Session 5.02
New approaches to risk assessments (ESTIV-Session)

In Vitro-In Vivo Extrapolation of Toxic Potencies for Hazard and Risk Assessment – Problems and New Developments

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Summary
The aim of toxicological hazard assessment is to characterise the dangerous properties of chemicals for man and the environment. Information on both (a) the toxic potential, i.e. the spectrum of toxic effects a chemical can produce, and (b) the toxic potency, i.e. the quantitative relationship between dose/concentration and toxicity, are essential to characterise the toxic hazard. Toxicological risk assessment comprises hazard assessment and aims to characterise the likelihood and severity of adverse effects occurring to man or the environment following exposure to a chemical under defined conditions.
Two fundamental problems hamper the application of in vitro assays for hazard assessment: firstly, the endpoints of toxic action detectable in vitro are less complex and, importantly, mostly different from those assessed in vivo (toxicodynamic problem). Secondly, toxic concentrations determined in vitro are not equivalent to toxic doses or concentrations in vivo. This is due to important differences in biokinetics and bioavailability of chemicals in vitro and in vivo (toxicokinetic problem).
This contribution is focussed on the second aspect. It is demonstrated, how predictions can be made of the toxic concentrations in human serum or the aquatic environment, which are equivalent to toxic concentrations in vitro. This can be achieved by the application of a recently developed quantitative extrapolation model, taking into account substance and system specific parameters important for the bioavailability of chemicals. It appears that this approach represents a real progress in solving part of the "toxicokinetic problem".

Keywords: bioavailability in vitro, free concentrations, in vitro – in vivo prediction model, equivalent exposure approach, acute toxicity

Introduction

Toxicological hazard and risk assessment until today largely rely on animal toxicity data. Animal toxicity tests are performed to identify the toxic effects that a chemical can induce (hazard identification) and to determine the toxic potency, i.e. the quantitative relationship between external exposure level (administered dose, environmental concentration) and the incidence and severity of toxic effects (dose-response assessment). Both components of hazard assessment are essential for toxic hazard classification and labelling, determination of safe exposure levels and, in connection with exposure assessment, for risk assessment (fig. 1). The aim of toxicological risk assessment is to characterise the likelihood, the nature and the severity of adverse effects occurring to man or the environment caused by defined exposure to a chemical. If *in vitro* methods shall be used for toxicological hazard and risk assessment and for safety evaluation, they must provide information which can be used to reliably assess the kinds of toxic hazards and the corresponding toxic potencies.

Despite considerable efforts during the last about 20 years, *in vitro* methods have only been accepted for hazard identification and classification in a few cases, e.g. in testing for genotoxicity/mutagenicity, acute local skin and eye toxicity and phototox-

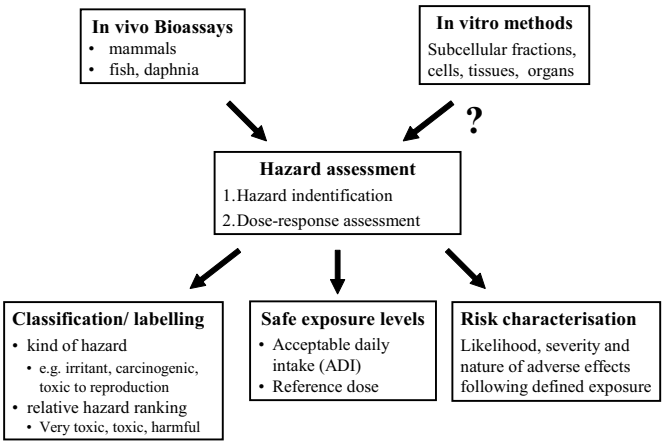

Fig. 1: *In vitro* methods in hazard and risk assessment.

icity. Currently, no *in vitro* methods are available that can be used to identify more complex toxic hazards, like carcinogenicity, reproductive toxicity, endocrine disruption, immunotoxicity or acute and chronic systemic toxicity.

The two fundamental problems which hamper the application of *in vitro* toxicity data for hazard assessment are well known. The first problem, which may be called the "toxicodynamic problem", is that the types of toxicity assessed *in vitro* and *in vivo* are not the same. Endpoints of toxic action detectable *in vitro* are much less complex and, importantly, mostly different from those assessed *in vivo*. No correlates for responses of organisms like death or disease, reproductive and developmental failure, cancer development or impairment of nervous, endocrine or immune system functions can be assessed *in vitro*. The diversity of mechanisms and interactions between tissues and organs involved in these complex toxic endpoints cannot be modelled by simple *in vitro* systems. *In vitro*, interactions or responses at cellular or subcellular level can be measured which are at best mechanistically connected to the observed *in vivo* phenomena. It is widely assumed that this toxicodynamic problem may be solved, at least in part, by the use of *in vitro* test batteries and tiered testing schemes, covering diverse mechanisms of action (e.g. Seibert et al., 1994; Seibert et al., 1996; Worth and Balls, 2002). The question here is how to identify relevant in vitro tests and how to combine their data.

The second problem, which may be called the "toxicokinetic problem", is that the exposure levels that characterise the potencies *in vitro* and *in vivo* are not equivalent. Exposure levels *in vivo* are given, for instance, as concentrations in the ambient air or water, or as administered doses, while exposure levels *in vitro* are most often given as nominal concentrations in the medium surrounding the cells or subcellular components. *In vivo* the effectivity of exposure and the bioavailability of chemicals is largely determined by biokinetic processes, which are either absent or quite different *in vitro*. That is why *in vitro* and *in vivo* exposure levels are not equivalent. If *in vitro* toxic potency data shall be used for *in vivo* toxic potency assessment, they have to be transformed into the external doses or concentrations *in vivo*, which are equivalent to the toxic exposure levels *in vitro*.

A further complication is that the nominal effective concentration used to characterise the *in vitro* toxic potency of a chemical for a given cell type and endpoint is not necessarily representative for the activity of that chemical but may vary with the composition of the *in vitro* system used, for instance with the albumin or serum concentration in the culture medium or the cell density (e.g. Bohets et al., 1994; Finlay and Baguley, 2000; Hestermann, 2000; Gülden et al., 2001; Seibert et al., 2002; Heringa et al., 2004). Thus, the question is: which *in vitro* potency measurement should be used for extrapolation to *in vivo* potency?

This contribution is focussed on some aspects of the "toxicokinetic problem". Firstly, a recently developed method (equilibrium distribution modelling) is described which is used to obtain composition independent, representative *in vitro* potency measurements. Secondly, a strategy is outlined for extrapolation of the internal or external exposure levels, which are equivalent to *in vitro* toxic concentrations (equivalent exposure approach). Finally, two examples for the application of this approach are presented to demonstrate its practical value.

Distribution model for chemicals in cell cultures

Cell cultures consist of at least three components: culture vessel, cells, and medium, the latter of which is frequently supplemented with 5-20% serum. It is known that chemicals can be bound to proteins and accumulated in lipids and that this occurs to a great extent in serum. Additionally, cells can concentrate chemicals via diverse mechanisms. Using "negligible depletion" solid-phase microextraction (nd-SPME) it was shown that *in vitro* considerable amounts of added chemicals can be bound by serum or cells and thus become unavailable for toxic action (Vaes et al., 1997; Heringa et al., 2004). Only the aqueous concentration of the freely dissolved chemical is considered to be related to the intensity of a pharmacological or toxic effect. The above-mentioned dependence of *in vitro* toxic potencies on albumin, serum or cell concentration in the *in vitro* systems is easily explained by an altered availability of the nominal concentrations of the chemicals when the concentration of the binding components is changed. A distribution model for chemicals in cell cultures has been developed to gain a composition independent measure of *in vitro* toxic potency, i.e. the free effective concentration (Gülden and Seibert, 1997; Gülden et al., 2001; Seibert et al., 2002).

This model is based on the assumptions that the total substance added is equilibrated between cells and extracellular components, the equilibrium concentrations are constant throughout the exposure period, and the total cell volume is negligible compared to the volume of the extracellular medium. Furthermore, it is assumed that chemicals can be freely dissolved in the extracellular and cellular water, can be bound to extracellular proteins and can partition into cellular and extracellular lipids. Any substantial influence of cell binding other than partitioning into cellular lipids is neglected. Cellular and extracellular lipids are treated as a single compartment.

Then, the nominal effective concentration (EC_{50}) can be expressed as a function of the free effective concentration

(ECu_{50}), the protein bound concentration (Cb), the concentration in the lipid compartment (C_L) and the relative volume of lipid (V'_L) in the *in vitro* system:

$$EC_{50} = ECu_{50} + Cb + C_L \bullet V'_L \quad (1)$$

Applying the Nernst distribution law to describe partitioning into lipids using the octanol/water partition coefficient (K_{ow}) and the mass action law to describe binding to extracellular proteins represented by albumin, the most abundant and most important binding protein in serum, the following equation was developed:

$$ECu_{50} = \frac{EC_{50} - B \bullet P}{1 + K_{ow} \bullet V'_L} \quad (2)$$

Provided that the albumin concentration in the medium (P), the relative lipid volume in the *in vitro* system, the specific binding of the chemical to albumin (B) and its octanol/water partition coefficient are known, this equation enables the calculation of the free effective concentration from the nominal effective concentration. The specific binding to albumin can be determined from EC_{50}-measurements at different albumin concentrations in the medium (Gülden et al., 2003).

The distribution model has been applied to characterise the distribution and availability of cytotoxic concentrations of a variety of chemicals in the mouse Balb/c 3T3 cell cytotoxicity test system (Gülden et al., 2002, Gülden and Seibert, 2005). Despite the low albumin concentration of about 18 µM in culture medium supplemented with 5% foetal bovine serum and the low lipid content ($V'_L = 10^{-4}$ l/l), various compounds have been found, whose availability is considerably, in some cases drastically, lowered by albumin binding and/or partitioning into lipids (tab. 1).

In vitro-in vivo extrapolation of toxic potencies: The equivalent exposure concept

In order to use *in vitro* toxic potency data for *in vivo* toxic potency assessment, the differences in biokinetics *in vitro* and *in vivo* have to be taken into account. *In vivo* biokinetic processes, like resorption, biotransformation, excretion and distribution, determine the relation between the administered toxic dose or concentration of a chemical in the ambient air or water and the internal dose or the actually effective free concentration at the target site (fig. 2). As outlined above, the free effective and not the nominal effective concentrations must be considered to be the representative, composition independent *in vitro* potency measurement. From a toxicodynamic point of view, nominal concentrations and doses of a chemical can be regarded to be equivalent if they are connected to the same free concentration of that chemical. Thus, it can be taken as a basic demand on an *in vitro-in vivo* extrapolation model for toxic potencies that it is aimed at determining external or internal doses/concentrations of a chemical *in vivo* that result in a free concentration which is comparable to the *in vitro* free effective concentration. We call this the equivalent exposure concept.

Tab. 1: Nominal (EC_{50}) and free (ECu_{50}) cytotoxic concentrations of chemicals and their free fractions (fu) in the Balb/c 3T3 cytotoxicity test system (Gülden et al., 2002; 2003).

Substance	EC_{50} (µM)[a]	ECu_{50} (µM)[b]	fu[c]
Pesticides			
p,p´-DDE	34.4	0.034	0.001
p,p´-DDT	30.6 ± 2.4	0.035	0.0011
Dieldrin	33.3 ± 7.3	9.27	0.28
Lindane	65.3	35.5	0.54
Malathion	60.7	53.7	0.88
Paraquat	74.6	74.6	1.0
2,4-Dichlorophenoxy acetic acid	877	803	0.92
Phenols			
4-Octylphenol	18.6 ± 3.6	2.73	0.15
4-Nonylphenol	24.1 ± 6.9	3.36	0.14
Hexachlorophene	4.06	0.027	0.0067
Pentachlorophenol	39.2 ± 13	≤ 2	≤ 0.050
2,4,5-Trichlorophenol	64.4	17.7	0.27
2,4-Dichlorophenol	281	235	0.84
4-Chlorophenol	219 ± 44	192	0.88
Phenol	1140	1140	1.0

a : Data are means of at least two independent measurements ± SD if n ≥ 3.
b: Calculated with Eq. (6) using a relative lipid volume $V'_L=10^{-4}$ l/l, an albumin concentration P = 18 µM, the pH 7.4 adjusted values for K_{ow} obtained from the literature and the values for B, the specific binding to albumin, as determined from EC_{50}-measurements in the presence of different albumin concentrations.
c : $fu = ECu_{50}/EC_{50}$

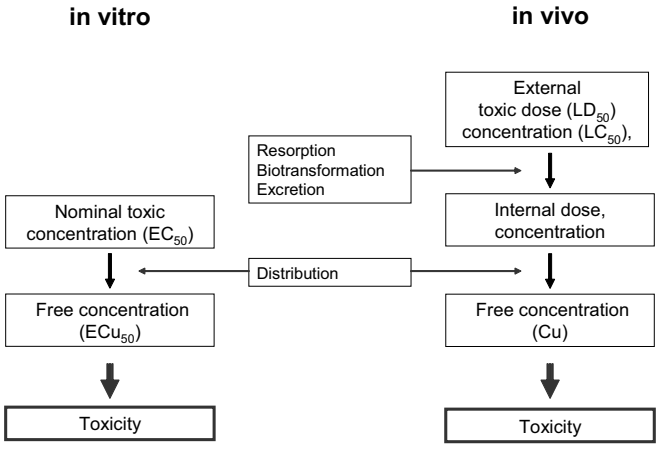

Fig. 2: Influence of biokinetic processes on the bioavailability of chemicals *in vitro* and *in vivo*.

In the following, using cytotoxic potency data obtained with the mouse Balb/c 3T3 cytotoxicity test system, the application of this approach to predict (a) fish acute toxic potencies and (b) toxic human serum concentrations is demonstrated.

Application example 1: Prediction of acute aquatic toxicity to fish

Fish acute toxicity tests are performed in large numbers for toxicological hazard and risk assessment of chemicals in the aquatic environment. Some efforts have been undertaken to develop *in vitro* cytotoxicity tests with fish cell lines as alternatives to the acute fish test (for review see Segner, 1998; Castano et al., 2003). Using cell death as an endpoint of cytotoxicity, various fish cell lines have been used to measure the cytotoxic potency of a variety of chemical compounds. In any case, fish cell line tests showed a clearly lower sensitivity than the *in vivo* fish tests when *in vitro* cytotoxic concentrations (EC_{50}-values) of groups of chemicals were compared with their acute toxic concentrations (LC_{50} values) (Castano et al., 2003; Segner, 2004). The low sensitivity of fish cell line tests hampers their use as alternatives to acute fish tests.

An example using cytotoxicity data obtained with the rainbow trout R1 cell line (data from Segner and Lenz, 1993; Segner, 2004) is shown in figure 3. It can be seen that only for a few substances the *in vitro* cytotoxic concentrations are close to the acute toxic concentrations or somewhat lower. In most cases the cytotoxic concentrations are higher, up to more than a factor of 100. The deviations between LC_{50}- and EC_{50}-values become more frequent and more pronounced with increasing potency.

Meanwhile, various studies have shown that this "low sensitivity" is not a unique feature of fish cell lines (Segner 2004, Castano and Gómez-Lechon, 2005, Gülden et al., 2005). In fact, the sensitivity of mammalian and human cell lines towards the cytotoxic action of chemicals, in general, is comparable to that of fish cell lines, provided that the experimental conditions are similar. The sensitivity of *in vitro* cytotoxicity tests using cell lines, however, can be increased if cell growth inhibition instead of cell death is used as endpoint for cytotoxicity (Gülden et al., 2005). Nevertheless, the principal relation between *in vitro* and *in vivo* assays, i.e., the lower sensitivity of *in vitro* assays especially for chemicals with higher toxicity, remains.

We have investigated whether a reduced availability of chemicals *in vitro* can account for the remaining lower sensitivity of *in vitro* cytotoxicity test systems (Gülden and Seibert, 2005). Fish cell lines are usually cultured in the same basal culture media as mammalian cell lines, also supplemented with 5-10% bovine serum. It has been shown above that, under these conditions, considerable fractions of nominal effective concentrations of chemicals can be bound to extracellular serum albumin and can be accumulated in cellular and extracellular lipids. The bioavailable effective concentrations of chemicals (ECu_{50}), thus, can be much lower than their nominal effective concentrations (EC_{50}). On the other hand, the fish acute toxic concentrations (LC_{50}) ideally refer to the bioavailable aqueous concentration of the freely dissolved chemicals. This is warranted by quantitative analytical chemistry and/or proper design of the fish acute toxicity test if performed according to the OECD test guidelines. This suggests that the free and not the nominal effective concentrations *in vitro* are the appropriate *in vitro* potencies for aquatic toxicity assessment.

To test this hypothesis, nominal cytotoxic concentrations of chemicals determined with the Balb/c 3T3 proliferation inhibition assay were converted into free effective concentrations using the *in vitro* distribution model outlined above (Eq. 2). The octanol/water partition coefficients were taken from the literature and values for the specific binding to albumin were determined from EC_{50}-measurements in the presence of different albumin concentrations with the Balb/c 3T3 test system. Organic chemicals covering a wide range of cytotoxicity and lipophilicity were selected for which acute fish toxicity data were avail-

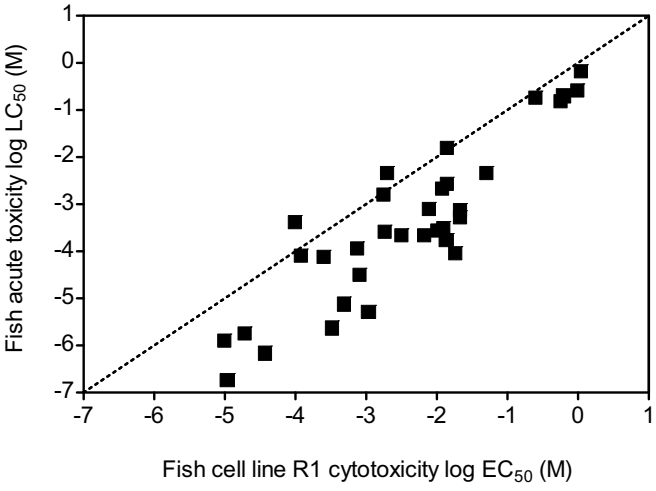

Fig. 3: Comparison of the aquatic acute fish toxic concentrations of chemicals with their *in vitro* cytotoxic concentrations in the R1 fish-cell line. Data from Segner and Lenz (1993) and Segner (2004).

able in the Ecotoxicology Database (ECOTOX) of the US EPA for at least one of the three fish species, medaka (*Oryzias latipes*), rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*).

The results show that the sensitivity of the Balb/c 3T3 cytotoxicity assay and the correspondence between *in vivo* and *in vitro* toxic potencies can clearly be increased when the free cytotoxic concentrations instead of the nominal cytotoxic concentrations are used as measure of cytotoxic potency. An example is shown in figure 4 with data taken from Gülden and Seibert (2005). The few remaining prominent underestimations of acute fish toxic potencies can be explained by more specific mechanisms of acute toxic action than basal cytotoxicity. Chemicals, like the tested nicotine or the pesticides dieldrin and lindane are known to exert acute toxic action via specific interference with neuronal transmission. If a chemical exerts acute toxicity via interference with specific cell functions at concentrations lower than those that are cytotoxic, its acute toxic potency must be higher than its cytotoxic potency. That means, even if the toxicokinetic problem is solved, cytotoxicity testing alone cannot be expected to safely assess the acute aquatic toxicity to fish. However, since cytotoxicity can be considered with certainty to be a cause for acute toxicity, cytotoxicity testing *in vitro* may well be suited to assess the minimal acute fish toxic potency of chemicals.

Practical *in vitro* test strategy for estimating minimal aquatic toxic potency

Based on these results and earlier investigations on the impact of protein binding and partitioning into lipids on the availability and toxic potency of chemicals *in vitro* (Gülden et al., 2001 and 2003; Seibert et al, 2002), the following practical *in vitro* testing strategy for estimating the minimal aquatic toxic potency of chemicals is proposed.

1) Determination of nominal EC_{50}-values for growth inhibition using a mammalian cell line.

2) Determination of the distribution relevant composition of the *in vitro* test system used, like albumin concentration in the medium (P) and the relative volume of cellular plus extracellular lipids (V'_L).

3) Determination of the octanol/water-partition coefficient (K_{ow}) of the tested chemicals. If the octanol/water partition coefficient of the chemical is lower than the reciprocal of the relative lipid volume in the *in vitro* system ($Kow < 1/V'_L$), partitioning into lipids is not likely to affect the availability of the chemical in the *in vitro* system.

4) If the EC_{50}-value of a chemical is much higher than the albumin concentration in the *in vitro* system ($EC_{50} \gg P$), quantitatively relevant binding to albumin is not likely to occur. Then, the free effective cytotoxic concentration *in vitro* is given by the following equations:

a) If $K_{ow} < 1/V'_L$

$$ECu_{50} = EC_{50} \quad (3)$$

b) If $K_{ow} \geq 1/V'_L$

$$ECu_{50} = \frac{EC_{50}}{1 + K_{ow} \bullet V'_L} \quad (4)$$

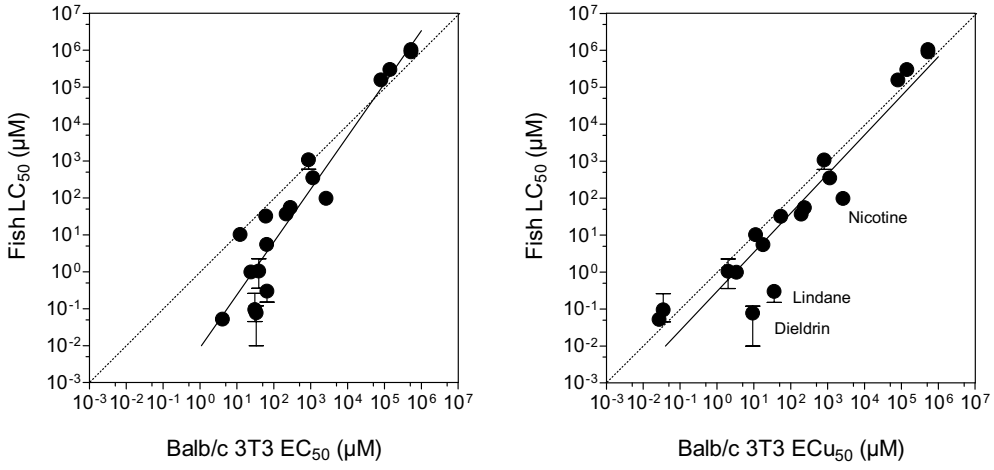

Fig 4: Comparison of the aquatic acute toxic concentrations of chemicals to fathead minnow (*Pimephales promelas*) with their nominal cytotoxic (left) and free cytotoxic (right) concentrations measured *in vitro* using the mouse Balb/c 3T3 cell proliferation inhibition assay. Data are from Gülden and Seibert (2005). The dotted lines indicate 1:1 correlation. The solid lines represent the linear regression lines:
left: $\log LC_{50} = -2.06 + 1.43 \times \log EC_{50}$, $r^2 = 0.911$
right: $\log LC_{50} = -0.52 + 1.06 \times \log ECu_{50}$, $r^2 = 0.901$

(5) Otherwise, the specific binding to albumin (B) has to be measured and the free effective concentration is given by:

a) If $K_{ow} < 1/V'_L$

$$ECu_{50} = EC_{50} - B \bullet P \quad (5)$$

b) If $K_{ow} \geq 1/V'_L$

$$ECu_{50} = \frac{EC_{50} - B \bullet P}{1 + K_{ow} \bullet V'_L} \quad (2)$$

Application example 2: Prediction of human serum concentrations which are equivalent to cytotoxic concentrations *in vitro*

The albumin and lipid contents of animal and human serum are considerably higher than those of the extracellular media in *in vitro* tests systems. Consequently, the availability of lipophilic and albumin bound chemicals in serum must be lower than *in vitro*. To attain the same free concentration of a chemical, in such cases, higher nominal concentrations have to be achieved. On the basis of the equilibrium distribution model described above, we have developed the following algorithm to extrapolate the serum concentrations (EC_{serum}) of chemicals which are equivalent to the *in vitro* effective concentrations (Gülden and Seibert, 2003):

$$EC_{serum} = (EC_{50} - B \bullet P_{invitro}) \frac{1 + K_{ow} \bullet V'_{L,\,serum}}{1 + K_{ow} \bullet V'_{L,\,invitro}} + B \bullet P_{serum} \quad (6)$$

where $V'_{L,invitro}$ and $V'_{L,serum}$ are the relative lipid volumes, and $P_{invitro}$ and P_{serum} the albumin concentrations in the medium of the *in vitro* system and in serum, respectively.

This extrapolation model was applied to cytotoxic concentrations determined *in vitro* with the Balb/c 3T3 proliferation inhibition test in order to estimate equivalent human serum concentrations. In total 33 chemicals covering a wide range of cytotoxic potency and lipophilicity were investigated. Octanol/water partition coefficients were available for all of these chemicals. The specific binding to albumin was determined by EC_{50}-measurements in the presence of different albumin concentrations (Gülden et al., 2002, 2003).

The concentration of albumin in human serum (~ 600 µM) is about 33-times higher than in the medium of the Balb/c 3T3 test system used, and the relative volume of lipids in serum, with a mean of $6x10^{-3}$ l/l, is about 60-times higher.

The application of the extrapolation model revealed that for 13 of the 33 compounds the equivalent serum concentrations were equal to the nominal EC_{50}-values obtained *in vitro* (fig. 5).

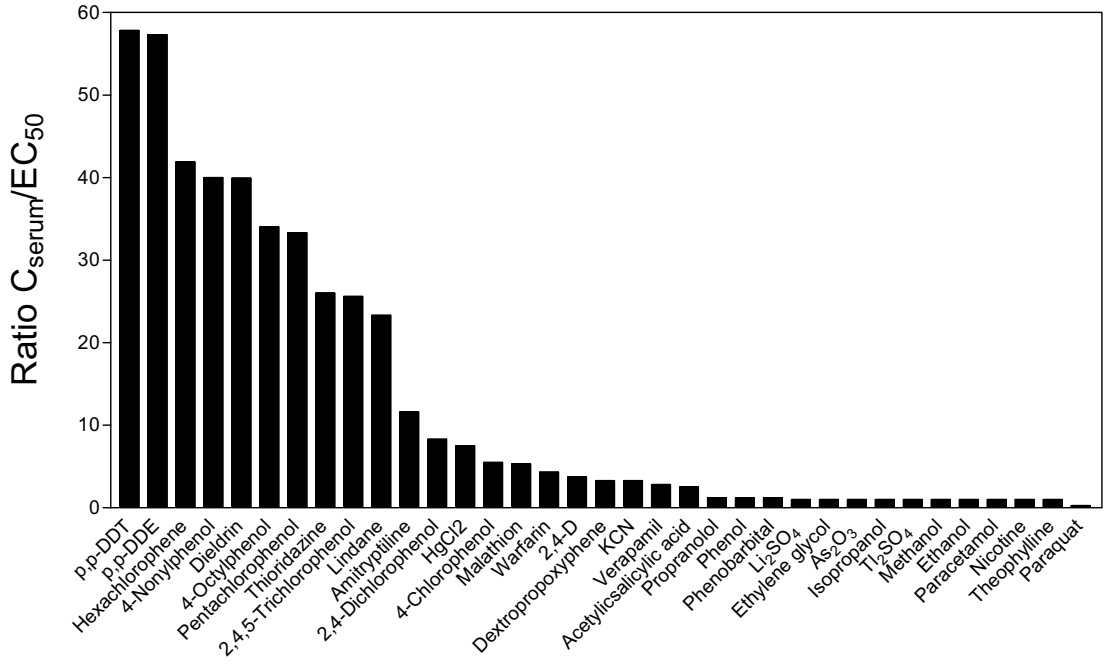

Fig. 5: Ratios of the equivalent human serum concentrations and the nominal cytotoxic concentrations *in vitro* determined with the mouse Balb/c 3T3 cell proliferation inhibition assay. The equivalent serum concentrations (C_{serum}) were calculated from the nominal cytotoxic concentrations *in vitro* (EC_{50}) using the extrapolation model Eq. (6), albumin concentrations *in vitro* and in serum of 18 µM and 600 µM, and lipid volume fractions *in vitro* and in serum of 10^{-4} and $6x10^{-3}$ l/l, respectively.

Neither lipophilicity nor albumin binding capability of these chemicals were high enough to affect their availability in the *in vitro* system or in serum. The equivalent human serum concentrations of the other chemicals were substantially higher, by factors of 2.5 to 58, compared to the nominal EC_{50}-values. The lipophilicity and/or protein binding capacity of these chemicals were at least high enough to affect their bioavailability in serum.

A further analysis of the results indicated that differences in the availability of chemicals become more frequent with increasing potency. In case of chemicals with low toxic potency neither lipophilicity nor albumin binding is high enough to have relevant influence on their availability. Neglecting differences in the availability of chemicals *in vitro* and *in vivo*, thus, will systematically bias the correlation between *in vitro* and *in vivo* toxic potencies. First, the slopes of the regression lines describing the correlations between the log transformed potencies must clearly deviate from the 1:1 correlation and be lower than 1. Second, the scatter of data points around the regression line must increase with increasing *in vitro* potencies. Both these phenomena are seen when acute toxic potencies (e.g. LD_{50}) are correlated with cytotoxic potencies determined *in vitro* (Garle et al., 1994; Halle, 2003).

Recently, the validity of this extrapolation model was evaluated. The results indicate that the extrapolation model is well suited to predict equivalent concentrations in serum from *in vitro* effective concentrations (Gülden et al., 2006).

Practical strategy for extrapolating effective serum concentrations from *in vitro* test results

From these results and theoretical considerations on the impact of protein binding and partitioning into lipids on the bioavailability of chemicals in serum and in *in vitro* test systems, some pragmatic rules for the extrapolation of equivalent chemical concentrations in human serum can be deduced (see Gülden and Seibert, 2003). These rules are not restricted to cytotoxic potencies but can be applied to any biological activity of chemicals determined *in vitro*. The premise is that the distribution relevant composition (V'_L = lipid volume fraction, P = albumin concentration) of both the *in vitro* system and the serum concerned and the octanol/water partition coefficients of the chemicals are known.

1) If the octanol/water partition coefficient of the chemical is lower than the reciprocal of the relative lipid volume in serum ($Kow < 1/V'_{L,serum}$), partitioning into lipids is not likely to affect the availability of the chemical in serum or *in vitro*. In case of human serum, with a relative lipid volume of $V'_{L,serum} \leq 10^{-2}$ l/l, this is the case if $K_{ow} \leq 10^2$.

2) If the EC_{50}-value of a chemical measured *in vitro*, either in the absence or the presence of comparatively low serum protein concentrations (i.e. up to 20% serum in the medium), is higher than the albumin concentration in the serum concerned for extrapolation ($EC_{50} > P$), a quantitative impact of protein binding on the availability of the chemical is not to be expected. For human serum this is the case if the EC_{50} is higher than 600 µM. Then, the equivalent serum concentration (EC_{serum}) is given by the following equations:

a) If $K_{ow} < 1/V'_{L,serum}$

$$EC_{serum} = EC_{50} \quad (7)$$

b) If $K_{ow} \geq 1/V'_{L,serum}$

$$EC_{serum} = EC_{50} \frac{1 + K_{ow} \bullet V'_{L,serum}}{1 + K_{ow} \bullet V'_{L,invitro}} \quad (8)$$

3) Otherwise, the specific binding (B) to albumin has to be measured, and the equivalent serum concentration is given by

a) If $K_{ow} < 1/V'_{L,serum}$

$$EC_{serum} = EC_{50} + B(P_{serum} - P_{invitro}) \quad (9)$$

b) If $K_{ow} \geq 1/V'_{L,serum}$

$$EC_{serum} = (EC_{50} - B \bullet P_{invitro}) \frac{1 + K_{ow} \bullet V'_{L,serum}}{1 + K_{ow} \bullet V'_{L,invitro}} + B \bullet P_{serum} \quad (6)$$

Conclusion

In conclusion, taking into consideration the elementary differences in the bioavailability of chemicals between *in vitro* and *in vivo* systems improves the usage of *in vitro* generated toxicity data in the process of toxicological hazard and risk assessment. The concept of equivalent exposure for *in vitro-in vivo* extrapolation of toxic potencies takes such differences in the availability into account. Applying this concept to predict acute fish toxicity and toxic concentrations in human serum from *in vitro* cytotoxic concentrations demonstrates the practical value of this approach.

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