Prioritised substance group: Phthalates & DINCH

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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.

Please note: Not all policy questions are listed – only those which have been addressed until now with notably results. You can find all 13 PQs in the scoping document.

Policy Question	Short Summary of Results
Exposure characteristics	
1. Which are the most sensitive, reliable and cost-effective methods and biomarkers to measure phthalates and Hexamoll® DINCH®?	In WP9 a prioritised list with most suitable biomarkers, matrices and analytical methods has been elaborated. In total 26 suitable biomarkers representing exposure to 14 parental compounds were selected. Two methods have been evaluated as being suitable to measure the metabolites: GC-MS-MS for measuring DPHP metabolites only and LC-MS-MS for all other biomarkers. Urine has been selected as matrix of choice for all compounds. No information has been found for DiPeP, DHNUP and DMEP. Hence, no methods or biomarkers could be selected (see D9.2). Furthermore, a final list of the parameters that will be included in the ICI/EQUAS 2018 has been elaborated in substance specific working groups based on the existence of solid and reliable analytical methods and the availability of reference material. The following compounds have been selected to be obligatory: DEP, BBzP, DiBP, DnBP, DCHP, DnPeP, DEHP, DnOP, DiNP, DiDP, DINCH. Additionally, DMP and DPHP can be included on a voluntary basis. In WP 9.3 “Development of new methods”, a feasibility study was conducted that identified new, valuable urinary exposure biomarkers for EU-labelled, reprotoxic phthalates currently not covered in HBM analytical methods (Cat C phthalates): Di-isopentyl phthalate (DiPeP), Di-C7-11-(linear and branched)alkyl phthalate (DHNUP), Di-n-hexyl phthalate (DnHexP) and Di-(methoxyethyl) phthalate (DMOP). Biomarkers for these phthalates will soon be implemented in a new phthalate multi-method and tested in WP9.3 with a small round robin test. A Quality Assurance/ Quality Control Programme was implemented in order to establish a European database of candidate laboratories that are equally qualified for exposure biomarker analysis within HBM4EU. For this an interlaboratory comparison investigation/external quality assurance scheme (ICI/EQUAS) scheme and evaluation criteria were developed (see Deliverable 9.4). Throughout the ICI/EQUAS exercise, in combination with training and knowledge exchange, a substantial increase in capable laboratories being able to analyse DINCH/phthalates within HBM4EU was achieved: Aafter the 4th round ICI/EQUAS a total of 20 laboratories from 14 countries could be identified, that successfully participated for the analysis of phthalates biomarkers. More

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	than half of the laboratories qualified for the analysis of DEHP, DEP, DiBP, DnBP and BBzP biomarkers (≥11/ 20). For the Cat B and C phthalates also an increasing number of approved laboratories was achieved, ranging from six to ten laboratories. For DINCH metabolites, 8 laboratories from 8 countries did successfully participate of which all qualified for the analysis of OH-MINCH and almost all for cx-MINCH (7/8). The most current list of candidate laboratories can be found on the HBM4EU online library (https://www.hbm4eu.eu/online-library/).

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2. What is the extent of the current exposure of the EU population to the 16 phthalates (Cat A, B and C) and their substitute Hexamoll® DINCH®? **&** **3. Do the exposure levels differ significantly between the countries?** **&** **5. What are the high exposure groups? (Is there a statistically significant and toxicological relevant difference in mean concentration between adults and children? […] between occupational exposed and non-exposed adults? […] between male and female?**	In preparation for answering the policy questions, WP10 has developed a general and a substance-specific statistical analysis plan for phthalates and DINCH. Variables, which are needed for the statistical analyses to address substance-specific research questions on general exposure levels, time trends, geographic comparisons, and exposure determinants and reference values were defined. A requirement for the statistical analyses is the sharing of data as laid down in the Data Management Plan. The HBM4EU DMP gives details on the procedures that ensure that data are transferred and used in a secure setting; the use of the data is compliant with ethical and legal requirements, and that the use of data gathered within and before HBM4EU is done in agreement with the Data Contact Point. (see Revised DMP, D10.7). In WP7.1 a gap analysis has been carried out to get an overview how many studies of the priority substances, including phthalates and DINCH are available within the participating countries and has been summarised in a report (see D7.1). 42 studies in 12 different countries have been conducted or are initiated/ongoing, with measurements of phthalates and/or DINCH exposure over all age groups (New-borns, Children, Adolescents, Adults and Elderly). In general, most studies on this substance group have been carried out in the Northern or Eastern European-defined regions. 32 of the 42 studies reported to have biobanked samples and 6 of these studies are representative at national level. For the phthalate and DINCH substance group, most of the studies reported were with children and these studies were mostly conducted in Western Europe. Data owners/providers of 23 different studies on phthalates and DINCH have already provided their metadata to the HBM4EU repository and the data will continuously be included. Up to now, metadata of 49 different datasets, which measured phthalates from 20 different countries are included into IPCHEM. As these existing data collections are heterogeneous in terms of sampling time, biomarkes, matrices and study populations, WP10 developed an R-script in order to be able to obtain harmonised aggregated data of these different data sets. 27 data collections from 12 different countries shared harmonised aggregated data for phthalates and 1 data set for DINCH (German ESB) could be harmonised based on this script. Exposure distributions of the obtained merged harmonised aggregated data output files was visualised by using box plots based on different percentiles (P5, 10, 25, 50, 75, 90, 95). For more details, please see D10.6. Gaps in EU-representative data on exposure to phthalates and DINCH (e.g. missing regions and/ or exposure biomarkers) are being filled in by targeted analyses of biobanked samples and/or by studies of planned or ongoing HBM studies in the participating countries with 50% of HBM4EU funding. A sampling frame to obtain EU wide coverage with recent HBM exposure data was developed in WP8 (See D8.1). For phthalates and DINCH biobanked urinary samples will be analysed, already analysed data shared and new data will be collected in children aged 6-11 years and for teenagers aged 12-19 years for all geographical regions. For the Northern region Norway and Sweden will analyse biobanked samples from a national study, whereas Denmark will collect new samples in a regional study. In the Eastern region, Hungary and Slovakia will analyse biobanked samples and collect new samples in national studies, whereas Poland contribute with biobanked samples from a regional study. For the Southern region, Slovenia, Greece and Italy will collect new samples in national and regional (Italy) studies and Spain contribute with biobanked samples from a national study. In the Western region, France will contribute with the analysis of biobanked samples from a national study and Germany will share data from a national study. The Netherland will collect new samples from the HBM4EU Specimen study to be analysed for phthalates and DINCH metabolites in the alignment of studies and Belgium will contribute with new samples collected from a

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	regional study (see D8.4). Until now, datasets on phthalates and DINCH metabolites in teenagers have been received from Sweden, Spain, Belgium and Germany and for children from Denmark (see: https://www.hbm4eu.eu/online-library/). In WP10 different research protocols have been developed to investigate the difference in exposure to phthalates and DINCH between European countries; to identify high exposure groups as well as identify exposure determinants from existing data collection as well as from the new data collections done in Task 8.1 “Alignment of studies”. More information can be found in the updated Statistical Analysis Plan (D10.10) and on in the WP10 internal webpages of the HBM4EU website.
4. What are the main sources of exposure and the reasons for differences in exposure (different regulations in different countries) to phthalates and Hexamoll® DINCH®?	WP7 has developed a concept for a study protocol for recruitment and sampling to ensure harmonised recruitment, sampling and questionnaire implementation. This harmonised procedure aims at obtaining comparable results across countries involved in the HBM4EU targeted studies. A substance-specific questionnaire for phthalates/DINCH was developed to collect all the necessary information concerning individual characteristics of the participants (sociodemographic, dietary, occupational, lifestyle, environmental and health factors) with the aim to characterise as well as identify possible sources and routes of exposures to these substances (see D7.3 and D7.6). In WP 10.4 a protocol has been developed that will investigate exposure determinants on existing data sets on phthalate exposure for several age groups and different phthalates metabolites (see: https://www.hbm4eu.eu/online-library/). In addition, the 2nd occupational study will investigate by measuring urinary phthalates metabolites in workers of companies from 10 different countries, among others, what are the most relevant compounds in e-waste processing. Thereby, giving insights in important occupational exposure sources in the recycling sector.
Monitoring the success of existing policy actions and assessing the needs for further regulation	
6. Are there different time trends for unregulated (DEP, DMP, DCHP, DPHP) and regulated phthalates (DEHP, BBzP, DnBP, DiBP, DinP, DnOP) and Hexamoll® DINCH®?	In WP 8.2 will evaluate time trends for DINCH and phthalates in one or to age-groups and 4 European geographical areas by comparing three different time points (D8.4). For the first time point (2006-2010) already published exposure data will be used, for the second time point (2011-2013) new analysis of DEMOCOPHES samples will be conducted and for the third time point (2014-2020) data from the alignment of studies will be used. So far, no published information on DINCH exposure in children were found for the first time point, and only little information for adults. It will be explored whether biobanked samples can be accessed to gain more information on DINCH exposure for the first time point as several biobanks from all 4 geographical regions exist, even though not for each age group of interest. Information for several phthalate metabolites are available in the literature. For the second time point, DEMOCOPHES samples will be analysed. Up to now, already 9 partners have replied positive to perform a new analysis with 50% cofounding from 3 geographical regions. The analysis of DINCH in these samples is already agreed upon. For the detailed description of planned measurements for the third time point, please see above and D8.4.
7. How effective have the different mitigation steps and regulations been for phthalates?	A protocol for examination of the temporal trends of phthalate exposure has been elaborated (WP10, task 10.4). Germany and Denmark have available and suitable data collections to be used in the time trend analysis (cross-sectional studies with repeated measurement design). Since there is only very few European studies with such a design data on young adults from two countries will be used to compare time trends in Cat A and Cat B phthalates and DINCH between 2000 and today. Based on the analysis a first picture of the temporal trends of exposure to regulated and unregulated phthalates will be drawn. In addition, a protocol under

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	WP 8 has been developed in order to evaluate time trends for DINCH and phthalates (see results for PQ 6). On that account, it can be monitored how successful the existing policy actions have been and assessed where there are needs for further regulation.
Impact on human health	
8. Is the exposure to phthalates and their substitutes of health relevance for the general population and vulnerable groups? **What part of the population has exposure levels exceeding the HBM guidance values or TDI?** **&** **9. Is the health-relevance dependent on age or gender?**	In WP 5.3 an exercise was conducted for several phthalates for the general population if risk assessments could be improved by Human Biomonitoring data, and what the strengths and limitations are in using HBM in RA (D.5.5). For DEHP and the alternative plasticizer DINCH, RCRs were calculated based on metabolite concentrations in the DEMOCOPHES study using the HBM-GVs derived in task 5.2 and these were compared to RCR calculated in the restriction dossier from ECHA and the Danish EPA, 2016. As a result, RCR were in generally higher for children and lower for mothers when using the HBM-GVs. Finally, employing HBM data to monitor the implementation and effectiveness of the REACH restriction, and for studying time trends of the four restricted phthalates as well as the substitute phthalates are discussed. The work concerning the occupational population covered DiNP, DiDP and DPHP, because their use has not been extensively restricted in the occupational field and they are widely used in plastic product manufacturing. The calculated RCRs were well below one for DiNP and DiDP, based on a rough Biomonitoring Equivalents (BE) approach. The urinary concentration of the DPHP metabolite OH-MPHP is roughly 40x lower than the provisional EU HBM-GV of 0.9 mg/L, indicating a low occupational risk for this individual phthalate, based on conservative assumptions. Population exposure to phthalates and their substitutes is of outermost relevance for both the general population and especially vulnerable groups including pregnant women, children and adolescents. This is due to three main reasons: 1) Exposure is ubiquitous and virtually all the population is exposed to phthalate metabolites in a daily basis; 2) Recent systematic reviews are showing that current levels of exposure to specific phthalate families are associated with reproductive, neurodevelopmental and other health endpoints[1]; 3) The described exposure-health associations in the population are supported by toxicological knowledge. WP 14 conducted an extensive literature review in order to have a detailed overview of existing biomarkers of effect for phthalates (D14.2). This included both, long established “traditional” effect biomarkers and less studied “novel” biomarkers of effect. Several effect biomarkers of different health outcomes, such as cancer, effects on reproduction, neurobehavioral changes, endocrine disruption, allergy or effects on immune system, allergy and cardiovascular or metabolic endpoints has been inventoried. For phthalates a strategy for the selection of effect biomarkers for specific chemicals, health outcomes and window of exposure (i.e., biomarkers of reproductive effects associated with phthalate exposure in children/adolescents), as a proof of concept has been conducted jointly between WP

[1] Ribeiro CM, Beserra BTS, Silva NG, Lima CL, Rocha PRS, Coelho MS, Neves FAR, Amato AA. Exposure to endocrine-disrupting chemicals and anthropometric measures obesity: a systematic review and meta-analysis. BMJ Open. 2020;10(6):e033509. doi: 10.1136/bmjopen-2019-033509.
Golestanzadeh M , Riahi R , Kelishadi R. Association of phthalate exposure with precocious and delayed pubertal timing in girls and boys: a systematic review and meta-analysis. Environ Sci Process Impacts. 2020;22(4):873-894. doi: 10.1039/c9em00512a.
Radke EG, Braun JM, Nachman RM, Cooper GS. Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence. Environ Int. 2020;137:105408. doi: 10.1016/j.envint.2019.105408.
Golestanzadeh M, Riahi R, Kelishadi R. Association of exposure to phthalates with cardiometabolic risk factors in children and adolescents: a systematic review and meta-analysis. Environ Sci Pollut Res Int. 2019;26(35):35670-35686. doi: 10.1007/s11356-019-06589-7.
Radke EG, Glenn BS, Braun JM, Cooper GS. Phthalate exposure and female reproductive and developmental outcomes: a systematic review of the human epidemiological evidence. Environ Int. 2019;130:104580. doi: 10.1016/j.envint.2019.02.003.

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	14 and WP 13 and published (doi: https://doi.org/10.1016/j.envres.2019.05.013). Here, an overview of effect biomarkers for reproductive toxicity are presented that are substantiated with mechanistic information (e.g. AOPs). WP 13 did give a detailed overview of the available knowledge on AOPs for phthalates (D13.4) and subsequently summarised key knowledge gaps emerging from previous research. Specific activities, studies or other relevant steps to fill the missing information was proposed (D13.5). As a result, the following target receptors are proposed that may initiate events leading, among others to impaired male and female fertility: PPARα, PPARγ and GR. In a second step a thorough process in prioritising the best suited biomarkers of effect to be utilised in human epidemiological studies were conducted (D14.3). For phthalates several novel and traditional biomarkers of effect are proposed to be implemented in the HBM4EU aligned studies (WP8) for the following endpoints measured in children and adolescents: neurodevelopment, asthma and allergy, sexual maturation, testicular function and metabolism and BMI. This will serve as proof of principle to examine that the implementation of specific effect biomarkers will complement the interpretation of exposure biomarker measurements and thereby support the weight of evidence of exposure health-relationships. WP12 has developed and improved a methodology for exposure reconstruction to deliver external exposure estimates from available (existing) HBM data (see AD12.6). Aggregated HBM data from 13 different countries for different age groups including young children, young adults. seniors and (pregnant) mothers were used for the assessment. Daily intake estimates could be established for DEHP, DINP, BBzP, DnBP and DINCH. For DEHP, DINP and DnBP, in most of the studies included mean daily intakes were close or above 1 µg/kg bw/ d, whereas for DINCH estimates were lower and for BBzP markedly lower. These values are below EFSA's TDIs for the respective single compounds (DnBP = 10; BBzP = 500; DEHP = 50; DINP and DIDP = 150 µg/kg bw/d) (see AD12.5). Within WP10 research protocols have been developed to investigate what proportion of the population from children and adolescents (data obtained via Task 8.1 "Alingment of studies") as well as which population from exisiting data collection does exceed HBM-GVs and also whether the mean concentration values differ with age or gender. More information can be found in the updated Statistical Analysis Plan (D10.10) and on in the WP10 internal webpages of the HBM4EU website.
10. Can EU-wide accepted HBM guidance values be derived for single substances and for the additively acting phthalates?	WP5.1 elaborated a concept document on the strategy for the derivation of health-based guidance values for the general population (HBM-GVGenPop) and for occupationally exposed adults (HBM-GVWorkers), thereby referring to the statement of the German Human Biomonitoring Commission on the basic principles for the derivation of HBM values as well as to the statement of the National Public Agency for Food, Environment and Occupational Health and Safety the occupational aspect of HBM values derivation. The strategy was applied for DINCH and DEHP metabolites and consolidated HBM guidance values were derived for DINCH & DEHP. For DEHP HBM-GVsGenPop of the sum of the metabolites 5-oxo-MEHP and 5-OH-MEHP or alternatively the sum of 5cx-MEPP and 5-OH-MEHP has been derived for adults and children (see D5.2): Σ [5-oxo-MEHP and 5-OH-MEHP] in urine: Children (6 - 13 y): 340 µg/L Adults: 500 µg/L Σ [5cx -MEPP and 5-OH-MEHP] in urine: Children (6 - 13 y): 380 µg/L

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	Adults: 570 µg/L. For DINCH HBM-GVsGenPop of the sum of the metabolites OH-MINCH and cx-MINCH has been derived for adults and children (see D5.2): Σ [OH-MINCH and cx-MINCH] in urine: Children: 3 µg/L Adults: 4.5 µg/L. In addition, for the exposure in the workplace an HBM-GVWorkers of 0.62 mg/L has been derived for the metabolite 5cx-MEPP in urine at the end of the work shift. Currently, the derivation of HBM-GV for the following phthalates is being published in a peer-reviewed journal: DPHP, DnBP, DiBP and BBzP. In addition, the concept for a harmonised HBM-GV derivation developed under HBM4EU has been submitted in a peer-reviewed journal and is currently under review- HBM-GV are derived on the basis of toxicological studies. The values represent the concentration of a substance in human biological material below which there is no risk for adverse health effects and, consequently, no need for action. Hence, they are an important tool to easily assess whether the exposure of a population/subpopulation (e.g. reference values) is of health-relevance and whether policy actions are needed. Additionally, for each HBM-GV an level of confidence (LoC) is given, reflecting the underlying the uncertainties in the underlying database. Thereby, HBM-GVs can also help to identify research needs. These values will together with the result of WP10 feed also into addressing the research question 8.
11. How can cumulative risks of phthalates and other anti-androgenic substances be assessed for their health relevance? Are their additive effects relevant for regulation?	WP15 has developed case studies of mixture effects of pollutants within the HBM4EU project. The case studies should focus on exposures and on health endpoints of concern and include different approaches from both toxicology and epidemiology. Phthalates are included in a case study that will evaluate the potential for a human health risk due to the present exposure to complex mixtures of 'anti-androgenic' chemicals based on our current knowledge. The group of anti-androgenic chemicals is expected to be very diverse, including phthalates, phenolic substances, certain pesticides and pharmaceuticals such as analgesics. Firstly, the known and widespread 'antiandrogenic' chemicals and drugs which humans are exposed to will be identified and gathered through existing literature on 'antiandrogenic' mixtures. Afterwards, hazard data from available sources including in vitro assays (AR reporter gene assay and the H295R steroidogenesis assay), ex vivo assays and, if available, in vivo data will be collected. Subsequently, relevant exposure data from available sources (human exposure levels, Cmax values for drugs, µM internal exposure levels) will be compiled. The Hazard Index approach will be employed and hazard quotients will be calculated. The analysis will be refined in the light of data on the likelihood of co-exposures. The work will be linked to investigations of antiandrogenic effects in placenta extracts which is ongoing in WP14 (in AR reporter gene assay and H295R steroidogenesis assay). In addition, a joint work between WP5.2 and WP15.3 has been started in which a mixture risk assessment of five selected phthalates will be conducted.
Usage of HBM4EU results for policy making	
12. How can HBM4EU results feed into the regulatory decisions of ECHA and EFSA?	HBM guidance values for phthalates and DINCH (WP5) are a useful tool to determine if a concern to human health might exist for the exposure to phthalates and DINCH and therefore measures, e.g. policy actions need to be taken. In order to ensure a good

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	interaction between Task 5.2 and the EU Policy Board it is now foreseen to include the EU Policy Board into the consultation process for the different HBM-GVs to be derived to allow for input during the consultation period. The investigation of exposure sources by the statistical analysis group (WP10) can help identifying major exposure sources for each substance in the group. Hence, specific risk reduction measures can be implemented in policies to ensure safe exposure from major exposure sources, e.g. food and food contact materials or by restriction of the use of substances in articles, medicines or personal care products and cosmetics or the authorisation of substances. The improved use of HBM data in health risk assessment (HRA) and in health impact assessment (HIA) for phthalates has been explored. So far, HBM data was used for the following evaluations under the REACH regulation: • Application for authorisation on formulation of recycled soft PVC containing DEHP in compounds and dry-blends • Application for Authorisation for DnBP used as an absorption solvent in a closed system in the manufacture of maleic anhydride • Restriction of DEHP, BBzP, DiBP, DnBP in toys and childcare articles (See D5.1) In 2018 a case study on phthalates and bisphenols were conducted under WP 5.4 in which a structured and participatory process were developed to facilitate the use of HBM data and results by decision makers and also stakeholders. For phthalates specifically, it was mentioned that a major milestone achieved so far is the use of HBM data from the DEMOCOPHES project for health impact assessment, which severed the basis for the restriction proposal of 4 phthalates (DEHP, BBzP, DiNP and DnBP). This restriction would further restrict the use of these phthalates in consumer products in addition to the already restriction in place for childcare articles. It also made clear that substantiated data is needed to support policy making as former efforts to restrict or ban this substance group failed due to lack of data. Among the concerns were that the regulatory process still is very slow and only for some single substances progress has been made. In addition, the scientific methodology for assessing the risk of combined effect to this large group of chemicals is still under debate and too less individual data is made available to risk assessors in order to be able to make a detailed analysis to give a better insight in the actual cumulative exposure. It was also noted, that its regulatory policies are divided into domains which makes it rather difficult to prevent exposures if not all domains are implementing a restriction or ban. Representatives from the industry stated that EU companies are committed to innovation also because of stricter regulations. However, enforcement has been a bottleneck in the past. As a conclusion it can be said, that the follow-up of exposure trends of phthalates would help the EU agencies to evaluate whether the current regulations are effective enough or need to be adapted (e.g. are sources not adequately controlled by current regulation?). Also, it should be laid a focus on following up of how the substitution of the regulated phthalates develop in order to make prospective policy decisions. On that account, new methods for assessing the health risks to related substitutes but also for mixtures of phthalates and possibly other anti-androgenic substances should be developed. Furthermore, HBM4EU did participate in the open consultation processes of SCHEER on the “Preliminary guidelines on the benefit-risk assessment of the presence of phthalates in certain medical devices covering phthalates which are carcinogenic, mutagenic, toxic to reproduction (CMR) or have endocrine-disrupting (ED) properties” and of ECHA on the “Public consultation on behalf of the Commission: Update of Annex XIV entries of four phthalates” to feed in the expertise of the Consortium and ensure that results are directly fed into the regulatory processes.