1 Key messages

The most important source of exposure for the general population in Europe to acrylamide is the diet.

- Carcinogenic and neurotoxic effects are suspected, but further investigation is necessary to address health impacts in the general population.
- HBM4EU Aligned Studies¹ (2014-2021) have allowed for the collection of exposure data from four and six countries, covering Northern, Western and Southern Europe for children and adults respectively.
- They revealed an exceedance of a health-based guidance value in about 96% when compared to the biomonitoring equivalents (16µg/g crt). However, when compared to HBM Orientation Values (321.7 µg/L and 291.4µg/L), only 2% of children and 7% of adults had

¹ The HBM4EU Aligned Studies are a survey aimed at collecting HBM samples and data as harmonised as possible from (national) studies to derive current internal exposure data representative for the European population/citizens across a geographic spread.

acrylamide values higher than HBM Orientation Values (321.7 µg/L and 291.4 µg/L). Since the HBM4EU Aligned Studies are not representative of the European regions and the participating cohorts were not representative of the country or region, further investigation needs to be addressed to calculate current values of exposure for the whole EU.

- Children seem to be the most vulnerable and sensitive group to the adverse effects of acrylamide.
- Gestational acrylamide during pregnancy seems to be associated with adverse effect on the foetal growth.
- Mitigation measures were not visible until 2017 but there is an indication of a first effect of the 2017 EU regulation in adults only.
- Societal concern is mainly related to the discovery that acrylamide is produced in processed foods rich in carbohydrates like chips, making acrylamide exposure widespread.

2 Introduction

HBM4EU is a project funded under Horizon 2020 and runs from 2017 until June 2022. It generates knowledge to inform about the safe management of chemicals, and hence protect human health in Europe. HBM4EU uses human biomonitoring (HBM) to monitor the actual human exposure to chemicals and resulting health impacts to build upon existing evidence bases and improve chemical risk assessment. HBM4EU compares data from across Europe which allows an understanding of regional differences and can help to identify vulnerable groups in order to inform targeted measures to reduce exposure. The results of the HBM4EU project are aimed at supporting policy development, by providing a key evidence base in the understanding of exposure and impacts to toxic chemicals.

If you would like to read more about the project itself, please visit the HBM4EU [website](#).

2.1 How to use this document

This document provides a summary of the known and suspected adverse human health effects of acrylamide and describes the main exposure pathways for humans. It also indicates where HBM could be of value in the development of EU policy, along with the remaining challenges in determining human acrylamide exposure. This substance report is intended to inform scientists, relevant stakeholders and policy makers on the value of HBM to establish the EU population's exposure to acrylamide.

This substance report is based largely on the HBM4EU [scoping document](#) for acrylamide, first draft produced in 2019 and updated regularly, as well as the accompanying reports on [legislative mapping](#) and [policy questions](#). Where necessary, additional information has been used from the European Chemical Agency (ECHA) documents including the Classification and Labelling (C&L) Inventory, and legislative text for relevant EU policy areas, have also been used for this report.

2.2 Overview of acrylamide

Acrylamide is a highly water-soluble and nonpersistent organic compound used for different purposes in the chemical industry and within research laboratories. Its primary use is to manufacture polyacrylamides (used as flocculants²) that are used for the production of dyes, organic chemicals, permanent-press fabrics, textiles, pulp, and paper products. Acrylamide and its

² Flocculants are chemicals which are mainly used to clarify drinking water and to treat wastewater by creating a floc (grouped particles) that can be filtered.

polyacrylamides are further used in the oil industry as a flow control agent to enhance oil production from wells. Acrylamide was widely used in the building and construction sector e.g. as a grouting agent and soil stabiliser for the construction of tunnels (Kassotis et al., 2015) until its use as a grouting agent was restricted in 2012. If you would like to read more about the regulatory context with regards to acrylamide, please see [Section 5](#).

In addition to its commercial uses, since 2002 acrylamide has also been identified to form unintentionally during everyday cooking activities, with further associated health risks. Acrylamide is formed in certain high carbon food during high-temperature cooking (> 120 °C) under low moisture conditions such as baking, frying or roasting³. Food products in which acrylamide can be formed are predominantly starch-based such as potato products, biscuits, soft or crispy bread and cereals. In addition, it can be found in roasted coffee, roasted nuts, olives in brine, prunes, dates and baby food. Protein-based foods (such as meat) probably contain low amounts of acrylamide (David & Gooderman 2018). If you would like to read more about acrylamide in food, please visit [section 3](#) of this report or the European Food safety Authority (EFSA) [website](#).

3 Human exposure to acrylamide

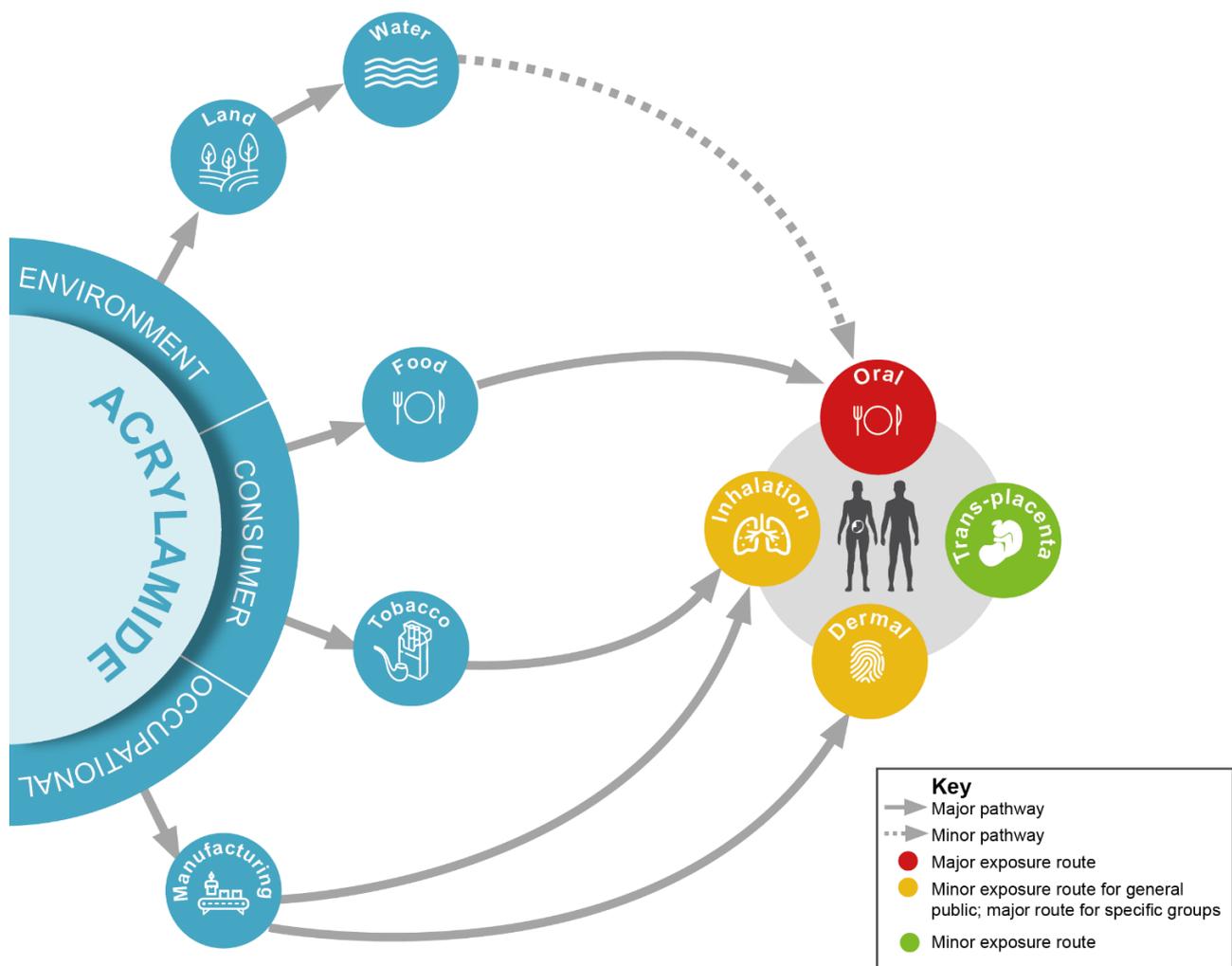
Acrylamide exposure is assumed to be widespread amongst the European Union population. The main source of acrylamide exposure for the general population is through the diet. Additionally, exposure from occupational settings may be significant for certain individuals or groups depending on the specific settings for manufacture and use.

An overview of main sources of exposure (environmental, occupational, consumer) and exposure pathways (oral, inhalation, dermal) is provided in Figure 1.1 below.

Additional information on these sources and pathways is provided in [Appendix 1](#).

Figure 1.1 Overview of exposure routes and pathways for acrylamide

³ This reaction is called Maillard reaction. Heat induces forming of new substances which cause new flavours and the browning of the food. One of the formed substances is acrylamide. https://www.efsa.europa.eu/sites/default/files/acrylamide_en.png



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3.1 Environmental exposure

Direct releases of acrylamide to the environment from intentional use occur during its manufacture, or where it is used as an intermediate in other production processes, for example in the production of thermoplastics. Primary emissions are likely to be to air with deposition to soil and water. Indirect environmental contamination occurs using acrylamide as a flocculant in water treatment and as a grouting agent in construction (EC, 2002). However, further note that where acrylamide has a high-water solubility it is unlikely to bind to the soil itself, but instead can be expected to be mobile, passing through soil layers and into ground water, or horizontally through the soil into surface water (Kassotis et al., 2015). The risks of drinking-water contamination by acrylamide are considered as low (EC, 2002).

Within the environment ambient concentrations of acrylamide are primarily found in water, with air the next most significant environmental compartment (Pratt et al. 2000). However, monitoring data for acrylamide is limited, with information on water concentrations on a geographic basis providing an incomplete picture. It is also further worth noting that based on its physical properties, acrylamide is considered as non-persistent and non-bioaccumulative. Therefore, the ambient concentrations witnessed in the environment can be expected to come from new use/generation, rather than legacy persistence issues.

3.2 Occupational exposure

According to ECHA, acrylamide is imported and manufactured in the European Economic Area in 100 000 – 1 000 000 tonnes per year bracket. The Roadmap on carcinogens initiative estimates that more than 50 000 workers in the EU are potentially exposed to acrylamide at their workplace. They are exposed through dermal contact with acrylamide itself or inhalation of dust and vapor during production (Roadmap on Carcinogens, 2020). If you would like to read more about the Roadmap on carcinogens initiative and their proposed prevention measures with regards to occupational acrylamide exposure, please visit their [website](#).

3.3 Consumer exposure

The main exposure route for the general population is through the diet. However, the exposure varies among the population and ultimately depends on the quantity of high carbon food products consumed and cooking techniques used. According to EFSA, the most important dietary source depends on the age. Whereas adults are mainly exposed to acrylamide through fried potato products such as French fries and coffee, children are mainly exposed through soft bread, breakfast cereals and crackers. Infants are predominately exposed through baby food such as rusks and biscuits. EFSA recommends avoiding excessive crisping and burning as well as a balanced diet to reduce acrylamide in food (EFSA 2020). If you would like to read more about dietary exposure, please visit EFSA's [website](#).

The Food Drink Europe⁴ Acrylamide Toolbox is the result of a cooperation between the food industry, national authorities, and the European Commission. The toolbox highlights ways to lower levels of acrylamide in manufactured food. Promising techniques include the reduction of sugars in agronomy, recipe adaptations and processing recommendations.

In 2017, the EC has implemented and approved a mitigation law aiming to monitoring the levels of acrylamide in food produced by the food industry. ([Regulation \(EU\) 2017/2158](#)). If you would like to read more about the regulatory context with regards to acrylamide, please visit [section 5](#).

In addition to food products, acrylamide is present in tobacco smoke. The overall acrylamide exposure of smokers is 50 % higher than for non-smokers (Mojska et al., 2016).

4 Health impacts of acrylamide

4.1 Overview of key health impacts from acrylamide

Acrylamide does not bioaccumulate but once ingested is extensively metabolised. The human health effects due to the exposure to acrylamide are presented in Table 4.1. Based on reviewing the data held in Table 4.1, it becomes evident that there is limited evidence available concerning human health impacts caused by acrylamide. In particular, EU-wide HBM data to map population level exposure to acrylamide is very scarce.

Due to the suspected health impacts of acrylamide, it is listed under the REACH Regulation⁵ as a substance of very high concern, with IARC⁶ also reaching the same conclusion (consider a substance of very high concern). An overview of current EU (ECHA C&L Inventory) classifications

⁴ European food industry organisation, also a member of EFSA's Stakeholder Consultative Platform

⁵ [Regulation \(EC\) No 1907/2006](#) on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

⁶ International Agency for Research on Cancer

of acrylamide is provided in the Figure 1.1⁷. If you would like to read more about acrylamide, please visit the referenced ECHA info card in the far-right hand column of Table 1.1.

Table 1.1 Overview of CLP classifications for acrylamide

Substance	Properties of concern				Category according to CLP criteria								ECHA info card
	Carcinogenicity	Mutagenic	Skin sensitising (SS)	Reproductive Toxicity	Carcinogenicity	Acute Toxicity	Specific target organ tox (repeated exposure)	Reproductive Toxicity	Mutagenic	Eye Damage/ Eye Irritation	Skin Sensitivity	Skin Corrosion/ Irritation	
Acrylamide					1B* 2A**	3, 4*	1*	2*	1B*	2*	1*	2*	Link

* Harmonised classification under the CLP Regulation. (Other classifications are those notified to the CLP inventory but without harmonised EU classification.); ** Based on IARC classification. Blank cells denote a lack of classification.



Confirmed
Suspected
Some data

Most hazardous Least hazardous



⁷ An explanation of the categorisation of the strength of evidence for the health effects presented in Figure 1.1 is provided in Appendix 2.

Figure 2.1 Overview of health effects associated with exposure to acrylamide

Target organ of the body	Effects	Relevant Substances	 Adults (men)	 Adults (women)	 Infants / Foetuses
Non-organ specific/ whole body 	Cancer	Acrylamide	●	●	●
	Disturbance of foetal growth	Acrylamide	○	○	●
Brain/neurological system 	Neurotoxic effects	Acrylamide	●	●	●
Endocrine system 	Endocrine disruptor	Acrylamide	●	●	●
DNA 	DNA damage	Acrylamide	●	●	●
	Reproductive toxicity	Acrylamide	●	●	●

Key: ● Strong evidence ● Suspected ● Evidence lacking ○ Not applicable

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4.2 Vulnerable target groups

Children are thought to be the most vulnerable and sensitive group to the adverse effects of acrylamide as children have higher (food) intake per kg body weight. In addition, pregnant and lactating women and hence, foetuses may be more vulnerable and sensitive to the effects of acrylamide as these are the periods of the human brain development. The epidemiologic evidence for transplacental exposure is insufficient but expected (Annola et al., 2008, Duarte-Salles et al., 2013).

In addition, workers on industrial sites and in manufacturing are vulnerable target groups as they undergo prolonged and repeated exposure over time.

4.3 Societal concerns

Societal concerns are predominately related to acrylamide formation in processed high carbon food products resulting in a widespread exposure. Therefore, NGOs such as Safe Food Advocacy Law, Changing Market and Sum Of Us demand mandatory EU limits of acrylamide in food⁸.

The NGO Chemsec (International Chemical Secretariat) is also seeking for the substitution of toxic chemicals and advocates for more progressive chemical legislation. Chemsec has included acrylamide in the SIN (Substitute It Now) List. The list comprises chemicals that have adverse health impacts⁹. Health concerns are also reflected in the ranking of acrylamide in the third place (of 334 substances) on the Trade Union Priority List for REACH Authorisation¹⁰.

Given that the knowledge on occupational exposure is limited, the Roadmap on carcinogens initiative demands proper exposure measurements and training of exposed workers¹¹.

5 EU policies on acrylamide

Several policy measures have already been introduced at European Union level to address potential human exposure to acrylamide, which includes management of the risks identified in this document. In general, the existing EU policies cover i) regulations on chemicals; ii) consumer products; iii), the environment and iv) occupational exposure. An overview of these regulatory measures at EU level is provided in Table 5.1.

Table 2.1 Overview of EU policies relating to acrylamide

Chemicals	<ul style="list-style-type: none"> Acrylamide is registered under REACH (Regulation (EC) No 1907/2006) and included in the candidate list of substances of very high concern. Acrylamide is part of the registration list in Annex XVII and should not be placed on the market or used as a substance or constituent of mixture in a concentration equal or greater than 0.1 % by weight for grouting applications. Acrylamide is subject to EU harmonised classification and labelling under CLP (Directive on the classification, labelling and packaging of substances and mixtures) (Regulation (EC) No 1272/2008) – see list of classifications above. 	<p><i>Food</i></p> <ul style="list-style-type: none"> Regulation (EU) 2017/2158 provides for mitigation measures and benchmark levels in food (e.g., roast coffee 400 µg/kg). Acrylamide is banned in plastic material and articles intended to come in contact with food as required under Regulation (EU) No 10/2011. <p><i>Cosmetics</i></p> <ul style="list-style-type: none"> Acrylamide is subject to Regulation (EC) No 1223/2009 on cosmetic products. Its use in products is prohibited under Annex II. 	Consumer
Environmental	<p><i>Water</i></p> <ul style="list-style-type: none"> The Drinking Water Directive (98/83/EC) limits the concentration of acrylamide in water for human consumption to 0.10 µg/L. 	<ul style="list-style-type: none"> Acrylamide is subject to Directive (EC) 2004/37 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work. 	Occupational

⁸ <https://www.safefoodadvocacy.eu/wp-content/uploads/2020/06/Acrylamide-Position-paper-2020.pdf>

⁹ <https://sinsearch.chemsec.org/chemical/79-06-1>

¹⁰ https://www.etui.org/sites/default/files/TU_List_REACH_UK.pdf

¹¹ <https://roadmaponcarcinogens.eu/acrylamide>

6 Policy questions for acrylamide

6.1 Introduction

For each of the HBM priority substances stakeholders were asked to identify policy related questions that HBM4EU should address in order to contribute to the strengthening of policy ambitions on acrylamide. Further background detail on acrylamide and how the policy questions were selected is available in the [scoping document](#) and the [report on stakeholder consultation and mapping of needs](#).

6.2 What is the current exposure of the EU population to Acrylamide?

New data for 2 acrylamide biomarkers (AAMA and GAMA) is available from the HBM4EU Aligned Studies in children (6-11 years) and adults (20-39 years). The Aligned Studies in children collected data between 2014-2021 across 4 sampling sites in Europe (Norway, Italy, Germany and France) representing 1198 individuals. The Aligned Studies in adults collected data between 2014-2021 across 5 sampling sites in Europe (Iceland, Portugal, France, Germany and Luxembourg) representing 1180 individuals. P50 of urinary AAMA concentrations are in the range of 51.44 - 83.88 µg/g crt across studies in children and 28.78 - 91.70 µg/g crt across studies in adults. P95 of urinary AAMA concentrations are in the range of 126.39-220.50 µg/g crt across studies in children and 90.82-503.92 µg/g crt across studies in adults. P50 of urinary GAMA concentrations are in the range of 8.32 - 30.74 µg/g crt across studies in children and 7.13 - 25.01 µg/g crt across studies in adults. P95 of urinary GAMA concentrations are in the range of 17.72-65.16 µg/g crt across studies in children and 13.51-48.82 µg/g crt across studies in adults. For AAMA the share of individuals with exposure levels exceeding the BE-value of 16 µg/g crt ranges from 98.33%-100% for children and 90.20%-100% for adults. However, compared to the HBM orientation values (OV)- calculated according to EFSA BMDL10 value of 0.43 mg/kg b.w. per day for peripheral neuropathy in rats- 7% of adults (France and Portugal) had acrylamide values higher than the HBM-OV (291.4µg/L for adults with a body weight of 70 kg) and 2% of children had acrylamide values higher than the HBM-OV (321.7 µg/L for children).

6.3 Does the exposure to acrylamide differ significantly between countries and population groups? Are the main reasons for these differences related to different dietary habits or to other factors?

The exposure to acrylamide in the HBM4EU studies showed that the levels of acrylamide exposure measured in urines are higher in the participating studies from Southern Europe when compared to Northern and Western studies in both children and adults. The reasons for these differences are still unclear and were not fully revealed through our investigation. Certain non-dietary determinants might explain these geographical differences in adults.

Children have slightly higher levels of exposure than adults suggesting that they might be a susceptible group. In both children and adults, gender seems not a determinant of exposure levels. Smoking was confirmed to be a strong determinant of acrylamide exposure in all ages. In adults we found that acrylamide levels were higher in relation to high consumption of potato fried and coffee but lower in relation to increase in body mass index, intake of fruit/vegetables and cereals. These results may indicate to strengthen the policy strategies strategy to reduce acrylamide in certain foods as well as make the citizen more aware of healthy choices.

6.4 Which population groups are more at risk? Are there other sources of exposure of acrylamide that need to be discovered (e.g. smoking habits or other food sources)?

In children, higher levels of acrylamide were found in relation to low socioeconomic factors, living in cities but lower in relation to increasing age. These results found in children might indicate that the awareness of acrylamide, and in general, healthy lifestyle/choices, among certain groups exposed to low socioeconomic education or living in cities (where the access to fast food might be easy and of easy choice) is still low. Also, these results may suggest that urinary biomarkers of acrylamide may not capture fully dietary exposure of acrylamide in children.

We also found an association between higher levels of acrylamide with increasing sampling year (2014-2017) in children, suggesting that the typical food eaten by children is still high in acrylamide. These results are also confirmed by the time trends analysis (below explained).

6.5 Is there an impact from the mitigation for the production in food processing and manufacturing and REACH restrictions on the distribution of acrylamide exposure? Do we need to implement other restrictions to decrease the level of exposure of acrylamide?

Findings from this protocol are important to understand whether the measures adopted to decrease acrylamide formation in food have been effective in lowering the levels of exposure to acrylamide in the European population. Of note, results from this project will be helpful to evaluate the current exposure of acrylamide in the European population.

Results from analysis of aggregated data indicate an overall increase of acrylamide exposure between the year 2000 and 2017 in non-smokers. Such a trend results also from statistical analysis of individual data from HBM4EU aligned studies by comparing yearly means of single studies from children. Studies focusing on adults with samples collected after 2018 do not show increasing exposure or even declining values. Regional differences appear to affect absolute values, but not the overall time-trend of exposure.

As in 2018, benchmark levels for acrylamide content in food have been adopted in Europe, our results may show first slight effects of these measures, but only indicated for adults as according to data are still missing for children. We encourage further biomonitoring studies with samples taken after 2019 in both children and adults to check furtherly the effect of these measures in the European population.

6.6 Are the exposure levels a concern for health? Is the exposure to acrylamide associated to cancer, neurological alterations and foetal growth in humans? Is the health risk dependent on long term or intermittent exposure to low quantity of acrylamide?

1) Acrylamide and cancer:

Geometric mean urinary AAMA concentrations in the HBM4EU aligned studies were in the range of 50-70 µg/L in children and 20-100 µg/L in adults. In previously published studies with the general population, the corresponding range has been 30-73 µg/L. Taking the European Food

Safety Authority (EFSA 2015) risk assessment for dietary intake of acrylamide as a basis, an acrylamide tumour risk of 1:1000 at 0.425 µg/kg bw/d corresponding 28.8 µg/L of AAMA (in 70 kg adult) was estimated. This means that acrylamide cancer risk for children varied from 1:570 to 1:464 and in adults from 1:1384 to 1:288, when biomonitoring data from HBM4EU aligned studies were used as a starting point. These risks correspond to mean acrylamide intakes of 0.75 – 0.92 µg/kg bw/d in children and 0.31 – 1.47 µg/kg bw/d in adults, respectively. These levels are in line with the EFSA estimates on acrylamide intake via food (mean intake 0.4 - 1.9 µg/kg bw/d). The risk assessment is based on the Benchmark Dose Lower Confidence Limit (BMDL10) of 0.17 mg/kg bw/d based on neoplastic effects in mice derived by EFSA (2015), it was concluded that acrylamide potentially increases the risk of developing cancer in all age groups of the general population, since the main exposure to acrylamide happens via dietary intake. EFSA (2015) derived for acrylamide a health-based limit value for dietary intake.

This risk assessment has quite high uncertainty and is conservative since the risk was based on the linear extrapolation to zero from the mice carcinogenicity data using the BMDL10 value of 0.17 mg/kg bw/d derived from the most sensitive tumour type as a POD.

Results from the meta-analysis, based on 31 epidemiological studies, showed that high dietary intake of acrylamide was not associated with an increased risk of any of the investigated specific cancers, including oral cavity, oesophageal, gastric, colon-rectal, pancreatic, prostate, bladder, lung, renal, lymphoma, myeloma, thyroid, brain, larynx, and melanoma. As a novel finding, we found that the potential shape of the association between different levels of dietary acrylamide and the risk of any of the specific cancers considered, if present, would be linear. Considering studies performed in Western geographical areas, a borderline increased risk of lymphoma was observed in relation to high intake of dietary acrylamide. In general, findings did not differ by smoking status except for lung cancer in smokers and melanoma for never smokers. In another meta-analysis, of epidemiological studies, performed under the HBM4EU project, investigating dietary acrylamide and gynecological cancers only, a slightly increased risk of endometrial and ovarian cancer was noted, particularly in never-smoking women, and an increased risk of premenopausal breast cancer risk. Most of the epidemiological studies identified on acrylamide and cancer were performed using dietary assessment of acrylamide. There is a lack of epidemiological studies investigating the risk of acrylamide in relation to cancer using HBM studies. This observation is of importance since it may explain the reason why epidemiological studies have failed to show an increased risk of cancer with acrylamide exposure. We encourage further high-quality epidemiological studies using biomarkers of acrylamide to understand better whether acrylamide is associated with cancer in humans.

Results from the mechanistic information on AOP:

Based on the literature review, increased risk of gastrointestinal cancer observed in experimental animals may be related to intermediate levels of acrylamide rather than low levels of exposure to acrylamide and may also be related to sex differences. No conclusions could be derived on whether the current exposure levels pose a concern for health or whether age plays a role on the risk of developing oesophageal, gastric, or colorectal cancer. Also, an increased risk of oesophageal cancer (based on 341 cases) emerged in subjects with intermediate levels as compared to low acrylamide intake. In relation to gender, acrylamide might be associated with colorectal cancer with specific somatic mutations, differentially in men (increased risk if activating KRAS mutation) and women (decreased risk if truncating APC mutation). No mechanistic information was found on the pathways induced by acrylamide for the development of other types of cancers examined including breast, endometrial, pancreatic, lung and prostate. No conclusions could be derived on whether the current exposure levels pose a concern for health or whether age plays a role in the risk of developing oesophageal, gastric, or colorectal cancer.

2) Acrylamide and neurological alteration:

Health based guidance values are available to which the newly generated HBM data in the HBM4EU aligned studies can be compared. The critical endpoints are nerve damage or peripheral neuropathy in rats. For AAMA the share of individuals with exposure levels exceeding the Biomonitoring equivalent (BE-value) of 16 µg/g crt ranges from 98.33%-100% for children and

90.20%-100% for adults. However, compared to a more recently derived HBM orientation values (OV)- calculated according to the EFSA BMDL10 value of 0.43 mg/kg b.w. per day for peripheral neuropathy in rats- 7% of adults (France and Portugal) had acrylamide values higher than the HBM-OV (291.4µg/L for adults with a body weight of 70 kg) and 2% of children had acrylamide values higher than HBM OV (321.7 µg/L for children).

There are no studies on neurodevelopmental functional effects in the general population exposed to acrylamide. Moreover, evidence from animal studies indicate that acrylamide exposure during neural development has the potential to affect key events known to lead to cognitive impairment in children.

The lowest “low observed adverse effect level” (LOAEL) reported for neurodevelopmental toxicity in rats was 0.5 mg/kg bw/day after maternal exposure of acrylamide in drinking water up to 3.0 mg/kg bw/day [starting at GD6 until 2 years of age]. However, in most cases the neurodevelopmental NOAEL was below the dose levels of acrylamide tested in the studies and therefore unknown. It is thus currently not established a NOAEL for developmental neurotoxicity of acrylamide.

Furthermore, most in vitro studies were performed using concentrations far above the levels estimated from the daily dietary intake in the general population.

Thus, there is an urgent need for further research to examine whether pre- and perinatal acrylamide exposure might impair neurodevelopment in humans. Also, to be able to elucidate which of the mechanisms attributed to acrylamide exposure are relevant for humans performing long-term studies at real-life concentrations in human relevant neural stem cell models are needed. The higher exposure in children than in adults makes it particularly important to assess the potential pre- and postnatal neurodevelopmental effects.

Since it is currently not possible to estimate a threshold dose for induction of neurodevelopmental toxicity in experimental animal studies for use in risk assessment, there is consequently also insufficient evidence to conclude whether the developing brain is more susceptible to acrylamide toxicity than the adult brain. We did not identify any studies of neurodevelopmental effects in humans. Possible gender differences in acrylamide neurodevelopmental toxicity therefore need to be based on animal experiments, and there is not enough evidence to conclude on gender differences in relation to effects on the developing brain.

3) acrylamide and foetal growth:

Based on a dose-response meta-analysis of epidemiological studies, we observed that higher gestational acrylamide exposure was associated with lower birth length, head circumference, and weight, and with a higher risk of SGA. Results were stronger and more consistent for the studies pooling the biomarkers. Moreover, whenever it was possible to investigate the shape of the relationship (only with birth weight), we found no clear evidence of departure from linearity. Based on the results of our current meta-analysis, we encourage the development of further epidemiological studies to confirm these results. In light of the Developmental Origins of Health and Disease hypothesis, our meta-analysis suggests that prenatal acrylamide exposure may have detrimental effects at a later age. However, in the meantime, based on the current body of evidence, we think a strategy to reduce the acrylamide intake of pregnant women through targeted public health education is warranted.

6.7 Are the health risks dependent on age and gender?

See answer for policy question above

7 HBM4EU outputs to date

7.1 Categorisation

Substances under HBM4EU have been categorised depending on availability of HBM data. The categorisation indicates the information gaps allowing the development of targeted activities to fill the knowledge gaps. Substances will pass from Category E over D, C, B towards Category A as more information becomes available. Fully characterised substances should end up as category A substances.

Table 3.1 HBM4EU categorisation for acrylamide

Category	Priority substance(s)	Details
B	HBM data exist but not for across Europe Acrylamide	The exposure pathways are identified. Limited knowledge on health impacts is available. First HBM studies in the EU have been performed, but at insufficient level to provide an overall picture of exposure in Europe.

7.2 Key outputs

The main outputs from the HBM4EU to date include the following:

Current exposure

To answer these specific policy questions on current exposure, geographical differences and exposure determinants in the EU population, two research protocols have been developed (Task 10.4). An extensive search has been conducted to identify HBM studies performed in EU (n=25). Few studies were identified with HBM data available for acrylamide and the invitation response was very low (0.2%). The European HBM dashboard has 1 dataset with acrylamide exposure data integrated.

Definition of the best biomarkers, matrices, and analytical method for monitoring acrylamide in the general population.

Identification of the laboratories with experience and capacities to analyse acrylamide in human samples and their inclusion in the [European network of HBM laboratories](#).

Already published studies reported determinants of exposure in certain foods such as coffee (and solid coffee substitute), fried potato products (including potatoes and vegetables crisps), biscuits, cereals and other products such as roasted nuts, olives in brine, prunes and dates and baby food. Very few European studies have investigated other exposure determinants of acrylamide exposure in the general population. To our knowledge no European biomonitoring studies have investigated exposure determinants of acrylamide in newborns, only one study in children. Hence, one of the proposed research protocols aimed to investigate the most relevant determinants of acrylamide exposure among European adults, children and whether the exposure determinants may differ among children and adults. The project will not cover newborns as this study population is not included in the aligned studies and there is no response from the only available study (NewGeneris) considering this age group. We also investigated whether the exposure determinants are different for dietary or non- dietary regional differences. The statistical analysis was performed with individual data from existing European studies performed in the general population of adults and children and included cohorts from the following geographical areas (North, West, South), available information on biomarkers of acrylamide in urine (AAMA and GAMA) and a concomitant assessment of other variables considered as possible determinants of

the exposure of acrylamide. HBM4EU data were obtained from the following countries: 4 cohorts of children from Italy, France, Germany and Norway and 6 cohorts of adults from Portugal, Spain, France, Germany, Luxembourg and Iceland. Each variable selected as exposure determinants (dietary and non-dietary determinants) was assessed in relation to urine levels of urinary acrylamide exposure biomarkers using adequate regression quantile models. Analysis were performed for children and adults separately. Each variable was tested in univariate and multiadjusted models.

A second protocol for analysis of current exposures to acrylamide in the European population (together with time trends) has been developed and applied on the data of the HBM4EU aligned studies.

The aligned studies made use of a variety of materials produced in WP7 that provide the groundwork for a harmonised approach to study planning and conduct of HBM studies in Europe. For Acrylamide, questionnaires for adults and children are available.

New data for 2 acrylamide biomarkers (AAMA and GAMA) is available from the HBM4EU Aligned Studies in children (6-11 years) and adults (20-39 years). The Aligned Studies in children collected data between 2014-2021 across 4 sampling sites in Europe (Norway, Italy, Germany, and France) representing 1198 individuals. The Aligned Studies in adults collected data between 2014-2021 across 5 sampling sites in Europe (Iceland, Portugal, France, Germany, and Luxembourg) representing 1180 individuals.

Mitigation

Strategies for mitigation and reduction of acrylamide in food and foodstuff have been carried out since the discovery in 2000.

Currently, no country has set legally binding maximum AA levels for foods. Only in 2017, the first EU regulation 2017/2158 was released with the aim to establish mitigation measures and benchmark levels for the reduction of AA in food. So far, no studies have been performed at European level to evaluate time trends of acrylamide exposure in the general population. Moreover, the awareness of acrylamide exposure has been shown to differ by European countries.

Within the Task 10.4, one of the research protocols aim to investigate whether the adopted measures at European level have been effective to decrease the exposure of acrylamide from 2002 up to now in the European population. Moreover, we investigate whether these trends are equally observed in all European countries. This study is performed using the aggregated data from published European studies with available biomarkers of acrylamide measured in urine or blood during the period 2002-2017 and aligned studies with available biomarkers of acrylamide measured in urine covering the period from 2014 up to now. We utilize data on AA and GA concentrations in blood and urine. Published studies have data available on biomarkers measured in urine and blood whereas the aligned studies will only have urinary biomarker data. Due to the heterogeneity in the measurements of acrylamide within participating studies, data are harmonised to obtain comparable values using a transmatrix calculation. Time points are treated as continuous. Time-line analysis for aggregated data were performed to evaluate the relationship between time points and the mean of distribution of urine acrylamide biomarkers. The results are finalized, and a manuscript has been drafted.

Health risks

To answer the policy questions whether the exposure levels for acrylamide are a concern for health, specifically for cancer, neurological alteration and foetal growth, and whether the health risk is dependent on long-term or intermittent exposure to low quantities of acrylamide, activities are ongoing within WP13/WP14.

To address these policy questions, Task 13.1 on mechanistic information for AOPs and 13.2 on the epidemiological evidence in relation to acrylamide and cancer, neurological and early developmental disorders and foetal growth have been implemented.

1) Acrylamide and cancer: We performed a systematic literature review and meta-analysis of epidemiological studies evaluating the shape of the association between increasing levels of acrylamide and the risk of subtypes of cancer (Task 13.2). The meta-analysis has been finalized.

A manuscript has been submitted (Please see column 4).

Information on the AOPs leading to the development of oesophageal, gastric, breast, lung, ovarian, endometrial pancreatic, prostate, renal and colorectal Cancer (Task 13.1) were also gathered. The molecular initiating event of the AOP involves the formation of adducts between acrylamide and its epoxide metabolite glycidamide and DNA. Acrylamide affects hormonal balances in animals, leading to increased occurrence of mammary gland tumours in rats. The main route of exposure is dietary acrylamide meaning that the gastrointestinal tract is exposed to considerable amounts of the agent; however, since the acrylamide molecule is small and hydrophilic, it reaches every organ and virtually every tissue in the body. In addition, we have conducted an animal experiment to test the effects of dietary acrylamide on colon tissue. For this purpose, 10 male Balb/c mice (6 weeks old) were randomized to one mock-treated and one acrylamide treated group. The mice in the treatment group received oral gavage of 0.1 mg/kg acrylamide (AA) daily for 4 weeks. At the end of the 4-week period, tissue samples (brain, epididymis + Vas and colon) were obtained from mice following euthanization. Brain and epididymis + Vas samples were snap frozen and sent to the Norwegian Institute of Public Health for further processing. Colon samples were sent to "ATLAS Biolabs GmbH" (Berlin, Germany) for RNA sequencing, as a subcontract service. A list of differentiated genes and related pathways were identified in the acrylamide-treated group compared to the control group. Further data analysis is underway and will be completed by the end of the project.

2) Acrylamide and neurological alteration: For Task 13.1 (mechanistic information for AOPs relevant for neurological and early developmental disorders including sex related disorders) and 13.2, critical assessment of possible associations with neurological alterations in cohorts, a literature search has been performed by NIPH Library on developmental neurotoxicity of acrylamide (AA) (human cohorts, occupational, animals and in vitro studies) resulting in approximately 500 abstracts after filtration (out of an initial search of 1389 abstracts; search was updated Nov 2020). A literature summary of cohort and mechanistic studies was performed. Different neurodevelopmental adverse effects in animals and humans as well as key event information from in vitro studies are summarized in forest plots and various adverse outcome pathways / networks are explored, selecting the impairment of learning, memory and cognitive function as adverse outcome.

3) Acrylamide and foetal growth: a dose response meta-analysis summarizing the results of individual data available in this field has been performed. The study is the most up-to-date meta-analysis covering this topic, including 5 epidemiological studies. Among them, three were performed with estimated dietary acrylamide exposure assessed through dietary questionnaires and the remaining two with validated biomarkers of acrylamide i.e. hemoglobin adducts of acrylamide and glycidamide. Compared to the previous meta-analysis published by Zhag et al. in 2020, we added one study performed using biomarkers, one additional birth outcome (birth length) and we evaluated the shape of the relationship using the novel approach of dose-response meta-analysis whenever possible.

The meta-analysis is finalized, and a manuscript has been recently submitted.

Implementation of specific effect biomarkers related with human exposure to AA through proteomic profiling (WP13 and WP14) has been performed. This proteomic panel aimed to identify new circulating markers of effect related to acrylamide exposure in the existing European EuroMix human biomonitoring study (n=141). Olink was used for a proteomics analysis of inflammation (92 proteins) and neurological markers.

Risk assessment of acrylamide has been performed in WP 5.3, based on the aligned study data on urinary levels of acrylamide mercapturic acid (AAMA) and glycidamide mercapturic acid (GAMA). The risk assessment covers general dietary intake of acrylamide in the general population and cancer risk.

7.3 Key data gaps

HBM4EU is a five-year project, that kicked off in 2017 and will run until June 2022. HBM4EU has helped to identify a number of specific data gaps that are needed to give policy makers relevant and strategic data to establish appropriate regulations and improve chemical risk management. However, some gaps and needs for action will remain after the end of HBM4EU which should be addressed in the future:

- Long-term monitoring of exposure levels to evaluate differences between countries and population groups, time trends, and age-related differences in exposure.
- Evaluation of the risk of cancer, neurological development and foetal growth in relation to acrylamide exposure through generation of new HBM data. Consistent use of validated biomarkers would significantly enhance studies on acrylamide exposure. **In particular, an urgent call is for further investigations on the link between acrylamide exposure and cancer risk**
- **Investigations on prenatal exposure to acrylamide and neurodevelopmental functional effects (learning, memory and cognitive function)**
- Identification of biomarkers of effects which may be useful in strengthening of causality and mode of action analysis in epidemiological studies linking exposures to health effects.
- Further investigation of the biological pathways behind the possible risk of cancers and neurotoxicity and fetal growth in humans due to acrylamide exposure. A better understanding of the mixture of effects of acrylamide and other dietary carcinogens.
- Although a new risk assessment has been released by EFSA¹² in May 2022, we encourage a constant risk assessment, expressed as margin of exposure, for both occupational settings and general population. If you would like to read more about the margin of exposure approach, please visit EFSA's website¹³.
- Experimental research to support the development of human biomarkers which may be measured in specimens which are easy to collect. This will facilitate the screening of acrylamide exposure, and in turn the monitoring of exposure levels, in the general population and in the occupational setting.
- Constant monitoring of the measures developed to reduce the level of acrylamide in order to measure the effectiveness and, if needed, to adapt the measures.

8 Future recommendations

Based on the gap of knowledge remaining together with the results obtained during this project, we highly recommend continuing to monitor the levels of acrylamide exposure in foods and develop a strategy to make the citizen of all ages and groups more aware of the acrylamide issue through public health educational campaign. Also, we encourage to implement more urgent research in the areas highlighted in the section above.

¹² <https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2022.7293>

¹³ <https://www.efsa.europa.eu/en/topics/topic/margin-exposure>

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Appendix 1: Additional information on exposure routes

Source of exposure	References
<p>Environmental</p> <p>Release to soil and water through man-made sources.</p>	Scoping Document
<p>Occupational</p> <p>Used in industrial processes e.g. production of organic chemicals, dyes, grouting agent.</p> <p>Used in health service and scientific research.</p>	Pennisi et al. (2013)
<p>Consumer</p> <p>Forms during everyday high-temperature cooking.</p> <p>Present in tobacco smoke.</p>	David et al. (2018) EFSA (2020)

Route of exposure	Reference
<p>Oral</p> <p>Oral uptake through the ingestion of food is the primary source. The uptake of contaminated water is a minor exposure source for the general population.</p>	Scoping document EFSA (2020)
<p>Dermal</p> <p>For occupational exposure, inhalation and dermal contact at the workplace where acrylamide is used or produced is another important route of acrylamide exposure.</p>	Scoping document Pennisi et al. (2013)
<p>Inhalation</p> <p>For occupational exposure, inhalation and dermal contact at the workplace where acrylamide is used or produced is another important route of acrylamide exposure. The general population may be exposed to acrylamide through cigarette smoke inhalation.</p>	Scoping document Pennisi et al. (2013)
<p>Trans-placenta</p> <p>Transplacental exposure should also be taken in consideration for the risk assessment, although more investigation is needed.</p>	Scoping document Annola et al. (2008)

Appendix 2: Additional information on health effects

Human health effect	Category	Justification for category	References
Non-organ specific (cancer)	Strong	Based on harmonised listing of 1B.	See table 4.1
Non-organ specific (disturbance of foetal growth)	Suspected	As noted in the scoping document – a limited number of epidemiological studies suggest adverse impacts on foetal growth.	Scoping document Duarte-Salles et al. (2013)
Brain/neurological system (neurotoxic effects)	Suspected	As noted in the scoping document – neurotoxic effects are suggested and occupational exposure may be a potential harm.	Scoping document Pennisi et al. (2013)
Endocrine system (endocrine disruptor)	Evidence lacking	As noted in the scoping document – there is a lack of consistent evidence in humans.	Scoping document Kassotis et al. (2015)
DNA (DNA damage)	Strong	Based on harmonised listing of 1B.	See table 4.1
DNA (reproductive toxicity)	Suspected	Based on harmonised listing of 2.	See table 4.1

For the categorisation of the strength of evidence for human health effects, the following criteria has been used:

- **Strong** – where the health effect is confirmed by either a harmonised classification indicating that there is a known effect (e.g. 1A or 1B for CMRs) (see Table 4.1), or where there is no applicable C&L classification, a statement in the Scoping Document that concludes there is strong evidence (or where a significant body of evidence is presented in the scoping document).
- **Suspected** – where there is either (a) a harmonised classification indicating that there is a suspected effect (e.g. category 2 CMRs or similar); (b) notified classification for that effect, or (c) where there is no applicable C&L classification, a statement in the Scoping Document (or other references presented in the Table above) that there is a suspected health impact.
- **Evidence lacking** – where a health effect is noted in the Scoping Document (or other evidence sources referenced in the Table above), but it is stated that evidence is currently lacking or there are uncertainties or inconsistencies in the available evidence.
- **Not applicable** – where a health effect does not apply to a specific group/gender