

# Modeling Chronic Toxicity: A comparison of experimental variability with read across predictions

Christoph Helma<sup>1</sup>, David Vorgrimm<sup>1</sup>, Denis Gebele<sup>1</sup>, Martin Gtlein<sup>2</sup>, Benoit Schilter<sup>3</sup>, Elena Lo Piparo<sup>3</sup>

E-mail:

<sup>1</sup> in silico toxicology gmbh, Basel, Switzerland

<sup>2</sup> Inst. f. Computer Science, Johannes Gutenberg Universitt Mainz, Germany

<sup>3</sup> Chemical Food Safety Group, Nestl Research Center, Lausanne, Switzerland

## Introduction

Relying on standard animal toxicological testing for chemical hazard identification and characterization is increasingly questioned on both scientific and ethical grounds. In addition, it appears obvious that from a resource perspective, the capacity of standard toxicology to address the safety of thousands of untested chemicals (Fowler, Savage, and Mendez 2011) to which human may be exposed is very limited. It has also been recognized that getting rapid insight on toxicity of chemicals in case of emergency safety incidents or for early prioritization in research and development (safety by design) is a big challenge mainly because of the time and cost constraints associated with the generation of relevant animal data. In this context, alternative approaches to obtain timely and fit-for-purpose toxicological information are being

developed. Amongst others, non-testing, structure-activity based *in silico* toxicology methods (also called computational toxicology) are considered highly promising. Importantly, they are raising more and more interests and getting increased acceptance in various regulatory (e.g. (ECHA 2008, EFSA (2016), EFSA (2014), Health Canada (2016), OECD (2015))) and industrial (e.g. (Stanton and Krusezewski 2016, Lo Piparo et al. (2011))) frameworks.

For a long time already, computational methods have been an integral part of pharmaceutical discovery pipelines, while in chemical food safety their actual potentials emerged only recently (Lo Piparo et al. 2011). In this later field, an application considered critical is in the establishment of levels of safety concern in order to rapidly and efficiently manage toxicologically uncharacterized chemicals identified in food. This requires a risk-based approach to benchmark exposure with a quantitative value of toxicity relevant for risk assessment (Schilter et al. 2014). Since most of the time chemical food safety deals with life-long exposures to relatively low levels of chemicals, and because long-term toxicity studies are often the most sensitive in food toxicology databases, predicting chronic toxicity is of prime importance. Up to now, read across and quantitative structure-activity relationship (QSAR) have been the most used *in silico* approaches to obtain quantitative predictions of chronic toxicity.

The quality and reproducibility of (Q)SAR and read-across predictions has been a continuous and controversial topic in the toxicological risk-assessment community. Although model predictions can be validated with various procedures, to review results in context of experimental variability has actually been rarely done or attempted. With missing information about the variability of experimental toxicity data it is hard to judge the performance of predictive models objectively and it is tempting for model developers to use aggressive model optimisation methods that lead to impressive validation results, but also to overfitted models with little practical relevance.

In the present study, automatic read-across like models were built to generate quantitative

predictions of long-term toxicity. Two databases compiling chronic oral rat lowest adverse effect levels (LOAEL) as endpoint were used. An early review of the databases revealed that many chemicals had at least two independent studies/LOAELs. These studies were exploited to generate information on the reproducibility of chronic animal studies and were used to evaluate prediction performance of the models in the context of experimental variability.

An important limitation often raised for computational toxicology is the lack of transparency on published models and consequently on the difficulty for the scientific community to reproduce and apply them. To overcome these issues, all databases and programs that have been used to generate this manuscript are made available under GPL3 licenses.

A self-contained docker image with all programs, libraries and data required for the reproduction of these results is available from <https://hub.docker.com/r/insilicotox/loael-paper/>.

Source code and datasets for the reproduction of this manuscript can be downloaded from the GitHub repository <https://github.com/opentox/loael-paper>. The lazar framework (A. Maunz et al. 2013) is also available under a GPL3 License from <https://github.com/opentox/lazar>.

A graphical webinterface for **lazar** model predictions and validation results is publicly accessible at <https://lazar.in-silico.ch>, models presented in this manuscript will be included in future versions. Source code for the GUI can be obtained from <https://github.com/opentox/lazar-gui>.

## Materials and Methods

The following sections give a high level overview about algorithms and datasets used for this study. In order to provide unambiguous references to algorithms and datasets, links to source code and data sources are included in the text.

## Datasets

### Nestl database

The first database (Nestl database for further reference) originates from the publication of (P. Mazzatorta et al. 2008). It contains chronic ( $> 180$  days) lowest observed effect levels (LOAEL) for rats (*Rattus norvegicus*) after oral (gavage, diet, drinking water) administration. The Nestl database consists of 567 LOAEL values for 445 unique chemical structures. The Nestl database can be obtained from the following GitHub links: [original data](#), [unique smiles](#), [-log10 transformed LOAEL](#).

### Swiss Food Safety and Veterinary Office (FSVO) database

Publicly available data from pesticide evaluations of chronic rat toxicity studies from the European Food Safety Authority (EFSA) (EFSA 2014), the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (WHO 2011) and the US EPA (US EPA 2011) were compiled to form the FSVO-database. Only studies providing both an experimental NOAEL and an experimental LOAEL were included. The LOAELs were taken as they were reported in the evaluations. Further details on the database are described elsewhere (Zarn, Engeli, and Schlatter 2011, Zarn, Engeli, and Schlatter (2013)). The FSVO-database consists of 493 rat LOAEL values for 381 unique chemical structures. It can be obtained from the following GitHub links:

[original data](#), [unique smiles](#) and [mmol/kg\\_bw/day units](#), [-log10 transformed LOAEL](#).

## Preprocessing

Chemical structures (represented as SMILES (Weininger 1988)) in both datasets were checked for correctness. When syntactically incorrect or missing SMILES were generated from other

identifiers (e.g names, CAS numbers). Unique smiles from the OpenBabel library (OBoyle et al. 2011) were used for the identification of duplicated structures.

Studies with undefined or empty LOAEL entries were removed from the datasets. LOAEL values were converted to mmol/kg\_bw/day units and rounded to five significant digits. For prediction, validation and visualisation purposes -log10 transformations are used.

## Derived datasets

Two derived datasets were obtained from the original databases:

The *test dataset* contains data from compounds that occur in both databases. LOAEL values equal at five significant digits were considered as duplicates originating from the same study/publication and only one instance was kept in the test dataset. The test dataset has 375 LOAEL values for 155 unique chemical structures and was used for

- evaluating experimental variability
- comparing model predictions with experimental variability.

The *training dataset* is the union of the Nestl and the FSVO databases and it was used to build predictive models. LOAEL duplicates were removed using the same criteria as for the test dataset. The training dataset has 998 LOAEL values for 671 unique chemical structures.

## Algorithms

In this study we are using the modular lazar (*lazy structure activity relationships*) framework (A. Maunz et al. 2013) for model development and validation. The complete **lazar** source code can be found on [GitHub](#).

lazar follows the following basic *workflow*:

For a given chemical structure lazar

- searches in a database for similar structures (*neighbors*) with experimental data,
- builds a local QSAR model with these neighbors and
- uses this model to predict the unknown activity of the query compound.

This procedure resembles an automated version of *read across* predictions in toxicology, in machine learning terms it would be classified as a *k-nearest-neighbor* algorithm.

Apart from this basic workflow *lazar* is completely modular and allows the researcher to use any algorithm for similarity searches and local QSAR modelling. Within this study we are using the following algorithms:

## Neighbor identification

Similarity calculations are based on [MolPrint2D fingerprints](#) (Bender et al. 2004) from the OpenBabel chemoinformatics library (OBoyle et al. 2011).

The MolPrint2D fingerprint uses atom environments as molecular representation, which resemble basically the chemical concept of functional groups. For each atom in a molecule it represents the chemical environment using the atom types of connected atoms.

MolPrint2D fingerprints are generated dynamically from chemical structures and do not rely on predefined lists of fragments (such as OpenBabel FP3, FP4 or MACCs fingerprints or lists of toxocophores/toxicophobes). This has the advantage they may capture substructures of toxicological relevance that are not included in other fingerprints. Unpublished experiments have shown that predictions with MolPrint2D fingerprints are indeed more accurate than other OpenBabel fingerprints.

From MolPrint2D fingerprints we can construct a feature vector with all atom environments of a compound, which can be used to calculate chemical similarities.

The [chemical similarity](#) between two compounds A and B is expressed as the proportion between atom environments common in both structures  $A \cap B$  and the total number of atom

environments  $A \cup B$  (Jaccard/Tanimoto index, Equation 1).

$$sim = \frac{|A \cap B|}{|A \cup B|} \quad (1)$$

The threshold selection is a trade-off between prediction accuracy (high threshold) and the number of predictable compounds (low threshold). As it is in many practical cases desirable to make predictions even in the absence of closely related neighbors, we follow a tiered approach:

First a similarity threshold of 0.5 is used to collect neighbors, to create a local QSAR model and to make a prediction for the query compound. If any of this steps fail, the procedure is repeated with a similarity threshold of 0.2 and the prediction is flagged with a warning that it might be out of the applicability domain of the training data.

Compounds with the same structure as the query structure are automatically **eliminated from neighbors** to obtain unbiased predictions in the presence of duplicates.

## Local QSAR models and predictions

Only similar compounds (*neighbors*) above the threshold are used for local QSAR models. In this investigation we are using **weighted random forests regression (RF)** for the prediction of quantitative properties. First all uninformative fingerprints (i.e. features with identical values across all neighbors) are removed. The remaining set of features is used as descriptors for creating a local weighted RF model with atom environments as descriptors and model similarities as weights. The RF method from the **caret** R package (Kuhn 2008) is used for this purpose. Models are trained with the default **caret** settings, optimizing the number of RF components by bootstrap resampling.

Finally the local RF model is applied to **predict the activity** of the query compound. The RMSE of bootstrapped local model predictions is used to construct 95% prediction intervals

at  $1.96 \times \text{RMSE}$ .

If RF modelling or prediction fails, the program resorts to using the **weighted mean** of the neighbors LOAEL values, where the contribution of each neighbor is weighted by its similarity to the query compound. In this case the prediction is also flagged with a warning.

## Applicability domain

The applicability domain (AD) of lazar models is determined by the structural diversity of the training data. If no similar compounds are found in the training data no predictions will be generated. Warnings are issued if the similarity threshold has to be lowered from 0.5 to 0.2 in order to enable predictions and if lazar has to resort to weighted average predictions, because local random forests fail. Thus predictions without warnings can be considered as close to the applicability domain and predictions with warnings as more distant from the applicability domain. Quantitative applicability domain information can be obtained from the similarities of individual neighbors.

Local regression models consider neighbor similarities to the query compound, by weighting the contribution of each neighbor is by its similarity. The variability of local model predictions is reflected in the 95% prediction interval associated with each prediction.

## Validation

For the comparison of experimental variability with predictive accuracies we are using a test set of compounds that occur in both databases. Unbiased read across predictions are obtained from the *training* dataset, by **removing all information** from the test compound from the training set prior to predictions. This procedure is hardcoded into the prediction algorithm in order to prevent validation errors. As we have only a single test set no model or parameter optimisations were performed in order to avoid overfitting a single dataset.



Results from 3 repeated 10-fold crossvalidations with independent training/test set splits are provided as additional information to the test set results.

The final model for production purposes was trained with all available LOAEL data (Nestl and FSVO databases combined).

## Availability

**Public webinterface** <https://lazar.in-silico.ch>

**lazar framework** <https://github.com/opentox/lazar> (source code)

**lazar GUI** <https://github.com/opentox/lazar-gui> (source code)

**Manuscript** <https://github.com/opentox/loael-paper> (source code for the manuscript and validation experiments)

**Docker image** <https://hub.docker.com/r/insilicotox/loael-paper/> (container with manuscript, validation experiments, **lazar** libraries and third party dependencies)

## Results

### Dataset comparison

The main objective of this section is to compare the content of both databases in terms of structural composition and LOAEL values, to estimate the experimental variability of LOAEL values and to establish a baseline for evaluating prediction performance.

### Structural diversity

In order to compare the structural diversity of both datasets we evaluated the frequency of functional groups from the OpenBabel FP4 fingerprint. Figure 1 shows the frequency of functional groups in both datasets. 139 functional groups with a frequency > 25 are depicted,

197 the complete table for all functional groups can be found in the supplemental material at  
198 [GitHub](#).

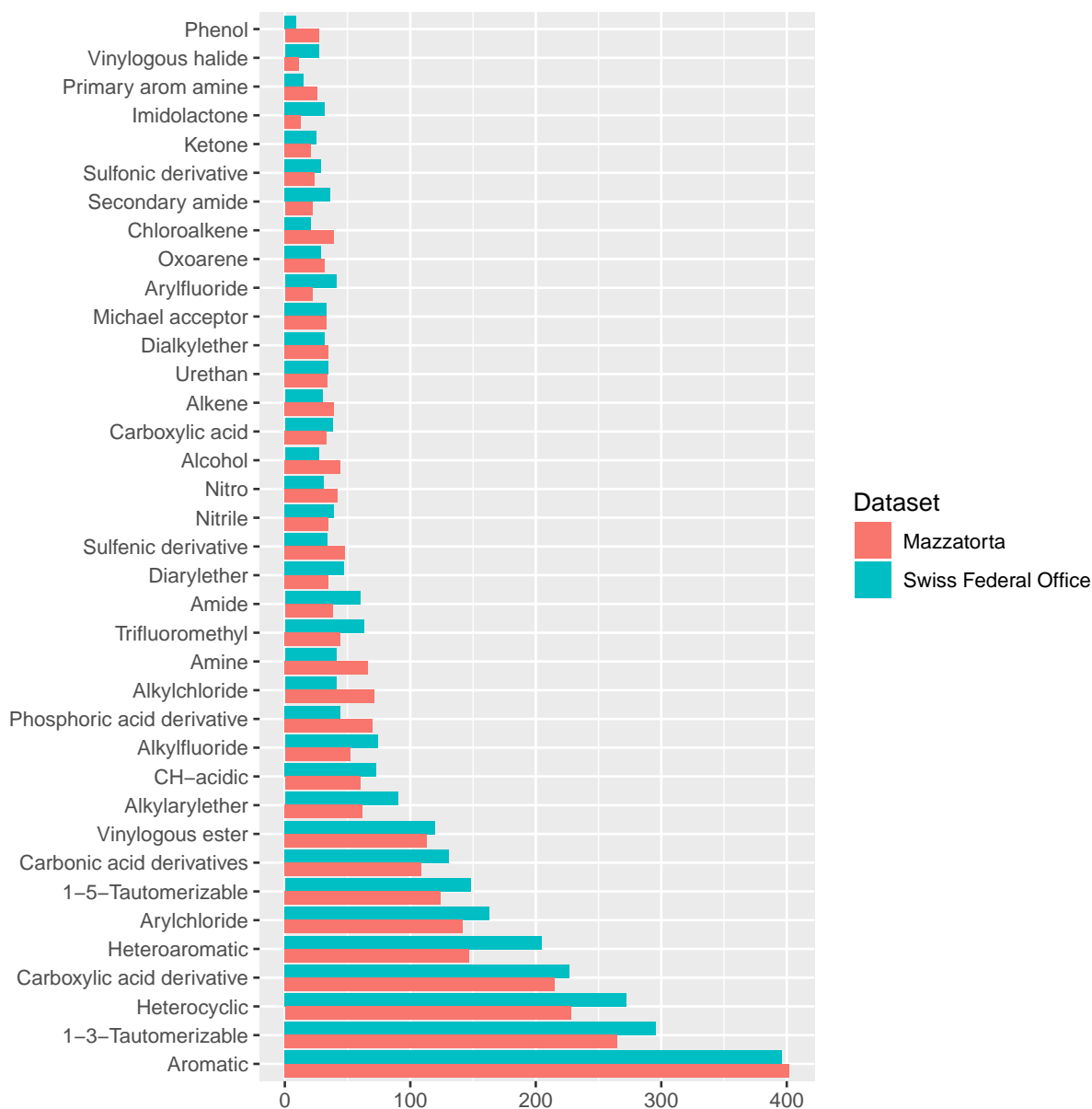


Figure 1: Frequency of functional groups.

199 This result was confirmed with a visual inspection using the [CheS-Mapper](#) (Chemical Space  
200 Mapping and Visualization in 3D, Gütlein, Karwath, and Kramer (2012)) tool. CheS-Mapper  
201 can be used to analyze the relationship between the structure of chemical compounds, their  
202 physico-chemical properties, and biological or toxic effects. It depicts closely related (similar)

compounds in 3D space and can be used with different kinds of features. We have investigated structural as well as physico-chemical properties and concluded that both datasets are very similar, both in terms of chemical structures and physico-chemical properties.

The only statistically significant difference between both datasets, is that the Nestl database contains more small compounds (61 structures with less than 11 atoms) than the FSVO-database (19 small structures, p-value 3.7E-7).

### Experimental variability versus prediction uncertainty

Duplicated LOAEL values can be found in both datasets and there is a substantial number of 155 compounds with more than one LOAEL. These chemicals allow us to estimate the variability of experimental results within individual datasets and between datasets. Data with *identical* values (at five significant digits) in both datasets were excluded from variability analysis, because it is likely that they originate from the same experiments.

### Intra database variability

The Nestl database has 567 LOAEL values for 445 unique structures, 93 compounds have multiple measurements with a mean standard deviation (-log10 transformed values) of 0.32 (0.56 mg/kg\_bw/day, 0.56 mmol/kg\_bw/day) (P. Mazzatorta et al. (2008), Figure 2).

The FSVO database has 493 rat LOAEL values for 381 unique structures, 91 compounds have multiple measurements with a mean standard deviation (-log10 transformed values) of 0.29 (0.57 mg/kg\_bw/day, 0.59 mmol/kg\_bw/day) (Figure 2).

Standard deviations of both datasets do not show a statistically significant difference with a p-value (t-test) of 0.21. The combined test set has a mean standard deviation (-log10 transformed values) of 0.33 (0.56 mg/kg\_bw/day, 0.55 mmol/kg\_bw/day) (Figure 2).

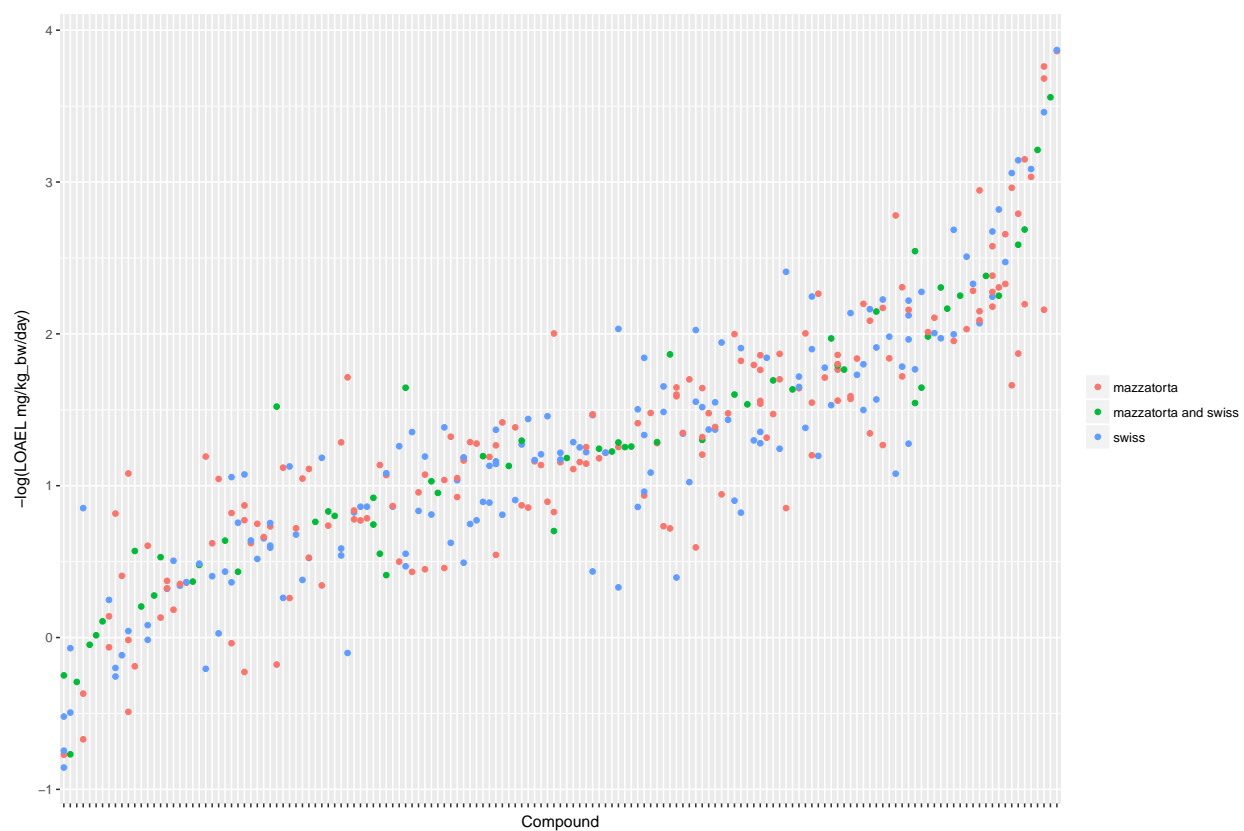


Figure 2: Distribution and variability of LOAEL values in both datasets. Each vertical line represents a compound, dots are individual LOAEL values.

## Inter database variability

Figure 4 shows the experimental LOAEL variability of compounds occurring in both datasets (i.e. the *test* dataset) colored in red (experimental). This is the baseline reference for the comparison with predicted values.

Figure 3 depicts the correlation between LOAEL values from both datasets. As both datasets contain duplicates medians were used for the correlation plot and statistics. It should be kept in mind that the aggregation of duplicated measurements into a single median value hides a substantial portion of the experimental variability. Correlation analysis shows a significant ( $p\text{-value} < 2.2\text{e-}16$ ) correlation between the experimental data in both datasets with  $r^2$ : 0.52, RMSE: 0.59

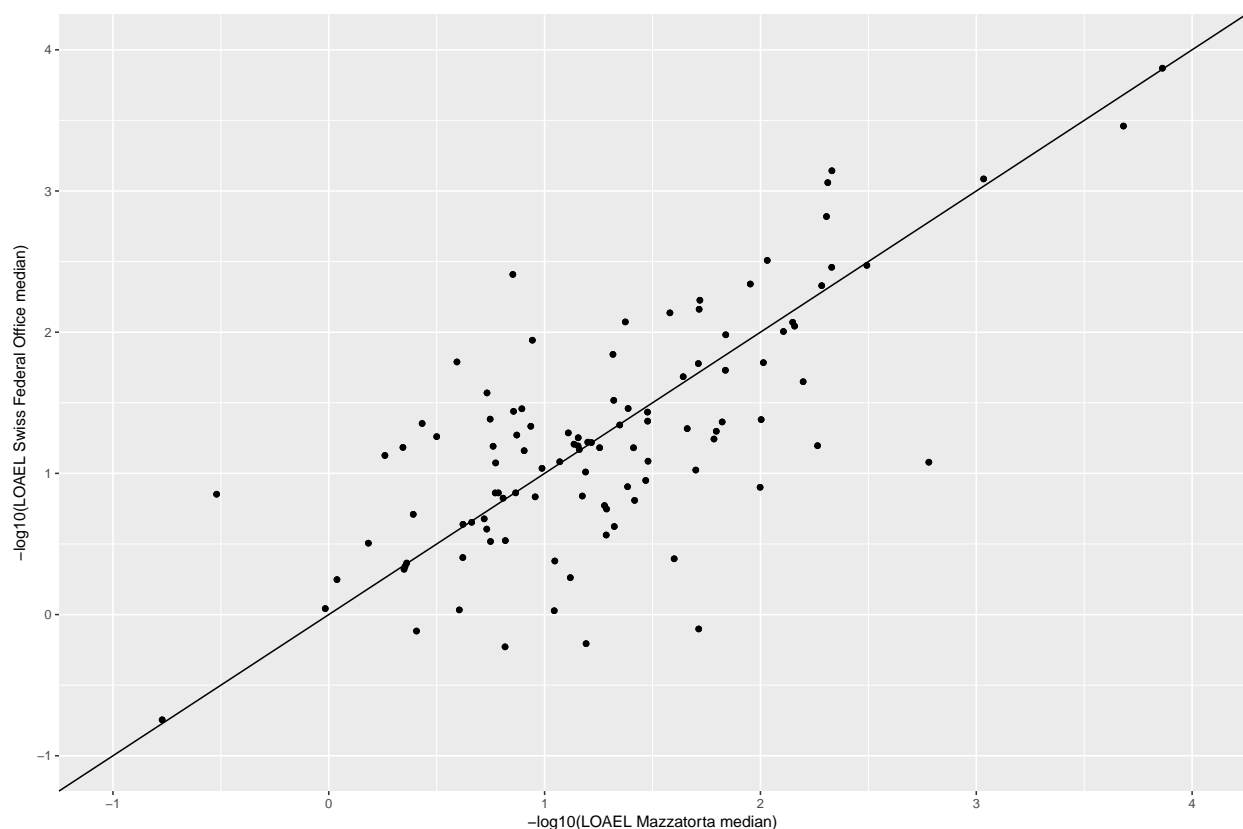


Figure 3: Correlation of median LOAEL values from Nestl and FSVO databases. Data with identical values in both databases was removed from analysis.

## Local QSAR models

In order to compare the performance of *in silico* read across models with experimental variability we are using compounds that occur in both datasets as a test set (375 measurements, 155 compounds). **lazar** read across predictions were obtained for 155 compounds, 37 predictions failed, because no similar compounds were found in the training data (i.e. they were not covered by the applicability domain of the training data).

Experimental data and 95% prediction intervals overlapped in 100% of the test examples.

Figure 4 shows a comparison of predicted with experimental values. Most predicted values were located within the experimental variability.

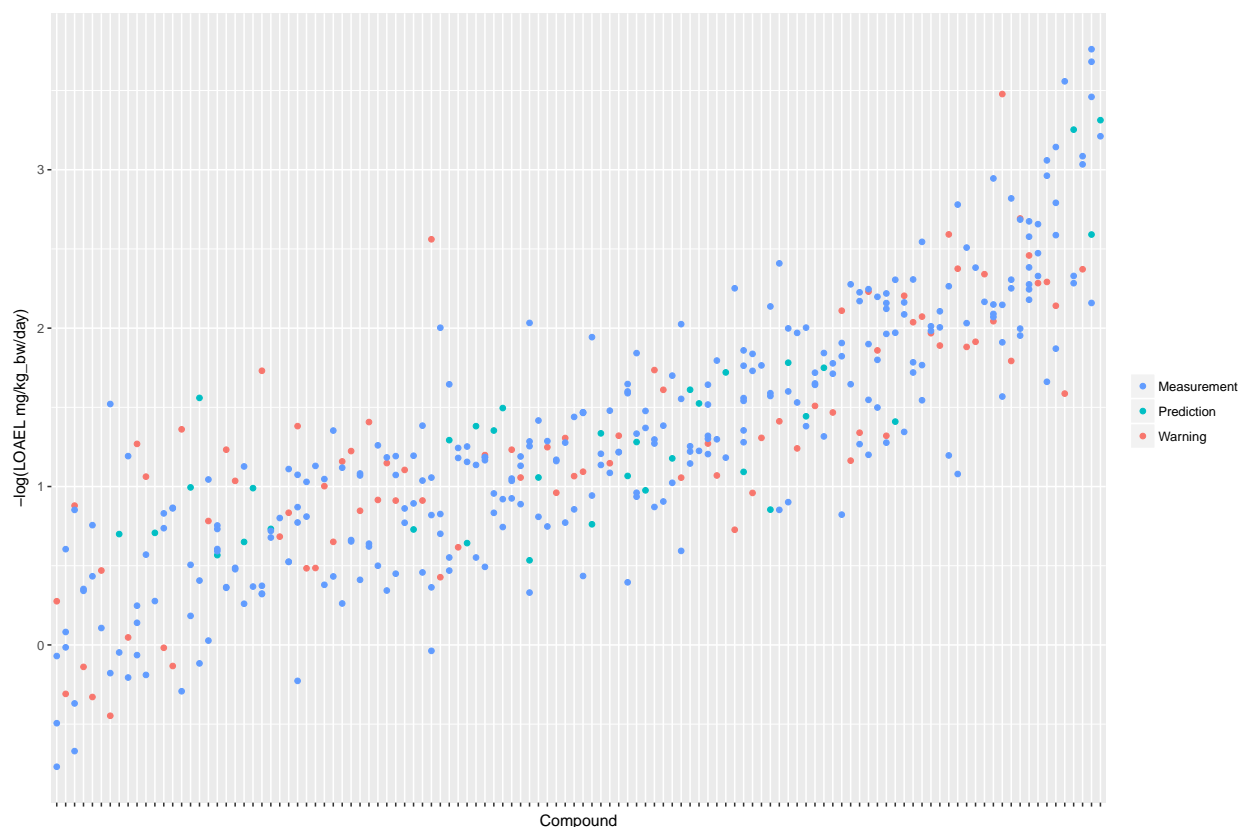


Figure 4: Comparison of experimental with predicted LOAEL values. Each vertical line represents a compound, dots are individual measurements (blue), predictions (green) or predictions far from the applicability domain, i.e. with warnings (red).

Correlation analysis was performed between individual predictions and the median of exper-

245 imental data. All correlations are statistically highly significant with a p-value  $< 2.2\text{e-}16$ .  
 246 These results are presented in Figure 5 and Table 2. Please bear in mind that the aggregation  
 247 of multiple measurements into a single median value hides experimental variability.

Table 1: Comparison of model predictions with experimental variability.

Comparison	$r^2$	RMSE	Nr. predicted
Nestl vs. FSVO database	0.52	0.59	
AD close predictions vs. test median	0.48	0.56	34/155
AD distant predictions vs. test median	0.38	0.68	84/155
All predictions vs. test median	0.4	0.65	118/155

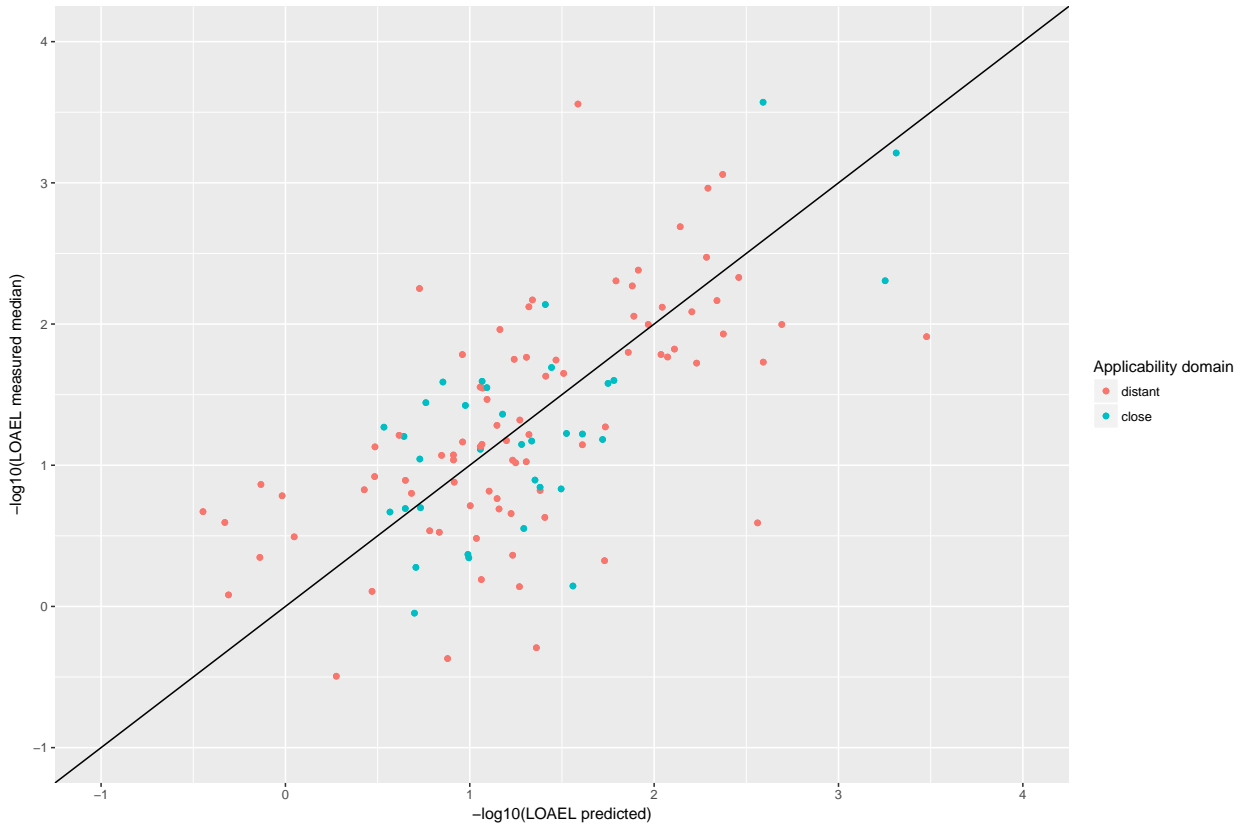


Figure 5: Correlation of experimental with predicted LOAEL values (test set). Green dots indicate predictions close to the applicability domain (i.e. without warnings), red dots indicate predictions far from the applicability domain (i.e. with warnings).

For a further assessment of model performance three independent 10-fold cross-validations were performed. Results are summarised in Table 2 and Figure 6. All correlations of predicted with experimental values are statistically highly significant with a p-value  $< 2.2\text{e-}16$ . This is observed for compounds close and more distant to the applicability domain.

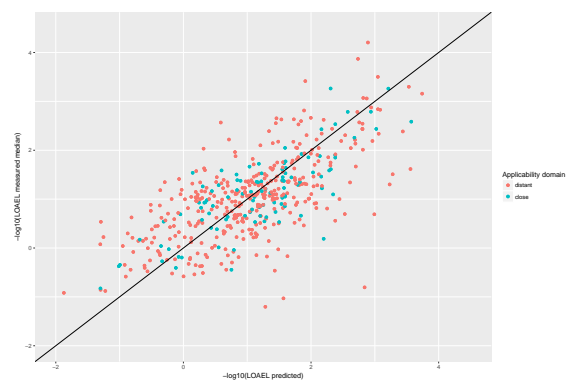
Table 2: Results from 3 independent 10-fold crossvalidations

Predictions	$r^2$	RMSE	Nr. predicted
AD close	0.61	0.58	102/671
AD distant	0.45	0.78	374/671
All	0.47	0.74	476/671
AD close	0.59	0.6	101/671
AD distant	0.45	0.77	376/671
All	0.47	0.74	477/671
AD close	0.59	0.57	93/671
AD distant	0.43	0.81	384/671
All	0.45	0.77	477/671

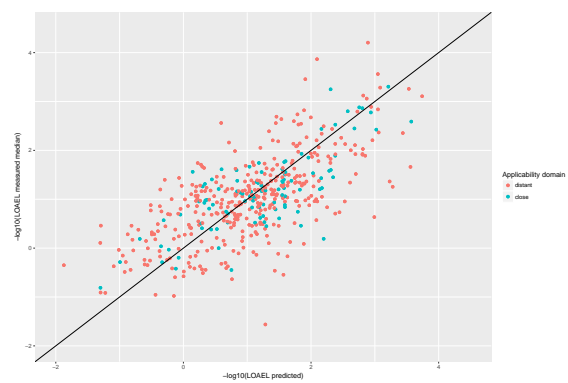
## Discussion

It is currently acknowledged that there is a strong need for toxicological information on the multiple thousands of chemicals to which human may be exposed through food. These include for examples many chemicals in commerce, which could potentially find their way into food (Stanton and Krusezewski 2016, Fowler, Savage, and Mendez (2011)), but also substances migrating from food contact materials (Grob et al. 2006), chemicals generated over food

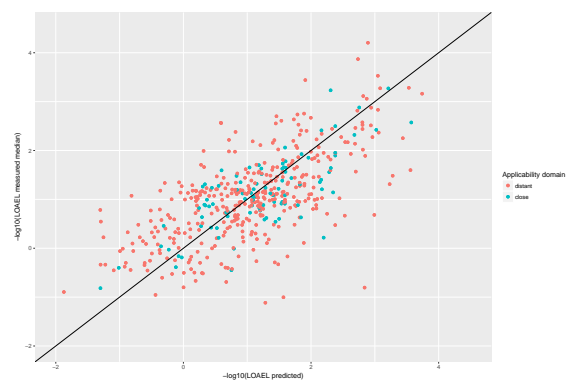




(a)



(b)



(c)

Figure 6: Correlation of predicted vs. measured values for three independent crossvalidations with MP2D fingerprint descriptors and local random forest models.

processing (Cotterill et al. 2008), environmental contaminants as well as inherent plant  
toxicants (Schilter, Constable, and Perrin 2013). For the vast majority of these chemicals,  
no toxicological data is available and consequently insight on their potential health risks is  
very difficult to obtain. It is recognized that testing all of them in standard animal studies is  
neither feasible from a resource perspective nor desirable because of ethical issues associated  
with animal experimentation. In addition, for many of these chemicals, risk may be very  
low and therefore testing may actually be irrelevant. In this context, the identification of  
chemicals of most concern on which limited resource available should be focused is essential and  
computational toxicology is thought to play an important role for that.

In order to establish the level of safety concern of food chemicals toxicologically not charac-  
terized, a methodology mimicking the process of chemical risk assessment, and supported by  
computational toxicology, was proposed (Schilter et al. 2014). It is based on the calculation  
of margins of exposure (MoE) between predicted values of toxicity and exposure estimates.  
The level of safety concern of a chemical is then determined by the size of the MoE and its  
suitability to cover the uncertainties of the assessment. To be applicable, such an approach  
requires quantitative predictions of toxicological endpoints relevant for risk assessment. The  
present work focuses on prediction of chronic toxicity, a major and often pivotal endpoint of  
toxicological databases used for hazard identification and characterization of food chemicals.

In a previous study, automated read-across like models for predicting carcinogenic potency  
were developed. In these models, substances in the training dataset similar to the query  
compounds are automatically identified and used to derive a quantitative TD50 value.  
The errors observed in these models were within the published estimation of experimental  
variability (Lo Piparo et al. 2014). In the present study, a similar approach was applied  
to build models generating quantitative predictions of long-term toxicity. Two databases  
compiling chronic oral rat lowest adverse effect levels (LOAEL) as endpoint were available  
from different sources. Our investigations clearly indicated that the Nestl and FSV databases

are very similar in terms of chemical structures and properties as well as distribution of experimental LOAEL values. The only significant difference that we observed was that the Nestl one has larger amount of small molecules, than the FSVO database. For this reason we pooled both dataset into a single training dataset for read across predictions.

An early review of the databases revealed that 155 out of the 671 chemicals available in the training datasets had at least two independent studies/LOAELs. These studies were exploited to generate information on the reproducibility of chronic animal studies and were used to evaluate prediction performance of the models in the context of experimental variability. Considerable variability in the experimental data was observed. Study design differences, including dose selection, dose spacing and route of administration are likely explanation of experimental variability. High experimental variability has an impact on model building and on model validation. First it influences model quality by introducing noise into the training data, secondly it influences accuracy estimates because predictions have to be compared against noisy data where “true” experimental values are unknown. This will become obvious in the next section, where comparison of predictions with experimental data is discussed. The data obtained in the present study indicate that **lazar** generates reliable predictions for compounds within the applicability domain of the training data (i.e. predictions without warnings, which indicates a sufficient number of neighbors with similarity  $> 0.5$  to create local random forest models). Correlation analysis shows that errors (RMSE) and explained variance ( $r^2$ ) are comparable to experimental variability of the training data.

Predictions with a warning (neighbor similarity  $< 0.5$  and  $> 0.2$  or weighted average predictions) are more uncertain. However, they still show a strong correlation with experimental data, but the errors are larger than for compounds within the applicability domain. Expected errors are displayed as 95% prediction intervals, which covers 100% of the experimental data. The main advantage of lowering the similarity threshold is that it allows to predict a much larger number of substances than with more rigorous applicability domain criteria.

As each of this prediction could be problematic, they are flagged with a warning to alert risk assessors that further inspection is required. This can be done in the graphical interface (<https://lazar.in-silico.ch>) which provides intuitive means of inspecting the rationales and data used for read across predictions.

Finally there is a substantial number of chemicals (37), where no predictions can be made, because no similar compounds in the training data are available. These compounds clearly fall beyond the applicability domain of the training dataset and in such cases predictions should not be used. In order to expand the domain of applicability, the possibility to design models based on shorter, less than chronic studies should be studied. It is likely that more substances reflecting a wider chemical domain may be available. To predict such shorter duration endpoints would also be valuable for chronic toxicity since evidence suggest that exposure duration has little impact on the levels of NOAELs/LOAELs (Zarn, Engeli, and Schlatter 2011, Zarn, Engeli, and Schlatter (2013)).

Elena: Should we add a GUI screenshot?

## Summary

In conclusion, we could demonstrate that **lazar** predictions within the applicability domain of the training data have the same variability as the experimental training data. In such cases experimental investigations can be substituted with *in silico* predictions. Predictions with a lower similarity threshold can still give usable results, but the errors to be expected are higher and a manual inspection of prediction results is highly recommended.

## References

- Bender, Andreas, Hamse Y. Mussa, Robert C. Glen, and Stephan Reiling. 2004. "Molecular Similarity Searching Using Atom Environments, Information-Based Feature Selection, and a Naïve Bayesian Classifier." *Journal of Chemical Information and Computer Sciences* 44 (1): 170–78. doi:10.1021/ci034207y.
- Cotterill, J.V., M.Q. Chaudry, W. Matthews, and R. W. Watkins. 2008. "In Silico Assessment of Toxicity of Heat-Generated Food Contaminants." *Food Chemical Toxicology*, no. 46(6): 1905–18.
- ECHA. 2008. "Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.6: QSARs and Grouping of Chemicals." ECHA.
- EFSA. 2014. "Rapporteur Member State Assessment Reports Submitted for the EU Peer Review of Active Substances Used in Plant Protection Products." <http://dar.efsa.europa.eu/dar-web/provision>.
- EFSA. 2016. "Guidance on the Establishment of the Residue Definition for Dietary Assessment: EFSA Panel on Plant Protect Products and Their Residues (PPR)." *EFSA Journal*, no. 14: 1–12.
- Fowler, B., S. Savage, and B. Mendez. 2011. "White Paper: Protecting Public Health in the 21st Century: The Case for Computational Toxicology." ICF International, Inc. icfi.com.
- Grob, K., M. Biedermann, E. Scherbaum, M. Roth, and K. Rieger. 2006. "Food Contamination with Organic Materials in Perspective: Packaging Materials as the Largest and Least Controlled Source? A View Focusing on the European Situation." *Crit. Rev. Food. Sci. Nutr.*, no. 46: 529–35. doi:10.1080/10408390500295490.
- Gütlein, Martin, Andreas Karwath, and Stefan Kramer. 2012. "CheS-Mapper - Chemical Space Mapping and Visualization in 3D." *Journal of Cheminformatics* 4 (1): 7.

doi:[10.1186/1758-2946-4-7](https://doi.org/10.1186/1758-2946-4-7).

Health Canada. 2016. <https://www.canada.ca/en/health-canada/services/chemical-substances/chemicals-management-plan.html>.

Kuhn, Max. 2008. “Building Predictive Models in R Using the Caret Package.” *J. of Stat. Soft.*

Lo Piparo, E., A. Maunz, C. Helma, D. Vorgrimmmler, and B. Schilter. 2014. “Automated and Reproducible Read-Across Like Models for Predicting Carcinogenic Potency.” *Regulatory Toxicology and Pharmacology*, no. 70: 370–78.

Lo Piparo, E., A. Worth, A. Manibusan, C. Yang, B. Schilter, P. Mazzatorta, M.N. Jacobs, H. Steinkelner, and L. Mohimont. 2011. “Use of Computational Tools in the Field of Food Safety.” *Regulatory Toxicology and Pharmacology*, no. 60(3): 354–62.

Maunz, Andreas, Martin Gütlein, Micha Rautenberg, David Vorgrimmmler, Denis Gebele, and Christoph Helma. 2013. “Lazar: A Modular Predictive Toxicology Framework.” *Frontiers in Pharmacology* 4. Frontiers Media SA. doi:[10.3389/fphar.2013.00038](https://doi.org/10.3389/fphar.2013.00038).

Mazzatorta, Paolo, Manuel Dominguez Estevez, Myriam Coulet, and Benoit Schilter. 2008. “Modeling Oral Rat Chronic Toxicity.” *Journal of Chemical Information and Modeling* 48 (10): 1949–54. doi:[10.1021/ci8001974](https://doi.org/10.1021/ci8001974).

OBoyle, Noel M, Michael Banck, Craig A James, Chris Morley, Tim Vandermeersch, and Geoffrey R Hutchison. 2011. “Open Babel: An Open Chemical Toolbox.” *Journal of Cheminformatics* 3 (1). Springer Science and Business Media: 33. doi:[10.1186/1758-2946-3-33](https://doi.org/10.1186/1758-2946-3-33).

OECD. 2015. “Fundamental and Guiding Principles for (Q)SAR Analysis of Chemicals Carcinogens with Mechanistic Considerations Monograph 229 ENV/JM/MONO(2015)46.”

377 In *Series on Testing and Assessment No 229*.

378 Schilter, B., R. Benigni, A. Boobis, A. Chiodini, A. Cockburn, M.T. Cronin, E. Lo Piparo, S.  
 379 Modi, Thiel A., and A. Worth. 2014. "Establishing the Level of Safety Concern for Chemicals  
 380 in Food Without the Need for Toxicity Testing." *Regulatory Toxicology and Pharmacology*,  
 381 no. 68: 275–98.

382 Schilter, B., A. Constable, and I. Perrin. 2013. "Naturally Occurring Toxicants of Plant  
 383 Origin: Risk Assessment and Management Considerations." In *Food Safety Management: A  
 384 Practical Guide for Industry*, edited by Y. Motarjemi, 45–57. Elsevier.

385 Stanton, K., and F. H. Krusezewski. 2016. "Quantifying the Benefits of Using Read-Across  
 386 and in Silico Techniques to Fullfill Hazard Data Requirements for Chemical Categories."  
 387 *Regulatory Toxicology and Pharmacology*, no. 81: 250–59. doi:[10.1016/j.yrtph.2016.09.004](https://doi.org/10.1016/j.yrtph.2016.09.004).

388 US EPA. 2011. "Fact Sheets on New Active Ingredients."

389 Weininger, David. 1988. "SMILES, a Chemical Language and Information System. 1.  
 390 Introduction to Methodology and Encoding Rules." *Journal of Chemical Information and  
 391 Computer Sciences* 28 (1): 31–36. doi:[10.1021/ci00057a005](https://doi.org/10.1021/ci00057a005).

392 WHO. 2011. "Joint FAO/WHO Meeting on Pesticide Residues (JMPR) Publications."  
 393 <http://www.who.int/foodsafety/publications/jmpr-monographs/en/>.

394 Zarn, J. A., B. E. Engeli, and J. R. Schlatter. 2011. "Study Parameters Influencing NOAEL  
 395 and LOAEL in Toxicity Feeding Studies for Pesticides: Exposure Duration Versus Dose  
 396 Decrement, Dose Spacing, Group Size and Chemical Class." *Regul. Toxicol. Pharmacol.*, no.  
 397 61: 243–50.

398 ———. 2013. "Characterization of the Dose Decrement in Regulatory Rat Pesticide Toxicity  
 399 Feeding Studies." *Regul. Toxicol. Pharmacol.*, no. 67: 215–20.