Prioritised substance group: Acrylamide

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Short overview of results of the activities carried out within HBM4EU in 2020 to answer the policy questions with reference to corresponding deliverables.

Policy Question	Short Summary of Results
1. What is the current exposure of the EU population to Acrylamide? **+** **2. 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?** **+** **3. 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)?**	To answer to these specific policy questions on current exposure, geographical differences and exposure determinants in the EU population, two research protocols has 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 low. Among them, only one study (NewGeneris) was eligible for inclusion. Determinants of the exposure have been identified 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 general population. No European biomonitoring studies have investigated exposure determinants of acrylamide in newborns and children. Hence, one of the proposed research protocols aims to investigate the most relevant determinants of acrylamide exposure among European adults, children and newborns and whether the exposures determinants may differ among newborns, children and adults. Also, we will investigate whether the exposure determinants may differ for dietary or non- dietary regional differences. The study will use individual data from existing European studies performed in the general population of adults and children including both sexes with sufficient representative coverage of at least one EU geographical area (North, West, East, South), available information on biomarkers of acrylamide in urine or blood and a concomitant assessment of other variables considered as possible determinants of the exposure of acrylamide. Acrylamide metabolites will be quantified in urine for children and adults and in cord blood for newborns. Individual data from NewGeneris for newborns, aligned studies for children (including GERV) and adults will be used. Each variable selected as exposure determinants will be assessed in relation to blood or urine levels of acrylamide using adequate regression models. Each analysis will be adjusted for sex, age and country. Further, a multivariable-adjusted model will be performed including in the model all the variables considered. Stratification analysis will also be performed by sex and geographical location (North, East, South and West). Currently we are waiting for the data transfer from NewGeneris and the measurements of biomarkers of acrylamide in the aligned studies. Results will allow identifying groups of people at higher risk of acrylamide exposure as well as the most relevant determinants of acrylamide exposure and hence, provide the basis for possible guidelines.

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4. 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?	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 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 will investigate whether these trends are equally observed in all European countries. This study will be performed using the aggregated data from published European studies with available biomarkers of acrylamide measured in urine or blood during the period 2002-2014 and aligned studies with available biomarkers of acrylamide measured in urine covering the period from 2014 up to now. We will utilise data on AA and GA concentrations in blood and urine. Published studies will 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 will be harmonised to obtain comparable values. Time points will be treated as a continuous or categorical variable. Three categories of time period will be created according to the mitigation measures: before 2014, 2014-2018, and after 2018. Moreover, since the published studies have measurement of biomarkers of acrylamide before 2014 and aligned studies cover the period from 2014 and on, we will also consider to use this year as single cut-off time period. Time-line analysis for aggregated data will be performed to evaluate the relationship between time points and the mean of distribution of urine acrylamide biomarkers. Preliminary results based on published studies have shown a slightly clear increase of level of biomarkers of acrylamide for each year increase (p0.00). Findings from this study will be important to understand whether the measures adopted to lower acrylamide formation in food have been effective in lowering the levels of exposure to acrylamide in the European population.

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5. Are the exposure levels a concern for health? Is the exposure to acrylamide associated to cancer, neurological alterations and fetal growth in humans? Is the health risk dependent on long term or intermittent exposure to low quantity of acrylamide? **+** **6. Are the health risks dependent on age and gender?**	To answer the policy questions whether the exposure levels for acrylamide are a concern for health, specifically for cancer, neurological alteration and fetal 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 evidences in relation on acrylamide and cancer, neurological and early developmental disorders and fetal growth have been implemented. 1) Acrylamide and cancer: We conducted a literature search on acrylamide and cancers including studies performed in humans, with acrylamide exposure measured through dietary assessment and/or biomarkers of acrylamide and all types of cancers as outcome (Task 13.2). A total of 65 papers were identified though Pubmed. In addition, a recent search through Scopus and Web of Science rendered 19 additional eligible papers to be included in the critical review. The extraction of the results for further interpretation from the selected papers is ongoing. 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 preliminary observation is of importance since may explain the reason due to the epidemiological evidences have failed to show an increase risk of cancer with acrylamide exposure. Information on the AOPs leading to the development of oesophageal, gastric, 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 tumors 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. Based on the preliminary findings: increased risk of gastrointestinal cancer 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 (on the basis of 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) (9). 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. Stronger conclusions on acrylamide exposure and its relation to cancer in the general and potential vulnerable subgroups of the population will be produced when we integrate the further results of AOP with those of the review/meta-analysis. 2) Acrylamide and neurological alteration: Literature search has been performed on acrylamide and neurotoxicity (human cohorts, occupational, animal and in vitro studies). The final database consists of 460 studies. Among them, 22 were classified as human studies, 375 as animal studies and 63 as in vitro studies. Based on the preliminary results of the review, very few cohort studies on the association between dietary acrylamide exposure and neurological outcomes are performed, and data gap exist since no studies up to now have addressed neurodevelopmental cognitive alterations in children (only fetal growth, please see below for details). Seventeen occupational studies have been identified in the literature search (excluding smaller case studies), showing acrylamide-related (mainly peripheral) neurotoxic alterations, but also impaired cognition. The variety of reported symptoms show involvement of the peripheral and the central nervous system, as well as the autonomic nervous system, including muscular weakness, paraesthesia,

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	numbness in hands, feet, lower legs and arms, and unsteadiness. Acute and high exposures to acrylamide more often result in early CNS involvement, while longer exposures are associated with peripheral neuropathy. In addition, sensory impairment in the form of reduced color vision and light sensitivity has been reported and more recently impaired hearing has been suggested to be associated with acrylamide exposure. Both short term, higher exposures and prolonged lower exposures are shown to induce neurotoxicity in humans. The potential influence of age and gender with respect to acrylamide susceptibility is not clear. Differences in acrylamide metabolism may be of importance but has not been explored in relation to neurological impairments. Due to the exquisite sensitivity of the developing nervous system, a risk of early life exposure can be postulated (data gap). This concern is augmented by the likely effect of acrylamide on fetal growth (presented below). Acrylamide exposure is in some cases associated with an unhealthy diet (high fat diet) and animal experiments suggest a combined effect of acrylamide and high fat diet at least with respect to male reproductive toxicity. This may also be the case for neurodevelopmental toxicity as diet influences several of the postulated MoA/targets of acrylamide in the brain. AOP evaluation is ongoing. A proposed molecular initiating event (MIE) for peripheral neurotoxic effects of acrylamide is binding to cysteine residues in presynaptic membrane proteins. 3) Acrylamide and fetal growth: From the literature search of epidemiological studies on acrylamide exposure during pregnancy and fetal growth (Task 13.2) five publications including a recent meta-analysis based on these studies were identified and critically assessed. All of these studies used a food frequency questionnaire (FFQ) to estimate acrylamide intake and one study additionally measured hemoglobin adduct levels in umbilical cord blood. The epidemiological evidence for an inverse relationship between prenatal acrylamide exposure and reduced fetal growth is quite strong. However, with epidemiological research the question is always whether the association represents a cause and effect relationship. These preliminary findings indicate the urgent need to gather further data on the potential important effects of acrylamide on human prenatal and postnatal development. More studies along the line of the presented studies should be performed and it would also be helpful to try and interrogate the causality of the observed inverse association by investigating the biological plausibility (mechanism of action) using biomarkers of effect (such as growth factors) and susceptibility (genetic variants in acrylamide-metabolising genes). Based on the current knowledge, there are no AOP for fetal growth (Task 13.1). The construction of a new AOP on fetal growth might be complex but one can may be think to more specifically define an endpoint under the umbrella of fetal growth, such as thyroid disruption.