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Inhalation threshold of toxicological concern (TTC) — Structural alerts discriminate high from low repeated-dose inhalation toxicity☆,☆☆

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ABSTRACT

The threshold of toxicological concern (TTC) of a compound represents an exposure value below which the associated human health risk is considered negligible. As such, this approach offers assessing the risk of potential toxicants when little or no toxicological information is available. For the inhalation repeated-dose TTC, the goal was to derive structural alerts that discriminate between high- and low-toxic compounds. A further aim was to identify physicochemical parameters related to the inhalation-specific bioavailability of the compounds, and to explore their use as predictors of high vs low toxicity. 296 compounds with subacute, subchronic and chronic inhalation toxicity NOEC (no-observed effect concentration) values were subdivided into three almost equal-sized high-, medium- and low-toxic (HTox, MTox, LTox) potency classes. Whereas the derived 14 HTox and 7 LTox structural alerts yield an only moderate discrimination between these three groups, the high-toxic vs low-toxic mis-classification is very low: LTox-predicted compounds are not HTox to 97.5%, and HTox-predicted compounds not LTox to 88.6%. The probability of a compound being HTox vs LTox is triggered further by physicochemical properties encoding the tendency to evaporate from blood. The new structural alerts may aid in the predictive inhalation toxicity assessment of compounds as well as in designing low-toxicity chemicals, and provide a rationale for the chemistry underlying the toxicological outcome that can also be used for scoping targeted experimental studies.

1. Introduction

In the last decade, the call for transforming the chemical toxicity assessment from routine in vivo animal testing to a focus on mechanistic in vitro and in vivo information (Collins et al., 2008; Hartung, 2009) has fostered research into molecular initiating events and pathways of toxicity. To this end, systems biology (Krewski et al., 2014; Zhang et al., 2014) and systems chemistry (Prescher and Bertozzi, 2005) offer routes for research into the exposome (Rappaport, 2012; Rappaport and Smith, 2010; Wild, 2005, 2012), complemented by computational chemistry to unravel prevalent pathways of metabolic activation (Ji and Schüürmann, 2012, 2013).

Accordingly, alternative approaches designed for reducing or replacing animal testing such as structural alerts, read-across and computational toxicology have gained an increased importance. In this context, the threshold of toxicological concern (TTC) offers an exposure-oriented procedure for the risk assessment of compounds with little or even no experimental information (Hennes, 2012; Munro et al., 2008), and is defined as threshold of exposure below which there is no significant risk for human health.

Depending on the route of exposure, oral or inhalation TTCs are derived from compound-specific NOEL or NOEC (no-observed effect level or concentration) values for a given species (e.g. rat and mouse). Safety factors to account for inter- and intraspecies differences (e.g. 100 for oral exposure) are employed to convert the 5-percentile of the associated distribution function into a contaminant intake rate that is considered to exert no harmful effects. The NOEC or NOEL assessment refers to a substantial part of the lifetime, and takes into account survival data, body weight and food consumption changes, biochemical, clinical, ophthalmological, neurotoxicological and immunotoxicological analyses, organ weights, and further necropsy and histopathological findings (OECD, 2009a). Uncertainty factors are also considered to address shortcomings associated with exposure durations lower than life time.

In 1995, the US FDA set a "Threshold of Regulation" at 1.5 μg/person/day for substances of food-packing materials without toxicological information. Subsequently, Munro et al. (1996) employed the Cramer classification for a structure-based discrimination between compounds of low, medium and high intrinsic toxicity (Cramer et al., 1978), and proposed TTC values for oral exposure of 1800, 540 and 90 μg/person/day (for Cramer classes 1, 2 and 3, respectively) derived from repeated-dose

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toxicity NOELs of 613 compounds. The respective data distribution is shown in Fig. 1 left with concentrations converted to mmol/person/day.

Later, specific oral TTCs were delineated for neurotoxic organophosphates and potential genotoxicants (18 and 0.15 μg/person/day) (Munro et al., 2008). In this context, structure-activity reasoning gains increasing importance for identifying high-concern compounds such as potentially genotoxic agents with specific associated TTCs, which holds in particular for read-across that has recently been proposed to aid in the predictive assessment of repeated-dose toxicity (Berggren et al., 2015).

The TTC methodology has also been applied to the inhalation pathway (Carthew et al., 2009; Escher et al., 2010). 203 industrial compounds of the RepDose database with repeated-dose subacute, subchronic and chronic no-observed effect concentrations (NOECs) led to inhalation TTCs of $1.5 \cdot 10^{-3}$ and $2.2 \cdot 10^{-5}$ ppm for Cramer classes 1 and 3, respectively, corresponding to body doses of 71 and 4 μg/person/day that are substantially lower than their Munro oral counterparts (Escher et al., 2010). Moreover, a statistically significant inhalation TTC could not be derived for Cramer class 2 because of too few respective compounds (see Fig. 1 bottom right). One aspect contributing to the less clear separation between Cramer classes 1 and 3 was considered to be caused by different impacts of local vs systemic effects upon uptake through the inhalation pathway. As shown below, however, the differentiation between local and systemic NOEC values does not improve a compound grouping according to toxicological potency.

A further indication for the need to improve the structure-based evaluation of compound toxicity was pointed out when analyzing oral NOEL values for 521 substances as collected in the TTC part of the RepDose database (Tluczkiewicz et al., 2011). The latter showed a similar overlap between the three Cramer classes that was manually reduced through re-allocation of some compounds considering structural similarity, eventually yielding class-specific TTCs similar to the original Munro values. Moreover, analysis of a regulatory dataset with 824 compounds and associated oral repeated-dose NOAEL (no-observed adverse effect level) values demonstrated the generally conservative performance of the Cramer scheme, allocating 90% to the high-toxic Cramer class 3 as opposed to only 22% classified as high-toxic according to the Globally Harmonized System (GHS) (Kalkhof et al., 2012).

In the present study, a more comprehensive strategy was envisaged for predicting the inhalation toxicological potency of compounds from chemical structure. To this end, 296 compounds with inhalation NOECs collected in RepDose were subdivided into high-, medium- and low-toxicity categories, and subjected to structural analyses employing our atom-centered fragment (ACF) approach (Kühne et al., 2009) that has already proven useful for the read-across prediction of environmental toxicity (Schüürmann et al., 2011). Subsequent refinement led to 14 and 7 structural alerts identifying high and low inhalation toxicity that may aid in the predictive hazard assessment of repeated-dose inhalation NOEC values. Moreover, pertinent physicochemical properties could be identified with trigger values indicating a high and low inhalation toxicological potency, thus complementing the structural alert scheme and enabling a consensus modeling approach.

2. Materials and methods

For the statistical analyses, the RepDose (http://fraunhofer-repdose.de/) subset of 296 compounds with repeated-dose inhalation toxicity data in terms of NOEC (no-observed effect concentration) [ppm] values covering subacute (mainly 28 days), subchronic (90 days) and chronic (1 year) exposure times (OECD, 2009a, 2009b, 2009c) has been subdivided into the three subgroups of 110 high-toxic (HTox: NOEC < 0.75 ppm), 92 medium-toxic (MTox: 0.75 ppm ≤ NOEC ≤ 12 ppm) and 94 low-toxic (LTox: NOEC > 12 ppm)

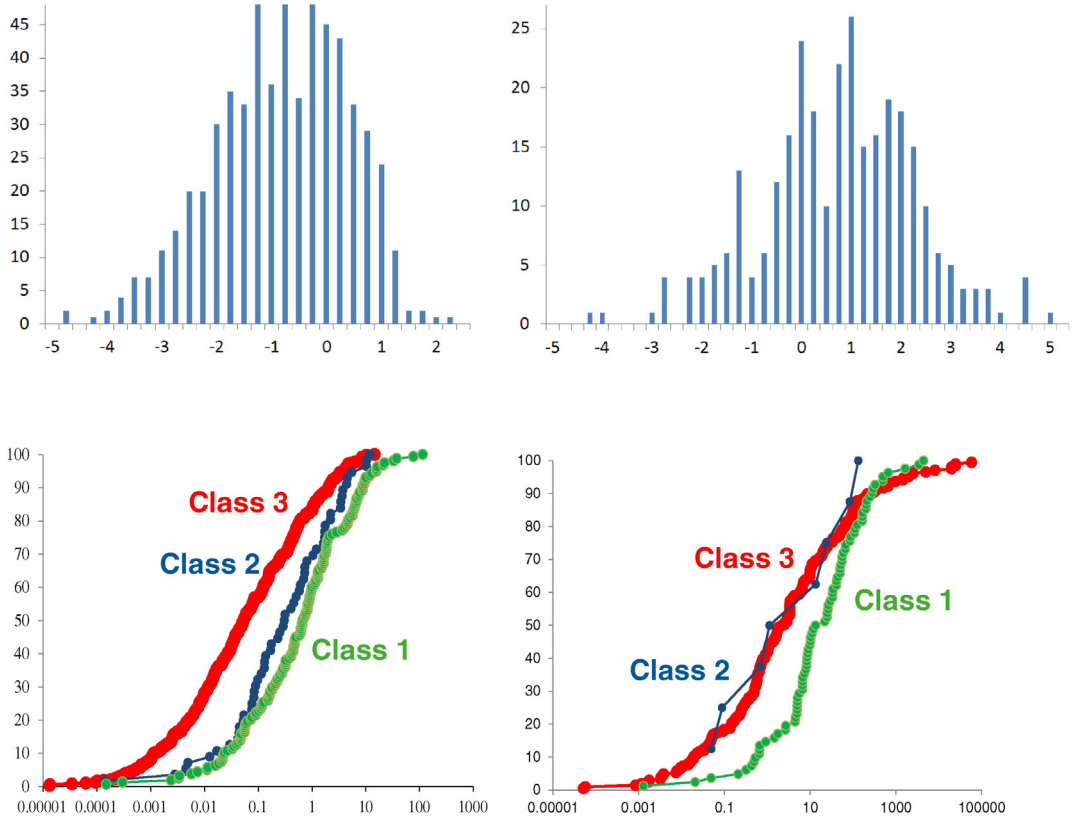


Fig. 1. Oral repeated-dose toxicity NOEL [mol/kg/day] values (left, Munro et al., 1996) and inhalation repeated-dose toxicity NOEC [ppm] values of 296 compounds covering subacute, subchronic and chronic exposure (right). Top: Frequency of occurrence of compounds per NOEL or NOEC value interval of 0.25 log units vs log NOEL or NOEC. Bottom: Associated cumulative frequency distribution per Cramer class (3 = left = red, 2 = middle = blue, 1 = right = green), indicating the percentage of compounds with NOELs or NOECs below or equal to the x axis NOEL or NOEC.

compounds. The respective thresholds of 0.75 and 12 ppm have been selected through analysis of Fig. 1 top right with the goal to obtain subsets of at least similar size (see Tluczkiewicz et al., 2015, submitted, for toxicological details underlying the NOEC values).

The performance of the structural alert models for discriminating between LTox, MTox and HTox compounds has been characterized in terms of the following three statistical parameters:

$$\text{Concordance} = \frac{1}{N_{\text{tot}}} = \sum_{i=1}^{N_{\text{cat}}} T_{\text{pred}}(i) \quad (1)$$

where N_{tot} denotes the total number of compounds (here: 296), N_{cat} the number of categories (here: 3 potency categories HTox, MTox and LTox), and $T_{\text{pred}}(i)$ the number of truly (correctly) predicted compounds to belong to category i.

$$\text{Sensitivity}\ (i) = \frac{T_{\text{pred}}(i)}{n_{\text{exp}}(i)} \quad (2)$$

$$\text{Predictivity}\ (i) = \frac{T_{\text{pred}}(i)}{T_{\text{pred}}(i) + F_{\text{pred}}(i)} = \frac{T_{\text{pred}}(i)}{n_{\text{pred}}(i)} \quad (3)$$

In Eqs. (2)–(3), $n_{\text{exp}}(i)$ is the number of experimental compounds in category (i), $F_{\text{pred}}(i)$ the number of compounds falsely predicted to belong to that category, and $n_{\text{pred}}(i)$ the total number of compounds predicted to belong to category (i). The sensitivity thus quantifies the fraction of compounds correctly recognized by the model (category-specific recognition power) through dividing the number of correctly predicted compounds (T_{pred}) by the number of compounds actually belonging to this category (n_{exp}), whereas the predictivity yields the fraction of correctly predicted compounds (category-specific prediction power) through dividing T_{pred} by the total number of compounds predicted to belong to this category (n_{pred}).

For evaluating the chemical domain of the inhalation NOEC dataset as compared to existing compound inventories, the atom-centered fragment (ACF) approach has been employed (Kühne et al., 2009). In short, a given chemical structure is decomposed into substructural units such that each non-hydrogen atom forms the center of a substructure that is constructed through including non-hydrogen neighbor atoms along each bonding direction up to a pre-defined topological distance. The structural (ACF-defined) similarity between any two compounds is then obtained as ratio of joint ACFs over the total number of ACFs occurring in both compounds. The ACF methodology has also been used for an initial similarity-based discrimination between HTox, MTox and LTox compounds that formed the starting point for a slight manual refinement yielding the structural alerts as described below.

3. Results

3.1. Data set features

For the 296 compounds, RepDose includes 107 subacute, 104 subchronic and 85 chronic inhalation no-observed effect concentration (NOEC) values with an overall rat-to-mouse percentage ratio of 90:10 (267:29 compounds), covering the elements C, H, F, Cl, Br, I, O, N, S, P, Si, Na with overall 214 non-aromatic and 76 aromatic substances (see Table S1 for more details).

Analysis of the chemical domain through the atom-centered fragment (ACF) approach (Kühne et al., 2009) reveals significant differences to the compounds of the Munro oral TTC dataset (Munro et al., 1996). According to this ACF criterion, only 19% of the 296 inhalation NOEC chemicals are inside the Munro domain, with 53% outside and 28% borderline cases (Table 1).

To put this into a broader perspective, we have performed corresponding analyses with the Bursi mutagenicity data set (Kazius et al., 2005) and the Duluth acute fish toxicity database (AQUIRE, Aquatic toxicity information retrieval database and US EPA, Environmental Protection Agency, 2011; Russom et al., 1997) that contain 4225 and 692 organic compounds, respectively. Here, 33% of the present 296 compounds would be considered outside or borderline outside the 14-fold larger Bursi set. At the same time, 33% are inside the ACF domain of the 2.3-fold larger Duluth set as opposed to 53% outside that domain (Table 1). These results suggest that from the viewpoint of structural chemistry, the presently analyzed inhalation NOEC compound set contains quite novel features as compared to established larger databases of compounds with experimental information about human and environmental toxicity endpoints.

Table 1
Chemical domain of the inhalation NOEC dataset in relation to the Munro oral TTC dataset and to two further datasets concerning mutagenicity and acute fish toxicity.[a]

ACF-based domain belonging type	Oral TTC (Munro)	Mutagenicity (Bursi)	Fish toxicity (Duluth)
In	57 (19.3%)	179 (60.5%)	98 (33.1%)
Borderline in	32 (10.8%)	20 (6.8%)	15 (5.1%)
Borderline out	50 (16.9%)	36 (12.2%)	27 (9.1%)
Out	157 (53.0%)	61 (20.6%)	156 (52.7%)

[a] The numerical entries indicate the numbers of compounds (and percentages) of the inhalation NOEC (no-observed effect concentration) data associated with a certain type of domain belonging as specified in the left-most column defined through the ACF (atom-centered fragment) approach (Kühne et al., 2009). The data set sizes are as follows: inhalation NOEC data: 296; oral TTC (Munro): 613 (Munro et al., 1996); Ames test mutagenicity (Bursi): 4225 (Kazius et al., 2005); acute fish toxicity towards the fathead minnow *Pimephales promelas* (Duluth): 692 (AQUIRE, Aquatic toxicity information retrieval database and US EPA, Environmental Protection Agency, 2011; Russom et al., 1997).

3.2. Bioavailability vs toxicological potency

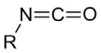
Inspection of Fig. 1 top right suggested a manual subdivision of the total NOEC range of more than 9 orders of magnitude into high-toxicity (HTox: NOEC < 0.75 ppm), medium-toxicity (MTox: 0.75 ppm ≤ NOEC ≤ 12 ppm) and low-toxicity (LTox: NOEC > 12 ppm) subsets covering 110, 92 and 94 compounds, respectively.

To explore the potential impact of bioavailability on the toxicological potency of the compounds in the repeated-dose inhalation regime, high and low value ranges of corresponding physicochemical properties were analyzed with respect to their capability for discriminating between HTox and LTox compounds (Table S2). Interestingly, compounds with large and small molecular weight (MW) are predominantly associated with high and low inhalation toxicity (MW > 200 D: 62.5% HTox vs MW < 80 D: 66.0% LTox). The corresponding discrimination is still more pronounced with experimental vapor pressure P_v (ChemProp, 2014) that yields the highest degree of association with HTox at its low-end range (log P_v [Pa] < −1: 74.5% HTox vs log P_v > 4.6: 78.3% LTox).

Similar results are obtained with the partition coefficients air–water (K_{aw} = Henry's law constant in dimensionless form), octanol–air (K_{oa}) and blood–air (K_{ba}), with log K_{oa} < 2.5 yielding the largest degree of association with LTox (80%). Note further that quantitative linear correlations between log NOEC and any of these physicochemical properties are negligible ($r^2 \le 0.33$), that QSAR (quantitative structure-activity relationship) models as implemented in ChemProp (2014) had been used for predicting log K_{aw}, log K_{oa} and log K_{ba}, and that the NOEC compound set is essentially outside the application domain of the log K_{ba} model. The latter should thus be considered as (at best) tentative and remained included only because of its potentially high mechanistic relevance.

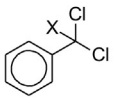
Overall these results indicate that the compound potency for inhalation toxicity is triggered by the high-end and low-end value ranges of properties related to their evaporation tendency from the liquid (blood) phase, keeping in mind that K_{aw} and K_{oa} may capture the water and

HT1: Isocyanates



R = C

HT2: Benzylic Chlorides



X = H or Cl

HT3: Organophosphorus Compounds

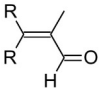
Z = O or S

Y = H, Cl, O, N, S or P

HT4: Primary Amines

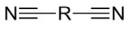
$R-NH_2$ R = C (aromatic or aliphatic)

HT5: Michael Acceptor Aldehydes



R = H or aliphatic C

HT6: 1,ω-Dinitriles



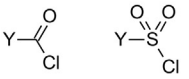
R = alkyl chain

HT7: Secondary and Tertiary Aliphatic Amines

R_1 = aliphatic C

R_2 = H or aliphatic C

HT8: Carbonyl and Sulfuryl Chlorides



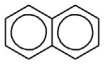
Y = C, O, Cl

HT9: Amides and Amide Derivatives

Y_1 = C, H, O, N

Y_2 = C, H, S

HT10: Polycyclic and Heterosubstituted Aromatics



additional fused aromatic rings possible

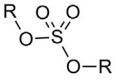
Z = O, N, S; multiple substitutions at ring possible

HT11: Vicinal Dihalogenides

X = Cl,Br

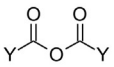
Y = C, H, F, Cl, O

HT12: Sulfates



R = C

HT13: Anhydrides

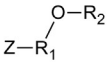


Y = C, N

HT14: Aliphatic and Aromatic Polyols

HO−Y−OH Y = alkyl , phenyl, may contain N or O

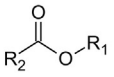
LT1: Saturated Ethers and Glycol Ethers



R_1, R_2 = alkyl

Z = H or OH

LT2: Saturated Carboxylic Acid Esters and Lactates



R_1 = C

R_2 = H or C

LT3: Nonaromatic Ketones

R_1, R_2 = alkyl

LT4: Aliphatic Monoaldehydes

R = H or alkyl

LT5: Aliphatic Hydrocarbons (Including Fluorinated / Chlorinated)

X = H, F, Cl

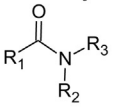
R = alkyl

R' = H or alkyl

LT6: Aliphatic Monoalcohols

R—OH R = alkyl

LT7: Acyclic Tertiary Amides



R_1 = H, alkyl

R_2, R_3 = alkyl

Fig. 2. Structural alerts for identifying HTox (high-toxic) and LTox (low-toxic) compounds in terms of repeated-dose inhalation NOEC values, featuring 14 HTox rules HT1–14 and 7 LTox rules LT1–7, respectively. Compounds meeting no HT and no LT rule are classified as MTox (medium-toxic).

protein phase of blood, respectively. In other words, compounds with a high rate of absorption from air to blood (low P_v, low K_{aw}, high K_{oa}) show a correspondingly high bioavailability for initiating a toxicological process, whereas a high tendency to escape from blood to the air phase (high P_v, high K_{aw}, low K_{oa}) appears to translate into lowering the inhalation toxicity.

Regarding molecular weight, its rough high-end and low-end capability for discriminating between high and low toxicity could be interpreted as reflecting a respective relationship with volatility (that generally increases with decreasing MW). Interestingly, however, the intercorrelation of MW with the other properties is relatively low ($r^2 < 0.5$) as opposed to significantly higher r^2 values among log P_v, log K_{oa} and log K_{ba} (r^2 around 0.8), with log K_{aw} being the second outlier with a trend significantly different from MW (r^2 0.02) and still with low to moderate overlap with log P_v, log K_{oa} and log K_{ba} (r^2 around 0.5; Table S3). In particular, the high-MW and low-MW subsets do not yield increased intercorrelations, which may be confounded by the fact that r^2 mathematically depends on the experimental value range and tends to decrease with decreasing data set size (Schüürmann et al., 2008).

Overall, these statistics suggest that combining two bioavailability-related properties with an at most moderate intercorrelation has scope in improving the possible HTox vs LTox discrimination. Examples are the following two-parameter bioavailability triggers, employing MW, experimental log P_v and QSAR-predicted log K_{aw} and log K_{oa} (with n = number of compounds meeting the property range and if applicable also the HTox or LTox category as indicated below):

HTox prevalence:

$$\text{Log } P_v \text{ [Pa]} < -1 \text{ and MW} > 120 : n = 45, \text{HTox } n = 36\ (80\%) \text{ vs LTox } n = 3\ (6.7\%)$$

$$\log K_{oa} > 6.5 \text{ and MW} > 120 : n = 60, \text{HTox } n = 45\ (75\%) \text{ vs LTox } n = 4\ (6.7\%)$$

LTox prevalence:

$$\log P_v \text{ [Pa]} > 4.6 \text{ and } \log K_{aw} > -1 : n = 35, \text{HTox } n = 1\ (2.8\%) \text{ vs LTox } n = 31\ (88.6\%)$$

$$\log P_v \text{ [Pa]} > 4.6 \text{ and } \log K_{aw} > -0.5 : n = 30, \text{HTox } n = 0\ (0\%) \text{ vs LTox } n = 28\ (93.3\%)$$

$$\log K_{aw} > -0.5 \text{ and } \log K_{oa} < 2.5 : n = 35, \text{HTox } n = 1\ (2.8\%) \text{ vs LTox } n = 30\ (85.7\%).$$

Overall, it appears that the bioavailability-related physicochemical properties predict the potency for inhalation toxicity at their high and low end of value ranges, and in this way may contribute to a predictive evaluation of the expected HTox vs LTox category of a compound under investigation.

3.3. Structural alert discrimination between HTox, MTox and LTox

Initially, our focus was on exploiting the experimental knowledge of local vs systemic effects at the LOEC. In this context, local effects are defined as referring to organs contacted first upon inhalation (lung, trachea, larynx, pharynx, bronchi, nose, eye) with an assumed causative relationship to chemical reactivity, whereas systemic effects cover all other target organs examined in the repeated-dose toxicity studies (e.g. liver, kidney, spleen, thymus). Of the total set of 296 compounds, 17 had a local and 113 a systemic LOEC, 137 showed both local and systemic effects at the LOEC, and 29 showed neither local nor systemic effects regarding the organs analyzed (but still some toxicity sufficient for deriving a NOEC). The subsequent search for structural alerts as discriminators between high and low toxicity was performed with the following two subsets: Local LOEC subset comprising 154 compounds with either only local (17) or local and systemic (137) effects at the LOEC, and the systemic LOEC subset consisting of 250 compounds that show either only systemic (113) or both systemic and local (137) effects at the LOEC.

Table 2
Structural alert performance of the 14 HTox and 7 LTox rules.[a]

Structural alert	Match	T_{pred}(HL)	F_{pred}(HL)	Category-specific F_{pred}	
High-toxicity rule				MTox	LTox
HT1	5	5	0	0	0
HT2	3	3	0	0	0
HT3	15	12	3	2	1
HT4	17	11	6	5	1
HT5	4	3	1	1	0
HT6	3	2	1	1	0
HT7	21	8	13	9	4
HT8	5	5	0	0	0
HT9	12	4	8	5	3
HT10	64	40	24	22	2
HT11	20	9	11	6	5
HT12	2	2	0	0	0
HT13	5	4	1	1	0
HT14	10	5	5	1	4
All (HT1–14)	186	113	73	53	20
Low-toxicity rule				MTox	HTox
LT1	39	23	16	15	1
LT2	14	10	4	4	0
LT3	6	6	0	0	0
LT4	4	3	1	0	1
LT5	20	19	1	1	0
LT6	25	14	11	11	0
LT7	4	2	2	2	0
All (LT1–7)	112	77	35	33	2

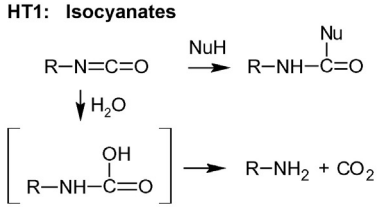
[a] Structural alerts HT1–14 and LT1–7 as defined in Fig. 2. Match = number of compounds containing the structural alert; T_{pred}(HL) = number of compounds truly (correctly) predicted as HTox (high-toxic) or LTox (low-toxic); F_{pred}(HL) = number of compounds predicted falsely (wrongly) as HTox or LTox; category-specific F_{pred} = number of compounds falsely (wrongly) predicted, counted separately for the two cases of either belonging actually to the neighbor category (experimentally MTox (medium-toxic) vs HTox or LTox prediction) or to the fully opposite category (experimentally LTox vs HTox prediction, or experimentally HTox vs LTox prediction).

Application of our ACF methodology resulted in 14 HTox (high-toxicity) and 7 LTox (low-toxicity) rules in terms of distinct structural features that turned out to be quite similar for the local and systemic subsets. Importantly, there was no indication for a particular relevance of chemical reactivity for the local HTox compounds, and the few cases with only local or only systemic HTox vs LTox discriminators could be traced back to respective differences in the subset compositions (in other words, the local and systemic subsets differed slightly regarding the representation of compound classes).

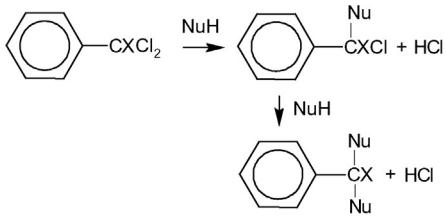
Considering these findings, the initial separation between local and systemic effects was abandoned as model basis, and the originally defined subsets of HTox (NOEC < 0.75 ppm) and LTox (NOEC > 12 ppm) each pooling both local and systemic effects at the LOEC were used for the subsequent analysis. Slight further adaptation and generalization of the structural rules discriminating between high and low toxicity led to the list of 14 HTox (high-toxicity) and 7 LTox (low-toxicity) structural alerts shown in Fig. 2. Taking HT1 (HTox structural alert no. 1) as example, isocyanates (R–NCO) are expected to yield an HTox NOEC regarding repeated-dose inhalation exposure, and aliphatic ketones (R_2CO, LT3 = LTox rule no. 3) are likely associated with a NOEC in the LTox range. In this scheme, MTox (medium-toxicity) compounds are defined implicitly through their lack of both HTox and LTox structural features.

At first sight, application of these structural alerts to the 296 compounds without and with subdivision into local and systemic LOEC values yields an only moderate classification performance with

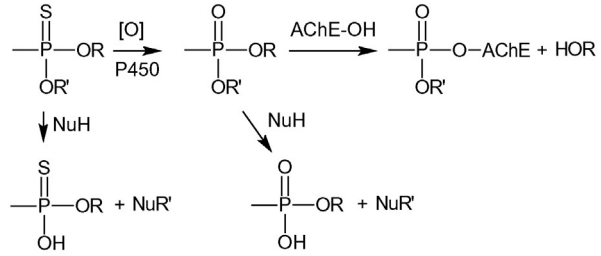
HT1: Isocyanates



HT2: Benzylic Chlorides

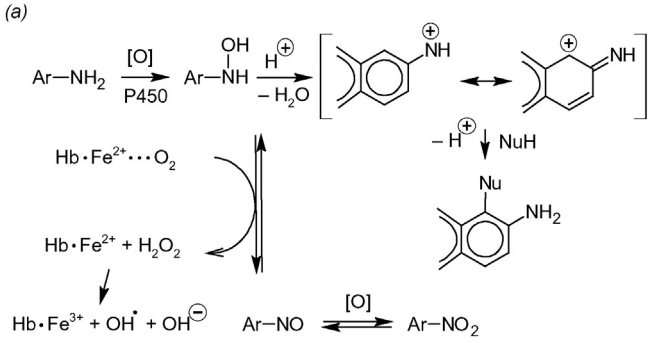


HT3: Organophosphorus Compounds

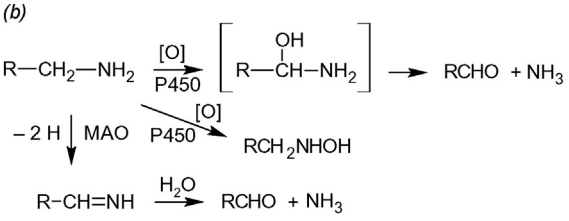


HT4: Primary Amines

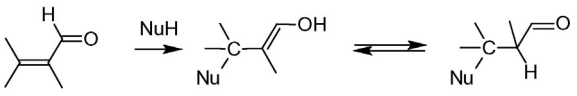
(a)



(b)

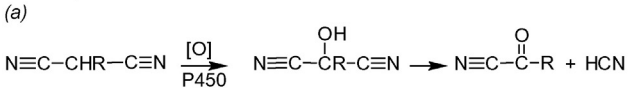


HT5: Michael Acceptor Aldehydes

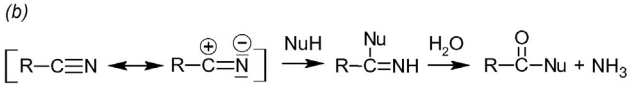


HT6: 1,ω-Dinitriles

(a)

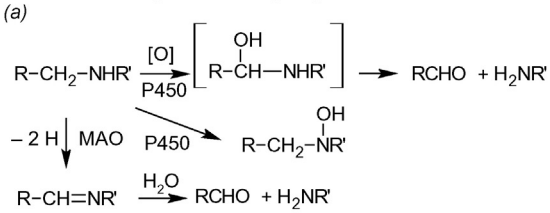


(b)

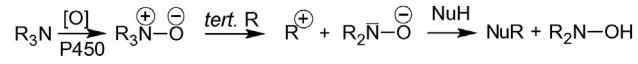


HT7: Secondary and Tertiary Aliphatic Amines

(a)

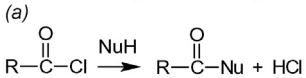


(b)

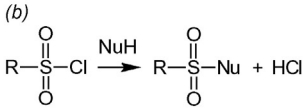


HT8: Carbonyl and Sulfuryl Chlorides

(a)

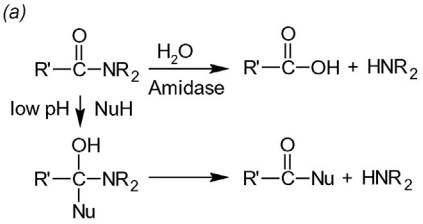


(b)

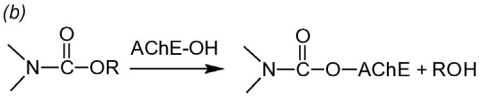


HT9: Amides and Amide Derivatives

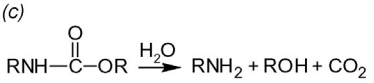
(a)



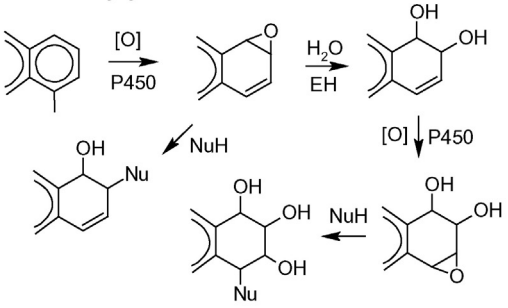
(b)

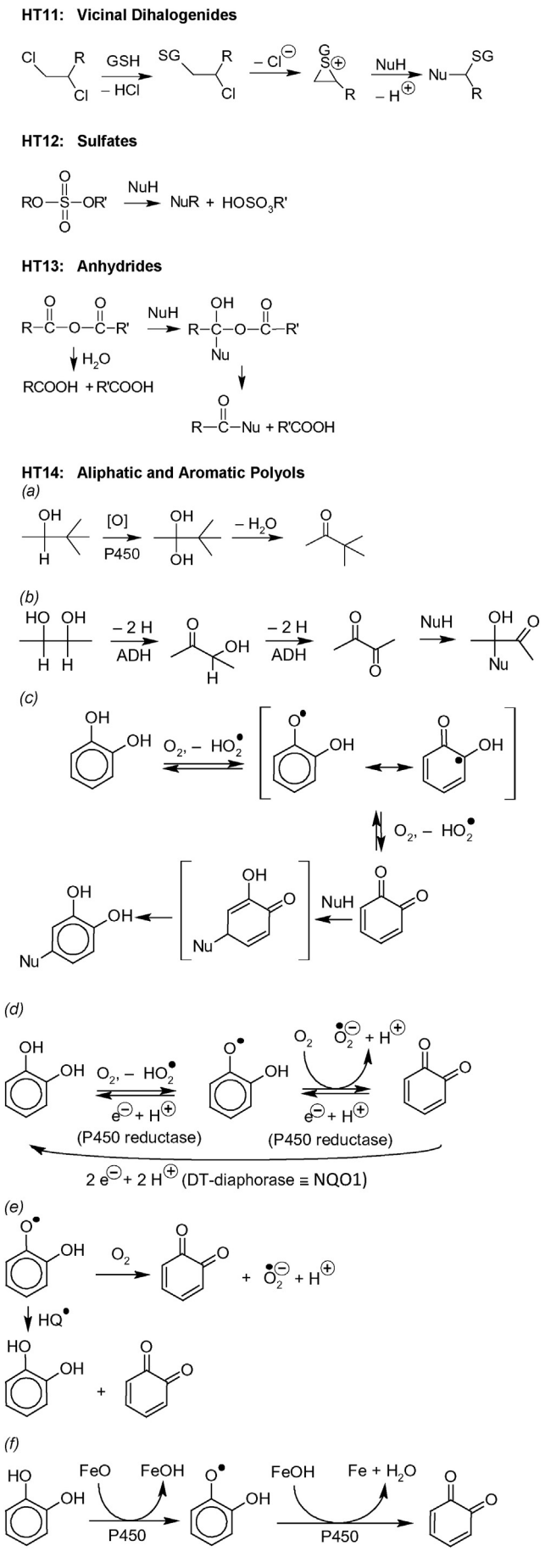


(c)



HT10: Polycyclic and Heterosubstituted Aromatics





concordances of 57–58% (Tables S4 and S5). Merging systemic and local effects, 59 of the 82 LTox compounds predicted as LTox actually belong to this experimental category (Table S4), which corresponds to a prediction power of 72% (=0.720, Table S5). Conversely, (only) 59 of the 110 LTox compounds are recognized as such (Table S4) through LT1–LT7, implying a (relatively low) sensitivity of 53.6% (0.536, Table S5). When discriminating LTox from the combined group of 74 MTox and 140 HTox compounds, the latter subgroup is correctly recognized to 88% (sensitivity) and predicted to 76%. Correspondingly, combination of the 82 LTox and 74 MTox compounds yields recognition and prediction powers for this pooled subgroup of 71% and 92%, respectively.

So far, however, the classification statistics have been analyzed only in gross form, ignoring differences in the degree of mis-classification. Taking HT10 (fused or heterosubstituted aromatics, Fig. 2) as an example, 40 of the 64 compounds predicted (through this structural alert) as HTox are actually HTox, but 22 of the 24 wrongly classified compounds belong to the neighboring category MTox, and only 2 compounds are actually LTox. From this viewpoint, only 3% of the 64 compounds meeting the HT10 condition actually belong to the LTox category, and 97% are either HTox or at least MTox.

Corresponding analysis of all structural alerts (Table 2) reveals that there are only two cases where an LTox-predicted compound is actually HTox (LT1 and LT4). In all other cases, false LTox predictions refer to compounds that experimentally belong to the MTox category and are thus not HTox. The latter is particularly pronounced for LT6 with a mis-classification rate of 44% (11 of 25 LTox-predicted compounds are not LTox), with none of the wrongly predicted compounds belonging to the HTox category.

From this viewpoint, LT1–7 appear to be powerful for identifying compounds that are at least not HTox, which is true in 108 out of 110 cases (98%), keeping in mind that various LTox compounds meet more than one LTox structural rule. With HT1–14, the total number of matches is 186 (again with various HTox compounds meeting several HTox rules), of which in only 20 cases the compound actually belongs to the LTox category, leaving 166 cases (89%) with compounds being at least MTox.

In terms of compound counts, the accordingly loosened degree of accuracy when accepting MTox for both LTox and HTox predictions (but neither HTox for LTox prediction nor LTox for HTox prediction) yields the following statistics: 80 of the 82 LTox-predicted compounds actually are LTox or MTox (and only two compounds HTox; Table S4), which corresponds to an accordingly generalized predictivity of 97.5% (that is slightly different from the above-mentioned 98% referring to the sum of individual LTox structural alert matches). Moreover, of the 140 HTox-predicted compounds 124 actually belong to the HTox or MTox category, thus indicating a generalized predictivity of 88.6% (where only 16 HTox-predicted compounds are experimentally LTox; Table S4).

These results demonstrate that while the presently introduced structural alert model yields an only moderate performance in discriminating between LTox, MTox and HTox as three separate categories, it appears to be quite powerful in avoiding LTox vs HTox mis-classifications. As such, the structural alerts may be useful for the initial assessment of the likely potency of inhalation toxicants, and for providing guidance about the extent of experimental investigation in case more detailed information is required.

Fig. 3. Mechanistic rationale for the 14 HTox rules HT1–14 from Fig. 2 in terms of possibly underlying reaction pathways involving endogenous nucleophiles NuH (see text; AChE-OH = acetylcholine esterase, ADH = alcohol dehydrogenase, GSH = glutathione, Hb · Fe^{2+} = hemoglobin, Hb · Fe^{3+} = methemoglobin, MAO = monoamine oxidase, NQO1 = NAD(P)H: quinone oxidoreductase 1, P450 = cytochrome P450; P450 reductase = NADPH cytochrome P450 reductase).

4. Discussion

The structural alerts HT1–14 listed in Fig. 2 provide mechanistic hypotheses for the chemistry underlying the molecular initiating events that eventually lead to high inhalation toxicity. In the following sections, potentially relevant mechanisms of reaction of the HTox test compounds with nucleophilic sites of endogenous molecules (peptides, proteins, membrane components, DNA) are outlined (Fig. 3), thus offering a rationale for the observed inhalation NOEC. Whereas most of these reaction mechanisms are based on general knowledge regarding the toxicological action of chemicals (Eisenbrand et al., 2005; Klaassen, 2008), they have so far not been invoked as a means for discriminating between high and low repeated-dose inhalation toxicity.

4.1. High-toxicity structural alerts HT1–3

HT1 specifies isocyanates that contain an electrophilic carbon with double bonds to both nitrogen and oxygen (R–NCO). Besides hydrolysis to yield an alkyl amine and CO_2, nucleophilic attack at this activated carbon may result in an acylated functionality of the respective endogenous nucleophile (peptide, protein, DNA), thus confounding its physiological function (Fig. 3). Benzylic chlorides (HT2) are particularly reactive as S_N2 electrophile, because cleavage of the halide as good leaving group is supported further by stabilizing the reaction intermediate through delocalization of the charge developing temporarily at the benzylic carbon. From this viewpoint, benzylic bromides and iodides are also suspect of belonging to the HTox class, keeping in mind that respective derivatives are not present in our database.

Organophosphorus compounds (HT3) are used as insecticides inhibiting the acetylcholine esterase (AChE) at the synaptic gap through attack by their electrophilic P atom (after P450-mediated oxidation in case of phosphorothionates) at the serine OH of the enzyme, which holds in the same manner for rodents, mammals and humans (Schüürmann, 1992). This compound class serves also as flame retardants and plasticizers, with indoor concentrations yielding substantial internal exposure of metabolites in humans (Carignan et al., 2013; Cequier et al., 2015; Fromme et al., 2014).

Besides inhibition of synaptic AChE, delayed neurotoxicity may take place (Johnson, 1975) that could be linked to their additional capability as alkylating agents. The latter proceeds through dealkylation of one of the ester functions, implying that only organophosphates with at least one ester function could exert this delayed mode of action (with phosphinates $R_2P(O)X$ being a class that does not meet this condition (Johnson, 1975). Hydrolysis of the phosphorylated enzyme may cleave one ester function resulting in a (dissociated and thus particularly stable) phosphoric acid (a process called aging), whereas the alternative hydrolytic pathway to reactivating the enzyme is usually very slow.

Interestingly, calculated NMR ^{17}O shifts for aromatic phosphorothionates $(RO)_2P(S)OArX$ demonstrate an intramolecular impact of the aromatic substituents X on both the aromatic leaving-group oxygen ($\rightarrow^-OArX$ upon AChE phosphorylation) and the oxygen of the dealkylating side chain (P–O bond fission, see HT3) with a significant but moderate intercorrelation (Schüürmann and Schindler, 1993), providing an explanation for the structural impact on delayed neurotoxicity through the dealkylation route.

4.2. High-toxicity structural alerts HT4–7

HT4 comprises aliphatic and aromatic amines that are known for quite distinct modes of toxicological action. Whereas both compound classes may undergo P450-catalyzed N-hydroxylation, the further metabolic activation to electrophilic diazonium and nitrenium cations usually requires facilitation through delocalization to the aromatic moiety attached to N. Moreover, aromatic amines are also prominent methemoglobinemia formers through redox-cycling between the hydroxyl amine and nitroso metabolites, thus reducing the oxygen transport capability of the red blood cells (Fig. 3, HT4a).

Aliphatic amines are significantly more basic than their aromatic counterparts (conjugate acid pK_a ca. 10 vs 5), implying pH-mediated irritation and corrosion. In addition, oxidation through MAO (monoamine oxidase) yields an imine metabolite that can readily dissociate (without enzymatic action) into a carbonyl as hard electrophile and an amine with one less alkyl group than initially. In case of P450-catalyzed N-hydroxylation, the resultant aliphatic hydroxylamine may react with amino groups of DNA bases or proteins, resulting in N-hydroxylated endogenous compounds and (again basic and thus pH stress inducing) ammonia (HT4b).

HT5 represents Michael-acceptor aldehydes that may attack endogenous nucleophiles. Because of their soft electrophilicity at the β-carbon, such α,β-unsaturated carbonyls react preferably with protein sites, contrasting with epoxides as harder electrophiles that are known for forming covalent adducts with DNA bases. Regarding aliphatic 1-ω dinitriles (NC-R-CN), a possible route could be P450-catalyzed C-hydroxylation to form a cyanohydrin that is prone to dissociate into cyanide (CN^-) and a carbonyl (HT6a). In addition, direct attack of the electron-poor CN carbon at endogenous nucleophilic sites appears possible as outlined in Fig. 3 (HT6b).

HT7 comprises secondary and tertiary aliphatic amines. Whereas the former may be subject to the same reaction mechanisms as outlined for primary amines (HT7 a), P450-catalyzed oxidation of the latter leads to N-oxides ($R_3N^+–O^-$) whose chemistry underlying their toxicological action appears to be less clear. A speculative reasoning, however, would be that under certain conditions the N-oxide may cleave one of their (e.g. tertiary) alkyl groups as carbenium ion (R^+), the latter of which could alkylate endogenous nucleophiles with parallel formation of an N-hydroxylamine (HT7b).

4.3. High-toxicity structural alerts HT8–10

Carbonyl and sulfuryl chlorides become strong electrophiles upon elimination of Cl^- (HT8a and b). Amides (HT9a) are usually considered to be chemically stable under physiological conditions except if they are good substrates for amidases, resulting in a basic amine and a carboxylic acid. A speculative further route could be that at low pH with a correspondingly increased degree of protonation of the carbonyl oxygen ($>C^+–OH$), electrophilic attack at endogenous nucleophiles would form α-C-hydroxylated adducts that could probably cleave amines and convert to acylated products (Fig. 3).

HT9 includes also carbamates ($RO–C(O)–NR_2$) that may inhibit AChE through its carbamylation (HT9b) (Kuhr and Dorough, 1976) in a way similar to the AChE phosphorylation by organophosphorus compounds (see HT3 above), except that in mammals and rodents the enzyme reactivation upon hydrolysis is usually significantly faster. Interestingly, the hydrolytic stability of carbamates ranges from seconds-days (RHN-CO-OAr) to ca. 50,000 years (no H at amide N; Tinsley, 2004). Thus, a speculative further pathway is through hydrolysis to basic amine, alcohol and CO_2 (HT9c) that could jointly yield a pronounced toxicological potency.

Polycyclic aromatics and heteroaromatics (HT10) may form electrophilic epoxides upon P450-catalyzed oxidation, but can also serve as aryl hydrocarbon receptor (AhR) ligands confounding the metabolic homeostasis.

4.4. High-toxicity structural alerts HT11–14

Regarding vicinal dihalogenides (HT11), a prominent feature of their toxicological profile is the formation of episulfonium metabolites after initial GSH conjugation followed by elimination of the second halide. Whereas our current dataset does not include iodine as respective substituent, its ability as excellent S_N2 leaving group suggests its inclusion in HT11. Dialkyl sulfates (HT12) are a further group of alkylating agents,

in this case through their capability of delocalizing the temporary excess charge upon liberation of one of their alkyl substituents as carbenium ion. With anhydrides (HT13), electrophilic attack at endogenous nucleophiles competes with hydrolysis (where water is the nucleophilic reaction partner).

Finally, HT14 comprises aliphatic and aromatic polyols. Whereas the OH substituent is not ready for S_N2 reactions because of its poor leaving group ability, a possible molecular initiating event for exerting enhanced toxicity could be a P450-catalyzed hydroxylation at one (of the several) OH-bonding carbon atoms, forming a geminal diol that could eliminate water (dehydration) to yield a carbonyl with a hard electrophilic carbon (Fig. 3, HT14a). The probability for this activation pathway is likely to increase with increasing number of OH groups already present, providing a rationale for the HTox profile of polyhydroxylated aliphatics.

In case of vicinal aliphatic diols such as the highly toxic ethylene glycol ($HOCH_2CH_2OH$), metabolic activation proceeds through two consecutive ADH-catalyzed oxidations (ADH = alcohol dehydrogenase) to a glyoxal derivative (glyoxal as smallest dialdehyde, O═C(H)–C(H)═O, would result from ethylene glycol) that is readily electrophilic for reacting with endogenous nucleophiles (HT14b).

By contrast, polyhydroxylated benzenes are redox-active agents and thus capable of exerting oxidative stress as well as to act as Michael acceptors in their oxidized quinone form. Redox cycling between ortho (and para) di-hydroxy benzenes (hydroquinone, H_2Q) and their quinone counterpart (Q) can proceed through autocatalysis (HT14c) as well as with enzymes (HT14d) (Bolton et al., 2000; Brunmark and Cadenas, 1989; O'Brien, 1991). Here, DT-diaphorase (NQO1 = NAD(P)H: quinone oxidoreductase 1) may catalyze a direct 2-electron $Q \rightarrow H_2Q$ reduction without passing through the intermediate semiquinone HQ• that appears to be particularly prone for generating superoxide anion ($HQ\bullet + O_2 \rightarrow O_2^{-}\bullet + H^+$) and subsequent reactive oxygen species (ROS), or may recover quinone upon disproportionation (HT14e). Alternatively, hydroquinone autoxidation may yield hydrogen superoxide radical HOO• as further ROS ($H_2Q + O_2 \rightarrow HQ\bullet + HOO\bullet$) that possibly decomposes to the more stable $O_2^{-}\bullet$ ($HOO\bullet \rightarrow O_2^{-}\bullet + H^+$).

Whereas the consecutive 1-electron reductions are catalyzed by NADPH cytochrome P450 reductase or NADH cytochrome b_5 reductase, the reverse 1-electron oxidations can be catalyzed by the monooxygenase cytochrome P450 involving consecutively its so-called compound I as prominent oxidant (FeO) and compound II (FeOH; HT14f), which has been evaluated through computational chemistry for the specific case of paracetamol (acetaminophen) (Ji and Schüürmann, 2015).

HT14 also includes bisphenol A (BPA) and its 2,2′,6,6′-tetrabromo derivative (TBBPA). BPA has been used as plasticizer and fungicide and is a known endocrine disruptor that is also suspected to impair thyroid function (Boas et al., 2012; Gentilcore et al., 2013; Rezg et al., 2014; Sheng et al., 2012). Regarding the flame retardant TBBPA, there is a controversial discussion concerning its activity as endocrine disruptor (Gentilcore et al., 2013; Colnot et al., 2014) with some reports indicating a specific anti-thyroid action (Sun et al., 2009; Kitamura et al., 2005). Interestingly enough, the repeated-dose oral toxicity of TBBPA appears to be relatively low (Colnot et al., 2014), contrasting with our present result of a high-potent repeated-dose toxicant when exposed through the inhalation pathway, possibly because of respective differences in the toxicokinetics.

5. Conclusions

The present results demonstrate the scope of structure-activity reasoning for complex endpoints such as the repeated-dose inhalation toxicity. The derived structural alerts enable a screening-level discrimination between high- (or medium-) vs low-toxic and low- (or medium-) vs high-toxic compounds. Accordingly, they represent a non-test tool for evaluating the long-term hazard of chemicals, may support scoping experimental studies if deemed necessary, and provide a rationale for designing less harmful compounds. In the risk assessment context, the HTox vs LTox structural characteristics yield a mechanistic basis for deriving respective TTC values, which in turn could be used as more specific thresholds for identifying levels of exposure that are unlikely to yield harmful effects.

In particular, the structural alerts elucidate the chemistry underlying the likely molecular initiating events. As such, they inform about potentially relevant toxicological mechanisms, and yield a reaction-chemistry basis for extending the rules to compounds sufficiently similar to the present database such that their allocation to one of the described mechanistic domains becomes sufficiently probable. The derived physicochemical triggers for high and low inhalation toxicity characterize the tendency of the substances to escape from blood to air or interact with endogenous compounds (proteins, lipid, DNA), and thus likely represent the impact of bioavailability on the repeated-dose toxicity outcome. Besides applying them as standalone-tool, they can also be used in combination with the structural alerts, indicating a way forward to a consensus model approach that may be subject to future investigations.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.12.005.

References

AQUIRE (Aquatic toxicity information retrieval database), US EPA (Environmental Protection Agency), 2011U. Duluth, national health and environmental effects research laboratory. http://cfpub.epa.gov/ecotox/ (accessed 2011/1/21).

Berggren, E., Amcoff, P., Benigni, R., Blackburn, K., Carney, E., Cronin, M., Deluyker, H., Gautier, F., Judson, R.S., Kaas, G.E.N., Keller, D., Knight, D., Lilienblum, W., Mahony, C., Rusyn, I., Schultz, T., Schwarz, M., Schüürmann, G., White, A., Burton, J., Lostia, A.M., Munn, S., Worth, A., 2015. Environ. Health Perspect. 123, 1232–1240. http://dx.doi.org/10.1289/ehp.1409342.

Boas, M., Feldt-Rasmussen, U., Main, K.M., 2012. Thyroid effects of endocrine disrupting chemicals. Mol. Cell. Endocrinol. 355, 240–248.

Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J., 2000. Role of quinones in toxicology. Chem. Res. Toxicol. 13, 135–160.

Brunmark, A., Cadenas, E., 1989. Redox and addition chemistry of quinoid compounds and its biological implications. Free Radic. Biol. Med. 7, 435–477.

Carignan, C.C., McClean, M.D., Cooper, E.M., Watkins, D.J., Fraser, A.J., Heiger-Bernays, W., Stapleton, H.M., Webster, T.F., 2013. Predictors of tris(1,2-dichloro-2-propyl) phosphate metabolite in the urine of office workers. Environ. Int. 55, 56–61.

Carthew, P., Clapp, C., Gutsell, S., 2009. Exposure based waiving: the application of the toxicological threshold of concern (TTC) to inhalation exposure for aerosol ingredients in consumer products. Food Chem. Toxicol. 47, 1287–1295.

Cequier, E., Shakhi, A.K., Marcé, R.M., Becher, G., Thomsen, C., 2015. Human exposure pathways to organophosphate triesters — a biomonitoring study of mother-child pairs. Environ. Int. 75, 159–165.

ChemProp, 2014. Version 6.2, UFZ Department of Ecological Chemistry. http://www.ufz.de/index.php?en=6738 (accessed 2015/7/20).

Collins, F.S., Gray, G.M., Bucher, J.R., 2008. Transforming environmental health protection. Science 319, 906–907.

Colnot, T., Kacew, S., Dekant, W., 2014. Mammalian toxicology and human exposures to the flame retardant 2,2′,6,6′-tetrabromo-4,4′-isopropylidenediphenol (TBBPA): implications for risk assessment. Arch. Toxicol. 88, 553–573.

Cramer, G.M., Ford, R.A., Hall, R.L., 1978. Estimation of toxic hazard — a decision tree approach. Food Cosmet. Toxicol. 16, 255–276.

Eisenbrand, G., Metzler, M., Hennecke, J., 2005. Toxikologie für Naturwissenschaftler und Mediziner. third ed. Weinheim, VCH-Wiley.

Escher, S.E., Tluczkiewicz, I., Batke, M., Bisch, A., Melber, C., Kroese, E.D., Buist, H.E., Mangelsdorf, I., 2010. Evaluation of inhalation TTC values with the database RepDose. Regul. Toxicol. Pharmacol. 58, 259–274.

Fromme, H., Lahrz, T., Kraft, M., Fembacher, L., Mach, C., Dietrich, S., Burkhardt, R., Völkel, W., Göen, T., 2014. Organophosphate flame retardants and plasticizers in the air and dust in German daycare centers and human biomonitoring in visiting children (LUPE 3). Environ. Int. 71, 158–163.

Gentilcore, D., Porreca, I., Rizzo, F., Ganbaatar, E., Carchia, E., Mallardo, M., de Felice, M., Ambrosino, C., 2013. Bisphenol A interferes with thyroid specific gene expression. Toxicology 304, 21–31.

Hartung, T., 2009. Toxicology for the twenty-first century. Nature 460, 208–212.
Hennes, E.C., 2012. An overview of values for the threshold of toxicological concern. Toxicol. Lett. 211, 296–303.
Ji, L., Schüürmann, G., 2012. Computational evidence for α-nitrosamino radical as initial metabolite for both the P450 dealkylation and denitrosation of carcinogenic nitrosamines. J. Phys. Chem. B116, 903–912.
Ji, L., Schüürmann, G., 2013. Model and mechanism: N-hydroxylation of primary aromatic amines by cytochrome P450. Angew. Chem. Int. Ed. 52, 744–748. Angew. Chem. 125, 772–776.
Ji, L., Schüürmann, G., 2015. Computational biotransformation profile of paracetamol catalyzed by cytochrome P450. Chem. Res. Toxicol. 28, 585–596.
Johnson, M.K., 1975. Organophosphorous esters causing delayed neurotoxic effects. Arch. Toxicol. 34, 259–288.
Kalkhof, H., Herzler, M., Stahlmann, R., Gundert-Remy, U., 2012. Threshold of toxicological concern values for non-genotoxic effects in industrial chemicals: re-evaluation of the Cramer classification. Arch. Toxicol. 86, 17–25.
Kazius, J., McGuire, R., Bursi, R., 2005. Derivation and validation of toxicophores for mutagenicity prediction. J. Med. Chem. 48, 312–320.
Kitamura, S., Kato, T., Ida, M., Jinno, N., Suzuki, T., Ohta, S., Fujimoto, N., Hanada, H., Kashiwagi, K., Kashiwagi, A., 2005. Life Sci. 76, 1589–1601.
Klaassen, C.D. (Ed.), 2008. Casarett and Doull's Toxicology, seventh ed. The Basic Science of Poisons. McGraw-Hill, New York.
Krewski, D., Weshtphal, M., Andersen, M.E., Paoli, G.M., Chiu, W.A., Al-Zoughool, M., Croteau, M.C., Burgoon, L.D., Cote, I., 2014. A framework for the next generation of risk science. Environ. Health Perspect. 122, 796–805.
Kühne, R., Ebert, R.-U., Schüürmann, G., 2009. Chemical domain of QSAR models from atom-centered fragments. J. Chem. Inf. Model. 49, 2660–2669.
Kuhr, R.J., Dorough, H.W., 1976. Carbamate Insecticides: Chemistry, Biochemistry, and Toxicology. CRC Press, Cleveland.
Munro, I.C., Ford, R.A., Kennepohl, E., Sprenger, J.G., 1996. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of toxicological concern. Food Chem. Toxicol. 34, 829–867.
Munro, I.C., Renwick, A.G., Danieleweska-Nikiel, B., 2008. The threshold of toxicological concern (TTC) in risk assessment. Toxicol. Lett. 180, 151–156.
O'Brien, P.J., 1991. Molecular mechanisms of quinone cytotoxicity. Chem. Biol. Interact. 80, 1–41.
OECD, 2009a. Chronic toxicity studies. Test Guideline No. 452. OECD Guidelines for the Testing of Chemicals,. OECD, Paris, France.
OECD, 2009b. Subacute inhalation toxicity: 28-day study. Test Guideline No. 412. OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
OECD, 2009c. Subchronic inhalation toxicity: 90-day study. Test Guideline No. 413. OECD Guidelines for the Testing of Chemicals. OECD, Paris, France.
Prescher, J.A., Bertozzi, C.R., 2005. Chemistry in living systems. Nat. Chem. Biol. 1, 13–21.
Rappaport, S.M., 2012. Discovering environmental causes of disease. J. Epidemiol. Community Health 66, 99–102.
Rappaport, S.M., Smith, M.T., 2010. Environment and disease risks. Science 330, 460–461.
Rezg, R., El-Fazaa, S., Gharbi, N., Mornagui, B., 2014. Bisphenol A and human chronic diseases: current evidences, possible mechanisms, and future perspectives. Environ. Int. 64, 83–90.
Russom, C.L., Bradbury, S.P., Broderius, S.J., Hammermeister, D.E., Drummond, R.A., 1997. Predicting modes of toxicity action from chemical structure: acute toxicity in the fathead minnow (*Pimephales promelas*). Environ. Toxicol. Chem. 16, 948–967.
Schüürmann, G., Schindler, M., 1993. Fish toxicity and dealkylation of aromatic phosphorothionates — QSAR analysis using NMR chemical shifts calculated by the IGLO method. J. Environ. Sci. Health A28, 899–921.
Schüürmann, G., Ebert, R.-U., Chen, J., Wang, B., Kühne, R., 2008. External validation and prediction employing the predictive squared correlation coefficient — test set activity mean vs training set activity mean. J. Chem. Inf. Model. 48, 2140–2145.
Schüürmann, G., Ebert, R.-U., Kühne, R., 2011. Quantitative read-across for predicting the acute fish toxicity of organic compounds. Environ. Sci. Technol. 45, 4616–4622.
Schüürmann, G., 1992. Ecotoxicology and structure–activity studies of organophosphorus compounds. In: Fujita, T., Draber, W. (Eds.), Rational Approaches to Structure, Activity and Ecotoxicology of Agrochemicals. CRC Press, Boca Raton, pp. 485–541.
Sheng, Z.-G., Tang, Y., Liu, Y.-X., Yuan, Y., Zhao, B.-Q., Chao, X.-J., Zhu, B.-Z., 2012. Low concentrations of bisphenol a suppress thyroid hormone receptor transcription through a nongenomic mechanism. Toxicol. Appl. Pharmacol. 259, 133–142.
Sun, H., Shen, O.-X., Wang, X.-R., Zhou, L., Zhen, S-q., Chen, X-d., 2009. Anti-thyroid hormone activity of bisphenol A, tetrabisphenol A and tetrachlorobisphenol A in an improved reporter gene assay. Toxicol. in Vitro 23, 950–954.
Tinsley, I.J., 2004. Chemical Concepts in Pollutant Behaviour. second ed. Wiley, Hoboken.
Tluczkiewicz, I., Buist, H.E., Martin, M.T., Mangelsdorf, I., Escher, S.E., 2011. Improvement of the Cramer classification for oral exposure using the database TTC Repdose — a strategy description. Regul. Toxicol. Pharmacol. 61, 340–350.
Tluczkiewicz, I., Kühne, R., Ebert, R.U., Batke, M., Schüürmann, G., Mangelsdorf, I., Escher, S.E., 2015. Inhalation TTC values: A new integrative grouping approach based on structural, toxicological and mechanistic features. Regulat. Toxicol. Pharmacol.
Wild, C.P., 2005. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol. Biomark. Prev. 18, 1847–1850.
Wild, C.P., 2012. The exposome: from concept to utility. Int. J. Epidemiol. 41, 24–32.
Zhang, Q., Bhattacharaya, C.R.B., Clewell, H.J., Kaminski, N.E., Andersen, M.E., 2014. Molecular signaling network motifs provide a mechanistic basis for cellular threshold responses. Environ. Health Perspect. 122, 1261–1270.