Chemical Analysis and Biological Testing of a Polar Fraction of Ambient Air, Diesel Engine, and Gasoline Engine Particulate Extracts

Michael Strandell,¹ Susanne Zakrisson,¹ Tomas Alsberg,¹ Roger Westerholm,² Lars Winquist,³ and Ulf Rannug³

¹Laboratory for Analytical Environmental Chemistry, Institute of Applied Environmental Research, University of Stockholm, Solna, Sweden; ²University of Stockholm, Department of Analytical Chemistry, Stockholm, Sweden; ³University of Stockholm, Department of Toxicology Genetics, Wallenberg Laboratory, Stockholm, Sweden

Extracts of gasoline and diesel vehicle exhaust and ambient air particles were fractionated into five fractions according to polarity on a silica gel column. Two medium polar fractions showing high genotoxic activity in the Ames test were further subfractionated, using normal-phase high-performance liquid chromatography. Chemical analyses were performed by means of gas chromatography combined with mass spectrometry and flame ionization and detection. The crude extracts, fractions, and subfractions were assayed with the Ames test, with and without S9, and the most abundant compounds in the subfractions are reported. — Environ Health Perspect 102(Suppl 4):85-92 (1994).

Key words: diesel exhausts, gasoline exhausts, ambient air particulate, HPLC, polar compounds, direct-acting mutagens, oxygenated polycyclic aromatic compounds

Introduction

It is well known that both gasoline and diesel vehicles are significant sources of polycyclic aromatic compounds (PAC) in ambient air (1). However, the compounds responsible for most of the mutagenicity of urban air particulate and many combustion emissions are still unknown.

A number of researchers have shown that the main part of the direct-acting mutagenicity of particle extracts is found in polar or moderately polar fractions. The work has been focused on fractions mainly constituted by oxygenated and nitrated PAC (2–10).

In this study, chemical analysis was combined with the Ames mutagenicity test to find the most potent mutagens in active subfractions. A combination of an open-column system and straight-phase high-pressure liquid chromatography (HPLC) was used. First, the extract was divided into five (I–V) fractions using an open tube silica column. Those fractions were subjected to the Ames test. Priority was given to fraction IV, because of its high mutagenicity for air particulate extracts (Table 1). This fraction, from gasoline and diesel exhausts as well as air particle extracts, was subfractionated with normal-phase HPLC and again Ames tested, followed by chemical analysis.

Materials and Methods

Sampling

The diesel vehicle was a Volvo 240 with a four cylinder precombustion engine. The gasoline vehicle was a Volvo 245 station wagon (noncatalyst) with a four-cylinder carburetor engine and a swept volume of 2 L. The fuel was leaded 96 octane (RON) commercial gasoline. Samples were collected using a dilution tunnel built according to the specifications set by the US Federal Register (11). The particle emissions were collected on Teflon-coated filters (Pallflex T60/A20), which prior to use were washed in ethanol 99% and heated to 200°C for 1 hr. The driving cycle was according to USA-73 specifications. Particle emission values for the diesel vehicle were 0.23 g/km and for the gasoline vehicle 0.021 g/km.

The airborne particles were collected during wintertime at a downtown Stockholm street with heavy traffic. The sampling equipment consisted of a high-volume sampler (S-20 Gebruder Becker, Wuppertal Germany) with a glass-fiber filter (C-305-GF). Prior to sampling, the filter was washed with ethanol 99% and heated to 550°C for 24 hr. The sample volume was 3600 m³. Particle weight was not measured due to the high content of fine sand in the particulate.

Table 1. Mutagenicity of fraction I to V.

| Fraction | Air ⁹ | Gasoline ⁹ | Diesel ⁹ |
|----------|-------|------------|----------|
|          | -S9   | +S9        | -S9      | +S9 | -S9 | +S9 |
| I        |       | 0          | 0        | 0  | 0   | 0   |
| II       |       | 0          | 3        | 1  | 42  | 0   |
| III      |       | 1          | 3        | 7  | 12  | 98  |
| IV       |       | 20         | 14       | 14 | 12  | 20  |
| V        |       | 14         | 11       | 2  | 3   | 8   |
| Crude    | 30    | 94         | 31       | 68 | 222 | 96  |

⁹The mutagenicity is given in revertants per cubic meter. ⁹The mutagenicity is given in revertants per meter.

This paper was presented at the Symposium on Risk Assessment of Urban Air: Emissions, Exposure, Risk Identification and Risk Quantitation held 31 May–3 June 1992 in Stockholm, Sweden.

We thank Karl-Erik Egeblick, the National Swedish Environmental Agency, and Volvo for supplying the test vehicles. Peter Haglund at the Special Analytical Laboratory, National Swedish Environmental Agency, Stockholm, is acknowledged for supplying the airborne particle sample. This study was financially supported by the National Swedish Environmental Agency.

Address correspondence to Michael Strandell, Laboratory for Analytical Environmental Chemistry, Institute of Applied Environmental Research, University of Stockholm, S-171 85 Solna, Sweden. Telephone 46 8 7091588. Fax 46 8 267629.
Table 2. PAH concentrations in fraction II (ng/m or ng/m$^3$).

| PAH                | Diesel engine | Gasoline engine | Air engine |
|--------------------|---------------|-----------------|------------|
| Phenanthrene       | 54.8          | 1.8             | 15.3       |
| Anthracene         | 4.6           | 0.6             | 4.2        |
| Fluoranthene       | 43.7          | 10.4            | 46.8       |
| Pyrene             | 35.1          | 19.2            | 48.4       |
| Benzo[a]pyrene     | 10.5          | 8.8             | 16.7       |
| Benzo[b,j]fluoranthene | 4.0   | 16.5            | 10.2       |
| Benzo[k]fluoranthene | 2.0    | 5.1             | 12.4       |
| Chrysene           | 3.7           | 7.7             | 10.4       |
| Benzo[a]pyrene     | 5.0           | 5.6             | 8.1        |
| Benzo[b,k]pyrene   | 1.5           | 3.7             | 7.9        |
| Indeno[c,d]pyrene  | 1.2           | 4.2             | 13.5       |
| Benzo[a,p]perylene | 2.3           | 18.9            | 23.6       |
| Coronene           | –             | 29.5            | 25         |

Extraction

Soxhlet extraction of the exhaust and airborne particles was carried out for 24-hr with dichloromethane (DCM) (Merck, pro analysis, redistilled in glass).

Cleanup and Fractionation

The crude extracts of the exhaust samples were fractionated on a silica gel column 10 x 120 mm bed (Merck Kieselgel 60, 70–230 mesh ASTM). The silica was heated to 500° C for 16 hr prior to deactivation with 10% distilled water (1 g H$_2$O/10 g gel).

Five fractions were collected from the silica gel column: fraction I, eluted with 10 ml of hexane; fraction II, 10 to 50 ml hexane; fraction III, 0 to 50 ml of 25% DCM in hexane; fraction IV, 0 to 50 ml of DCM; fraction V, 0 to 112 ml of methanol. The crude extract of ambient air particles was fractionated in the same way, but the silica gel bed was scaled up to 15 x 120 mm, and the volumes of the eluents were increased accordingly.

PAH Analysis

Fraction II was used for the quantification of the PAHs with β, β-binaphthyl added as an internal standard and gas chromatography–flame ionization detector (GC-FID) analyses performed as described below. The results from the PAH analysis appear in Table 2.

Subfractionation

HPLC was used for the subfractionation of fraction IV. A liquid chromatograph, consisting of two Constantmetric III pumps (Laboratory Data Control, Milton Roy, FL) was used. The effluent was monitored using UV absorption at 289 nm. For the normal-phase separation, a laboratory-packed Lichrosorb DIOL (Merck) column (10 x 100 mm) was fitted with the liquid chromatograph. The mobile phase was hexane and all runs were gradient programmed as follows: 0 to 30% DCM in hexane (Rathburn, pro analysis) during 40 min. The flow rate was 3.33 ml per min, and the injection volume was 100 L.

To choose the cutoffpoints for the subfractions of fraction IV, three subfractions were obtained from each extract in a pilot study, and they were assayed in the Ames test. Subfraction IV:2 was found to contain the main part of the mutagenicity. In this part of the chromatogram, another four cutoffpoints were chosen and the procedure was repeated, thus resulting in six subfractions: IV:1, 2A, 2B, 2C, 2D, and 3 as shown in Figure 1.

The reconstituted sample used for making an estimation of the recovery was obtained by letting fraction IV merely pass through the HPLC column.

Analysis

Gas Chromatography–Flame Ionization.

The gas chromatograph (GC) was a Hewlett Packard model 5790 equipped with a split/splitless injector and a 10-m SE-54 capillary column. The temperature program was: 70° C for 1 min; increase 7° C/min to 300° C; isothermal for 4 min. The injector temperature was 275° C. The GC was connected to a flame ionization detector.

Gas Chromatography–Mass Spectrometry (Electron Impact). The GC was a Hewlett Packard model 5790 equipped with a split/splitless injector and a 10-m SE-54 capillary column, and connected to a JEOL JMS-D300 mass spectrometer (MS) controlled by a Finnigan Incos computer.

Gas Chromatography–Mass Spectrometry (Chemical Ionization). The GC-chemical ionization (NCI)-instrument was a Finnigan 4021 with an Incos 2000 data system. The reagent gas was methane and the GC conditions were as described earlier.

Biological Testing

Mutagenicity tests were performed as described by Ames et al. (12) with Salmonella typhimurium TA98NR. All samples were tested in the presence and absence of a metabolic system (S9).

The S9 fraction was prepared from Aroclor-pretreated male Sprague-Dawley rats according to the Ames test. The nitro reductase-deficient strain TA98NR was also used in order to indicate the presence of nitro aromatic mutagens.

Results and Discussion

The HPLC chromatograms from the subfractionations of fraction IV are shown in Figure 1. The subfractions were further analyzed by means of GC-FID and GC-MS. Figures 2 to 7 show the GC-chromatograms of each subfraction. The pro-
Figure 2. Gas chromatography–flame ionization detector chromatograms of subfraction 1.

Figure 3. Gas chromatography–flame ionization detector chromatograms of subfraction IV:2A.
Figure 4. Gas chromatography–flame ionization detector chromatograms of subfraction IV:2B.

Figure 5. Gas chromatography–flame ionization detector chromatograms of subfraction IV:2C.
Figure 6. Gas chromatography–flame ionization detector chromatograms of subfraction IV/2D.

Figure 7. Gas chromatography–flame ionization detector chromatograms of subfraction IV/3.
posed molecular weights (mw) are marked on the top of the corresponding peaks. All the molecular weights are confirmed with GC-NCI analysis. The internal standard (β, β-biphenylthyl) is in the range of 1.1 ng/m³ for the vehicle exhaust samples and 3.1 ng/m³ for the air particulate sample. Internal standard is marked by "1.5." phthalates are marked by "ph" and "A" represents polar long-chained hydrocarbons.

The predominating compounds that were quantified against standards are listed in Table 3. Other compounds are in the range of less than 1 ng/m (or per cubic meter for the air sample), and are reported as tentatively identified.

The results from the mutagenicity tests are presented in Table 4.

**Subfraction IV:1**

The activity of these subfractions measured with TA98–S9 was in the range between 3 and 17% of the mutagenicity of the reconstituted sample.

In the diesel extract the main component was fluorenone (mw = 180). Components with mw = 194 and 196 were found, which were interpreted as dibenzofuranaldehyde and phenanthrene. Those compounds were also found in the corresponding subfractions of extracts from ambient air particles and particles emitted from gasoline engines, although at a lower concentration.

One component that is common for all three sample types is benzantrone, mw = 230. Other components with mw of 230 (e.g., benzo[b]fluorenone) can be found in subfraction IV:2D.

**Subfraction IV:2A**

This subfraction is quite potent for diesel (12% of the Ames activity of the reconstituted sample), but does not show the same activity for the corresponding subfractions in gasoline and air (2 and 6%). However, although there was about the same amount of the major compound benzo[c,d]pyrene in these subfractions, the mutagenic activity was different. This excludes this compound as a probable major direct-acting mutagen. The higher direct-acting mutagenicity in the diesel subfraction could result from dinitro-PAHs, as indicated by the lower response with strain TA98NR. However, we have not been able to trace any of those.

Other compounds tentatively found in these fractions are naphthopyrane (mw = 184), xanthone (mw = 182), and a ketone (mw = 242) derived from a PAH with m/z = 226, possibly cyclopenta[c,d]pyrene.

Diesel and gasoline exhausts have several compounds in common (e.g., naphthofuran-dione [mw = 198], xanthone [mw = 196], a ketone [mw = 278] from PAH with mw = 264).

The subfraction from gasoline exhausts contains unknown compounds with molecular weights of 222, 224, and 248.

**Subfraction IV:2B**

The mutagenicity (TA98–S9) for these subfractions was approximately 12% of the reconstituted sample mutagenicity.

The compounds in common in these fractions are several isomers of methyl- and dimethylbenzofuranones (mw = 148 and 162). There are also some phenalenone and xanthone isomers.

The diesel fraction also has a great number of alkane derivatives. In the gasoline fraction, several high-molecular-weight oxygen-containing heterocyclics were found. It is known that cyclopenta[c,d]pyrene is easily degraded, and these may be such degradation products. We could not trace those compounds in the air particle subfraction.

**Subfraction IV:2C**

For gasoline, this subfraction showed 10% of the reconstituted mutagenicity—for diesel 4% and for air 8%. Here, the most abundant component in all three fractions is phenalenone, reported as a nonmutagen in low concentrations (4). Here are also found two isomers of an anhydride, probably methylbenzaldehyde-carboxylic acid anhydride. Other components in common are naphthopyrane, anthracene, and naphthopyrane-dione.

**Subfraction IV:2D**

This subfraction showed 13 to 18% of the reconstituted mutagenicity for fraction IV with strain TA98–S9. The mass spectra obtained from analysis of this subfraction indicate that there are some PAH-dicarboxylic acid anhydrides in these subfractions.

The major compound is naphthalene dicarboxylic acid anhydride (mw = 198). Others are anthracene/phenanthrene dicarboxylic acid anhydride (mw = 248), and pyrene dicarboxylic acid anhydride (mw = 272). The latter is reported as a weak mutagen (13). Two isomers of benzoquinone (mw = 230), are also found in this fraction.

In diesel and gasoline subfractions there are equal amounts of a compound showing an intense 179-fragment, phenalenone (mw = 180), which is basically in subfraction 2C, and naphthopyrane (mw = 184); the peak with mw = 210 may be methylxanthene. Small amounts of anthrone (mw = 194) are present in subfraction 2D of gasoline and air.

A component with mw = 208 present in diesel is anthracene/phenanthrene-dione (Table 3). Present only in this subfraction of gasoline are: mw = 272, possibly pyredicarboxylic acid anhydride; mw = 254, a possible isomer of benzo[c,d]pyrene.

**Subfraction IV:3**

This subfraction is the most polar, and also shows the highest mutagenicity for all the three samples: 51% of the reconstituted mutagenicity for gasoline, 39% for diesel, and 23% for air. It contains mainly low-boiling components. Some phenol derivatives (e.g., nitrophenols) were tentatively identified. Several nitrophenols have also been reported identified by Nishihoka et al. in air particle extracts (19). Further fractionation of this subfraction in order to explain

---

**Table 3. Contents of PAC.**

|          | Diesel | Gasoline | Air |
|----------|--------|----------|-----|
| Xanthone | 1.6    | 2.5      | -   |
| Anthrone | 1.9    | 0.4      | -   |
| Phenalenone | 17    | 17       | 11  |
| Anthraquinone | 4.6   | 14       | 39  |
| Benzol[2,3]fluorenone | 0.8  | 1.4      | -   |
| Benzo[c,d]pyrene | 4.0  | 4.1      | 11  |

*The contents are given in mg/m³ of PAC.*

---

**Table 4. Mutagenicity.**

| Subfractions of fraction IV | Air  | Gasoline | Diesel |
|----------------------------|------|----------|--------|
|                            | S9   | S9       | S9     |
|                            | a    | NR       | NR     |
|                            | S9   | S9       | S9     |
|                            | a    | NR       | NR     |
| 1                          | 1.8  | 3.2      | 0.8    |
|                            | 0.4  | 0.6      | 0.5    |
|                            | 2.7  | 1.6      | 0.7    |
| 2                          | 1.0  | 0.8      | 0.6    |
|                            | 0.3  | 0.2      | 1.9    |
|                            | 1.0  | 1.0      | 10     |
| 3                          | 2.0  | 1.5      | 1.2    |
|                            | 1.3  | 0.7      | 1.3    |
|                            | 1.9  | 0.2      | 1.2    |
| 1                          | 1.4  | 0.8      | 0.8    |
|                            | 1.5  | 0.2      | 1.5    |
|                            | 0.6  | 0.6      | 0.3    |
| 2                          | 3.3  | 1.3      | 0.9    |
|                            | 2.0  | 0.8      | 1.5    |
|                            | 2.0  | 0.5      | 1.0    |
| Sum                       | 4.1  | 2.5      | 2.5    |
|                            | 7.8  | 3.3      | 3.7    |
|                            | 6.1  | 1.5      | 4.1    |
| Rec                       | 18   | 16       | 16     |
|                            | 15   | 2.8      | 9.4    |
|                            | 16   | 2.5      | 8.8    |
| Crude                     | 15   | 13       | 7.8    |
|                            | 12   | 4.3      | 9.0    |
|                            | 23   | 2.3      | 13     |

*The mutagenicity is given in revertants per cubic meter. The mutagenicity is given in revertants per meter.*
the mutagenic activity seems necessary because of its complexity.

It should be pointed out that the silica gel fraction investigated here, fraction IV, should not contain any mononitro-PAH, whereas dinitro-PAH may be present. In order to determine the presence of dinitro-PAH in the subfractions, these fractions were subjected to GC-NCI analysis and screened for dinitrated fluorene and pyrene, respectively. However, these compounds were not detected in any of the analyzed samples (detection limit for dinitropyrene in the diesel sample was approximately 10 pg/m). The level of 1-nitropyrene in fraction III of the diesel exhaust sample corresponded to an emission of 4.2 ng/m, and in fraction III of the air particle extract the concentration was 0.2 pg/m². This compound was not detected in the gasoline exhaust sample (detection limit 50 pg/m). The analysis of 1-nitropyrene was carried out with an HPLC-method described earlier (17).

A reduction in mutagenic response when using nitro-reductase-deficient strains, (e.g., TA98NR), as compared to the response with the corresponding nitro-reductase-containing strain (i.e., TA98), is usually interpreted as a measure of the importance of nitro-PAH for the mutagenicity of the tested sample. In Table 4, the response of TA98S9 is compared with that of TA98NR, as well as with that of TA98S9, for the subfractions. A reduction using TA98 NR is seen for all of the diesel subfractions, and all the air subfractions, but only for subfractions 2C, 2D (minor reduction), and 3 from the gasoline sample.

The mutagenicity profiles of the diesel and gasoline subfractions are very similar in their TA98NR-S9 response, but differ in their TA98-S9 response. Thus, it seems that the composition of the two sample types are similar, except for some nitro-reductase-dependent mutagens in subfractions 1, 2A, and 2B of the diesel extract. Such compounds are also present in all of the air subfractions. One group of compounds that recently has been investigated (20) is nitrated aromatic ketones (e.g., nitrobenzopyranois [NDBPs]), which are formed by atmospheric transformation of phenanthrene. Several isomers of NDBP have been identified in air particles, and one isomer (2-NDBP) has been found in diesel and gasoline particle extracts. We have not traced any of those isomers, but they could possibly be in fraction IV:3, where nitrophenols can be found. However, the contribution of NDBPs to the direct mutagenicity in the Ames plate incorporation assay are reported to be negligible (J. Lewtas and M. Nishioka, submitted).

With the addition of a metabolizing system (+S9), the distribution patterns from the air and gasoline subfractions are very similar, showing high activities in subfractions 1 and 3, in addition to a small peak in subfraction 2B. The reduction of the direct mutagenicity that can be observed in nearly all subfractions has also been reported by others (18). They suggest that the main reason for this reduction is enzymatic deactivation of direct-acting mutagens.

Other possibilities for this observation are a reduction by nonspecific bindings to lipophilic compounds in the S9 suspension, or the activation of some and deactivation of other compounds.

For diesel vehicle exhaust, as reported before, nitro-PAHs contribute to a varying degree to the mutagenicity (14–16). In this investigation, the distribution pattern of the mutagenic activity is similar for the three samples, although an enhanced activity could be seen in subfraction 2A of the diesel extract. This indicates that the compounds responsible for the effects are similar, possibly the same. For subfraction 2A, the higher direct mutagenicity in the diesel particle extract may result from dinitro-PAHs.

Summary

This paper describes a method for the analysis of particle extracts from ambient air, gasoline engines, and diesel engines. Extracts were fractionated, then tested for mutagenicity. The most potent fraction was further subfractionated and characterized with chemical analysis and mutagenicity testing. The distribution of the mutagenic response over the different subfractions was quite similar for the three extracts. However, the diesel extract showed a somewhat elevated mutagenicity in a subfraction that possibly contained dinitro-PAHs. Chemical analysis also showed many similarities between the subfractions of the different extracts. The main components in all subfractions were tentatively identified as oxygenated derivatives of PAHs.

REFERENCES

1. Nielsen T, Seitz B, Ramdahl T. Occurrence of nitro-PAH in the atmosphere in a rural area. Atmos Environ 18:2159–2165 (1984).
2. Schulze J, Hartung A, Kieb H, Kraft J, Lies K-H. Identification of oxygenated polycyclic aromatic hydrocarbons in diesel particulate matter by capillary gas chromatography and capillary gas chromatography-mass spectrometry. Chromatographia 19:391–397 (1984).
3. Dorie LD, Bagley ST, Leddy DG, Johnson JH. Characterization of mutagenic subfractions of diesel exhaust modified by ceramic particulate traps. Environ Sci Technol 21:757–765 (1987).
4. Alsborg T, Stenberg U, Westerholm R, Strandell M, Rannug U, Sundvall A, Romert L, Bernsson U, Petterson B, Tofgård R, Franzen B, Jansson M, Gustavsson JA, Eggback KE, Teije G. Chemical and biological characterization of organic material from gasoline exhaust particles. Environ Sci Technol 19:43–50 (1985).
5. Jensen TE, Schuetzle D, Prater TJ, Ball JC, Salmen I. Biological and chemical characterization of a composite heavy-duty diesel particle sample accumulated over a long period of time. In: Polyfunctional Aromatic Hydrocarbons: Mechanisms, Methods, and Metabolism (Cooke M, Dennis AJ, eds). Columbus, OH:Batelle Press, 1985;643–661.
6. Schuetzle D, Jensen T, Ball J. Polar polyaromatic aromatic hydrocarbon derivatives in extracts of particulates: biological characterization and techniques for chemical analysis. Environ Int 11:169–181 (1985).
7. Matsumoto H, Inoue K. Mutagenicity of a polar portion in the neutral fraction separated from organic extracts of airborne particulates. Arch Environ Contam Toxicol 16:409–416 (1987).
8. Yu ML, Hites RA. Identification of organic compounds on diesel engine soot. Anal Chem 53:951–954 (1981).
9. Moller M, Hagen I, Ramdahl T. Mutagenicity of polycyclic aromatic compounds (PAC) identified in source emissions and ambient air. Mutat Res 157:149–156 (1985).
10. Manabe Y, Kinouchi T, Ohsishi Y. Identification and quantification of highly mutagenic nitroareoxypropenes and nitrohydroxyprenes in diesel-exhaust particles. Mutat Res 158:3–18 (1985).
11. Federal Register. Code of federal regulations, parts 81 to 99. Rev. July 1, 1986.
12. Ames BN, McCann J, Yamazaki E. Biological testing with Salmonella typhimurium. Mutat Res 31:347–364 (1975).
13. Rappaport S, Wang Y, Wei E, Sawyer R, Watkins B, Rappaport H. Isolation and identification of a direct-acting mutagen in diesel-exhaust particulates. Environ Sci Technol 14:1505–1509 (1980).
14. Draper WM. Quantification of nitro- and dinitropolycyclic aromatic hydrocarbons in diesel exhaust particulate matter. Chemosphere 15:437–447 (1986).
15. Newton DL, Erickson MD, Toner KB, Pellizzi ED, Gentry P, Zweidinger RB. Identification of nitroaromatics in diesel exhaust particulate using gas chromatography negative ion chemical ioniza-
16. Paputa-Peck MC, Marano RS, Schuetzle D, Riley TL, Hampton CV, Prater TJ, Skewes LM, Jensen TE, Ruehle PH, Bosch LC, Duncan WP. Determination of nitrated polynuclear aromatic hydrocarbons in particulate extracts by capillary column gas chromatography with nitrogen selective detection. Anal Chem 55:1946–1954 (1983).

17. Tejada SB, Zweidinger RB, Sigsby JE. Analysis of nitroaromatics in diesel and gasoline car emissions. SAE Paper No. 820775. Society of Automotive Engineers, 1982.

18. Ball JC, Greene B, Young WC, Richert JFO, Salmeen IT. S9-Activated Ames assay of diesel-particle extracts. Detecting indirect-acting mutagens in samples that are direct acting. Environ Sci Technol 24:890–894 (1990).

19. Nishioka MG, Howard CC, Contos DA, Ball LM, Lewtas J. Detection of hydroxylated nitro aromatic and hydroxylated nitro polycyclic aromatic compounds in an air particulate extract using bioassay-directed fractionation. Environ Sci Technol 22:908–915 (1988).

20. Helmig D, Arey J, Harger WP, Atkinson R, Lopez-Cancio J. Formation of mutagenic nitrobenzopyranones and their occurrence in ambient air. Environ Sci Technol 26:622–624 (1992).