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The Threshold of Toxicological Concern for prenatal developmental toxicity in rats and rabbits

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ABSTRACT

The Threshold Toxicological Concern (TTC) is based on the concept that in absence of experimental data reasonable assurance of safety can be given if exposure is sufficiently low.

Using the REACH database the low 5th percentile of the NO(A)EL distribution, for prenatal developmental toxicity (OECD guideline 414) was determined. For rats, (434 NO(A)ELs values) for maternal toxicity, this value was 10 mg/kg-bw/day. For developmental toxicity (469 NO(A)ELs): 13 mg/kg-bw/day. For rabbits, (100 NO(A)ELs), the value for maternal toxicity was 4 mg/kg-bw/day, for developmental toxicity, (112 NO(A)EL values): 10 mg/kg-bw/day. The maternal organism may thus be slightly more sensitive than the fetus. Combining REACH- (industrial chemicals) and published BASF-data (mostly agrochemicals), 537 unique compounds with NO(A)EL values for developmental toxicity in rats and 150 in rabbits were evaluated. The low 5th percentile NO(A)EL for developmental toxicity in rats was 10 mg/kg-bw/day and 9.5 mg/kg-bw/day for rabbits. Using an assessment factor of 100, a TTC value for developmental toxicity of 100 μg/kg-bw/day for rats and 95 μg/kg-bw/day for rabbits is calculated. These values could serve as guidance whether or not to perform an animal experiment, if exposure is sufficiently low. In emergency situations this value may be useful for a first tier risk assessment.

1. Introduction

The Threshold of Toxicological Concern (TTC) concept is a risk assessment tool that is based on the idea that reasonable assurance of safety can be given even in the absence of chemical-specific toxicity data if the exposure is negligible. An important prerequisite is a sufficiently low intake to define an exposure level below which no significant risk to human health can be expected (JECFA, 2006). The TTC refers to the development of generic human exposure threshold values for groups of chemicals below which no appreciable risk to human health is assumed (Barlow et al., 2001). The concept of a TTC evolved from the review (Munro, 1990) of the Threshold of Regulation as applied by the FDA for food contact chemicals and was refined (Munro et al., 1996, 1999), based on an extensive analysis of available chronic oral toxicity data of substances.

The approach was adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to evaluate flavoring substances (JECFA, 1993, 1995; 1999; Munro et al., 1999) and has been used already from 1997 for the safety evaluation of 1259 flavoring substances (Renwick, 2004). A TTC decision tree developed by Kroes et al. (2004) provides a systematic structured approach for the consistent application of the TTC approach to chemicals that are present in food at low concentrations.

The application of the TTC concept in the absence of chemical-specific data is a pragmatic approach that allows the safety evaluation of chemicals to which humans are exposed via food and the environment. Thus, the TTC can be considered to be a first-tier assessment. If exposure levels are below the TTC value then no immediate action is required. If exposure levels are above the TTC value then more information on the hazard potential is needed. Initially this may include structure-activity approaches, read-across to similar chemicals or *in vitro* assays. The strength of this approach is that unnecessary animal studies are not performed because it identifies those chemicals that need additional testing. JECFA considered extension of the TTC concept to other substances present in the diet in small amounts (e.g., processing aid residues, packaging materials, and contaminants) and recommended development of guidelines for the application of the approach in the risk assessment of such substances for which full toxicological

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datasets are not available or are unnecessary (JECFA, 2005). Regulatory TTC values have been defined for migrant substances from packaging material in food (Food and Drug Administration, 1995), flavoring substances in food (JECFA, 2003; Renwick, 2004), genotoxic impurities in pharmaceutical preparations (EMEA, 2003, 2004; Müller et al., 2006) and were proposed for cosmetic ingredients (Kroes et al., 2007). The approach is used by the European Food Safety Authority to evaluate flavoring substances (EFSA, 2004) and has been endorsed by the WHO International Program on Chemical Safety for the risk assessment of chemicals (IPCS, 1998) and by the EU Scientific Committee on Toxicology, Ecotoxicology and the Environment. The approach has also been suggested for application to aquatic environmental exposure (De Wolf et al., 2005), constituents of consumer products (Blackburn et al., 2005) and occupational exposure in drug manufacturing (Dolan et al., 2005). More recent and novel applications for the TTC concept include its use to derive tolerable concentrations for "non-relevant metabolites" formed from plant protection products (Melching-Kollmuß et al., 2010).

Most of the reported work within the context of TTC development refers to chronic toxicity and carcinogenicity following oral exposure. Only a limited number of reports deal with end point-specific TTC values, i.e. TTC values for other toxicological end points than chronic toxicity and carcinogenicity. One area of particular interest is prenatal developmental toxicity. The reason for this is manifold; irreversible changes to the unborn are considered as the most grave in toxicology, theoretically such changes may be induced by a single or only a few exposures and under REACH extensive animal testing for reproduction toxicity is required. Therefore, a solid database to derive an acceptable TTC value for developmental toxicity could serve as a starting point in the assessment if (further) studies are needed from a regulatory perspective and could give risk managers a tool that could be used in emergency situations. Unfortunately, only few data evaluations relating to a TTC value for prenatal developmental toxicity in rats (OECD, 2001) have entered the scientific literature (Kroes et al., 2004; Bernauer et al., 2008; Laufersweiler et al., 2012). To increase the number of data available for TTC consideration for developmental toxicity we have published the NOAEL and LOAEL values of 93 different OECD 414 guideline studies in rats performed in our laboratories over the last two decades (van Ravenzwaay et al., 2011). Even less data are available with respect to the second species used for the determination of prenatal developmental toxicity, i.e. the rabbit. We have contributed to this database by publishing BASF's database, which contains 48 compounds for which the NOAEL and LOAEL values for maternal and developmental toxicity based on the OECD 414 guideline. ECHA's public database of REACH registration dossier offers a large toxicology dataset useful for assessing the outcome of toxicological studies for different end-points (Luechtefeld et al., 2016a). Here, we have collected the NO(A)EL and LO(A)EL values obtained for prenatal developmental toxicity studies in rats and rabbits. We have examined the studies for adherence to regulatory guidelines and used the data to establish TTC values for developmental toxicity for both species. Subsequently we have compared the obtained TTC values for these largely industrial chemicals with the TTC values obtained from our database, which consist mainly of active ingredients.

2. Materials and methods

2.1. Identification of developmental toxicity studies

The database for these analyses was created from ECHA dossier pages as described (Luechtefeld et al., 2016a). Automated extraction by linguistic search engines of data from ECHA online dossiers enables analysis of diverse chemical study data. Extracted REACH data were stored as a query-able collection of documents in a Mongo database (Chodorow, 2013; Godbillon, 2015). The public release of the REACH database is under discussion with ECHA; at this stage, it is available for collaborative analyses from the authors. Analyses for endpoints such as oral acute toxicity, eye irritation and skin sensitization have been published earlier (Luechtefeld et al., 2016b, 2016c, 2016d).

Briefly, data was downloaded from ECHA using HtmlUnit in an iterative manner in order not to hinder data flow, using an open source Java "Guiless browser" library (Bowler, 2002). Implementation of ECHA dossier download automation used the functional programming language SCALA (Odersky et al., 2004). A MongoDB database (https://www.mongodb.org/) was generated from REACH data (Chodorow, 2013). Extracted REACH data is stored as a query able collection of documents in this Mongo database. The database was generated by automated data extraction from ECHA dossier URLs via the SCALA driver ReactiveMongo (Godbillon, 2015).

Every document is identified by a unique set of three fields:

- ECNumber: Substance identifier ("415-890-1")
- Type: Study description (e.g., "Exp Key Eye irritation")
- Num: disambiguates repeat studies (1, 2, 3, …)

The constructed database, downloaded December 17, 2014, contains 816,048 such documents with 9801 unique substances (identified by ECNumber) and 3609 unique study descriptions.

The prenatal developmental toxicity study data and NO(A)EL/LO(A)EL values extracted from the ECHA files were carried out according to OECD guideline 414. For some performed after 1997, also according to U.S. EPA Health Effects Test Guidelines OPPTS 870.3700. The studies evaluated, were performed in the period from 1971 to 2014 according to the REACH database. Studies carried out in rats and rabbits were evaluated for maternal- and developmental-toxicity. Only studies in which maternal and developmental NOAEL/LOAEL or NOEL/LOEL values were reported in units of "mg/kg bw/d" were recruited for the analysis. The most frequently used rat strains were Sprague-Dawley and Wistar and for rabbits the New Zealand White, Himalayan and Dutch strains. The numbers of selected studies reported NOAEL/LOAEL and NOEL/LOEL values of maternal and developmental toxicity are illustrated in Fig. 1 (rat) and in Fig. 2 (rabbit).

2.2. Statistical analysis

A total of 480 chemicals tested in rats (477 of which contained developmental toxicity data) and 112 (all with developmental toxicity data) in rabbits were finally obtained taking into account the criteria mentioned above and used for evaluation. In some cases several NOAEL/LOAEL values for maternal- and developmental toxicity were available for the same chemical due to multiple studies. In this situation, the lowest NOAEL and LOAEL values were used as the input parameter for the evaluation of the chemical in question, thus resulting in a lower LOAEL/NOAEL values than potentially correct. Therefore, the TTC for oral prenatal toxicity values are determined in our analysis represent a conservative evaluation. The cumulative distribution functions of the NOAEL/LOAEL values were calculated and are displayed. Additionally, the median values as well as the 5th, 10th, 90th and 95th percentiles were determined. Since some studies merely reported LOEL or NOEL values, the same analyses were performed to compare the results when LOEL and NOEL values were included in the distribution. In this paper, NO(A)EL/LO(A)EL denotes the combination of