EVALUATION AND INTERPRETATION OF TOXICITY: 4-CHLOROPHENOL EQUIVALENT FOR ORGANIC CONTAMINANTS

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ABSTRACT

Environmental risk of contaminated environment can be characterized by measuring the contaminant concentration and the **adverse effects of environmental samples**. These adverse effects are usually quantified by the **ED_{20} and ED_{50} values** (the dose of the environmental sample which causes 20% and 50% decrease in the measured endpoint). To get easily understandable and usable adverse-effect test results we introduce here the **"equivalent evaluation and interpretation" and "equivalent calibration" tool for organic contaminants**.

The essence of this evaluation and interpretation methodology is that **the inhibition of environmental samples is compared to the toxicity of a reference (calibrating) compound**, which is the same in case of each measuring set and finally the inhibition of samples is expressed in an equivalent unit of the selected reference substance. This way organic compounds contaminated soils' toxicity is compared to 4-chlorophenol (4CP) toxicity and expressed in 4CP equivalent: **TE_{Q4CP50} (mg 4CP /kg soil)**. This technical support tool is similar to those are used by many chemical analytical methods, that need calibration between measured endpoint (e.g. colour) and the concentration of a chemical substance.

INTRODUCTION

In this presentation we describe the application of 4CP equivalent for direct contact toxicity testing of contaminated soils. The main advantage of direct contact toxicity test is that the results can integrate all the interactions between different substances, substances and environmental matrix, the substances and the biota by association of testorganism and the tested soil *in vitro*. Otherwise the consequences of these interactions are hardly predictable only on the basis of the chemical analytical results (Gruiz et al. 2001).

To calculate the equivalent – similar to chemical analytical calibration methods – a calibration series is prepared and measured parallel to the unknown environmental sample. This calibration series can be prepared in water, or in soil. If it is prepared in soil it is important to use an uncontaminated soil, which is the same or similar to the soil sample to be tested. If it is prepared in water, we apply **a correction factor ($f_{soil/water}$)** for simulating matrix effect of soil; sediment or other solid-phase containing samples, with the application of a correction factor. This correction factor was determined from the measured toxicity of 4CP in water and in different type of soils.

MATERIALS AND METHODS

For interpreting the toxicity of soils contaminated with an unknown organic chemical substances we compared the measured effect to the toxicity of 4-chlorophenol (4CP) and expressed it in "4CP equivalent" TEQ_{4CP50} (mg 4CP /kg soil). With the idea of using the measured toxicity of 4CP series as a calibration curve, the toxicity of a contaminated soil can be expressed as if it were caused only by 4CP in the case of organic substance contaminated soils.

Calibration Series and bioassays

By this time we worked out the calibration series for three different toxicity tests with different end points, which are listed in Table 1. The applied initial 4CP solutions concentrations for *Vibrio fischeri* bioluminescence inhibition test and the *Tetrahymena pyriformis* growth inhibition test are made in water, but for *Folsomia candida* the required 4CP concentration was made in the artificial EOCD soil (OECD, 1984) .

Table 1 4CP calibration series for toxicity tests

Testorganism	Test endpoint	4CP Calibration series
Vibrio fischeri	bioluminescence inhibition	200; 100; 50; 25; 12.5 mg 4CP/l in water
Tetrahymena pyriformis	growth inhibition	500; 100; 50; 25; 12.5 mg 4CP/l in water
Folsomia candida	mortality	500; 100; 50; 10 mg 4CP/kg OECD soil

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References

Gruiz, K., Horváth, B. and Molnár, M. (2001) *Environmental Toxicology* (in Hungarian), Műegyetem Publishing Company, Budapest

Organization of Economic Cooperation and Development (1984) OECD guidelines for Testing chemicals, Guideline 207, Earthworm acute toxicity test, OECD, Paris, France

RESULTS

Interpretation of bioassay results in TE_{Q4CP50}

After measuring the unknown sample along with the calibration series, the dose of the contaminated soil and 4CP was plotted against the rate of inhibition or mortality or other inhibition effect in relative percent, then sigmoid curve is fitted with Origin 8.0 program, and ED_{50} is to be read. The ED_{50} (the dose causing 50% effect) is calculated from the dose-effect curves.

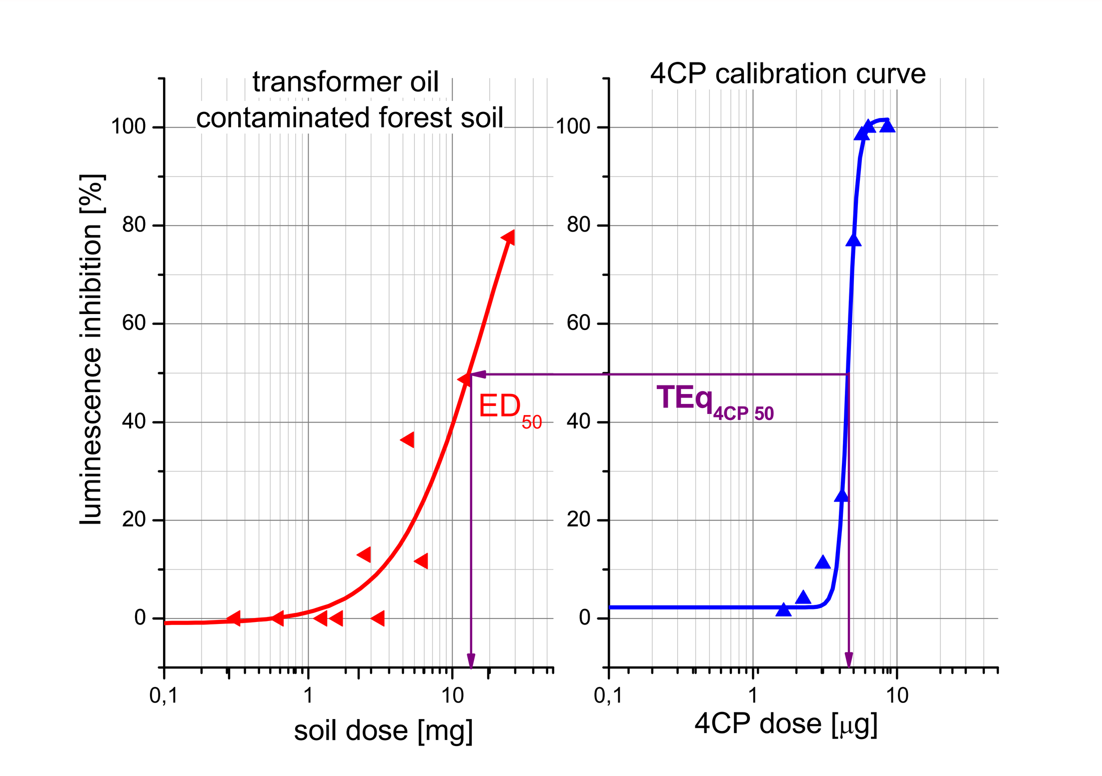

Figure 1 Reading off a contaminated soil's ED_{50} and 4CP's ED_{50}

Secondly we have to calculate the 4CP equivalents according to the formula below:

$$TEQ_{4CP50}\left[\frac{mg\ 4CP}{kg}\right] = \frac{ED_{4CP50}\left[\mu g\ 4CP\right]}{ED_{sample50}\left[g\ sample\right]} * f_{soil/water}$$

The suitable $f_{soil/water}$ correction factor was chosen according to the type of the contaminated soil.

Determination of $f_{soil/water}$

We measured 4CP calibration curves in water and in different types of soils spiked with 4CP to determine the equivalent and the correction factor ($f_{soil/water}$) between the toxicity of 4CP in water and in different soils. Finally the correction factors for two typical soil types were calculated: sandy soil with humus content of 0.45 % and brown forest soil with humus content of 1.3%.

Our aim was the use of these determined correction factors when the unknown, contaminated soils toxicity 's is expressed in 4CP equivalent.

Table 2 Determination of $f_{soil/water}$ correction factor for luminescence inhibition test

Vibrio fischeri bioluminescence inhibition test			
Tested sample name	4CP calibration series	500 mg 4CP contaminated forest soil	500 mg 4CP contaminated sandy soil
Unit of measure	mg 4CP/l	mg 4CP/kg soil	mg 4CP/kg soil
EC_{50}	9.64	19.22	15.55
Unit of measure	mg 4CP/mg 4CP	mg 4CP/kg soil	mg 4CP/kg soil
TEQ_{4CP50}	1.00	0.50	0.62
		$f_{FS/w}$ (4CP in soil/4CP in water)	**$f_{SS/w}$ (4CP in soil/4CP in water)**
Soil correction factor		**2.0**	**1.6**

CONCLUSIONS

The calibration tool is able to characterise and integrate the effect of different soil types, the different sensitivity of testorganisms and the effect mechanisms of contaminants. Using the toxicity equivalent method the different bioassay results can be compared which each other and used for quantitative risk assessment and decision making.

For the calculation of the Risk Quotient (RQ) a no effect concentration (PNEC) is needed, not the dose of the contaminated environmental compartment, however for an unknown pollution we can only measure the effective dose.

With the application of a calibration curve drawn by 4CP and the calculation of 4CP equivalent we get an independent effective concentration value that we can use to calculate a PNEC data for the purpose of quantitative risk assessment and risk based decision making.