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Sensitivity of different generations and developmental stages in studies on reproductive toxicity

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HIGHLIGHTS

- Introduction of the new FeDTex database for prenatal development and reproductive toxicity studies.
- Analysis of the most responsive generation and developmental stage.
- Determination of the most affected critical targets in reproduction studies.
- Identification of F1 or F2 exclusive effects.

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ABSTRACT

Numerous studies on reproductive toxicity are expected to be necessary under the EU program on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Therefore, it is important to analyse existing testing strategies including also the recently implemented extended one-generation reproduction toxicity study (EOGRTS, OECD guideline 443). For this purpose the responsiveness of the different generations and developmental stages in studies on reproductive toxicity is analysed and critical targets of reproductive toxicity are identified by using the Fraunhofer FeDTex database.

The F1 generation is identified as most responsive generation in more than 50% of one-generation and multi-generation reproduction studies. Within the F1 generation the adult stage is mostly affected compared to the prenatal or postnatal stage. The target analysis in F1 has revealed alterations in body weight as highly sensitive for all developmental stages. Other important targets are the liver, kidney, testes, prostate, sperm parameters as well as developmental landmarks. The findings in the F2 generation have shown a higher responsiveness than F1 only in 3% of the studies. Although in 29 studies new effects are observed in F2 offspring compared to F1 irrespective of dose levels, overall no severe new effects have emerged that would change classification and labelling and justify an F1 mating.

The presented data support the importance of F1 for risk assessment and demonstrate that the study design of the EOGRTS is a suitable alternative to two-generation studies. However, compared to a conventional one-generation study the EOGRTS may identify additional effects but will change risk assessment with respect to NOELs only in rare cases.

1. Introduction

Currently, the EU Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program claims for the (re)evaluation of the toxicity of up to 100,000 chemicals until 2018 (Rovida and Hartung, 2009), including developmental and reproductive toxicity for industrial chemicals imported or manufactured at ≥10 tons per year according to mandatory endpoints mentioned in annexes VIII–X of the European REACH Regulation (EC, 2006). The required offspring studies are estimated to be responsible for approximately 90% of animal use and 70% of toxicity testing costs under REACH (Rovida and Hartung, 2009). Given the short time frame, this ambitious goal seems only feasible if existing data are utilised at their best, current testing strategies are optimised and new alternative *in vitro* and *in silico* methods are developed. This also contributes to the 3R-principle (Reduction, Refinement and Replacement of animal testing) originally published more than 50 years ago (Russell and Burch, 1959), primarily for ethical reasons but also due to cost savings and to allow a more rapid toxicity evaluation. Toxicity databases are integrated as useful tools into this process. The main task consists hereby in organising study data in an analysable format without losing information. Afterwards, the data pool can be used to analyse compound related toxicological properties and to refine toxicity testing as follows:

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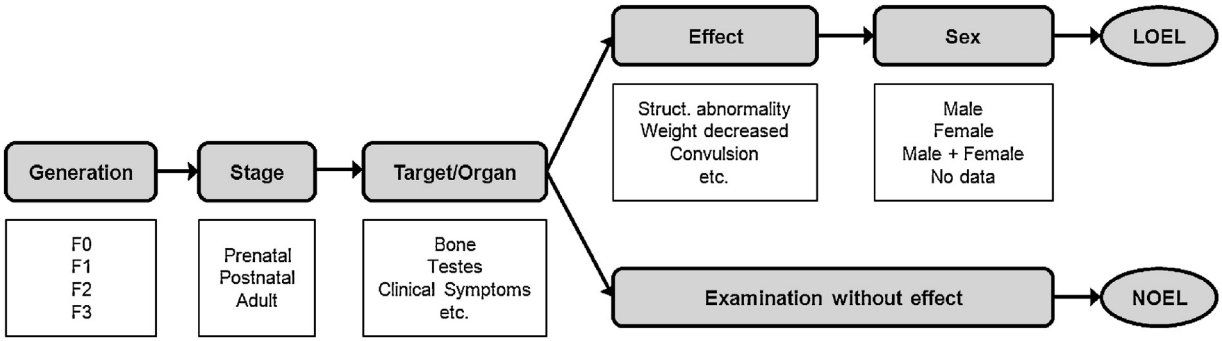

Fig. 1. Scheme of effect data entries.
Effect data entries are defined by the affected generation, subdivided into different developmental stages and corresponding organs or targets. The LOEL is documented gender-specifically. Examinations without effect result in a target-specific NOEL.

(I) Identification of critical targets in studies on reproductive and developmental toxicity to identify most responsive generations and developmental stages for (a) improvement of current *in vivo* testing strategies, (b) development of alternative *in vitro* methods and (c) identification of cellular level based adverse outcome pathways (AOPs).
(II) Improvement and extension of current *in silico* models to predict the hazard of untested chemicals as trigger for the need of further testing or waiving of dispensable evaluations.

Based on our recently developed FeDTex Database (Fertility and Developmental Toxicity in experimental animals database) critical targets in reproductive and developmental toxicity studies are identified and the most responsive generation and developmental stage in multi-generation reproduction studies are determined. The database also provides an extensive data pool for subsequent enhancement of *in vitro* and *in silico* models.

2. Materials and methods

2.1. Database structure of FeDTex

The FeDTex DB was developed using Microsoft Access® and was integrated into a MySQL™-based online platform. The database design distinguishes between three major parts: reference data, study design and toxicological data. The reference covers author, journal, volume and pages. Study design comprises general study data and provides major information on test substance, study type, species used including strain, sex and number of animals per dose group, exposure including dosage, route of application and duration, scope of examination and sacrifice. The toxicological data contain the results of the studies. Effects are assigned to associated targets/tissues and are characterised by their corresponding LOELs, differentiated to the affected developmental stage. For studies with an effect-free dose level the study NOEL is documented in the database. Examinations with no apparent effect on the target are documented additionally with their corresponding NOELs. Entry of toxicological data is described in Section 2.4.

2.2. Selection criteria for chemicals and studies

FeDTex DB focuses on studies of organic compounds like industrial chemicals, pesticides, food additives and pharmaceuticals conducted in rodents (i.e. rat or mouse) and rabbits. Inorganic chemicals are included only to a minor extent. Metal compounds and mixtures as well as studies in other species are excluded.

Prenatal development toxicity studies, one- and multi-generation reproduction studies (i.e. two- or three-generation reproduction studies and studies following the continuous breeding protocol) are generally accepted as study types. Oral and inhalation studies are preferred and represent more than 90% of the database content. Injection and dermal studies are included to a minor extent. All FeDTex DB entries are based on peer-reviewed publications. Common search engines like PubMed, Web of Science and SciFinder are used for literature research, in particular to screen for studies overlapping with the in-house database on repeated dose toxicity RepDose (Bitsch et al., 2006). To assure a suitable test design, e.g. duration of exposure, endpoints examined, number of dose levels tested, studies following OECD, U.S. EPA, ICH and/or Japanese MAFF guidelines are selected. To increase the amount of studies, studies with a comparable scope to guideline studies are additionally included.

2.3. Data entry standardisation

To ensure consistent database entries and to facilitate queries for a comparative analysis of chemicals, study data and toxic effect data have to be standardised. Therefore, uniform glossaries are implemented into the database. Pick lists are notably available for the type of study, application route, species, strain, and examined generations. The treatment of animals and the scope of examination are further specified by unique tick-sheets. Information on treatment covers the affected sex, exposure concerning different life stages, and necropsies performed according to the developmental stage. Examinations are selected by setting of check marks for the respective generation. Additional information can be provided using free text fields. The toxicological effects and their related targets are also selected from corresponding pick lists. Furthermore, specific effects are attributed to their respective targets, therefore assuring a consistent data entry (i.e. the effect "hormone status (changed)" is solely available for the target "endocrine system"). The data entry standardisation is permanently validated and new terms can be added to the pick lists when necessary.

2.4. Toxicological data

Effects are entered into FeDTex DB when statistical significance was proven, when a dose-response relationship was observed or the incidence was beyond the historical control range. Adverse and non-adverse effects are not distinguished. Thus the database provides NOELs and LOELs. All entries are cross-checked by the four-eye principle. Debatable effects (e.g. effects lacking a clear dose-response relationship) are labelled with a specific flag. This provides the opportunity to exclude these effects from evaluation. The effects finally entered into FeDTex DB follow a specific organisation chart (Fig. 1) and are dependent of the corresponding generation (i.e. F0, F1, F2 or F3), developmental stage (i.e. prenatal, postnatal up to puberty or adult), and target/organ. The prenatal stage covers foetal assessment and birth weight as markers of prenatal development. The postnatal stage covers all following examinations after birth up to puberty. Every effect is finally characterised by a specific LOEL. To be able to assess different susceptibility of the sexes, LOELs are provided for both sexes. As different effects can occur at a distinct target/organ, the target/organ LOEL is defined by the lowest effect LOEL in this target/organ and is documented in the database. A LOEL for each developmental stage and generation as well as an overall study LOEL is analogically generated and documented. Examinations without detected effect lead to a corresponding NOEL.

2.5. Comparison of FedTex DB and ToxRefDB data

To compare the content of FeDTexDB with the Toxicity Reference Database (ToxRefDB), the latest available ToxRefDB-version (i.e. toxrefdb_2010q1b) from the U.S. EPA homepage was used for analysis.

2.6. Analysing the chemical domain of FeDTex DB using the QSAR Toolbox

The chemical domain of FeDTex DB was analysed by using the OECD QSAR Toolbox V2.3. The Toolbox is an open source software intended to be used for grouping approaches such as read across and category definition. Several grouping tools are provided. It is possible to group according to (1) predefined groups such as categories derived from the US EPA New chemical or the OECD HPV program; (2) mechanistic aspects e.g. DNA binding or biodegradation; (3) endpoint specific aspects e.g. based on a certain reactivity observed in *in vitro*/*in vivo* assays; and (4) empiric methods e.g. chemical elements or organic functional groups. The substances of the FeDTex DB were grouped by using the organic functional group (OFG) profile provided in the Toolbox. The profiling system allows a classification of the characteristic structural fragments and different functionalities of organic chemicals and can be used to identify structurally similar chemicals. As substances may contain several functional groups, one single substance may also be assigned to more than one OFG.