Chapter 9

In Silico Models for Repeated-Dose Toxicity (RDT): Prediction of the No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) for Drugs

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Abstract

The preclinical stage in drug development requires the determination of repeated-dose toxicity (RDT) in animal models. The main outcome of RDT studies is the determination of the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL). NOAEL is important since it serves to calculate the maximum recommended starting dose (MRSD) which is the safe starting dose for clinical studies in human beings. Since in vivo RDT studies are expensive and time-consuming, in silico approaches could offer a valuable alternative. However, NOAEL and LOAEL modeling suffer some limitations since they do not refer to a single end point but to several different effects and the doses used in experimental studies strongly influence the final results. Few attempts to model NOAEL and LOAEL have been reported. The available database and models for the prediction of NOAEL and LOAEL are reviewed here.

Key words Repeated-dose toxicity, NOAEL, LOAEL, Drug safety, In silico models, Chronic toxicity

1 Introduction

Repeated-dose toxicity (RDT) studies are designed to determine the effects of repeated oral, dermal, or inhalation exposure to a substance over a specific period of time [1]. Characterization of the toxicological profile of the test substance after repeated exposure is the primary goal of RDT study. RDT tests provide detailed information to identify the adverse effects, the potential target organs or systems (reproductive system, liver, kidney, central nervous system, endocrine system), and the persistence or reversibility of the effects [2].

Toxicity after repeated dosing must also be tested to contribute to the development of safe medicinal products that are to be given repeatedly [3].

Drug development is a long, complex, and expensive process. The typical procedure comprises three major steps: discovery,

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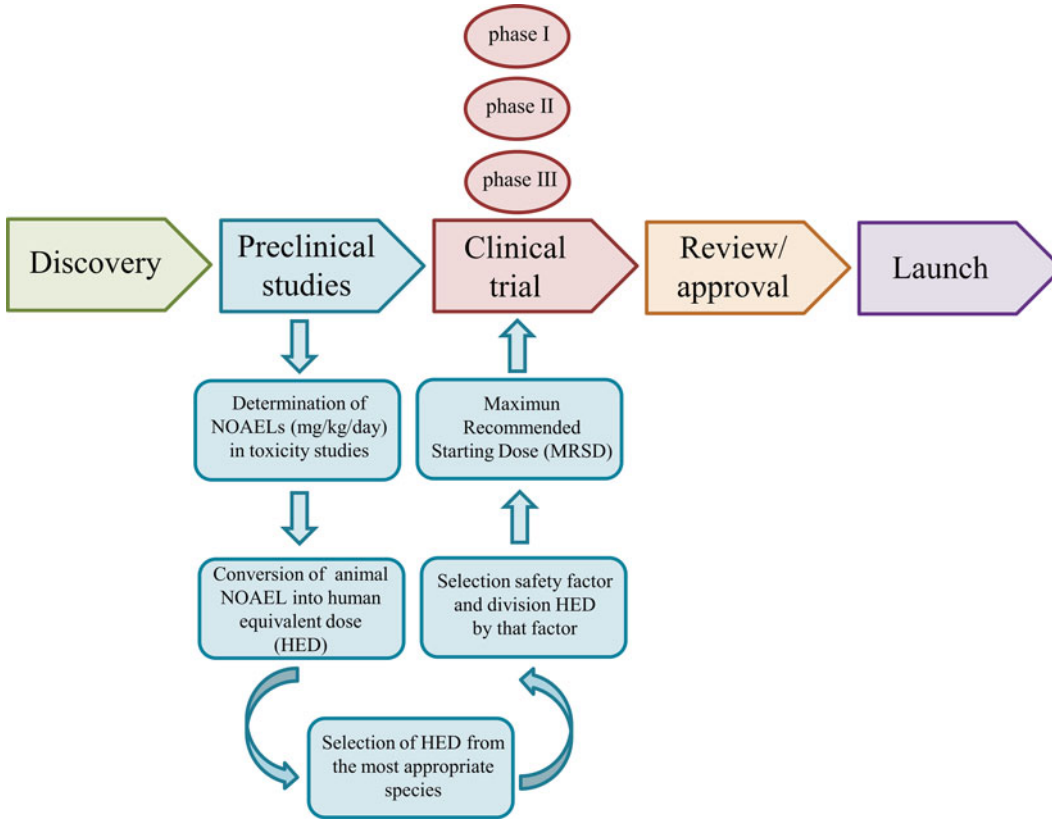

Fig. 1 Scheme of the typical drug development

preclinical development, and clinical trial [3, 4] (Fig. 1). Clinical trials involving daily chronic dosing require RDT studies on animal models (two species, one non-rodent) in the preclinical stage [3]. The no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL), the main outcomes of these studies, are of the utmost importance in the non-clinical risk assessment. Although the definitions of NOAEL and LOAEL are debated [5], generally, NOAEL is the highest dose without any biologically significant adverse effects, while LOAEL refers to the lowest exposure at which adverse effects are seen (Fig. 2). NOAEL, determined in non-clinical safety studies in the most appropriate animal species, gives important information for the first dose in humans [6]. NOAEL is essential to calculate the maximum recommended starting dose (MRSD), the dose used in the first human study (clinical trial) [7] (Fig. 1).

Besides pharmaceuticals [8, 9], other regulatory contexts require RDT testing to assess the potential risks of a substance: industrial chemicals [10], agrochemicals [11, 12], biocides [13], and cosmetics [1, 14].

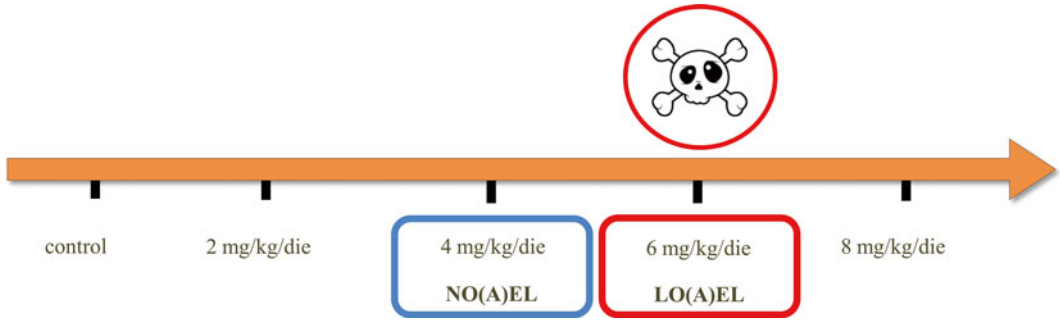

Fig. 2 Identification of the lowest observed adverse effect level (LOAEL) and no observed adverse effect level (NOAEL)

Considering the high cost of drug failure and withdrawal due to toxicity found in the development process, the potential toxicity of a drug needs to be determined as soon as possible [15]. The importance of the results of RDT studies for the evaluation of the safety of chemicals is undeniable, but the in vivo tests are time-consuming and very expensive [16]. The possibility of obtaining the same information using non-testing methods is tempting, though considering the peculiar nature of NOAEL and LOAEL, their computational modeling is a challenge. Few attempts have been made to model NOAEL and LOAEL. A review of the software, databases available, and published models is presented here.

2 LOAEL and NOAEL Databases

Databases containing NOAEL and LOAEL values are available, with a high percentage of overlap between the different sources (Table 1). Generally, for NOAEL and LOAEL, the measurement unit is expressed as mg/kg body weight/day. In order to build accurate computational models, the quality of the chemical structures and data is crucial [17]. In addition, for LOAEL and NOAEL, not only is the final number important but other supporting information is too, such as route and duration of exposure, species and strain used, space between doses, and organ level effects, in order to properly assess the quality and the potential use of these data for modeling.

The RepDose database, developed by Fraunhofer ITEM as part of a project funded by the European Chemical Industry Council (CEFIC), contains experimental NOAEL and LOAEL values for 655 chemicals related to oral (gavage, feeding, and drinking water) or inhalation studies in rodents exposed to the substance over at least 14 days. The chemicals in the database have a limited number of functional groups since complex and multi-functional chemical structures such as pharmaceuticals, inorganic

Table 1
RDT databases for modeling

Name	No. of substances	Developers	Exposure route	Exposure duration	Animal models	Available at	Copyright	Drugs
RepDose	655	Fraunhofer ITEM	Oral, inhalation	At least 14 days	Rodents	http://fraunhofer-repdose.de/	Yes	No
Munro	613	Munro et al. (1996)	Oral	Up to 910 days	Rodents, rabbit	QSAR OECD Toolbox	No	No
HESS	500	NITE	Oral	28–120 days	Rats	QSAR OECD Toolbox	No	No
IRIS	Over 500	US EPA	Many	Many	Rodents, non-rodents	http://www.epa.gov/iris/intro.htm	No	No
COSMOS	1660	COSMOS project	Many	Many	Rodents, non-rodents	http://cosmosdb.cosmostox.eu	No	Yes
ToxRef	310	US EPA	Many	Many	Rodents, non-rodents	QSAR OECD Toolbox	No	Yes

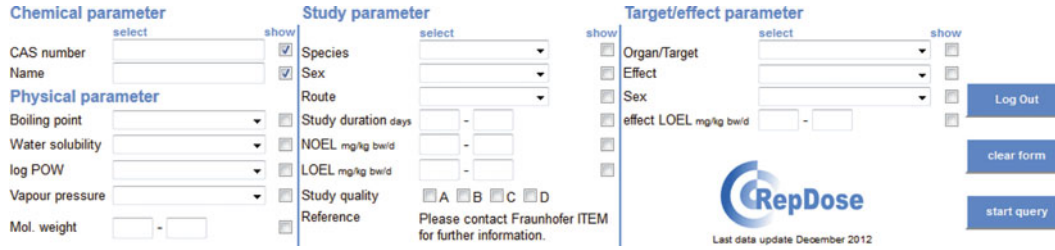

Fig. 3 Query form of the RepDose database

or metal compounds, and mixtures were eliminated [18]. A score (A, B, C, D) indicating the data quality is also provided. Details on the animals used (strain, sex, number per dose group) and the exposure (duration and route, postexposure observation period, and dose groups) are also provided. The database includes toxicological (effect data include all target organs with all associated effects and corresponding LOAEL) and physicochemical (molecular weight, solubility in water, physical state, boiling point, dissociation constant, octanol-water partition coefficient, and vapor pressure) data. The RepDose database is available at http://fraunhofer-repdose.de/, and access is free on registration by the user. A user-friendly query screen (Fig. 3) puts several questions regarding the influence of structural features and physicochemical data on LOAEL, target organs, and effects [18]. Although all the data in the database are displayed, their use is restricted.

Munro et al. [19] provide NOAEL and LOAEL values for 613 organic compounds related to sub-chronic, chronic, reproductive toxicity, and teratogenic studies in rodents and rabbits. For each compound the chemical name, CAS number, structural classification using the decision tree of Cramer et al. [20], species, sex, route of exposure, doses tested, study type, duration, end points, NOAEL, and LOAEL references are reported. The data come from four sources: US National Toxicology Program (NTP) technical reports (post-1984), the toxicological monographs prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Integrated Risk Information System (IRIS) database, and the Developmental and Reproductive Toxicology (DART) database. The compounds in the Munro database represent a variety of chemicals (e.g., pesticides, food additives, industrial chemicals). To demonstrate that a study is rigorous enough to detect toxic effects, a compound needs to have both NOAEL and LOAEL to be included in the database; however, in some cases, the LOAEL is not available because the substances are major food ingredients and had no toxicity at the highest dose tested in well-conducted studies [19]. The database is downloadable from the QSAR OECD Toolbox; otherwise, the publication provides a paper version of the database.

The Hazard Evaluation Support System (HESS) database comprises 500 chemicals for which RDT data were obtained from test reports of Japanese CSCL by the Ministry of Health, Labour and Welfare, the National Institute of Technology and Evaluation (NITE), and the Ministry of Economy, Trade and Industry (METI) and from reports produced by the US NTP [21]. All these tests were conducted in compliance with GLP principles. This database contains detailed RDT data related to sub-chronic and chronic (28–120 days) oral exposure in rats. The HESS database, freely downloadable from QSAR OECD Toolbox, provides information for the target compounds such as CAS number, chemical name, SMILES, exposure route and duration of the studies, animal used (strain, sex), toxicological data (organ, tissue, effects, largest and smallest doses used) and NOAEL/LOAEL values.

The Integrated Risk Information System (IRIS) is a publicly available repository, developed by the US Environmental Protection Agency (EPA) that contains information on over 500 chemicals. It provides descriptive and quantitative chronic health information on chemicals found in the environment in order to support risk decision-making [22]. Two main categories of effects are present in IRIS database: non-cancer (oral reference doses and inhalation reference concentrations: RfDs and RfCs) and cancer effects. NOAEL and LOAEL are reported with a detailed summary of the studies containing information on the species used, route and duration of exposure, concentrations tested, and target organs. The user can consult data on the EPA website (http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList); substances are listed in alphabetical order.

The COSMOS database [23] contains 12,538 toxicological studies for 1660 compounds. Two datasets are available: US FDA PAFA and oRepeatToxDB. The first contains 12,198 studies across 27 end points including both repeated-dose (in this case the lowest effect level, LEL, is reported) and genetic toxicity data. ORepeatToxDB, assembled by the COSMOS consortium, contains 340 in vivo repeated-dose toxicity studies from different sources (EC REACH project, US NTP) for 228 chemicals. It reports observed toxicological effects together with the sites at which the effect occurred. Figure 4 reports the typical output of a COSMOS database query. The user needs to be registered for a free account. The COSMOS database was built in the context of the EC project SEURAT-1, partly funded by Cosmetics Europe.

The Toxicity Reference Database (ToxRefDB), developed by US EPA [24], comprises thousands of animal toxicity studies (reporting NOAEL and LOAEL) after testing hundreds of different chemicals. ToxRefDB is freely downloadable from the QSAR OECD Toolbox or can be consulted at the US EPA website (http://actor.epa.gov/toxrefdb/faces/Home.jsp).

Although none of these databases contains only NOAEL and LOAEL data for drugs, some of them cover pharmaceuticals.

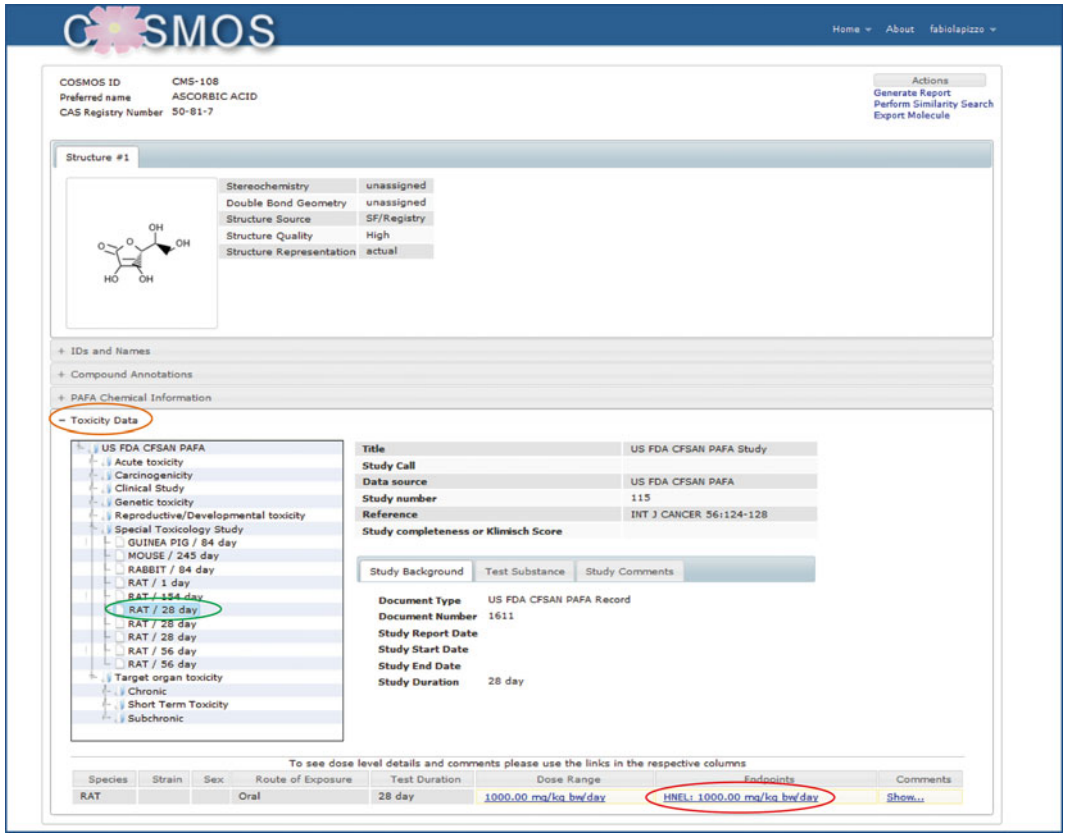

Fig. 4 Typical output of a query using the COSMOS database. In the toxicity data section (*orange*), the exposure duration and the animal used for the in vivo experiment (*green*) are indicated, and the RDT study is reported at the bottom of the screen (*red*) as highest no effect level (HNEL)

3 In Silico Models for the Prediction of LOAEL and NOAEL

A limited number of in silico models are available for the prediction of LOAEL and NOAEL. Published models and software are reviewed here.

3.1 Published Models

The models described here were not built primarily to predict NOAEL and LOAEL for pharmaceuticals; indeed, the compounds used for modeling came from different industrial and environmental contexts. The performances are close to acceptability and do offer a good starting point for the development of a reliable model that can be used in a multidisciplinary context. Table 2 provides a general overview of the literature-based models.

One of the most recent models for the prediction of RDT is described in Toropov et al. [25]. They modeled NOAEL for 113 organic compounds using the Monte Carlo method and three molecular descriptors. The dataset was split three times and the average performances in the training set (97 compounds) in terms

Table 2
General overview of published models for the prediction of LOAEL and NOAEL

Reference	End point	Modeling method	Descriptors	Data used	Training set size	Test set size	Drugs
Toropov et al. (2015)	NOAEL	Monte Carlo	3	28 and 90 days oral exposure in rats	97	16	No
Gadaleta et al. (2014)	LOAEL	*k*-NN	Fingerprints + 3 structural keys	90 days oral exposure in rats	254	179	No
Toropova et al. (2014)	NOAEL	Monte Carlo	8	28 days oral exposure in rats	~180	~21	No
Sakuratani et al. (2013)	LOAEL	Read-across	None	From 28 to 90 days oral exposure in rats	500	None	No
Mazzatorta et al. (2008)	LOAEL	GA-PLS for selecting descriptors and LOO-SMLR for model generating	19	Longer than 180 days oral exposure in rat	445	None	No
Garcia-Domenech et al. (2006)	LOAEL	Furnival-Wilson algorithm for selecting descriptor; MLR for (LOO cross-validation) model generating	11	Chronic studies in rats	87	16	No
De Julián-Ortiz et al. (2005)	LOAEL	Stepwise procedure for selecting descriptor; LDA for model generating	15	Chronic studies	86	17	No
Matthews et al. (2004)	MRTD/NOEL	Structural alerts	None	Human, oral route, 3–12 months	1309	Leave more out	Yes

of R^2 and RMSE were, respectively, 0.52 and 0.61. In the test set (16 compounds), the performance in terms of R^2 and RMSE ranged from 0.62 to 0.73 and from 0.44 to 0.52, respectively.

Gadaleta et al. [26] using the *k* nearest neighbors (*k*-NN) algorithm, a computational technique based on the concept of similarity, built a model for the prediction of LOAEL. However, to improve the performance, the basic algorithm was refined by setting additional conditions, and a target chemical must fulfill all those rules to be considered reliably predicted. The training and test sets of the model comprised, respectively, 254 and 174 organic compounds, and R^2 for the two sets ranged from 0.632 to 0.769 and from 0.552 to 0.682, considering the different *k*. This model will be implemented in the VEGA (http://www.vega-qsar.eu/) platform and will be freely available.

Toropova et al. [27] modeled 218 NOAEL data (28 days of oral exposure in the rats) using the Monte Carlo method. *R2* for the training and test sets ranged from 0.679 to 0.718 and from 0.61 to 0.66, respectively, considering the different splits.

Sakuratani et al. [28] identified 33 chemical categories related to individual types of toxicity on the basis of mechanistic knowledge starting from a training set of 500 chemicals with RDT data related to oral exposure between 28 and 120 days in rats. Chemicals were assigned to a given category, and then the LOAEL was derived as the result of a data gap-filling approach by read-across on other chemicals in the category. This model does not provide figures for the LOAEL but can be used to identify the target organ most likely to be affected by the target chemical. The category library has been implemented and is available through the Hazard Evaluation Support System (HESS) integrated computational platform.

A further model for the prediction of LOAEL was developed by Mazzatorta et al. [29], applying an integrated approach of genetic algorithm (GA) and partial least squares (PLS). Selected descriptors (19 from DRAGON) were used to develop a LOAEL predictive model through a leave-one-out stepwise multiple linear regression (LOO-SMLR) starting from a set of 445 chronic toxicity data (180 days or more of oral exposure in rats) selected from several sources. The final dataset included pesticides, drugs, and natural products. This model performed as follows: R^2 0.570 and RMSE 0.700. No external validation was done, so the real predictive model's power is not known. However, the performances of LOO cross-validation were q^2 0.500 and RMSE 0.727.

De Julián-Ortiz et al. [30] used a dataset of chronic LOAEL data for 234 compounds compiled from different sources (US Environmental Protection Agency, EPA, and National Cancer Institute/National Toxicology Program, NTP) to build a multilinear regression model (MLR). They selected 15 topological descriptors by a Furnival-Wilson algorithm from among those in the

DESCRI program. MLR and the Furnival-Wilson algorithm were also applied to a smaller but more homogeneous dataset (86 compounds). The results on the first 234 compounds were quite poor (R^2 0.524 and RMSE 0.74). However, the performance on the second dataset (86 compounds) was significantly better (R^2 0.647 and RMSE 0.66). In both cases no external validation was done.

García-Domenech et al. [31] applied the same techniques (Furnival-Wilson for descriptor selection and MLR for model building) on the same 86 molecules used by De Julián-Ortiz et al. [30]. The model, based on six descriptors, was validated on 16 external chemicals. Performances in the training set were R^2 0.795 and RMSE 0.517; q^2 0.719 and RMSE 0.564 in LOO cross-validation and R^2 0.712 and RMSE 0.853 in external validation.

To the best of our knowledge, Matthews et al. [32], Toropova et al. [27], and Toropov et al. [25] are the only studies that report attempts at NOAEL modeling.

Matthews et al. [32] used Maximum Recommended Therapeutic Dose (MRTD) data for 1309 pharmaceutical substances for classification modeling. The MRTD (or Maximum Recommended Daily Dose, MRDD) was determined from clinical trials that employed an oral route of exposure and daily treatments, usually for 3–12 months. The MRTD is derived from human clinical trials and is an upper dose limit beyond which the efficacy of a drug does not increase and/or adverse effects start to outweigh the beneficial ones [33]. MRTD and NOEL for drugs are directly related in humans [32]. An analysis of the MRTD database indicated that most drugs do not show efficacy or adverse effects at a dose approximately ten times lower than the MRTD. Based on this observation, Matthews et al. [32] calculated NOEL as MRTD/10. Chemicals with low MRTD/NOEL were considered strongly toxic, whereas those with higher values were labeled as safe, and structural alerts were identified on this basis. The predictive ability of this model was evaluated through leave-more-out external validation (40 compounds were removed from the training data set of 120 selected test chemicals), and the results showed that the model gave good predictions of toxicity for the test chemicals; the positive predictivity and specificity were high, at, respectively, 92.5 % and 95.2 %, whereas the sensitivity was lower (74.0 %).

3.2 Software

Two software are available for the prediction of LOAEL, both commercial. The first is Toxicity Prediction by Komputer Assisted Technology (TOPKAT), developed by Accelrys®. The TOPKAT model aims to predict chronic oral LOAEL in rats (studies lasting 12 or more months were considered) and has been described in Mumtaz et al. [34]. Starting from a dataset of 234 heterogeneous chemicals, the model was built using a stepwise regression analysis with 44 descriptors selected from an initial pool of electronic,

topological, symmetry descriptors and molecular connectivity indices. The performance of the model was tested comparing the predicted with the experimental LOAEL. About 55 % of the compounds were predicted within a factor of 2 and more than 93 % within a factor of 5 [34].

Over the years the TOPKAT model for LOAEL prediction has been refined, including more data in the training set. Using the expanded training set (393 chemicals), models for five chemical classes were developed (acyclics, alicyclics, heteroaromatics, single benzenes, and multiple benzenes). Venkatapathy et al. [35] tested the predictive performance of the five sub-modules using a large dataset of 656 substances and the R^2 ranged between 0.78 (multiple benzenes) and 0.98 (alicycles). TOPKAT was further validated by Tilaoui et al. [36] using 340 chemicals not included in the TOPKAT training set. TOPKAT correctly predicted (with an error lower than 1 log unit) only 33 % of these chemicals [16].

Another software for LOAEL prediction has been developed by Molcode Ltd. using RDT data in the rat. Information about this model is available from the QSAR Model Reporting Format (QRMF) document. The model is proprietary, but the training and test sets are available. The model was developed using multilinear regression, and the descriptors were chosen through a stepwise selection. There were 76 compounds in the training set, and in order to validate the real ability of the model to predict LOAEL, an external dataset containing 18 compounds was used. In terms of R^2, the performance of the Molcode model gave, respectively, 0.79 and 0.725 for the training and test set; a definition of applicability domain was also provided.

These software are not built using only pharmaceutical compounds. However, they can be used for the prediction of LOAEL for drugs.

4 Uncertainty of LOAEL and NOAEL Data

The development of non-animal testing for RDT is difficult mainly because the complex underlying processes, which include effects on different organs and tissues and different time scales [2]. NOAEL and LOAEL have been criticized as conceptually inappropriate for providing quantitative estimates for toxicity, and it has been proposed to replace them with the benchmark dose [37].

Besides the fact that many organs and tissues are involved, other aspects make the NOAEL and LOAEL data uncertain. NOAEL and LOAEL are not derived or calculated from a dose-effect curve but can only be identified from the doses. This means that they both depend on the study design, particularly the spaces between doses. Consequently, different NOAEL and LOAEL

values may be obtained for the same substance using different study designs or different exposure doses. There is a further intrinsic uncertainty in LOAEL experimental data. The "true" LOAEL (the real dose of the substance that starts to generate an effect) may be anywhere between the NOAEL and the LOAEL.

This uncertainly is probably big, but how big cannot be measured. This is another problem of the NOAEL and LOAEL approach, as in risk assessment quantifying the uncertainties involved is crucial for establishing protective human exposure limits [38]. The variability of the responses between animals in the dose groups, the definition of the "adversity" of an effect, and the statistical methods supporting this definition are other aspects that raise the level of uncertainty of NOAEL and LOAEL [39].

5 Conclusion

The NOAEL and LOAEL of substances are required for human health hazard assessments under different regulatory contexts (pharmaceutical, biocides, REACH, cosmetics) [2]. In vivo RDT studies are very expensive and time-consuming and involve a large number of animals. In vivo RDT has been banned for the safety assessment of cosmetics [1], and REACH legislation [10] requires to use as few animals as possible to evaluate the toxicity of substances. Therefore, there is a pressing need to find a valid alternative.

However, considering the uncertainty of NOAEL and LOAEL values, the in silico models are extremely complex because all this uncertainty will be implicitly transferred into the data predicted by a model. Moreover, considering the QSAR approach, there is a no solid mechanistic basis to support the statistical association between a set of molecular descriptors and the systemic effects [2].

Despite the limitations of each single alternative approach, the combination and interpretation of data from different alternative techniques, such as QSARs, physiologically based pharmacokinetic modeling (PBPK), read-across, threshold of toxicological concern (TTC), and in vitro methods, may be useful to gain more reliable predictions of NOAEL and LOAEL.

Acknowledgments

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