

In vitro - in vivo Extrapolation toxischer Dosen: Das „equivalent exposure“ Konzept

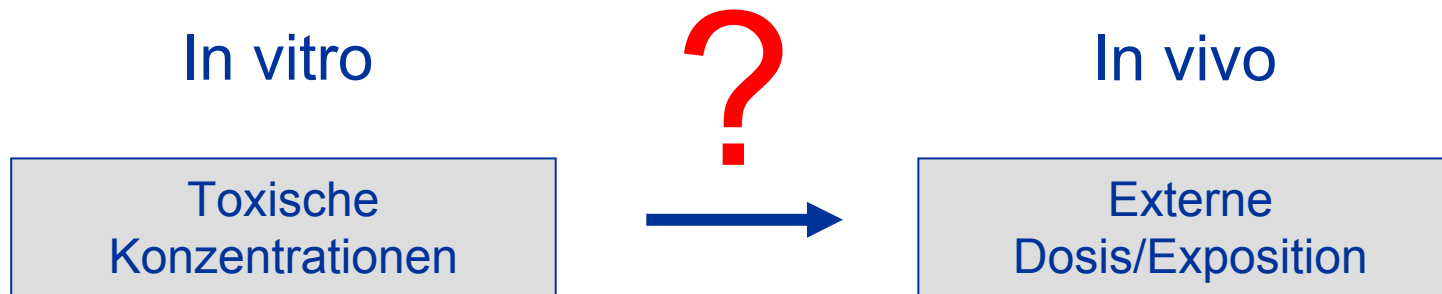
Michael Gülden

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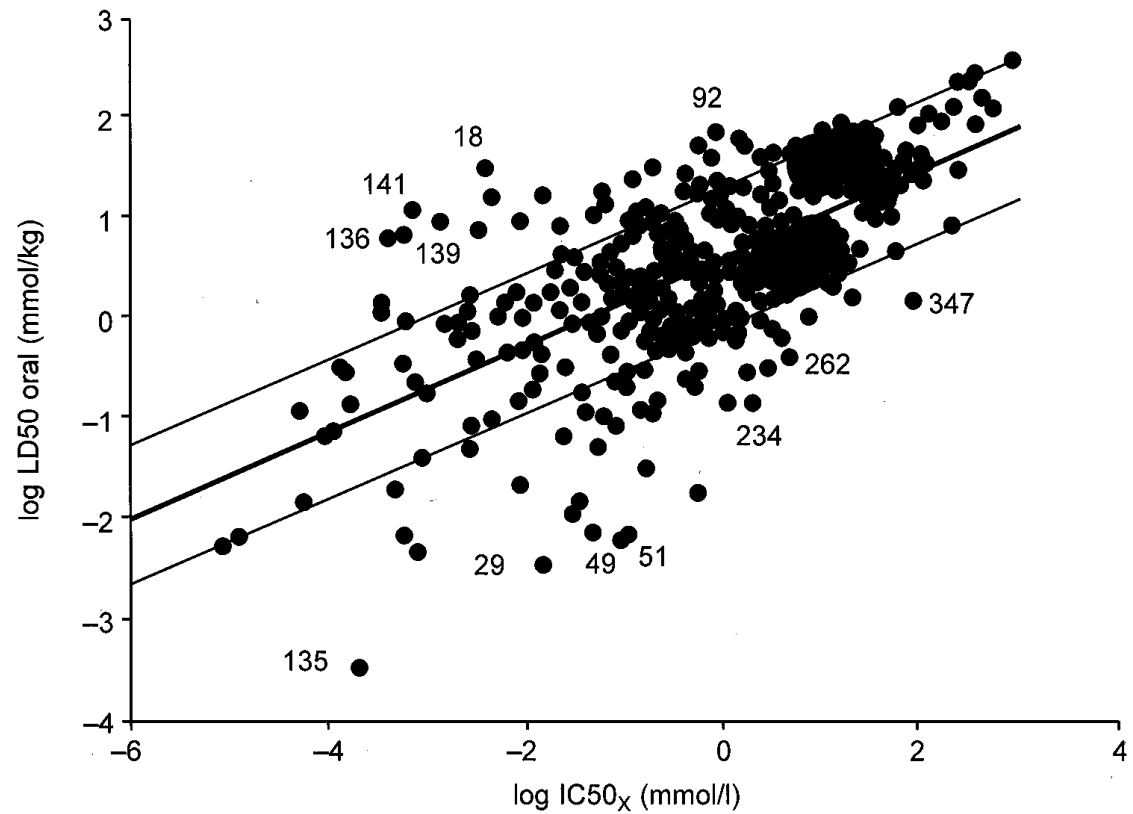
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Quantitative in vitro – in vivo Extrapolation

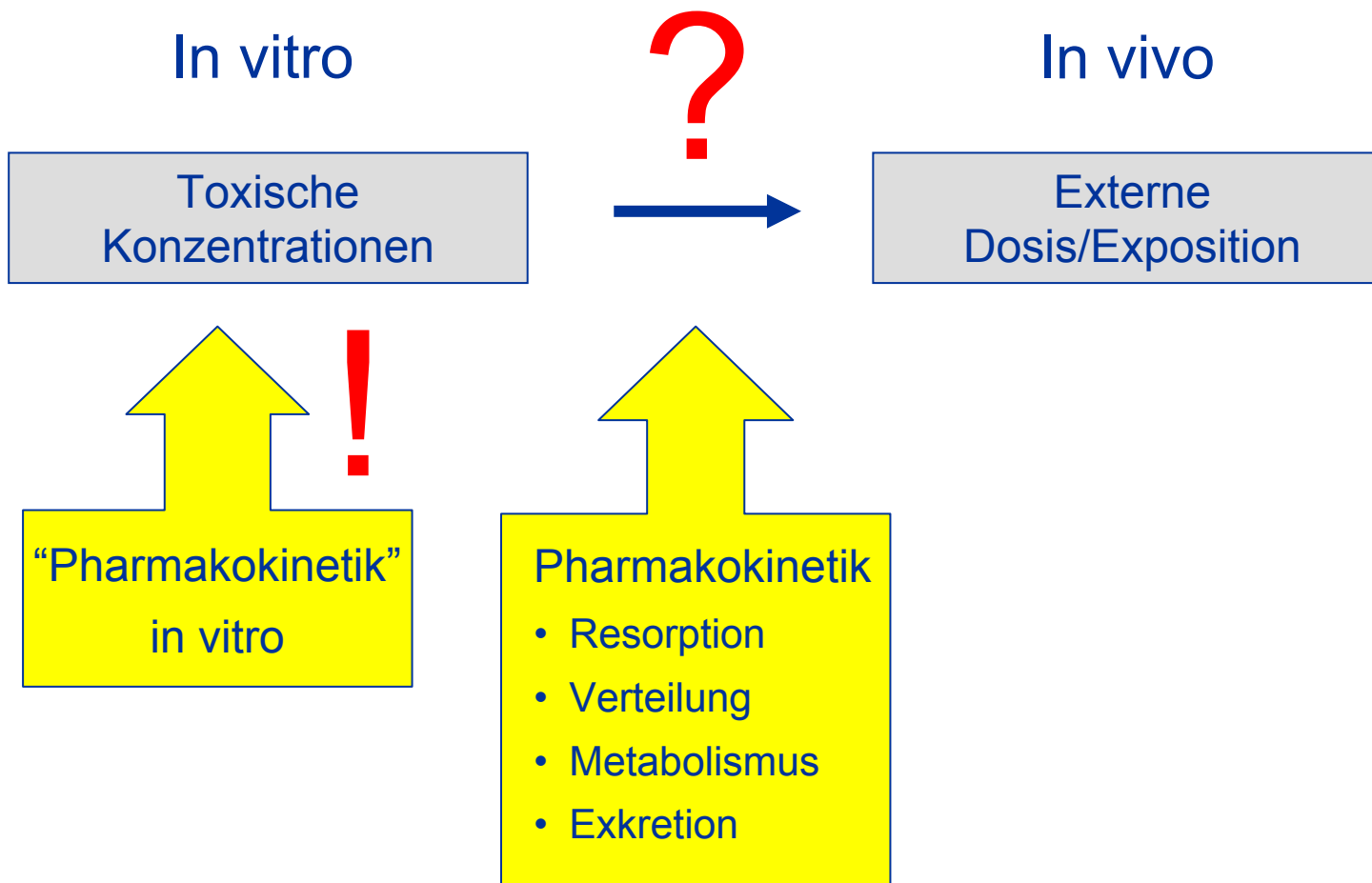


Keine einfachen Lösungen des Problems



W. Halle (2003) The Registry of Cytotoxicity: Toxicity Testing in Cell Cultures to Predict Acute Toxicity (LD50) and to Reduce Testing in Animals. ATLA 31, 89-198

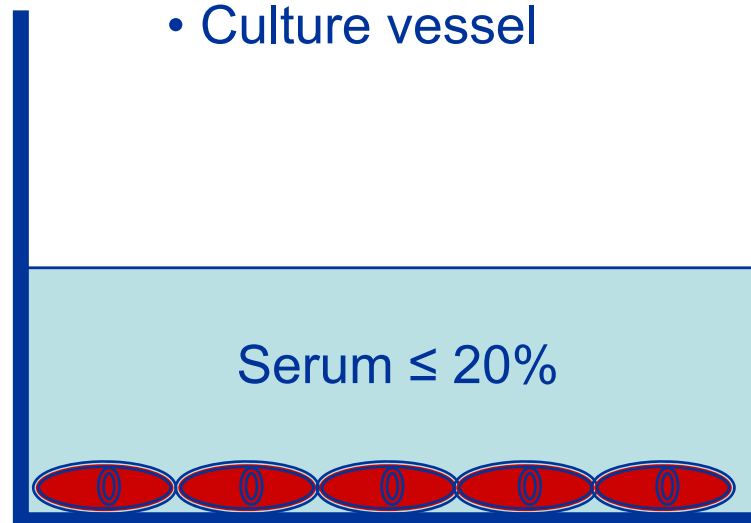
Quantitative in vitro – in vivo Extrapolation



In vitro Systeme

Principal components:

- Cells
- Culture medium
- Culture vessel

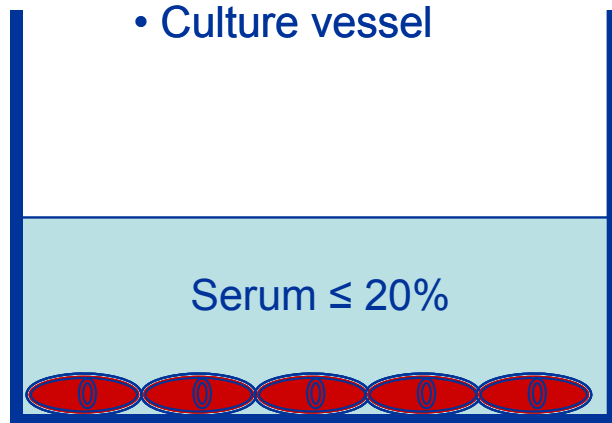


Relative cell volume « 1% of total volume

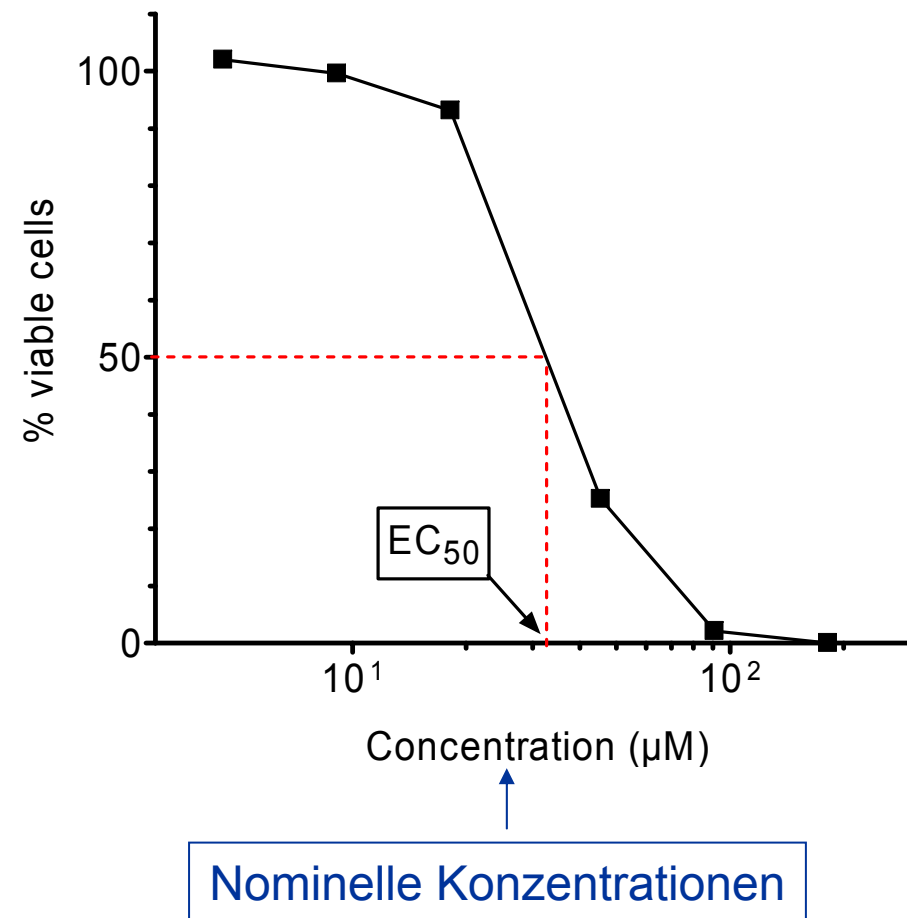
In vitro bestimmte Konzentrations-Wirkungsbeziehungen basieren auf nominellen Konzentrationen

Principal components:

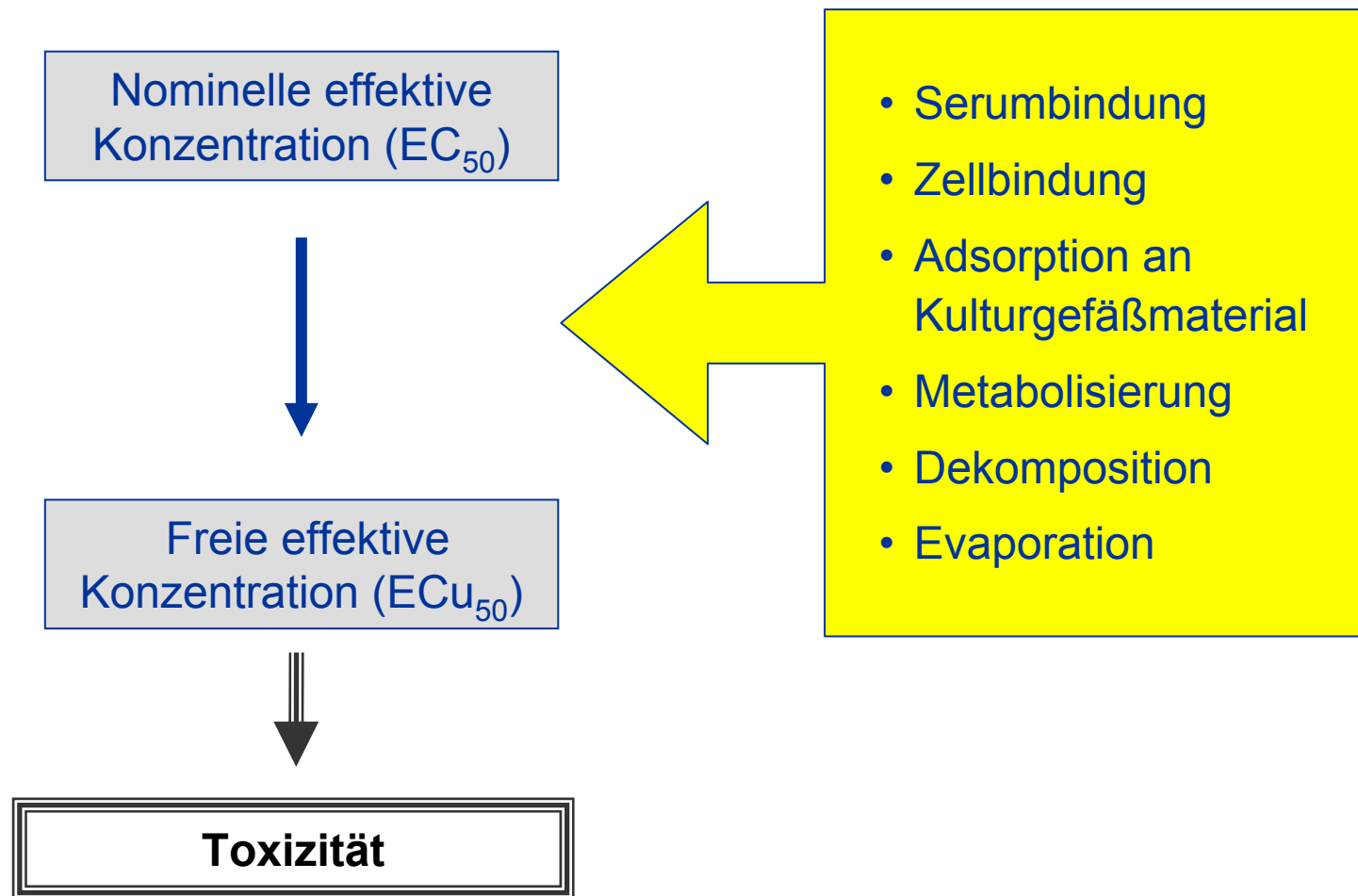
- Cells
- Culture medium
- Culture vessel



Relative cell volume \ll 1% of total volume



Verteilung und Elimination in vitro



Binding of drugs to microsomes

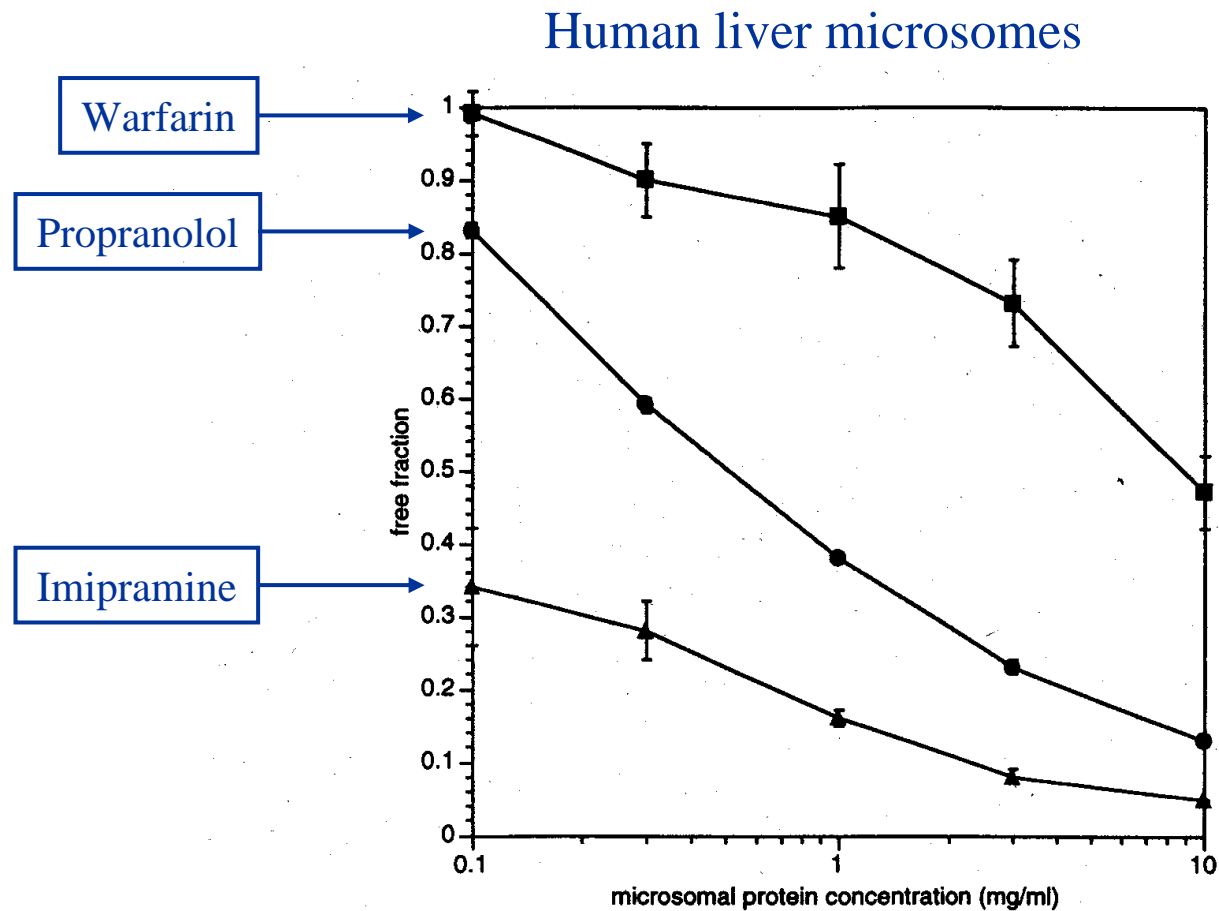
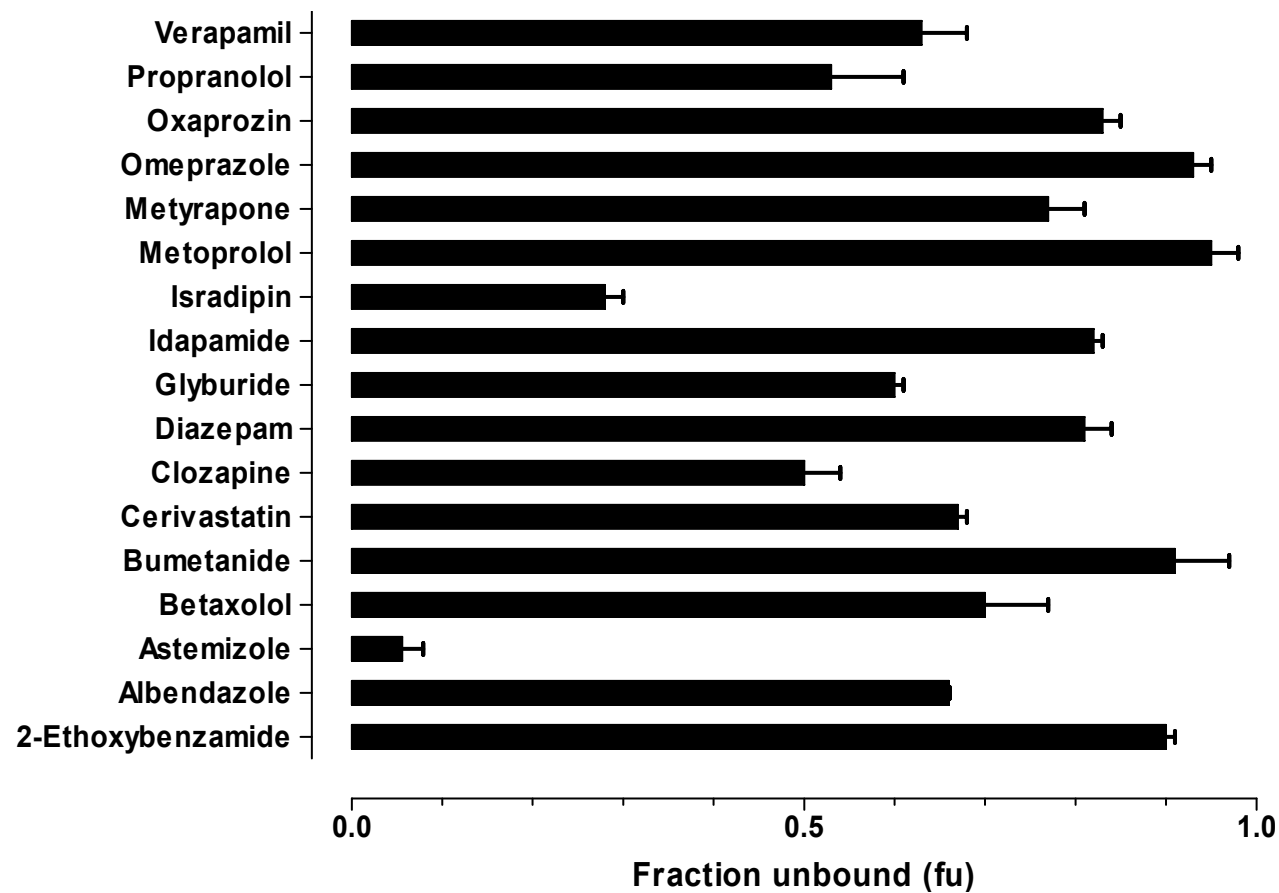


FIG. 2. Binding of warfarin, imipramine, and propranolol to pooled human liver microsomes as a function of microsomal protein concentration. [^{14}C]Warfarin (■) at $10\ \mu\text{M}$ and [^3H]propranolol (●) and [^3H]imipramine (▲) at $1.0\ \mu\text{M}$ were dialyzed vs. human liver microsomes for 5 hr. Points represent the mean \pm SD of triplicate determinations.

(Obach R.S., Drug Metab. Dispos. 1997, 25, 1359-1369)

Binding of drugs to hepatocytes

(3 μ M, 10^6 cells/ml)



(Austin et al., Drug Metab Dispos 2005, 33 (3), 419-425)

Binding of octylphenol to serum

Estrogenicity reporter gene assay using a human cell line

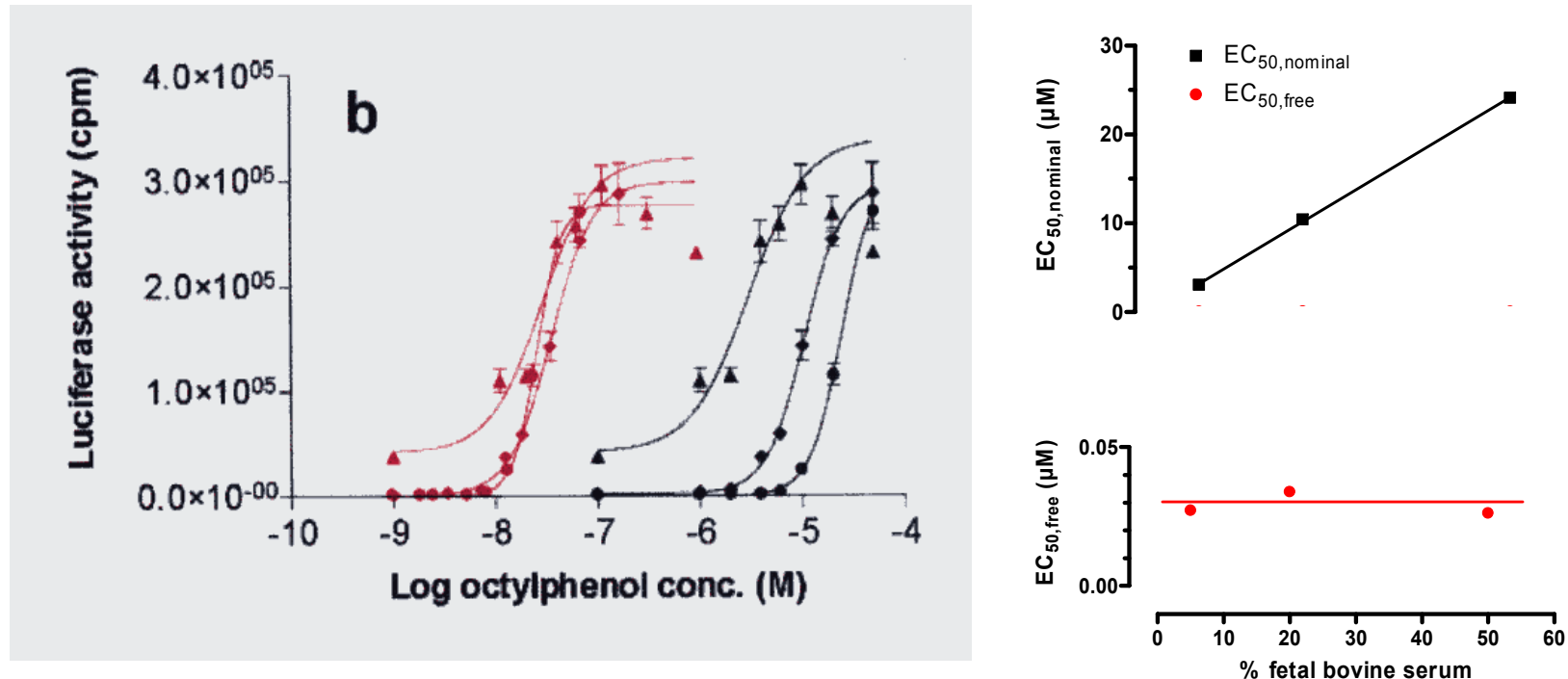
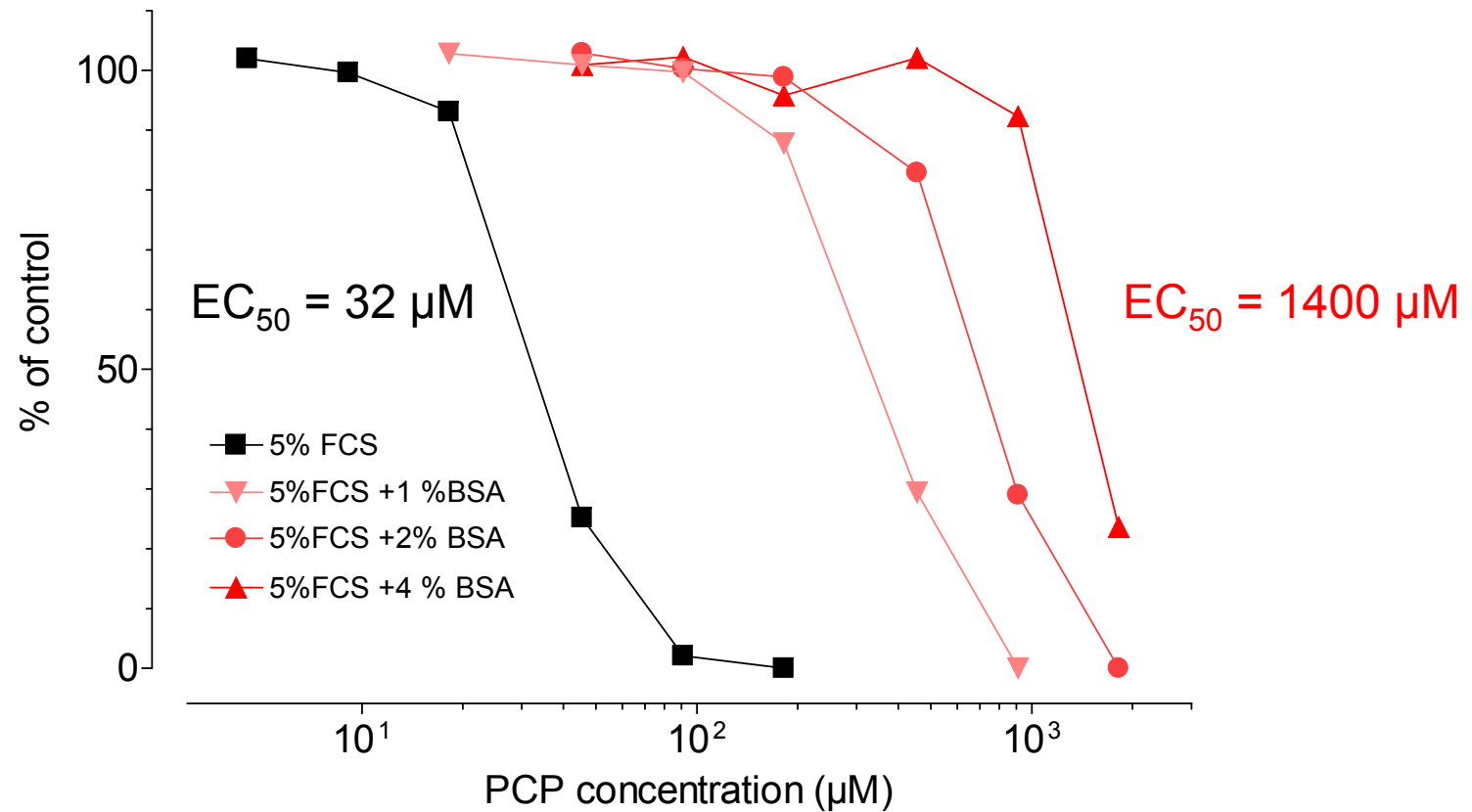


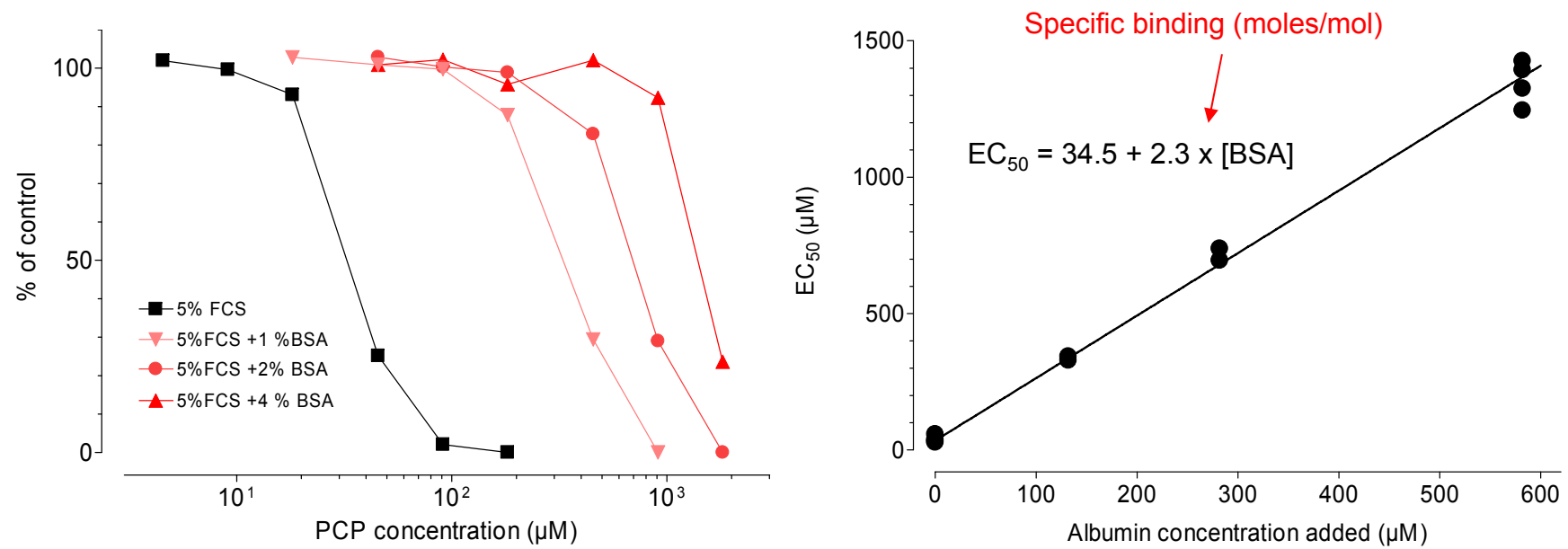
FIGURE 5. Dose–response curves of [³H]estradiol (a) and *p*-octylphenol (b) in an estrogenicity reporter gene assay, and the resulting EC₅₀ values as a function of serum content (panels c and d for estradiol and octylphenol, respectively). Dose–response curves were measured in the presence of 1% (■), 5% (▲), 20% (◆), or 50% (●) serum in the culture medium. Black data are based on nominal concentrations; red data are based on measured free concentrations. The first data point of every curve is a control. Error bars show standard errors of the mean ($n = 3$; panels a and b) or 95% confidence intervals (panels c and d).

(Heringa et al., Environ Sci & Technol. 2004, 38, 6263-6270)

Influence of **albumin concentration** on the cytotoxic potency of pentachlorophenol in proliferating Balb/c 3T3 cells

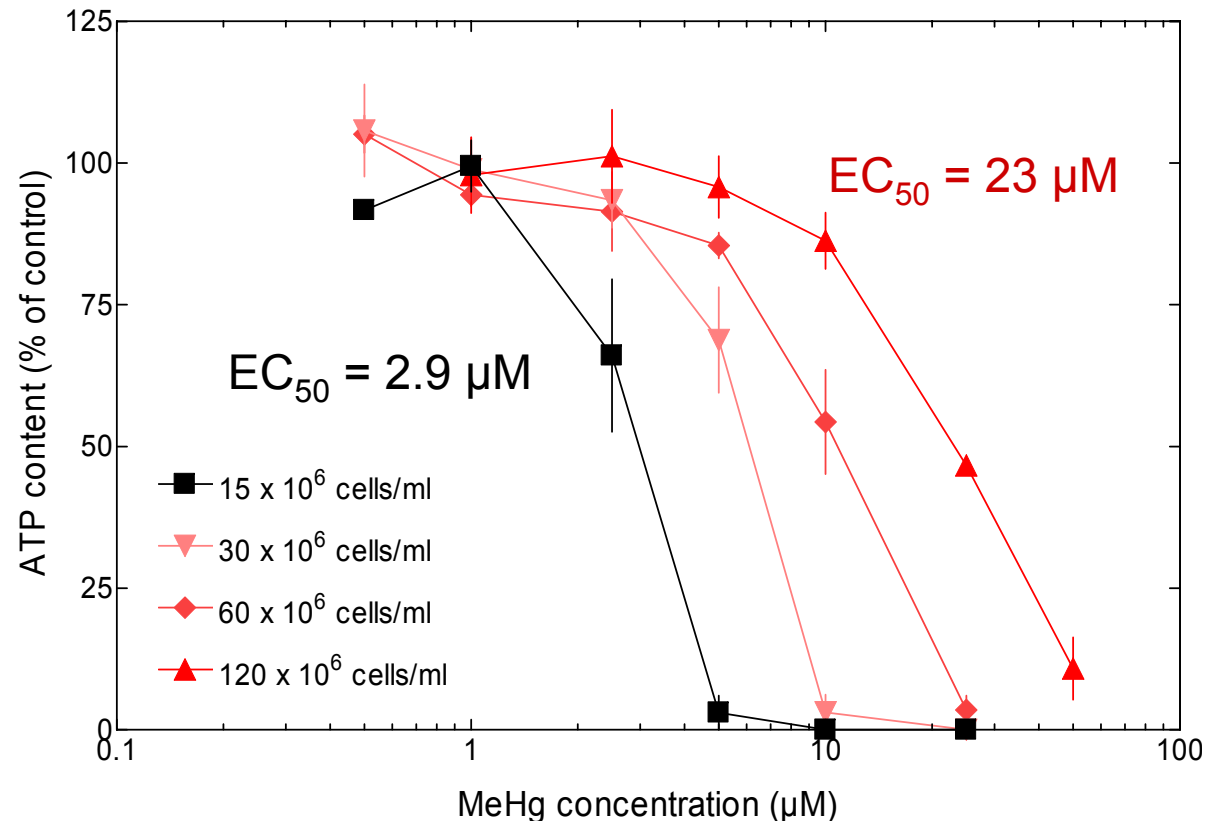


Influence of **albumin concentration** on the cytotoxic potency of pentachlorophenol in proliferating Balb/c 3T3 cells

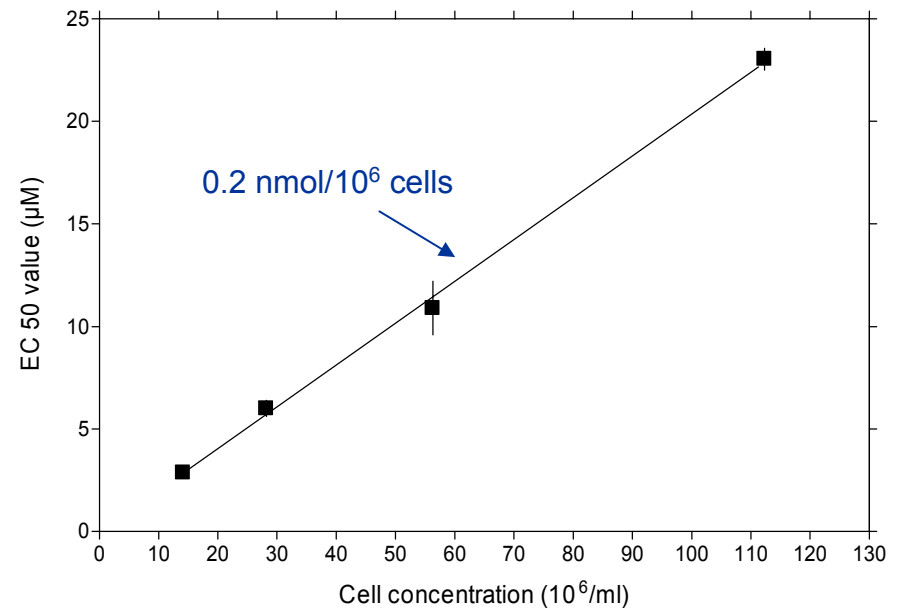
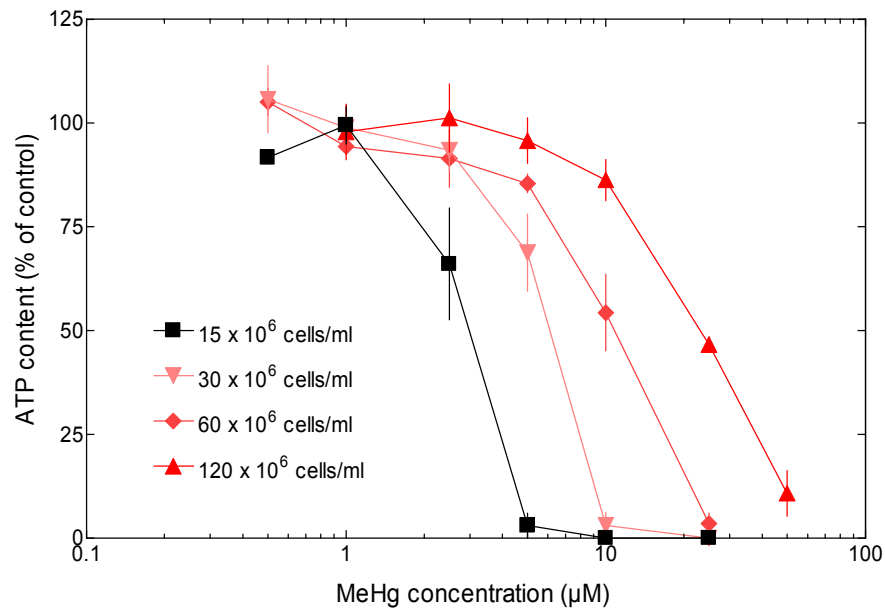


(Gülden et al., 2002. Toxicology 175, 201-213)

Influence of **cell concentration** on the cytotoxic potency of methylmercury in bull sperm cells



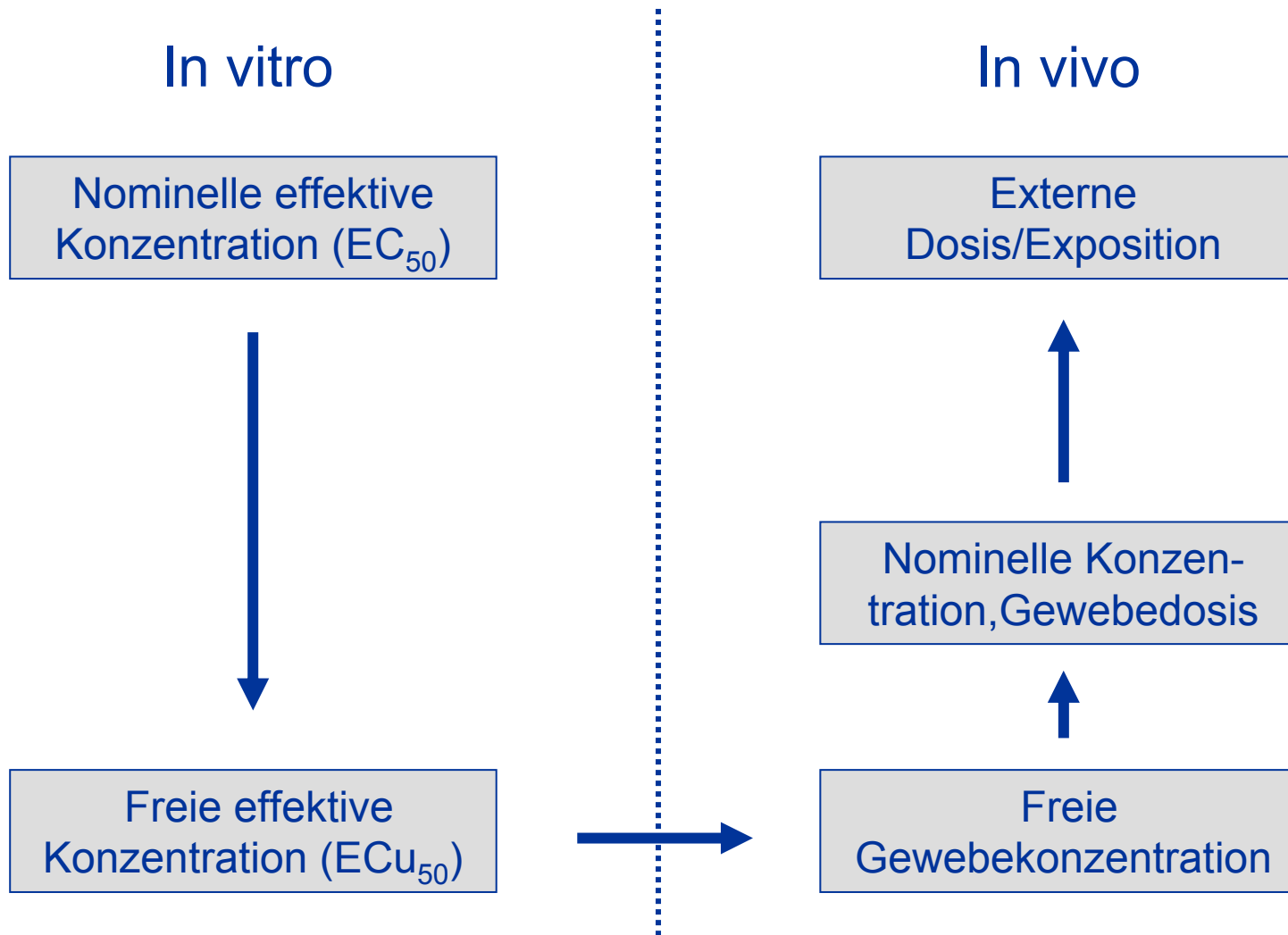
Influence of **cell concentration** on the cytotoxic potency of methylmercury in bull sperm cells



(Gülden et al., 2001. Toxicol. in Vitro 15, 233-243)

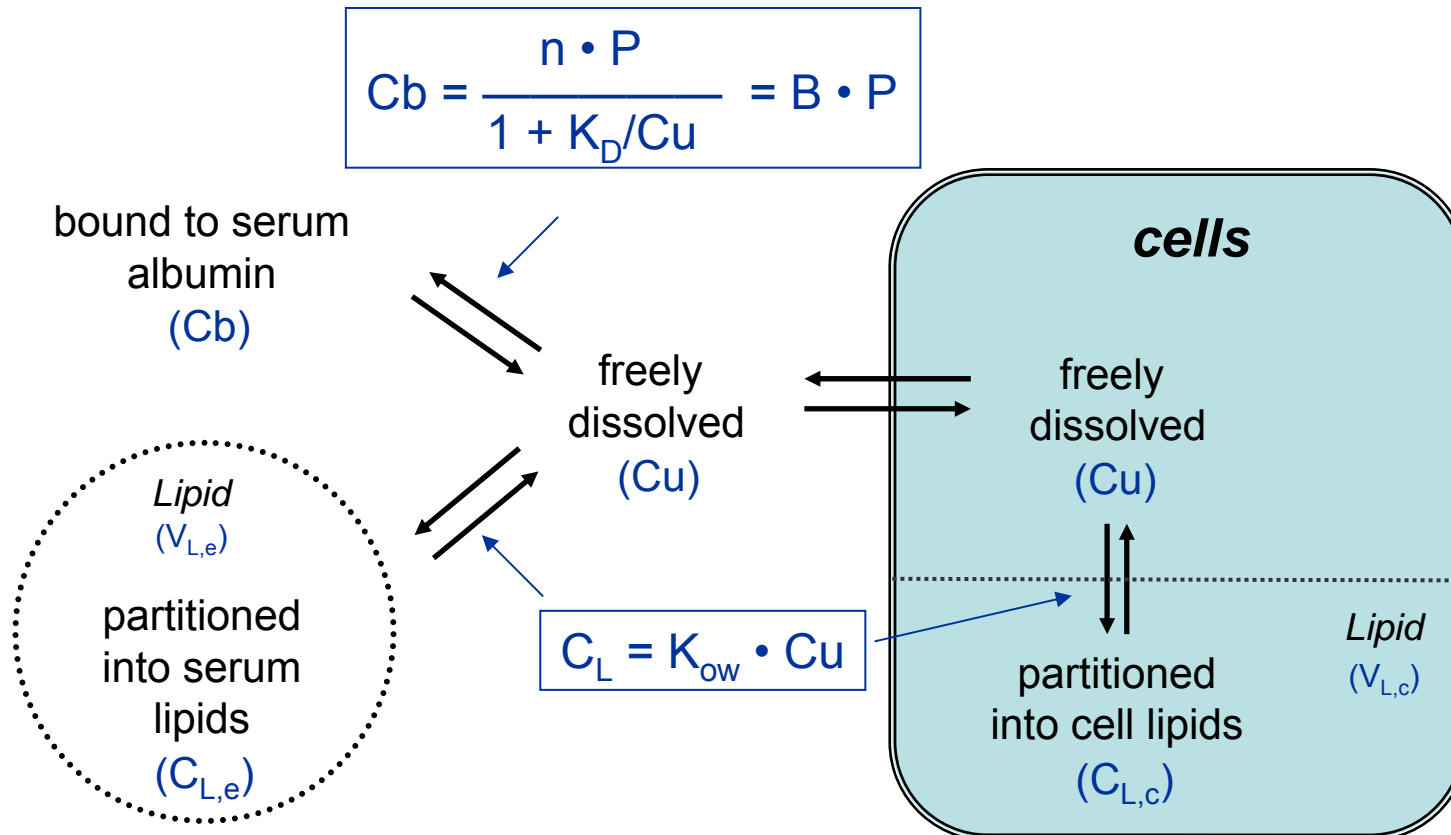
Quantitative in vitro – in vivo Extrapolation

Das „equivalent exposure“ Konzept



Distribution model for chemicals in vitro

extracellular medium



B: Specific binding (moles/mole)

Distribution equation

System specific factors

Relative lipid volume in the in vitro system

Albumin concentration in the medium

$$EC_{50} = ECu_{50}(1 + K_{ow} \cdot V'_L) + B \cdot P$$

Substance specific factors

Octanol/water partition coefficient

Specific binding to albumin

Bioavailability of chemicals in an in vitro system

Example: Balb/c 3T3 test system

Composition

Cell concentration at seeding:

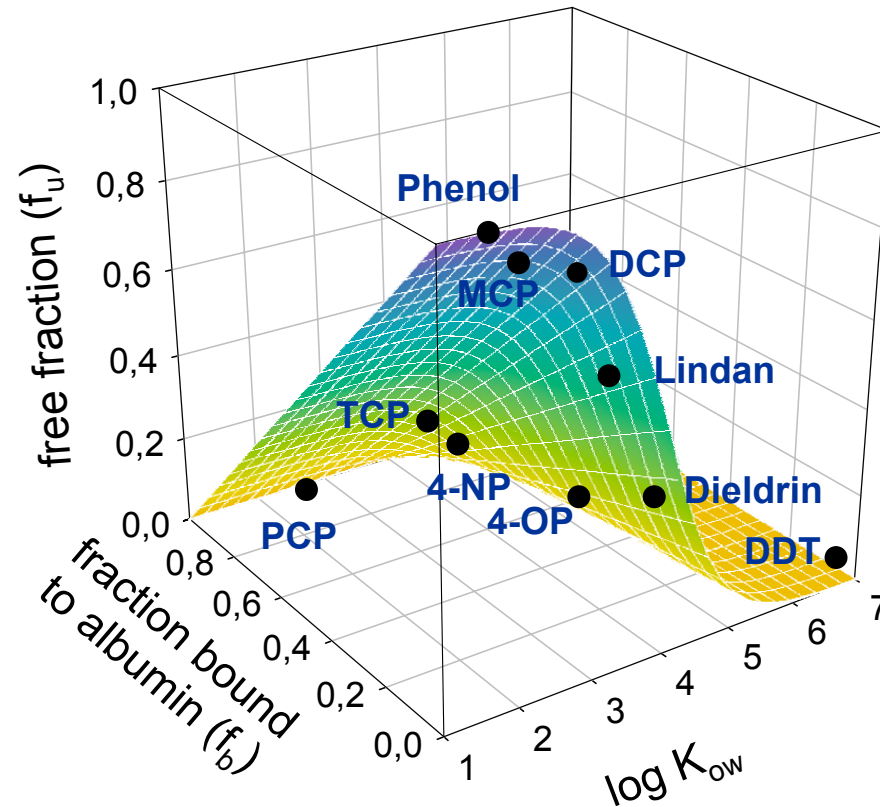
$3 \times 10^4/\text{ml}$

Albumin concentration in the culture medium (5% FBS):

$18 \mu\text{M}$

Relative lipid volume:

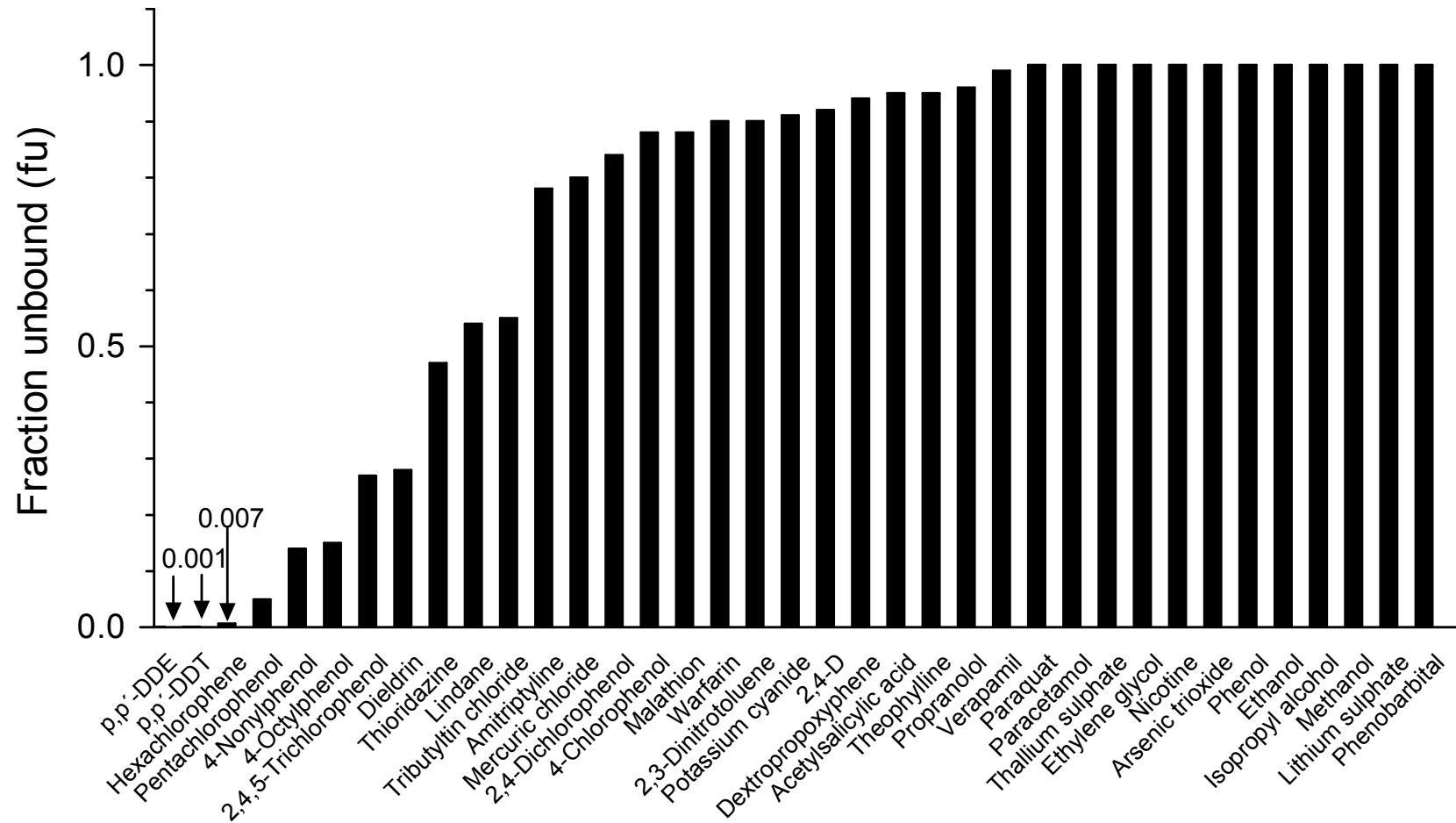
$0.1 \mu\text{l}/\text{ml}$



(Gülden & Seibert, 2005. Aquatic Toxicology 72, 327-337)

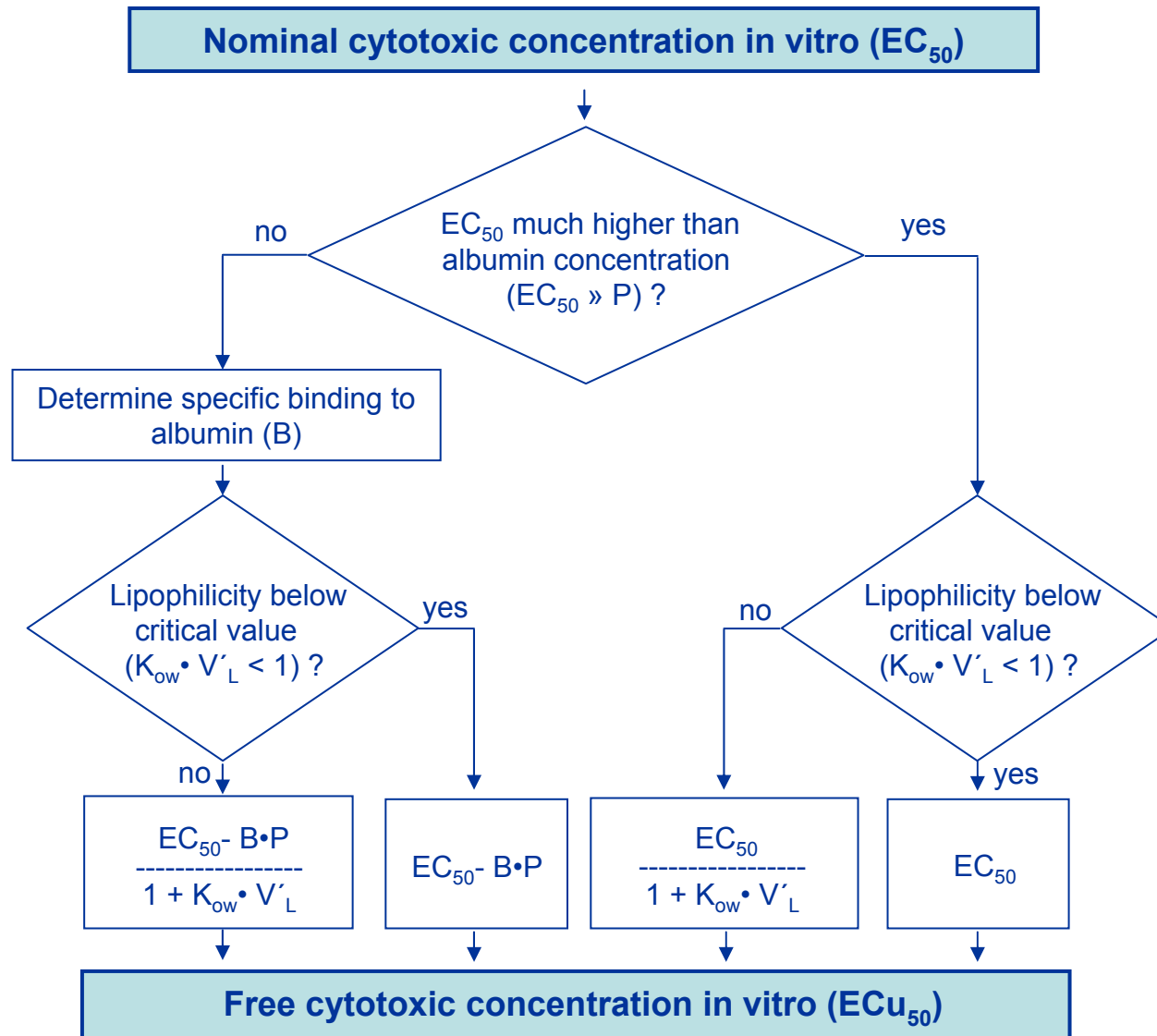
Bioavailability of chemicals in an in vitro system

Example: Balb/c 3T3 test system



(Gülden & Seibert, 2007. ATLA 35, 39-46)

Application of the distribution model to estimate free cytotoxic concentrations

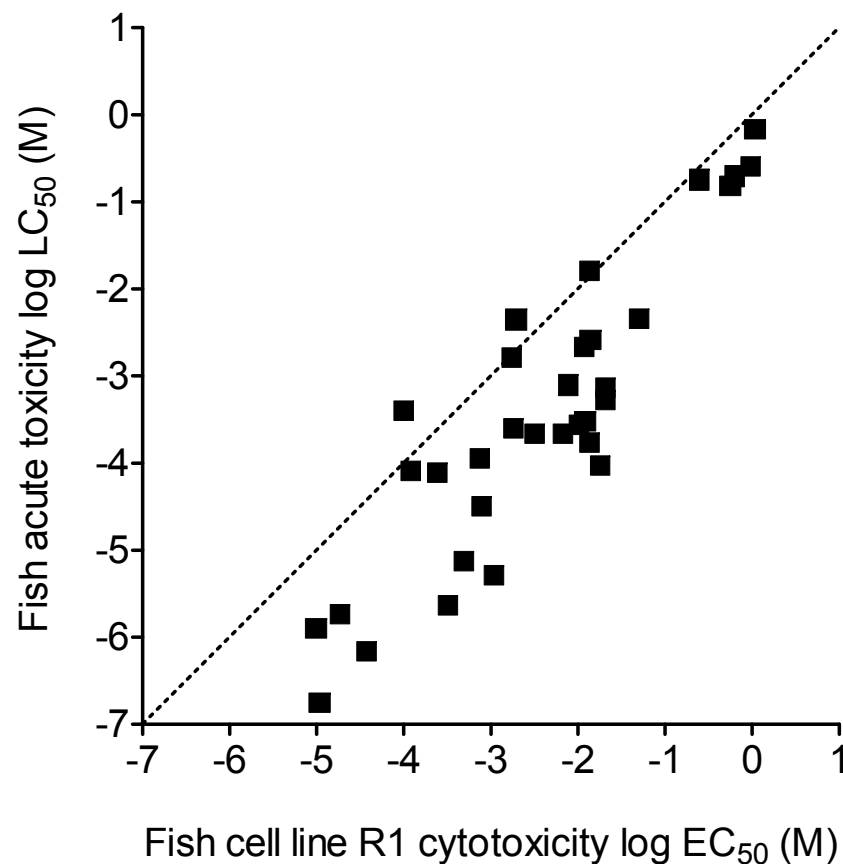


„Equivalent exposure“ Konzept

Anwendungsbeispiel

Verwendung von in vitro
Zytotoxizitätsdaten zur Vorhersage der
akuten Toxizität für Fische

Fish acute toxic potency vs. cytotoxic potency



LC₅₀

- Endpoint: death
- Free aquatic concentrations (OECD guideline test)

EC₅₀

- Endpoint: cell death
- Nominal concentrations
- Medium with 10% FBS

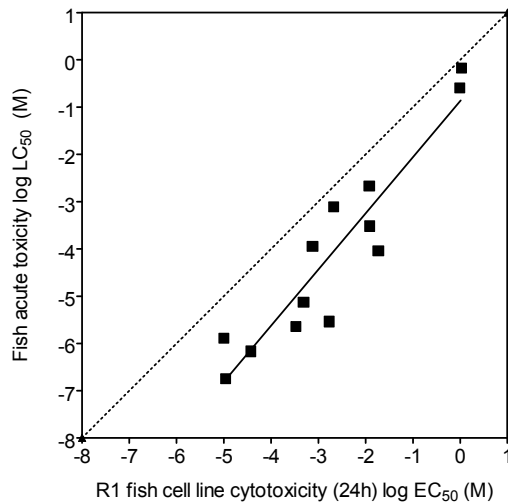
Segner H. & Lenz D., 1993. Cytotoxicity assays with the rainbow trout R1 cell line. *Toxicology in Vitro* 7, 537-540

Segner H., 2004. Cytotoxicity assay with fish cells as an alternative to the acute lethality assay with fish. *ATLA* 32, 375-382

Improvement of in vitro toxicity testing for aquatic toxicity assessment

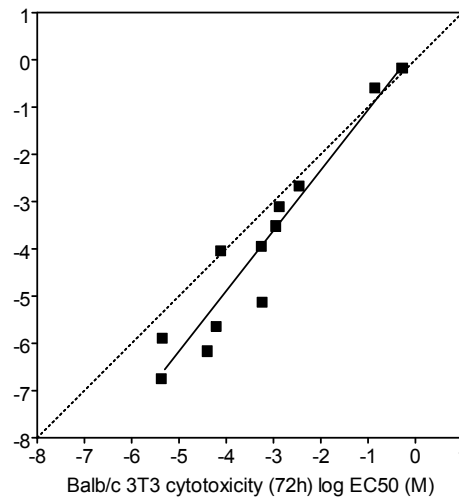
Fish cell line: R1

- cell death
- 24 hours exposure
- nominal EC_{50} -values

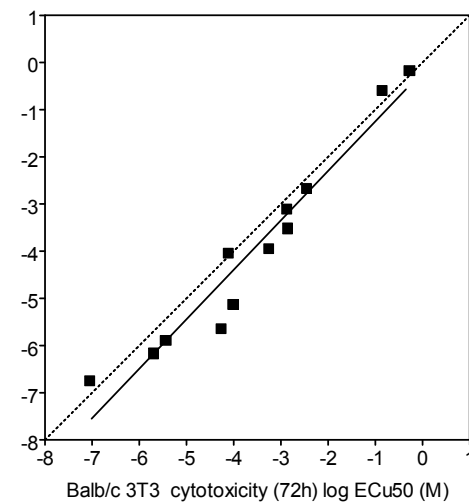


Mammalian cell line: Balb/c 3T3

- cell growth inhibition
- 72 hours exposure
- nominal EC_{50} -values



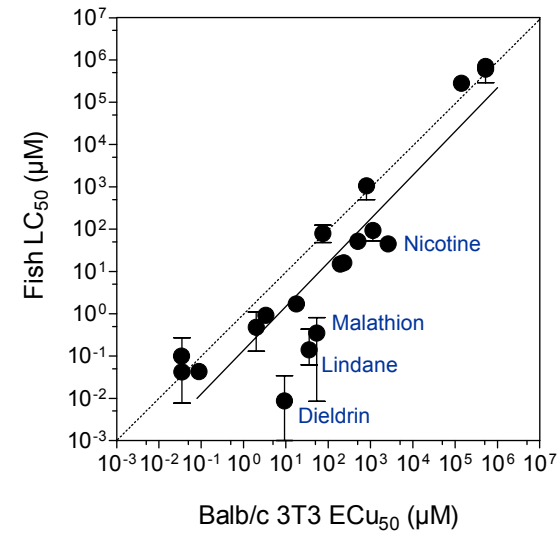
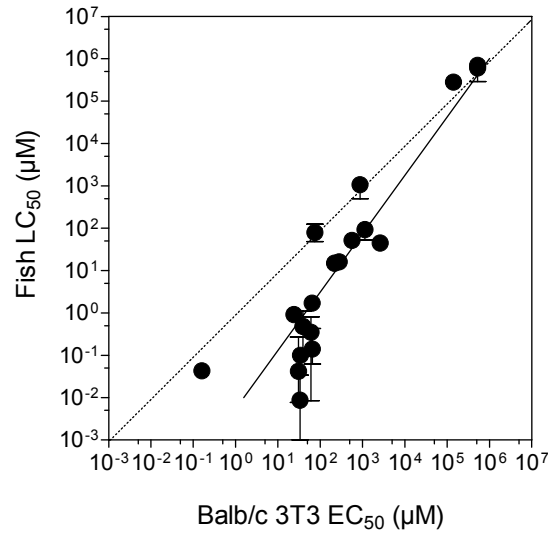
- free ECu_{50} -values



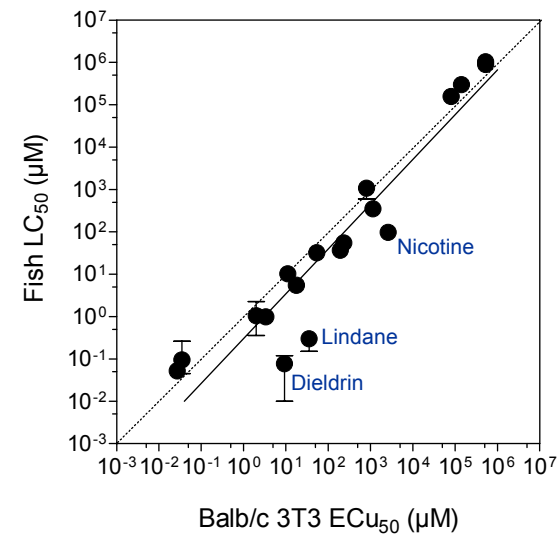
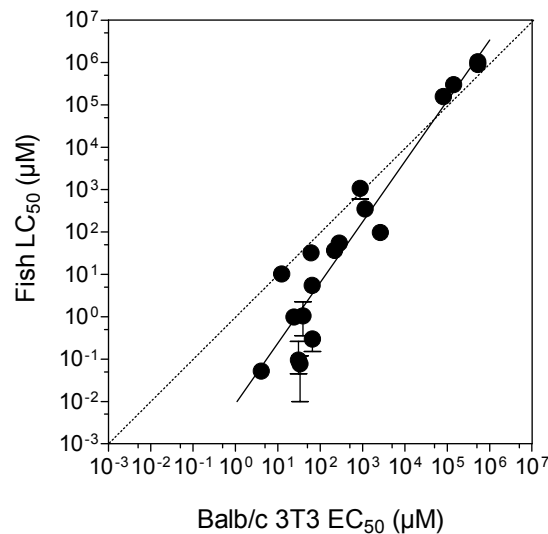
Segner H., 2004. ATLA 32, 375-382

Improvement of in vitro toxicity testing for aquatic toxicity assessment

Rainbow trout



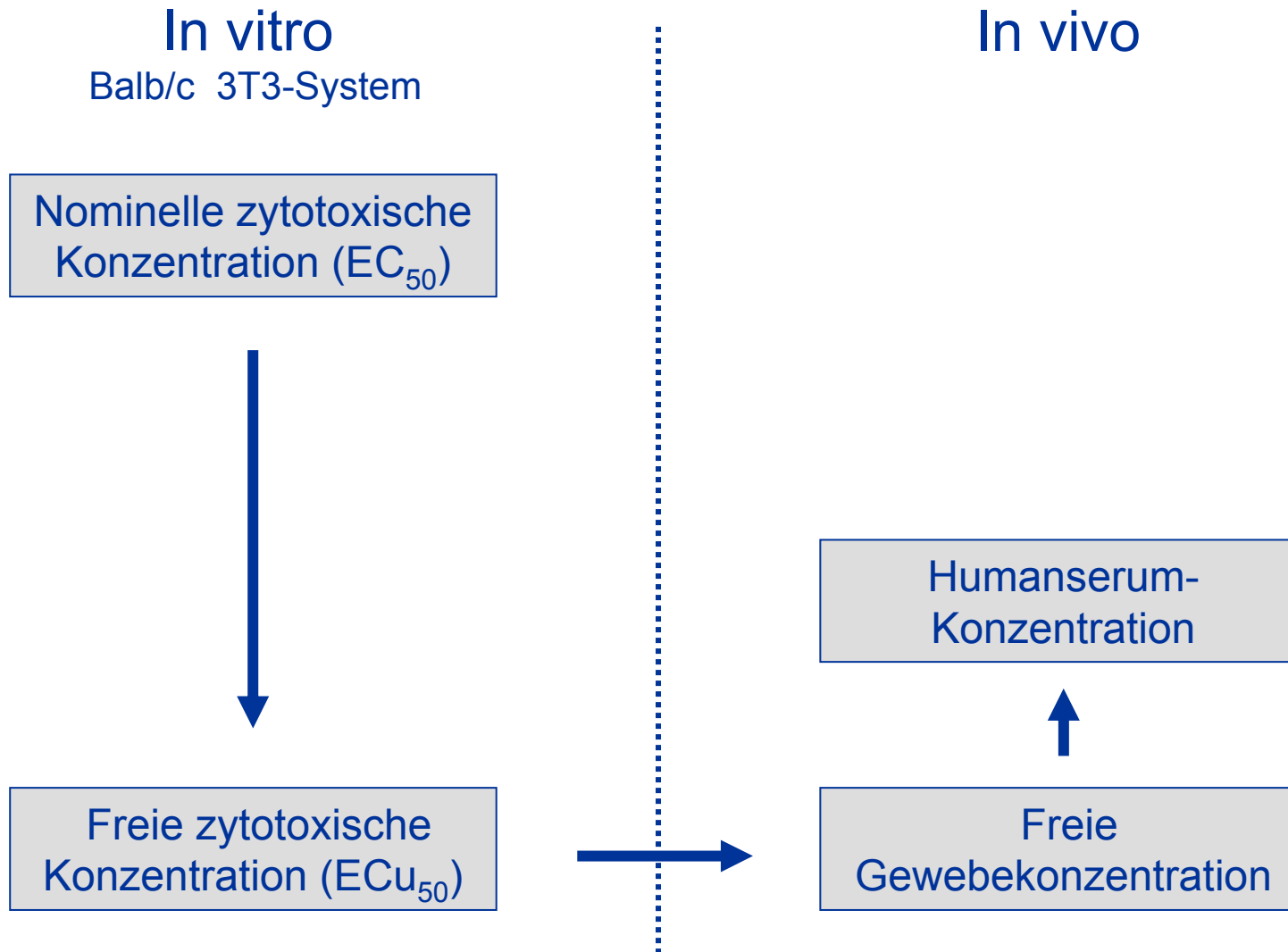
Fathead minnow



(Gülden & Seibert, 2005.
Aquatic Toxicology 72,
327-337)

Quantitative in vitro – in vivo Extrapolation

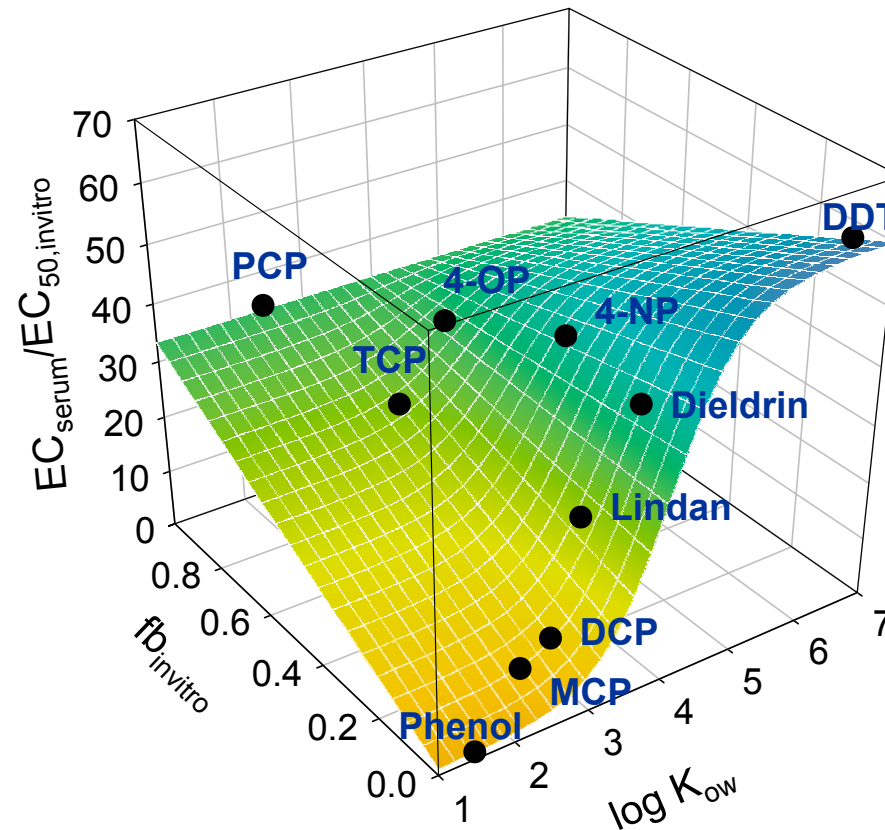
Das „equivalent exposure“ Konzept



Differences in composition of human serum and in vitro systems

	Balb/c 3T3 cell system	Human serum	Ratio
Albumin concentration	18 μM	600 μM	33
Lipid volume fraction	0.1 ml/l	6 ml/l	60

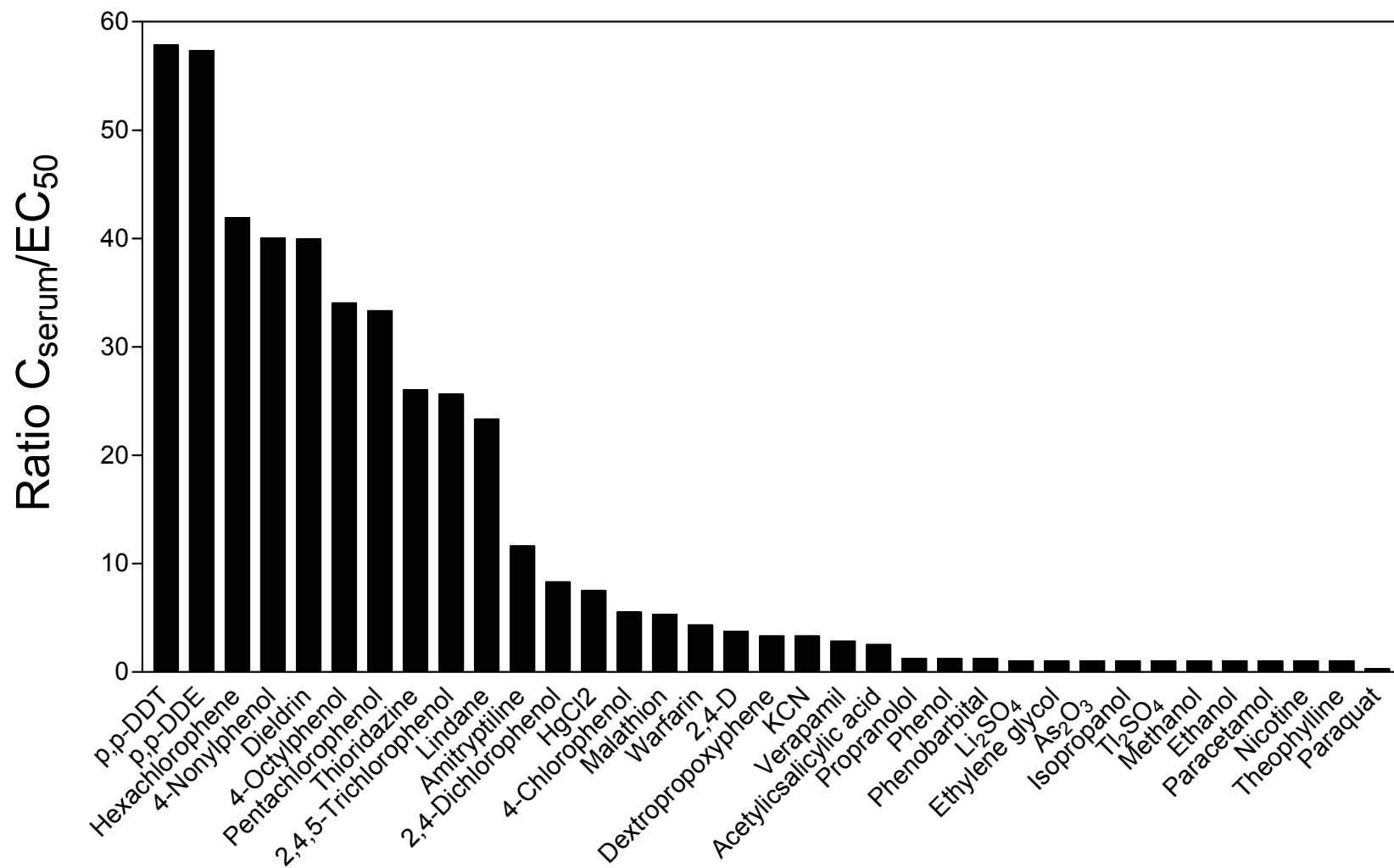
Prediction model for equivalent human serum concentrations



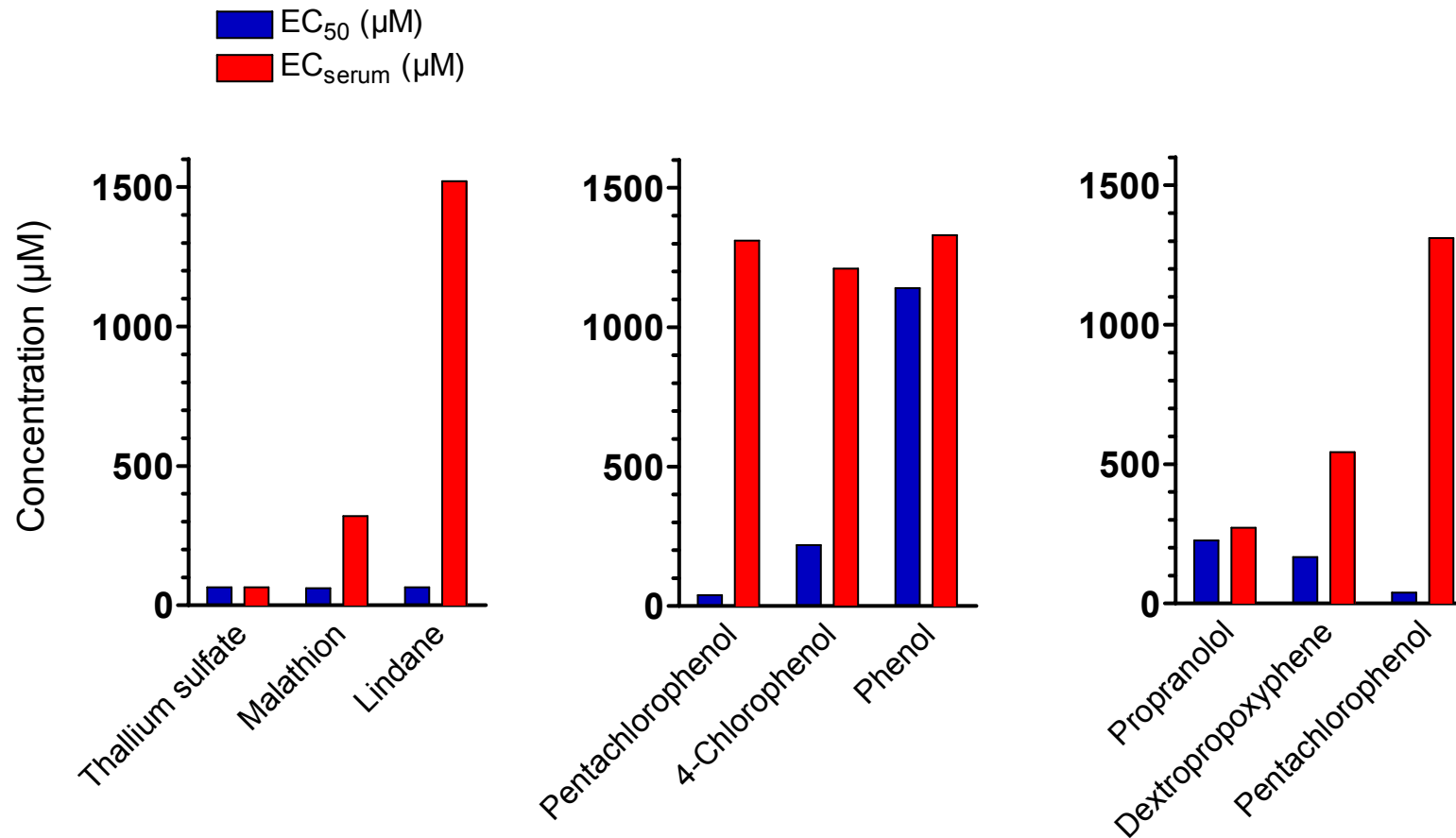
$$EC_{\text{serum}} = (EC_{50} - B \cdot P_{\text{invitro}}) \frac{1 + K_{\text{ow}} \cdot V'_{\text{L,serum}}}{1 + K_{\text{ow}} \cdot V'_{\text{L,invitro}}} + B \cdot P_{\text{serum}}$$

(Gülden et al. 2003, Toxicology 189, 211-222)

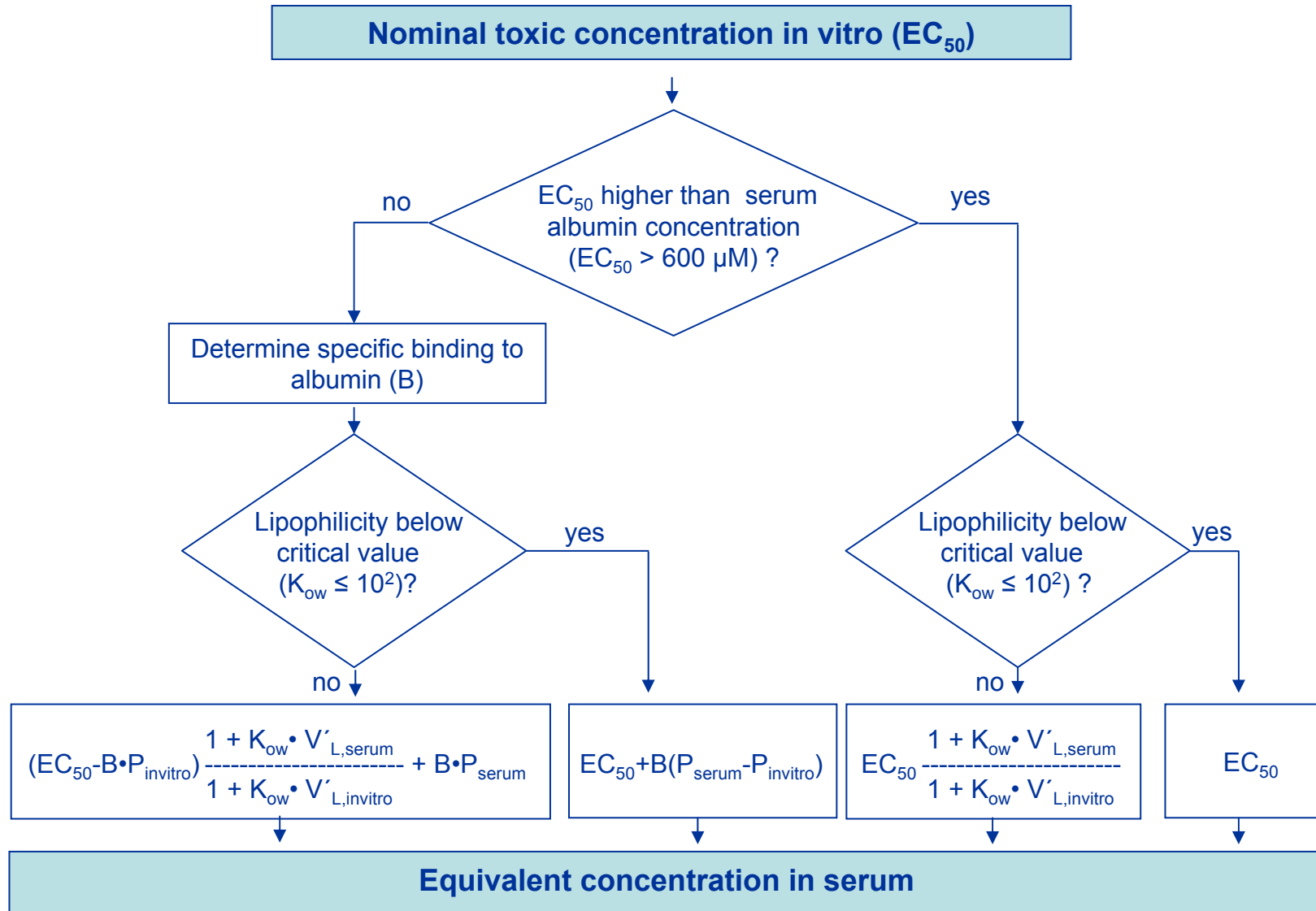
Human serum concentrations equivalent to EC₅₀-values in Balb/c 3T3 cell cultures



Relative potency (potency ranking) in vivo may be completely different compared to in vitro



Strategy for extrapolation of equivalent serum concentrations



(Gülden et al., 2006. Toxicol. in Vitro 20, 1114-1124)

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