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Human biomonitoring reference values: Differences and similarities between approaches for identifying unusually high exposure of pollutants in humans

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ABSTRACT

In exposure and risk assessment, the indication of unusually high exposure levels in humans to chemicals has been considered as an important objective for decades. To realize this objective, reference values (RV) need to be derived. However, while there is a tendency towards using the 95th percentile as a basis for deriving these reference values there is still no consensus. Moreover, side approaches have evolved including deriving RVs based on other percentiles, reporting multiple RVs or only reporting percentiles. The purpose of this article is to give an overview of the current literature, to point out differences and similarities between existing approaches, and to highlight important criteria for the derivation of RVs. We observe the majority of studies to base RVs on the 95th percentile and its 95% confidence interval which can been justified by statistical paradigms, present arguments for a single defined reference value, and discuss characteristics which call for more consistency. To conclude, our overview provides a first step towards a more homogenous and standardized derivation procedure to identify unusually high exposures in exposure science.

1. Introduction

Exposure of the general population to ubiquitous health-relevant pollutants via the environment, food or consumer products is one of the most important concerns in public health. To be able to identify comparatively high levels of exposure of the population, reference values need to be statistically derived from empirical studies. From a human biomonitoring (HBM) and public health research perspective, a reference value (RV) at the upper end of the exposure distribution is desirable to detect individuals who are highly exposed to a substance of interest and might need increased attention in risk assessment (Angerer et al., 2011; Saravanabhavan et al., 2017).

As pointed out by Bevan et al. (2013), individuals and sub-groups with exposure beyond RVs suggest that further investigation is needed to elucidate routes and determinants of these exposures as key reasons for these enhanced levels as compared to the general population. In addition, detecting highly exposed sub-populations is of particular interest for substances which are carcinogenic, have no known effect threshold, or cannot be assessed by means of the health-based HBM guidance values (values not available or cannot be derived) (Angerer et al., 2011). RVs can also be used to track changes in exposures to substances in a population over time.

Two aspects are important in understanding the role of RVs: First, it is not in the scope of reference values to provide criteria for identifying health risks or health-related decisions. The German HBM Commission has pointed out that reference values are strictly statistically derived values (Bundesgesundheitsbl., 2009). Thus, reference values have a different function than health-based HBM guidance values (HBM-I/-II, BE (Biomonitoring Equivalent), or TDI (Tolerable Daily Intake)/ADI (Acceptable Daily Intake); Angerer et al., 2011) and provide complementary information, especially when the latter are not available. Second, RVs are not suitable to describe the distribution of a substance in a sample because they only focus on the upper end of the distribution and point to individuals in the general population who are exposed to unusually high levels. When the intention is to describe the exposure distribution in a sample other statistical parameters such as mean, standard deviation and a set of several percentiles should be used (cf. quartiles, median and outlier in boxplots).

The aim of this article is to outline and compare current approaches to derive RVs and to indicate important criteria for the derivation of RVs.

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2. Derivation of a reference value

The underlying statistical measure of reference values, the percentile, separates sample members who are less exposed (or less and equal, depending on the definition) than a specific exposure from those with higher exposures which are assumed to be of particular interest in risk management. Considering that the derived percentile underlies statistical errors, the confidence interval (CI) for this percentile should be computed. In addition, the variability around the percentile estimate should be considered, for example by evaluating a coefficient of variation of the data used for RV derivation, as described by Saravanabhavan et al. (2017).

With respect to their exposure distribution, a variety of pollutants in human and environmental samples have been observed to be approximately log-normally distributed which can be explained by a successive random dilution process (Ott, 1990).

The definition of the reference value of the German HBM Commission – RV95 – is the 95th percentile of the substance of interest at a specific time point rounded off within its 95% CI (Bundesgesundheitsbl., 2009) and agrees with the International Union of Pure and Applied Chemistry (IUPAC) guideline (Poulson et al., 1997). The choice of the 95th percentile and 95% CI can be motivated by the convention in hypothesis testing where the 5% most extreme sample values indicate unusual values (Cowles and Davis, 1982). We would like to point out that describing reference populations has been defined differently in the literature. For example, reference ranges denote the values within the 95% of the sample distribution which cut-off 2.5% of the lower and upper distribution and indicate the “general” sample values (Reed et al., 1971). However, since the desired exposure to a pollutant is zero, a 2.5% cut-off at the lower end might be an adequate threshold for substances where low concentrations are undesired (e.g., physiological measures).

For further information on comparatively high exposure in subpopulations, RVs can also be easily derived for sub-groups (e.g., Ewers et al., 1999). Depending on the chemical substance and well-known sources/routes, the German HBM Commission removes specifically exposed sub-groups (e.g., smokers) to be separated from the majority of background exposed individuals before calculating RVs or derives additional RVs for these sub-groups. Other exclusion criteria might be based on substance characteristics. For example the German HBM Commission – in line with the WHO – recommends the exclusion of urine creatinine values smaller than 0.3 g/L or larger than 3.0 g/L (Bundesgesundheitsbl., 2005). In Canada, using data from the nationally representative Canadian Health Measures Survey (CHMS), a standard procedure has been developed to check the necessity for deriving RVs for sub-groups (e.g., sex, age group, and smoking status) as opposed to one general RV for the whole reference population. This is tested by calculating the RV for a sub-group, computing the proportion of these highly exposed individuals in the general sample, and comparing it to beforehand defined limits (for details see Khoury et al., 2018; Saravanabhavan et al., 2017). In addition, before calculating RV95s, the Canadian approach removed extreme values with Tukey's approach and excluded substance-specific concentration confounders (e.g., smoking, fish consumption in last 24 h, fasting, and amalgam fillings for metals and trace elements) based on expert knowledge and regression tests (Saravanabhavan et al., 2017). Since reference values cut-off the portion of values at the higher end, this is important to know when interpreting RVs.

3. Approaches to deriving RVs

The German HBM Commission has developed RV95s for a number of environmental chemicals including metals, persistent organic pollutants and emerging contaminants in the German population (Apel et al., 2017; Schulz et al., 2011). Quite a number of countries also used the RV95: The Czech Republic and Spain used the 95th percentile levels and its 95% CI for reference values on metals and trace elements, respectively (Batariova et al., 2006; Pena-Fernandez et al., 2014). Similarly, the UK derived RVs from the 95th percentile for a broad range of substances including metals and phthalates (Bevan et al., 2013). Publications with HBM substances in the CHMS used the 95th percentile as the reference value for example to investigate metals, trace elements, persistent organic pollutants (POPs), organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), perfluoroalkyl substances (PFAS), and non-persistent chemicals (Haines et al., 2017; Khoury et al., 2018; Saravanabhavan et al., 2017). Studies in Brazil and the Democratic Republic of Congo also reported reference values on metal exposure in humans based on the 95th percentile's upper limit of the 95% CI (Freire et al., 2015; Kuno et al., 2013) and with the 95% CI (Tuakuila et al., 2015), respectively.

There are also studies which reported several percentiles emphasizing the 95th percentile or comparing it to the RV95 of the German HBM Commission (e.g., South Korea for heavy metals; Lee et al., 2012). Another example is an Italian study on internal doses of metals which reported various percentiles and their CIs (Alimonti et al., 2011), but the researchers pointed out that it is the 95th percentile which helps to conclude whether contaminant levels are unusually high.

Yet another body of studies based their RV on different percentiles: For example, studies in Belgium did not use RV95 but several percentiles as reference values and the RV of the 97.5th percentile and its 90% CI or the 90th percentile with or without its 95% CI (Den Hond et al., 2013, 2015; Hoet et al., 2013). As an exception among National Health and Nutrition Examination Survey (NHANES) reports, a study with NHANES data explicitly used the term reference value in a publication on lead concentration by the Centers for Disease Control and Prevention (CDC) which uses the 97.5th percentile and recommended it for future publications on child blood lead levels (CDC, 2012). A different approach was taken in a Chinese study on PFOS and PFOA where the 90th percentile was used as RV (Liu et al., 2012).

Some of the studies cited in the previous paragraphs do not define the reference value neither with the percentile nor with the upper margin of the percentile's CI but a value in between the percentile and its lower or upper confidence interval limit.

Overall, European studies show a preference for using the 95th percentile for defining reference values with some variance in specifics on how defining reference values (i.e., reporting the percentile per se and which, its CIs, values in between, or upper CI). Outside of Europe, there also seems to be a slight tendency towards using the 95th percentile as a basis in HBM studies as well. However, the definition and operationalization of reference values varies even more, depending on the national study.

4. Discussion and identified criteria for the use of reference values

New projects offer a unique opportunity to derive HBM reference values for various countries and for a broad variety of chemical substances in a harmonized way. That is exactly the goal of the European HBM4EU initiative which harmonizes HBM data from up to 28 countries (Ganzleben et al., 2017). In such far reaching projects, a standard and uniform definition of RVs is particularly important.

We have identified the following criteria as especially important when deriving reference values and when further harmonizing the derivation approach: First, as a general aspect, only a single RV for each reference population or sub-population should be defined. In order to facilitate regulatory decision-making, it is necessary to have one value that specifies from which level onwards an exposure to a substance is “unusually high”. Additionally, the value should suggest which subgroups of individuals might need intervention or at least increased attention in risk management. From a regulatory perspective of the research community on HBM, using one defined RV (e.g., RV95) enables a straightforward interpretation and comparability of RVs across studies, countries, substances, time, samples and sub-groups, etc. However,

while defining a universal RV would enable comparisons with future studies, already published studies could sometimes not be related to reference values published in the future.

Second, it could be helpful to report further typical percentiles in addition to the 95th. Beneficial, however, is to use these to describe the distribution of the sample[1] in more detail (e.g., the normal range) and not to support the identification of unusually high exposures.

Third, previous work by IUPAC and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) already provided useful guidelines to check before deriving RVs (Saravanabhavan et al., 2017): sample selection, sample size, time period, exclusion criteria, partitioning criteria, and the achieved analytical quality. To illustrate the handling of sample selection and sample size, to provide valid information for the general population, RVs need to be derived from population-representative samples (or representative for a specific subpopulation of interest) with sufficient sample sizes. In France, national metropolitan representativeness in the ELFE cohort study was achieved by basing the cohort on a study from census data and by selecting regional units by their size (Vandentorren et al., 2009). In the French study ESTEBAN national representativeness was achieved by a three-degree sampling plan with respect to geographical area and degree of urbanization (Balicco et al., 2017). Another feasible way of defining such a nationally representative sample was chosen for the German Environmental Survey (GerES). Gender, age, and community size (i.e., German municipalities) were used as sampling characteristics and a basis for case weighting (Schulz et al., 2012). With respect to a sufficient sample size, the German HBM Commission suggests a minimum sample size of 72 (Mekel et al., 2007) to 120 individuals (based on the suggestion from the National Committee for Clinical Laboratory Standards, NCCLS, 2000; IFCC, Solberg, 2004; and Reed et al., 1971)[2] necessary for a reliable derivation of reference values for a sub-group (e.g., men and women).

Fourth, RV95 and the 95% CI of the underlying 95th percentile emerged as the preferred and mathematically justified basis for deriving RVs. In addition, for determining the exact value of the RV it is necessary to develop criteria with respect to mathematical and analytical characteristics of the substance (e.g., scaling and precision). For example, to have a specific value for unusually high exposure, the upper limit of the 95% CI of the 95th percentile can be used as the reference value. In line with this reasoning, it is necessary to consider how to round off the RV95 to make it easily applicable in public health. When harmonizing the process of RV derivation, one should be aware that – depending on the software – different algorithms can be used to calculate a percentile (Hyndman and Fan, 1996).

Fifth, since some studies (e.g., CHMS; Saravanabhavan et al., 2017) exclude sub-groups with behavioral properties (e.g., smoking or fish consumption) which might per se cause unusually high exposure to substances (or groups of substances) specifically a uniform convention is needed as to whether and how extreme values and values of specific groups are handled.

Finally, it should be noted that this list is not exhaustive and further criteria such as the handling of missing values and number of values below the detection/quantification limit can influence the reference value and should also be taken into appropriate account (Zeghnoun et al., 2007). Moreover, here outlined considerations can also be taken into account when deriving reference values in concentrations of other environmental matrices (e.g., air, water).

In summary, the main criteria that should be considered when deriving RVs are to derive a single reference value to have a distinct threshold to high exposure. There is a tendency towards basing the RV within the 95% CI of the 95th percentile which can easily be justified by established mathematical paradigms. To achieve a standard definition the RV95 should be calculated based on samples of at least 72 to 120 individuals which are representative with respect to beforehand defined criteria. If possible, a standardized procedure to exclude sub-groups (such as active smokers) for a better reflection of background exposure should be developed. Keeping in mind the here identified criteria helps exposure and risk assessment and public health to progress towards a more uniform and comparable approach to identify unusually high exposures.

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[1] Additional percentiles could be used as rough approximates for comparability with already published studies with differently defined RVs but clearly separating them from RV95.

[2] The sample size of 120 is based on the minimum sample size for a reliable derivation of a reference value for the 90% CI of normal range estimates (Reed et al., 1971; Solberg, 2004) and a different sample size could be needed to derive the 95% CI of the 95th percentile.

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