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Genotoxic and mutagenic activity of environmental air samples in Flanders, Belgium

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Abstract

Atmospheric pollution is assumed to play a role in the incidence of respiratory diseases and cancers. Airborne particles are able to penetrate deep into the lung, they are composed of complex chemical mixtures, including mutagens and carcinogens such as polycyclic aromatic compounds (PACs). The present study reports mutagenic and genotoxic activities associated with ambient air collected near a busy street in Borgerhout, at an industrial site in Hoboken and in Peer, a rural community 70 km east of Antwerp in Flanders, Belgium. Airborne particulates (PM10) and semi-volatile organic compounds were sampled during winter and summer. Samples were collected with a high-volume sampler using quartz filters (QF) and polyurethane foam (PUF) cartridges. The mutagenic and genotoxic potency of the organic extracts was determined using the Salmonella test / standard plate incorporation assay and the Vitotox® assay. Concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) in the extracts were determined by reversed-phase high performance liquid chromatography (HPLC). The mutagenicity assay, using Salmonella typhimurium strain TA98, demonstrated direct-acting mutagenicity of up to 58 rev/m³ for the QF extracts and low or no mutagenic activity in the PUF extracts. Metabolic activation of the samples resulted in high indirectacting mutagenicity for both QF and PUF extracts: up to 96 rev/m³ were found in QF samples and 62 rev/m³ in PUF samples. Genotoxic effects of the filter extracts was assessed with the Vitotox® assay, some direct-acting genotoxic effects were noted but almost no effects were observed after metabolic activation. Without metabolic activation most PUF extracts were bacteriotoxic. With metabolic activation this toxicity disappeared, but genotoxic effects were not observed. By use of PLS analyses the observed biological effects correlated well with the PAH concentrations.

Keywords: Airborne particles (PM10); Ames mutagenicity assay; genotoxicity; polycyclic aromatic hydrocarbons; Vitotox® assay.

Introduction

Residents of industrialized and densely populated regions, like Flanders (Belgium), are exposed to ambient air pollution arising primarily from industrial activities, traffic sources and heating. Epidemiological studies, carried out to investigate the health risk related to air pollution, suggest that ambient air pollution may be responsible for increased rates of lung cancer [1,2,3]. Particulate matter with a mean diameter less then 10 μ m (PM10), is associated with adverse health effects including increased respiratory problems, cancer and mortality [4]. Indeed PM10 particles have the ability to penetrate and deposit on the tracheobronchial and alveolar regions of the respiratory tract [5]. Various air pollutants/organic compounds, that are present in the air in a gaseous and semi-volatile state are known to adsorb onto the surface of these airborne particles. Of primary concern are the PAHs and their derivatives, such as nitro-PAHs (NPAHs) and oxy-PAHs, collectively know as polycyclic aromatic compounds (PACs). Several PAHs and NPAHs are considered as probable (2A) or possible (2B) human carcinogens by the Agency for Research on Cancer (IARC) [6].

Identifying and estimating human exposure to these mutagenic and genotoxic compounds is imperative in evaluating public health risk. However, the complexity and potential interactive effects of airborne toxic compounds cannot be adequately ascertained using chemical analysis. Therefore, biomonitoring of environmental air for it is mutagenic activity in addition to conventional chemical monitoring, is receiving increasing attention for evaluating potential risks to public health [7,8].

Little or no information is available on the levels of airborne mutagenicity/genotoxicity in Flanders. In the present study the mutagenic and genotoxic activities associated with ambient air were evaluated using two bioassays: the Salmonella test/ plate incorporation assay and the Vitotox® test. Airborne particles (PM10) and semi-volatile organic compounds were

collected during winter and summer near a busy street in Borgerhout, at an industrial site in Hoboken and in Peer, a rural community 70 km east of Antwerp. These samples were analyzed for PAH and bioassays were performed to determine their genotoxic and mutagenic potency. In addition, the suitability of the bioassays for monitoring purposes were evaluated. Finally, PAHs concentrations and mutagenic/ genotoxic activies were statistically compared to investigate potential relations between observed biological effects and PAH concentrations.

2. Experimental: Materials and methods

2.1 Sampling and sample preparation

The air samples were collected during winter 2000-2001 and summer 2001. Three different sites, representative of different sources of emissions, were selected. The urban site in "Borgerhout" was located close (3m) to a busy approach road to the centre of Antwerp, with intense traffic including many diesel vehicles. The industrial site "Hoboken", a suburb of Antwerp, was located next to a large non-ferrous smelter; Hoboken also harbours metallurgic, printer and electronic equipment industries. "Peer", situated in a rural area 70 km east of Antwerp, was selected to represent background conditions in Flanders with low inputs from industry and heavy traffic (no polluting industries within 15 km, no highway). Each of these sites (urban, industrial and rural) was sampled 3 times in winter and 3 times in summer, resulting in 36 samples (18 QF and 18 PUF, see below).

The samples were taken using a high-volume air sampler (Digitel) with a size selective inlet (PM10). Sampler inlet was located at street level (1.8 m height). Particulate matter and semi-volatile compounds were collected on quartz filters (QF20 Schleicher & Schuell) and polyurethane foam (PUF) cartridges, placed in series. The more volatile compounds, which were not trapped on the filter, were retained in the PUF cartridges. These were pre-cleaned by 24-h soxhlet extractions using acetone (CAS 67-61-1). The samples were taken at a flow rate of 30 m³/h (500l/min) during 48 hours, resulting in a total air volume of about 1300 m³. All samples were taken during a week day starting at about 9 a.m. The quartz filters were changed automatically after 24 hours. The same PUF cartridge, however, was used during the entire 48 hour sampling. The weight of the collected particulate matter on the filters was determined by

weighing the filters before and after sampling (prior to weighing, 24 hours conditioning at 20° c in desiccator). Sampled filters were stored at -18° C until extraction.

QF and PUF were extracted separately by a soxhlet apparatus during 24 hours with 100 ml and 2000 ml acetone (CAS 67-61-1). QF and PUF blanks were extracted in an identical manner. Individual extracts were concentrated to 5.0 ml under a gentle N_2 stream and using a turbovap concentrator. A 0.5 ml aliquot was taken for HPLC (high-pressure liquid chromatography) analyses, the remaining volume was evaporated and dissolved in 4.5 ml dimethylsulfoxide (DMSO) (CAS 67-68-5) and evaluated using the bioassays. The extracts were stored at -80° C prior to bioassay execution.

2.2. PAH analyses

The organic extracts of the QF and PUF cartridges were analyzed for 16 PAHs using high performance liquid chromatography (HPLC) connected to a fluorescence detector [9]. The detected and quantified PAHs (3- to 5- rings) were: naphthalene (N), acenaphtylene (ACY), acenaphtene (ACE), fluorene (FL), phenantrene (PH), anthracene (AN), fluoranthene (F), pyrene (P), benz(a)anthracene (B[a]A), chrysene (CH), benzo(b)fluoranthene (B[b]F), benzo(k)fluoranthene (B[k]F), benzo(a)pyrene (B[a]P), dibenz(a,h)anthracene (DB[ah]A), benzo(ghi)perylene (B[ghi]PY) and indeno(1,2,3-cd)pyrene (I[cd]P).

A 0.5 ml aliquot of each organic extract was solvent exchanged into 1 ml acetonitrile (CAS 75-05-8). The HPLC system consisted of a liquid chromatographic system (Waters, Milford, MA, USA). Separation of the PAHs was accomplished using a Vydac 201TP column (250 x 4,6 mm), with a gradient elution ranging from 50/50 acetonitrile-water mixture to 100% acetonitrile (CAS 45-05-8) in 20 min. The PAHs were monitored with a fluorescence detector (Perkin-Elmer LC240), the wavelengths were adjusted automatically for each

compound according to the retention time. The excitation wavelength ranged between 260 and 300 nm, the emission wavelength between 380 and 465 nm. The standard reference material SRM1647 (NIST) was used for the calibration of the quantification method.

2.3. Bioassays

The organic extracts of QF and PUF cartridges, dissolved in DMSO, were tested for their mutagenic and genotoxic activity using two different bioassays.

The Salmonella test is a well-known bacterial mutagenicity test where the reverse His \rightarrow His⁺ mutations are visualized by plating *Salmonella typhimurium* bacteria in a histidine poor growth medium [10]. The assays were performed on both the QF and PUF extracts using the standard late incorporation test [11,12] with Salmonella typhimurium strain TA 98, in the presence and absence of an exogenous metabolic activation system (S9). The effects measured following metabolic activation are considered the indirect-acting activity. The effects measured without activation are regarded as direct-acting activity. Dimethylsulphoxide (DMSO) was used as solvent controls, whereas 4-nitroquionline-N-oxide (7µg/plate) (CAS 56-57-5) and benzo(a)pyrene (5 µg/plate) (CAS 50-32-8) were used as positive control, without (-S9) and with (+S9) exogenous metabolic activation, respectively. For the exogenous metabolic activation a 10% S9 mix was prepared with Acrolor 1254 induced rat liver S9 homogenate (ICN) [12]. Each sample was assayed in three concentrations (due to limited test material) using three replica plates per concentration. Revertants were counted using Quantity One quantification software in combination with Gel Doc2000 equipment (Bio-Rad), after 48 h incubation at 37°C. The data obtained are presented as revertants per m³ of air sampled, calculated from the dose-response curves. Samples with a two-fold increase (for the highest test concentration) compared to the average yield of spontaneous revertants (TA98: number of spontaneous revertants between 20 and 50) and with a concentration-related response, were regarded as a positive [12].

The Vitotox® test is a bacterial assay, similar to the SOS chromotest [13], based on the expression of repair genes induced by genotoxic agents. This bacterial SOS system is a DNA repair mechanism that reacts directly and immediately to DNA damage. The bacterial strain used for this assay is the TA104 RecN2-4 [14], which has a *lux* operon of the luminescent marine micro-organism *Vibrio fischeri* under transcriptional control of the recN gene, that is part of the SOS repair system. Under the influence of a genotoxic compound, the RecN promotor is depressed which results in expression of the lux operon and light production. Some chemicals directly interfere with the light emission system and stimulate light emission without any genotoxic potency (false positives). Other chemicals are bacteriotoxic and decrease light intensity because the micro-organisms are killed. In this case genotoxic effects might be masked (false negatives). To estimate these effects, the test substance is simultaneously tested on a constitutive bacterial strain, TA 104 pr1 [15]. This construct provides the organisms with a background light emission. Substances that interfere directly with light emission will be detected with this strain and the genotoxic response can be corrected for this direct interference.

Vitotox[®] test is performed in 96 multiwell plates, filled with the bacterial mix and dilutions of the test substance. Dilution series are prepared in the presence and absence of a microsomal enzymatic mixture S9 (see above). Luminescence is measured with an automated spectrophotometer. Results are analyzed with a standard software package (Microsoft-Excel), which calculates for each concentration:

the maximal signal to noise ratio for the *Rec* strain (=maximal luminescence of the treated cells, divided by the maximal luminescence of the untreated cells)

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- (2) the maxima signal to noise ratio for the *pr1* strain (=maximal luminescence of the treated cells, divided by the maximal luminescence of the untreated cells)
- (3) the ratio of (1) and (2), which is used to correct for toxicity and for direct induction of the *Lux* genes (max S/N(recN2-4)/ max S/N(pr1)).

Genotoxicity is assumed if calculation (1) and (3) are equal or larger than 1.5 and if there is a good concentration-effect relationship [15].

2.4. Statistics and correlation analyses

For data analyses of the environmental samples, non-parametric tests were selected because these do not require a normal distribution and a homogeneous variance. The non-parametric Wilcoxon Matched Pairs test was used for testing paired data, correlations were tested using the non-parametric Spearman test, (Statistica, Statsoft 2000) [16]. The mutagenic and genotoxic responses were related to the analyses of the 16 PAHs using partial least squares projections to latent structures (PLS) models Simca-P 9.0 software, (Umetrics, Umeå, Sweden) [17]. PLS models allow an investigation of correlations between numerous, often correlated input and process variables (X) and several result variables (Y). PLS analysis results in model coefficients for the variables, called weights. The weights for the X-variables indicate the importance of these variables in the modeling of Y. The R² of the model is a measure for the variance explained by the model. The resulting model can be used to predict the mutagenic or genotoxic responses based on measured chemical concentrations. [18,19]. A first model was developed with all 16 input variables (PAHs). Next, the known indirect-acting mutagens, i.e. 10 of 16 analyzed PAHs, were related with the indirect-acting activity (+S9).

Resulting models were evaluated with respect to their correlation coefficients (R^2) and prediction properties (Q^2) .

3. Results

3.1. Particulate matter

The amount of airborne particles (PM10) collected on the filters are given in Table 1. The amount per m³ ranged from 12 to 57 μ g/m³ with an average of 32.8 ± 3.6 μ g/m³. The average PM10 concentrations were not significantly different among the three sites. There was no significant difference between the PM10 concentrations in winter and in summer. Observed difference in the collected amounts are due to differences in the meteorological conditions (i.e. rain, wind).

3.2. Chemical analyses

The concentrations of the 16 PAHs in the organic extracts (QF + PUF) are shown in Table 2. PAHs concentrations in winter were significantly higher (except for ACE) than in summer (P=0.0006). Analysis of the PAH profiles showed that concentrations of the low molecular weight (up to pyrene, mw 202.26) were 5 to 10 times higher than the high molecular weight (from B[a]A, mw 228) PAHs. FL, PH, F and P were the most abundant low molecular weight PAHs. CH, B[b]F and I[cd]P were most abundant high molecular weight PAHs. The PAH concentrations varied between the different locations. For the winter samples, the mean (n=3) total concentration (Σ 16 PAHs) ranged from 66.5 ± 31.3 ng/m³ at the industrial site to 108.5 ± 34.7 ng/m³ in the urban location and 154.2 ± 101.8 ng/m³ to 77.6 ± 10.7 ng/m³ and 34.1 ± 18.2 ng/m³, respectively.

The concentrations of the 16 PAHs found in the filter extracts differed from those in the PUF extracts. The partitioning of the individual PAHs over the QF and PUF are shown in Fig.1. For the winter samples the 2-4 ring PAHs (from N to P) were mainly present in the PUF extract, whereas the heavier 4 and 5 ring PAHs (starting with B[a]A) were mainly retained in the filter extracts. In summer the low molecular weight PAHs were almost exclusively found in the PUF extracts, whereas the high molecular weight PAHs were found both on the QF and in the PUF extracts. In summer, concentrations of DB[a,h]A, B[ghi]P and I[cd]P were higher in the PUF extracts than in the QF extracts.

3.3. Mutagenic potency, Salmonella test

Mutagenicity assay results of the QF and PUF extracts are shown in Table 3. Results are expressed as specific mutagenicity, i.e. rev/ m^3 (± 95% confidence limits) obtained from the dose-response curves by regression analyses (Statistica, Statsoft 2000) and rev/ µg PM10 (QF samples only). Due to limited amount of test material only three test doses were used, therefore the resulting regression analyses (slope) could be influenced. The raw data (mean number of revertants per dose ± standard deviation) is available on the site: http://allserv.ugent.be/~vdufour.

The direct- (-S9) and the indirect-acting (+S9) mutagenic potency differed with sample type (QF or PUF) and season. Most filter (except Industrial summer 3 and Rural winter 3, without activation) extracts showed mutagenic activity, with and without metabolic activation. All PUF extracts exhibited indirect-acting activity and a few also showed direct-acting activity. In winter samples, the indirect-acting activity of the filters was significantly higher than the direct-acting activity (p = 0.018). No significant difference was observed

between their direct- and indirect-acting potency in the filter samples during summer. Addition of S9 to the PUF extracts resulted in higher potencies, both for samples taken during winter and summer (p = 0.002).

The mutagenic activity in winter and summer depended on the sample type; the QF samples had the highest activity in winter whereas the PUF samples had the highest activity in summer (p = 0.028). However, the total (QF+PUF) direct- and indirect-acting activity was significantly higher (p = 0.028) in winter than in summer. The mean total activity for the three sites, considering both direct- and indirect-acting activities, was 45.8 ± 24.4 rev/m³ during winter and 29.1 ± 19.2 rev/m³ in summer.

The average mutagenic potency of the ambient air at the three locations was very similar; only with respect to direct mutagenic activity in the summer period a higher activity of $39.6 \pm 3.7 \text{ rev/m}^3$ was measured in Borgerhout (urban) compared to $18.8 \pm 14 \text{ rev/m}3$ in Hoboken (industry) or $19.6 \pm 19.8 \text{ rev/m}^3$ in Peer (rural).

The highest total responses were found at the rural location with metabolic activation in winter (100.7 rev/m³) and summer (82.5 rev/m³). Two QF winter samples, taken at the industrial location, were toxic, since no bacterial growth was observed.

The mutagenic activity of the QF samples can also be expressed by the activity per μ g PM10 collected on the filters (Table 3). Similar trends as for the activity per meter cube were obtained. In winter indirect activity was higher than direct activity (p = 0.028), whereas in summer there was no significant difference. Activity in winter was higher than in summer (p = 0.019).

3.4. Genotoxic potency: Vitotox® assay

In the Vitotox assay, the samples were tested at four doses; 0.13 %, 0.25 %, 0.50 % and 1 % of the organic extracts or, respectively, 0.032, 0.065, 0.128 and 0.250 m³ of air sampled. The evaluation for genotoxicity (Lowest Observed Effect Concentration, LOEC) and toxicity (Effect Concentration, EC50) is given in Table 4. Based on the calculations, as explained in the Material and Method section, the genotoxic effects are presented as the lowest test dose giving a genotoxic result (LOEC) and the response (signal to noise ratio of the *Rec* and *Pr1* strain) per m³ air. The raw data is available on the site http://allserv.ugent.be/~vdufour.

All PUF samples without metabolic activation were toxic. Although addition of S9 resulted in detoxifications of these samples, only one sample (urban, winter 2) gave a genotoxic response. In contrast to the PUF extracts, the QF extracts gave genotoxic responses and less toxicity was observed, i.e. only three samples exhibited toxicity. The direct-acting activity in winter was significantly higher (p = 0.0006). Metabolic activation (+S9) significantly reduced the genotoxic responses in QF samples, i.e. only the three samples with the highest direct-acting response were still genotoxic after addition of S9.

Responses differed between winter and summer for the three locations. In winter, the highest activity was found at the rural site and lower at the urban and industrial site. During summer, the highest activity was found at the urban site and almost no activity was found at the two other sites.

3.5. Modelling mutagenicity/ genotoxicity

Correlation analysis using PLS modelling was used to relate the mutagenic and genotoxic activity with the chemical composition of the samples (Table 5). Models based on the PAH concentrations and the Ames test of the filter samples predicted the indirect-acting mutagenicity ($Q^2 = 0.702$) better than the direct-acting activity ($Q^2 = 0.488$). Relating the indirect mutagenicity with the 10 mutagenic PAHs improved the model ($Q^2 = 0.741$). The models correlating the mutagenicity and chemical analyses of the PUF samples predicted direct- ($Q^2 = 0.826$) and indirect-acting ($Q^2 = 0.544$) activity quite well. The correlations or predictions were not improved by relating the indirect mutagenicity with the 10 mutagenic PAHs only.

For the total (QF+PUF) indirect-acting mutagenic activity of 18 samples and the corresponding PAH (16) concentrations a good model was obtained ($Q^2=0.74$). The observed versus predicted total mutagenic response (rev/m³) obtained by this model is shown in Fig 2. Using only 10 PAH the correlation and the prediction capability was significantly reduced. A similar model for the total direct-acting activity was less powerful ($Q^2=0.454$).

A good model ($Q^2=0.898$) was developed for effects measured in the Vitotox® test based on the direct-acting activity of QF samples. Other models based on the indirect-acting genotoxicity data were not relevant because almost no effects were observed with activation (+S9) or the samples showed severe toxic effects.

4. Discussion

This study reports genotoxic and mutagenic activity of ambient air in Flanders in combination with chemical analyses. The study was performed to evaluate the need to include biological testing in the Flemish environmental survey network and to assess health effects of environmental pollutants in an integrated way.

The complex mixtures of organic compounds to which we are exposed through air pollution are difficult to identify and to quantify. Therefore, chemical analyses in this study were limited to 16 PAHs, most of them are biologically active [20-23] or even carcinogenic [6]. The seasonal and spatial pattern of these 16 PAHs was investigated. In winter, the individual PAH concentrations were higher (except for Ace) than in summer. Similar seasonal trends were also observed in previous studies [24-26]. Higher PAH concentrations in winter are due to the contribution of domestic heating and to the specific meteorological conditions, i.e. sunlight intensities, temperatures and ozone concentration. These parameters under summer conditions increase volatilisation, the rates of chemical degradation and atmospheric dispersion [26]. Resulting in an artificial decrease of the measured concentrations at higher ambient temperatures. The total (Σ 16) PAHs concentrations were in the range 43.1-268.7 ng/m³ in winter and 20.9-88.9 ng/m³ in summer. These values correspond to values reported for polluted urban areas throughout the world [27-29].

Also the PAH profile was in agreement with those of other studies [26,28,30] i.e. lower molecular weight PAHs were the most abundant. The highest concentrations were measured for phenanthrene with levels up to 66.8 ng/m³, comparable to those previously reported (60 ng/m³) by Muller [28]. The levels of the heavier PAHs (5-6 rings) were 10 to 100 times lower, however these are because of their mutagenic and genotoxic potency of higher concern.

In many studies B[a]p has been used as indicator compound for PAH exposure because of its strong carcinogenicity and close correlation with other PAHs [31,24]. With average concentrations of 1.4 ng/m³ (0.1-5.3) in winter and 0.1 ng/m³ (0.02-0.1) in summer, B(a)p levels in our study were among the lowest values reported in Europe. In Naples for instance, average concentrations were 2.67 ng/m³ (0.09-12.18) during winter and 1.09 ng/m³ (0.03-4.02) in summer [24], and concentrations comparable to those were found in Prague (Czech) [32] Rome (Italy) [24], Thessaloniki (Grece) [27] and Teplice (Czech) [32].

The total mutagenicity (QF+PUF) was higher in winter than summer but the difference between both seasons was not as pronounced as in other studies. For example Cerna [33] reported activities between 2 and 107 rev/m³ in winter and between 2 and 23 rev/m³ for samples taken in summer. Comparable values were reported by Nardini [34]; 19 - 82 rev/m³ in winter and between 3 - 39 rev/m³ in summer. Others like Binkova [35] and Zhoa [36] described lower activies but still with a considerable difference between winter and summer, activities ranged between 13-25 rev/m³ in winter and 4-8 rev/m³ in summer and between 7-24 rev/m³ in winter and 2-8 rev/m³ in summer, respectively. In our study activities in winter were at the same level (4-100 rev/m³) but the activities measured during summer were 2 to 4 times higher then those reported elsewhere. This might be explained by the use of PUF cartridges next to the QF filters in our sampling campaign. Most papers reporting mutagenic activity of ambient air only used filters [33-40]. For the summer samples, 43% of the total activity was found in de PUF extracts whereas only 10% was found in winter. This was confirmed by PAH concentrations in the different samples: in winter samples the higher molecular weight PAHs were almost exclusively found in the filter extracts. Conversely, in summer PUF extracts contained substantial amounts of these higher molecular weight PAHs, indicating that the higher total activities during summer are probably caused by these mutagens.

Indirect-acting mutagenicity is usually attributed to PAHs [20], since these compounds need metabolic activation in order to exert effect. Next to the indirect-acting compounds other chemicals like nitro-PAHs and oxy-PAHs have direct mutagenic effect and do not need any metabolic activation [38]. In the air samples both type of mutagens were present: most extracts (mainly QF) showed direct and indirect mutagenicity.

Seasonal differences in mutagenic potency showed that the chemical composition or at least the ratio between the mutagens with direct or indirect activities are not identical in winter and summer. For the samples taken in winter, the indirect-acting mutagenicity was significantly higher than the direct-acting activity, suggesting a considerable contribution of the PAHs. Indeed total level of the 10 carcinogenic PAHs as well as that of the individual PAHs, were 2 to 7 times higher in winter than in summer. As discussed above, substantial activities were also measured in summer and the mutagenicity levels were on average 60% of the levels observed during winter. The mutagenicity level in summer did not increase with metabolic activation, indicating that during summer the direct-acting mutagens contribute more to the total activity. It can be assumed that the levels of the nitroderivatives are higher in summer due to increase atmospheric reactions of PAHs with nitrogen oxides [33, 39].

Fl, P, B[a]A, CH, B[b]F, B[k]F, B[a]P, DB[a,h]A, B[ghi]P and I[cd]P all showed specific activity (rev/ μ g) in the Ames test [20-23]. The sum of the concentrations of these 10 indirect-acting PAHs correlated quite well with the indirect-acting (+S9) total mutagenic activity (r=0.73, p=0.0004). As discussed above, the levels of benzo(a)pyrene measured in our study were rather low. However, the mutagenic activity in Flanders was higher than expected based on the benzo(a)pyrene concentrations. The mutagenic activity (+S9) of air samples taken in a busy streets in Copenhagen was on average 13.4 revertants per ng B[a]P for filter samples [40]. The values in our study were much higher with an average of 62.7 revertants \pm 38.8 per

ng B[a]P. In summer, the activity per ng B[a]p was much higher, 899 revertants \pm 932 per ng B[a]p, a value rendered less precise by the low B[a]p concentrations.

The air samples from the industrial and urban sites were expected to contain mutagenic compounds with higher potency and/or in higher quantities than those from the rural sites. However, the highest activity, both for QF and PUF samples, and the highest PAH concentrations were found in samples taken at the rural location, selected as a background site. This indicates that pollution is widespread in Flanders and that in developed Western nations exposure to pollution may be quite homogeneous. The presence of a military airport located a 4 miles from the sample site might have contributed to higher than expected PAH concentrations and mutagenic potency. This assumption is corroborated by the observation that the highest activity was found in samples taken when there was a east north east wind, which is the direction from the airport to the sampler. A similar situation was described by Zhoa [36], where high activity measured at a control site was explained by the presence of an international airport.

Two QF samples taken at the industrial location showed severe toxic effects in both the Ames test and the Vitotox assay. Addition of S9 reduced the toxic effect but the resulting mutagenic response was masked by lower bacterial growth. This toxic effect cannot be explained by the chemicals analyses as no increased PAHs concentration were measured in these two samples. Probably other compounds, such as heavy metals, originating from nearby metallurgic industry, caused this high toxicity.

There was no significant correlation between the results (QF samples) of the Vitotox® assay and the Ames test, although samples exhibiting a indirect-acting genotoxic activity also had a high mutagenic response (>50 rev/m³). The strong decline of the observed genotoxic potency in the presence of S9 suggests that the Vitotox® assay is mainly sensitive for direct-

acting compounds, of which the reductive activation pathway can be disturbed by the oxidation enzymes in S9.

The Vitotox results of the PUF samples without metabolic activation showed toxic effects for all samples, masking any possible genotoxic response. Toxicity was evaluated by the use of the bacterial strain TA 104 pr1 [15] which provide a background light emission. All PUF samples (-S9) showed a reduction of the cell activity resulting in a strong decline of this background emission with increasing test dose. For the highest test doses (0.5 and 1 %) with most samples the cell activity dropped too low to detect any genotoxic effect. At the lowest dose (0.13%) genotoxicy was detectable as little or no toxicity was found, however no genotoxic signals were observed, making further dilutions of no use.

With the PLS analyses we investigated possible correlations between the concentrations of 16 PAHs and the measured activities. The resulting models indicate which PAHs have an important contribution to the observed mutagenicity/ genotoxicity. The model based on the direct-acting mutagenicity of the filter samples showed a similar contribution for all 16 PAHs. The significant correlations with and predictions of direct-activity based on the concentrations of indirect-acting PAHs indicates that these PAH concentrations are correlated with indirect-acting mutagens like oxy- and nitro-PAHs. Contrary to this, the model using the indirect-acting mutagenicity data showed that FL, P, B[a]A, CH, B[b]F, B[k]F, B[a]P, DB[a,h]A, B[ghi]P and I[cd]P were the most important variables. These 10 PAHs are known indirect-acting mutagens, only asserting their effects in the presences of exogenous metabolic activation enzymes. The second PLS model, based on these 10 PAH, was developed and gave better correlations and predictions with the indirect mutagenicity results.

In conclusion, our work suggests that the Ames test is able to measure mutagenic activity of QF and PUF extracts and is suitable to use in a monitoring network. For both sample types, reproducible direct- and indirect-acting effects were found. With the Vitotox® assay, severe problems with toxicity of the PUF extracts were encountered and genotoxic activity could only be detected in the presence of metabolic activation.

Compared to data from other regions the mutagenicity levels in Flanders were high, especially in summer. That our measurements during summer resulted above those reported most studies can be explained by the use of PUF cartridges next to QF filters. In summer, substantial amounts of higher molecular weight PAHs were found on the PUF cartridges, indicating that they were not sufficiently retained on the QF filters. As such, the use of a high-volume sampler for evaluating the mutagenic potency of ambient air in combination with only QF filters underestimates the mutagenic effects, especially during summer conditions.

The PAHs analysed in this study do not have any direct-acting activity and the 10 known mutagen PAHs were only responsible for a small part (1-2%) of the observed indirect activity: the B[a]P concentrations for example were very low compared with the measured activities. Using PLS analysis making predictions of the observed activities based on the chemical analyses was possible. Further improvement of these models require more samples with different compositions [18]. Chemical analyses provide essential information, but in order to come to an integrated assessment health effects of environmental pollution, bioassays need to be included. In an environmental monitoring network chemical analyses are restricted (practical and technical) to a limited number of compounds, only indicating the level of pollution. Bioassay allow us to assess the potential hazard of this complex environmental samples.

In this first study to measure the mutagenic/genotoxic potency of ambient air in Flanders three different types of sites were selected. Results showed significant activities at all sites, with unexpected high values at the rural location, indicating that human exposure outside urban agglomeration are still significant.

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Figure Legends

Figure 1

Relative concentration of the 16 analyzed PAHs on the quartz filter (QF) and polyurethane foam (PUF) cartridge in winter and summer. PAHs; N: naphthalene, ACY: acenaphtylene, ACE: acenaphtene, FL: fluorene, PH: phenantrene, AN: anthracene, F: fluoranthene, P: pyrene, B[a]p: benzo(a)anthracene, CH: chrysene, B[b]F: benzo(b)fluoranthene, B[k]f: benzo(k)fluoranthene, B[a]p: benzo(a)pyrene, DB[ah]A: dibenzo(a,h)anthracene, B[ghi]P: benzo(ghi)perylene and I[cd]P: indeno(1,2,3-cd)pyrene.

Figure 2

The predictive capacity of the PLS model indicating observed versus the predicted total (sum activity measured in QF and PUF sample) indirect-acting mutagenicity.





Figure 1



Figure 2

Tabel 1

Location	Season ^a	Total (mg)	Conc (µg/m3)
Urban	W 1	48.0	25.4
	W 2	57.8	45.3
	W 3	42.1	32.9
	S 1	52.4	41.6
	S 2	45.8	36.1
	S 3	39.5	31.4
Industrial	W 1	58.5	45.7
	W 2	44.5	34.2
	W 3	34.7	27.1
	S 1	48.3	38.3
	S 2	44.2	34.9
	S 3	15.9	12.1
Rural	W 1	39.6	30.3
	W 2	75	56.9
	W 3	18.3	14.3
	S 1	62.0	49.4
	S 2	24.7	19.4
	S 3	20.0	15.7

Amount of particle matter (PM10) collected on filters

^a Each locations was sampled 3 times in W: winter and S: summer.

Table 2

PAHs	Mean. ^b $(ng/m^3) \pm S.D.$									
	Url	oan	Indu	istrial	Rural					
	winter	summer	winter	summer	winter	summer				
Napthalene	2.96 ±2.94	4.32 ±1.51	0.55 ±0.31	1.66 ±0.94	6.52 ±10.53	1.34 ± 1.02				
Acenapthylene	5.64 ±4.88	0.58 ± 0.48	2.88 ±3.09	0.23 ±0.20	23.7 ±31.5	0.17 ±0.16				
Acenaphtene	0.39 ±0.6	0.59 ±0.17	0.04 ±0.03	0.39 ±0.33	0.15 ±0.09	1.17 ±1.67				
Fluorene	11.5 ±3.41	6.36 ±2.01	8.78 ±5.17	3.57 ±2.05	16.1 ±11.64	3.33 ±1.71				
Phenantrene	49.4 ±16.05	51.9 ±6.68	27.8 ±8.95	31.3 ±21.25	44.8 ±19.03	21.1 ±9.41				
Anthracene	6.22 ±2.32	1.31 ±0.08	2.5 ±1.67	0.4 ±0.19	6.05 ±3.22	0.29 ± 0.1				
Fluoranthene	9.76 ±2.45	5.85 ±3.11	7.56 ±2.81	2.81 ±1.43	17.9 ±8.8	3.38 ±1.89				
Pyrene	13 ±4.38	3.11 ±0.66	6.8 ±2.06	1.64 ±0.16	11.8 ±5.58	1.4 ±0.64				
Benz(a)anthracene	0.41 ±0.05	0.19 ±0.14	0.37 ±0.54	0.59 ±0.9	1.76 ±1.57	0.13 ±0.14				
Chrysene	1.84 ±0.5	0.41 ±0.2	1.6 ±1.65	0.53 ±0.46	5.39 ±4.38	0.15 ±0.10				
Benzo(b)fluoranthene	1.46 ±0.45	0.3 ±0.14	1.58 ±1.61	0.15 ±0.08	4.04 ±2.61	0.21 ±0.18				
Benzo(k)fluoranthene	0.84 ±0.29	0.15 ±0.07	0.8 ±0.65	0.05 ±0.04	1.9 ±1.29	0.07 ± 0.04				
Benzo(a)pyrene	0.82 ±0.31	0.07 ± 0.01	0.76 ±1.04	0.03 ±0.02	2.53 ±2.41	0.03 ±0.02				
Dibenz(a,h)anthracene	0.43 ±0.18	0.42 ±0.26	0.6 ±0.7	0.54 ±0.47	0.88 ±0.38	0.53 ±.79				

0.72 ±0.24 1.76 ±1.76

 0.42 ± 0.25

Concentrations (mean ± standard deviation) of PAHs in the organic extracts (QF+PUF)^a

^a Sample types: QF: quartz filter, PUF: polyurethane foam

Indeno(1,2,3-cd)pyrene 1.62 ± 0.54

Benzo(ghi)perylene 2.18 ±0.77 1.3 ±0.26 1.82 ±1.34 0.72 ±0.5

^b Values are the mean \pm standard deviation of 3 samples.

3.63 ±2.64 0.48 ±0.56

 0.33 ± 0.34

4.41 ±3.3

Table 3

Results Salmonella test, mutagenicity (response \pm 95% confidence limits) of QF and PUF samples with (+S9) and without (-S9) metabolic activation^a

Location	Season ^b	Mutagenic activity ^c									
		QF (re	ev/m³)	QF (rev/µ	ıg PM10)	PUF (rev/m ³)					
		-89	+89	-89	+S9	-89	+S9				
Urban	w 1	44.4 ± 10.5	52.6 ± 3.5	1.75 ± 0.41	2.07 ± 0.14	-	4.3 ± 0.9				
	w 2	36 ± 3.4	$50.8\pm~3.2$	0.79 ± 0.07	1.12 ± 0.07	-	23.3 ± 1.7				
	w 3	38.2 ± 2.8	49 ± 9.1	1.16 ± 0.08	1.43 ± 0.28	-	$7.7\pm\ 0.9$				
	s 1	35.3 ± 2.3	17 ± 2.9	0.85 ± 0.05	0.41 ± 0.07	8.6 ± 1.0	19.1 ± 2.4				
	s 2	23.8 ± 1.6	14.7 ± 1.3	0.66 ± 0.04	0.41 ± 0.04	13.6 ± 0.9	17.5 ± 2.5				
	s 3	21.3 ± 3.1	25.3 ± 2.2	0.68 ± 0.1	0.80 ± 0.07	16.3 ± 1.1	12 ± 1.6				
Industrial	w 1	х	7.6 ± 1.7	Х	0.17 ± 0.04	-	4.7 ± 0.5				
	w 2	36.6 ± 2.8	54.1 ± 5.5	1.07 ± 0.08	1.58±0.16	-	5.5 ± 0.9				
	w 3	x	25 ± 3.1	х	0.92 ± 0.11	-	3.1 ± 0.3				
	s 1	13.7 ± 0.7	8.9 ± 1.3	0.36 ± 0.02	0.23 ± 0.03	7.4 ± 0.5	12.4 ± 2.1				
	s 2	15.1 ± 0.8	14.2 ± 1.6	0.43 ± 0.02	0.41 ± 0.04	16.6 ± 1.5	32.3 ± 1.8				
	s 3	-	4.7 ± 0.8	-	0.39 ± 0.07	-	4.9 ± 0.5				
Rural	w 1	42.3 ± 2.9	55.2 ± 6.2	1.4 ± 0.1	1.82 ± 0.2	-	3.7 ± 0.3				
	w 2	58.2 ± 4.2	96.6±11.3	1.02 ± 0.07	1.7 ± 0.2	-	4.1 ± 0.5				
	w 3	-	10.9 ± 0.7	nd	nd	-	3.5 ± 0.5				
	s 1	22.7 ± 2	21.2 ± 1.3	0.46 ± 0.04	0.43 ± 0.3	19.8 ± 2.7	60.3 ± 7				
	s 2	6.6 ± 0.5	9.1 ± 0.9	0.34 ± 0.03	0.47 ± 0.05	-	3.7 ± 0.6				
	s 3	5.8 ± 0.9	5.2 ± 0.8	0.37 ± 0.06	0.33 ± 0.05	-	6.5 ± 0.7				

^a Sample type; QF: quartz filter, PUF: polyurethane foam.

^b Each locations was sampled 3 times in W: winter and S: summer.

^c (-): no mutagenic respons, (x): toxic, no bacterial growth, (nd): not determined.

Tabel 4

Location	Season ^b			QF ^a					PUF ^a		
		LO	EC	(rec/pr1) per m ³	EC50	LO	DEC	(rec/pr1) per m ³	EC50
		-S9	+S9	-S9	+S9		-S9	+S9	-S9	+S9	
Urban	W 1	0.25%	1%	28.39	4.60	0.3	Х	-	Х	-	0.1%
	W 2	0.5%	+	22.12	6.10	0.12%	х	1%	х	7.4	0.13%
	W 3	0.5%	-	21.62	-	0.18%	х	-	х	-	0.07%
	S 1	0.5%	-	19.65	-	0.21%	х	-	х	-	0.21%
	S 2	+	-	7.53	-	0.37%	х	-	х	-	0.11%
	S 3	1%	-	9.00	-	0.28%	х	-	Х	-	0.31%
Industrial	W 1	х	-	х	-	0.07%	х	-	Х	-	0.10%
	W 2	0.25%	1%	29.35	6.80	0.20%	х	-	х	-	0.12%
	W 3	х	-	х	-	0.07%	х	-	х	-	0.12%
	S 1	+	-	11.19	-	0.24%	х	-	Х	-	0.09%
	S 2	-	-	-	-	0.21%	х	-	х	-	0.26%
	S 3	х	-	х	-	0.16%	х	-	Х	-	nd
Rural	W 1	+	-	9.37	-	0.35%	х	-	Х	-	0.24%
	W 2	0.13%	0.50%	69.60	13.30	0.17%	х	-	Х	-	0.19%
	W 3	0.25%	-	31.05	-	0.40%	х	-	х	-	0.40%
	S 1	0.10%	-	7.52	-	0.78%	х	-	х	-	0.45%
	S 2	-	-	-	-	0.91%	х	-	х	-	0.47%
	S 3	-	-	-	-	0.72%	X	-	х	-	0.64%

Results Vitotox® assay, genotoxicity of QF and PUF samples with (+S9) and without (-S9) metabolic activation^c

^a Sample type; QF: quartz filter, PUF: polyurethane foam.

^b Each locations was sampled 3 times in W: winter and S: summer.

^c LOEC: lowest observed effect concentration, EC50: concentration where cell activity has dropped to 50%,(S/R=0.5), (+): probably genotoxic (Rec strain >1 while the Pr strain decreases), (-): no genotoxic effect, (nd) : not determined, (x): toxic, no bacterial growth.

Table 5

	No. of		
Result variable Y	X var.	r ²	Q ²
Ames, +S9, QF	16	0.757	0.702
Ames, +S9, QF	10	0.754	0.741
Ames, -S9, QF	16	0.527	0.488
Ames, +S9, PUF	16	0.894	0.544
Ames, +S9, PUF	10	0.613	0.407
Ames, -S9, PUF	16	0.896	0.854
Vito, -S9, QF	16	0.898	0.84
Ames, +S9, QF+PUF	16	0.84	0.74
Ames, +S9, QF+PUF	10	0.499	0.459
Ames, -S9, QF+PUF	16	0.724	0.454

Summary of the PLS models developed, relating PAHs concentrations and mutagenic/genotoxic responses

Y: mutagenic/genotoxic responses, X : PAH concentrations,

r²: correlation, Q² variance.