Substance report

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Phthalates and Hexamoll® DINCH

HBM4EU science and policy for a healthy future

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Authors and Acknowledgements

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First version (December 2019) by RPA consultants, based on scoping documents produced by the chemical group leader (CGL) and colleagues.

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
CGL	Chemical Group Leader
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
HBGV	Health based guidance values
HBM-GV	Human Biomonitoring guidance value
HMW	High molecular weight
IARC	International Agency for Research on Cancer
LMW	Low molecular weight
OEL	Occupational Exposure Limits
PVC	Flexible polyvinyl chloride
RCR	Risk Characterisation Ratio
REACH	The ‘Registration, Evaluation, Authorisation and Restriction of Chemicals’ Regulation Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals
SVHC	Substances of Very High Concern
TDI	Tolerable Daily Intake
WP	Work package
WPL	Work package leader
Phthalate compounds and substitutes	
BBzP	Benzyl butyl phthalate

DCHP	Dicyclohexyl phthalate
DEHP	Bis(2-ethylhexyl) phthalate
DEP	Diethyl phthalate
DiBP	Diisobutyl phthalate
DiDP	di-iso-decyl phthalate
DINCH	Diisononyl cyclohexane-1,2-dicarboxylate
DiNP	Diisononyl phthalate
DiPeP	Di-isopentyl phthalate
DMEP	Di(methoxyethyl) phthalate
DMP	Dimethyl phthalate
DnBP	Di-n-butyl phthalate
DnHP	Di-n-hexyl phthalate
DnOP	Di-n-octyl phthalate
DnPeP	Di-n-pentyl phthalate
DPHP	Dipropylheptyl phthalate

1 Key messages

- HBM4EU has generated EU-wide HBM data on the internal human exposure on ten different phthalates and the substitute plasticiser DINCH from 12 countries in a harmonised way through the HBM4EU Aligned Studies
- The HBM4EU Aligned Studies (2014-2021) have established baseline levels of recent internal exposure to phthalates and DINCH for European children (6-11 years) and teenagers (12-18 years)
- The highest average exposures observed were for DEHP and DiBP in children and for DEP and DEHP in teenagers.
- For some of the strictly regulated reprotoxic phthalates (BBzP, DiBP, DEHP) and phthalate substitute DINCH, higher levels were found in the group of children as compared to teenagers. On the other hand, higher levels of DEP, DiDP and DiNP were measured in teenagers.
- Geographical differences in internal exposure were observed between European countries and regions.
- Results show that despite extensive regulatory measures for many phthalates, children and teenagers in Europe are still exposed to multiple phthalates and/or DINCH simultaneously.
- The ubiquitous exposure to health-impacting phthalates and their substitutes is of concern for the general population, especially for children and teenagers. In a single substance risk assessment, 4 % of the European children and 4-7% of the European teenagers exceeded the human biomonitoring guidance value (HBM-GV) for DnBP, and 4 % of the children and

2-3 % of the teenagers the HBM-GV for DiBP. The HBM-GV for DEHP was exceeded as well, although only in a smaller number of cases (at least 0.32 % of all children and teenagers investigated).

- A mixture risk assessment revealed a toxicologically undesirable high exposure of ~17 % of the European children and teenagers from cumulative health effects of five reprotoxic phthalates (DEHP, DiBP, DnBP, BBzP, DiNP). The main drivers of the mixture risk are DiBP and DnBP.
- The HBM4EU analyses show decreasing time trends for most regulated phthalates since the 2000s, illustrating the effectiveness of policy action. At the same time, an increasing trend for the substitutes DINCH and DEHTP was observed. Further monitoring of phthalates and their substitutes is needed to assess the further effectiveness of recent regulatory measures (ban of DEHP, DiBP, DnBP, BBzP in consumer articles since 2020) to ensure levels below HBM-GVs and to prevent regrettable substitution.
- Societal concern regarding phthalates and their reprotoxic properties underlines the importance of investigating their impacts on citizens and the environment.

2 Introduction

HBM4EU is a project co-funded under Horizon 2020 and runs from 2017 until 2022. It generates knowledge to inform about the exposure of European citizens, the revealed risks for health, and safe management of chemicals, and hence protect human health and wellbeing in Europe. HBM4EU uses human biomonitoring (HBM) to monitor the actual internal 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 higher exposed and vulnerable groups 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 Phthalates and Hexamoll® DINCH (DINCH) 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 phthalates exposure. This substance report is intended to inform policy makers and relevant stakeholders on the value of HBM to establish the EU population's exposure to phthalates and DINCH.

This substance report is based largely on the HBM4EU scoping document for Phthalates and Hexamoll®DINCH, first draft produced in 2017 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 Phthalates and Hexamoll® DINCH

Phthalates (also named phthalate esters or esters of phthalic acid), and their substitute DINCH, are a group of plasticizers with a production volume of millions of tons per year. They are widely used

in the manufacture of plastics, to make them soft and flexible, and in personal care products. They can be found in common products such as soaps, suntan lotion, soft plastic toys, plastic bottles, raincoats, shoes and food packaging.

Due to their endocrine disrupting properties, some phthalates have been assigned use restrictions since the late 1990s. There is societal concern due to their toxicity to reproduction and presence in biological matrices of humans.

Depending on their molecular structure, phthalates can be differentiated into low molecular weight orthophthalates (LMW) and high molecular weight (HMW) with different physico-chemical properties resulting in different applications.

The majority of HMW are used in flexible polyvinyl chloride (PVC) products such as flooring, wires and cables, sport equipment, toys, coated textiles, footwear, synthetic leather and others. DEHP is also used in PVC medical devices (Koch & Angerer, 2011). HMW phthalates include: DEHP, DiNP, DiDP and DPHP.

The LMW phthalates are more volatile and have plasticising and solvent-like properties. Therefore, they have various other applications in addition to PVC products such as gelling plasticizers**,** paints, dispersions, and adhesives, but also as solvents in insect repellents (DMP) and in cosmetics (DEP). They comprise of: DiBP, BBzP, DnBP, DEP and DMP.

DEP and DnBP are also used in enteric-coated tablets/capsules as enteric film-coating materials or matrix binder (Wittassek, 2011).

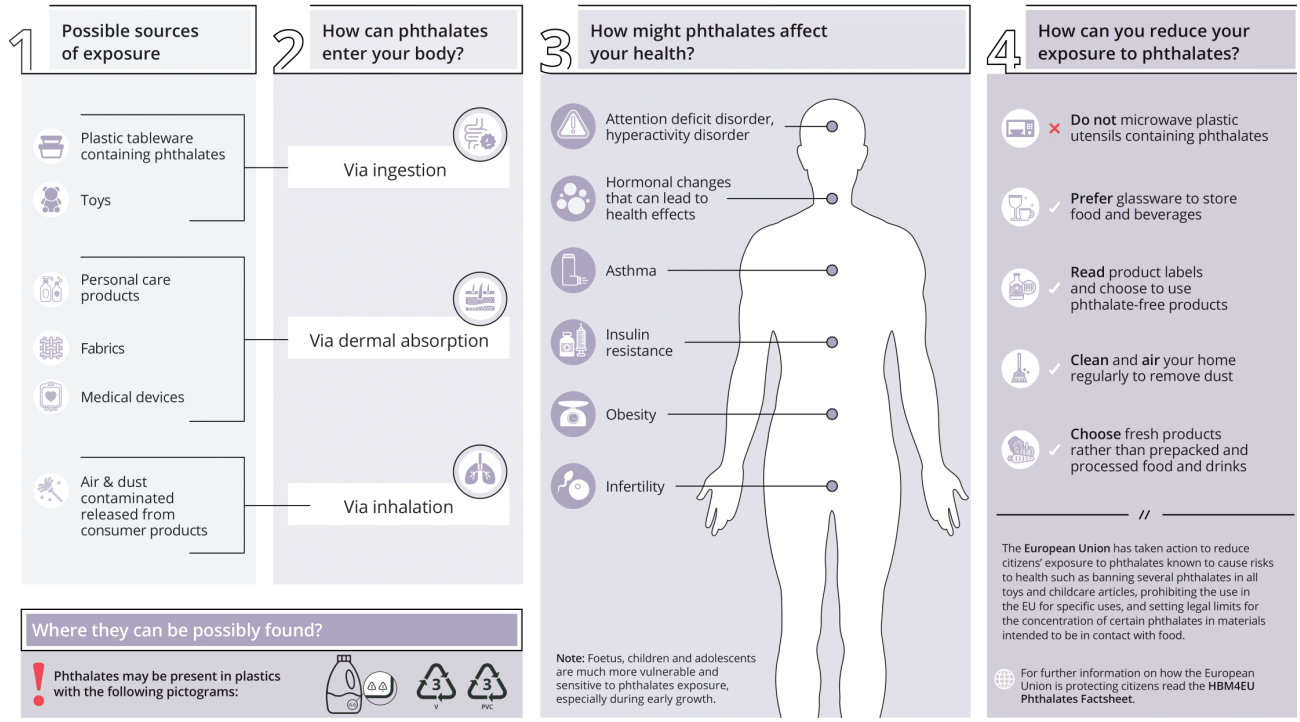
DINCH, due to its low toxicity and low migration rate, is used in soft PVC-containing medical devices such as blood bags, in food contact materials, such as artificial wine corks, in sports equipment and textile coatings, in wallpaper, paints and inks, adhesives and in cosmetics and toys. In the latter, DINCH is thought to be the most used plasticiser alternative.

3 Human exposure to Phthalates and Hexamoll® DINCH

Plasticizers can be taken up by ingestion, inhalation and dermal contact. Whereas for HMW phthalates the main source of exposure is via food originating from contamination, e.g. via food contact materials (Wittassek et al. 2011), especially for DEHP but also for DiNP, the exposure to LWM phthalates from food contribute to the overall exposure to a minor degree (Koch & Angerer, 2011). Inhalation of indoor air, exposure via ingestion of house dust by children and dermal contact with articles and dust can also be sources of exposure, especially for the short chain phthalates. Fromme et al., 2013 found significant correlations between phthalate concentrations in dust samples and urinary levels of DnBP, BzBP, but also for DEHP.

In addition, medical treatment can lead to high exposure towards certain phthalates. For example, an exposure source for DEHP can be medical devices, such as tubes in blood transfusion. Long-term treatment with enteric-coated tables/capsules can lead also to high exposure of DEP and DnBP.

Figure 3.1 Overview of exposure sources, exposure pathways and health effects for Phthalates and Hexamoll® DINCH



3.1 Environmental exposure (general population)

Since phthalates are not chemically bound to the (plastic) materials, they can leach, migrate or evaporate into indoor air and atmosphere, foodstuff or other materials and so are of ubiquitous presence in the environment (Heudorf et al. 2007).

Several human-biomonitoring studies in the EU, US and Asia were conducted, showing that the ubiquous use of phthalates lead to a continuous internal exposure of the general public. Phthalate metabolites are being detected in a high percentage of the study population, sometimes present in each urine sample investigated (Schwedler et al., 2020; Berman et al., 2013; Huang et al., 2016; Koch et al., 2017; Ye et al., 2008;).

Comparison of exposure estimates between studies from the DEMOCOPHES project revealed a clear age difference. Levels of metabolites were in general higher in children than in mothers, which is confirmed also by other studies (Lemke et al., 2021; ECHA, 2016; Hartmann et al., 2015; Frederiksen et al., 2013; Becker et al., 2009; Geens et al., 2014). The relative metabolite levels differed among countries, with Swedish children having higher urinary MBzP levels than the European average, Slovak children having two times higher concentrations of DEHP metabolite levels than the European average and Polish children showed highest levels of MnBP and MiBP. In Spain, average MEP levels were six times higher than the European average. However, exceedance of health-based guidance values, in particular HBM-I values and BE values, were only reported for few cases, i.e. for DEHP metabolites in mothers and children (Den Hond, 2015).

In the Restriction Report of ECHA (2016) risk characterisation ratios (RCRs) for the health of the general public were calculated based on DEMOCOPHES data revealing that in 13 out of 15 Member States of the EU RCRs for combined 95th percentile exposure for DEHP, DnBP, BBzP and DiBP are at or above 1 for children (ECHA, 2016). This stresses the fact, that cumulative risk assessment is crucial to accurately determine the hazards originating from phthalates exposure.

Since endocrine active phthalates can act in a dose additive manner and humans simultaneously are exposed to multiple phthalates, cumulative exposure to phthalates might exceed health-based guidance values and therefore pose a risk to the public health.

Nonetheless, phthalate use in the industry and in the consumer environment has changed dramatically during the past decade due to regulatory restrictions. Recently, Koch et al., investigated the time trend of phthalates exposure using urinary samples from the German Environment Specimen Bans (ESB) regularly taken in the time frame of 1988 until 2015. They showed that the exposure to old, well-known and restricted phthalates (DEHP, DnBP, BBzP) has decreased. When comparing DEMOCOPHES data to older studies, a significant decline in exposure was also seen for Germany and Denmark (ECHA, 2016). Exposure to the metabolites of the substitute DINCH has increased in Germany and in Sweden (Koch et al, 2017; Gyllenhammar et al., 2017).

The population may also be significantly exposed to substitute phthalates like DiNP and DPHP and one can assume that the European population will still be exposed to restricted phthalates in the future, e.g. due to long lifetimes of articles, recycled PVC and from remaining authorised uses or uses that are not restricted. Concerning occupational exposure there is only limited data on the exposure of workers to different phthalates in the plastic industry, but the same trends as for consumers are considered relevant.

3.2 Occupational exposure

In regard to occupational exposure there is only limited data on the exposure of workers to different phthalates in the plastic industry. Also, phthalate use in the industry has changed dramatically during the past decade due to regulatory restrictions, which means, that the exposure to old, well known phthalates (DEHP, DnBP etc.) in the industry has decreased. However, the workers may be significantly exposed to newer phthalates like DiNP and DPHP or substitutes like DINCH.

3.3 Consumer exposure

DnBP, DiBP, BBzP, DEHP, DMEP, DnPeP, DiPeP are generally not allowed to be placed on the EU-market, when used as individual substances or in mixtures for supply to the general public when concentration limits are equal to or exceed 0,3 %. In addition, DEHP, DnBP, BBzP, DEMP, DnPeP, DiPeP and DHNUP are prohibited for use in cosmetics in the European Union. Consumer articles from outside EU/EEA (e.g. Asia or USA) can contain phthalates since there is no such strict regulation for the use of phthalates in consumer articles in every country. China has in recent years enacted restrictions of certain phthalates in toys and food contact materials. Nevertheless, it has been shown that restricted phthalates are found in a high number of products imported into the EU and had mostly unknown origin or were imports from China (ECHA, 2018).

4 Health impacts of Phthalates and Hexamoll® DINCH

4.1 Overview of key health impacts from Phthalates and Hexamoll® DINCH

Phthalates can cause a variety of adverse effects in humans and in laboratory animals (Koch and Calafat 2009, Mariana et al. 2016) the most prominent of which are the endocrine disrupting and reproductive effects. It has been shown that some phthalates, such as DEHP, BBzP, DnBP and DiBP induce the so-called phthalate syndrome already at low doses, which covers different reproductive abnormalities in male offspring of rats exposed during pregnancy with the critical time window of gestation day 15-17.

The effects of phthalates are, among others, malformations of the testes, epididymides and Gubernaculum Testis, cryptorchism, hypospadias, reduced semen count and others caused by interference of the development of fetal Leydig cells, reduced or inhibited testicular testosterone production and reduced production of insulin-like 3 peptide hormone (HBM Commission, 2011). Not all phthalates exhibit the reprotoxic and developmental effects described above and not all have the same endocrine disrupting potency.

In addition, several epidemiological studies conjecture an association between phthalate exposure and overweight, insulin resistance, asthma, attention deficit disorder and attention deficit hyperactivity disorder (Hatch et al., 2010; Engel et al., 2010; Wang et al., 2015, Franken et la., 2017). In terms of risk assessment it is important to note that mixtures of the above mentioned phthalates have direct additive effects (Howdeshell et al. 2017), but also additive effects with other endocrine disrupting chemicals has been demonstrated, even though they function via a different mode of action (Gray et al., 2006; Rider et al., 2010).

Due to increased knowledge of the endocrine disrupting effects of the above mentioned phthalates, less harmful plasticisers became more important over the last decade including DPHP and DINCH. DPHP due to its molecular structure is thought to have no anti-androgenic effects, but only minimal data is available. DINCH was introduced into the market in 2002 as a substitute mainly for DEHP and DiNP.

4.2 Vulnerable target groups

Vulnerable groups include pregnant women, children and teenagers.

4.3 Societal concerns

Societal stakeholders have been expressing concerns for decades, with Greenpeace (Greenpeace, 2006) having conducted several studies addressing phthalates in consumer products and the potential health effects emerging from its endocrine disrupting effects in.

Many websites inform the public worldwide about consumer products free of phthalates. Furthermore, efforts have been made to reduce the phthalate uses in cosmetics and toys beyond the scope of European regulation as in the US, Japan and China. In addition, industry already substituted many of the endocrine disrupting phthalates with less potent or no endocrine disrupting substances, such as DINCH. All phthalates discussed here, except DPHP and the substitute DINCH are included in the SIN list.

In 2019 a HBM4EU workshop was held in Brussels to create a space for dialogue on available evidence and how it might serve today's policy agenda's (D5.4).

5 EU policies on Phthalates and Hexamoll® DINCH

DEHP, DnBP, DiBP, BBzP, DnPeP, DiPeP, DHNUP, DnHP and DMEP are classified as reproductive toxicants category 1B under Annex VI to the Classification Labelling and Packaging (CLP) regulation (EC 1272/2008). In addition to their reprotoxic properties, DEHP, DnBP, DiBP, and BBzP also have endocrine disrupting properties and have been classified as substances of very high concern (Annex XIV EC 1907/2006) and therefore included in the candidate list for the inclusion in Annex XIV of the REACH regulation (Annex XIV of REACH EC 1907/2006). DEHP, BBzP, DiBP and DnBP are already subject to authorisation (Directive (EU) 2015/863). Since February 2015 they must not be used within the European Union without authorisation. There are Commission decisions on some authorisations, others are currently under evaluation. In addition, DiNP, di-n-octyl phthalate (DnOP), DiDP are **restricted for all children's toys and childcare articles** that can be placed in children's mouth with a concentration limit of 0.1 % by entry 52 of Annex XVII to REACH.

However, imported goods do not come under the authorisation requirement. Since June 2017, three other phthalates are included in the Authorisation List: DiPeP, DMEP and DnPeP with a sunset date of July 2020.

Reprotoxic substances, such as DEHP, BBzP, DnBP, DiBP, DnPeP, DiPeP, DCHP, DHNUP, DnHP and DMEP are generally not allowed to be placed on the market, in the EU as individual substances or in mixtures for supply to the general public when concentration limits are equal or exceed 0,3 %. Furthermore, the use of DEHP, DnBP, DiBP and BBzP is restricted in plasticised materials of all toys and childcare articles with a concentration limit of 0.1 % by entry 51 of Annex XVII to REACH. In addition, DiNP, di-*n*-octyl phthalate (DnOP), DiDP are restricted for all children's toys and childcare articles that can be placed in children's mouth with a concentration limit of 0.1 % by entry 52 of Annex XVII to REACH.

The use of DEHP, DnBP, DiBP and BBzP are restricted under REACH Annex XVII in consumer products on the EU market since July 2020. Several phthalates (DEHP, BBzP, DiBP, DnBP, DiPeP, DHNUP, DMEP, DnPeP and DnHP) cannot be used in the EU without authorisation for specific uses.

DEHP, BBzP, DnBP, DiNP, DiDP and DINCH have specific migration limits (SMLs) in foodstuff.

Other product-specific legislation which regulate certain phthalates include:

- Cosmetic Products' Regulation (EC/1223/2009)
- Regulation on plastic materials and articles intended to come into contact with food (EC 935/2004 and Directives 80/590/ECC & 89/109/ECC)
- Regulation for Plastics Implementation Measure (10/2011/EC)
- EFSA has set a tolerable daily intake (TDI) for the concentration of certain phthalates (for DnBP, BBzP, DEHP, DiNP and DiDP) in food contact materials.

6 Policy questions for Phthalates and DINCH

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 Phthalates and DINCH. Further background details on Phthalates and DINCH 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 Which are the most sensitive, reliable and cost-effective methods and biomarkers to measure phthalates and DINCH?

HBM4EU has elaborated a prioritised list with most suitable biomarkers, matrices and analytical methods. 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. In addition, a feasibility study was conducted that identified new, valuable urinary exposure biomarkers for EU-labelled, reprotoxic phthalates currently not covered in HBM analytical methods. This work provides a harmonized method covering biomarkers for all EU-regulated phthalates.

The HBM4EU Quality Assurance/ Quality Control Programme was implemented for MEP, MBzP, MiBP, MnBP, MCHP, MnPeP, MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MnOP, OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP, OH-MINCH, cx-MINCH.

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: After 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 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.

6.3 What is the extent of the current exposure of the EU population to the 16 phthalates (Cat A, B and C) and their substitute DINCH?

HBM4EU Aligned Studies (2014-2021) have generated new baseline levels of internal phthalate and DINCH concentrations in urine samples of the European general population for children (6-11 years) and teenagers (12-18 years). Almost every child and teenager investigated is internally exposed to some phthalate and DINCH metabolites (e.g. for DEHP, DiBP, DnBP, BBzP), whereas other phthalates such as DnOP , DCHP, DnPeP were only rarely detected. Highest levels were observed for DEP and DnBP in both age groups.

P50 concentrations for the Sum (5-oxo + 5-OH-MEHP) are in the range of 11.14-39.07 µg/g crt across studies in children and 6.49-24.08 µg/g crt across studies in teenagers. P95 of urinary Sum

(5-oxo + 5-OH-MEHP) concentrations are in the range of 42.18-100.38 µg/g crt across studies in children and 21.31-105.28 µg/g crt across studies in teenagers.

P50 concentrations for the Sum (5-cx-MEPP + 5-OH-MEHP) are in the range of 14.45-47.98 µg/g crt across studies in children and 8.95-25.17 µg/g crt across studies in teenagers. P95 of urinary Sum (5-cx-MEPP + 5-OH-MEHP) concentrations are in the range of 49.36-152.49 µg/g crt across studies in children and 33.56-107.78 µg/g crt across studies in teenagers.

All teenagers had detectable levels of MEP in their urine samples. P50 of urinary MEP concentrations are in the range of 8.89-63.51 µg/g crt across studies in children and 13.05-64.42 µg/g crt across studies in teenagers. P95 of urinary MEP concentrations are in the range of 45.49-514.93 µg/g crt across studies in children and 99.02-484.41 µg/g crt across studies in teenagers.

P50 of urinary MBzP concentrations are in the range of 1.36-11.88 µg/g crt across studies in children and 0.86-6.91 µg/g crt across studies in teenagers. P95 of urinary MBzP concentrations are in the range of 9.20-60.89 µg/g crt across studies in children and 5.11-51.61 µg/g crt across studies in teenagers.P50 of urinary MiBP concentrations are in the range of 15.76-48.33 µg/g crt across studies in children and 14.33-27.93 µg/g crt across studies in teenagers. P95 of urinary MiBP concentrations are in the range of 51.44-261.03 µg/g crt across studies in children and 50.74-142.37 µg/g crt across studies in teenagers. P50 of urinary MnBP concentrations are in the range of 13-40.38 µg/g crt across studies in children and 11.14-53.14 µg/g crt across studies in teenagers. P95 of urinary MnBP concentrations are in the range of 45.96-250.40 µg/g crt across studies in children and 32.21-203.35 µg/g crt across studies in teenagers.

P50 of urinary MCHP concentrations are < detection limit (0.05-0.22 µg/L) in 6 studies or < quantification limit (0.1-0.2 µg/L) in 4 studies across studies in children and are < detection limit in (0.05-0.2 µg/L) in 6 studies or < quantification limit (0.2 µg/L) in 3 studies across studies in teenagers. P95 of urinary MCHP concentrations are in the range of 0.18-16 µg/g crt across studies in children (with 4 studies with a P95 value < detection limit (0.05-0.22 µg/L) and 2 studies with a P95 value < quantification limit of 0.2 µg/L) and 0.11-1.00 µg/g crt (with 5 studies with a P95 value < detection limit (0.05-0.2 µg/L) and 3 studies with a P95 value < quantification limit of 0.2 µg/L). P50 of urinary MnPeP concentrations are < detection limit (0.04-0.25 µg/L) in 6 studies or < quantification limit (0.1-0.2 µg/L) in 4 studies across studies in children and are < detection limit in (0.05-0.2 µg/L) in 4 studies or < quantification limit (0.2 µg/L) in 3 studies across studies in teenagers. P95 of urinary MnPeP concentrations are in the range of 0.21-15 µg/g crt across studies in children (with 4 studies with a P95 value < detection limit (0.07-0.25 µg/L) and 3 studies with a P95 value < quantification limit of 0.1-0.2 µg/L) and 0.50-1.41 µg/g crt (with 2 studies with a P95 value < detection limit (0.07 µg/L) and 3 studies with a P95 value < quantification limit of 0.2 µg/L).

P50 of urinary MEHP concentrations are in the range of 0.73-3.51 µg/g crt across studies in children and 0.81-2.15 µg/g crt across studies in teenagers. P95 of urinary MEHP concentrations are in the range of 3.40-14.44 µg/g crt across studies in children and 2.91-11.81 µg/g crt across studies in teenagers.P50 of urinary 5OH-MEHP concentrations are in the range of 6.57-20.30 µg/g crt across studies in children and 3.93-20.18 µg/g crt across studies in teenagers. P95 of urinary 5OH-MEHP concentrations are in the range of 24.48-62.38 µg/g crt across studies in children and 13.02-88.27 µg/g crt across studies in teenagers. P50 of urinary 5oxo-MEHP concentrations are in the range of 4.60-18.08 µg/g crt across studies in children and 2.49-7.53 µg/g crt across studies in teenagers. P95 of urinary 5oxo-MEHP concentrations are in the range of 16.99-53.87 µg/g crt across studies in children and 8.47-29.61 µg/g crt across studies in teenagers.

All children had detectable levels of 5cx-MEPP in their urine samples. P50 of urinary 5cx-MEPP concentrations are in the range of 8.22-28.16 µg/g crt across studies in children and 4.02-13.21 µg/g crt across studies in teenagers. P95 of urinary 5cx-MEPP concentrations are in the range of

26.61-90.43 µg/g crt across studies in children and 15.28-45.68 µg/g crt across studies in teenagers.

P50 of urinary MnOP concentrations are < detection limit (0.03-0.5 µg/L) in 6 studies or < quantification limit (0.1-0.2 µg/L) in 4 studies across studies in children and are < detection limit in (0.05-0.5 µg/L) in 6 studies or < quantification limit (0.2 µg/L) in 3 studies across studies in teenagers. P95 of urinary MnOP concentrations are in the range of 0.56-0.60 µg/g crt across studies in children (with 5 studies with a P95 value < detection limit (0.033-0.5 µg/L) and 4 studies with a P95 value < quantification limit of 0.1-0.2 µg/L) and 0.27-0.42 µg/g crt (with 5 studies with a P95 value < detection limit (0.07-0.5 µg/L) and 3 studies with a P95 value < quantification limit of 0.2 µg/L).

P50 of urinary OH-MiNP concentrations are in the range of 1.95-7.63 µg/g crt across studies in children and 2.35-9.44 µg/g crt across studies in teenagers. P95 of urinary OH-MiNP concentrations are in the range of 7.16-50.05 µg/g crt across studies in children and 6.59-60.95 µg/g crt across studies in teenagers.

P50 of urinary cx-MiNP concentrations are in the range of 0.34-11.59 µg/g crt across studies in children and 1.21-8.20 µg/g crt across studies in teenagers. P95 of urinary cx-MiNP concentrations are in the range of 0.68-73.51 µg/g crt across studies in children and 5.28-66.36 µg/g crt across studies in teenagers.

P50 of urinary OH-MiDP concentrations are in the range of 0.67-3.20 µg/g crt across studies in children and 0.34-1.54 µg/g crt (with 1 study with a P50 < detection limit of 0.20 µg/L) across studies in teenagers. P95 of urinary OH-MiDP concentrations are in the range of 2.65-11.28 µg/g crt across studies in children and 0.59-10.16 µg/g crt across studies in teenagers.P50 of urinary cx-MiDP concentrations are in the range of 0.50-1.33 µg/g crt (with 1 study with P50 < detection limit of 0.37 µg/L) across studies in children and 0.25-0.82 µg/g crt across studies in teenagers. P95 of urinary cx-MiDP concentrations are in the range of 1.60-7.09 µg/g crt across studies in children and 0.93-4.28 µg/g crt across studies in teenagers.

P50 of urinary OH-MINCH concentrations are in the range of 1.20-4.04 µg/g crt across studies in children and 0.55-2.1 µg/g crt across studies in teenagers. P95 of urinary OH-MINCH concentrations are in the range of 7.39-32.78 µg/g crt across studies in children and 3.93-25.86 µg/g crt across studies in teenagers.

6.4 P50 of urinary cx-MINCH concentrations are in the range of 0.51-2.81 µg/g crt across studies in children and 0.44-1.19 µg/g crt across studies in teenagers. P95 of urinary cx-MINCH concentrations are in the range of 1.53-21.26 µg/g crt across studies in children and 2.12-8.37 µg/g crt across studies in teenagers.Do the exposure levels differ significantly between the countries?

HBM4EU Aligned Studies revealed geographical differences in average internal exposure to phthalates and DINCH up to a factor of 9 between the different studies. Children from France, Italy and Slovenia, on average, have the highest levels (geometric means) in phthalates and DINCH compared to the European average, and Denmark, Hungary, and Belgium have the lowest

concentrations. Teenagers, from France, Slovakia, and Norway are exposed the highest (geometric mean) and Belgium, Poland, and Sweden are the least exposed to phthalates and DINCH.

HBM4EU also found differences between European regions in most phthalates and DINCH. For example, in children, there were no differences in DnBP and secondary metabolites of DEHP. But Eastern Europe had lower levels in DiNP, BBzP, and DiDP compared to at least one other European region. For DINCH, Eastern and Western Europe had lower levels than Southern and Northern Europe. Regional differences for children indicate, however, a complex result pattern where pairwise comparisons between European regions depend on the phthalate or substitute studied.

6.5 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?

Higher levels of phthalates BBzP, DiBP, DEHP, and DINCH are found in children compared to teenagers who, in turn, have higher overall levels in DEP, DiDP and DiNP. In the analyses of existing HBM studies since 2005 including all regions, HBM4EU finds an indication for age differences (age groups between 3 to 60+) where, for example DnBP and DiDP levels children's levels are higher than for teenagers and these, in turn, are higher than adult's levels.

Occupational exposure to phthalates was studied among E-waste workers in HBM4EU E-waste study. Results will be available soon.

6.6 What are the main sources of exposure and the reasons for differences in exposure (different regulations in different countries) to phthalates and DINCH?

Results from the analyses of the newly generated data from the HBM4EU Aligned Studies identified the daily use of body care and beauty products being important exposure determinants. However, depending on the physicochemical properties of the phthalates, other exposure pathways might get of importance, such as indoor dust through ingestion or inhalation of phthalates in gaseous and particles phase.

In general, main common exposure determinants for children and teenagers are fast food, plastic food packaging, PVC floor, drinks in plastic bottle, cosmetics and hygiene products (fragrances, eyes make-up, body lotion) urbanization and education.

Important exposure determinants for children are physical activities (DEHP) (via dermal contact with sports equipment and increased inhalation of indoor dust) and handheld electronical device usage > 4 hours per weekdays; also frequent plastic containers usage for food heating in microwaves (DINCH).

Important exposure determinants for teenagers are the frequent use of local food (DINCH) and waste incineration nearby home; also a home construction year in the time between 2001 and 2006 (DEHP).

The main source of exposure for phthalates and DINCH according to bottom-up calculations using the INTEGRA computational platform, where exposure data that account for multi-pathway and multi-route exposure have been used, as well as from regression analyses of the aligned studies, is diet through food contact materials.

Existing data (adults) also show similar findings regarding daily use of beauty products and possible phthalates intake via diet because of food contact materials (packaging etc.). There is a limitation regarding existing data because of lack of information on all exposure determinants.

Reasons for differences in exposures between countries with regards to national regulations and product content/quality control/monitoring (with focus on products from non-EU counties, e.g., e-market/shops) cannot be answered yet on the results obtained in HBM4EU. In general, regulatory measures to limit exposure to phthalates via food contact materials and cosmetics may be insufficient.

6.7 Are there different time trends for less regulated (DEP, DMP, DCHP, DPHP), regulated phthalates (DEHP, BBzP, DnBP, DiBP, DiNP, DnOP) and the phthalates substitute DINCH?

HBM4EU data analyses from Danish and German time trend studies in young adults show decreasing 24-hour excretion since the 2000s in the more regulated phthalates DEHP, BBzP, DnBP, and DiBP (in the range of 9-17 % yearly decrease, depending on the phthalate), and stability in DiNP and DiDP/DPHP. Time trends since 2006 for less regulated phthalates (DEP, DMP) show decrease by about 17 % per year and phthalate, and substitutes DINCH and DEHTP show strong increases as determined in 24-hour excretion. These data seem to illustrate the effectiveness of policy action or lack/gaps thereof in case of phthalate substitutes.

Data from existing HBM studies since 2005 from various European regions, available via the European HBM Dashboard, analysed in HBM4EU indicate decreases in the more regulated phthalates DEHP, DnBP, DiBP, DiDP, BBzP, but also the less regulated phthalates DEP and DMP.

HBM4EU also evaluated time patterns for phthalates and DINCH in children and four European geographical areas by comparing HBM data from different studies from three different time points. When data for children from the DEMOCOPHES survey collected in the years 2010-2012 were compared to data from the HBM4EU Aligned Studies collected between 2014-2021, in general a lower exposure of DiBP in children was observed at the later time point, whereas DINCH exposure was, as expected, higher at the later time point. The HBM4EU Aligned Studies data will form baseline European exposure levels for phthalates and DINCH in teenagers, allowing follow up studies to monitor increased or decreased usage.

As a result of the data presented, further monitoring of phthalates and its substitute DINCH (and DEHTP) is needed i) to assess the longer term success of recent regulatory measures and its impact to yield decreases in the exposure particularly towards DiBP and DnBP , and ii) follow up on increasing exposure levels of substitutes that are not (yet) a priority in single substance risk assessment to prevent regrettable substitution.

6.8 How effective have the different mitigation steps and regulations been for phthalates?

See answer for Policy Question 6.7.

6.9 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?

Single substance risk assessment

Comparison of the newly generated HBM4EU Aligned Studies data with HBM-GVs showed that currently, exposure to DnBP and DiBP is a health concern in some countries, since the percentage of children and teenagers exceeding these values is highest for these highly regulated phthalates (up to 4% of European children and 2 - 7% of European teenagers, depending on the substance). The exposure to BBzP, DEP and DINCH is well below the corresponding health-based guidance values for both children and teenagers. One child exceeds the HBM-GV for BBzP. Some participants exceed the HBM-GV for DEHP. An impact on health cannot be excluded for these children and teenagers.

For the phthalates studied (DEHP, DiNP, BBzP, DnBP and the replacement DINCH) daily intake from exposure reconstruction based on data from the HBM4EU Aligned Studies was on average in the range of 0.1 to 1 µg/kg bw/d in most studies, far below the existing regulatory thresholds.

Occupational exposure to DiNP, DiDP and DPHP was estimated to result in internal exposure levels that are not of health concern.

Mixture risk assessment

A mixture risk assessment of 5 selected phthalates (DEHP, DnBP, DiBP, BBzP and DiNP) is being carried out in HBM4EU Aligned Studies, since animal mixture toxicity studies revealed that some of the anti-androgenic phthalates can act in an additive manner. Results show that approximately 17% of the European children and teenagers are at risk from possible adverse effects of combined exposure of the 5 anti-androgenic phthalates. Furthermore, the mixture risk assessment revealed that for most European children and teenagers combined risks are driven by multiple phthalates. Main drivers of the mixture risk are DnBP and DiBP. The mixture risk for the majority of children and teenagers would have gone unnoticed in single substance risk assessment. Furthermore, case studies are being carried out to evaluate a proof-of-concept for the identification of mixture health effects, one case study being a mixture risk assessment for male reproductive health with a focus on semen quality, that includes certain phthalates. Final results will be available soon.

Associations with health effects

Combined toxicological data, effect biomarkers assessed in epidemiological studies and biological plausibility captured in several Adverse Outcome Pathways indicate associations between phthalate exposures and reproductive disorders in males.

Additional evidence (also including limited number of human cohort studies) points to development of certain estrogen-dependent cancers, and possible metabolic-, neurodevelopmental- and

immune-related health outcomes. In general, targeted human studies assessing exposures and health outcomes are limited and there is a clear gap and need for the future to confirm causality.

Statistical analyses on HBM4EU Aligned Studies data are currently performed to look into the pathways linking exposure to phthalates/DINCH to health effects (neurodevelopment, asthma and allergy, sexual maturation, testicular function and metabolism and BMI) via established and novel effect biomarkers.

6.10 Is the health-relevance dependent on age or gender?

See answer for Policy Question 6.9.

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 7.1 HBM4EU categorisation for Phthalates and Hexamoll® DINCH

Category		Priority substance(s)
A	HBM data are sufficient to provide an overall picture of exposure levels across Europe, and interpretation of biomonitoring results in terms of health risks is possible.	DEHP, BBzP, DnBP, DiBP, DEP and DiNP
B	HBM data exist but not sufficiently to have a clear picture across Europe.	DiDP, DnOP, DMP, DnPeP, DCHP, DPHP, Hexamoll®DINCH
C	HBM data scarcely or doesn't exists. Efforts to develop an analytical method to obtain relevant HBM results need to be done	DiPeP, DHNUP, DMEP and DnHP

7.2 Key outputs

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

Methods and biomarkers

- 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 has been included in the HBM4EU ICI/EQUAS 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 QA/QC programme included: MEP, MBzP, MiBP,

MnBP, MCHP, MnPeP, MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MnOP, OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP, OH-MINCH, cx-MINCH.

- 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 be implemented in a new phthalate multi-method and tested 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).

Exposure levels

In WP7.1 a gap analysis has been carried out to get an overview how many studies of 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 (Newborns, Children, Teenagers, 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.

In WP10, up to now, metadata of 55 different existing datasets, which measured phthalates are included into IPCHEM. Covering information from 17 different EU countries and Israel. Data owners/providers of 33 different studies have already provided their data on phthalates and DINCH in harmonized format to the European HBM dashboard created by HBM4EU which provides summary statistics for HBM data from different European countries.

WP10 developed an R-script in order to be able to obtain aggregated data in a standardised and comparable way of these different data sets. 32 data collections from 14 different countries shared harmonised aggregated data for phthalates and 12 data sets for DINCH 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) in the European HBM dashboard.

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 the HBM4EU Aligned Studies. 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 have been analysed, already analysed data were shared and new data for 15 phthalate markers MEP, MBzP, MiBP, MnBP, MCHP, MnPeP, MEHP, 5OH-MEHP, 5oxo-MEHP, 5cx-MEPP, MnOP, OH-MiNP, cx-MiNP, OH-MiDP, cx-MiDP) and 2 markers for phthalate substitute DINCH (OH-MINCH, cx-MINCH) have been collected in the HBM4EU Aligned Studies for children aged 6-11 years and for teenagers aged 12-18 years for all geographical regions.

For children samples were collected between 2014-2021 across 12 sampling sites in Europe (Norway, Denmark, Greece, Slovenia, Italy, Slovakia, Poland, Czech Republic, France, Belgium, The Netherlands and Germany) representing 2880 individuals. The HBM4EU Aligned Studies data in teenagers were collected between 2014-2021 across 11 sampling sites in Europe (Norway, Sweden, Greece, Slovenia, Spain, Slovakia, Poland, Czech Republic, France, Belgium and

Germany) representing 2799 individuals. Not all biomarkers were analysed in all contributing studies, therefore number of sampling sites and data points can vary per biomarker.

In order to answer questions on exposure levels and sources and further support current and future HBM studies, WP7 has produced a variety of materials to provide the groundwork for a harmonised approach to study planning and conduct in Europe. These materials are:

- data platform with information on existing, ongoing and planned general and occupational HBM studies in the HBM4EU consortium
- manuals/guidelines for study planning and conduct
- Standard Operating Procedures for qualified recruitment of participants, fieldwork, sampling and exchange of samples
- different kinds of questionnaires for various age groups and substance (groups)
- influence of thawing and freezing procedures on the integrity of biobanked samples in the literature and supplied a respective concept for a quality study
- templates for the communication with participants

When identifying exposure levels and sources as well as groups at risk, WP7 questionnaires are of great value. They can be used to set up new studies and allow the harmonized collection of data on a participant's individual characteristics and their potential exposure pathways from different sources (sociodemographic characteristics, residential environment/home exposures, dietary habits, lifestyle, occupational exposure and health status) with Phthalates and DINCH. For Phthalates and DINCH, questionnaires for adults, teenagers and children are available. Questionnaires for the 2nd occupational study on e-waste (incl. some plasticisers as biomarkers) have also been developed.

WP9 designed and implemented a QA/QC program to ensure the comparability of the analytical results obtained in the HBM4EU Aligned Studies. Furthermore, the Quality Assurance Unit (QAU) of WP9 revised the data of some aligned studies that analyzed their samples before the HBM4EU QA/QC programme and so, data generated by laboratories that were not approved for the analysis of phthalates and/or DINCH metabolites. After a critical revision, the QAU elaborated some recommendations on how to use the analytical data of these laboratories in HBM4EU.

WP10 has developed a general and a substance-specific statistical analysis plan for phthalates and DINCH for existing and new HBM data collections. Variables which were 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. More information can be found in the Statistical Analysis Plans (D10.10 and D10.12).

HBM based indicators for phthalates/DINCH with indicator graphs on time patterns, geographical differences and health impact are being developed under WP5 (statistical analysis in progress).

Exposure sources

Comparable HBM results have been obtained under the harmonized premises and QA/QC of WP7, WP8 and WP9 to support the robustness of the conclusions derived from them.

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 to investigate exposure determinants on existing data sets on phthalate exposure for adults for different phthalates metabolites. Also for the HBM4EU Aligned Studies in children and teenagers exposure determinants were investigated as described in the statistical analysis plan D10.12.

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.

Time trends and regulations

Recent and harmonized HBM data throughout Europe are indispensable to not only assess the populations risk from chemical exposure but to evaluate the suitability and success of current legislations and political measures in place.

WP 8.2 and WP 10.4 studied existing data sets on phthalate exposure and evaluated time patterns for DINCH and phthalates in children and 4 European geographical areas by comparing three different time points (D8.4 & D10.12). For the first time point (2006-2010) already published exposure data were used, for the second time point (2011-2013) new analysis of DEMOCOPHES samples were conducted and for the third time point (2014-2020) data from the HBM4EU Aligned Studies are used.

No published information on DINCH exposure in children was found for the first time point. Information for several phthalate metabolites is available in the literature. For the second time point, DEMOCOPHES samples were analysed from 4 geographical regions. (North: SE, DK East: PL, CZ, South: ES, CY, West: LX, DE, BE). For the second timepoint DINCH data of 8 out of 9 countries were made available to T8.2 and WP10 for time trend analysis. For the detailed description of planned measurements for the third time point, please see above and D8.4.

In addition, a protocol in WP 10 has been developed to investigate time trends in studies with at least 3 repeated measurement points with individual data from Danish and German young adults (cross-sectional studies with repeated measurement design).

Guidance values and vulnerable groups

HBM-GVs were derived for five phthalates (DEHP, DnBP, DiBP, BBzP and DPHP) and for the non-phthalate substitute DINCH (Lange et al., 2021). For the adult general population (including teenagers from 14 years onwards), the HBM-GVs for the specific metabolite(s) of the respective parent compounds in urine are the following: 0.5 mg/L for the sum of 5-oxo-MEHP and 5-OH-MEHP; 0.19 mg/L for MnBP, 0.23 mg/L for MiBP; 3 mg/L for MBzP; 0.5 mg/L for the sum of oxo-MPHP and OH-MPHP and 4.5 mg/L for the sum of OH-MINCH and cx-MINCH. HBM-GVs for children (which are lower than for adults) and for workers were also specified.

Within WP10 research protocols have been developed to investigate what proportion of the population from children and teenagers in the HBM4EU Aligned Studies as well as which population from existing 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).

In addition, WP 5.3 evaluated the strengths and limitations of using HBM data in risk assessment (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.

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 teenagers. 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); 3) The described exposure-health associations in the population are supported by toxicological knowledge.

WP14 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. A strategy for the selection of effect biomarkers substantiated with mechanistic information (e.g. AOPs)., health outcomes and window of exposure (i.e. biomarkers of reproductive effects associated with phthalate exposure in children/teenagers) has been conducted jointly between WP 14 and WP 13 as a proof of concept and published. WP13 made 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, a number of health outcomes and associated effect biomarkers were proposed for further evaluation in HBM4EU. 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 were proposed to be implemented in the HBM4EU Aligned Studies (WP8) for the following endpoints measured in children and teenagers: neurodevelopment, asthma and allergy, sexual maturation, testicular function and metabolism and BMI. Measurements of nuclear receptors, BDNF, Kisspeptin, oxidative stress, classical effect biomarkers, neurodevelopment, pubertal development/sexual maturation, and adverse metabolic effects in the HBM4EU aligned studies 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. These exposure-effect associations are also described in the statistical analysis plan of the aligned studies (D10.12). A Directed Acyclic Graph was adopted for each exposure-effect association as visual aid to check for relevant confounders and for completeness of the statistical model. Statistical analyses are now being initiated by selected partners.

WP12 has developed and improved a methodology for exposure reconstruction to deliver external exposure estimates from available (existing) HBM data. 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 modelled daily intakes were close or above 1 µg/kg bw/ d, whereas for DINCH estimates were lower and for BBzP markedly lower.

7.3 Key data gaps

HBM4EU is a five-and-a-half-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:

- For the phthalate and DINCH substance group, most HBM studies reported were on children and these studies were mostly conducted in Western Europe. In most instances, but not all, sampling is not representative of the entire national populations. However, in HBM4EU important gaps in EU-representative data on exposure to phthalates and DINCH (e.g. missing regions and/ or exposure biomarkers) have been filled 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).
- The majority of the available HBM data concerns data for well-known phthalates (such as DEHP), while substitute phthalates and alternatives (such as DINCH) have been studied less. For the newer phthalates and alternatives, it is important to monitor the trends.
- With regard to the study of health impacts linked to phthalate and DINCH exposure, targeted human studies assessing exposures and health outcomes are limited and there is a clear gap and need for the future to confirm causality.
- Mixture risk assessment is challenging for real-time mixtures of phthalates. The trend of phthalate use and exposure need to be addressed. The trends of phthalate use is one of decreasing use, however there is still high frequency of the detection of phthalates in human samples.
- Analytical measurement of HBM sampling is a hurdle that needs to be overcome (deliverable 9.2) in relation to separating isomers and separation of short-chain phthalates. The difficulty faced when measuring DINCH metabolites is that different peaks are produced during analysis; thus, particular expertise is required to identify the peaks that are relevant. Method development is also a challenge that needs to be overcome.
- The current biomarkers for longer chain phthalates (DiNP and DiDP) also have some issues in relation to defining the appropriate suite of urinary marker chemicals to best define exposures.
- Further consideration is also appropriate on possible re-exposure to phthalates though the circular economy. This could occur where products that contain phthalates are subject to recycling. Waste processing does not remove these phthalates leading to re-exposure when the recycled product(s) are used.

8 Future recommendations

Exposure of European children and teenagers is still of toxicological concern. A continuous Human Biomonitoring of both regulated and unregulated phthalates is needed to further assess if the existing regulation is apt to protect the population against health impacts or if additional measures are needed to reach this goal.

By repeated EU-wide HBM studies the progress made by the implementation of Green Deal, Zero Pollution Strategy and the Chemical Strategy for Sustainability should be assessed against the baseline defined by the HBM4EU Aligned Study results.

Human Biomonitoring of substances used as substitutes for the strictly regulated reprotoxic phthalates needs to be extended as a considerable number of substances is used in high tonnages for this purpose. These substances are less well toxicologically investigated than the substituted phthalates and enter the human body by the same pathways. Therefore, research has to be intensified to avoid regrettable substitution.

Mixture risk assessments is essential because of the additive effects of phthalates and include the mixture effects from simultaneous exposure to other antiandrogenic substances. Methodology for real life mixtures effects needs to be further developed, tested and included this into the regulatory process. For this purpose conventions for the integration of the mixture effects in regulatory risk assessments need to be established, for example a Mixture Assessment Factor.

Further investigations and statistical analyses to elucidate exposure determinants are required. These can include ambient monitoring and targeted studies such as food duplicate studies.

The group of phthalates which have or might have a health impact of concern is large. AOP work could help understanding the principles of effects which could justify regulation of a larger group of compounds on the basis of extended mechanistic understanding.

The public concern needs to be taken seriously. Therefore, additional target specific information materials, interactive formats, social media messages and the dialog with citizens needs to be continued.

All activities need to be located at the European level to safeguard that those regions with an exposure and risk above the average become as well protected as the rest of Europe and preferably lower.

9 References

Becker, K., T. Goen, M. Seiwert, A. Conrad, H. Pick-Fuss, J. Muller, M. Wittassek, C. Schulz and M. Kolossa-Gehring (2009). “GerES IV: phthalate metabolites and bisphenol A in urine of German children.” Int J Hyg Environ Health 212(6): 685-692 http://www.ncbi.nlm.nih.gov/pubmed/19729343.

Berman, T., R. Goldsmith, T. Goen, J. Spungen, L. Novack, H. Levine, Y. Amitai, T. Shohat and I. Grotto (2013). “Urinary concentrations of environmental contaminants and phytoestrogens in adults in Israel.” Environ Int 59: 478-484 https://www.ncbi.nlm.nih.gov/pubmed/23962452.

Den Hond, E., E. Govarts, H. Willems, R. Smolders, L. Casteleyn, M. Kolossa-Gehring, G. Schwedler, M. Seiwert, U. Fiddicke, A. Castano, M. Esteban, J. Angerer, H. M. Koch, B. K. Schindler, O. Sepai, K. Exley, L. Bloemen, M. Horvat, L. E. Knudsen, A. Joas, R. Joas, P. Biot, D. Aerts, G. Koppen, A. Katsonouri, A. Hadjipanayis, A. Krskova, M. Maly, T. A. Morck, P. Rudnai, S. Kozepesy, M. Mulcahy, R. Mannion, A. C. Gutleb, M. E. Fischer, D. Ligocka, M. Jakubowski, M. F. Reis, S. Namorado, A. E. Gurzau, I. R. Lupsa, K. Halzlova, M. Jajcaj, D. Mazej, J. S. Tratnik, A. Lopez, E. Lopez, M. Berglund, K. Larsson, A. Lehmann, P. Crettaz and G. Schoeters (2015). “First steps toward harmonized human biomonitoring in Europe: demonstration project to perform human biomonitoring on a European scale.” Environ Health Perspect 123(3): 255-263 https://www.ncbi.nlm.nih.gov/pubmed/25493439.

European Chemicals Agency and Danish Environmental Protection Agency (2016) ”Annex XV Restriction Report – Proposal for a restriction on four phthalates (DEHP, BBP, DBP, DIBP)”

Engel, S.M., Miodovnik, A., Canfield, R.L. et al. (2010). “Prenatal Phthalate Exposure is Associated with Childhood Behavior and Executive Functioning”. Environ Health Perspect 118: 565-571.

Franken, C., N. Lambrechts, E. Govarts, G. Koppen, E. Den Hond, D. Ooms, S. Voorspoels, L. Bruckers, I. Loots, V. Nelen, I. Sioen, T.S. Nawrot, W. Baeyens, N. Van Larebeke, G. Schoeters. (2017). “Phthalate-induced oxidative stress and association with asthma-related airway inflammation in adolescents”. Int J Hyg Environ Health 220:468-77.

Frederiksen, H., J. K. Nielsen, T. A. Morck, P. W. Hansen, J. F. Jensen, O. Nielsen, A. M. Andersson and L. E. Knudsen (2013). “Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother-child pairs.” Int J Hyg Environ Health 216(6): 772-783 http://www.ncbi.nlm.nih.gov/pubmed/23528233.

Fromme, H., A. Schütze, T. Lahrz, M. Kraft, L. Fembacher, S. Siewering, R. Burkardt, S. Dietrich, H. M. Koch and W. Völkel (2016). “Non-phthalate plasticizers in German daycare centers and human biomonitoring of DINCH metabolites in children attending the centers (LUPE 3).” International Journal of Hygiene and Environmental Health 219(1): 33-39 http://www.sciencedirect.com/science/article/pii/S1438463915001030.

Geens, T., L. Bruckers, A. Covaci, G. Schoeters, T. Fierens, I. Sioen, G. Vanermen, W. Baeyens, B. Morrens, I. Loots, V. Nelen, B. N. de Bellevaux, N. V. Larebeke and E. D. Hond (2014). “Determinants of bisphenol A and phthalate metabolites in urine of Flemish adolescents.” Environ Res 134: 110-117 https://www.ncbi.nlm.nih.gov/pubmed/25127521.

Gray, L.E., Wilson, V.S., Stoker, T. et al. (2006). “Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals.” Int J Androl 29:96-104.

Greenpeace Report: Michelle Allsopp, D.S., Ulrike Kallee & Martin Hojsík (2006)

Gyllenhammar, I., et al., “Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs?”. Environ Res, 2017. 153: p. 48-54.

Hatch, E.E., Nelson, J.W., Stahlhut, R.W., Webster, T.F. (2010). “Association of endorcrine disruptors and obesity: perspectives from epidemiological studies”, Int J Androl 32: 324-269.

Hartmann, C., M. Uhl, S. Weiss, H. M. Koch, S. Scharf and J. König (2015). “Human biomonitoring of phthalate exposure in Austrian children and adults and cumulative risk assessment.” International Journal of Hygiene and Environmental Health 218(5): 489-499 http://www.sciencedirect.com/science/article/pii/S1438463915000504.

HBM (Human Biomonitoring) Commission, 2011. “Stoffmonographie für Phthalate – Neue und aktualisierte Referenzwerte für Monoester und oxidierte Metabolite im Urin von Kindern und Erwachsenen”. Gesundheitsschutz 54:770-785.

Heudorf, U., V. Mersch-Sundermann and J. Angerer (2007). “Phthalates: toxicology and exposure.” Int J Hyg Environ Health 210(5): 623-634 http://www.ncbi.nlm.nih.gov/pubmed/17889607.

Howdeshell, K. L., A. K. Hotchkiss and L. E. Gray, Jr. (2017). “Cumulative effects of antiandrogenic chemical mixtures and their relevance to human health risk assessment.” Int J Hyg Environ Health 220(2 Pt A): 179-188 https://www.ncbi.nlm.nih.gov/pubmed/27923611.

Huang, P.C., et al., “Early Phthalates Exposure in Pregnant Women Is Associated with Alteration of Thyroid Hormones”. PLoS One, 2016. 11(7): p. e0159398

Koch, H. M. and J. Angerer (2012). Phthalates: Biomarkers and Human Biomonitoring. Biomarkers and Human Biomonitoring Knudsen and D. F. Merlo, RSC Publishing. 1: 179-233.

Koch, H. M. and A. M. Calafat (2009). “Human body burdens of chemicals used in plastic manufacture.” Philos Trans R Soc Lond B Biol Sci 364(1526): 2063-2078 http://www.ncbi.nlm.nih.gov/pubmed/19528056

Koch, H. M., M. Rüther, A. Schütze, A. Conrad, C. Pälmke, P. Apel, T. Brüning and M. Kolossa-Gehring (2016). “Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012.” Int J Hyg Environ Health https://www.ncbi.nlm.nih.gov/pubmed/27863804.

Lange, R., Apel, P., Rousselle, C., Charles, S., Sissoko, F., Kolossa-Gehring, M., & Ougier, E. (2021). The European Human Biomonitoring Initiative (HBM4EU): Human biomonitoring guidance values for selected phthalates and a substitute plasticizer. *International Journal of Hygiene and Environmental Health*, *234*, 113722.

Lemke N, Murawski A, Lange R, Weber T, Apel P, Dębiak M, Koch HM, Kolossa-Gehring M. Substitutes mimic the exposure behaviour of REACH regulated phthalates - A review of the German HBM system on the example of plasticizers. Int J Hyg Environ Health. 2021 Jul;236:113780. doi:

10.1016/j.ijheh.2021.113780. Epub 2021 Jun 11. Erratum in: Int J Hyg Environ Health. 2022 Jan 6;:113920. PMID: 34126298.
Mariana, M., J. Feiteiro, I. Verde and E. Cairrao (2016). “The effects of phthalates in the cardiovascular and reproductive systems: A review.” Environ Int 94: 758-776 https://www.ncbi.nlm.nih.gov/pubmed/27424259.
Rider, C.V., Furr, J.R., Wilson, V.S., Gray, L.E., Jr (2010). “Cumulative effects of in utero administration of mixtures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity.” Int J Androl 33:443-462.

Schwedler G, Rucic E, Lange R, Conrad A, Koch HM, Pälmke C, Brüning T, Schulz C, Schmied-Tobies MIH, Daniels A, Kolossa-Gehring M. Phthalate metabolites in urine of children and adolescents in Germany. Human biomonitoring results of the German Environmental Survey GerES V, 2014-2017. Int J Hyg Environ Health. 2020 Apr;225:113444. doi: 10.1016/j.ijheh.2019.113444. Epub 2020 Feb 12. PMID: 32058939.
Wang, I.J., W.J. Karmaus, S.L. Chen, J.W. Holloway, S. Ewart. (2015). “Effects of phthalate exposure on asthma may be mediated through alterations in DANN methylation”. Clin Epigenetics 7:27.
Wittassek, M., H. M. Koch, J. Angerer and T. Bruning (2011). “Assessing exposure to phthalates – the human biomonitoring approach.” Mol Nutr Food Res 55(1): 7-31 http://www.ncbi.nlm.nih.gov/pubmed/20564479.
Ye, X., et al., “Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: the Generation R study”. Environ Res, 2008. 108(2): p. 260-7.