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Relevance of non-guideline studies for risk assessment: The coverage model based on most frequent targets in repeated dose toxicity studies

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HIGHLIGHTS

- Coverage approach: evaluation of scope of examination of old repeated dose toxicity studies.
- Statistical model calculates probability that LOEL or the next higher dose (LOEL + 1) is determined.
- Evaluation based on Fraunhofer RepDose DB.
- Statistical model can be transferred to other datasets.

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ABSTRACT

A common challenge for human risk assessment is the quality of the available animal studies. Non-guideline studies are often limited for different aspects of study design and documentation. Within this publication the relevance of a limited scope of examination is discussed. Preliminary analyses of the RepDose database have shown that liver, body weight, kidney and clinical symptoms are frequently affected in oral repeated dose toxicity in rats and mice (Bitsch et al., 2006), while many other targets are seldom affected.

As most of these targets are investigated frequently also in non-guideline studies, it is likely that they provide a reliable NOEL, although the full spectrum of endpoints has not been covered. Based on RepDose data we investigate the relevance of individual targets for determining the LOEL and the consequences for risk assessment. The resulting coverage model for subchronic oral rat studies includes up to six targets and an additional assessment factor for LOEL extrapolation. It can be applied to assess the reliability of non-guideline studies with respect to the scope of examination. Furthermore the application of the coverage model to other databases will increase and/or specify the chemical domain and reveal respective targets as well as effects.

1. Introduction

In the evaluation of studies on existing chemicals, the risk assessor often faces the situation that repeated dose toxicity studies are available that are rather old, i.e. performed before the implementation of guidelines, or were not conducted according to guidelines. For these studies, less than three dose levels may be available or a low number of animals/dose group. In addition the scope of investigations may be reduced with regard to the number of organs investigated by histopathology. Further, organ weights, clinical chemical examinations, hematology or urine analysis may not be available.

In the REACH program, the quality of a study is determined by the Klimisch Code (Klimisch et al., 1997): Depending on the scope of examinations and quality of documentation studies are "reliable" (Klimisch Code 1), "reliable with restriction" (Klimisch Code 2) or "not reliable" (Klimisch Code 3). Many of the old, non-guideline studies can be assigned to reliability 2 or even 3. In the case of Klimisch Code 3, the study is usually considered as not sufficient for evaluation and new animal testing is necessary. However, with the aim of saving experimental animals, it is desirable to use existing studies in risk assessment as far as possible.

At Fraunhofer ITEM a database on repeated dose toxicity studies (RepDose) has been developed, that contains currently more than 1100 oral repeated dose toxicity studies, mainly on existing chemicals. These studies include subacute, subchronic and chronic studies with rats and mice with reliability 1 and 2, and

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Table 1
Datasets selected from RepDose for further analyses.

Study duration	Study type	No. of studies	
		Rats	Mice
Subacute	Comprehensive	52	≤10
Subchronic	Comprehensive	88	45
Subchronic	Old	56	≤10

also some studies with reliability 3. Preliminary analyses of the overall database have shown that liver, body weight, kidney and clinical symptoms are frequently affected in oral repeated dose toxicity in rats and mice (Bitsch et al., 2006), while many other organs are seldom affected. In inhalation studies the respiratory tract and especially the nose and lung are major targets in addition.

As most of these targets are investigated frequently also in non-guideline studies, it is likely that non-guideline studies provide a reliable NOEL, although the full spectrum of endpoints has not been covered in the respective study. Based on RepDose data, in this publication we investigate the relevance of individual targets for determining the LOEL and the consequences for risk assessment. A method is developed to calculate the probability that a certain set of targets provides a reliable reference value. A model consisting of up to six targets is proposed for subchronic oral rat studies.

2. Materials and methods

2.1. Analyses with RepDose

RepDose contains effects for different dose levels and general information on the study design as well as a reliability score. The reliability score assesses the study quality with respect to general design and scope of examination. Based on the reliability and the publication date two types of studies were discriminated: studies with "guideline-conform", OECD-guideline-like design and scope as well as "non-guideline" studies performed before the implementation of OECD guidelines in 1981. Additionally, the following criteria were applied to select the datasets from the RepDose DB:

- Oral studies (gavage studies, application via drinking water or food).
- Rats and mice.
- Subacute and subchronic studies.
- LOEL available.
- In addition NOEL, and LOEL + 1 (dose above LOEL) available (only guideline-conformstudies). If a NOEL was not available, also studies with only LOEL and LOEL+1 were included. Conditions were: Only one organ affected at the LOEL or mild effects at the LOEL

The datasets shown in Table 1 were derived. Datasets with 10 or less studies were not considered for further evaluation.

Most analyses were performed with the guideline-conformdataset on rat subchronic studies. Studies were analysed for the targets at their LOEL and at the LOEL + 1. Targets for effects are classical organs (histopathology or organ weight) in toxicological studies, i.e. liver, kidney, brain, etc. In addition, body weight, clinical symptoms, clinical chemistry and haematology are termed targets in our analysis.

In the RepDose database no distinction is made between LOELs and LOAELs. Any effect that is statistically different from the control is termed LOEL. Thus, effects that may be considered as non-adverse are also covered in our analyses. The references for the selected studies are given in supplementary material.

2.2. Statistics

The probability that a study detects the LOEL, or the LOEL + 1, when only a limited number of targets has been investigated in a study, can be calculated by methods of categorical data analysis, in particular loglinear modelling (Agresti, 2002; Bishop et al., 1975; Christensen, 1997; Fienberg, 1980; Powers and Xie, 1999). We aimed to identify the most suitable and generally usable model, avoiding overfitting. Models that overfit will predict badly in new situations: they have limited generalizability. We have found the independent organ model to be generally acceptable to calculate the probability that the LOEL or the LOEL + 1 were detected. The model selection is documented in supplementary material.

The multinomial linear model without interaction terms can be interpreted as the independent organ model. For example, at the LOEL, the fraction of studies with particular organ combinations affected can be modelled by multinomial probability parameters φ which are proportional to the exponential function of a linear expression of the target organs. Suppose L, K, C, and B are binary values (0 or 1) of the target organs: liver, kidney, clinical chemistry, and body weight. A value of 0 means not affected, or not reported to be affected, while a 1 means affected. Then probabilities φ are modelled as:

$$\phi \propto \exp(\beta_1 \cdot L + \beta_2 \cdot K + \beta_3 \cdot C + \beta_4 \cdot B).$$

The exponential function factorises into terms to be multiplied:

$$\begin{aligned} \phi &\propto \exp(\beta_1 \cdot L + \beta_2 \cdot K + \beta_3 \cdot C + \beta_4 \cdot B) \\ &= \exp(\beta_1 \cdot L) \cdot \exp(\beta_2 \cdot K) \cdot \exp(\beta_3 \cdot C) \cdot \exp(\beta_4 \cdot B) \\ &= \phi_L \cdot \phi_K \cdot \phi_C \cdot \phi_B \end{aligned}$$

Hence, the probability of a study with a certain organ pattern is the product of the probabilities of studies with each individual organ affected. The β-coefficients are estimated by the method of maximum likelihood.

Given the data in Table 3 the coverage at the LOEL of a study with liver, kidney and heart examined would be:

$$\text{Coverage} = 1 - (1 - 0.318) \times (1 - 0.239) \times (1 - 0.057) = 0.51$$

2.3. Simulation of coverage uncertainty

Coverage uncertainty was simulated through Bayesian Monte-Carlo sampling of the linear multinomial models, cf. (Albert, 2009). The details are explicated in supplementary material.

To sketch the procedure, note that the individual target fractions in Table 3 are estimated from observed fractions at the LOEL, and at LOEL + 1. Thus, the fraction 31.8% of studies showing Liver to be affected at the LOEL, as employed in the above three-organ coverage equation, is estimated from the ratio of studies with the organ affected to the total number of studies: 28/88 = 31.8%,

However, this is a so-called *point estimate*, without any uncertainty addressed.

Through Bayesian simulation of the model likelihoods, the uncertainty around these point estimates can be evaluated. This yields the uncertainty of the β-coefficients, as well as the estimated fractions of studies with a particular organ affected, and therefore of the individual organ and total coverage.

We found that in each organ model, the median of the simulated coverage distributions was very close to the point estimates.

3. Results

3.1. Number of target organs

Table 2 shows the number of organs affected in subacute or subchronic studies with rats or mice, at the LOEL, the dose level above the LOEL (LOEL + 1) or for all dose levels investigated. At the LOEL on average more than one target organ is affected in all studies types. With increasing dose levels the average number of targets increases from 1.6 to 4.2 for subacute rat studies and 1.8

Table 2
Number of targets affected for different study types.

Species	Study duration	Characterization	n	No. of targets[a]		
				LOEL	LOEL+1	Overall study
Rat	Subacute	Comprehensive	52	1.6 (1.8)	3.7 (2.0)	4.2 (2.0)
Rat	Subchronic	Comprehensive	88	1.8 (1.8)	3.4 (2.0)	6.5 (1.7)
Rat	Subchronic	Old	56	1.8 (2.0)	3.0 (2.0)[b]	3.6 (2.3)
Mouse	Subchronic	Comprehensive	45	1.7 (1.7)	2.3 (1.9)	4.2 (1.9)

[a] Geometric means and the respective geometric standard deviation in brackets.
[b] This figure refers only to 41 of the 56 old oral rat studies which have a LOEL + 1 dose.