

Development and Validation of a New Bitumen Fume Generation System which Generates Polycyclic Aromatic Hydrocarbon Concentrations Proportional to Fume Concentrations

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Bitumen fumes emitted during road paving and roofing contain polycyclic aromatic compounds (PACs) of potential health concern. Little information is available for an experimental device devoted to inhalation experiments with animals exposed to bitumen fumes, and in all studies the systems were never validated for a range of fume concentrations, which prohibited their use for toxicological concentration–effect studies. Therefore, the purpose of this study was to validate a new experimental device able to generate bitumen fumes at different total particulate matter (TPM) concentrations with a linear correlation between TPM and the concentrations of different PACs, thus allowing toxicological dose–response studies with fumes representative of those in the field. Atmosphere samples collected from an animal exposure chamber allowed the determination of TPM, toluene soluble matter, polycyclic aromatic hydrocarbons (PAHs) and semi-volatiles. The particulate size distributions were determined in order to assess the deposition pattern in the respiratory tract. The temperature of 170°C was chosen by analogy with the upper range of the temperature used during paving operations. The temperature of the air passing over the fume emission area was regulated to 20°C and stirring of the heated bitumen was restricted to 90 r.p.m. The data show that the objective of developing a static fume generation system that reproducibly produces fumes in the inhalation chamber for specified target concentrations (TPM) were successful. The within-day variation coefficients for TPM were between 2.5 and 6.1%. The day-to-day variations for TPM concentration were between 4.1 and 5.8%. The concentrations of the 4–5 ring PAHs and the polycyclic aromatic sulphur heterocycles were proportional to the TPM concentration. The 2 and 3 ring PAH concentrations showed a deviation from proportionality with the TPM, probably due to their re-evaporation during sampling. The mass median aerodynamic diameter of airborne particles varied from 1.4 µm at a fume concentration of 5 mg/m³ to 3.2 µm at 100 mg/m³. In conclusion, this equipment was suitable for nose-only inhalation studies in the 5–100 mg/m³ range of TPM. Bitumen fumes were generated with a good reproducibility under well-controlled conditions. Finally, the PAH profiles from atmospheric samples were in good agreement with those measured during road paving.

Keywords: bitumen fumes; inhalation equipment; polycyclic aromatic hydrocarbons/analysis

INTRODUCTION

Bitumen fumes emitted during road paving and roofing contain polycyclic aromatic hydrocarbons (PAHs). The two principal routes of worker exposure

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to bitumen fumes are skin deposition and inhalation. Although levels of exposure of workers to these fumes are generally low, they are of potential concern, because laboratory generated bitumen fume condensates have been found to be carcinogenic in mouse skin painting tests (Thayer *et al.*, 1981; Sivak *et al.*, 1989). More recently, DNA adducts in skin, lung and lymphocytes were found in rats after skin painting with undiluted bitumen fume condensates (Genevois *et al.*, 1996). PAHs [for example, benzo[*a*]pyrene (B[*a*]P) or 3-methylcholanthrene] generally administered to animals by the oral or intraperitoneal routes were shown to have toxic effects on reproductive function (MacKenzie and Angevine, 1981), the cardiovascular system (Penn *et al.*, 1981; Paigen *et al.*, 1985), the bone marrow (Legraverend *et al.*, 1983), the immune system (Hardin *et al.*, 1992) and the liver (Armstrong and Bonser, 1950); for a review see IARC (1983).

Little information is available for an experimental device devoted to inhalation experiments with animals exposed to bitumen fumes. Two inhalation studies have been carried out to evaluate the toxic effects of bitumen fumes on the lungs (Hueper and Payne, 1960; Simmers, 1964). However, the fumes were poorly characterized in terms of composition and never in terms of particle size. A recent study described a new inhalation system comprising a generation system and an inhalation chamber (Bonnet *et al.*, 2000; Brandt *et al.*, 2000) validated for fumes at 5 mg/m³. However, in all these studies the systems were never validated for a range of fume concentrations, which prohibited their use for toxicological concentration–effect studies. Recently, the National Toxicology Program (NTP, 1999) recognized the lack of concentration–response and time course data and the necessity of developing a bitumen fume generator for inhalation studies. A laboratory bitumen fume generator described by Brandt and De Groot (1999) produces bitumen fumes that are representative of those workers in the field are exposed to. However, because it produces only milligram amounts of bitumen fume condensates it is not suitable to be connected to an animal exposure chamber.

In personal exposure studies in paving or indoor mastic laying, the PAH concentrations measured in the condensed bitumen fumes were found to be independent of the total fume exposure in terms of benzene soluble matter (BSM) or total particulate matter (TPM) (see for example Brandt *et al.*, 1993, for exposures at the 1 mg/m³ TPM level; Brandt *et al.*, 2000, for exposures in the range 6–25 mg/m³). This means that the concentration of individual PAHs linearly relates to the particulate exposure measured as BSM.

The purpose of this study was to validate an experimental device able to generate different

bitumen fume concentrations with a linear correlation between PAH concentration and TPM values, thus allowing toxicological dose–response studies with fumes representative of those in the field. This equipment was tested by collecting atmospheric samples from the animal exposure chamber using a glassfibre filter followed by a XAD-2 resin. The glassfibre filter allowed the measurement of the aerosol phase, including the determinations of TPM, BSM as toluene soluble matter and polycyclic aromatic compounds (PACs). The XAD-2 resin allowed measurement of the gas phase, including the determination of semi-volatiles (2 ring, most of the 3 ring and some of the 4 ring PACs) which were not retained on the glass filter. The particulate size distributions were determined according to the method of Horton *et al.* (1992), in order to assess the deposition pattern in the respiratory tract.

MATERIALS AND METHODS

Product

A 1200 kg batch of bitumen (50/70 pen, Venezuelan origin) was used for this experiment.

Bitumen fume generator

All the information concerning the shape and dimensions of the bitumen fume generator is given in Fig. 1. The fume generator comprised a 10 l bitumen vessel and a fume chamber. The bitumen vessel was heated to the desired temperature (170°C) by an electric hot plate. For temperature control, two calibrated probes were arrayed inside the bitumen vessel, one for temperature regulation and one for actual temperature measurement. The bottom of the fume chamber was perforated at the dimensions of the bitumen vessel diameter. When bitumen fumes were generated the upper rim of the vessel was at the same level as the bottom of the fume chamber. A detail of the adjustment of the bitumen vessel in the fume chamber is given in Fig. 2. Each desired concentration in the exposure chamber was obtained by choosing an aluminium tube with a different inside diameter (5.0, 17.0 and 23.3 cm), corresponding to evaporation areas of 19.6, 227 and 426 cm². This defines the area from which the fume is emitted (emission area). As soon as the temperature reached ~170°C an electric stirrer was started in order to obtain a uniform mixture within the bitumen vessel during the generation period; the stirrer was regulated at a constant rate (90 r.p.m.). A stainless steel stirring rod passed through the top of the chamber via a gas tight seal. The rod extremity, submerged in the hot bitumen, was equipped with a thin stainless steel blade (240 × 60 mm) perforated with 12 holes (each 14 mm diameter). After starting the stirrer, the compressed air (filtered through an Ultrafilter GmbH filter, model PEG.0006; Ultrafilter Int., Germany)

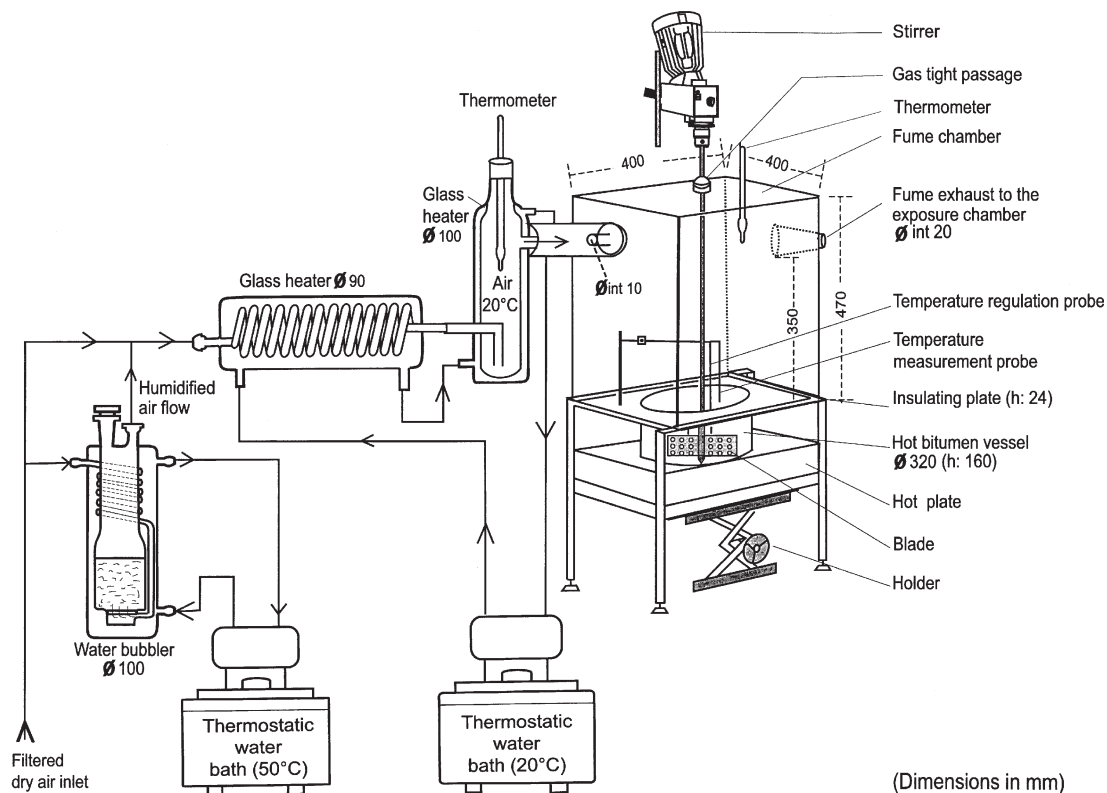


Fig. 1. Schematic representation of the bitumen fume generator.

was admitted into the fume chamber at a flow rate of 23 l/min: 20 l dry air and 3 l water-saturated air in order to obtain an ~40% relative humidity in the animal exposure chamber. Before entering the fume chamber this air was regulated to 20°C by passing through two glass heaters connected to a cryothermostatic water bath. This filtered and conditioned air flowed horizontally through the fume chamber at a distance of 350 mm from the bitumen surface. Bitumen emissions were then introduced into the inhalation chamber (a thermometer also measured the temperature in the fume chamber).

Inhalation chambers

Two identical inhalation chambers were used for this experiment (Bonnet *et al.*, 2000). The first, connected to the bitumen fume chamber, was placed inside a hood for safety reasons. The second was placed outside the hood and was devoted to exposure of control animals to filtered air. During the exposure, the temperature of the chambers was $25 \pm 2^\circ\text{C}$ and the relative humidity was $40 \pm 5\%$. In order to prevent leakage of test fumes, a negative pressure of ~6 mm water was maintained in the exposure chamber by extracting air with a fan at a constant speed. Each chamber, made of stainless steel with a glass door, had an internal volume of 37 l. The 23 l/min air flow containing the bitumen fumes was

introduced at the top of the chamber and exhausted at the bottom. The air exhaust was linked to a series of cleaning devices before being released into the main exhaust pipe. The door included several ports for atmosphere sampling and for connecting the plethysmographs used for nose-only exposures (rats or mice).

Sampling and analysis of the atmosphere in the inhalation chamber

In order to validate the equipment, atmospheric samples were collected in the breathing zone of animals. Analyses were performed by two laboratories: INRS (Vandoeuvre, France) and IST (Institut Universitaire Romand de Santé au Travail, Lausanne, Switzerland). Filter analysis included quantitative determination of TPM, soluble matter (BSM), B[a]P and other PACs (IST only). Analyses of the XAD-2 resins included quantitative determination of semi-volatile and volatile PACs.

Particulates were collected by drawing air through a 37 mm pre-weighed glass filter (Whatmann) at a rate of 1 l/min. Each filter was fitted in a standard 37 mm closed-face cassette (Millipore). The vapour and semi-volatile phases were collected on a XAD-2 adsorption tube placed in series with the filter cassette. Sampling volumes depended on TPM concentrations.

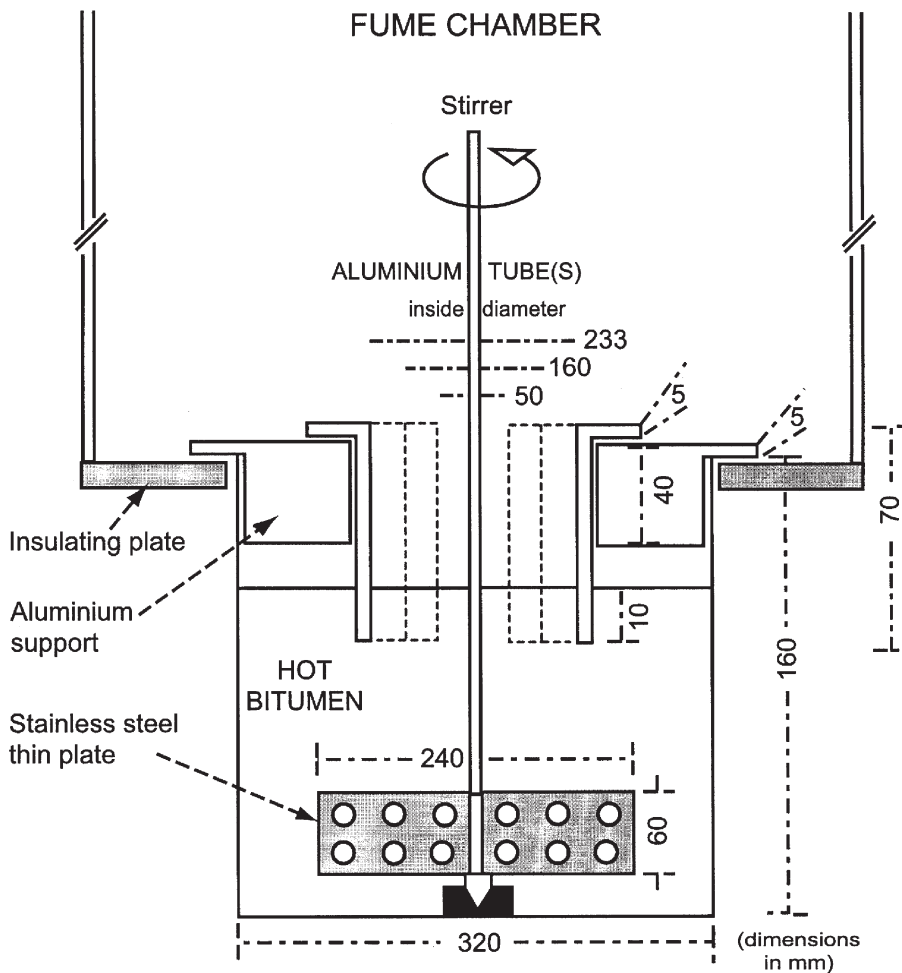


Fig. 2. Details of the adjustment of the bitumen vessel in the fume chamber.

Stability of the emission over time

In the exposure chamber the stability of the emission over time was followed by analysis of TPM and B[a]P and by monitoring the variations in aerosol mass concentration with a HundTM photometer.

The TPM measurements were performed by weighing filters on a 10 µg accuracy electronic balance (Sartorius MC 210S; Goettingen, Germany) before and immediately after sampling, as previously described (Bonnet *et al.*, 2000). This fast weighing allowed a regular control of stability during the daily 6 h generation period.

The B[a]P analysis by INRS was performed using the working principle described in Simon *et al.* (2000), but with some modifications. The filter was extracted twice with 4 ml of methanol for 15 min in an ultrasonic bath. Then the extract was directly injected into a liquid chromatographic switching system equipped with three pre-columns (two diol Nucleosil 5 µm, one polyNO₂ Polygosil 5 µm; Macherey Nagel) and one analytical Si-C₁₈ column

(Chromasil; Ekanobel). Detection was carried out with a Shimadzu RF 551 fluorimeter set to 365 nm for excitation and 420 nm for emission.

A HundTM data photometer (Helmut Hund GmbH, Wetzlar, Germany) monitored the aerosol concentration in the exposure chamber by measuring the light scattered by airborne particles inside the sensing volume of the instrument (Görner *et al.*, 1995). It was also used to determine the optimal time to start the inhalation exposure.

Determination of the particle size distribution

The particle size distribution was investigated according to Fabries *et al.* (2000). Briefly, two instruments, positioned near the inhalation chamber, were used: a quartz crystal microbalance (QCM) impactor (model 3000; California Measurements, Sierra Madre, CA) and an Aerosizer[®] particle size analyser (Amherst Process Instruments Inc., Hadley, MA). The QCM impactor was a 10 stage cascade impactor that used coated piezoelectric quartz crystal col-

lection substrates. The sampling time was limited to 10 s at 5 mg/m³ and 5 s only at high aerosol concentrations (50 and 100 mg/m³). The sampling time for each measurement sequence was fixed to 2 min with the Aerosizer.

Analysis of PAHs and sulphur heterocycles (PASHs)

PAH profiles and PASHs in raw bitumen and in bitumen fumes were determined by IST with GC-MS further to a multistep sample treatment and automatic clean-up procedure, essentially based on the method described by Vu Duc *et al.* (1995). Briefly, the internal standard, indeno[1,2-*c,d*]fluoranthene, was added to 0.1–0.3 g raw bitumen or to bitumen fume filter samples obtained by extracting the filter by sonication twice with 25 ml of toluene for 10 min. The evaporated extract was submitted to a liquid–liquid partition between cyclohexane and dimethyl formamide:H₂O according to the procedure of Grimmer (1972) and Grimmer *et al.* (1983), before elution of the cyclohexane extract on a SiO₂ micro-column. The eluent was filtered on 0.45 µm Acrodisc filters, evaporated to ~200 µl and fractionated by HPLC. The clean-up phase was performed as an automatic process. The PAHs fraction of interest, which also contained PASHs, was separated on a capillary column and analysed by GC ion trap MS using the single ion storage mode for optimized sensitivity. For raw bitumen, a double liquid–liquid partition was found necessary to reduce the aliphatic hydrocarbon background to achieve the sensitivity required for some PAHs.

The identification of PAHs and PASHs was made by comparison of the spectrum of the unknown peak with that of a standard compound at the same retention time. Quantification was made relative to the respective calibration curves between compounds of interest and the internal standard. The performance of the method has been assessed in inter-laboratory comparisons and certifications of reference materials (European Commission, 1994).

Quality assurance was according to procedures described in standard EN 45000, for which the laboratory is accredited.

The semi-volatiles PAHs (SV-PAHs) were determined after separate desorption of the two distinct parts of the XAD-2 with 4 ml of CS₂ by sonication for 30 min. One microlitre of the combined filtered solution was adjusted to 5 ml and then injected into a GC column and quantified by ion trap MS. Standard reference compounds were used for comparisons of respective spectra.

RESULTS

Operating parameters of the fume generator and resultant TPM concentrations in the exposure chamber

Emission areas used to obtain different TPM concentrations in the exposure chamber and the temperatures measured in the different components of the exposure system are given in Table 1. Three emission areas were studied: 19.6, 227 and 426 cm². When using 19.6 cm², the volume sampled was 180 l and the TPM concentration varied between 5.45 and 6.6 mg/m³. When using 227 cm², the volume sampled was 80 l and the TPM concentration varied from 50.4 to 56.6 mg/m³. When using 426 cm², the volume sampled was 40 l and the TPM concentration varied from 88.8 to 106.9 mg/m³. The within-day variation coefficients for TPM were between 2.5 and 6.1% and the day-to-day variations for TPM concentration were between 4.1 and 5.8% (from raw data, not shown). The temperatures corresponded to the means of the temperatures measured every 15 min over 6 h/day. Results showed that the bitumen temperature was well regulated and that the fume chamber temperature increased from ~50 to 63°C between 5 and 100 mg/m³ target TPM. The variations in temperature in the inhalation chamber were very small (<1.4°C).

Analytical results of some TPM and the corresponding BSM and B[a]P are given in Table 2. The ratio BSM/TPM ranged from 73 to 83%.

Figure 3 shows a typical HundTM record for a target TPM concentration of 100 mg/m³. The instrument was mainly used to check the stability of aerosol concentration, but not to accurately monitor this parameter. The reason is that the signal measured by

Table 1. Operating parameters of the fume generation system (data from INRS and IST)

Aluminium tube inside diameter (cm)	Emission area (cm ²)	TPM (mg/m ³) (mean ± SD)	Bitumen temperature (°C) (mean ± SD)	Fume chamber temperature (°C) (mean ± SD)	Exposure chamber temperature (°C) (mean ± SD)
5	19.6	6.18 ± 0.35 (n = 8)	169.4 ± 0.65 (n = 120)	50.1 ± 0.69 (n = 120)	24.9 ± 1.08 (n = 120)
	Range	5.45–6.60	168.0–171.0	48.5–51.4	22.2–27.0
17	227	53.6 ± 2.19 (n = 16)	169.2 ± 0.48 (n = 120)	59.6 ± 0.74 (n = 120)	25.3 ± 1.23 (n = 120)
	Range	50.4–56.6	168.0–170.0	58.5–61.5	22.0–27.7
23.3	426	97.7 ± 5.63 (n = 47)	169.8 ± 0.64 (n = 230)	63.4 ± 0.62 (n = 230)	26.3 ± 0.85 (n = 230)
	Range	88.8–106.9	168.0–171.5	61.0–68.0	23.5–29.0

the photometer depends not only on aerosol concentration but also on particle size distribution. The accurate measurement of aerosol concentration is performed by sampling the aerosol on glassfibre filters and direct weighing. The concentration was stabilized 120 min after the beginning of the experiment ($t = 0$) and was maintained within a range of 88.8–91.5 mg/m³. A peak concentration was observed at $t = 90$ –120 min, corresponding to an integrated concentration measured by sampling at 117 mg/m³.

Assessment of particle size distribution

The particle size distribution in the exposure chambers was measured at 5, 50 and 100 mg/m³ TPM concentrations. Both techniques, QCM and Aerosizer®, gave similar results. Figure 4 shows that the mass median aerodynamic diameter (MMAD) of particles increased with fume concentration: the average MMAD value was 1.4 µm at 5 mg/m³, 2.6 µm at

50 mg/m³ and 3.2 µm at 100 mg/m³. The average value of geometric standard deviation, calculated from the percentiles 15.87, 50 and 84.13% of the cumulative mass distribution, was 1.6 according to QCM measurements and 1.3 for the Aerosizer, corresponding to a weakly polydisperse aerosol.

The particle size distribution did not show any significant intra-day variation after a stabilization period of 120 min.

Determination of PACs on the filters and XAD-2 resins

Table 3 presents the concentrations of the PAHs and PASHs analysed on the filter, on the XAD-2 cartridge and the corresponding total concentration. The PASHs have been presented as a separate group, allowing a comparison of the PASHs and of the PAHs with TPM concentrations.

PAHs with 4–5 rings and PASHs. When the mean TPM concentration increased by a factor of 8.5 (from

Table 2. Analytical results of TPM, BSM and B[a]P (all data from IST)

	TPM (mg/m ³)	BSM (mg/m ³)	B[a]P (ng/m ³)
Mean ± SD	6.30 ± 0.22 ($n = 3$)	4.6 ± 0.46 ($n = 3$)	36.3 ± 3.21 ($n = 3$)
Range	6.05–6.45	4.10–5.0	34.0–40.0
Mean ± SD	53.5 ± 2.72 ($n = 6$)	42.7 ± 2.02 ($n = 6$)	140 ± 15.6 ($n = 6$)
Range	50.4–56.6	41–46.6	114–157
Mean ± SD	98.7 ± 6.12 ($n = 17$)	82.4 ± 4.89 ($n = 17$)	198 ± 50.4 ($n = 17$)
Range	88.8–106.9	76–93	147–299

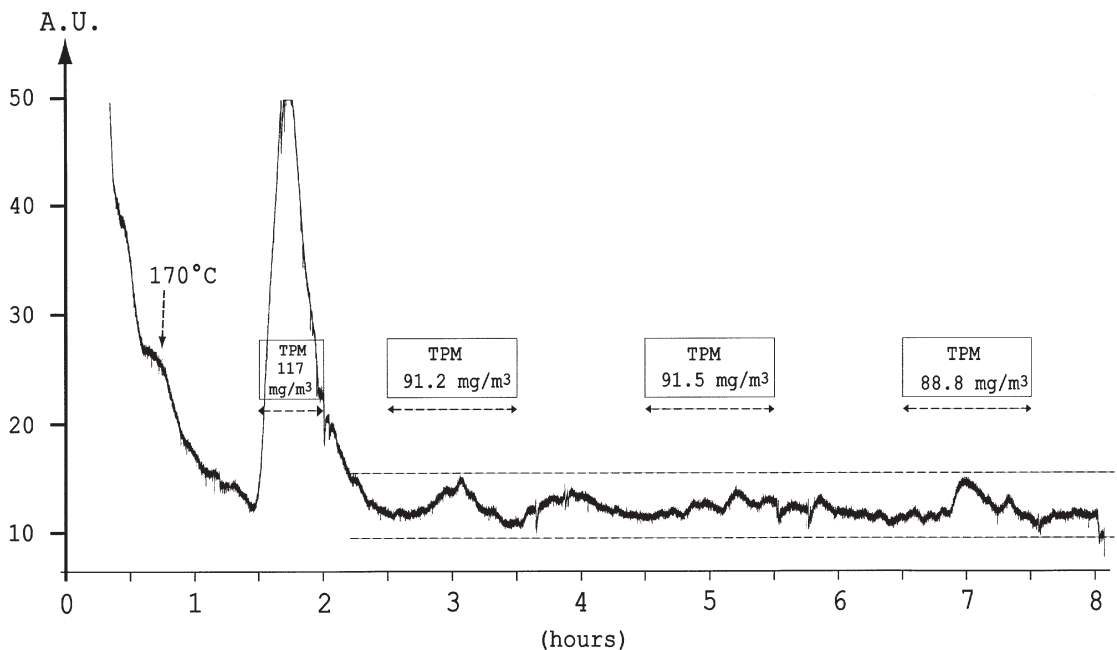


Fig. 3. Hund™ signal (arbitrary units) for a bitumen fume generation experiment for a target concentration of 100 mg/m³.

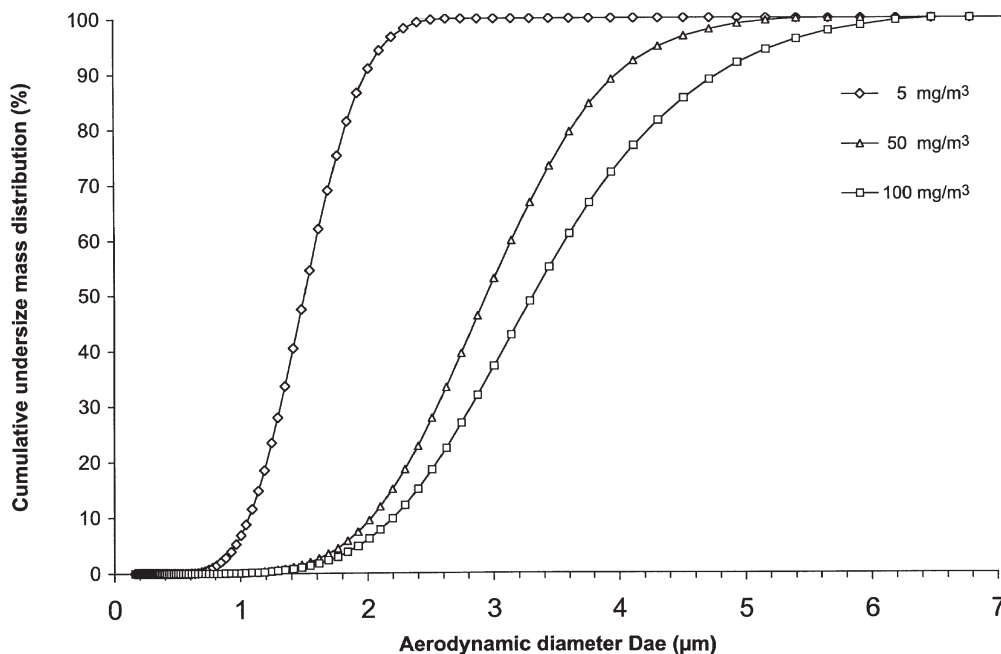


Fig. 4. Cumulative undersize particle mass distribution of bitumen fumes for three concentrations obtained for one measurement sequence with the Aerosizer technique.

6.3 to 53.5 mg/m³) the mean sum of 4–5 ring PAHs increased by a factor of 6.7 and the mean sum of 3–4 ring PASHs increased by a factor of 7.9. When the mean TPM concentration increased by a factor of 15.7 (from 6.3 to 98.7 mg/m³) the mean sum of 4–5 ring PAHs increased by a factor of 14.5 and the mean sum of PASHs increased by a factor of 19.7. Therefore, we can conclude that there is good agreement between the increase in 4–5 ring PAHs and 3–4 ring PASHs and the increase in TPM concentration.

PAHs with 2–3 rings. The data demonstrate a lack of proportionality between the TPM concentrations and the concentrations of 2 and 3 ring PAHs. The sums for the 3 ring PAHs increased between 6.3 and 53.5 and 53.5 and 98.7 mg/m³, but not in proportion, and those of the 2 ring PAHs did not increase at all in the 53.5–98.7 mg/m³ range.

Exhaustion of volatile compounds due to the fume generation process

In bitumen. Table 4 presents the PAH contents in the bitumen 50/70 pen before and after 7 h fume generation at 170°C during 5, 50 and 100 mg/m³ target TPM concentration experiments. There is no sign of exhaustion for any of the PAHs analysed in all cases.

In the fumes. Table 5 presents the PAH concentrations for the 100 mg/m³ target TPM sampled at the beginning and end of a day from the same batch of bitumen. No statistical differences were observed with a one-way Anova for the interaction between the

first and the third samplings during the same day ($P = 0.45$). However, there is a tendency for 2 ring PAHs to be lower at the end of the day and a two-way Anova analysis of the interaction between sampling and number of rings confirmed a statistical difference ($P < 0.001$). This difference disappeared when the data for naphthalene and monosubstituted naphthalene were excluded ($P = 0.44$).

DISCUSSION

We have developed an inhalation exposure system based on a newly developed fume generator and an exposure chamber used in a previous bitumen fume inhalation study (Bonnet *et al.*, 2000). The development of a new fume generator was necessary, because with the system used in the previous study, a fume generator developed by the Heritage Research Group, only the fumes generated at a 5 mg/m³ TPM concentration were representative of fumes workers are exposed to in paving or roofing operations; fumes generated at the 50 mg/m³ level were not (Brandt *et al.*, 2000).

With our current system, a static fume generator has been used, the fume emission area of which can be varied. In this way, different fume concentrations can be delivered to the inhalation chamber without changing the bitumen temperature (170°C, chosen by analogy with the upper range of the temperature used during paving operations). Due to stirring, a cone was

Table 3. Concentrations of PAHs and PASHs in the filter and XAD-2 tubes for the three target TPM concentrations (5, 50 and 100 mg/m³) (all data from IST)

Compound	n	Concentration (µg/m ³)								
		Target TPM 5 mg/m ³ (measured 6.3 mg/m ³) (n = 3)			Target TPM 50 mg/m ³ (measured 53.5 mg/m ³) (n = 6)			Target TPM 100 mg/m ³ (measured 98.7 mg/m ³) (n = 17)		
		Filter	XAD-2	Sum (mean ± SD)	Filter	XAD-2	Sum (mean ± SD)	Filter	XAD-2	Sum (mean ± SD)
<i>PASH</i>										
Benzo(b)naphtho- [1,2-d]thiophene	4	0.099		0.099 ± 0.0081	0.804		0.804 ± 0.021	2.030		2.030 ± 0.073
Benzo(b)naphtho- [2,1-d]thiophene	4	0.327		0.327 ± 0.0222	2.909		2.909 ± 0.086	7.585		7.585 ± 0.281
Benzo(b)naphtho- [2,3-d]thiophene	4	0.045		0.045 ± 0.0035	0.305		0.305 ± 0.014	0.721		0.721 ± 0.025
Dibenzothiophene	3	2.645		2.645 ± 0.1867	20.552		20.55 ± 0.914	51.225		51.23 ± 2.599
<i>PAHs and SV-PAH</i>										
Naphthalene	2	0.104	53.1	53.2 ± 3.34	0.199	139.5	139.7 ± 11.97	0.306	104.5	104.8 ± 8.15
1-Methylnaphthalene	2	0.022	49.8	49.8 ± 1.47	2.181	197.1	199.3 ± 12.34	5.983	211.0	217.0 ± 13.42
2-Methylnaphthalene	2	0.027	83.8	83.8 ± 1.48	2.423	334.5	337.0 ± 22.32	6.593	295.2	301.8 ± 19.90
1-Ethylnaphthalene	2		20.4	20.4 ± 0.42		91.5	91.5 ± 5.11		86.1	86.1 ± 3.82
1,2-Dimethylnaphthalene	2	0.053	15.8	15.9 ± 0.48	2.610	63.1	65.7 ± 3.73	7.718	79.4	87.1 ± 12.08
1,3- + 1,4- + 1,5- Dimethylnaphthalene	2	0.282	34.0	34.0 ± 1.09	10.778	138.8	149.6 ± 8.14	31.809	142.8	174.6 ± 6.99
2,3-Dimethylnaphthalene	2	0.020			1.538		1.5 ± 0.11	4.525		4.53 ± 0.31
2,6-Dimethylnaphthalene	2	0.052	54.2	54.3 ± 1.77	2.491	223.2	225.7 ± 11.34	7.255	208.9	216.1 ± 17.15
2,3,5-Trimethyl- naphthalene	2	0.271	26.9	27.2 ± 0.77	5.200	71.2	76.4 ± 4.55	14.309	69.6	83.9 ± 3.22
Biphenyl	2		4.3	4.32 ± 0.34		16.1	16.1 ± 0.73		16.7	16.7 ± 0.70
2-Methylbiphenyl	2		0.8	0.81 ± 0.05		3.3	3.3 ± 0.17		3.36	3.36 ± 0.17
Acenaphthylene	3		1.7	1.68 ± 0.08		5.6	5.6 ± 0.39		6.76	6.76 ± 0.27
Acenaphthene	3		4.1	4.10 ± 0.57		11.0	11.0 ± 0.56		11.8	11.8 ± 0.57
Anthracene	3	0.012	0.5	0.48 ± 0.03	0.400	0.43	0.83 ± 0.07	1.032	3.15	4.18 ± 1.92
Fluorene	3	0.144	12.1	12.24 ± 0.46	3.011	27.5	30.5 ± 1.88	8.478	25.3	33.7 ± 2.25
Phenanthrene	3	1.846	7.7	9.50 ± 0.478	12.399	7.1	19.5 ± 1.16	30.607	5.9	36.5 ± 1.48
1-Methylphenanthrene	3	1.622	1.4	2.98 ± 0.084	8.549	0.74	9.29 ± 0.246	22.033	0.55	22.58 ± 0.945
2-Methylanthracene	3	1.576	1.3	2.85 ± 0.080	8.446	0.69	9.14 ± 0.320	22.076	0.52	22.60 ± 1.056
3,6-Dimethyl- phenanthrene	3	0.155	nd	0.16 ± 0.004	1.012	nd	1.012 ± 0.029	2.812	nd	2.812 ± 0.133
Benzo[a]anthracene	4	0.060		0.060 ± 0.002	0.287		0.287 ± 0.008	0.622		0.622 ± 0.018
Benzo[a]fluorene	4	0.171		0.171 ± 0.007	1.087		1.087 ± 0.026	2.473		2.473 ± 0.062
Benzo[b]fluorene	4	0.007		0.007 ± 0.003	0.165		0.165 ± 0.005	0.442		0.442 ± 0.017
Chrysene	4	0.116		0.116 ± 0.008	0.820		0.820 ± 0.030	1.827		1.827 ± 0.053
Fluoranthene	4		nd			nd			nd	
Pyrene	4	0.141	nd	0.141 ± 0.005	0.991	nd	0.991 ± 0.030	2.801	nd	2.801 ± 0.122
3-Methylchrysene	4	0.071		0.071 ± 0.014	0.377		0.377 ± 0.011	0.875		0.875 ± 0.027
4-Methylchrysene	4	0.080		0.080 ± 0.008	0.765		0.765 ± 0.030	1.441		1.441 ± 0.024
5-Methylchrysene	4	0.083		0.083 ± 0.006	0.546		0.546 ± 0.029	1.083		1.083 ± 0.030
1-Methylpyrene	4	0.194		0.194 ± 0.009	1.265		1.265 ± 0.042	2.804		2.804 ± 0.071
Benzo[a]pyrene	5	0.036		0.036 ± 0.002	0.141		0.141 ± 0.006	0.198		0.198 ± 0.012
Benzo[e]pyrene	5	0.061		0.061 ± 0.005	0.441		0.441 ± 0.018	0.715		0.715 ± 0.022
Benzo[k]fluoranthene	5	0.059		0.059 ± 0.003	0.322		0.322 ± 0.006	0.407		0.407 ± 0.0386
Sum of 2 ring SV-PAHs			343	344		1278	1306		1218	1296
Sum of 3 ring SV-PAHs			29	34		53	87		53.9	141
Sum of 4–5 ring PAHs				1.08			7.21			15.7
Sum of PASHs				3.12			24.6			61.6

Quantification by GC-MS.

Table 4. PAH content in the 50/70 bitumen before and after heating for 7 h at 170°C during fume generation at 5, 50 and 100 mg/m³ TPM target concentration

Compound	Rings	Concentration (ng/mg)					
		Target TMP 5 mg/m ³		Target TMP 50 mg/m ³		Target TMP 100 mg/m ³	
		0 h heating (112 mg assay)	7 h heating (178 mg assay)	0 h heating (115 mg assay)	7 h heating (98 mg assay)	0 h heating (97 mg assay)	7 h heating (114 mg assay)
<i>PASH</i>							
Dibenzothiophene	3	2.8	3.5	3.1	2.7	2.6	2.2
Benzo(b)naphtho- [1,2- <i>d</i>]thiophene	4	1.7	1.7	1.3	1.2	1.2	0.9
Benzo(b)naphtho- [2,1- <i>d</i>]thiophene	4	6.1	6.2	4.8	4.4	4.7	4.0
Benzo(b)naphtho- [2,3- <i>d</i>]thiophene	4	0.6	0.7	0.2	0.1	0.5	0.1
<i>PAH</i>							
1,2-Dimethylnaphthalene	2	0.16	0.21	Trace	Trace	Trace	Trace
1,3-Dimethylnaphthalene	2	0.73	0.72	0.68	0.77	0.64	0.56
1,4-Dimethylnaphthalene	2	Trace	Trace	Trace	Trace	Trace	Trace
1,5-Dimethylnaphthalene	2	0.07	0.07	Trace	Trace	Trace	Trace
1-Methylnaphthalene	2	0.40	0.40	0.30	0.27	0.08	0.09
2,3,5-Trimethylnaphthalene	2	0.23	0.27	0.17	0.20	0.06	0.07
2,3-Dimethylnaphthalene	2	0.08	0.11	Trace	Trace	Trace	Trace
2,6-Dimethylnaphthalene	2	0.16	0.19	0.13	0.12	Trace	Trace
2-Methylnaphthalene	2	0.56	0.53	Trace	Trace	Trace	Trace
Naphthalene	2	0.27	0.23	Trace	Trace	Trace	Trace
1-Methylphenanthrene	3	2.84	3.37	5.84	6.33	5.65	5.66
2-Methylanthracene	3	2.62	3.06	2.82	3.06	2.73	2.91
3,6-Dimethylphenanthrene	3	0.32	0.49	0.26	0.17	0.12	0.19
Anthracene	3	Trace	Trace	Trace	Trace	Trace	Trace
Fluorene	3	0.10	0.18	0.52	1.57	0.36	0.44
Phenanthrene	3	2.28	2.71	2.96	3.19	2.35	2.30
1-Methylpyrene	4	1.40	1.70	2.35	2.68	2.70	2.62
1-Methylpyrene	4	1.39	1.68	2.47	2.73	2.70	2.62
3-Methylchrysene	4	1.22	1.02	1.12	1.60	1.27	1.36
4-Methylchrysene	4	2.43	1.84	1.16	1.06	0.99	0.93
5-Methylchrysene	4	1.75	1.36	1.76	1.46	1.73	1.83
Benzo[<i>a</i>]anthracene	4	0.66	0.62	0.76	0.59	0.58	0.57
Benzo[<i>a</i>]fluorene	4	0.99	1.13	0.87	0.69	0.67	0.65
Benzo[<i>b</i>]fluorene	4	0.15	0.19	0.16	0.23	0.22	0.19
Chrysene	4	1.94	1.78	1.26	1.83	1.75	1.85
Pyrene	4	0.88	1.07	1.34	1.36	1.32	1.31
Benzo[<i>a</i>]pyrene	5	0.66	0.50	0.62	0.61	0.67	0.78
Benzo[<i>e</i>]pyrene	5	3.37	3.22	2.54	2.64	2.31	2.45
Benzo[<i>k</i>]fluoranthene	5	1.23	1.58	0.69	0.94	0.78	0.81

Trace, < 0.1 ng/mg.

formed at the surface of the hot bitumen. Therefore, in order to prevent leakage of fumes from the whole surface of the vessel or part of it, it was important to immerse the tube 10 mm into the hot bitumen before stirring. This positioning ensured reproducible analytical results and a steady TPM. The amount of bitumen is such that during the time of the experi-

ment no exhaustion takes place even at the highest rate of fume generation. In Table 6 it is demonstrated that for PAHs with a boiling point above say 350°C the PAH profile (expressed as p.p.m.w. in BSM) is virtually independent of the amount of fumes (TPM) produced, as is the case with field fumes sampled during personal exposure studies (Brandt *et al.*,

Table 5. PAH content ($\mu\text{g}/\text{m}^3$) of the particulate matter in the first and the final third filter samples during the 6 h bitumen fume generation at a target TPM concentration of $100 \text{ mg}/\text{m}^3$ ($n = 6$)

	Start (mean \pm SE)	End (mean \pm SE)
TPM (mg/m^3)	101 \pm 2.6	96 \pm 2.5
Naphthalene	0.45 \pm 0.09	0.18 \pm 0.02
1-Methylnaphthalene	7.3 \pm 1.22	4.5 \pm 0.45
2-Methylnaphthalene	8.2 \pm 1.43	4.8 \pm 0.49
1,2-Dimethylnaphthalene	7.9 \pm 1.14	7.0 \pm 0.75
1,3+1,4+1,5-Dimethylnaphthalene	33 \pm 5.0	28 \pm 3.0
2,3-Dimethylnaphthalene	4.6 \pm 0.67	4.1 \pm 0.43
2,6-Dimethylnaphthalene	7.9 \pm 1.24	6.1 \pm 0.71
2,3,5-Trimethylnaphthalene	14 \pm 1.7	14 \pm 1.5
Anthracene	0.9 \pm 0.09	1.1 \pm 0.09
Fluorene	8.0 \pm 0.93	8.2 \pm 0.84
Phenanthrene	28 \pm 2.6	31 \pm 2.7
1-Methylphenanthrene	20 \pm 1.6	22 \pm 1.8
2-Methylanthracene	20 \pm 1.8	23 \pm 2.0
3,6-Dimethylphenanthrene	2.6 \pm 0.21	2.8 \pm 0.25
Benzo[<i>a</i>]anthracene	0.6 \pm 0.03	0.6 \pm 0.03
Benzo[<i>a</i>]fluorene	2.4 \pm 0.08	2.5 \pm 0.14
Benzo[<i>b</i>]fluorene	0.4 \pm 0.02	0.5 \pm 0.04
Benzo[<i>e</i>]pyrene	0.7 \pm 0.03	0.8 \pm 0.05
Chrysene	1.7 \pm 0.08	1.9 \pm 0.10
Pyrene	2.6 \pm 0.21	2.8 \pm 0.22
3-Methylchrysene	0.8 \pm 0.05	0.9 \pm 0.05
4-Methylchrysene	1.4 \pm 0.04	1.5 \pm 0.04
5-Methylchrysene	1.0 \pm 0.05	1.1 \pm 0.05
1-Methylpyrene	2.7 \pm 0.10	2.8 \pm 0.14
Benzo[<i>a</i>]pyrene	0.2 \pm 0.02	0.2 \pm 0.02
Benzo[<i>k</i>]fluoranthene	0.39 \pm 0.07	0.41 \pm 0.07
Sum of 2 ring SV-PAH	84 \pm 12.4	69 \pm 12.4
Sum of 3 ring SV-PAH	81 \pm 7.2	88 \pm 7.2
Sum of 4–6 ring PAHs	15 \pm 0.5	16 \pm 0.5

2000). For the deviation from proportionality of the measured concentrations of PAHs boiling below 350°C with the TPM concentration the following explanation can be given. During sampling some of the material, including the lower molecular weight PAHs that were primarily deposited on the filter re-evaporates (Brandt *et al.*, 1985; Bonnet *et al.*, 2000), are transferred to the XAD-2 adsorption tube connected in series with the filter. Brandt *et al.* (1985) demonstrated that approximately constant amounts of material were removed per volume of air. Because for the $5 \text{ mg}/\text{m}^3$ target TPM 180 l was sampled, more material was transferred to XAD-2 than with the 50 and $100 \text{ mg}/\text{m}^3$ target TPM, where 80 and 40 l were sampled, respectively. This is confirmed by the increase in the BSM:TPM ratio with increasing TPM; 73, 80 and 83% for the 5, 50 and $100 \text{ mg}/\text{m}^3$ target TPM, respectively. In the working up of the filters and preparation for PAH analysis evaporation losses will also occur. Because

the XAD-2 adsorption tubes are only extracted prior to analysis in this step, no evaporation losses will occur. Hence, material transferred to the XAD-2 resin during sampling will be analysed without loss, whereas material remaining on the filter will be lost by evaporation before analysis. This mechanism indicates that the deviations from proportionality for the 2 and 3 ring PAHs is an analytical artefact.

The data in Table 2 show that the fumes can be generated in a reproducible way.

A comparison of the profiles [again as p.p.m.w. in BSM, because this is independent of the fume (TPM or BSM) concentration] of the PAHs that have been analysed in both our study and during personal exposure studies of road paving operations has been made (Table 7). This comparison shows that the profiles of our laboratory generated fumes are similar, especially those for the 4 and 5 ring PAHs. In the field samples, due to re-evaporation of material already deposited on the filter, losses of material with

Table 6. PAH profiles (p.p.m.w. in BSM) for the three target concentrations

Compound	BP	<i>n</i>	PAH concentration (p.p.m.w. in BSM)		
			Target TPM 5 mg/m ³	Target TPM 50 mg/m ³	Target TPM 100 mg/m ³
<i>PAH</i>					
Sum of 2 ring PAHs			181	642	953
Anthracene	340	3	3	9	13
Fluorene	294	3	31	71	103
Phenanthrene	338	3	401	290	371
1-Methylphenanthrene	355	3	353	200	267
2-Methylanthracene	363	3	343	198	268
3,6-Dimethylphenanthrene		3	34	24	34
Sum of 3 ring PAHs			1164	792	1056
Benzo[<i>a</i>]anthracene	425	4	13	7	8
Benzo[<i>a</i>]fluorene		4	37	25	30
Benzo[<i>b</i>]fluorene		4	2	4	5
Chrysene	431	4	25	19	22
Pyrene	393	4	31	23	34
Benzo[<i>a</i>]pyrene	496	5	8	3	2
Benzo[<i>e</i>]pyrene		5	13	10	9
Benzo[<i>k</i>]fluoranthene	481	5	13	8	5
Sum of 4–5 ring PAHs			235	169	190
<i>PASH</i>					
Benzo(b)naphtho[1,2- <i>d</i>]thiophene		4	22	19	25
Benzo(b)naphtho[2,1- <i>d</i>]thiophene		4	71	68	92
Benzo(b)naphtho[2,3- <i>d</i>]thiophene		4	10	7	9
Dibenzothiophene	332	3	575	481	622

Table 7. PAH profiles of laboratory generated fumes compared with those from personal exposure studies (p.p.m.w. in BSM/TSM)

	This laboratory inhalation study			Personal exposure measurements		
	Fume generation temperature 170°C			Three road paving surveys, asphalt temperatures 139–176°C		
	Target TPM 5 mg/m ³	Target TPM 50 mg/m ³	Target TPM 100 mg/m ³	Average	Minimum	Maximum
Naphthalene	23	5	4	nd	nd	nd
Acenaphthene	0	0	0	1.3	0.0	2.7
Fluorene	31	71	103	0.8	0.4	1.3
Phenanthrene	401	290	371	44	13	92
Anthracene	3	9	13	5.6	5.6	5.6
Fluoranthene	nd	nd	nd	36	1.3	74
Pyrene	31	23	34	37	6.4	83
Benzo[<i>a</i>]anthracene	13	7	8	4.3	1.0	6.4
Chrysene	25	19	22	80	16	203
Benzo[<i>k</i>]fluoranthene	13	8	5	3.4	2.4	4.0
Benzo[<i>a</i>]pyrene	8	3	2	2.7	2.3	3.1
Sum of 3 ring PAHs	435	370	487	52	19	102
Sum of 4–5 ring PAHs	90	60	71	163	30	373

the highest vapour pressures (i.e. the 2 and 3 ring PAHs and other molecules of similar volatilities) can occur. In the laboratory samples this re-evaporation

will be less, because a higher concentration of these PAHs will be present in the vapour phase than in the field (Brandt and De Groot, 1999).

Under our new generation conditions, the MMAD varied from 1.4 μm at a concentration of 5 mg/m^3 to 3.2 μm at 100 mg/m^3 . The particle size distribution measured in the exposure chamber was close to that reported by Brandt *et al.* (1985), with a Bird and Tole cascade centripeter in three different field surveys and in the laboratory. Bitumen fumes were shown to be relatively fine aerosols, with a particle size ranging from 0.1 to 20 μm and a mass MMAD of ~ 2.3 μm . In any case, measurement of the particles generated using our new system proved them to be in a size range that permitted a deposit throughout the entire respiratory tract of animals (Ménache *et al.*, 1995).

In conclusion, our objective of developing an inhalation exposure system that enables us to perform inhalation experiments on exposure to bitumen fumes of varying concentrations in the range 5–100 mg/m^3 TPM has been reached. The system is reproducible and the bitumen fumes sampled from the inhalation chamber are representative of the fumes workers are exposed to in asphalt paving operations, both in terms of PAH profile and particle size distribution.

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