Prioritised substance group: Aprotic Solvents

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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.

Not all policy questions are listed – only those which have been addressed until now with notably results. All 19 PQs can be found in the scoping document.

N-methyl-2-pyrrolidone (NMP), N-ethyl-2-pyrrolidone (NEP), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAC)

Policy Question	Short Summary of Results
2. What is the current internal exposure of the workers in EU to reprotoxic aprotic solvents, especially with respect to female workers at reproductive age, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist? **3. Are there geographical differences and differences caused by industrial sector in the exposure of workers in EU to reprotoxic aprotic solvents?** **4. What is the current exposure of the general EU population to reprotoxic aprotic solvents, especially with respect to females at reproductive age**	Statistical analysis plan (SAP) for aprotic solvents is developed within WP10. The aim of the SAP is to set procedure for answering the exposure related research questions defined in the scoping document. The general part of the SAP includes statistical plans for the evaluation of time trends, geographic comparisons, evaluation of exposure determinants, a strategy for the calculation of EU reference values and a plan for conducting uncertainty analysis. It is assumed that urine (urine-spot, urine-24h, urine-morning) will be the obligatory matrix to be used for determination of chosen NMP, NEP, DMF and DMAC metabolites. Optionally a number of parameters characterising urine will be determined - total volume of urine collected, urine density of the sample, concentration of creatinine in urine of the sample, osmotic concentration of urine of the sample, specific gravity of urine (ratio of urine density compared with water density). Certain obligatory or optional variables characterising participants of the study will be applied – age, sex, education, current labour status, industrial sector of occupation, life style (frequent use of chemical household products (for cleaning, etc.) or focus on natural „ecological“ products) and consumption patterns (frequency of usage of cosmetics). Study protocols, standard operating procedures (SOPs) and guidelines, tailored and transferred questionnaires for recruitment and sampling have been elaborated within WP7 for 2th priority substances including aprotic solvents.

Policy Question	Short Summary of Results
as well as mothers and their young children, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist?	

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9. Are there differences in exposure of the general EU population to regulated and non-regulated reprotoxic aprotic solvents (banned use in cosmetics)? **10. Are there differences in exposure of the workers in EU in relation to regulated and non-regulated reprotoxic aprotic solvents after the restriction for NMP will enter into force after 9 May 2020?** **11. What are differences in profiles of reprotoxic aprotic solvents observed in exposure assessment regarding occupational environment and in relation to general public taking into account spatial and temporal distribution?**	
13. What are the best indicator`s substances (markers) to identify hazardous exposures to aprotic solvents as a whole? **14. What are the analytical options available with respect to aprotic solvents (gas chromatography-mass spectrometry versus liquid chromatography-tandem mass spectrometry for biological matrices, other methods in addition, methods for environmental media)**	In WP9 a suggested list with most suitable biomarkers, matrices and analytical methods has been elaborated based on published data. 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-N-methylsuccinimide (2-HMSI) are specific biomarkers of NMP for analysing urine samples and sufficiently low detection limits can be achieved. 5-hydroxy-N-ethyl-2-pyrrolidone (5-HNEP) and 2-hydroxy-N-ethylsuccinimide (2-HESI) are specific biomarkers of NEP exposure and are suitable for the monitoring of non-occupationally exposed populations. For both NMP and NEP GC-MS or GC-MS/MS analyses reached the lowest LODs and can currently be regarded the methods of choice. Urine is the preferred matrix for exposure characterisation for both NMP and NEP. Further need for method development in relation to NEP metabolites is identified due to scarcity of published data on NEP. The following exposure biomarkers have been described for DMF: DMF in urine, N-methylformamide (NMF) in urine, N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC) in urine, 3-methyl-5-isopropylhydantoin hemoglobin adducts (NMVal/NMHb) in red blood cells. NMF in urine is the preferred option for occupational exposure by application of GC-NPD analytical method, however, it seems unfit to capture environmental background exposure levels, AMCC in urine seems to be the best option for exposure assessment in the general population but the sensitivity of the method should be improved. AMCC is usually measured by LC-MS/MS. It shall be taken into account that AMCC may also be formed from the dietary uptake of methyl-isocyanate which is a component of wine and cruciferous vegetables such as cabbage, turnips and cress. Therefore, AMCC may lack specificity to reflect the environmental

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	exposure of DMF. The haemoglobin adducts might serve as indicators of longer term exposure, however their use in biomonitoring seems questionable due to complicated sample (isolated globin) preparation. The following exposure biomarkers have been identified for DMAC: the DMAC itself, N-methylacetamide (NMAC), N-hydroxymethyl-N-methylacetamide (DMAC-OH) and S-(acetamidomethyl) mercapturic acid (AMMA). Nevertheless, methods for the quantification of DMAC metabolites in human matrices are not well established. Urine seems to be the best matrix for analyses of DMAC metabolites. The DMAC biomarkers have been measured by GC-MS, GC-NPD, GC-FPD, LC-MS or UHPLC-MS/MS. UHPLC-MS/MS gave the best sensitivity for all four biomarkers. DMAC-OH is known to be converted to NMAC under high temperatures in the GC injector, but this problem can be avoided by LC analysis. The detection and quantification of DMAC metabolites needs further method improvement in order to enhance the sensitivity for low exposed general population. Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA) has held an advanced training course on method improvement for NMP and NEP on 23rd November 2018 in in Bochum, Germany (the 2nd HBM4EU training school). In total, 14 candidate laboratories for the analysis of aprotc solvents have been identified in 7 countries