THE LIMITATIONS OF COMMONLY USED ECOTOXICITY QSARS—EXPERIMENTS IN MODEL VALIDATION

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ABSTRACT

QSARs are becoming increasingly important tools to screen and, in some cases, assess the fate and effects of large groups of organic compounds. Tens of thousands of organic compounds are in the process of being screened for hazard classification under several international regulatory initiatives. A reliance on QSARs is likely; however, the limitations of the available models may be underestimated and unrecognized. Two commonly used QSAR models are the Könemann equation and ECOSAR, software available as part of Syracuse Research Corporation's EPIWIN Suite. ECOSAR uses a number of log K_{OW} based QSARs to estimate the ecotoxicity of organic compounds for several structural classes often resulting in ecotoxicity estimates of a variety of endpoints. In an effort to demonstrate the validity of the outputs of these models, a set of 44 fragrance materials was selected to compare the models' output against measured data for lethality (96 hour-LC_{50}) for *Pimephales promelas* (Fathead minnow). A method was developed to separate narcosis compounds from non-narcosis compounds. The two methods used to separate the compounds were the determination of the mode of action using a classification system reported by Verhaar *et al.* and the development of a log K_{OW} based toxicity relationship based on the work of DiToro *et al.* Twenty-four fragrance materials, using both methods, were categorized as definite narcosis compounds. The remainder did not fit both definitions of narcosis compounds. The correlations, using either the Könemann equation or the ECOSAR model, were very good (r^2 of 0.84 and 0.93, respectively) for the set of definite narcosis compounds and very poor for the remaining fragrance materials (r^2 of 0.065 and 0.063, respectively). It is apparent that these models are capable of both underpredicting and overpredicting toxicity.

INTRODUCTION

Narcosis is a non-specific type of reaction resulting in anesthesia of the organism. There is no direct interaction with specific receptors on the organism. Veith and Broderius define narcosis as “a reversible state of arrested activity of protoplasmic structures caused by a wide variety of organic chemicals.” Narcosis, exists as two types, nonpolar, or Type I, and polar, or Type II. Nonpolar or baseline narcosis is defined by Veith and Broderius as “characterized by progressive lethargy, unconsciousness, and death without any sustained symptoms.” Polar narcotics are narcotic chemicals that are found to be more toxic than baseline QSARs would otherwise predict. Veith and Broderius continue to explain that Type II narcosis may be the result of the presence of strong hydrogen bonding groups on the molecule; where Type I narcosis results from hydrophobic bonding of the chemical to enzymes and/or cell membranes. With increasing log K_{ow}, hydrophobic bonding exerts a greater influence on the toxicity of the toxicant than does hydrogen bonding.

The model used in the RIFM Framework to estimate toxicity for the PEC/PNEC is a very general model developed by Hans Könemann.

$$\log 1/LC_{50} = 0.871 \log K_{ow}\ 4.87 \qquad r = 0.988$$

This equation has become a widely accepted QSAR for narcosis chemicals and is the recommended QSAR found in the European Union's Technical Guidance Document and is used in the USEPA's QSAR computer model ECOSAR.

Könemann developed this QSAR based upon 50 industrial chemicals and the mortality of the guppy, *Poecilia reticulata*. The compounds selected fell into several categories: benzene, alkyl substituted benzenes, and chlorinated benzenes/alkyl substituted benzenes; chlorinated alkanes and alkenes; alcohols and glycols; and some miscellaneous compounds. These were 7 day static renewal tests performed on 2-3 month old guppies. Mortality was the endpoint of this study. He noted this worked best for narcosis type chemicals with log K_{ow} less than 6.

The RIFM Framework applies very large assessment factors to compensate for the indiscriminate results that may be provided by using the Könemann equation or ECOSAR. The Könemann equation is based on a series of narcosis toxicants; ECOSAR predicts toxicity for many structural classes and for many toxic endpoints, predominately freshwater acute and chronic standard tests (e.g., 48 hour *Daphnia*) and, infrequently, marine and terrestrial endpoints. In ECOSAR the QSARs used can be built from as few as one or no measured values (i.e., modifications of other QSARs but with no available data to support the equation).

In an effort to demonstrate the disparity of these results, a set of 44 fragrance materials was selected to compare the output of these two models against measured data for lethality (LC_{50}) for *Pimephales promelas* (Fathead minnow) in standard 96 hour tests. *P. promelas* is a freshwater organism commonly used for reporting the effects of chemicals to regulatory authorities. For example, the acute toxicity of *P. promelas* is often part of the base set used to determine a predicted no-effect concentration to ascertain the potential ecological risk of organic chemicals in the European Union.

METHOD

A search of the RIFM/FEMA Database indicated that there are 44 fragrance materials reported that have measured 96 hour LC_{50} endpoints for *Pimephales promelas*. These fragrance materials were divided into two sets. In an attempt to separate narcosis from non-narcosis toxicants, these fragrance materials were:

1. plotted against a log K_{ow} based—96 hour *P. promelas* line developed from known narcosis compounds, and
2. were divided into two sets based on a method developed by Verhaar.

Development of a K_{ow} based relationship for Pimephales promelas data

DiToro *et al.* in developing the Target Lipid Model used the critical body burdens to calculate LC_{50}s. Through a series of regression analyses, they found they could derive a cross species universal narcosis slope corrected for chemical classes. They recognize that the intercept will vary species to species, yet the slope will remain essentially unchanged. DiToro *et al.* offer the explanation that the slope of the relationship between LC_{50} and log K_{ow} in their derivation should be the same “regardless of species since it is a chemical property of the target lipid—the slope of the LFER.” Thus a linear relationship does exist, for narcosis chemicals, between log K_{ow} and LC_{50}. It is from the 96 hour LC_{50} *Pimephales promelas* data used in this model that a K_{ow} based QSAR is developed as a second approach for identifying narcosis fragrance materials. Figure 1 shows the relationship developed from these data. Figure 2 plots the 44 fragrance materials against this QSAR and its 95% confidence interval.

Fig. 1: narcosis QSAR based on the work of DiToro et al.

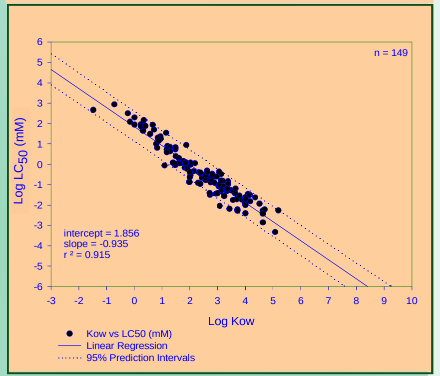
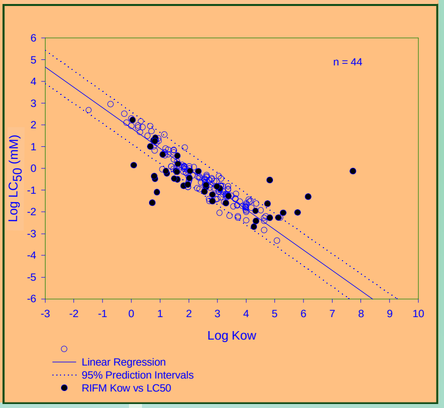


Fig. 2: fragrance materials plotted on the narcosis line



Verhaar Classification

Verhaar *et al.* describe a method to classify chemicals in one of five major classes (baseline, less inert, unspecific activity, specific mechanism and unclassifiable) with up to 6 different sub types. Table 1 shows an abbreviated set of the rules established in this paper that are applicable to the fragrance materials in this study.

Table 1: Verhaar rules for classifying modes of action

Toxicity	Rule Number	Element Present (in addition to C and H)		Rules
		N	O	
Baseline	1.1	N	N	no ionic groups
	1.2.2	N	Y	aliphatic alcohols/ not allylic or propargylic
	1.2.4	N	Y	ketones - not a,ß -unsaturated (1-butenone, acetophenone)
Less inert	2.1	Y	Y	non- or weakly acidic phenols (one nitro substituent or alkyl substituent)
Unspecific activity	3.1			allylic/propargylic activation (good leaving group a to double or triple bond)
	3.2			benzyl activation (good leaving group a to aromatic bond)
	3.8			acid anhydrides, lactones, ketenes, aldehydes, isocyanates, thiocyanates, isothiocyanates, disulphides, sulphonic acids, sulphuric esters, cyclic sulphuric/sulphonic esters
Unclassifiable	5.0			

Determination of narcosis fragrance materials

Tables 2 and 3 separate the 44 fragrance materials into “definite” narcosis compounds and potentially non-narcosis compounds.

Definite narcosis compounds (table 2):

- fit a rule for narcosis toxicity from Verhaar **and**
- fall within the 95% confidence interval around the QSAR developed from the data available for 96 hour LC_{50}s for *P. pimephales* from the Target Lipid Model of DiToro *et al.*

Potential non-narcosis compounds (table 3)

- either fit a Verhaar narcosis rule **or**
- fall within the 95% confidence interval of the QSAR **or**
- fit neither rule

Table.2: Materials that are classified as narcosis using both Verhaars rules and the Narcosis QSAR

Material	log K_{ow} (Calc indicates estimated and not measured)	Mean 96-h LC_{50} (mM) (n=1 unless otherwise noted)	Verhaar Class
Isobutyl alcohol	0.77	19.293	1.2.2
Isopropyl alcohol	0.05	169.085 (n=5)	1.2.2
Butyl alcohol	0.84	24.690 (n=4)	1.2.2
Lauryl alcohol	5.13	0.0054 (n=2)	1.2.2
Butyl acetate	1.82	0.155	1.1
Hexyl acetate	2.83	0.031	1.1
Diethyl sebacate	4.33	0.0109 (n=2)	1.1
Ethyl hexanoate	2.83	0.062	1.1
Ethyl salicylate	3.09	0.118	1.1
d-.alpha.-Pinene	4.27	0.00206	1.1
d-Limonene	4.83	0.00522 (n=2)	1.1
l-.beta.-Pinene	4.35	0.00368	1.1
Terpinolene	3.5, 5.3	0.0089	1.1
2-Methyl-3-butanone	0.67 Calc	10.055 (n=2)	1.2.4
Diethyl ketone	0.82	17.880	1.2.4
d-Camphor	2.34 Calc	0.723	1.2.4
2,4-Xylenol	2.61 Calc	0.139	2.1
2,6-Xylenol	2.61 Calc	0.170	2.1
cis-3-Hexenol	1.61 Calc	3.803	1.2.2
p-Cresol	1.97	0.176	2.1
p-Ethylphenol	2.55	0.0851 (n=2)	2.1
4-Methoxyphenol	1.59	0.679	2.1
Phenol	1.50	0.335 (n=7)	2.1
Thymol	3.30	0.025 (n=2)	2.1

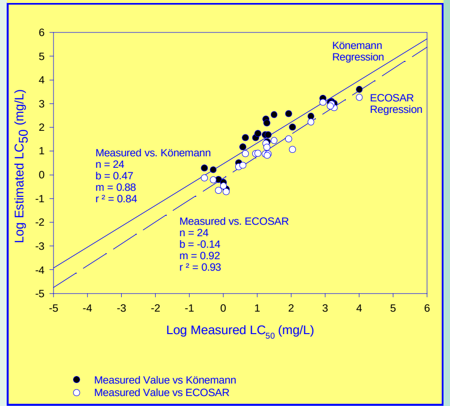
Table 3: Materials that are classified as potentially non-narcosis (either Verhaar or QSAR only or neither)

Material	log K_{ow}	Mean 96-h LC_{50} (mM) (n=1 unless otherwise noted)	Verhaar Class (Narcosis Y/N)	QSAR Narcosis
Acetic acid	0.09	1.391 (n=2)	5.0 (N)	N
Hexanoic acid	2.05	0.758	5.0 (N)	Y
Valeric acid	1.56	0.754	5.0 (N)	Y
Oleic acid	7.73	0.726	5.0 (N)	Y
Benzyl alcohol	1.10	4.254	3.2 (N)	Y
1-Hexen-3-ol	1.61	0.304	3.1 (N)	Y
Ethyl acetate	0.73	0.026	1.1 (Y)	N
Propyl acetate	1.24	0.587	1.1 (Y)	N
Diethyl malonate	0.9	0.080 (n=2)	1.1 (Y)	N
trans-Anethole	3.39	0.052	5.0 (N)	Y
dl-Limonene (racemic)	4.83	0.283	1.1 (Y)	N
p-Mentha-1,3-diene	4.75	0.023	1.1 (Y)	N
Longifolene	6.16	0.050	5.0 (N)	N
Furfural	0.83	0.333	5.0 (N)	N
Acetyl Cedrene	5.8	0.0093	5.0 (N)	N
Acetophenone	1.63	1.581 (n=5)	5.0 (N)	Y
Eugenol	2.99	0.146	5.0 (N)	Y
Resorcinol	0.80	0.424 (n=2)	2.1 (Y)	N
Vanillin	1.21	0.733 (n=5)	3.8 (N)	N
Quinoline	2.03	0.356	5.0 (N)	Y

DISCUSSION

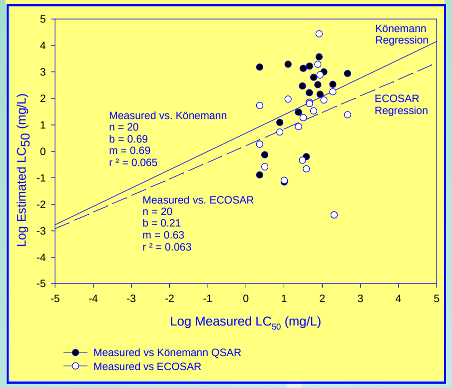
- Twenty-four fragrance materials, using both methods, were identified as definite narcosis compounds (table 2). These were predominantly alcohols, hydrocarbons, and esters.
- The remaining 20 compounds identified as potential non-narcosis compounds include polycyclic hydrocarbons, aldehydes, acids, esters, alcohols, and compounds with more than one structural moiety (e.g., vanillin contains both aldehyde and alcohol groups).
- These remaining twenty fragrance materials (table 3) that did not fit both definitions of narcosis compounds may be part of this set because:
  - the Verhaar method does not provide a clear descriptor for the particular class of compound (e.g., group 5.0)
  - an estimated log K_{ow} may be incorrect and, therefore, the data may be improperly plotted against the log K_{ow} line, or
  - the material is truly a non-narcosis chemical.

Figure 3: comparison between fragrance materials identified as narcosis chemicals: measured vs. QSAR



Figures 3 and 4 plot the measured endpoints against their respective predictions using the Könemann equation and ECOSAR.

Figure 4: comparison between fragrance materials identified as non-narcosis chemicals: measured vs. QSAR



- For the set of definite narcosis compounds the correlations, using either the Könemann equation or the ECOSAR model, are very good (r^2 of 0.84 and 0.93, respectively)
- For those identified as potentially non-narcosis compounds the correlations are very poor (r^2 of 0.065 and 0.063, respectively).

CONCLUSIONS

- These models both underpredict and overpredict toxicity
  - These errors in prediction included structural classes these models were developed from (e.g., alcohols).
  - Both plots contain some chemicals from the same chemical classes (e.g., alcohols and esters)
  - Both these models are frequently used to predict toxicity in a regulatory context.
- Compounds that are considered “non-narcosis” because of model predictions may, in fact, exhibit narcosis toxicity
- If an effort is not undertaken to determine the applicability of the model's domain to the chemical under study, the accuracy of the estimation is questionable.
  - Kaiser *et al.* raises some important points about log K_{ow} based QSAR models. They note that “much of the work has been done using small, highly congeneric datasets.” They point out that due to the restrictive and narrow ranges of compounds used in the development of these models, prediction becomes problematic because a compound of interest may not fit into the prescribed structural ranges of the QSAR or it may be difficult to ascertain what single structural group it may belong to because of multiple functional groups on the molecule.
- As fragrance materials are an example of a large and diverse set of organic compounds, and in light of regulatory initiatives to screen compounds for hazard based characteristics (i.e. PBT), the use of model estimations, while valuable, should be approached with prudence.
  - Minimally, the applicability of the model's domain should be reviewed.

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