

# 1-Nitropyrene as a Marker for the Mutagenicity of Diesel Exhaust-Derived Particulate Matter in Workplace Atmospheres

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The use of 1-nitropyrene (1-NP) as a marker for the occupational exposure to diesel exhaust (DE) mutagens was investigated in workplace atmospheres contaminated with DE from a variety of emission sources, such as power supplies, forklifts, trucks, caterpillar vehicles, trains, ships' engines, and vehicles in city traffic. Total suspended particulate matter was collected by area sampling. The 1-NP content of acetone extracts of these samples as determined by gas chromatography-high resolution mass spectrometry varied from 0.080 to 17  $\mu\text{g/g}$  acetone extractable matter, corresponding to air concentrations of 0.012 to 1.2  $\text{ng/m}^3$ . A sample collected in a rural area contained 0.0017  $\text{ng/m}^3$  1-NP. The mutagenicity of the extracts was tested in the *Salmonella typhimurium* strains TA98 and TA1538, using the microsuspension assay with and without metabolic activation by an exogenous metabolizing system (rat liver

S9-fraction). In addition, the *S. typhimurium* strains YG1021 and YG1024 were used because of their high sensitivity towards the mutagenicity of nitro polycyclic aromatic hydrocarbons. When plotting the mutagenic potency of the air sample extracts as determined in the absence of liver S9 versus the particle-associated 1-NP level, a relatively high correlation ( $r = 0.80\text{--}0.91$ ) was observed in all of the *S. typhimurium* strains. High correlations ( $r = 0.80\text{--}0.93$ ) were also observed when plotting the results of mutagenicity testing after activation by S9 versus the outcome of chemical analysis. These results show that the 1-NP content of workplace air samples is associated with their mutagenic potency, suggesting that 1-NP may be used as a marker for occupational exposure to DE-derived particle-associated mutagens.

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**Key words:** nitro polycyclic aromatic hydrocarbons, occupational exposure, microsuspension assay, mutagenic potency, *Salmonella typhimurium* YG-strains

## INTRODUCTION

The emissions of diesel-powered engines may cause exposure of operators of those engines to diesel exhaust (DE) constituents. Especially in indoor workplaces the use of diesel engines may give rise to occupational exposure [Wheeler et al., 1981; Reger et al., 1982; Waller et al., 1985; Ulfvarson et al., 1987; Froines et al., 1987; Hammond et al., 1988; Blome et al., 1990; Lehmann et al., 1990; Bauer et al., 1991]. Epidemiological studies have supplied evidence of elevated levels of lung cancer among railroad workers exposed to DE [Howe et al., 1983; Garshick et al., 1987, 1988] and some other occupational categories. Evaluation of these data together with observed lung tumor induction in experimental animals have led to the classification of whole DE as a possible carcinogenic risk factor [IARC, 1989; Deutsche MAK Kommission, 1987; NIOSH, 1988].

An important problem in the epidemiological studies that have been conducted and a pitfall in risk assessment is the lack of accurate and specific exposure estimates. Several methods have been proposed for environmental monitoring of exposure to DE. Some gas phase compounds, such as

$\text{NO}_2$  and total aldehydes, have been used as indicators of DE exposure [Wheeler et al., 1981; Reger et al., 1982; Ulfvarson et al., 1987]. Because the exposure to DE particles and not the gas phase was found to be associated with tumor induction in experimental animals, more attention has been given to the determination of the exposure to airborne particulate matter. The use of the air concentrations of total and respirable suspended particulate matter (TSPM/RSPM) as surrogates for DE [Reger et al., 1982; Guillemin et al., 1992] is a rather non-specific approach. In railroad studies adjustments were made to account for the contribution of environmental tobacco smoke to respirable particle exposure [Hammond et al., 1988; Woskie et al., 1988]. This is a rather crude method because the corrections made were based on the nicotine content of the cigarette smoke determined from a limited number of brands. This correction is

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not very accurate because the nicotine content may vary considerably between cigarette brands [Adams et al., 1987]. In the workplace, besides tobacco smoke, there may be many other sources of respirable dust that contribute to the respirable dust fraction (welding fumes, silica dust, and particles derived from other nondiesel combustion sources). The use of this approach was not reported in workplaces other than those connected to railroads.

Because of the relatively high content of organic extractable matter of DE particles, the use of the soluble organic fraction (SOF) of the particles has been proposed as a surrogate for DE exposure, using cyclohexane [Wheeler et al., 1981] or dichloromethane [Hammond et al., 1988; Froines et al., 1987] as extraction solvents. This method is unspecific for DE exposure since other exposures may also yield considerable amounts of organic soluble compounds. In addition, this method does not provide information about the biological (toxicological) activity of the SOF. A practical drawback is that in personal air sampling during a working period, only very small weights of SOF can be collected. Accurate gravimetric determination of these small weights is laborious and requires highly sophisticated instrumentation.

Additionally, specific polycyclic aromatic hydrocarbons (PAHs) in the SOF have been used to characterize exposure to DE: benzo[*a*]pyrene [Ulfvarson et al., 1987; Waller et al., 1985; Guillemain et al., 1992]; pyrene, benzo[*e*]pyrene, benzo[*ghi*]perylene, anthanthrene, and coronene [Waller et al., 1985]; phenanthrene [Schenker et al., 1992]; or the sum of PAH [Guillemain et al., 1992]. When using phenanthrene as a marker, the contribution of tobacco smoke-derived PAHs appeared to be an important confounder [Schenker et al., 1992].

In another approach, the exposure to DE particles was estimated by determination of the carbon content by colorimetric determination of carbon dioxide after oxidizing the collected dust particles [Blome et al., 1990; Lehmann et al., 1990; Bauer et al., 1991]. Because of the carbon core present in DE particles, this determination would be expected to reflect largely exposure to DE-derived soot. However, in this method airborne particulate matter not derived from DE but with a high carbon content, such as coal, organic carbon, and inorganic carbonate, could interfere with the exposure estimate. Attention should also be given to a possible contribution of pyrolytically generated elemental carbon derived from particle-associated organics.

An implicit consequence of choosing exposure to carbon as a surrogate for DE exposure is neglecting the chemical composition of the adsorbed organics and their possible contribution to the carcinogenic activity. Genotoxicity, expressed as the bacterial mutagenic potency, may be used to characterize carcinogenic combustion products, such as combustion-derived PAHs, that are adsorbed into airborne particles [Lewtas, 1993]. For PAHs, the mutagenic potency in bacterial *in vitro* tests is highly correlated to the carcino-

genic potency as observed *in vivo* in experimental animals [Glatt et al., 1981; Utesch et al., 1987]. Therefore, the mutagenic potency of the SOF may also account for the possible potentiating and/or synergistic toxic effects of organics that are desorbed from DE particles residing in the lungs. In inhalation studies in rats approximately half the dose of [<sup>14</sup>C]-labeled B[*a*]P and [<sup>3</sup>H]-labeled 1-nitropyrene (1-NP) adsorbed on diesel soot was retained in the lungs, with half-lives of 8–36 days [Bond et al., 1986a,b; Sun et al., 1984]. The slow release and metabolism of these compounds causes exposure of the surrounding lung tissue to genotoxic intermediates capable of covalent binding to macromolecules [Sun et al., 1982, 1984; Bevan and Ruggio, 1991]. In rats exposed to DE, increased DNA-adduct formation was observed [Wong et al., 1986].

If the genotoxic potency of the extractable organics is acknowledged as a toxicologically relevant parameter, the next objective would be to define (a group of) chemicals that may represent these genotoxic properties. This approach is the so-called "marker" approach. Markers can be used for source apportionment, exposure assessment, and effect. If single chemical substances are selected as markers, gas chromatography-mass spectrometry-based analytical chemical procedures may provide reliable, specific, and sensitive means of quantitation. Other investigators have pointed out the possible value of (1-NP) as a marker for source apportionment because of its characteristic appearance in DE [Schuetzle and Frazier, 1986]. There are several reasons for proposing 1-NP as a representative of nitro-PAH and as marker for DE exposure:

1. Until now 1-NP is reported to be the most abundant nitro-PAH in DE particulate extracts [Paputa-Peck et al., 1983; MacCrehan et al., 1988]. The abundance of 1-NP is associated with the presence of dinitropyrenes, hydroxy- and acetoxynitropyrenes, which together explain a considerable part (30–90%) of the direct-acting mutagenic activity of the DE particulate extract [Pederson and Siak, 1981; Nakagawa, 1983; Salmeen et al., 1984].
2. 1-NP as identified from ambient airborne particulate matter (APM) extracts originates primarily from (diesel) combustion sources [Arey et al., 1986; Zielinska et al., 1986]. We did not find convincing evidence of a significant contribution of 1-NP by atmospheric (photo)chemical conversions; analysis of APM and environmental chamber studies revealed 2-nitropyrene (2-NP) and not 1-NP as the nitro isomer of pyrene formed under simulated atmospheric conditions. To our knowledge 2-NP has not been identified in DE [Nielsen et al., 1984; Arey et al., 1986; Pitts et al., 1985].
3. Because of the specific conditions required for the formation of 1-NP and other nitro-PAHs, there are only a limited number of nondiesel combustion pro-

**TABLE I. Specifications of Air Sampling Equipment and Sampling Conditions\***

	TSPM	RSPM	ISPM
Sampled fraction	Stationary	Stationary	Personal
Type of air sampling	Open face	Cyclone	IOM
Type of sampler	125–600	5–30	0.5–1
Sampling volume (m <sup>3</sup> )	4–10	4–10	4–8
Sampling time (hr)	$1.5 \times 10^{-2}$	$2.0 \times 10^{-3}$	$4.9 \times 10^{-4}$
Filter surface (m <sup>2</sup> )	PTFE/PS	PTFE/PS	PTFE
Filter type			

\*TSPM = total suspended particulate matter; RSPM = respirable suspended particulate matter; ISPM = inhalable suspended particulate matter; IOM = Institute of Occupational Medicine, Edinburgh, Scotland; PTFE = polytetrafluoroethylene; PS = polystyrene.

cesses that have been identified as sources of 1-NP or nitro-PAH emissions, usually at a much lower rate than DE [McCarthy et al., 1986; Kinouchi et al., 1988; Gibson, 1982; Tokiwa et al., 1985]. Emission sources of pyrolysis products encountered in workplaces, such as coke oven emissions or bitumen fumes, do not cause significant emissions of nitro-PAH [Williams et al., 1986]. Cigarette smoke is a source of respirable dust (co)exposure frequently encountered in workroom atmospheres. Cigarette smoke does not contain significant amounts of 1-NP [El-Bayoumy et al., 1985; Williams et al., 1986], suggesting that the use of 1-NP as a marker for DE exposure would eliminate environmental cigarette smoke as a confounder in the exposure assessment of workers potentially exposed to DE.

In a previous study we have demonstrated that 1-NP is consistently observed in the atmospheres of workplaces associated with the use of different types of diesel-powered engines [Scheepers et al., 1994a]. In another study it was shown that the frequency of test runs of diesel engines was consistent with the magnitude of the 1-NP levels in the workplace atmosphere [Scheepers et al., 1994b]. In the present study we have investigated the value of 1-NP as a possible exposure marker and indicator of the mutagenic potency of extracts of DE-derived particles sampled from workplace atmospheres.

## MATERIALS AND METHODS

### Air Sampling

The equipment used for air sampling and the sampling conditions are specified in Table I. Total suspended particulate matter (TSPM) was sampled at a flow rate of  $\sim 1.0$  m<sup>3</sup>/min using a high volume sampler equipped with an open face sampler head. Respirable suspended particulate matter (RSPM) was collected using a cyclone with a 50% cut-off diameter of  $\sim 5$   $\mu$ m at a flow rate of 0.050 m<sup>3</sup>/min [Vrins and Hofschreuder, 1983]. A more detailed description of these air sampling procedures can be found in Scheepers et al. [1994a]. Inhalable suspended particulate matter (ISPM) was sampled at a flow rate of 0.002 m<sup>3</sup>/min with personal air sampling equipment consisting of an IOM sampler head (Institute of Occupational Medicine, Edinburgh, Scotland) connected to a small battery-operated pump worn on a waist-belt (Ametek, Tampa, FL). The sampling of ISPM is in accordance with the inhalable convention of the European standard

(EN481, July 1993). The collected RSPM fraction slightly overestimates the fraction recommended in the respirable convention of the EN481 standard.

### Workplaces, Reference Location, and Weather Conditions

At 12 sites, 27 workplaces were selected; an additional site was included as a reference location (see Table II). At six of these sites, workplaces were indoor or at least partly indoor. The other six sites included mainly outdoor activities. The following types of engines were included in the study: power supplies, lawn mowers, light duty forklifts (<4,000 kg lift capacity), heavy duty forklifts (>4,000 kg lift capacity), air cargo lift platforms, tractors, trucks, caterpillar vehicles, trains, ships' main engine and power aggregate, and traffic in a city on a busy street crossing (private cars, vans, trucks, buses, and taxis). The reference sample was collected in the open air. The sampling location was situated in a forest and moor area ("Hoog Buurlo") approximately 15 km SW of Apeldoorn in the centre of The Netherlands. The sampling location was 5 km from the nearest road minimizing the direct influence of passing traffic.

The study was conducted in the period February–July 1992. For every day of the survey that included outdoor measurements, the weather conditions of a nearby weather station were registered. Measurements of wind speed and wind direction (see Table II), temperature, and relative humidity (results not shown) were conducted at the workplaces.

### Chemicals

1-Aminopyrene (1-AP, 98.7%) and 1-NP (97%) were supplied by Aldrich Europe (Bornem, Belgium). Benzo[a]pyrene (B[a]P, 98%) was obtained from Sigma (St. Louis, MO). 1-Nitro-[<sup>2</sup>H<sub>9</sub>]-pyrene (1-N[<sup>2</sup>H<sub>9</sub>]P, >99%) was obtained from Chemsyn (Lenexa, KS). Sodium hydrosulfide hydrate (NaSH, 73% in water) was supplied by Aldrich (Steinheim, Germany). Heptafluorobutyric anhydride (HFBA) and 2-nitrofluorene (2-NF, 98%) were obtained from Janssen Chimica (Geel, Belgium). Isooctane (HPLC-grade) was supplied by Lab-Scan Analytical Sciences (Dublin, Ireland). Dimethylsulfoxide (DMSO, p.a.) was obtained from Merck (Darmstadt, Germany). Demineralized (demi) water (tap water treated in a Milli RO system, Millipore) and aqua pure (demi water treated in a Nanopure system, Barnstead, Boston, MA) were used. Other chemicals used were of the highest purity available.

### Filter Extraction

The particulate samples were acetone extracted because this solvent is known to extract mutagens from airborne particulates very efficiently [Krishna et al., 1983; Lee et al., 1991; Montreuil et al., 1992]. The mutagens were extracted by sonication which generally results in a better repeatability in mutagenicity testing of ambient air and diesel particulate matter extracts as compared to Soxhlet extraction [Krewski et al., 1992]. The filters were extracted as described previously [Scheepers et al., 1994a].

TABLE II. Workplaces That Were Selected for Airborne Particulate Sampling

No.	Workplace	Description	Date	I/O <sup>a</sup>	Sources	Wind	
						Speed (m/s)	Direction
0	Reference	Forest area	21.07	O	Remote sources	7	SW
1	Gardening	Park in city centre	25.02	O	Traffic	5	ENE
2	Grass verge maintenance	Park in domestic area	20.05	O	Lawn mowers	6	ESE
3 a	Storage of chemicals	(Un)loading of trucks	10.03	I/O	Trucks/forklifts	11	SW
b		(Un)loading of trucks	11.03	I/O	Trucks/forklifts	10	WSW
c		(Un)loading of trucks	12.03	I/O	Trucks/forklifts	14	WSW
d		(Un)loading of trucks	13.03	I/O	Trucks/forklifts	14	W
4 a	Aluminum rolling	Transport of aluminum	24.04	I	Forklifts <sup>b</sup>	<2	—
b		Transport of aluminum	16.03	I	Forklifts <sup>c</sup>	<2	—
5 a	Galvanization work shop	Transport of iron	21.04	I/O	Forklifts <sup>d</sup>	5	SE
b		Lifting iron	22.04	I/O	Forklifts <sup>e</sup>	5	W
c		Transport of iron	23.04	I/O	Forklifts <sup>d</sup>	6	SW
6 a	Concrete manufacturing	Short distance transport	09.04	I	Forklifts	<2	—
b		Short distance transport	28.04	I	Forklifts	<2	—
7	Farming	Turning of hay	24.07	O	Tractor	7	SE/S/SW/W
8 a	Flower auction	(Un)loading trucks	11.06	I	Trucks	<2	—
b		(Un)loading trucks	12.06	I	Trucks	<2	—
9 a	Army driving lessons	Field exercise	17.03	O	Armoured cars	4	WSW
b		Training circuit	24.03	O	Armoured cars	6	N
10 a	Repair shop for trains	Locomotives	01.04	I	Locomotive engines	<2	—
b		Passenger trains	02.04	I	Passenger train engines	<2	—
11 a	Inland transportation of bulk chemicals	Nijmegen-Antwerpen	17.04	O	Ship's engine <sup>f</sup>	8	WSW
b		Antwerpen Harbour	18.04	O	Ship's engine <sup>g</sup>	9	W
c		Rotterdam-Nijmegen	19.04	O	Aggregate <sup>f</sup>	8	NW
12 a	Loading and unloading of air cargo	Intercontinental flights	14.04	O	Platform vehicles <sup>h</sup>	11	SW
b		Continental flights	15.04	O	Platform vehicles <sup>h</sup>	13	WNW
c		Intercontinental flights	09.06	O	Platform vehicles <sup>h</sup>	5	NE
d		Continental flights	10.06	O	Platform vehicles <sup>h</sup>	6	NE

<sup>a</sup>I = indoor workplace; O = outdoor workplace.

<sup>b</sup>Truck fueled with low sulfur diesel fuel (<0.01 w% S).

<sup>c</sup>Truck fueled with normal sulfur diesel fuel (<0.2 w% S).

<sup>d</sup>Old forklift (>15 yr).

<sup>e</sup>New forklift (<2 yr).

<sup>f</sup>During journey.

<sup>g</sup>During (un)loading of cargo.

<sup>h</sup>Other possible sources: airplane engines and power supplies.

Another frequently applied solvent for extraction of DE particles, dichloromethane, could not be used because it affected the polystyrene filter membranes during the sonication procedure in such a way that a filter-derived residue remained after evaporation of the solvent.

The amount of dry extract was determined gravimetrically. The dry extract was soluted in acetone, sonicated, and divided over four preweighed glass tubes. The portions were evaporated to dryness under a gentle flow of N<sub>2</sub> at 50°C and weighed. Two of the portions were used for mutagenicity testing and nitro-PAH analysis, respectively. The other portions were stored at -20°C for other purposes. The dry extracts were stored at -20°C in the dark, until further analysis.

## Analysis of 1-NP

Filter extracts were analyzed on gas chromatography-high resolution mass spectrometry (GC-HRMS) as described previously [Scheepers et al., 1994a]. Briefly, the internal standard 1-N[<sup>2</sup>H<sub>9</sub>]P was added to the extract. Next, the extract was pre-cleaned on Seppak Si cartridges (Millipore, Bedford, CA), dried under a stream of N<sub>2</sub>, and reduced with NaSH in ethanol and aqua pure. The amino analogues were derivatized with heptafluorobutyric anhydride and dried under N<sub>2</sub>. The residue was dissolved in isoctane and analyzed on GC-HRMS. The analysis of derivatized amino-

PAH was performed on a VG Autospec Q HRMS (VG Instruments, Altrincham, England) equipped with an HP5890 GC and an HP7673A autosampler (Hewlett Packard, Palo Alto, CA) at the Agricultural Research Department of the State Institute of Quality Control of Agricultural Products (RIKILT-DLO, Wageningen, Netherlands).

## Mutagenicity Testing

The *Salmonella typhimurium* strains TA98 and TA1538 were kindly supplied by Dr. B.N. Ames of the Department of Biochemistry, University of California, Berkeley. The *S. typhimurium* strains YG1021 and YG1024 were a gift of Dr. T. Nohmi of the National Institute of Hygienic Sciences, Tokyo, Japan. YG1021 is equal to TA98(pYG216) and YG1024 is equal to TA98(pYG219). The plasmid pYG216 encodes nitroreductase (NR) and provides the strain YG1021 with high nitroreductase activity [Watanabe et al., 1989]. The plasmid pYG219 encodes acetyl-CoA:N-hydroxyarylamine-O-acetyltransferase (OAT), providing overproduction of this enzyme. The enhanced enzyme activities in both YG strains increased the sensitivity for the detection of nitro-PAH. As compared to TA98, the increase in sensitivity towards 1-NP amounts to a factor of 23.7 for YG1021 and a factor of 6.6 for YG1024 [Einistö et al. 1991]. For the mutagenicity assay, only small portions of sample (corresponding to vol-

TABLE III. Mutagenicity in Revertants per Plate of References With and Without S9 mix\*

Compound	$\mu\text{g}/\text{plate}$	TA98		TA1538		YG1021		YG1024	
		+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Spontaneous	—	27 $\pm$ 1	27 $\pm$ 1	38 $\pm$ 7	38 $\pm$ 7	49 $\pm$ 6	49 $\pm$ 7	32 $\pm$ 3	32 $\pm$ 3
DMSO	—	29 $\pm$ 5	28 $\pm$ 4	24 $\pm$ 3	35 $\pm$ 7	44 $\pm$ 7	51 $\pm$ 6	43 $\pm$ 4	41 $\pm$ 5
1-NP	0.05	—	1,576 $\pm$ 155	—	1,527 $\pm$ 25	—	2,998 $\pm$ 192	—	2,858 $\pm$ 192
2-NF	0.5	—	1,107 $\pm$ 83	—	5,832 $\pm$ 52	—	1,624 $\pm$ 80	—	3,262 $\pm$ 160
B(a)P	7.5	690 $\pm$ 49	—	190 $\pm$ 52	—	325 $\pm$ 17	—	642 $\pm$ 43	—

\*Mean values of the determinations in triplicate ( $\pm$ SD).

umes of 30–150 m<sup>3</sup> of sampled air) were available. Therefore, the micro-suspension method [Kado et al., 1983] was used. This method has a higher sensitivity as compared to the standard plate-incorporation method [Aguere and Stensman, 1992].

Mutagenicity testing was limited to the particulate fraction of the DE since this fraction contains the substances that are held responsible for the induction of lung tumors in rats [Heinrich et al., 1986; Iwai et al., 1986; Ishinishi et al., 1986; Brightwell et al., 1986, 1989]. In a recent study in diesel engine repair shops, Hammond et al. [1993] demonstrated the absence of mutagenicity in vapor-phase samples.

The microsuspension assay was conducted according to Kado et al. [1983] with minor adjustments (for practical reasons): the concentrated bacteria were resuspended in cold Vogel-Bonner medium E [Vogel and Bonner, 1956], instead of phosphate-buffered saline (0.15 M, pH 7.4), to concentrations of 10<sup>10</sup> per ml. The liver S9 fraction was prepared from male Wistar rats, pretreated with Aroclor 1254 [Maron and Ames, 1983]. In Table III the spontaneous mutagenicity; the mutagenicity of the organic solvent; and the mutagenicity of 2-NF, 1-NP, and B[a]P in the four *Salmonella* strains are presented. The dry extracts of the air samples were reconstituted in DMSO and at least three dilutions were prepared from a stock. An approximate dilution factor was determined by comparison of the 1-NP content with the results from assaying 1-NP standard dilutions in DMSO. A good linear dose-response relationship ( $r = 0.999$ ) was observed in the range 4–40 ng 1-NP/plate (results not shown). Triplicate plates were poured from one tube for each dilution tested.

The samples that were collected from the workroom air of the aluminum rolling facility (site 4) were toxic to the bacteria at the lowest dose tested. The mutagenic potencies of these samples could not be determined. In the sample from the farm (site 7), no 1-NP was detected. These samples were excluded from further analysis.

## Calculations

From determinations of the mutagenicity in triplicate, arithmetic means were calculated. Dose-response curves were constructed from the mutagenicity data that were acquired at different dose levels. The slope of the linear component was used as an estimate of the mutagenic potency according to Krewski and coworkers [1992]. Linearity was judged from the regression coefficient. At  $r$ -values greater than 0.95 the assumption of linearity was accepted. From each series of samples collected during one working period, a time-weighted average (TWA) of TSPM, RSPM, ISPM, 1-NP, and mutagenicity data was calculated over that period. The mutagenic potencies (rev/m<sup>3</sup>) per day and per workplace were plotted against the 1-NP content of the particulate extracts. Linearity was evaluated by regression analysis.

## RESULTS

In this study we have collected samples of airborne particulate matter at workplaces contaminated with DE. The 1-NP content of the particulate extracts was determined by GC-HRMS. The mutagenic potency of each sample was tested

in *S. typhimurium* TA98, TA1538, YG1021, and YG1024. We have evaluated the association between the 1-NP content and the mutagenic potency of the extracts both in the presence and the absence of rat liver S9.

The time-weighted average TSPM, RSPM, ISPM, and 1-NP air levels are presented in Table IV. In all of the indoor workplaces 1-NP could be detected. In one of the outdoor workplaces 1-NP was not detected (site 7, turning of hay at the farm). The lowest detectable TWA air level was observed at the reference location (0.0017 ng/m<sup>3</sup>), whereas the highest TWA levels were observed in indoor workplaces associated with the use of forklifts (0.14–1.2 ng/m<sup>3</sup>, sites 3–6). The 1-NP content of the TSPM varied from 0.018 to 7.8  $\mu\text{g}/\text{g}$ . Expressed as ng/g acetone extractable matter, it varied from 0.076 in the reference sample to 17  $\mu\text{g}/\text{g}$  in a sample collected in the aluminum rolling workshop.

Air levels of inhalable dust in the breathing zones of the workers showed considerable variation but remained under 4.0 mg/m<sup>3</sup> at all workplaces. The amount of particulate matter collected on each of the filters and filter cassettes was too small for further mutagenicity testing.

The mutagenic potency of the sample extracts decreased after addition of S9 (Tables V–VIII). This was the case for most of the samples tested with all four strains. The mutagenic potency per microgram dust or dry extract showed considerable variation (up to two orders of magnitude). The mutagenicity of the air samples (expressed as rev/m<sup>3</sup>) varied over one order of magnitude. The results obtained in the strain YG1024 were 2–5 times higher than the mutagenicity detected in TA98. The mutagenicity of the extracts of indoor TSPM samples were significantly correlated with the 1-NP content of the extracts (see Figs. 1–4). This correlation was observed in all four *Salmonella* strains (with or without S9) that were used in this study. We did not observe a significant correlation between the mutagenic potency and TSPM or its SOF.

## DISCUSSION

We have investigated the suitability of 1-NP as a marker for DE mutagenicity. This compound is one of the most abundant nitro-derivatives of PAHs identified in DE. 1-NP is known to be associated with the exhaust particles and is not detected in the semivolatile or vapor fraction [IARC, 1989].

