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

Paul T.J. Scheepers, Marty H.J. Martens, Dick D. Velders, Peter Fijneman, Marco van Kerkhoven, Jan Noordhoek, and Rob P. Bos

Toxicology Department, University of Nijmegen, Nijmegen, The Netherlands

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 μg/g acetone extractable matter, corresponding to air concentrations of 0.012 to 1.2 ng/m^3. A sample collected in a rural area contained 0.0017 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 exogeneous 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 ($r = 0.80$–0.91) was observed in all of the *S. typhimurium* strains. High correlations ($r = 0.80$–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.

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 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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Address reprint requests to P.T.J. Scheepers, Toxicology Department, University of Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands.

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; Guillemin 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 [Guillemin 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 carcinogenic 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 [^{14}C]-labeled B[*a*]P and [^{3}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*

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

*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 ~1.0 m^3/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 ~5 μm at a flow rate of 0.050 m^3/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^3/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-[$^2H_9$]-pyrene (1-N[$^2H_9$]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[a]	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 maintainance	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[b]	<2	—
b		Transport of aluminum	16.03	I	Forklifts[c]	<2	—
5 a	Galvanization work shop	Transport of iron	21.04	I/O	Forklifts[d]	5	SE
b		Lifting iron	22.04	I/O	Forklifts[e]	5	W
c		Transport of iron	23.04	I/O	Forklifts[d]	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	Nijmegen-Antwerpen	17.04	O	Ship's engine[f]	8	WSW
b	of bulk chemicals	Antwerpen Harbour	18.04	O	Ship's engine[g]	9	W
c		Rotterdam-Nijmegen	19.04	O	Aggregate[f]	8	NW
12 a	Loading and unloading	Intercontinental flights	14.04	O	Platform vehicles[h]	11	SW
b	of air cargo	Continental flights	15.04	O	Platform vehicles[h]	13	WNW
c		Intercontinental flights	09.06	O	Platform vehicles[h]	5	NE
d		Continental flights	10.06	O	Platform vehicles[h]	6	NE

[a]I = indoor workplace; O = outdoor workplace.
[b]Truck fueled with low sulfur diesel fuel (<0.01 w% S).
[c]Truck fueled with normal sulfur diesel fuel (<0.2 w% S).
[d]Old forklift (>15 yr).
[e]New forklift (<2 yr).
[f]During journey.
[g]During (un)loading of cargo.
[h]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_2 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[$^2H_9$]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_2, and reduced with NaSH in ethanol and aqua pure. The amino analogues were derivatized with heptafluorobutyric anhydride and dried under N_2. The residue was dissolved in isooctane 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*

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

*Mean values of the determinations in triplicate (±SD).

umes of 30–150 m^3 of sampled air) were available. Therefore, the microsuspension method [Kado et al., 1983] was used. This method has a higher sensitivity as compared to the standard plate-incorporation method [Agurell 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^{10} 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^3) 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^3), whereas the highest TWA levels were observed in indoor workplaces associated with the use of forklifts (0.14–1.2 ng/m^3, sites 3–6). The 1-NP content of the TSPM varied from 0.018 to 7.8 μg/g. Expressed as ng/g acetone extractable matter, it varied from 0.076 in the reference sample to 17 μg/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^3 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^3) 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].

TABLE IV. Measured Concentrations (GM ± GSD) of TSPM, RSPM, and 1-NP in Air Sampled at Workplaces Associated With the Use of Diesel-Powered Engines*

Site No.	Description	Sources producing DE	N	1-NP (ng/m³)	TSPM (μg/m³)	RSPM (μg/m³)	ISPM (mg/m³) GM	ISPM (mg/m³) GSC	ISPM (mg/m³) n
0	Reference	Remote sources	1	0.0017	49	41	—	—	—
1	Gardening	Traffic	1	0.036	719	66	0.4	1.1	2
2	Grass verge maintenance	Lawn mowers	1	0.0066	660	166	0.5	1.0	2
3	Storage of chemicals	Forklifts	4	0.21 ± 2.0	92 ± 1.6	44 ± 1.5	1.8	1.6	11
4	Aluminum rolling	Forklifts	2	1.05 ± 1.2	162 ± 1.0	130 ± 1.0	0.6	1.6	6
5	Galvanization workshop	Forklifts	3	0.12 ± 1.3	378 ± 1.2	130 ± 1.2	0.6	1.5	6
6	Concrete manufacturing	Forklifts	2	0.52 ± 1.4	1,711 ± 1.7	245 ± 1.4	2.0	1.4	6
7	Farming	Tractor	1	ND	383	82	—	—	—
8	Flower auction	Trucks	2	0.081 ± 1.0	225 ± 1.1	88 ± 1.2	1.4	1.7	5
9	Army driving lessons	Armoured cars	2	0.012[a]	117 ± 1.1	53 ± 1.9	1.2	1.5	7
10	Repair shop for trains	Trains	2	0.41 ± 1.1	165 ± 1.1	104 ± 1.0	1.6	1.7	8
11	River vessel	Ship's engine	2	0.034 ± 1.9	51 ± 1.0	40 ± 1.3	0.8	1.7	3
		Power supply	1	0.79	104	79			
12	Airport platform	Platform vehicles	3	0.034 ± 1.4	82 ± 1.4	81 ± 1.1	0.6	1.9	18

*N = number of workplaces studied; n = number of workers studied; ND = not detected (see text for detection limits); — = Not determined.
[a]Value represents only 40% of working period.

TABLE V. Mutagenic Potency (±SD) of Extracts of Diesel Engine-Derived Particle Samples in *S. typhimurium* TA98

No.	Source	TSPM (rev/μg dust) +S9	TSPM (rev/μg dust) −S9	SOF (rev/μg extract) +S9	SOF (rev/μg extract) −S9	Air (rev/m³) +S9	Air (rev/m³) −S9
0	Remote sources	0.7 ± 0.1	1.2 ± 0.3	16 ± 3	26 ± 3	39 ± 3	55 ± 5
1	Traffic	1.6 ± 0.2	2.7 ± 0.4	49 ± 5	82 ± 13	121 ± 4	204 ± 3
2	Lawn mower	2.5 ± 0.2	2.9 ± 0.7	35 ± 3	42 ± 10	160 ± 1	186 ± 4
3 a	Forklifts	0.3 ± 0.0	0.6 ± 0.1	10 ± 1	21 ± 3	16 ± 5	47 ± 2
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
c		0.5 ± 0.3	1.5 ± 1.2	8 ± 5	25 ± 20	17 ± 5	21 ± 11
d		1.1 ± 0.1	1.2 ± 0.3	34 ± 3	40 ± 10	82 ± 1	95 ± 2
4 a	Forklifts	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
5 a	Forklifts	0.1 ± 0.1	0.1 ± 0.0	8 ± 2	13 ± 3	48 ± 26	29 ± 1
b		0.1 ± 0.0	0.3 ± 0.0	8 ± 3	43 ± 6	64 ± 8	121 ± 10
c		0.1 ± 0.0	0.1 ± 0.0	5 ± 1	6 ± 1	23 ± 9	22 ± 9
6 a	Forklifts	0.1 ± 0.0	0.1 ± 0.0	23 ± 4	25 ± 6	119 ± 22	141 ± 14
b		0.1 ± 0.0	0.2 ± 0.0	25 ± 4	60 ± 7	179 ± 31	295 ± 90
7	Tractor	0.0 ± 0.0	0.1 ± 0.0	1 ± 1	4 ± 1	57 ± 6	87 ± 1
8 a	Trucks	0.2 ± 0.1	0.4 ± 0.1	16 ± 6	32 ± 8	44 ± 6	110 ± 10
b		0.3 ± 0.0	0.6 ± 0.1	24 ± 3	48 ± 9	65 ± 9	161 ± 2
9 a	Armoured vehicles	0.2 ± 0.1	0.8 ± 0.1	7 ± 2	28 ± 2	7 ± 6	77 ± 2
b		0.3 ± 0.2	0.6 ± 0.1	7 ± 4	11 ± 3	45 ± 4	72 ± 1
10 a	Trains	0.6 ± 0.1	1.1 ± 0.1	32 ± 4	56 ± 6	106 ± 4	155 ± 2
b		0.4 ± 0.0	0.9 ± 0.1	36 ± 3	75 ± 8	75 ± 12	155 ± 13
11 a	Ship's engine	0.9 ± 0.3	1.5 ± 0.4	14 ± 4	24 ± 6	78 ± 1	90 ± 2
b		0.2 ± 0.0	0.3 ± 0.0	3 ± 1	6 ± 1	7 ± 1	19 ± 2
c		1.1 ± 0.6	1.9 ± 0.7	12 ± 7	22 ± 8	29 ± 2	52 ± 7
d	Power supply	2.6 ± 0.1	4.6 ± 0.3	164 ± 4	288 ± 22	267 ± 3	446 ± 58
12 a	Platform vehicles	0.5 ± 0.1	1.8 ± 0.5	5 ± 1	8 ± 1	16 ± 1	95 ± 6
b		0.3 ± 0.1	0.5 ± 0.0	8 ± 2	12 ± 1	29 ± 3	36 ± 5
c		0.3 ± 0.1	0.7 ± 0.1	6 ± 1	15 ± 2	20 ± 2	59 ± 6

[a]Sample exhibits cytotoxicity.

Extracts of DE particles exhibit a strong mutagenic potency in the Salmonella mutagenicity assay in the absence of an exogeneous metabolic system [Huisingh et al., 1978]. Nitro-PAHs were observed to contribute considerably to the direct-acting mutagenicity [Manabe et al., 1985]. 1-NP itself may not be one of the strongest mutagens among nitro-PAH [Rosenkranz and Mermelstein, 1985] and only a limited portion of the mutagenicity of the total extract can be attributed directly to 1-NP [Nakagawa et al., 1983; Lewtas, 1988]. Nevertheless, the abundance of 1-NP as a precursor

TABLE VI. Mutagenic Potency (± SD) of Extracts of Diesel Engine-Derived Particle Samples in *S. typhimurium* TA1538

No.	Source	TSPM (rev/μg dust) +S9	TSPM (rev/μg dust) −S9	SOF (rev/μg extract) +S9	SOF (rev/μg extract) −S9	Air (rev/m^3) +S9	Air (rev/m^3) −S9
0	Remote sources	0.3 ± 0.1	0.6 ± 0.1	6 ± 1	13 ± 2	16 ± 1	30 ± 3
1	Traffic	0.8 ± 0.1	1.7 ± 0.1	26 ± 3	52 ± 4	61 ± 0	119 ± 2
2	Lawn mower	0.0 ± 0.2	0.1 ± 0.0	0 ± 3	6 ± 2	0 ± 1	36 ± 7
3 a	Forklifts	0.5 ± 0.0	0.1 ± 0.1	18 ± 2	29 ± 3	43 ± 0	66 ± 5
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
c		0.5 ± 0.1	1.5 ± 0.9	9 ± 1	24 ± 25	20 ± 4	28 ± 2
d		2.0 ± 0.3	1.7 ± 0.1	45 ± 4	65 ± 9	116 ± 3	133 ± 9
4 a	Forklifts	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
5 a	Forklifts	0.1 ± 0.0	0.1 ± 0.0	10 ± 2	21 ± 2	71 ± 1	110 ± 47
b		0.1 ± 0.0	0.1 ± 0.0	13 ± 3	22 ± 5	32 ± 1	39 ± 13
c		0.1 ± 0.0	1.0 ± 0.0	4 ± 1	7 ± 1	38 ± 3	37 ± 4
6 a	Forklifts	0.1 ± 0.0	0.1 ± 0.0	27 ± 5	24 ± 4	143 ± 4	112 ± 6
b		0.1 ± 0.0	0.2 ± 0.1	22 ± 3	56 ± 32	197 ± 7	243 ± 16
7	Tractor	0.0 ± 0.0	0.1 ± 0.1	1 ± 1	7 ± 4	7 ± 5	14 ± 5
8 a	Trucks	0.2 ± 0.0	0.5 ± 0.0	19 ± 3	45 ± 4	54 ± 7	115 ± 6
b		0.2 ± 0.0	0.4 ± 0.0	17 ± 2	34 ± 4	55 ± 1	102 ± 6
9 a	Armoured vehicles	0.2 ± 0.1	0.8 ± 0.1	7 ± 2	28 ± 2	20 ± 6	45 ± 3
b		0.0 ± 0.1	0.2 ± 0.3	0 ± 3	5 ± 6	2 ± 22	48 ± 15
10 a	Trains	2.0 ± 0.5	0.9 ± 0.1	26 ± 8	42 ± 4	76 ± 2	109 ± 1
b		0.4 ± 0.0	0.6 ± 0.0	37 ± 4	53 ± 4	83 ± 16	107 ± 11
11 a	Ship's engine	1.1 ± 0.2	2.0 ± 0.3	19 ± 2	35 ± 7	33 ± 6	58 ± 5
b		0.3 ± 0.0	0.4 ± 0.0	6 ± 1	9 ± 1	23 ± 1	30 ± 2
c		0.2 ± 0.3	0.3 ± 0.0	10 ± 2	12 ± 0	33 ± 4	40 ± 6
d	Power supply	2.3 ± 0.0	2.5 ± 0.2	146 ± 3	159 ± 11	227 ± 32	239 ± 2
12 a	Platform vehicles	0.0 ± 0.4	0.2 ± 0.5	0 ± 5	3 ± 5	0 ± 21	33 ± 1
b		0.3 ± 0.1	0.3 ± 0.0	7 ± 2	12 ± 1	27 ± 4	30 ± 7
c		0.3 ± 0.1	0.3 ± 0.1	16 ± 4	6 ± 2	33 ± 2	71 ± 3

[a]Sample exhibits cytotoxicity.

of dinitropyrenes, hydroxynitropyrenes, and acetoxynitropyrenes was observed to be *associated* with the appearance of these strong mutagens [Manabe et al., 1985; Nakagawa et al., 1983; Schuetzle et al., 1985] and was *associated* with a considerable part of the DE mutagenicity [Pederson and Siak, 1981; Nakagawa et al., 1983; Salmeen et al., 1984; Austin et al., 1985].

In the workroom atmospheres studied, the highest air concentrations of 1-NP occurred in workplaces associated with the indoor use of forklifts (cf. Tables V–VIII). This is consistent with high emissions observed during transient load conditions (lifting heavy loads), but may also be caused by higher emissions of nitro-PAHs that are usually observed in light duty diesel engines with indirect fuel injection as compared to heavy duty engines with direct fuel injection [Yamaki et al., 1986]. In addition, most workplaces associated with forklifts were indoor (or at least partly indoor) locations that were poorly ventilated.

The levels of different fractions of suspended particulate matter (TSPM, RSPM, and ISPM) were not mutually consistent. We suggest that this may be caused by the different sources, with corresponding differences in particle size distributions, that were studied. In most cases, the respirable dust fraction constituted only a small portion of the TSPM because at several workplaces vast amounts of relatively large particles were suspended into the air (e.g., gardening, lawn-mowing, turning of hay, cross-country driving of armoured cars). In a few workplaces the collected dust consisted mainly of respirable particles. Activities at these workplaces mainly consisted of vehicle movements on paved roads (site 12, airport platform).

The mutagenicity of the sample extracts observed after testing with the strain YG1021 was only 30% increased as compared to the results obtained with TA98, independent of addition of S9. Apparently, the overcapacity in nitroreductase activity, such as is present in YG1021, did not cause much of an increase in the mutagenicity of the DE-derived extracts, and nitroreductase activity in strain TA98 was sufficient to activate the nitroaromatics present in the particulate extract. This is an interesting observation regarding the discrepancy of these results with the mutagenicity of individual nitro-PAHs. In that case, the bacterial strain YG1021 was much more sensitive to nitroarenes as compared to TA98 as shown in Table III [Einistö et al., 1990]. These results suggest that the metabolic activation of nitroaromatic compounds by nitroreduction is counteracted by other constituents in the DE particulate extract or that other mutagens are involved.

TABLE VII. Mutagenic Potency (±SD) of Extracts of Diesel Engine-Derived Particle Samples in *S. typhimurium* YG1021

No.	Source	TSPM (rev/μg dust) +S9	TSPM (rev/μg dust) −S9	SOF (rev/μg extract) +S9	SOF (rev/μg extract) −S9	Air (rev/m^3) +S9	Air (rev/m^3) −S9
0	Remote sources	0.6 ± 0.2	2.0 ± 0.1	13 ± 3	43 ± 1	32 ± 8	101 ± 4
1	Traffic	1.2 ± 0.1	1.9 ± 0.1	57 ± 4	52 ± 4	99 ± 29	136 ± 3
2	Lawn mower	1.0 ± 0.1	1.4 ± 0.1	8 ± 1	19 ± 1	35 ± 5	49 ± 0
3 a	Forklifts	1.1 ± 0.2	0.1 ± 0.1	38 ± 6	29 ± 3	89 ± 15	161 ± 7
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
c		0.6 ± 0.2	0.9 ± 0.2	10 ± 3	33 ± 8	17 ± 4	25 ± 3
d		1.4 ± 0.1	2.3 ± 0.1	45 ± 4	73 ± 4	98 ± 29	165 ± 0
4 a	Forklifts	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
5 a	Forklifts	0.2 ± 0.0	0.2 ± 0.0	12 ± 1	23 ± 1	86 ± 2	97 ± 1
b		0.1 ± 0.0	0.3 ± 0.0	28 ± 1	41 ± 2	76 ± 3	89 ± 8
c		0.1 ± 0.0	0.1 ± 0.0	9 ± 3	7 ± 1	12 ± 1	20 ± 2
6 a	Forklifts	0.1 ± 0.0	0.2 ± 0.0	32 ± 1	37 ± 5	153 ± 2	157 ± 4
b		0.1 ± 0.0	0.9 ± 0.1	12 ± 4	47 ± 2	174 ± 5	353 ± 14
7	Tractor	0.0 ± 0.0	0.1 ± 0.0	1 ± 2	3 ± 2	6 ± 2	27 ± 6
8 a	Trucks	0.3 ± 0.0	0.5 ± 0.0	24 ± 3	41 ± 2	62 ± 19	108 ± 2
b		0.2 ± 0.0	0.5 ± 0.0	18 ± 2	43 ± 3	53 ± 7	112 ± 22
9 a	Armoured vehicles	0.2 ± 0.0	0.6 ± 0.0	8 ± 1	22 ± 2	24 ± 4	61 ± 4
b		0.0 ± 0.2	0.2 ± 0.3	0 ± 3	4 ± 5	21 ± 12	16 ± 8
10 a	Trains	0.7 ± 0.0	0.8 ± 0.2	33 ± 3	36 ± 7	156 ± 9	155 ± 21
b		0.6 ± 0.0	1.0 ± 0.0	52 ± 3	90 ± 4	119 ± 8	203 ± 18
11 a	Ship's engine	1.3 ± 0.2	2.0 ± 0.1	22 ± 3	34 ± 1	46 ± 9	60 ± 4
b		0.2 ± 0.1	0.3 ± 0.0	5 ± 1	7 ± 1	12 ± 2	20 ± 2
c		1.4 ± 0.3	2.1 ± 0.2	16 ± 4	24 ± 3	45 ± 17	60 ± 3
d	Power supply	3.1 ± 0.4	4.7 ± 0.2	197 ± 22	298 ± 11	318 ± 5	493 ± 7
12 a	Platform vehicles	0.2 ± 0.4	0.5 ± 0.5	2 ± 5	5 ± 6	8 ± 2	34 ± 4
c		0.5 ± 0.1	0.7 ± 0.1	13 ± 3	18 ± 2	48 ± 1	59 ± 2
d		0.2 ± 0.1	0.9 ± 0.2	5 ± 2	19 ± 4	18 ± 9	73 ± 4

[a]Sample exhibits cytoxicity.

The highest mutagenicity was observed in strain YG1024. Enhancement of the mutagenicity in strain YG1024 up to a factor 3, relative to TA98 and YG1021, is believed to be caused by further *O*-acetylation of the *N*-hydroxylamines as a result of the 15–30 times increased levels of the bacterial OAT activity [Josephy, 1989; Einistö et al., 1990]. This confirms the prominent role of nitroaromatic compounds to the mutagenic potency of the workplace samples and makes a significant contribution of other mutagens (such as suggested above) unlikely. The higher sensitivity of strain YG1024 relative to TA98 was also observed in the analysis of outdoor air samples [Houk et al., 1992]. In the diversity of DE-derived samples collected in this study, enhanced OAT activity induced a much higher mutagenic potency than enhanced nitroreduction activity. Since the *O*-acetylation is also an essential activating biotransformation step in human metabolism, this could have implications for the human toxicity of DE-derived organics.

The correlation of the mutagenicity with the 1-NP content was studied in samples derived from a wide variety of mobile sources in different workplaces (Figs. 1–4). The regression analysis showed a high correlation between the 1-NP concentrations and mutagenic potencies (in four *S. typhimurium* strains) of the TSPM extracts. The regression equations (Table IX) suggest that the 1-NP content of the extracts of workplace-derived DE particulate samples can be used to assess the exposure to the DE-derived mutagenic potency. This high correlation was also observed in a study based on outdoor samples collected at different sampling sites in the Iwate Prefecture, Japan [Saitoh et al., 1990].

The mutagenicity level (in the presence of S9-mix) remaining when the 1-NP level is extrapolated to zero (equal to the intercept in Figs. 1–4) is relatively small (approximately two times the level of spontaneous mutagenicity, Table III). In the absence of an exogenous metabolizing system the residual mutagenicity is much higher, suggesting that other direct-acting mutagens not associated with the abundance of 1-NP may be present. These mutagens could be nitro-PAH from non-combustion sources, such as from photochemical origin or other direct-acting mutagens. Some investigators have shown that the microsuspension assay responds especially well to nitro compounds [Agurell and Stensman, 1992]. However, since nitroaromatics are also strong mutagens in the standard (plate incorporation) mutagenicity assay we consider the disappearance of the observed association under those conditions unlikely.

TABLE VIII. Mutagenic Potency (±SD) of Extracts of Diesel Engine-Derived Particle Samples in *S. typhimurium* YG1024

No.	Source	TSPM (rev/μg dust)		SOF (rev/μg extract)		Air (rev/m³)	
		+S9	−S9	+S9	−S9	+S9	−S9
0	Remote sources	1.7 ± 0.1	7.0 ± 0.3	35 ± 3	146 ± 6	80 ± 5	346 ± 12
1	Traffic	3.3 ± 0.2	6.3 ± 0.6	99 ± 6	191 ± 18	234 ± 21	453 ± 20
2	Lawn mower	0.8 ± 0.2	1.4 ± 0.2	11 ± 2	20 ± 2	52 ± 1	99 ± 15
3 a	Forklifts	0.4 ± 0.1	1.0 ± 0.2	12 ± 1	35 ± 6	28 ± 8	52 ± 7
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
c		0.4 ± 0.1	3.4 ± 0.1	24 ± 14	55 ± 24	34 ± 4	77 ± 3
d		3.6 ± 0.4	7.8 ± 0.3	115 ± 14	251 ± 9	283 ± 11	638 ± 25
4 a	Forklifts	—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
b		—[a]	—[a]	—[a]	—[a]	—[a]	—[a]
5 a	Forklifts	0.2 ± 0.1	0.7 ± 0.3	23 ± 2	146 ± 6	158 ± 13	383 ± 7
b		0.3 ± 0.1	0.9 ± 0.0	54 ± 12	134 ± 8	149 ± 3	362 ± 1
c		0.1 ± 0.0	0.3 ± 0.0	11 ± 4	23 ± 6	67 ± 3	133 ± 5
6 a	Forklifts	0.4 ± 0.0	0.6 ± 0.1	88 ± 3	129 ± 9	631 ± 16	977 ± 6
b		0.2 ± 0.0	0.5 ± 0.0	78 ± 5	168 ± 27	528 ± 35	971 ± 19
7	Tractor	0.2 ± 0.1	0.2 ± 0.0	9 ± 1	7 ± 1	71 ± 16	50 ± 17
8 a	Trucks	0.9 ± 0.1	1.8 ± 0.3	75 ± 4	147 ± 14	185 ± 3	380 ± 19
b		0.7 ± 0.1	1.9 ± 0.1	57 ± 8	158 ± 5	157 ± 4	466 ± 13
9 a	Armoured vehicles	0.9 ± 0.1	1.7 ± 0.9	34 ± 3	61 ± 32	60 ± 3	150 ± 21
b		0.4 ± 0.4	0.8 ± 0.1	8 ± 7	16 ± 3	52 ± 9	109 ± 14
10 a	Trains	1.7 ± 0.1	3.1 ± 0.2	90 ± 5	157 ± 9	293 ± 13	447 ± 27
b		2.0 ± 0.1	3.8 ± 0.2	163 ± 9	313 ± 14	330 ± 22	830 ± 31
11 a	Ship's engine	3.5 ± 0.3	8.3 ± 0.9	63 ± 5	146 ± 15	285 ± 14	659 ± 24
b		0.9 ± 0.0	1.0 ± 0.1	18 ± 1	20 ± 3	42 ± 5	48 ± 8
c		2.7 ± 0.4	6.9 ± 1.7	32 ± 5	80 ± 20	78 ± 3	208 ± 10
d	Power supply	8.0 ± 0.2	10.3 ± 0.2	505 ± 12	649 ± 15	847 ± 1	927 ± 22
12 a	Platform vehicles	0.8 ± 1.4	1.4 ± 1.0	9 ± 14	15 ± 10	8 ± 6	34 ± 4
c		0.4 ± 0.1	1.6 ± 0.2	11 ± 1	40 ± 4	40 ± 6	151 ± 10
d		1.1 ± 0.1	3.0 ± 0.5	24 ± 2	64 ± 11	66 ± 1	228 ± 7

[a]Sample exhibits cytotoxicity.

The scatter observed in the relation between mutagenic potency and 1-NP content may be caused by variations in the physico-chemical characteristics originating from engine type, use and maintenance, fuel characteristics, and sampling conditions [Salmeen et al., 1984]. Outdoor samples with a low 1-NP content exhibit a relatively poor association between 1-NP and mutagenic potency as compared to (indoor) samples with a higher 1-NP content (see Figs. 1–4). Beside artifacts introduced by sampling methods and analysis procedures (that may be more pronounced in samples with a lower 1-NP content and mutagenic potency), the presence of compounds originating from photo-induced chemical transformation, such as nitroarenes (other than 1-NP) and hydroxylated nitroarenes, may contribute to the mutagenic potency in the low range (outdoor) samples [Nielsen et al., 1984; Lewtas and Nishioka, 1990]. This may lead to a higher mutagenic potency than expected on the basis of the 1-NP content. Lewtas [1988] reported that in outdoor samples the relative contribution of 1-NP to the direct-acting mutagenicity in TA98 was much smaller compared to its contribution in sample extracts from vehicle emissions. However, this effect does not seem to have a major influence on the results presented in this study.

Some other sources of variability have been reported: Ambient factors such as irradiation of sunlight may cause photodecomposition of nitro-PAHs [Stärk et al., 1985]. The mutagenic potency of a single nitro-PAH was observed to be influenced by other constituents in the DE particulate extract [Matsushita et al., 1986]. The chemical composition and mutagenic potency may be related to the origin of the collected particulates and depend on the wind direction during sampling [Van Houdt et al., 1987]. Influence of the season has also been indicated as a time-dependent source of variation: In outdoor air samples collected during November and December at one fixed location in Tokyo (Japan), a correlation between the mutagenicity and the APM concentrations was observed [Houk et al., 1992]. In another study using data from five different locations in Japan during December–March considerable scatter was observed when plotting mutagenicity versus the APM concentration [Saitoh et al., 1990].

The great variability in the dust-specific mutagenic potencies (rev/μg dust) observed in our study (cf. Tables V–VIII) indicated that TSPM and RSPM levels cannot be used as markers for the mutagenic properties of extracts derived from samples collected in workroom atmospheres. Also, the

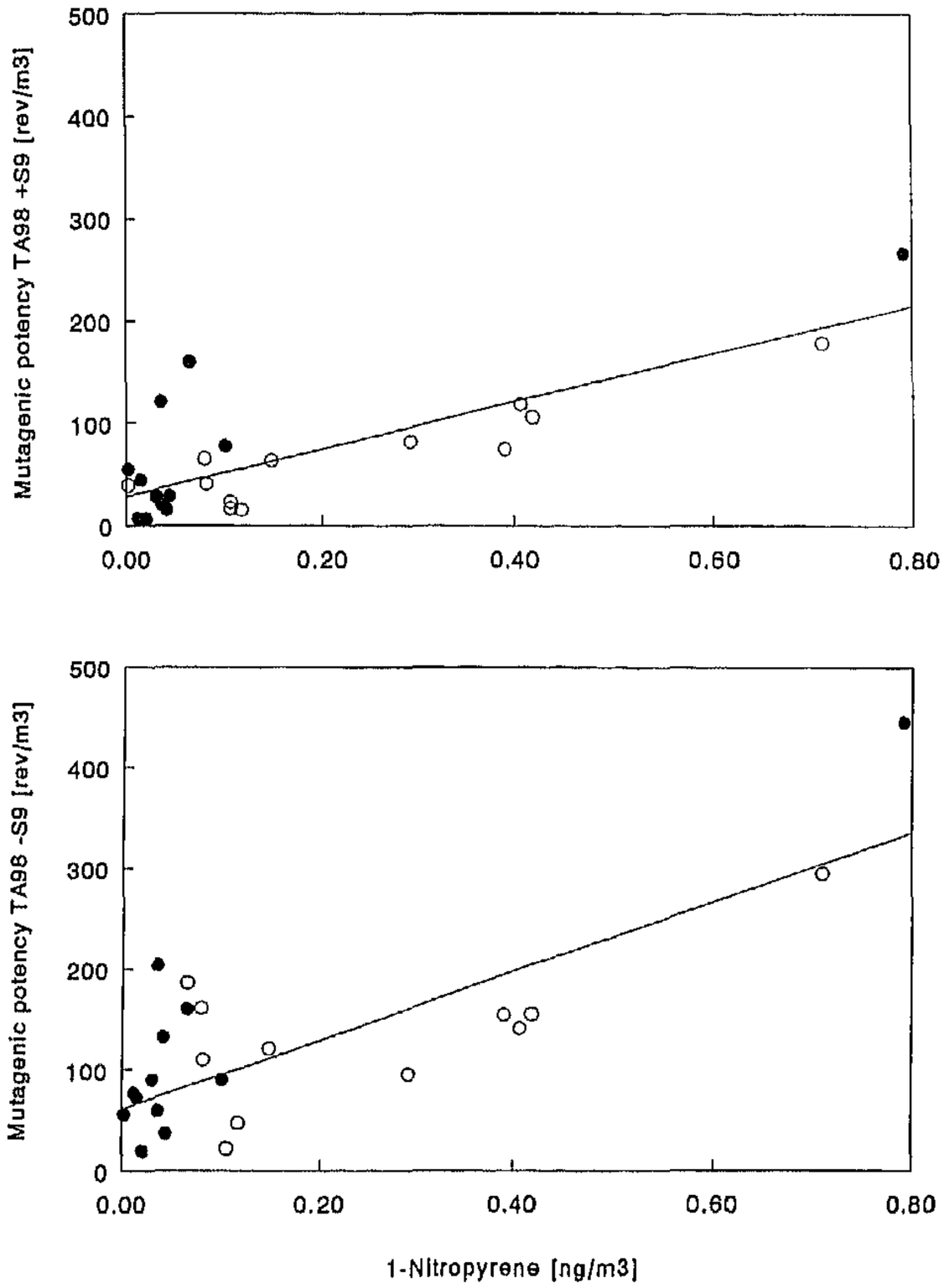

Fig. 1. Correlation of mutagenic potency and 1-NP content of TSPM samples collected from workplace atmospheres. Mutagenicity as determined in *S. typhimurium* TA98 with S9 **(upper panel)** and without S9 **(lower panel)**. Open circles represent indoor samples; closed circles represent outdoor samples.

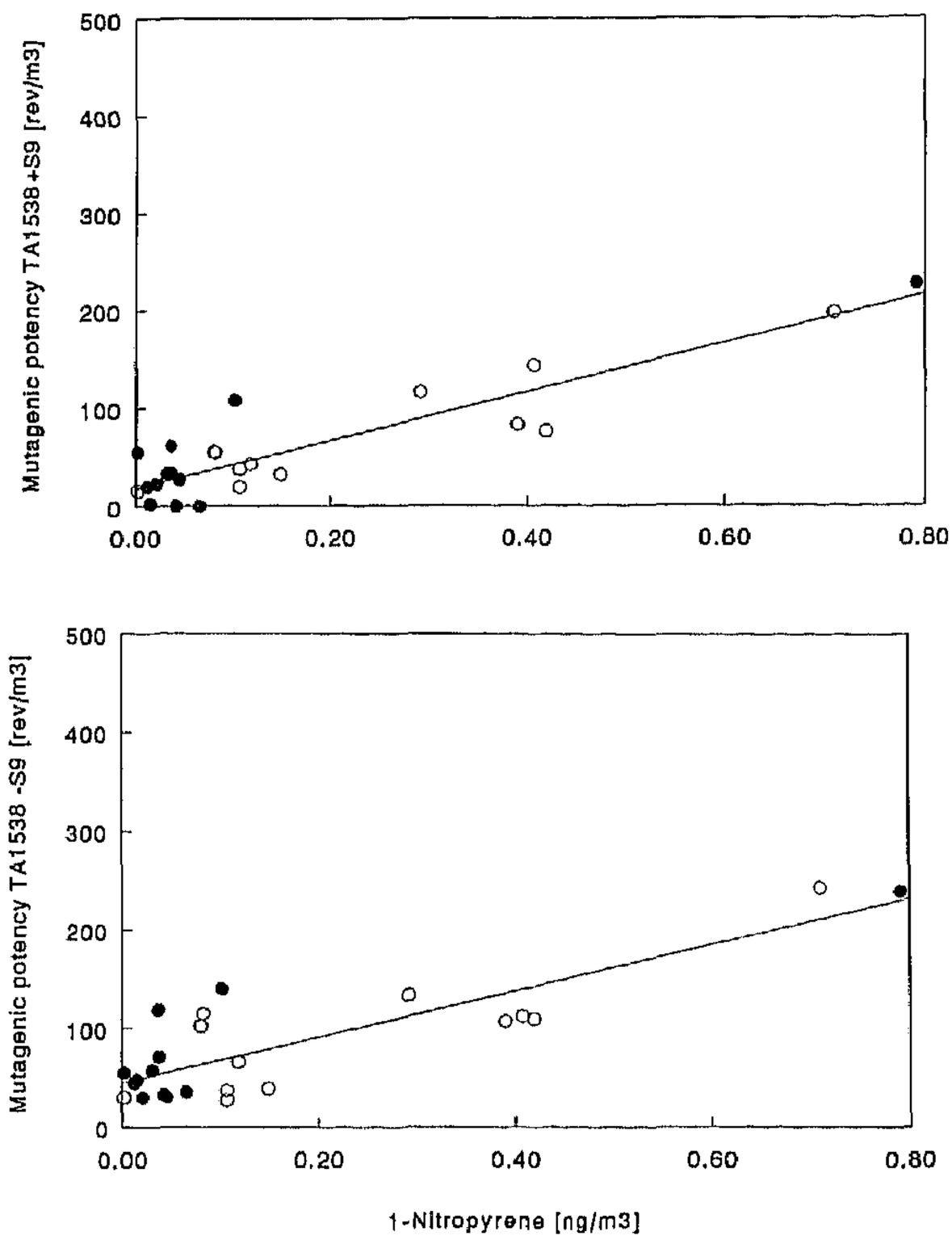

Fig. 2. Correlation of mutagenic potency and 1-NP content of TSPM samples collected from workplace atmospheres. Mutagenicity as determined in *S. typhimurium* TA1538 with S9 **(upper panel)** and without S9 **(lower panel)**. Open circles represent indoor samples; closed circles represent outdoor samples.

mutagenic potency expressed as rev/μg dry acetone extract showed a large variation. This may be caused by considerable emissions of soluble organic material derived from sources other than (diesel) combustion. Consequently, SOF cannot be used as a marker for DE-derived particle-extracted mutagens. In this study, the most important non-diesel aerosol sources were organic plant material and soil particles (gardening, grass verge maintenance, farming, flower auction, and army driving lessons), zinc chloride and ferrous oxides (galvanisation workshop), silicates (concrete manufacturing), and welding fumes (repair shop for trains).

The mechanism of DE carcinogenicity has not yet been elucidated. As a consequence, it is not possible to identify specific chemicals as key determinants of the carcinogenic risk. Nevertheless, Lewtas [1993] observed a correlation between the mutagenic potencies in TA98 (with S9) and the mouse skin tumor initiation potency of a range of combustion emissions. For emissions from coke ovens, roofing (coal) tar, and cigarette smoke condensate, the relative tumor initiating potency was found to be correlated with the human lung cancer potency. The unit risk estimate of DE for lung cancer (derived from a lifetime rodent inhalation carinogenesis study) fits in this linear relationship, suggesting that this model would also apply to DE [Nesnow, 1990]. Thus, for DE (and the other combustion emissions) the mouse skin tumor initiating potency could be a useful estimate of the lung cancer risk for human exposure. As a direct consequence, the mutagenic potency could also be used as an indicator of the human lung cancer risk.

In summary, we have studied the use of markers for the genotoxic properties of DE emissions by determination of the bacterial mutagenicity of acetone extracts of TSPM sampled from atmospheres of workplaces associated with the use of a variety of diesel engines. We have observed that TSPM air levels and their SOFs cannot be used as indicators of particle-associated mutagenicity because of interference by aerosols from non-DE sources. This study shows that, despite several known and unknown sources of interference and dispersion (source variability, photochemistry, and other atmospheric conditions), the mutagenic potency of extracts of DE-derived particles is significantly associated with the 1-NP content of the sample extract. Therefore,

Fig. 3. Correlation of mutagenic potency and 1-NP content of TSPM samples collected from workplace atmospheres. Mutagenicity as determined in *S. typhimurium* YG1021 with S9 **(upper panel)** and without S9 **(lower panel)**. Open circles represent indoor samples; closed circles represent outdoor samples.

Fig. 4. Correlation of mutagenic potency and 1-NP content of TSPM samples collected from workplace atmospheres. Mutagenicity as determined in *S. typhimurium* YG1024 with S9 **(upper panel)** and without S9 **(lower panel)**. Open circles represent indoor samples; closed circles represent outdoor samples.

TABLE IX. Parameters of the Correlation of the Mutagenic Potency Versus the 1-NP Content of Extracts of TSPM Samples (N = 24, $P < 0.0001$ for All Strains With and Without Activation)

Bacterial strain	S9	Intercept (rev/m^3)	Mutagenic potency (rev/ng 1-NP)	R
TA98	+	30 ± 10	231 ± 37	0.80
TA98	−	54 ± 16	356 ± 58	0.80
TA1538	+	16 ± 6	249 ± 21	0.93
TA1538	−	43 ± 8	230 ± 30	0.85
YG1021	+	25 ± 8	294 ± 29	0.91
YG1021	−	40 ± 12	454 ± 45	0.91
YG1024	+	47 ± 25	868 ± 90	0.90
YG1024	−	178 ± 48	1132 ± 175	0.81

1-NP can be used as a marker in exposure monitoring of particle-associated mutagens present in atmospheres contaminated with DE. For the assessment of the internal dose of DE-derived mutagenic constituents the suitability of 1-NP, as a dosimeter for exposure or biological effect, should be explored. We are currently developing methods for biomonitoring of occupational exposure to DE by the detection of metabolites of 1-NP in biological samples.

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