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# Toxicological Evaluation of Exposed SPMD Membranes

Vladimír Kočí*, Martin Mlejnek†, Lucie Kochánková‡

*Department of Environmental Chemistry, ICT, 166 28 Prague, Czech Republic*

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**Abstract:** This article is focused on bioassay evaluation of exposed SPMDs. Toxicity testing of SPMD extract is a suitable complementary parameter to chemical analysis. *Vtox* as an integrative parameter describing toxic properties of extract from membrane and thus a level of contamination of assessed ambient is presented here. Two examples of practical use of *Vtox* is demonstrated: a course of toxicity throw wastewater treatment plant with industrial inlets and increase of toxicity in surface water body caused by leakages of POPs from industrial plant.


*Keywords: passive sampling, POPs, SPMD, toxicity testing, Vtox*

## 1 Introduction

Persistent organic pollutants (POPs) belong to a group of serious environmental contaminants. POPs are wide variety of chemicals as polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), polychlorinated dibenzodioxins and furans PCDD/Fs, organochlorine pesticides (OCPs) with different functional groups and/or congeners and thus with different toxicological effects. As these trace-level contaminants are not easily to sample using conventional point sampling methodology some passive approaches are used.

A passive sampling method represents the measurement of a pollutant concentration in-situ as a weighted function of the sampling time. The exposure is being considered as integral contaminant level within particular sampling period. Many long-term monitor-

* E-mail: Vladimir.Koci@vscht.cz
† E-mail: Martin.Mlejnek@vscht.cz
‡ E-mail: Lucie.Kochankova@vscht.cz

ing studies have been based on some aquatic organisms to concentrate traces and ultra traces of persistent organic contaminants in waters in their fatty tissues, i.e. bioconcentration. All of organism-based samplers exhibit many limitation features due to lack of proportionality between concentration in their tissue and exposure concentration.

Analytical methods usually consider a selected list of toxic substances presumed to be present in a sample and/or assessed site, but not all toxicants actually present in the sample will be detected. The prediction of the real toxic hazard represented by the sample is not often possible, as only relatively few pure chemicals have been tested for toxicity. Moreover, the composition of a sample as determined by chemical analysis does not take into consideration the synergistic and antagonistic effects of pollutants in complex environmental mixtures, nor does it reflect the actual bioavailability of the pollutants. These are the main reasons why toxicity testing in parallel with analytical methods has become an important tool for characterizing complex environmental samples such as wastewaters, soil and sediments. Various aquatic bioassays are used in testing water and wastewater samples ([9]).

## 2 Monitoring of ambient POPs

Assessment of environmental pollutants exposure, particularly POPs, is closely connected with applications of an in-situ passive sampling approach. Passive dosimeters are mostly applied to monitor water environment. Presence of POPs in waters reflect serious risk to consequent transport to food chain through biota. The organism sampler limitations are given by physical stressors influence or migration for example. Passive sampling technology presents a lot of advantages to standard sampling methods. SPMD records low levels of contamination which is contrary to standard sampling methods needing large volumes of sampled media followed by pre-concentration with expensive analytical techniques (needed for reaching acceptable detection limits). The principal advantage of SPMD is its sampling of the truly-dissolved (bio available) phase of pollutants. It is not affected by accidental variation of pollutant concentration and therefore offers real environmental mean values. One such passive sampling method is the semi-permeable membrane device (SPMD) ([1], [7]).

Semipermeable membrane devices (SPMDs) are used with increasing frequency as samplers of organic contaminants in the environment. These devices are designed to sample non-polar, hydrophobic compounds that are easily bioavailable for most of the organisms. It is important to evaluate the final effect of these bioavailable compounds to the environment, not only a chemical composition of such a sample. SPMD sampler consists of a horizontal thin-walled tube of nonporous (with transient cavities) low-density polyethylene (LDPE), filled with 1 ml of synthetic lipid – triolein (1,2,3-tri-[*cis*-9-octacenoyl]glycerol) of high purity which is found in most organisms. Porosity (transient pores, approx. $10^{-9}$ m) of the LDPE is about the same all over the membrane. General dimension of the standard SPMD is: width 2.5 cm (horizontal), overall length 91 cm and thickness 75 $\mu$m.

A great advantage of SPMDs in environmental sampling is the possibility of conducting sampling and pre-treatment of the sample both for chemical analysis and bioassay. The chemical evaluation of SPMD sampling is finished with regard to method, especially with the use of values of effective sampling rates ($R_s$). This results in the possibility of good interpretation of obtained data to field conditions and offers the option of comparing results from different sites, projects and laboratories. The ambient water concentration ($c_w$) of the contaminant can be estimated based on the concentration in the SPMD's lipid ($c_{SPMD}$), the effective sampling rate ($R_s$) and the time of deployment (t) ([1], [2]):

$$c_w = \frac{c_{SPMD}}{R_S.t} \tag{1}$$

The effective sampling rates ($R_s$) of many hydrophobic compounds have already been determined and more research is under way to expand the number of compounds ([6]).

Such a use of SPMD sampling for bioassay is not yet widespread.

## 2.1 Toxicity testing of SPMD

A treatment of exposed SPMD membrane consists of few steps. Exposed SPMDs are quickly cleaned up with water removing dirt and peryphyton from surface. After that membranes are quickly washed in acetone and dried in air. This is followed by dialyzation with hexane (superpure quality) for 3 days including 2 solvent exchanges resulting in a 200 ml fraction. As the dialysis is accompanied with an extraction of pollutants bonded inside the LDPE membrane, we can also name this process as extraction. In fact both of these processes have to be accounted for; dialysis for those pollutant molecules bonded in the triolein, which the SPMD is filled with, and the extraction for molecules from the membrane itself. This is important for following interpretation of results from toxicity testing.

For bioassays evaluation dialysates and/or extracts are transferred into an acetone – DMSO (1:1) mixture ([4]). This offers good solubility and low background toxicity. Samples prepared by this way are then used for grounding of the dilution series for bioassays. The obtained acetone – DMSO extract is used as a toxicant for conventionally applied aquatic bioassays. According to an amount of obtained extract usually some small volume toxicity test (microbiotests) are provided. Good experience of this is seen with a test of inhibition of light production of bioluminescent bacterium *Vibrio fischeri* ([10], [11]), acute toxicity test with cladophora *Daphnia pulex* ([10]), test with fish rainbow trout *Oncorhynchus mykiss* ([8]) and test of inhibition of algal growth of *Scenedesmus subspicatus* and/or *Selenastrum capricornutum* ([5]).

## 2.2 Interpretation of toxicity tests of SPMDs

A principal obstacle in toxicological assessment with SPMDs is how to interpret obtained results – EC50 values of evaluated extract on the used organism. There is no one general

approach in interpretation of toxic response of the testing organism to the SPMD extract. Just to obtain a value of EC50 of extract is not enough. There are some other important factors involving the value of EC50 that must be accounted for.

The first factor is the duration of membrane exposure. If two membranes are exposed in the same conditions (same concentration of pollutants) but for different durations, the response of the testing organism to extracts obtained from both membranes will be different, resulting in different EC50 values. Thus, such an EC50 cannot be used as a parameter describing level of ambient contamination. For this reason the time of exposure has to be factored into a unit to be used as a parameter describing toxic properties of sampled site independently on time of deployment.

The second factor influencing value of EC50 of extract is the concentration of pollutants in the extract. This means how much of the sampled compounds were contained in a volume of Acetone – DMSO mixture and basically, how many membranes were used to obtain a millilitre of extract. EC50 expressed as concentration value based on ml of extract in L of media (for example fresh water) is not sufficient for use. The formerly used expression of EC50 as a concentration of triolein in media is also not recommended due to the adsorption of pollutants in the LDPE membrane as mentioned above. For this reason we recommend the use of an expression of EC50 as a number of whole membranes in media – pcs.$L^{-1}$.

Similarly as $V_{w-tox}$ is defined by Huckins ([3]), we present here unit *Vtox* in which both of the earlier mentioned factors are taken into account. *Vtox* represents a volume of media that is theoretically needed for dilution of all toxicants absorbed in one membrane during one average day of deployment to obtain a solution with a chosen effective concentration, for example EC50. The higher *Vtox* reflects the larger volume of toxicants absorbed and thus the higher contamination of the sampled site. The following formula defines *Vtox*, where (m) is the concentration of extracted membranes in solvent mixture expressed as number of membranes in ml of solvent mixture (pcs.$ml^{-1}$), (d) is duration of deployment of membrane during a sampling (days) and ECXX is an effective concentration of extract on the chosen organism, for example EC50 (ml.$L^{-1}$).

$$Vtox(50) = \frac{1}{m.EC50.d} \tag{2}$$

*Vtox* can be determined not only for EC50, but for other per cent values of effective concentration as EC20, EC10 etc. For this reason we recommend for every *Vtox* assign in brackets the per cent value used. Although unit of *Vtox* is L.$d^{-1}$, from practical point of view it is better express it as ml.$d^{-1}$. Similarly like toxicological unit TU, one of the benefits of *Vtox* is its property of easy demonstration of contamination level – the higher *Vtox* the higher ambient contamination.

## 2.3 Case studies

*Vtox* was successfully applied in several cases where the course of toxic properties of sampled sites was to be demonstrated. Here we present two examples of use of *Vtox*.

A big flood in August 2002 in Czech Republic presented a danger of leakage of POPs from Spolana chemical plant, which was monitored. A sampling based on SPMDs was conducted resulting in extracts from membranes being treated as described above and tested for toxicity on bacterium *Vibrio fischeri* and crustacean *Daphnia magna*. These ambient sites were sampled: River Labe up and downstream of Spolana plant and a lode in area of Spolana collecting possible leakages of POPs. For comparison data from year 2001 of River Labe downstream of Spolana are presented. As demonstrated on 1, parameter *Vtox* apparently shows an increase of toxicity of the River Labe due to the presence of the Spolana plant. The values of *Vtox*(50) of Labe River downstream of Spolana plant in year 2001 and upstream after the flood is comparative. Increase of toxicity caused by Spolana downstream after the flood is evident.

The second example is the evaluation of cleaning efficiency of a wastewater treatment plant (WWTP) in Ostrava city. This WWTP is used for cleaning of wastewater from three different sources: sewage water, sewage water with industrial wastewater and wastewater directly from a coking plant. Figure 2 show *Vtox* values from these inlets, Vtox of centrifuged water after second step of cleaning and *Vtox* of recipient Cerny Creek downstream of WWTP. Values of *Vtox* demonstrate a decrease of toxic potential in inlets during the course of cleaning and a good level of toxicity in the recipient comparative to an overall "toxicity" of surface water; see value of Labe River 2001 in former example I.

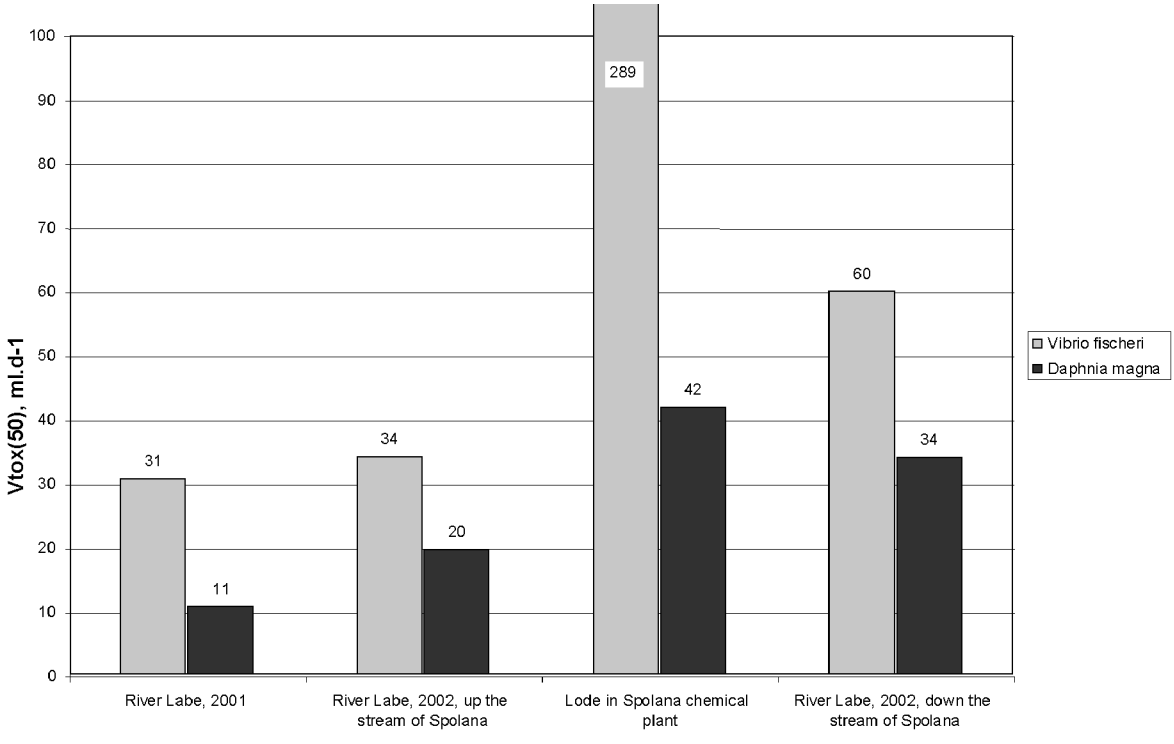


**Fig. 1** Contamination of Labe River with POPs originated in Spolana chemical plant.

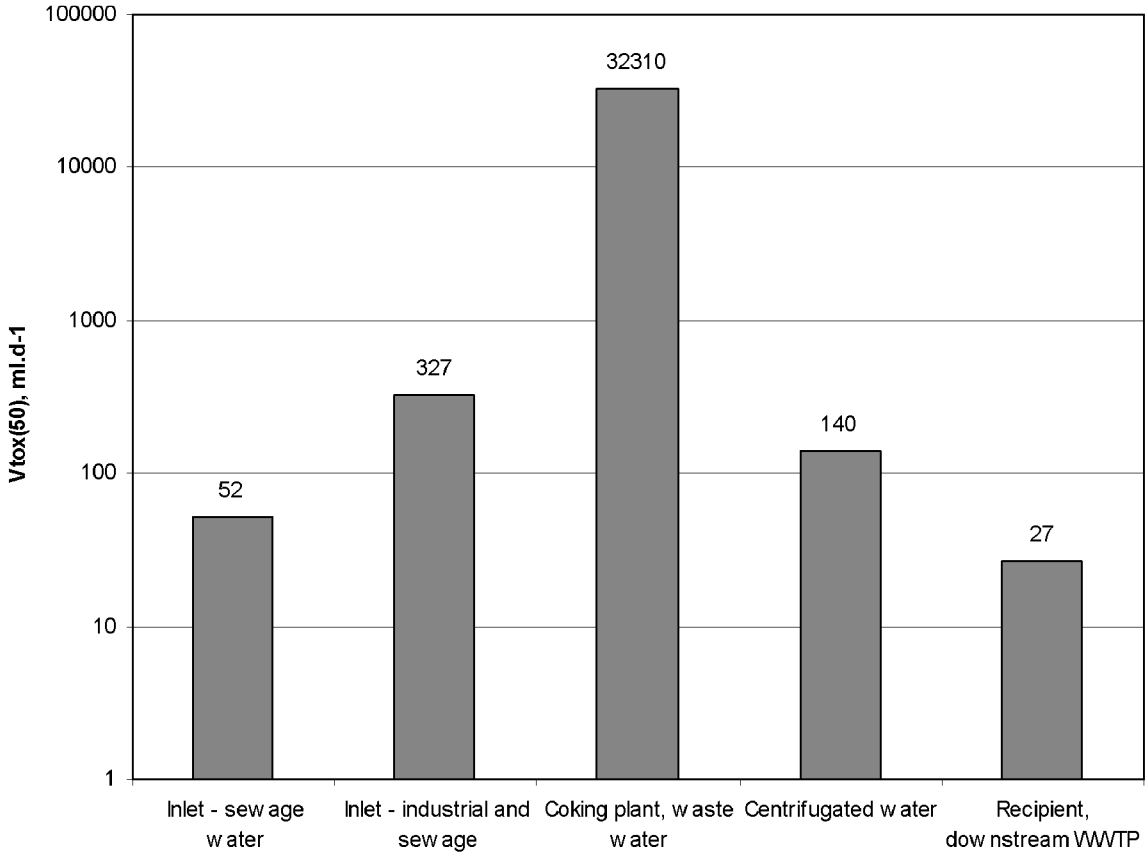


**Fig. 2** Waste water treatment plant. Values of Vtox determined based on bioassay with algae Scenedesmus subspicatus.

## 3 Conclusion

Herein is described a new way of result interpretation from toxicological evaluation of SPMDs. The parameter *Vtox* allows comparison of the toxicity of samples obtained from SPMDs with different exposure times, different sites, projects and laboratories. We did not find how to include sampling temperature in the toxicological unit of SPMD. The temperature is a factor influencing the rate of absorption of pollutants in triolein. As the sampling rates ($Rs$) are determined for large concentrations we recommend with *Vtox* assignment of a mean temperature during sampling and a temperature used (if done) for determination of $Rs$ in chemical assessment. An advantage of *Vtox* is its formal similarity to toxicological unit TU that means the higher contamination of the sampling site, the higher value of *Vtox*.

Application of toxicological tests for SPMD evaluation is highly recommended. It can distinguish different contamination levels of sampling sites. Bioassays also provide information about the action of monitored pollutants in the environment. Nevertheless chemical analysis of SPMDs samples is very important for complex classification of those samples.

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