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Inhalation TTC values: A new integrative grouping approach considering structural, toxicological and mechanistic features

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ABSTRACT

The present publication describes an integrative grouping concept to derive threshold values for inhalation exposure. The classification scheme starts with differences in toxicological potency and develops criteria to group compounds into two potency classes, namely toxic (T-group) or low toxic (L-group).

The TTC concept for inhalation exposure is based on the TTC RepDose data set, consisting of 296 organic compounds with 608 repeated-dose inhalation studies. Initially, 21 structural features (SFs) were identified as being characteristic for compounds of either high or low NOEC values (Schüürmann et al., 2016). In subsequent analyses these SF groups were further refined by taking into account structural homogeneity, type of toxicological effect observed, differences in absorption, metabolism and mechanism of action (MoA), to better define their structural and toxicological boundaries. Differentiation of a local or systemic mode of action did not improve the classification scheme.

Finally, 28 groups were discriminated: 19 T-groups and 9 L-groups. Clearly distinct thresholds were derived for the T- and L-toxicity groups, being 2×10^{-5} ppm (2 μg/person/day) and 0.05 ppm (4260 μg/person/day), respectively. The derived thresholds and the classification are compared to the initial mainly structure driven grouping (Schüürmann et al., 2016) and to the Cramer classification.

1. Introduction

The Threshold of Toxicological Concern (TTC) concept had originally been developed for risk assessment of lifelong and daily oral intake of chemicals, proposing exposure thresholds below which a risk to human health is negligible (Barlow, 2005; Munro et al., 2008; Hennes, 2012).

A threshold of regulation (ToR) was selected by FDA for food contact materials based on an analysis of the CPDB (Carcinogenic Potency Data Base) database, a large data repository of rodent carcinogenicity studies (Cheeseman et al., 1999). A linear extrapolation from TD_{50} values was used to conclude on a virtually safe dose of 0.5 ppb (1.5 μg/person/d). This threshold was later reduced to 0.15 μg/person/d (Cheeseman et al., 1999; Kroes et al., 2004) to cover more genotoxic compounds that do not rise the risk of cancer by one in a million. TTC values for non-genotoxic substances were derived by Munro et al. (1996). The authors applied the predefined Cramer classification to a broad database consisting of repeated-dose toxicity studies with oral exposure. The Cramer decision tree defines three structural classes of compounds most likely to be of low, moderate or high toxicity (Cramer et al., 1978). The 5th NOEL (No Observed Effect Level) percentile in each of the Cramer classes was used to derive thresholds of 1800 μg/person/day (Cramer class 1, low toxicity), 540 μg/person/day (Cramer class 2, moderate toxicity), and 90 μg/person/day (Cramer class 3, high toxicity), which are still relevant today (EFSA, 2012). Further, for one special structural group – organophosphates – a group-specific threshold of 18 μg/person/day was proposed (Kroes et al., 2004).

Thresholds for other routes, such as dermal application or inhalation exposure, are currently under investigation. The COSMOS project (http://www.cosmostox.eu) i.e. develops a TTC scheme for dermal application. In this concept, the oral TTC values are used and route differences are considered by differences in bioavailability/systemic exposure.

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In 2007, Drew and Frangos developed a screening tool for air toxicants by calculating a Concentration of No Toxicological Concern (CoNTC). The CoNTC of 0.03 μg/m^3 was derived from the ToR of 1.5 μg/person/d and was considered to be applied for carcinogens. Default assumption were used to account for human uptake and exposure e.g. a fraction of 50% apportioned to air, an average human body weight of 70 kg, 100% absorption in the lung, an inhalation and an average age weighted daily respiratory rate. A comparable approach was applied to the oral TTC values. The three Cramer classes resulted in thresholds of 15 μg/m^3 (Cramer class 1), 1.5 μg/m^3 (Cramer class 2) and 0.2 μg/m^3(Cramer class 3) (Drew, 2010). A comparison to 3274 compound specific guidelines values or OELs revealed that only 2 values were below 0.03 μg/m^3.

The application of the Cramer classification tree to a data set of 92 subacute or subchronic rat inhalation studies resulted in TTC values of 200 (class 1) and 67 μg/person/day (class 2) for local effects and of 980 (class 1) and 170 μg/person/day (class 3) for systemic effects (Carthew et al., 2009). In parallel, Escher et al. (2010) analyzed a data set of 203 repeated-dose inhalation studies from the Fraunhofer RepDose database (www.fraunhofer-repdose.de). TTC values of 71 (class 1) and 4 μg/person/day (class 3) were derived for the entire data set. Systemic TTC values were also higher than local TTC values with 95 (class 1) and 6 μg/person/day (class 3) compared to 12 (class 1) and 4 μg/person/day (class 3). This finding led to the hypothesis that this route-specific MoA may have to be considered in a classification scheme for inhalation exposure (Escher et al., 2010).

Only about 4% of the compounds in both data sets were assigned to Cramer class 2 (structures associated with moderate toxicity), a finding similar to what has been observed for Cramer class 2 for oral exposure.

NOEC values in Cramer classes 1 and 3 range over up to six orders of magnitude and show a considerable overlap, indicating that the current TTC classification does not well discriminate between groups of compounds with low and high toxicity (Escher et al., 2010).

This finding is not specific for inhalation exposure and has already been shown for chemicals administered via the oral route (Tluczkiewicz et al., 2011; Kalkhof et al., 2011). The structural rules of the Cramer decision tree were derived by theoretical considerations in the late 1970s with much less knowledge about toxicology than today. In addition, some of the Cramer rules are not purely structure-related, e.g. rules 1 and 22. Rule 1 asks if the substance is a normal constituent of the body or an optical isomer thereof. This question relies on lists of endogenous compounds. A positive answer classifies the substance into Cramer class 1, assuming low toxicity. As a result, formaldehyde, for example, is classified as low-toxic. Formaldehyde, however, is a reactive compound whose toxicity and risk assessment has been under controversial debate for years (Hauptmann et al., 2004; Bolt and Morfeld, 2013). These rules often lead to differences in classifications, e.g. by human experts or computational tools such as Toxtree or the OECD QSAR toolbox (Bhatia et al., 2015).

Overall, these findings indicate that the current Cramer classification scheme is not directly applicable to group compounds according to their toxicity observed after inhalation exposure.

The TTC concept is a screening tool. Compounds are grouped according to characteristic properties into broad potency classes, for which conservative thresholds are calculated by using the 5th percentile of the NOEL distribution as point of departure. The Cramer classification considers mainly structural properties but it has been noted that to some extent also knowledge on metabolism and mode of action is included (EFSA, 2015).

Recently, we developed an inhalation specific classification of compounds based on structural characteristics. This grouping resulted in 21 structural features (SF) being indicative for “toxic” (14 SFs) and “low” toxic (7 SFs) compounds (Schüürmann et al., 2016). Threshold values were not analyzed.

In the present publication we evaluated the applicability of additional parameters such as subgrouping based on additional structural features, toxicological profile (critical toxicological effects and target organs *in vivo* studies) as well as differences in absorption. In a very general way, differences in absorption were indicated by using a predicted blood–air partition coefficient, because compound specific experimental data such as retention time, ventilation rate and resulting systemic dose (maximum concentration, C_{max} or the area under the curve, AUC) were not available. In addition, published data on metabolism and MoA were considered, e.g. genotoxic reactivity (Benigni and Bossa, 2008). The distinction of local and systemic NOECs per compound did not result in an improvement of the grouping concept. The resulting 28 groups were again pooled according to their NOEC values into two broad toxicity groups, named “toxic” and “low toxic”. Thresholds were calculated by using the two experimental 5th NOEC-percentiles. These threshold values are compared to those derived from the grouping of Schüürmann et al. (2016) and to the Cramer classification (Carthew et al., 2009; Escher et al., 2010).

2. Material and methods

Atom-centered fragments (ACF) analysis (Schüürmann et al., 2016) and further refinement of the SF groups were performed based on the TTC RepDose data set consisting of 296 repeated-dose toxicity studies (RDT) with inhalation exposure in rodents (Appendix 1). Study details of the 296 RDT studies such as study design, observed toxicological effects and target organs are available online under www.fraunhofer-repdose.de.

2.1. Selection of RDT studies for the TTC RepDose dataset

Data were mainly derived from the Fraunhofer database RepDose (www.fraunhofer-repdose.de), which includes peer-reviewed publications and studies from reviews such as NTP (National Toxicology Program) reports, RA (Risk Assessment) reports, and OECD SIDS (Screening Information Data Set). Also studies testing organic compounds from the investigation of Carthew et al. (2009) were added to RepDose. The focus was placed on organic chemicals with a purity greater than 90% and with high-quality chronic, subchronic or subacute inhalation studies.

The TTC RepDose data set contains 296 chemicals with 608 appropriate inhalation studies. Studies therefore had to be prioritized according to their relevance (Tluczkiewicz et al., 2011; Escher et al., 2010). The TTC concept establishes threshold values for lifetime exposure, so that long-term studies were preferred to short-term animal studies. This resulted in the following order of preference:

1. Chronic ($\geq$365 days)
2. Subchronic ($\geq$84 and $\leq$ 98 days)
3. Subacute (14 days and $\geq$21 and $\leq$ 32 days)

Whenever more than one study of highest relevance was available, the study with the highest quality was included in the TTC data set. If more than one appropriate study was available, the lowest NOEC value was considered.

2.2. N(L)OECs for locally and systemically active compounds

Local and systemic N(L)OEC values were distinguished per study. Local LOECs (N = 154) were defined as lowest concentrations