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Evaluation of inhalation TTC values with the database RepDose

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ABSTRACT

The thresholds of toxicological concern (TTCs) define limit values for substances of unknown toxicity below which dietary intake is considered to be of no concern to human health. The TTC concept has already been used for risk assessment of e.g. food contaminants or flavoring substances and is in discussion to be applied to other classes of compounds such as cosmetic ingredients, household products, non-relevant metabolites in drinking water, and impurities in pharmaceuticals. The present publication aimed to evaluate whether the current TTC concept can also be applied to define limit values for inhalation exposure, using a data set of 203 industrial chemicals from the database RepDose.

It has been shown, that the NOEC values in classes 1, 2, and 3 are distributed over six orders of magnitude resulting in a considerable overlap between the distribution curves for the three classes. Inhalation thresholds for Cramer classes 1 (compounds likely to be of low-toxicity), 2 (compounds likely to be of moderate toxicity), and 3 (compounds suspect for high toxicity) were analyzed close to the approach described by Munro for oral TTCs. The 5th percentiles NOEC of Cramer classes 1–3 result in thresholds of 1.5×10^{-3} ppm for Cramer class 1 and 2.2×10^{-5} ppm for Cramer class 3. A threshold could not be derived for class 2 because of the small number of compounds available. If calculated as body doses, the inhalation thresholds for classes 1 and 3 (71 and 4 μg/person/d, respectively) are considerably lower than the oral thresholds derived by Munro (1800 and 90 μg/person/d). It has been shown that one reason for this difference is the high sensitivity of the respiratory tract to local effects.

In a next step, the values obtained were further refined. If organophosphates or compounds with structural alerts for genotoxicity are excluded, the TTC in Cramer class 1 increases, whereas the TTC in Cramer class 3 remains the same. Based on these analyses two inhalation TTCs for non-genotoxic compounds are proposed: 3.6×10^{-3} ppm (180 μg/person/d) for Cramer class 1 and 2.4×10^{-5} ppm (4 μg/person/d) for Cramer class 3.

1. Introduction

Thresholds of toxicological concern (TTCs) have been developed for risk assessment of compounds of known chemical structure for which no compound-specific toxicity data are available (Munro et al., 1996). Below the TTC value the risk to human health is assumed to be negligible. The TTC may be used as a substitute for substance-specific information in situations where there is limited or no information on the toxicity of a compound, and where human exposure is so low, i.e. below the corresponding TTC, that adverse effects are not to be expected.

In 2004, Kroes et al. proposed a detailed decision tree to identify the appropriate TTC value for an untested substance based on its structural features (Kroes et al., 2004). With this approach first "cancer" thresholds were assigned to certain groups of substances. For compounds with structural alerts for genotoxicity a TTC value of 0.15 μg/person/d was derived, and for non-genotoxic substances on which no further information is available a general TTC value of 1.5 μg/person/d was proposed. The general threshold was extrapolated from TD50 values in a database of more than 700 carcinogenic substances, for which a risk of 1 to 10^6 is assumed to be acceptable (Cheeseman et al., 1999). Recently, Felter et al. (2009) described the inclusion of data on genotoxicity like the AMES test to refine the very low TTC of 0.15 μg/person/d for substances with structural alerts for genotoxicity. Only few structural classes of highly toxic chemicals were identified not to be covered by the current TTC approach, e.g. steroids, polyhalogenated dibenzo-*p*-dioxins, polyhalogenated biphenyls/dibenzofurans, aflatoxin-like, *N*-nitroso-, or azoxy-compounds.

Abbreviations: LO(A)EL, lowest observed (adverse) effect level; NO(A)EL, no observed (adverse) effect level; N(L)OEC, no (lowest) observed effect concentration.

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For organophosphates (OPs) a special TTC value of 18 μg/person/d has been derived (Munro et al., 2008).

According to the Kroes decision tree, the non-genotoxic substances are then grouped into three broad structural classes using the Cramer decision tree (Cramer et al., 1978). Cramer class 1 contains "innocuous" structures for which metabolism and mode of action data suggest low-toxicity. Cramer class 2 contains less innocuous structures, and Cramer class 3 focuses on structures which can be assumed to be toxic.

In 1996, Munro and coworkers used a probabilistic approach to derive threshold values for each of the three Cramer classes. Munro used a database containing food substances, pharmaceuticals, industrial, environmental, agricultural, and consumer chemicals which had been tested in subacute to chronic repeated-dose toxicity studies with oral dosage. The TTCs for Cramer classes 1–3 are based on the 5th percentile NOAEL in each class, divided by an assessment factor of 100, and multiplied by an average human body weight of 60 kg to derive thresholds of 1800, 540, and 90 μg/person/d for Cramer classes 1, 2, and 3, respectively. This conservative approach gives a 95% probability that the risk for an untested substance is negligible, if the estimated intake does not exceed the TTC value. The database initially used by Munro to derive the above described TTCs for Cramer classes 1–3 included substances with structural alerts for genotoxicity as well as organophosphates. A recent publication stated that the very conservative TTC of Cramer class 3 was dominated by the NOAELs for OPs and organohalogens (Munro et al., 2008). Excluding OPs increases the threshold for Cramer class 3 to 180 μg/person/d, excluding both OPs and organohalogens raises it to 600 μg/person/d. The exclusion of "toxic" compounds from Cramer class 3 leads to a threshold which is comparable to that of Cramer class 2 (540 μg/person/d). Thus it has to be discussed what kind of thresholds will be the goal of TTC refinement: TTCs for specific categories of substances like for organophosphates and/or organohalogens, or generally applicable thresholds for structural classes defined as toxic, moderately toxic, and non-toxic according to Cramer, or an alternative method.

Up to now, the TTC values have been used for assessment of food contact materials by the US Food and Drug Administration (US FDA) and of flavoring substances by the European Food Safety Authority (EFSA), as well as for impurities in pharmaceuticals (Barlow, 2005; Cheeseman et al., 1999; Delaney, 2007; Kroes et al., 2000, 2004; Müller et al., 2006; Munro et al., 1998, 2008; Renwick, 2004). These applications are all characterized by very low exposure levels. Currently, it is under discussion whether the TTC can also be applied to the risk assessment of a broader variety of substances. Its use has been proposed for personal and household care products (Blackburn et al., 2005), cosmetic ingredients (Kroes et al., 2007), non-relevant metabolites from pesticides in drinking water (Melching-Kollmuß et al., 2010), and plant extracts (Re et al., 2009). Furthermore, inhalation TTCs have been derived for ingredients in consumer products (Carthew et al., 2009).

Inhalation is an important route of exposure for consumers (e.g. indoor air) and in the occupational context. Relevant *in vivo* data are, however, often not available for many compounds which are typically found in such environments. Therefore, it is of great interest to develop the TTC concept further and to derive inhalation-specific threshold values. We used the database RepDose that contains mainly existing chemicals (Bitsch et al., 2006, www.fraunhofer-repdose.de) to derive inhalation thresholds for Cramer classes 1–3 in a way close to the approach described by Munro et al. (1996) for oral TTCs. General inhalation thresholds for Cramer classes 1–3 were described, based on all available chemicals in RepDose which have been subject to inhalation studies. The 5th percentiles of NOEC values in ppm or mg/m^3 were used to determine inhalation TTCs. Furthermore, we evaluated how local and systemic toxicity influence the derived thresholds values. Local and systemic NOEC values were distinguished and target organs which occur at study LOEC were analyzed. Kroes et al. (2004) proposed to assign particular thresholds to genotoxic substances or organophosphates and exclude these from Cramer classes 1 to 3. The same procedure has been applied to the data set used to derive inhalation thresholds. Both groups, organophosphates and substances having structural alerts for genotoxicity, were excluded and thresholds were derived for the remaining substances in Cramer classes 1–3.

2. Materials and methods

2.1. Analysis of data and derivation of TTCs

The database RepDose (www.fraunhofer-repdose.de), developed at the Fraunhofer ITEM, has been used for the analyses. RepDose is a continuously growing database (Bitsch et al., 2006). For this report, the status of November 2009 was used. Currently, it contains over 650 mainly industrial chemicals and also some pesticides tested in repeated-dose toxicity studies with oral (gavage, diet, and drinking water) and inhalation exposure of rats and mice.

The classification of chemicals into Cramer classes was performed with the open source program Toxtree (http://ambit.acad.bg/toxtree).

As RepDose contains more than one study per chemical we prioritized the relevance of studies according to exposure duration. First, only studies with chronic exposure duration ($\geqslant$700 days) were considered. For substances which have not been investigated in a chronic study, subchronic (84–98 days) and then subacute (21–32 days) studies were used for the analysis. Whenever more than one study in one exposure category was available the study with the lowest NOEC value was analyzed. A total of 203 compounds were identified and analyzed (Appendix A). For values derived from short-term studies extrapolation factors of 2/6 for the corresponding subchronic/subacute studies were applied (ECHA, 2008). If a NOEC could not be identified by the procedure described above, it was extrapolated from LOEC to NOEC by applying a factor of 3 according to ECHA (2008).

Both ppm and mg/m^3 were used as dose measures for inhalation studies. If not provided in the individual studies, Eq. (1) was used to convert ppm to mg/m^3 and vice versa, as most of the RepDose compounds are vapors. The ppm values allow comparison of NOECs on a molar basis, whereas mg/m^3 have been used for calculation of body doses and derivation of threshold values that could be directly compared to TTCs derived from the oral route.

The geometric means, geometric standard deviations, medians, 5th and 95th percentiles of the distribution of the NOECs in the three Cramer classes were derived. Statistical analysis of the data was performed using the program STATISTICA from Statsoft.

TTC values were derived based on the 5th percentile NOEC as described by Munro et al. (1996) for the oral route. Thresholds for concentrations in air were calculated from the 5th percentile NOEC (ppm and mg/m^3) of Cramer classes 1–3 using Eq. (2). Furthermore, thresholds for daily exposure of humans (μg/person/d) were calculated from the NOEC in mg/m^3 using Eqs (3) and (4) to allow direct comparison to the corresponding values derived by Munro and coworkers for oral exposure.

Most animal studies use exposure durations of 6 h/d on 5 d/week. To account for exposure of consumers the 5th percentile was normalized to a daily exposure of 24 h and 7 days exposure per week (d_{exp}). Subsequently, a safety factor of 25 was used, which consists of an interindividual factor of 10 for interindividual