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REPDOSE: A database on repeated dose toxicity studies of commercial chemicals—A multifunctional tool

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Abstract

A database for repeated dose toxicity data has been developed. Studies were selected by data quality. Review documents or risk assessments were used to get a pre-screened selection of available valid data. The structure of the chemicals should be rather simple for well defined chemical categories. The database consists of three core data sets for each chemical: (1) structural features and physico-chemical data, (2) data on study design, (3) study results. To allow consistent queries, a high degree of standardization categories and glossaries were developed for relevant parameters. At present, the database consists of 364 chemicals investigated in 1018 studies which resulted in a total of 6002 specific effects. Standard queries have been developed, which allow analyzing the influence of structural features or PC data on LOELs, target organs and effects. Furthermore, it can be used as an expert system. First queries have shown that the database is a very valuable tool.

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1. Introduction

There are worldwide efforts on to evaluate the large number of Existing Chemicals. As the database is scarce for many compounds a large number of tests have to be performed. The completion of even the most basic toxicological testing of compounds of concern would take decades. A promising scientific alternative to minimize test requirements and to prioritize research needs could be the use of structure activity relationships (SARs) or quantitative structure activity relationships (QSARs). (Q)SAR approaches are increasingly applied by authorities, industry, and other institutions in the risk assessment of chemicals released to the environment and criteria are being developed for their use (Danish EPA, 2001; Eriksson et al., 2003; US EPA, 2000).

In the field of toxicology, several computer-aided commercial prediction systems are presently available, which use elements of (Q)SAR, e.g. TOPKAT, CASE/Multi-CASE, DEREK, ONCOLOGIC, and others. For their detailed description, critical evaluation and examples of worldwide use the reader is referred to the literature (e.g. Bristol et al., 1996; Cronin et al., 2003; Lewis et al., 1996; McKinney et al., 2000; Pöloth and Mangelsdorf, 1997; Richard, 1998; RIVM, 1999). Most of these (Q)SAR models/databases focus on mutagenicity and carcinogenicity, developmental toxicity, skin sensitization, respiratory sensitization, skin and eye irritation.

For repeated dose toxicity only limited tools are available. LOELs of repeated dose toxicity have been analyzed based on a database of 234 chemicals (Mumtaz et al., 1995). For over 93% of the data within the database the estimated LOELs were within a factor of 5 of the prediction. TOPKAT offers a QSAR module for the prediction of rat chronic LOELs (TOPKAT, 2003). A large reference database consisting of over 600 substances has been com-

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piled (Munro et al., 1996a,b). This database was the basis of the TTC concept (Threshold of toxicological concern, Kroes et al., 2004), which has proposed a threshold of human exposure below which “there would be not appreciable risk to human health”.

Substance group or category approaches have been used to assess repeated dose toxicity of certain groups of chemically related compounds (Greim et al., 1994, 1995) and common properties have been derived successfully. In single cases, special structural features have been related to specific target organs, for example the testes (ECETOC, 1995). However, the complex issue of relating specific organ toxicity, (e.g. cardiotoxicity, hepatotoxicity, renal toxicity) and/or specific toxic effects to certain structural features has not yet been addressed in a broader and systematic way.

Therefore, a database (REPDOSE) has been developed within the framework of the long-range research initiative of the Chemical Industry (CEFIC LRI). It focuses on adverse organ effects observed after repeated dosing and on the corresponding LOELs and NOELs. By applying specific queries, the relationships between structural features and other chemical properties and specific target organs and adverse effects can be assessed. The content and the function of the database are described in this publication.

2. Implementation of REPDOSE

The database is developed in Microsoft Access[®]. This program has been selected because it is commonly available and can be easily handled also by non-experts.

2.1. Selection criteria for chemicals and studies

Data on defined commercial organic chemicals with a limited number of functional groups have been used for constructing the database. Complex and multifunctional chemical structures like pharmaceuticals as well as inorganics, metal compounds and mixtures were excluded.

The chemicals which have been entered into REPDOSE have been selected by availability of suitable and reliable data. In consideration of the huge amount of data which is needed for a database comprising a large number of chemicals of different structures, peer-reviewed national and international documentations were used to pre-select chemicals with a well-founded data basis. These documents (i.e. German MAK¹-Documentations, BUA²-reports, EU Risk Assessments and EHC³-Documents) are based on publications/studies which are already approved for hazard

or risk assessment and cross-checked for their validity by expert groups. Relevant/important studies of high quality can be selected in an easy and time-saving way.

In general, studies were preferred, which follow the guidelines and/or were prepared for regulatory purposes. They should cover investigations of all critical organs for repeated dose toxicity as well as hematology, clinical chemistry, and histopathology. However, applying these criteria, the number of studies which could be entered into the database would be relatively low. Moreover, non-guideline studies addressing special questions may lead to lower LOELs on very specific target organs. Therefore, in addition to standard studies also special studies were included in the database. As indicator of study quality, studies are assigned to groups A, B, C or D, which are documented in a separate field for specification of the groups (see Table 1). A query selection can be used to distinguish between studies of different quality and scope.

Only oral or inhalation studies were entered into REPDOSE at the present stage. Dermal exposure is not given priority because information is available only for few compounds. Oral studies were further differentiated according to the way of administration: gavage, feeding, and drinking water studies. For inhalation studies, the body dose was calculated assuming 100% absorption.

The majority of repeated dose toxicity studies for industrial chemicals was performed in rats or mice. Therefore, to get comparable data about target organs, mechanisms of toxicity, and LOELs for different chemicals, the study selection was initially restricted to these two species.

Studies with exposure durations from 14 days up to lifetime exposure were selected. If several reliable studies were available, all were included in the database. This might for example be useful to cover different endpoints and to analyze the influence of the study duration on several endpoints. Moreover, by this selection strategy also contradictory results can be revealed.

2.2. Database content

2.2.1. Structure

In order to group structurally related compounds, chemicals were characterized by their functional groups. Obviously, one compound can have several functional groups. In addition, chemicals were assigned to categories. Categories are meant to include several functional groups, for example, aliphatic ethanol amines. One compound might belong to different categories.

2.2.2. PC-data

The following physico-chemical data were included in the database: molecular weight, solubility in water, physical state, boiling point, dissociation constant, octanol-water partition coefficient ($\log K_{ow}$) and vapor pressure. Physico-chemical data were obtained as far as possible from reviews, as well as from data compilations (i.e. Merck Index 12th ed., 1996; Handbook of Environmental Data

¹ MAK values: Maximum Concentrations at the Workplace recommended by the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area.

² BUA: German Advisory Board for Existing Chemicals.

³ EHC: Environmental Health Criteria published by the World Health Organization.

Table 1
Documentation of study quality

Group	Characterisation
A	Study according to OECD guidelines or study of similar quality
B	Study with some deficiencies, but nevertheless relevant for the overall evaluation of a chemical
C	Quality of a study cannot be assessed because of insufficient information. Information is considered relevant for the overall evaluation of the chemical
D	Special study designed for refined investigation of a certain endpoint

Table 2
Example for effect entry into database for one study

Organ	Effect	LOEL (mg/kg bw/d)
Blood	Anaemia	359
Body weight	Weight decreased	720
Clinical chemistry	Triglyceride	720
	Cholesterol	1540
Endocrine system	Changed hormone status	1540
Epididymis	Weight decreased	2964
Liver	Changed enzyme activity	359
	Changes in organ structure	359
	Proliferation	359
	Cholestasis	720
	Glycogen	720
	Eosinophilic structures	2964
Testes	Degeneration	720
	Spermatocytes abnormal	1540
	Spermiogenesis impaired	1540
	Weight decreased	1540
Overall LOEL		359
Overall NOEL		176

on Organic Chemicals, K. Verschueren, 3rd ed., 1996; Occupational Toxicants by DFG, 1991ff; The physical and theoretical chemistry laboratory at Oxford University–chemical and other safety information (<http://physchem.ox.ac.uk/MSDS>); International chemical safety cards (www.itcilo.it/english/actrav/telearn/osh/kemi/icsc1.htm)).

2.2.3. Toxicological data

Concerning the toxicological data it was distinguished between study data and effect data. Study data include the specification of the animals (strain, sex, number per dose group) and the exposure information (i.e. exposure duration, route of application, post-exposure observation period and dose groups). Effect data include all target organs with all associated effects and corresponding LOELs. Several effects may appear in one target organ at different dose levels; Table 2 shows an example of data for a single study, entered into the database. Besides the LOELs for the single effects, the overall lowest observed effect level (LOEL) and the overall no observed effect level (NOEL) are documented.

2.2.4. Standardization of entries

For a comprehensible analysis of chemical characteristics and toxic effects the database entries have to be uniform and standardized. For this purpose glossaries have been developed for all fields, which can be addressed by

standard queries (see chapter 3.5). They are available for the defined fields: category, functional group, mechanism, species, strain, sex, route of exposure, organ, and effect. Most of these glossaries have been adapted and completed during data entry and are still under validation. New entries can easily be added to the glossary. Fig. 1 gives an example for effects covered in the glossary. It is distinguished between general effects, which can occur in several organs, e.g. hyperplasia or irritation, and organ specific effects.

The values for physico–chemical endpoints show high discrepancies between different data sources. This raised problems for standardized data entries and reasonable query functions. Moreover, the endpoint water solubility, for example includes the chemical features insoluble or miscible. Therefore, data categories which cover a range were applied for all physico–chemical endpoints. In addition, the most realistic value obtained via expert judgement was entered into the database.

2.3. Query functions

For general and easy use, a query screen (see Fig. 2) has been developed, which allows several types of questions concerning the influence of structural features and physico–chemical data on LOELs, target organs and effects.

All possible query parameters (chemical category, functional group, physico–chemical data, species, route, organ/organ system, sex) can be combined in a logical AND manner. Combinations of more than one functional group are linked as logical AND, e.g. “aliphatic” AND “aldehyde”, to characterize chemicals from one category which contain both structural features. Different routes of application can be combined as logical OR, e.g. “food” OR “drinking water” OR “gavage”, to cover all oral application forms (see Fig. 2).

The query parameter sex can be used e.g. to select only those studies, which cover investigations in both sexes.

According to different query parameters, different query reports are generated.

3. Present status of the database

At present REPDOSE consists of 364 chemicals investigated in 1018 studies which resulted in 6002 specific effects.

Effect	Effect Number	Organ
hyperexcitability	260	clinical symptoms
hyperkeratosis	118	
hyperparathyroidism	135	thyroid gland
hyperplasia	74	
hypertrophy	147	
hypoactivity	254	clinical symptoms
hypoplasia	117	
hypotension	152	heart
hypothermia	348	clinical symptoms
infiltration	284	
inflammation	355	clinical symptoms
inflammation	5	
iodine accumulation	66	thyroid gland
irritation	96	
karyomegaly	31	
ketonuria	375	urine analysis
kidney stones	211	kidney

Fig. 1. Glossary for effects and organ in REPDOSE.

The screenshot shows the 'Query' interface of the REPDOSE database. It is divided into four main sections: Chemical Parameter, P/C-Parameter, Study-Parameter, and Organ/Effect-Parameter. Each section contains several input fields and dropdown menus for searching. A 'Main menu' button is located in the top right corner. The 'Chemical Parameter' section includes CAS, Category, and Fun. Group. The 'P/C-Parameter' section includes W.Solubility, Log POW, V.Pressure, and Molecular weight. The 'Study-Parameter' section includes Species, Route, Duration, Sex, mg/kg bw/d, oNOEL, mmol, mg/kg bw/d, oLOEL, mmol, and Reliability (A, B, C, D). The 'Organ/Effect-Parameter' section includes Organ, Organ-System, Sex, and Effect. There are also 'and' and 'or' operators and a 'fun. group-system' button.

Fig. 2. Query screen of REPDOSE.

The LOELs of all chemicals in the database so far range from 0.006 to 68,907 mg/kg body weight per day. Most chemicals have LOELs between 10 and 1000 mg/kg bw per day (see Fig. 3).

4. Application of the database

The very detailed information given in this database facilitates highly specific queries; thereby the database represents a multifunctional tool which can be used for several purposes:

Unidirected/monocausal SAR queries, i.e.:

- The relationship between chemical structures/functional groups and target organs for chemical toxicity.
- The relationship between chemical structures/functional groups and specific effects in target organs.
- The relationship between chemical structures/functional groups and NOELs/LOELs.
- The influence of physico-chemical properties (e.g. water solubility) on NOELs/LOELs.

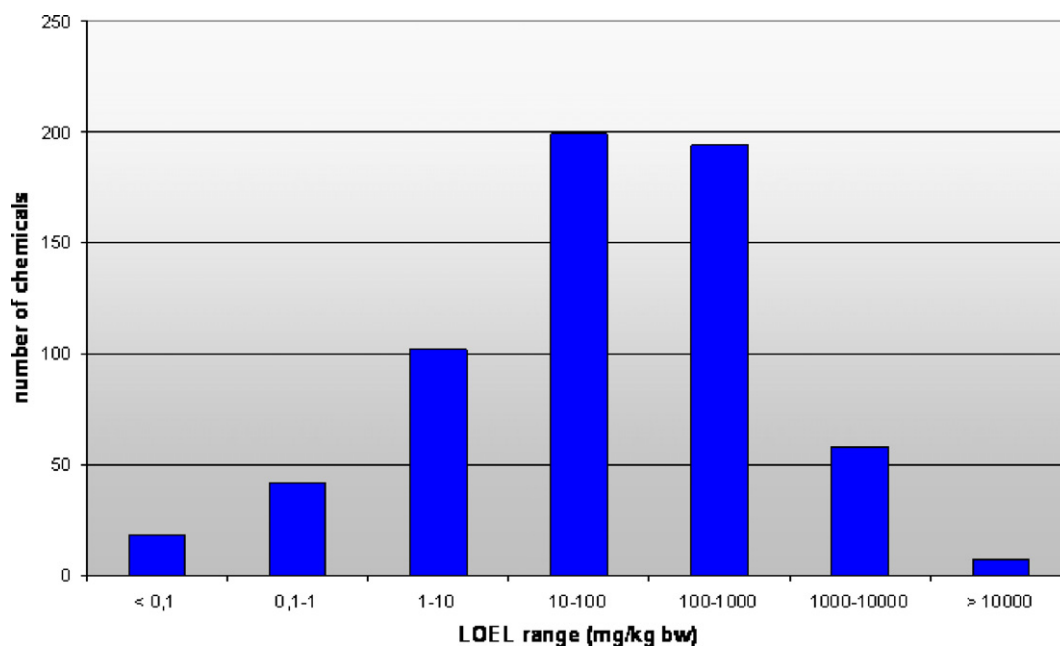


Fig. 3. Distribution of LOELs in REPDOSE (studies with rats and mice, oral and inhalation studies).

Complex SAR queries, i.e.:

- The relationship between combinations of chemical structures and physico-chemical properties and target organs and effects

Non-SAR queries, i.e.:

- Common target organs
- The sensitivity of certain target organs by their effect LOELs
- The influence of exposure duration on overall NOELs/LOELs
- The influence of exposure routes on target organs or NOELs/LOELs
- The sex-specificity of certain effects
- The susceptibility of different rat or mouse strains

As demonstrated by this list of queries, not only questions concerning SAR, i.e. the influence of structural features on target organs, effects, and NOELs, can be addressed, but also questions relating to the influence of physico-chemical data on target organs, effects and NOELs, as well as very general toxicological questions relating e.g. to the influence of exposure duration on overall NOELs/LOELs or the sex-specificity of certain effects.

Moreover, the database may serve as an expert system for evaluation of data within a group of structurally related substances. For example, effects which are often not significant in single studies (e.g. due to delayed appearance or due to lack of apparent time- or concentration-dependence) may also have been observed with structurally related substances, thereby increasing confidence on the results obtained.

Besides the queries, the database enables a comfortable, well structured, and transparent possibility for data compilation and data administration.

As the database can help to identify structures which can be suspected to have low NOELs, it can be used for the purpose of priority setting for further testing of chemicals in such a way that chemicals with suspected low NOELs should be tested prior to compounds with suspected high NOELs. By this way the database helps to structure the work on those 'existing chemicals', where repeated dose toxicity studies are missing.

Some examples for applications of the database are given in the following:

Some structurally related chemicals are known to have common toxicological properties. For example, it is known that the phthalates are liver toxicants and they also affect reproduction. This knowledge can also be reproduced with the REPDOSE database. Currently 7 phthalates are in the database. Only for 6 phthalates high quality studies (A) in rats are available. The main target organs of the phthalates in rats in comparison to the target organs in the overall database are shown in Table 3. All 6 phthalates produce effects in the liver, the kidney, and the blood. However, these targets are very common also for other chemicals. In addition, 4 phthalates cause effects in the testes such as impaired spermatogenesis and degeneration. As the testes are less frequently affected in the overall database, this is a clear indication that toxicity to the testes is a specific effect of the phthalates. Other, but less frequently reported targets, of the phthalates include the thyroid and the intestine showing effects on organ weight and/or histopathology.

Table 3
Main target organs and LOELs for 6 phthalates

Parameter	Intestine		Liver		Kidney		Blood		Thyroid		Testes	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Phthalates with organ affected/all Phthalates	3/6	50	6/6	100	5/6	83	4/6	67	2/6	33	4/6	67
Chemicals with organ affected/Chemicals in overall database	15/279	5	247/279	88	135/279	48	101/279	36	37/279	13	58/279	21

Parameters for analysis: Phthalates: Diallyl phthalate, diisodecylphthalate, di-*n*-butyl phthalate, diethyl phthalate, di-sec-octyl phthalate, butyl benzyl phthalate; Species: rat; Study quality: highest quality (reliability A); Study duration: any (not limited).

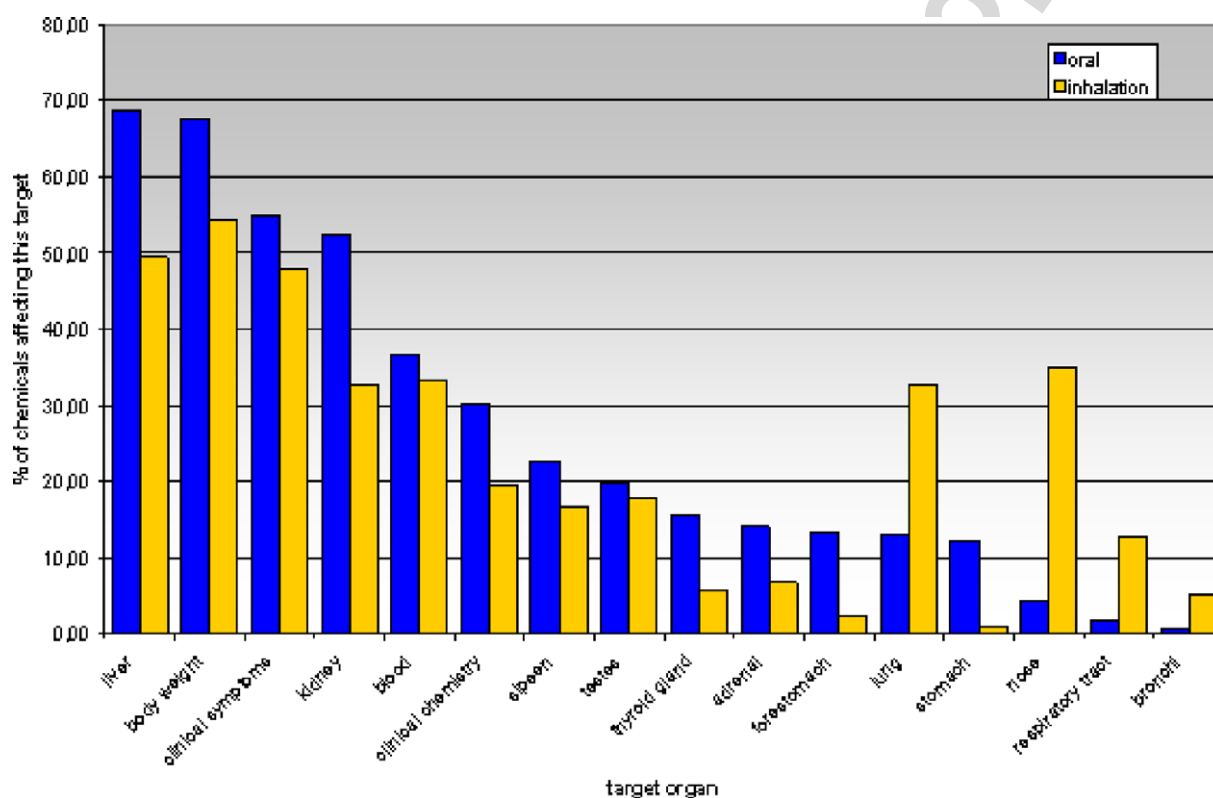


Fig. 4. Major targets in repeated dose toxicity studies with rats and mice.

Table 4
Sensitivity of reproductive organs in rats and mice in relation to other organs: a comparison on the basis of LOELs

	Testes		Uterus	
	Number of compounds	Number of studies	Number of compounds	Number of studies
Effect in testes/uterus	86	124	23	27
LOEL testes/uterus > overall LOEL*	53	66	14	16
LOEL testes/uterus = overall LOEL	46	58	10	10
Subsets (Studies analysed for additional effects in other target organs)				
LOEL testes/uterus = overall LOEL and LOEL other target organs = LOEL testes/uterus	40	48	7	7
LOEL testes/uterus as sole determinant of overall LOEL	8	9	3	3
LOEL data from special reproductive study	1	1	1	1

Overall LOEL: lowest LOEL within one toxicity study checking different targets (LOEL for this study).

In general, all those organs which are frequently affected by one specific chemical category but not from the overall compounds in the database demand attention.

Very general queries can also be performed. The liver is the most common target organ in repeated dose toxicity studies (Fig. 4). However, the body weight and the kidneys

are frequently affected. As expected, local effects in the lung/respiratory tract are observed in inhalation studies. Systemic effects occur frequently in the lung. In more than 10% of the oral studies, effects in the lung have been found.

Currently there is much concern on reproductive toxicity of chemicals. As described in Mangelndorf et al. (2003), results from repeated dose toxicity studies, such as increased/decreased testes weights and histopathology of the testes are sensitive indicators of male reproductive toxicity. In Table 4 it was investigated, whether reproductive endpoints are especially sensitive, i.e. that toxicity to the testes determines the overall toxicity. It turned out, that usually effects on the testes occur at higher dose levels than other effects. In several of the cases where the effects on the testes determined the overall LOEL Leydig cell tumors were described as critical effects. Some other studies had evaluated only effects on the testes. The only compound which really revealed the testes as most sensitive organ so far was ethoxy ethanol. Effects observed were degeneration of the germinal epithelium, decreased testes weights and atrophy of the testes. Therefore, it might be concluded that the toxicity to testes is not necessarily a very critical and sensitive effect in repeated dose toxicity studies.

The same analysis was performed for the uterus. Here the number of compounds/studies revealing effects was considerably lower.

Another general toxicological question, which can be addressed with our database relates to the influence of exposure duration on the NOELs/LOELs in toxicological studies. Fig. 5 shows the decrease in the overall LOELs with increasing exposure duration for the studies covered in our database. Although there are considerable differences in the LOELs for the individual chemicals, the

overall regression is significant. In a more specific analysis (Fig. 6), pairs of studies with different exposure durations for the same chemicals in the same species have been compared. On average the LOELs in subchronic studies is a factor of 1.5 lower than in subacute studies and the LOEL in chronic studies is by a factor of 2 lower than in subchronic studies. Similar values have been obtained in other analyzes (Kalberlah and Schneider, 1998).

5. Discussion

For refined (Q)SAR analyses it would be desirable to use only toxicity measurements made by a single laboratory following a single protocol (Cronin and Schultz, 2003). The existing data for repeated dose toxicity mostly do not fulfill this criterion. On the contrary, there is a broad variety in experimental design of a large number of tests.

Nevertheless, a suitable collection and meta-analysis of world-wide spread data may support a SAR analysis in several ways and is intended by the present database which provides numerous parallel datasets on substance characteristics and toxic effect levels.

In addition to the standard guideline studies also other peer-reviewed studies were included in the database because: (1) lower NOELs may be detected, (2) mechanisms may be indicated or (3) new target organs may be detected, (4) conflicting results in different studies may be detected or resolved. Moreover, the database on several compounds is not optimal even if full risk assessments have been performed.

It could be argued that consequential variations in the study quality may render queries less significant and pre-

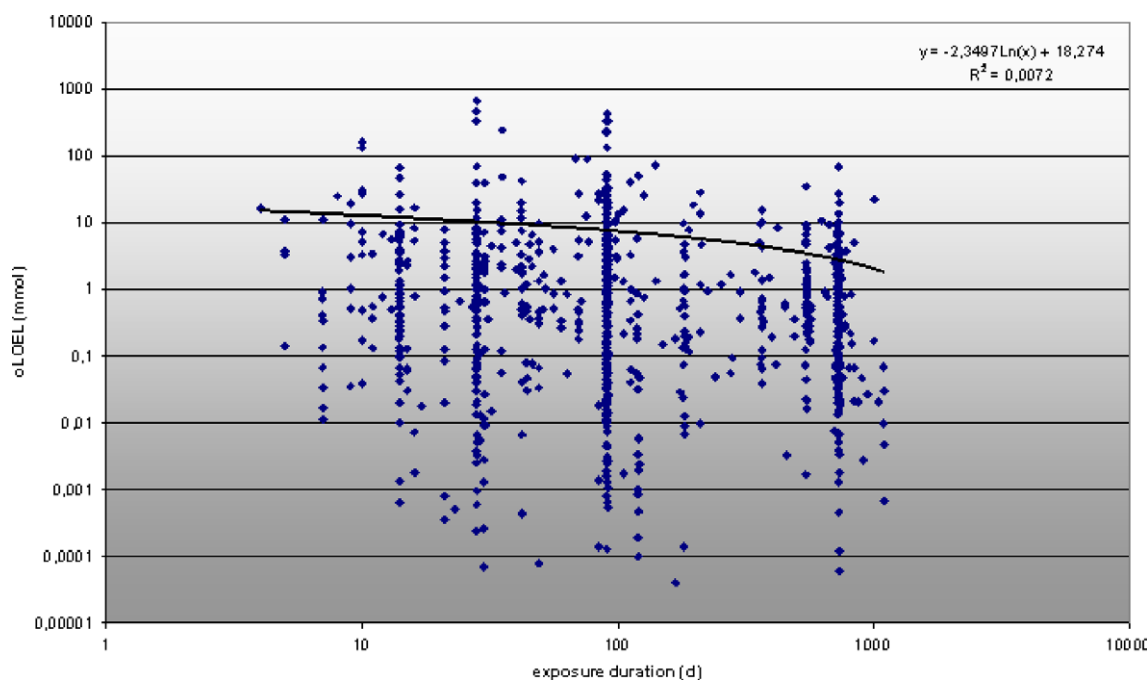


Fig. 5. LOELs depending on study duration (all chemicals, oral, and inhalation studies with rats and mice).

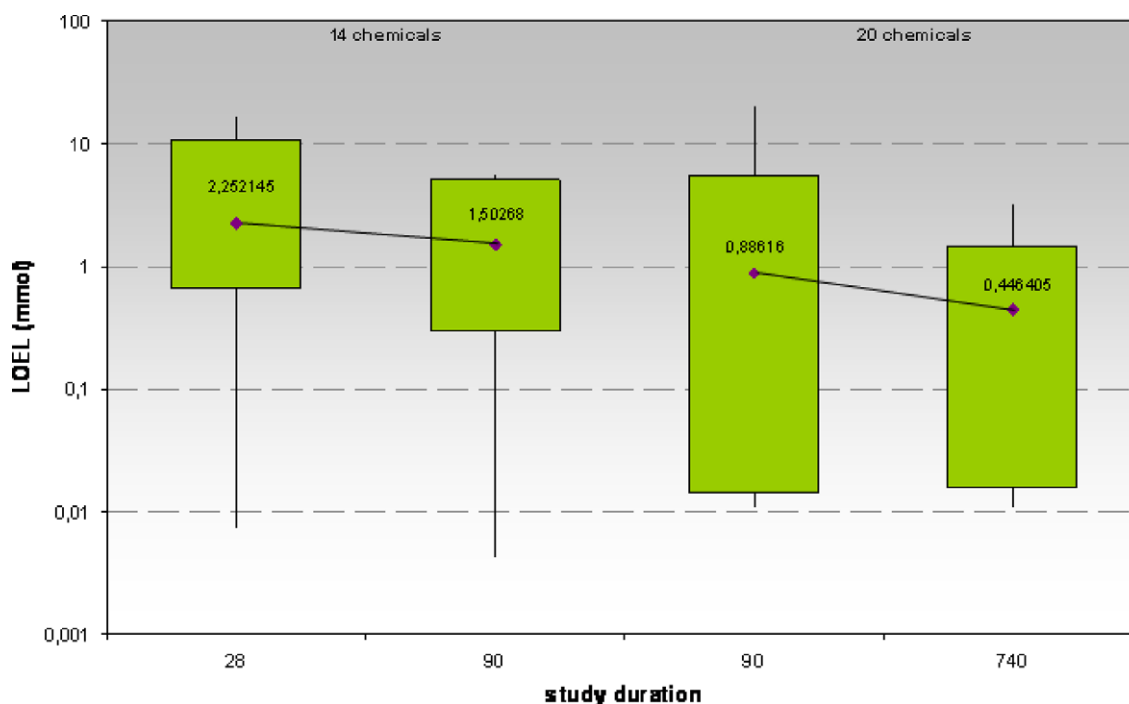


Fig. 6. LOELs depending on study duration (pairs of studies for individual chemicals, only studies in and with oral application have been evaluated).

dictive; therefore, studies were assigned to certain categories of quality or scope. It is a case-by-case decision whether a differentiation between studies of different quality or complexity should be done or not.

Another point concerning the quality of the entries relates to the fact that the study data were basically extracted from reviews. This use of pre-selected data with differing details of information might be a (major) disadvantage of the database. Nevertheless, this approach was accepted with regard to time economy for searching and evaluating relevant data. However, the detail of information may be sometimes weak and this has to be marked in the database. At a later stage those entries could be improved by consulting the primary reference.

The value and the usefulness of this SAR database depend very much on a standardized data entry which provides the basis for a comparability of individual data sets. Much effort was necessary for the development of appropriate glossaries. In particular, it was difficult to determine which level of detail is necessary to describe toxic effects precisely but still general enough to derive common rules. For example, several liver enzymes such as alanine-amino-transferase indicate liver damage. Instead of including every individual enzyme the more general term “liver enzymes changed” is used and an exact specification is given in the free text field “additional”.

The database includes both NOELs and LOELs. This allows assessing compounds where no NOEL could be derived, because effects occurred already at the lowest dose level, as well as compounds where no LOEL was found, because even the highest tested dose was without effect. Thus, our database allows analyzing all types of studies.

As demonstrated above, a considerable number of chemicals are already included in the database. However, it is desirable to base the SAR queries on a higher number of compounds in order to obtain statistically significant results. Interesting preliminary results which were obtained by our first queries already underline the considerable value of this project. These results will be published at a later stage, as soon as the number of studies covered has increased and allows statistical evaluation. Therefore, our primary improvement of the database will address the number of chemicals. In addition, a further extension of the general query function would create this feature more conveniently for other (internet) users.

Currently, chemical structures are captured by giving the formula and noting the functional groups which are contained in the structure. This allows only a very rough analysis of the impact of structural features on toxicity, as the neighbourhood which is essential for the chemical and toxicological properties of a chemical, is not captured. Therefore, to allow substructure searching it is planned to combine our database with another database which will allow search for chemical structures by drawing. The AMBIT database developed in another CEFIC LRI project is one that might be useful for this purpose. Furthermore, modern tools of similarity searching (Nikolova and Jaworska, 2003) will be applied to our database with the purpose of finding common properties for groups with similar structural or physico-chemical features.

It is planned to render the database publicly available in the Internet for registered users in the near future.

The database structure would allow easy extensions in future; additional endpoints like the sensitizing potential

of chemicals or reproductive toxicity could be evaluated. More specific substance-related information i.e. about metabolism and/or reactive metabolites could also be included in the SAR search. The inclusion of other species and routes of exposure would also be desirable to get more insight in route-to-route and interspecies variability.

Thus, the present database includes a lot of future potential with respect to useful query functions as well as to possible extensions.

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