



## Application of semipermeable membrane device (SPMD) to assess air genotoxicity in an occupational environment

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### ABSTRACT

Semipermeable membrane device (SPMD) is a passive sampler that sequesters lipophilic contaminants, mimicking the bioconcentration in the fatty tissue of organisms. This study was designed to assess the use of SPMD and biological tests (Comet assay and Ames test) for air monitoring. For this purpose an occupational environment with expected polycyclic aromatic hydrocarbons (PAHs) contamination (coke plant) was selected for a case study. The SPMDs were deployed in five occupational contaminated sites and in a control site. The SPMD dialysates were chemically analysed and examined for *in vitro* DNA-damaging activity in human cells (Jurkat) by Comet assay and for mutagenicity with the Ames test (TA98 strain, w/o S9). Total suspended particulates were also collected and analysed (GC–MS). No biological effect of SPMD extract was revealed in the control site. On the other hand, air samples collected with SPMDs within the coke plant showed variable degrees of genotoxic and mutagenic activity. The highest effects were associated with the highest PAH level recovered in the SPMDs extracts and in particulate samples.

Results obtained support the sensitivity of biological tests associated to SPMD sampling for evaluating the health risk of potentially contaminated work environments highlighting the usefulness of SPMDs for environmental air quality monitoring.

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### 1. Introduction

The exposure estimates to toxic and genotoxic environmental pollutants are a fundamental element of human hazard assessment (Petty et al., 2000a). Limitations in nearly all currently employed sampling techniques hinder comprehensive exposure assessment (Petty et al., 2000b). Generally, the risk assessment of airborne pollutants is based on the detection of their concentration with analytical chemistry methods and on the toxicity and genotoxicity evaluation of single compounds encountered.

This approach does not consider the synergistic, additive or antagonistic effects on biological systems of chemicals in complex air mixtures. Moreover, the determination of the bioavailable portion of pollutants is critical for the evaluation of air pollutant effects (Petty et al., 2000a). In addition it must be considered that the organisms bioconcentrate innocuous levels of contaminants to relatively high levels in their lipids.

Innovative sampling approaches are required to adequately define health effects of airborne chemicals exposure (toxic, mutagen,

carcinogen). Such methods should also be low-tech and cost-effective and should allow not only the direct monitoring of the fate and concentration of chemical pollutants, but also the evaluation of their effects and the assessment of the potential hazard for human health (Sabaliūnas and Sodergren, 1997). Passive samplers may offer a solution to any of these problems. They are preferred to conventional active air samplers since they do not require electricity, are less expensive and can sample for a long period of time (Söderström et al., 2005).

The semipermeable membrane device (SPMD), developed by Huckins et al. (1990), is a passive and integrative *in situ* sampler that is well known as potent concentrator of bioavailable organic contaminants including polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated dibenzodioxins (PCDDs), furans and poly chloro byphenils (PCBs) in the aquatic environment (Huckins et al., 2000, 2002). SPMD consists of a thin film of synthetic lipid triolein enclosed in thin walled layflat polyethylene tubing. The SPMD allows to measure not only the presence, but also the bioavailability and the bioconcentration potential of organic contaminants. The chemical compound diffusion through the polyethylene tubing mimics the passive diffusion of bioavailable organic contaminants through biomembranes. Furthermore the passive partitioning process mediating SPMD uptake of organic

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contaminants in triolein simulates, phenomenologically, the bio-concentration of organic contaminants in the fatty tissues of organisms (Petty et al., 2000b; Vrana et al., 2001). In addition, SPMD sequesters a broad spectrum of chemical classes, provides a time weighted sampling and allows to perform chemical and biological analyses (toxicity, mutagenicity and genotoxicity tests) guaranteeing the same sampling condition (Huckins et al., 1993). For a number of years SPMD has been successfully used for the monitoring of organic pollutants in the aquatic environment (Bergqvist et al., 1998; Rantailanen et al., 2000; Sabaliūnas et al., 2000; Gilli et al., 2005; Ellis et al., 2008) and in sediments (Lebo et al., 2000; Rantailanen et al., 2000). Despite the promising results reported by Prest et al. (1995) and the numerous attractive qualities of SPMD, the application of this passive sampler to the air monitoring has been studied for the identification and quantification of airborne chemicals accumulated by SPMD (Rantailanen et al., 1999; Söderström et al., 2005; Esteve-Turrillas et al., 2007; Esteve-Turrillas et al., 2008a,b) and for understanding the uptake kinetic and sampling rates for PAHs and PCBs (Ockenden et al., 1998; Bartkow et al., 2004; Cicenaitė et al., 2007), whereas few works have been carried out to evaluate the use of these passive air samplers in combination with biological tests (standard toxicity and genotoxicity assays) to study the pollutant health effect (Isidori et al., 2003).

The use of biological tests coupled with the chemical and physical analyses allows to better define the human environmental risk. The biological tests assess the biological effects of complex chemical contaminant mixtures. In particular mutagenicity tests performed in vitro or in vivo using prokaryotic or eukaryotic cells or organisms in toto can give an estimation of DNA damage resulting from environmental mutagen exposure. The most widely used bacterial mutagenicity bioassay is the *Salmonella typhimurium* assay (Ames test) (Maron and Ames, 1983). It is based on the reverse mutation of modified strains of *S. typhimurium* (TA98 and TA100) after the contact with the samples. Another short term bioassay system for detecting DNA-damaging agents is the Single-cell gel electrophoresis (SCGE) test or Comet assay. This is a sensitive, reliable, and rapid method for DNA double- and single-strand breaks, alkali-labile sites and delayed repair site detection in eukaryotic individual cells (Singh et al., 1988).

This study was designed to assess the use of SPMD and biological tests (Comet assay and Ames test) for air monitoring. This exposure assessing method was tested in a polluted occupational environment (coke plant).

## 2. Materials and methods

### 2.1. Air sampling

The monitoring was carried out in a coke plant in which PAH pollution is likely. The work at the coke plant is continuous. Within the plant 6 sampling sites have been identified (Fig. 1): an office located in a separated building near the coke plant was used as control site (site 1) and the other 5 sampling sites were selected as representative of different workplaces along the coke production plant; the site number 2 was located on the car charging coal into the coke oven batteries (outside the driver's cab), sites number 3, 4 and 5 were placed in different areas inside the coke oven gas treatment plant and the site number 6 was located in the finished coke sieving area. The sites 2, 3 and 6 were located in outdoor environment, while sites 4 and 5 were in closed areas. Airborne pollutant samplings were performed during winter season using 1 SPMD for each site. In the same day of SPMD deployment the total dust (total suspended particulate: TSP) sampling was performed in site 2 (which was supposed to present the highest pollution), 3 and 4 (where some pollution was in any case likely).

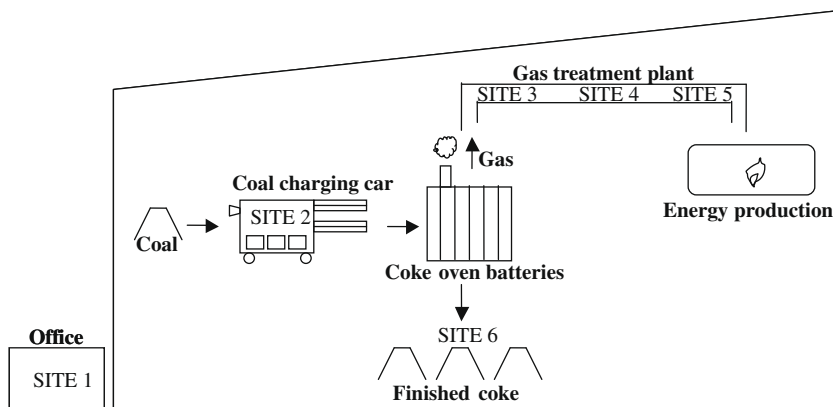
### 2.2. SPMD

#### 2.2.1. SPMD preparation

SPMDs were prepared as described by Huckins et al. (2000). Briefly, layflat low density polyethylene tubing (width 2.5 cm, thickness 75–95  $\mu\text{m}$ , provided by Novamont s.p.a.) was cut into 120 cm long segments and extracted with hexane (95% HPLC grade, Sigma) to remove potential contaminants. The segments were filled with 1 mL (0.915 g) of triolein (95% purity, from Sigma Chemical Company) configured to form a thin film and the tube ends were sealed. The effective length of the SPMD (distance between the two thermosealed ends of the segment) was 91.4 cm.

#### 2.2.2. SPMD deployment

SPMDs were suspended 1.5–2 m above the ground, at about the level of workers breathing zone, protected from the light. Air was sampled using a vertical perforated plastic container to protect the membranes against mechanical damage. After 24 d of sampling SPMDs were retrieved and preserved frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis. One control SPMD (Field blank SPMD) had accompanied the sampler during the transport, deployment, retrieval and treated as the exposed SPMDs.



**Fig. 1.** Scheme of the sampling sites selected in the coke plant (site 1: office located in a separated building near the coke plant; site 2: located on the car charging coal into the coke oven batteries; site 3: located in the area where the condensate separates out from the ammonia water; site 4: located near the saturator where the gas is bubbled through a bath of sulphuric acid solution to form ammonium sulphate; site 5: located in the area in which the gas was compressed; site 6: located in the finished coke sieving area.).

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