CHEMICAL EXPOSURE PROFILE IN HUMAN BLOOD, ARE WE AT RISK?

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Introduction

Humans are exposed to a variety of chemicals [1], of which only a few the toxicity is known. When a company manufactures, imports to or distributes a chemical over a certain volume within the EU market, the company is omitted to assesses the toxicity of the chemical and report it to the European Chemicals Agency (ECHA). Each chemical entering the market undergoes toxicity testing one by one to find a point of departure (POD) with a no observed effect concentration (NOEC) and/or a lowest observed effect concentration (LOEC). For each chemical, and for each endpoint, there might be many PODs in literature due to different experimental setups. Usually, when making a risk assessment, the lowest POD with no effect is used [2]. Today, researchers are trying to find a structure to risk assess the exposure to a mixture of chemicals, because ignoring the mixture effect could lead to underestimation of the risk [3-7]. When making a mixture risk assessment (MRA), a specific endpoint is selected and for that endpoint, extensive toxicity data is gathered for each chemical in the mixture. Thereafter, a ratio between the exposure and toxic concentrations is derived for each chemical and the ratios are combined into a mixture index. Examples of indices used in MRA are Hazard Index (HI) and Toxic Unit (TU). Specific endpoint herein refers to the biological endpoint studied during the toxicity test, preferably a molecular initiating event.

The preferred toxicity data to use for the risk assessment when having internal human blood concentrations, is human biomonitoring guidance values (HBM-GV). HBM-GVs give the internal dose in human tissues, such as blood or urine, below which no adverse effect can be seen [8]. HMB-GV can be derived using three different strategies, described here in the order of preference [9]. The first strategy is to associate health effects in epidemiology studies to internal exposures within the population [3]. The second strategy is to extrapolate the external animal dose to an internal dose using either a simple toxicokinetic (TK) model or a physiology-based TK (PBTK) model. When using animal studies for a risk assessment, the exposure is based on the administered dose, i.e. all the PODs are given as the external dose. The third and last option when deriving a HBM-GV is to use PODs from *in vivo* studies and apply assessment factors (AFs) to account for different uncertainties, such as inter- and intraspecies variations [2]. Additionally, it is important to assess the quality of the study *e.g.* by using assessment tools such as the Science in Risk Assessment and Policy (SciRAP) or the Toxicological data Reliability assessment Tool (ToxRTool) and to compare the study designs, *e.g.* regarding exposure timeframe (single, multiple exposure) and timepoint (life-stage) [9-11].

The German Human Biomonitoring Commission has derived several HBM-GV for blood and urine. These are categorized as human biomonitoring values I and II (HBM-I and HBM-II). The HBM-I value is defined as the concentration in blood or urine below which no observed adverse effect in humans can be seen, and thus no actions are needed to be taken [8]. HBM-II values are set at concentrations where a risk of health effect to a population cannot be excluded, and when concentrations are above or around this value, actions to decrease the exposure are needed [8]. HBM-II values are divided into two categories: general public and women in child-bearing age. The aim of this study was to investigate how comprehensive a human chemical MRA could be, performed on easily accessible existing exposure and toxicity data.

Materials and method

Literature searches were made gathering anthropogenic organic contaminants (OCs) analyzed in human blood using EBSCO Discovery Service and Google Scholar with the following search terms: human, (blood OR serum), (substance OR contaminant OR molecule OR chemical OR pollutant), published between 2000-2019. The literature search was conducted between 2019-2020, resulting in two databases, one focusing on OCs identified in human blood world-wide (Human Blood Database (HBDB)), and one specifically focusing on the exposure in Sweden (Swedish Exposure Database (SEDB)), where (Sweden OR Swedish) were added into the search terms. For the HBDB, the chemical's identity, year of sampling and country of blood origin were collected. For the SEDB, the concentration range (min-max), average concentration and cohort names (and details) were also extracted from the articles. Pharmaceuticals and metals were not included in the selection process and neither articles in other languages than English or Swedish. Chemical identifiers (such as CAS, SMILEs) were taken from SciFinder, ChemSpider and CompTox Chemical Dashboard. All concentrations in SEDB were converted to the same concentration unit, pg/mL in order to compare the data. The OCs were sorted based on their structural properties (aromatic, halogenated, phenolic or other). Only articles analyzing blood from the general public were included, i.e. studies analyzing chronically ill patients and studies on habitants living in the vicinity of a specific chemical leak were excluded.

In order to risk assess the chemical exposure using the blood concentrations, HBM-GV were collected. In total, nine chemicals with guidance values for blood concentrations were found (Table 1). From the German HBM Commission 2020,

five HBM-I values and four HBM-II values were found for the same OCs [8]. Apart from those, four separate studies deriving guidance values for human blood concentrations were found [12-15]. Even though the guidance values have different endpoints, a first tier MRA can give some information when using HBM-GV with a margin of safety (HBM-II) [16].

The *in vivo* toxicity data available was taken from USEPA's CompTox Chemicals Dashboard, using CAS and/or systematic names. NOEC, LOEC and benchmark dose (lowest confidence level) (BMDL) were collected for each compound when available. Relevant species for the project were selected, *i.e.* zebrafish (*Danio rerio*), brown trout (*Salmo trutta*), salmon (*Salmo salar*), rodents (all available), primates and humans. For each compound, the LOEC or NOEC value was chosen for each endpoint. However, the specific endpoints were not always specified. Additional searches concluded that some databases that Dashboard uses are more detailed than others. For this reason, only chemicals that had data published in two detailed databases, EFSA's OpenFoodTox and US EPA's ECOTOX were used. In these two databases, the chemicals in the SEDB were searched for the endpoints endocrine, developmental and reproductive and the species human, rat and mouse.

The hazard index (HI) is often used in MRA to assess the total chemical risk of a mixture. HI is the sum of hazard quotients (HQs) to estimate the risk. The HQs are the ratio when dividing the exposure concentration with the maximum acceptable concentration [17]. The maximum acceptable concentration can be a maximum daily intake (external dose), an *in vitro* concentration combined with AF or a HBM-GV. When the HI>1 there is a risk of adverse effects. Mixture effects can be identified and categorized in three ways, firstly when having individual chemicals above the safe levels (HQ>1), secondly when a few chemicals are the bad actors driving the risk, and thirdly when there are many chemicals contributing to the risk (all HQs<1 in similar levels) [16].

Results and discussion

The HBDB consists of 508 OCs (531 OCs when including isomers) analyzed in human blood world-wide. Typically, environmental pollutants are halogenated and aromatic, giving them the properties of being persistent and prone to bioaccumulation. The functional group of phenols (-OH) makes them more prone to be toxic. The database includes 436 halogenated and aromatic OCs, whereof 53 are also phenolic (Figure 1). Only 28 OCs are non-halogenic phenols. The SEDB consists of 154 OCs (171 OCs when including isomers) analyzed in Swedish blood. The database includes 85 halogenated, aromatic OCs, whereof 16 are phenolic (Figure 1). Only one compound, bisphenol A, is a non-halogenic phenol.

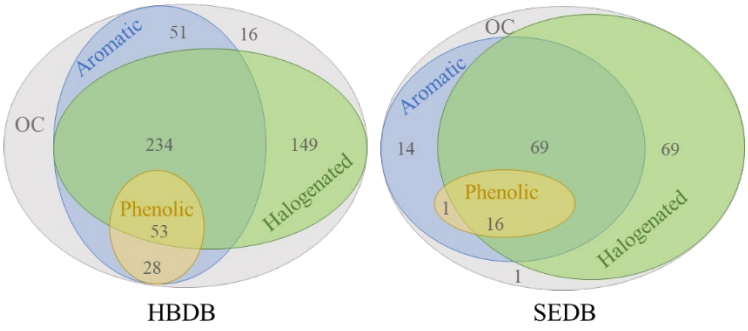

Figure 1. Chemical properties of the chemicals analyzed in human blood in Sweden (left) and worldwide (right).

In Table 1, the available HBM-GV are listed together with Swedish average blood and the HQs for the individual OCs. The concentrations of PFOS is still above the HBM-I GVs and PFOA was above the HBM-I GV until recently. PFOS have been above the HBM-II GV (20 000 pg/mL and 10 000 pg/mL for the general public and women in child-bearing age, respectively) previously and for some specific subpopulations, indicating that there has been a risk of adverse effects [8]. The concentration ranges cannot be combined into HI since the same population does not necessarily have the highest concentration of more than one chemical. However, it can be seen that there are certain subpopulations with concentrations reaching above the HBM-GV. When using the HI to assess the risk of chemical mixtures with HBM-GV, using HBM-I values when available, each HI is 2.8 during the last ten years. By only taking available data for the last five years (2015-2020), the HI is 1.9 with the PFOA and PFOS as the drivers of the risk. Looking at the time period 2010-2020, and excluding the chemicals with HQ>1, the HI for the remaining 6 OCs, is still 1.4 indicating that the included chemicals are contributing to mixture risk even though their HQs are all below 1.

Table 1. Guidance values (HBM-I/HBM-II) and average (min-max) blood concentrations from SEDB. First tier mixture risk assessment based on the mathematical model Hazard Index, using HBM-I GV. The HI is based on the average concentration as exposure with the min-max HQs in parenthesis.

Compound	Guidance value [pg/mL]	Average conc. (min-max) 2010-2020 [pg/mL]	Hazard quota (HQ) 2015-2020
BDE 99	3540 [13]	6.8 (0.31-170)	0.0019 (0.000087-0.048)
DDE+DDT	23 900 [12]	180 (20-6500)	0.0077 (0.00084-0.27)
ΣPCDD/F and DL-PCBs (TEQ)	0.211 [15]	0.00039 (0.00015-0.0048)	0.0019 (0.00071-0.023)*
HCB	170 [14]	49 (12-7400)	0.29 (0.069-44)
HBCDD	1600 [8]	1.4 (0.67-3.3)	0.00089 (0.00042-0.0021)
ΣPCBs (CB138,153,180)	3500/7000 [8]	120 (13-950)	0.033 (0.0036-0.27)
PFOA	2000/5000-10 000 [8]	1500 (290-8000)	0.77 (0.14-4.0)
PFOS	5000/10 000-20 000 [8]	4100 (510-17000)	0.82 (0.10-3.3)
HI = ΣHQs			**1.9**

*only CB118 and CB156

Out of the 154 OCs in SEDB, 56 chemicals (36%) had some toxicity data in CompTox Chemicals Dashboard. From the two more detailed databases, ECOTOX and EFSA 2020, 20 OCs had toxicity data. ECOTOX generated toxicity data for 18 OCs, and within the three selected endpoints there were 54 specific endpoints within 33 response tissues/sites. From the EFSA 2020 database, with the same species and endpoints, 6 OCs had toxicity data. Within this data, there were 5 specific endpoints, different exposure routes and 3 different types of PODs (NOAEL, LOAEL and BMDL10). AF could account for the different use of species and study design but since the toxicity data still had many different specific endpoints, it was not possible to make an MRA based on the available data. Adding to this, the concentrations for the toxicity values are usually the externally administered dose (oral or subcutaneous) and in order to compare these values with the internal blood concentrations in human, PK or PBTK modelling is needed.

The OCs that we do know the blood concentrations for in Sweden is not representing the whole chemical exposure profile since we are exposed to many more chemicals, and additionally, here we have only included blood levels. The number of OCs analyzed in Swedish blood are only a third of what have been analyzed world-wide. The preferred endpoints to use are the specific endpoints such as the molecular initiating events but depending on what tests have been done for a compound, the available toxicity data can vary a lot. A problem that arose during this study was how to categorize the available endpoints. One could include all endocrine toxicity studies, or for example only select thyroid hormone disruption or focus on one specific molecular initiating event within thyroid disruption, such as the TTR-binding. Strictly selecting one specific endpoint only yields a few chemicals.

An MRA of only these chemicals is not representable for the total complex mixture. There is today not a clear strategy to make a more holistic mixture risk assessment [5]. The question is then how can we estimate the risk of the chemical mixture we are exposed to when the data is not comparable? The AF derived by ECHA is one approach, however when the data is diverse, the AF can drive the assessment rather than the concentrations themselves. The external-internal dose extrapolation is too complex to only use an AF. Modelling using TK and PBK models is the correct way to solve the problem, but are only available for a few chemicals. There are new, less precise high-throughput PBK models that could be useful for this purpose. Generic PBK models are on the way from EFSA, JRC and US-EPA, hopefully making it easier to assess the risk for exposure to mixtures in the future [18]. Classifying risk assessments based on certainty and quality could be another approach, where the lowest level of certainty could include generic worst-case scenario AF for external-internal extrapolation.

When comparing the average concentrations in human blood in Sweden with the HBM-GV, all ages and sexes have been combined. Certain sub-populations and risk groups might therefore still be at risk. Adding to this, a recent study concluded that the underestimation of the risk when using single chemical exposure could be a factor ranging from 1 to 100 depending on the chemical [3]. Looking at the HI for the different time periods, the index is decreasing. Considering the numerous chemicals that we are exposed to, we here highlight the importance of a general guidance for mixture risk assessment today and the lack of derived HBM-GV for human blood.

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