Extrapolation of toxic indices among test objects

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ABSTRACT
Oligochaeta Tubifex tubifex, fish fathead minnow (Pimephales promelas), hepatocytes isolated from rat liver and ciliated protozoan are absolutely different organisms and yet their acute toxicity indices correlate. Correlation equations for special effects were developed for a large heterogeneous series of compounds (QSAR, quantitative structure-activity relationships). Knowing those correlation equations and their statistic evaluation, one can extrapolate the toxic indices. The reason is that a common physicochemical property governs the biological effect, namely the partition coefficient between two unmissible phases, simulated generally by n-octanol and water. This may mean that the transport of chemicals towards a target is responsible for the magnitude of the effect, rather than reactivity, as one would assume suppose.

KEY WORDS: QAAR; QSAR; extrapolation; acute toxicity; oligochaeta; fish; ciliate; hepatocytes

Introduction

The hazard of new chemicals in both environmental and human risk assessment attracts attention in all chemical safety programmes. To detect it is the responsibility of their manufacturers or distributors. The manufacturers, regulatory bodies or industrial research, are looking for an effective, rapid and inexpensive way of estimating a potential harm defined by various programmes of chemical safety. The standard tests of toxicity involve often the use of experimental animals. The concept of 3Rs (Replacement, Reduction and Refinement) (Russell & Burch 1959) call however for reduction of animals for experiments. Thus, alternative methods of toxicity testing & Burch 1959) call however for reduction of animals for experiments. Thus, alternative methods of toxicity testing are desired. Among the in vitro procedures (QSAR – quantitative structure-activity relationships) (Jaworska et al., 2003; Cronin et al., 2005) or on activities of rat hepatocytes (Tichy et al., 2010) were used for these studies.

To discover factors of toxicity variation among various species, acute toxicity data of many aquatic species and chemicals were analyzed by pattern recognition techniques (Vaal et al., 1997). Patterns in species sensitivity were found to be more diffuse because only part of the variance in species sensitivity could be explained. Most variations were due to differences in the toxicity of compounds and not to intrinsic differences among species.

Data on toxicity measured with several aquatic organisms such as Tetrahylena pyriformis, Vibrio fischeri or Tubifex tubifex (Schultz et al., 1986; Lipnick 1985; Bearden & Schultz 1997, 1998; Kaiser 1993; Cronin et al., 1991; Tichy et al., 2008), or on activities of rat hepatocytes (Tichy et al., 2010) were used for these studies. The extrapolation indicates that the accuracy of the estimated toxic activity was unaffected by the extrapolation. The estimated toxic activity was comparable with the activity measured with individual bioassays, at least for noncovalent acting chemicals (Dimirov et al., 2000).

In the present study, extrapolation of acute toxicity indices measured with oligochaeta Tubifex tubifex, fathead minnow (Pimephales promelas) and indices of metabolic disorder measured with primary rat hepatocytes are discussed. The data on EC50 inhibiting movement of the oligochaeta Tubifex in 3 mins (Tichy et al., 2007), EC50 of cell viability and EC50 influencing metabolic function of
primary rat hepatocytes, ureogenesis (Tichy et al., 2010), 
EC50 causing effect on fish *Pimephales promelas* in 96 
hours (Kaiser et al., 1997) were used.

**Material and methods**

**Chemicals**
The chemicals used were of pro-analysis grade purity, 
purchased from Fluka and Aldrich (Buchs, Switzerland) or 
Lancaster (Johnson Matthey, Ward Hill, Maryland, USA). 
Standard chemical manganese chloride was obtained 
from Merck (Darmstadt, Germany).

**Experimental organisms**
*Tubifex tubifex* also called sludge worm or sewage worm,
is a species of tubificid segmented worm that inhabits 
sediments of rivers, lakes and ponds on several contin-
ents. These worms ingest bacteria from the sediments and 
absorb molecules through their body wall. They can 
survive in areas heavily polluted with organic matter that 
almost no other organisms endure. They can survive with 
very little oxygen.

*Pimephales promelas*, fathead minnow, is a species 
of temperate fresh water fish belonging to the cyprinid 
family. The natural geographic range extends throughout 
much of North America including Canada. It is a golden 
or xanthic strain, known as the rosy-red minnow, and is a 
very common feeder fish sold in the United States. In its 
wild original form, the fathead minnow is generally dull 
olive-gray in appearance with a dusky stripe extending 
on the back and side and with a lighter belly. It inhabits 
muddy pools of headwaters, creeks and small rivers, 
ponds and lakes. It tolerates unsuitable turbid, hot or 
poorly oxygenated waters. EPA guidelines outline its use 
along the back and side and with a lighter belly. It inhabits 
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ponds and lakes. It tolerates unsuitable turbid, hot or 
poorly oxygenated waters. EPA guidelines outline its use 
for the evaluation of acute and chronic toxicity of samples 
or chemicals in vertebrate animals.

Hepatocytes are cells of livers forming liver tissue and 
are responsible for a majority of processes in the liver. 
They include a large amount of mitochondria, a devel-
oped Golgi complex, endoplasmatic reticulum and other 
organelles. They store glycogen, vitamins D, B12 and oth-
erwise. These worms ingest bacteria from the sediments and 
absorb molecules through their body wall. They can 
survive in areas heavily polluted with organic matter that 
almost no other organisms endure. They can survive with 
very little oxygen.

**Statistical analysis**
The QSARs were generated using a linear regression 
procedure providing the correlation coefficient (r), stan-
dard errors of the slope and of intercept and the residual 
standard deviation (SD) of the estimate (Origin 2003). 
The statistics of predictive parameters were performed 
as recommended (Eriksson et al., 2003) and the log 
P-based QSARs were validated in three ways (Tichy et al., 
2008).

**Toxicity indices**
The acute toxicity indices were taken from different 
published sources: *Tubifex tubifex* (Tt) (Tichy et al., 2007), 
fish *Pimephales promelas* (Pp) (Kaiser et al., 1997) and 
primary rat hepatocytes ure – ureogenesis, via – viability 
(Tichy et al., 2010).

**Physicochemical descriptor**
The logarithm of n-octanol-water partition coefficient, 
log P was taken from tables by Hansch et al. (1995).

**Results**
The primary data used are published in the relevant 
papers cited with the correlation equations (Tichy et al., 
2008; 2010). The correlation equations involving log P were found 
as follows:

\[
\log EC50(Pp) = -1.027 \log P - 1.107
\]

\[
\log EC50(Pp) = -0.941(\pm 0.083) \log P - 0.124(\pm 0.107)
\]

\[
\log EC50(Tt) = -0.848 \log P - 0.179
\]

The correlations of EC50 of various test objects with log 
EC50(Tt) result in (Tichy et al., 2010)

\[
\log EC50(ure) = -0.840(\pm 0.054) \log P - 0.303(\pm 0.065)
\]

\[
\log EC50(via) = -0.778(\pm 0.053) \log P - 0.199(\pm 0.065)
\]

These are just a few examples. It is possible to receive 
other intercorrelations. The predictivity and robustness 
of equations/models given above was checked by cross 
validation techniques. The squared correlation coefficient 
r^2 of the equation log EC50(via) vs. log EC50(Tt) changed 
from 0.956 to cross-validated squared correlation coeffi-
cient q^2=0.934, the predictivity index PRESS (Predictive 
Residual Sum of Squares) from 0.968 to 2.041 and PRESD 
(Predictive Standard Deviation) from 0.284 to 0.412. The 
parameters of the equation for ureogenesis changed also 
insignificantly: r^2=0.928 to q^2=0.824, PRESS from 2.102 
to 4.999 and PRESD from 0.419 to 0.645. The models are 
highly predictive and robust.

The predictive indices for the statistical evaluation of 
models were defined as (Eriksson et al., 2003):

\[
\text{PRESS} = \Sigma(\exp - \text{calcd})^2, \text{sum of differences squared between experimental and estimated (calculated) data},
\]

\[
\text{PRESD} = \left(\frac{\text{PRESS}}{n}\right)^{1/2}, \text{the second root of PRESS divided by the number of chemicals in the set.}
\]
The presence of log P in all log EC₅₀’s allows cross correlations. This is true at least of a series of chemicals of baseline toxicity (Schultz et al., 1994, Lipnick 1990). The correlation with log P makes it possible to use the rapid *Tubifex* assay (Tichy et al., 2007). Despite phylogenetic differences between the test objects and dramatic differences in test protocols, the QSARs for nonpolar narcosis are extremely consistent (Schultz et al., 1990). The concept of mechanism-based QSARs is successful even for freshwater benthic organisms as *Tubifex tubifex* and for acute exposure lasting minutes (Tichy et al., 2008).

The log P-based QSAR described for predicting acute toxicity index EC₅₀(Ti) measured with oligochaeta *Tubifex tubifex* can be recommended as an ecotoxicological model for predicting harmful effects of chemical compounds in ecological systems.

The fact that this assay can be used for determination of acute toxicity indices measured with higher organisms and under longer exposure periods supports the idea that the transport process is a prevailing step limiting the magnitude of an effect. The role of toxicokinetics can be the reason that results obtained with the *Tubifex* assay correlate with results obtained with higher organisms, and especially, including longer exposures (Tichy et al., 2007), when the involvement of biotransformation is considered (Veith et al., 1989; Lipnick et al., 1985). Although mechanisms of action of reactive chemical compounds were described (Veith et al., 1989; Lipnick et al., 1985), toxicokinetics may also play a role in the differences among biological test objects as well.

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