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Comparison of Exhaust Emissions from Swedish Environmental Classified Diesel Fuel (MK1) and European Program on Emissions, Fuels and Engine Technologies (EPEFE) Reference Fuel: A Chemical and Biological Characterization, with Viewpoints on Cancer Risk

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Diesel fuels, classified as environmentally friendly, have been available on the Swedish market since 1991. The Swedish diesel fuel classification is based upon the specification of selected fuel composition and physical properties to reduce potential environmental and health effects from direct human exposure to exhaust. The objective of the present investigation was to compare the most stringent, environmentally classified Swedish diesel fuel (MK1) to the reference diesel fuel used in the "European Program on Emissions, Fuels and Engine Technologies" (EPEFE) program. The study compares measurements of regulated emissions, unregulated emissions, and biological tests from a Volvo truck using these fuels. The regulated emissions from these two fuels (MK1 vs EPEFE) were CO (−2.2%), HC (12%), NO_x (−11%), and particulates (−11%). The emissions of aldehydes, alkenes, and carbon dioxide were basically equivalent. The emissions of particle-associated polycyclic aromatic hydrocarbons (PAHs) and 1-nitropyrene were 88% and 98% lower than those of the EPEFE fuel, respectively. The emissions of semi-volatile PAHs and 1-nitropyrene were 77% and 80% lower than those from the EPEFE fuel, respectively. The reduction in mutagenicity of the particle extract varied from −75 to −90%, depending on the tester strain. The reduction of mutagenicity of the semi-volatile extract varied between −40 and −60%. Furthermore, the dioxin receptor binding activity was a factor of 8 times lower in the particle extracts and a factor of 4 times lower in the semi-volatile extract than that of the EPEFE fuel. In conclusion, the MK1 fuel was found to be more environmentally friendly than the EPEFE fuel.

Introduction

Exhaust components emitted from vehicles are harmful to both human health and the environment (*1*−*3*). It is important, therefore, to reduce the potential health effects especially in densely populated areas such as larger cities. This may be achieved by the development of fuels that generate exhaust emissions that are less harmful. In Sweden two environmentally classified diesel fuels have been available on the market since 1991 (*4*). The market share in Sweden of the most environment-friendly diesel fuel, Environmental Class One (Miljöklass I, MK1) is currently (2000) more than 93% (*5*).

Exhaust emissions from vehicles generally consist of regulated and nonregulated constituents. Constituents regulated by law are carbon monoxide (CO), hydrocarbons (HC), nitrogen oxides (NO_x), and particulate material (PM) (*6, 7*). Other vehicle exhaust components belong to the group of unregulated constituents. There are several factors affecting the content of exhaust emitted from vehicles, such as driving conditions (*8*−*10*), engine type (*11*), ambient air temperature (*12, 13*), exhaust after-treatment devices, inspection and maintenance of the vehicle (*14*), and selection of the fuel (*15*). Several investigators have shown (*15*−*17*) that fuel specifications, selection of fuel, and fuel constituents also have an impact on the exhaust, affecting both regulated and unregulated components (*18*). Furthermore, the biological activity, i.e., mutagenicity and dioxin Ah-receptor affinity detected in exhaust samples, is also affected by influences of fuel parameters (*19*). Exhaust constituents such as carbon dioxide, methane, and nitrous oxide contribute to the global warming, or greenhouse, effect (*3*); however, this specific environmental problem is not within the scope of this investigation. The present publication is partly based on an internal report and focuses on a comparison between two fuels intended for use in diesel engines; viz., Swedish environmental class one (MK1) diesel fuel and a "European Program on Emissions, Fuels and Engine Technologies" (EPEFE) reference diesel fuel. The biological effects and chemical composition of both regulated and nonregulated components were compared per se, and are discussed with respect to associated cancer risks.

Experimental Procedures

The vehicle used in this investigation was a Volvo FH12 truck with a D12A 420 diesel engine. The engine had six cylinders with four valves per cylinder, and a swept volume of 12.1 L. The engine was equipped with a turbo, intercooler, and electronic fuel-injection system giving a maximum effect of 309 kW at 1800 rpm. The D12A 420 diesel engine is representative for heavy duty diesel vehicles in Europe and complies with the EURO 2 requirements. The vehicle was operated on a chassis dynamometer (Schenk, Germany). During the transient driving cycle, a flywheel capacity of

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TABLE 1. Fuel Characteristics

parameter measured	unit	analysis method	MK1	EPEFE
cetane number		ASTM D 613	53.8	52.7
cetane index		ASTM D 4737	53.1	51.7
density, 15 °C	kg/m^3	ASTM D 4052	816.9	839.6
viscosity, 20 °C	mm^2/s	ASTM D 445	2.93	4.20
viscosity, 40 °C	mm^2/s	ASTM D 445	2.03	2.68
CFPP	°C	DIN EN 116	−37	−16
cloud point	°C	ASTM D 2500	−34	−12
flash point	°C	ASTM D 93	67	78
ash content	wt %	ASTM D 482	<0.01	<0.01
carbon residue	%	ASTM D 524	0.09	0.14
water	mg/kg	ASTM D 1744	14	30
heat of combustion	MJ/kg	ASTM D 240	43.2	42.6
carbon	%	LECO	84.8	86.9
hydrogen	%	LECO	13.5	12.7
sulfur	mg/kg	ASTM D 3120	0.7	- - -
sulfur	mg/kg	ASTM D 4045	- - -	440
nitrogen	mg/L	ANTEK	2.4	- - -
nitrogen	mg/kg		- - -	70.5
distillation,	°C	ASTM D 86		
IBP	°C		188	184
5%	°C		206	210
10%	°C		212	219
20%	°C		220	234
30%	°C		226	247
40%	°C		233	260
50%	°C		239	271
60%	°C		245	282
70%	°C		252	294
80%	°C		260	306
90%	°C		271	320
95%	°C		280	332
FBP	°C		294	345
yield	%		98.5	98.5
residue	%		1.0	1.5
normal paraffins	wt%	GC OA3099	19.7	19.3
aromatics, D-FIA	%	NM 100	1.9	27.2
aromatics, total	%	IP 391/90	3.6	29.5
mono-aromatics	%		3.6	25.5
di-aromatics	%		<0.1	3.2
tri-aromatics	%		<0.02	0.8

13122 kg was used. The driving cycle, developed at the Technische Hochschule in Braunschweig (Braunschweig, Germany) is termed "Stochasticher Fahrcyclus für Stadtlinien Omnibusse" (bus cycle) and has previously been used by us (*20*, *21*). The duration of the driving cycle was 29 min and the driving distance was 11.0 km with a top speed of 58.2 km/h and average speed of 22.9 km/h, simulating city public transportation. Before testing, the engine was warmed so that the engine lubricating oil temperature was > 85 °C.

The diesel fuels investigated were a Swedish Environmental Classified diesel fuel (MK1) made by AB Svenska Shell (Sweden) and a European Program on Emissions, Fuels and Engine Technologies (EPEFE) reference fuel (CEC-RF-73-A93) made by Haltermann (Hamburg, Germany). The EPEFE fuel is identical to the fuel (from the same batch) used within the Auto Oil program (*22*). The main characteristics of the two tested fuels are presented in Table 1, and their PAH contents are given in Table 2. Fuel PAH analysis is described below.

Sampling and Analysis. All sampling was conducted on exhaust diluted in a dilution tunnel (alkenes omitted), which was designed to fulfill the specifications in the U.S. Federal Register (*6*). The flow of diluted exhaust was 72 m^3 per min, maintained by a venturi, and the average dilution ratio was approximately 40. Four consecutive tests were performed on each fuel investigated. Blank samples were taken when the engine was turned off and the vehicle was disconnected from the dilution tunnel. This is also valid for blanks for biological tests.

TABLE 2. PAH Content of the Fuels (mg/L, $n = 2$, ± standard deviation)

compound	MK1	EPEFE
phenanthrene	1.3 ± 0.1	188 ± 62
anthracene	0.1 ± 0.1	5.6 ± 4.9
3-methylphenanthrene	0.4 ± 0.01	210 ± 72
2-methylphenanthrene	0.5 ± 0.1	180 ± 40
2-methylanthracene	0.1 ± 0.1	2.8 ± 4.0
4H-cyclopenta(def)phenanthrene	<0.1	3.0 ± 4.2
9-methylphenanthrene	0.4 ± 0.1	86.2 ± 7.5
1-methylphenanthrene	0.6 ± 0.1	77.9 ± 10.6
2-phenylnaphthalene	0.1 ± 0.01	18.7 ± 0.1
3,6-dimethylphenanthrene	0.2 ± 0.1	74.2 ± 35.8
3,9-dimethylphenanthrene	0.7 ± 0.3	206 ± 22
fluoranthene	0.1 ± 0.01	3.8 ± 5.4
pyrene	0.2 ± 0.1	287± 34
benzo[*a*]fluorene	0.1 ± 0.01	<0.1
retene	0.2 ± 0.1	2.2 ± 3.1
benzo[*b*]fluorene	0.1 ± 0.01	<0.1
2-methylpyrene	0.2 ± 0.1	215 ± 22
4-methylpyrene	0.1 ± 0.1	60.3 ± 8.3
1-methylpyrene	0.1 ± 0.01	50.3 ± 3.5
benz[*a*]anthracene	<0.1	0.5 ± 0.1
chrysene	<0.1	3.6 ± 0.6
3-methylchrysene	<0.1	3.3 ± 0.4
2-methylchrysene	<0.1	1.6 ± 0.1
1-methylchrysene	<0.1	4.8 ± 0.5
benzo[*b*]fluoranthene	<0.1	0.2 ± 0.01
benzo[*k*]fluoranthene	<0.1	<0.1
benzo[*e*]pyrene	<0.1	0.3 ± 0.01
benzo[*a*]pyrene	<0.1	0.1 ± 0.01
benzo(ghi)perylene	<0.1	0.1 ± 0.01
coronene	<0.1	<0.1
sum PAHs	5.8 ± 0.6	1687 ± 196
dibenzothiophene	<0.1	0.3 ± 0.2

Regulated Emissions and Carbon Dioxide Emission Measurement. Following are the methods used for measurements of regulated emissions and carbon dioxide, in accordance with the test procedure described in the Federal Register (*6*): carbon monoxide (CO) and carbon dioxide (CO_2) with a nondispersive infrared analyzer (NDIR), total unburned hydrocarbons (HC) with a flame ionization detector (FID), and oxides of nitrogen (NO_x) with a chemiluminescence analyzer (Horiba Inc., Kyoto, Japan). The particulate emissions were measured using Teflon-coated glass-fiber filters (Pallflex T60A20, Pallflex Inc., CT).

Particle Associated Compounds. Filters, 50 × 50 cm, were used for collection of particulate emission samples (Pallflex T60A20). Before sampling, the filters, five at a time, were washed with 96% vol/vol ethanol (Kemetyl, Haninge, Sweden) (3 × 0.5 L), acetone (Merck, Darmstadt, Germany) (3 × 0.5 L), and dichloromethane (Riedel-deHaën, Sigma-Aldrich, Germany) (3 × 0.5 L), and dried at 200 °C for 1 h; this cleaning procedure is based on a method described elsewhere (*21*). After sampling, the filters were stored at −18 °C until extraction. The filters were Soxhlet extracted with dichloromethane (130 mL) for 18 h. Each dichloromethane extract was evaporated under reduced pressure to just dryness, diluted with acetone, and then stored at −18 °C until chemical analysis or biological testing. Four consecutive samples from each fuel were tested in the chemical analysis and biological analyses. Blank samples were collected and treated as described above.

Semivolatile Compounds. A specially designed sampling device with polyurethane foam (PUF) plugs (50 cm × 50 cm × 1.0 cm, 60 PPI; Specialplast AB, Sweden) was used for sampling of the gaseous fraction of semivolatile compounds. The PUF plugs were washed four at a time in a washing machine for 1 h at 85 °C and then centrifuged to near dryness.

Furthermore, one PUF plug at a time was washed with 5 L of distilled water, squeezed, washed with 1 L of 99% ethanol (Kemetyl, Haninge, Sweden), squeezed, and washed with 0.5 L of acetone (pro analysis; Merck, Darmstadt, Germany). In addition, the PUF plugs were extracted, one at a time, in a Soxhlet extractor (1.5 L) with toluene (Merck, Darmstadt, Germany) for 12 h and acetone for 24 h. The purified PUF plugs were stored separately in sealed plastic bags until exhaust sampling. After sampling, the PUF plug was transferred to a sealed glass jar and stored at −18 °C until extraction. The samples were Soxhlet extracted with acetone for 12 h, and the extract was then evaporated under reduced pressure and treated in the same way as the particulate extract. Four consecutive samples of each fuel were run for chemical analysis/biological testing. Blank samples were collected and treated as described above.

Aldehydes and Ketones. A chemisorption technique on an impregnated filter was used for the sampling of aldehydes and ketones (*23*). This method is based on the reaction of organic carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) at low pH to form corresponding hydrazones. The hydrazone derivatives are then analyzed by high performance liquid chromatography (HPLC) with UV-detection. Quantification was made with the corresponding hydrazones as external standards (*24*).

Alkenes. Analysis of ethene, propene, and 1,3-butadiene was performed by the use of a Fourier transform infrared (FTIR) spectroscopy instrument (Sesam I) using simultaneous real time measurements, in raw exhaust, developed by Siemens (Siemens AG, Germany). The instrument was calibrated for quantitation of compounds measured by external standard gas calibrations. The methodology used has been described in detail elsewhere (*25*).

Chemical Analysis and Quantification of Particulate and Semivolatile Polycyclic Aromatic Compounds (PACs). The particulate- and semivolatile- phase crude extracts were fractionated, according to polarity, into three fractions before chemical analysis. These fractions contained fraction I, "light" aliphatic hydrocarbons (not analyzed further); fraction II, "heavy" aliphatic hydrocarbons and PAHs; fraction III, nitro-PAHs. This procedure followed the one described by Alsberg et al. (*26*), although the solvent elution volumes were modified to fit the column size used.

Fraction II was analyzed using gas chromatography−mass spectrometry (GC−MS) for quantification of polycyclic aromatic hydrocarbons (PAHs). Prior to fractionation, internal standards were added (d_{10}-phenanthrene and 2,2′-binaphthyl) for quantification of identified compounds in fraction II. The gas chromatograph (Hewlett-Packard 5890 series II, Palo Alto, CA) was equipped with a split/splitless injector (injector temperature 285 °C) and a fused-silica capillary column (30 m × 0.25 mm i.d., HP5-MS, Hewlett-Packard, Palo Alto, CA). The temperature program was initial temperature 120 °C for 1 min, rate 7 °C /min, final temperature 300 °C for 10 min; the mass selective detector (Hewlett-Packard 5971A, Palo Alto, CA) interface temperature was set to 300 °C and the ion source temperature was 200 °C. The MS was operated in the electron impact (EI) ionization mode at 70 eV. The analysis of PAH in fraction II was made in selected ion monitoring (SIM) mode. A PAH standard mixture with internal standards was used for determination of retention times and response factor calculations. PAH standards and internal standards were of analytical grade and purchased from standard sources.

Fraction III containing nitro-PAH was analyzed for 1-nitropyrene by reversed-phase HPLC using "heart-cutting" and on-line reduction of 1-nitropyrene to 1-aminopyrene according to Tejada and co-workers (*27*). The system used in the present study is described in detail elsewhere (*21*).

PAC Analysis of Fuels. PAH analysis of the fuels was performed as follows. Internal standards d_{10}-phenanthrene and 2,2′-binaphthyl were added to 100 μL of fuel, and the volume was reduced using a gentle stream of nitrogen. The residue was dissolved in dichloromethane (DCM), transferred to 100 mg of deactivated silica gel (10% water) and the DCM was evaporated while heating under a gentle stream of nitrogen. The silica gel was put on the top of the silica gel column and the fuel sample was fractionated into two fractions according to the method previously described for the exhaust PAH. The PAH fraction, II, was further fractionated with HPLC according to Östman and Colmsjö (*28*) to separate "heavy aliphatic material" and PAH. The obtained PAH fraction was analyzed by GC−MS as described above.

Mutagenicity Tests. Mutagenicity tests were carried out using *Salmonella typhimurium* strains TA98, TA98NR, and TA100 according to Maron and Ames (*29*) with a slight modification in which histidine and biotin were added to the minimal medium instead of to the soft agar. TA98 and TA100 were tested with and without a liver preparation (S9) from Aroclor-1254 pretreated male Sprague−Dawley rats; this metabolizing system was used in an amount of 50 μL per plate. The particulate phase was tested as a crude extract in three concentrations per plate, corresponding to driving distances of 0.2, 0.4, and 0.8 m, respectively. Corresponding driving distances for the semivolatile crude extracts (PUF) were 1.0, 2.0, and 4.0 m, respectively. All acetone extracts were evaporated to nearly dryness under nitrogen and then diluted with dimethyl sulfoxide (DMSO) to known volumes and tested for mutagenicity. The volumes of each sample added to the soft agar were 25, 50, and 100 μL, respectively.

Dioxin Receptor Binding Assay. The crude extracts from both the particulate and the semivolatile phase (PUF) were assayed for relative dioxin receptor (Ah-receptor) binding affinity. The compounds used (i.e., 2,3,7,8-tetrachloro[1,6-^{3}H]dibenzo-*p*-dioxin ([^{3}H]TCDD) and 2,3,7,8-tetrachlorodibenzofuran) were purchased from Chemsyn Science Laboratories (Lenexa, KS). Hydroxyl-apatite (HAP) was purchased from Bio-Rad Laboratories (Richmond, CA). All other reagents were of analytical grade and were purchased from standard sources. The dioxin receptor binding affinities of samples were measured using a HAP adsorption assay, developed by Poellinger and co-workers (*30*), which is described in full elsewhere (*31*). Briefly, rat liver cytosol (a good source of dioxin receptor) was incubated with [^{3}H]TCDD and then added to a suspension of HAP. Adsorbed proteins were then separated by centrifugation. Pelleted material was washed with 75 mM phosphate buffer (pH 7.0). Receptor−ligand complexes were then extracted from the HAP by washes with 175 mM phosphate buffer (pH 7.0). Aliquots were taken before incubation with HAP to determine total added radioactivity. Specific binding was determined as the difference in the level of protein-bound ligand in the absence or presence of a 200-fold molar excess of the unlabeled ligand 2,3,7,8-tetrachlorodibenzofuran which is used as a competitor because of its similar affinity for the receptor but much higher solubility than that of TCDD. To determine the binding affinities of samples, competition experiments were carried out with increasing concentrations of sample against a standard concentration of radiolabeled TCDD. The relative binding affinities of the samples were then calculated from log−logit plots of the competition for TCDD-binding using linear regression analysis. Relative binding affinity was expressed as *IC*50, the concentration of sample required to compete for 50% of the [^{3}H]TCDD-binding sites.

Results and Discussion

In this results and discussion section only the mean values are compared and discussed. The fuel consumption originating from the MK1 fuel was 399 ± 4 g/km (n = 5) and from

TABLE 3. Exhaust Emission Factors from Regulated (g/km) and Carbon Dioxide Emissions (kg/km) (mean values ± standard deviation)

compound	MK1, $n = 5$	EPEFE, $n = 4$
CO, g/km	6.02 ± 0.40	6.16 ± 0.41
HC, g/km	0.37 ± 0.02	0.33 ± 0.01
NO_x, g/km	11.53 ± 0.27	13.01 ± 0.09
particulate, g/km	0.299 ± 0.016	0.336 ± 0.011
CO_2, kg/km	1.063 ± 0.012	1.095 ± 0.020

TABLE 4. Particle Analysis, Made and Reported by Ricardo Consulting Engineers, England (mg/km, $n = 3$, mean values ± standard deviation)

	MK1	EPEFE
fuel-derived hydrocarbons	23.3 ± 2.7	38 ± 1.8
lubricating-oil derived hydrocarbons	<1.1	1.5±<0.1
volatiles	37.2 ± 11.6	62.2 ± 0.7
carbon	230 ± 19.8	234 ± 2
soluble sulfates	0.5 ± 0.06	4.2 ± 0.4
sulfate-bound water	0.66 ± 0.08	5.5 ± 0.6
nitrates	3.6 ± 0.5	1.9 ± 0.1
sum of particle analysis contents[a]	296 ± 35	342 ± 5

[a] Sulfate-bound water excluded in sum because it is theoretically calculated.

the EPEFE fuel the consumption was 346 ± 4 g/km ($n = 4$). These results are in agreement with the heats of combustion, Table 1, and the carbon dioxide emissions, Table 3, of the respective fuels. Comparing the regulated exhaust emissions, Table 3, the HC emission from MK1 was found to be 12% higher that that from EPEFE. However, CO, NO_x, and particulate emissions from MK1 were lower by 2.3, 11, and 11%, respectively. Kleinschek and Röj (*32*) have reported reductions in MK1 diesel fuel emissions of NO_x by about 10% and of particles by 15 to 40% compared to other diesel fuels.

The particle analysis results are given in Table 4. The analysis comprises hydrocarbons derived from fuel and lubricating oil, particulate carbon, volatiles, soluble sulfates, and nitrates. Sulfate-bound water is calculated from soluble sulfates determined. Lubricating-oil-derived hydrocarbons and particle carbon are engine dependent and relatively constant, i.e., fuel independent. Compared to EPEFE, fuel-derived hydrocarbons are approximately 30% lower from the MK1 fuel. The corresponding value for volatiles is approximately 60% lower. The lower sulfur content in the MK1 fuel results in a reduction of soluble sulfates by approximately 90%, as expected. The relative high nitrate emissions from the MK1 fuel cannot be explained without further experiments.

The emissions of aldehydes and ketones are presented in Table 5. No major differences between the fuels in the individual aldehyde emissions were observed. However, a slightly (not significant) higher sum of aldehyde/ketone emissions may be seen to emanate from the MK1 fuel.

Alkene emissions are presented in Table 6. Again, no major difference was observed between the two fuels. A slightly higher (not significant) level of alkenes was observed in emission from EPEFE fuel. The relative contribution from 1,3-butadiene compared to ethene and propene emissions in the present fuel comparison is larger than previously reported (*33*). The present 1,3-butadiene emissions level is relatively close to the detection limit, i.e., <10 mg/km. However, the fact that there are no standards set for sampling and analysis of unregulated exhaust components from vehicles stresses the importance of intercomparisons and evaluation of methods.

TABLE 5. Aldehyde and Ketone Emissions (mg/km, $n = 3$, mean values ± standard deviation)

compound	MK1	EPEFE
formaldehyde	47.3 ± 5.3	44.5 ± 2.3
acetaldehyde	19.1 ± 1.9	15.5 ± 1.9
acrolein	1.5 ± 0.6	1.3 ± 0.3
propanal	3.2 ± 0.7	3.4 ± 1.1
crotonaldehyde	1.9 ± 0.6	1.9 ± 0.4
methacrolein	1.0 ± 0.3	1.1 ± 0.3
butyraldehyde	2.9 ± 0.2	3.4 ± 1.6
valeraldehyde	3.6 ± 0.9	2.8 ± 0.6
benzaldehyde	2.5 ± 0.9	1.9 ± 0.6
p-tolylaldehyde	0.6 ± 0.2	0.6 ± 0.3
hexanal	6.8 ± 3.2	3.7 ± 1.0
acetone	8.3 ± 2.3	5.4 ± 2.6
methylethyl ketone	6.6 ± 1.0	6.5 ± 1.9
sum	105	92

TABLE 6. Alkene Emissions (mg/km, $n = 4$, mean values ± standard deviation)

compound	MK1	EPEFE
ethene	10.6 ± 1.7	12.8 ± 1.7
propene	5.0 ± 1.6	7.5 ± 1.7
1,3-butadiene	10.0 ± 2.0	12.5 ± 0.6
sum	25.6	32.8

Summing up the major emission factors of compounds contributing to the HC signal, i.e., aldehydes/ketones and alkenes, in the exhaust, 35 mass % of HC may be explained for MK1 and 38 mass % for EPEFE.

From the PAH analysis of fuels, Table 2, it is evident that the MK1 diesel fuel has a substantially lower PAH content than the EPEFE fuel. The EPEFE fuel, most likely, contains PAHs in addition to those presented in Table 2, for example other dimethylphenanthrenes/ anthracenes (tentatively identified). However, these PAHs could not be conclusively identified and quantitated due to lack of standards. A previous investigation (*20*) has shown that the fuel content of PAHs has an impact on the content of PAHs in the diesel exhausts. As stated in this reference, a limitation of the PAH content in diesel fuels substantially reduces the emission of unburned fuel PAHs. As a result of this, it can be seen that MK1 has substantially lower PAH emissions than EPEFE in both the particulate and the semivolatile phase, Tables 7 and 8. Comparing the PAH emissions associated with the particles alone reveals that approximately 8.5 times more PAH is emitted from combustion of the EPEFE than of MK1. Predominant PAHs in EPEFE particulate emissions are pyrene, 2-methylphenanthrene, phenanthrene, and 2-methylanthracene, followed by 1-methylphenanthrene and fluoranthene. Corresponding predominant PAHs emanating from MK1 are phenanthrene, followed by fluoranthene, 1-methylphenanthrene, 2-methylanthracene, and pyrene. Making a similar comparison concerning semi-volatile PAHsd, Table 8, approximately 4.5 times higher emissions are obtained from the EPEFE fuel. Important PAHs are phenanthrene and pyrene, followed by 2-methylanthracene, 2-methylphenanthrene, fluoranthene, and anthracene. Pyrene is relatively abundant in the emission samples from both fuels, the same amounts of pyrene are emitted in the semivolatile phase from both fuels. Pyrene associated with the particles may originate from unburned pyrene from the fuel and semi-volatile pyrene may originate from pyrosynthesis of fuel constituents. The relatively large amounts of 1-nitropyrene determined in the particle crude extracts from the EPEFE fuel may be explained by the nitration of fuel-associated pyrene in the combustion and/or the

TABLE 7. Particle Associated PAC (μg/km, $n = 4$, mean values $\pm$ standard deviation)

compound	MK1	EPEFE
2-methylfluorene	0.6 ± 1.1	14.2 ± 6.5
phenanthrene	27.8 ± 1.1	108 ± 14
anthracene	2.1 ± 0.2	10.9 ± 3.2
2-methylanthracene	7.9 ± 2.2	94.5 ± 10.3
2-methylphenanthrene	6.8 ± 1.9	111 ± 24
1-methylphenanthrene	8.4 ± 2.9	62.6 ± 7.6
fluoranthene	9.9 ± 4.6	60.0 ± 22.1
pyrene	7.6 ± 3.8	140 ± 38
retene	<0.1	<0.1
2-methylpyrene	<0.1	9.1 ± 1.6
1-methylpyrene	<0.1	<0.1
benzo(ghi)fluoranthene	<0.1	1.3 ± 2.4
cyclopenta(cd)pyrene	<0.1	0.2 ± 0.3
benz[*a*]anthracene	<0.1	3.8 ± 1.4
chrysene/triphenylene	0.2 ± 0.3	1.4 ± 0.6
benzo(b&k)fluoranthene	<0.1	0.5 ± 0.3
benzo[*e*]pyrene	<0.1	<0.1
benzo[*a*]pyrene	<0.1	0.2 ± 0.2
perylene	<0.1	<0.1
indeno(1,2,3-cd)fluoranthene	<0.1	<0.1
indeno(1,2,3-cd)pyrene	<0.1	<0.1
dibenz(a,h)anthracene	<0.1	<0.1
benzo(ghi)perylene	<0.1	<0.1
coronene	<0.1	<0.1
sum PAH	71	618
dibenzothiophene	1.8 ± 0.5	2.3 ± 1.0
1-nitropyrene	0.009 ± 0.001	0.560 ± 0.044

TABLE 8. Semivolatile PAC (μg/km, $n = 4$, mean values $\pm$ standard deviation)

compound	MK1	EPEFE
2-methylfluorene	1.5 ± 0.5	22.5 ± 5.1
phenanthrene	11.9 ± 4.4	159 ± 34
anthracene	1.0 ± 0.3	17.3 ± 4.3
2-methylanthracene	1.2 ± 0.6	59.5 ± 14.8
2-methylphenanthrene	0.9 ± 0.9	54.8 ± 14.0
1-methylphenanthrene	1.4 ± 1.0	36.2 ± 9.1
fluoranthene	13.6 ± 0.6	22.5 ± 7.8
pyrene	62.7 ± 22.6	72.8 ± 28.3
retene	<0.1	<0.1
2-methylpyrene	0.9 ± 0.8	1.0 ± 0.4
1-methylpyrene	<0.1	<0.1
benzo(ghi)fluoranthene	6.2 ± 2.9	9.2 ± 4.3
cyclopenta(cd)pyrene	0.7 ± 0.8	0.2 ± 0.2
benz[*a*]anthracene	<0.1	0.1 ± 0.1
chrysene/triphenylene	0.3 ± 0.1	1.2 ± 1.5
benzo(b&k)fluoranthene	1.5 ± 0.1	0.2 ± 0.1
benzo[*e*]pyrene	0.3 ± 0.1	0.3 ± 0.2
benzo[*a*]pyrene	0.2 ± 0.1	0.4 ± 0.2
perylene	<0.1	0.2 ± 0.1
indeno(1,2,3-cd)fluoranthene	<0.1	<0.1
indeno(1,2,3-cd)pyrene	<0.1	<0.1
dibenz(a,h)anthracene	<0.1	<0.1
benzo(ghi)perylene	<0.1	<0.1
coronene	<0.1	<0.1
sum PAH	101	438
dibenzothiophene	1.1 ± 0.8	7.9 ± 4.1
1-nitropyrene	<0.005	0.026 ± 0.04

post combustion process in the silencer system. This is supported by the relatively larger NO_x emissions emanating from the EPEFE fuel. However, this statement needs to be investigated and verified by additional experiments. Other authors have speculated about the formation of 1-nitropyrene by the reaction between nitrous gases and pyrene (*34, 35*).

TABLE 9. Mutagenicity on *Salmonella typhimurium* Tester Strains in the Presence (+S9) and in the Absence of a Metabolizing System (−S9)(krev/km, $n = 4$, mean values $\pm$ standard deviation)[a]

strain	MK1	EPEFE
mutagenicity in particulate raw extract		
TA98−S9	10.3^{***} ±1.0	86.7^{***} ±4.8
TA98+S9	5.5^{**} ±0.9	52.6^{***} ±2.2
TA100−S9	55.3^{***} ±4.7	219.6^{***} ±17.4
TA100+S9	21.1^{*} ±4.3	135.2^{***} ±11.2
TA98NR−S9	6.0^{**} ±1.0	47.7^{***} ±2.7
mutagenicity in semi-volatile raw extract		
TA98−S9	1.7^{**} ±0.4	3.6^{*} ±0.9
TA98+S9	4.0^{*} ±1.1	10.7^{***} ±1.3
TA100−S9	11.2^{*} ±3.5	24.6^{**} ±4.4
TA100+S9	10.9 ± 4.5	22.6^{*} ±5.5
TA98NR−S9	1.9 ± 1.2	3.2 ± 1.0

[a] Significance levels: * $0.005 < p < 0.01$; ** $0.001 < p < 0.005$; *** $p < 0.001$, in difference from control values.

Biological Assays. *Mutagenicity Tests.* The mutagenicity data are shown in Table 9. It is evident that the mutagenicity determined in the particulate crude extracts is higher than the corresponding mutagenicity determined in the semi-volatile crude extracts. This is valid for both fuels. The mutagenicity emanating from the particle crude extract of the EPEFE is in general higher than the corresponding mutagenicity from the MK1 fuel. Depending on tester strain and whether the metabolic system is included, a difference by a factor of 4 (TA100−S9) to 9.6 (TA98+S9) was seen. For EPEFE the addition of S9 to TA98 and TA100 reduces the mutagenicity by approximately 40%. The corresponding values for MK1 are 47% (TA98) and 62% (TA100), respectively. We have previously reported a reduced mutagenicity of particle raw extracts of diesel exhausts in the presence of S9 (*17, 21*).

With tester strain TA98NR, deficient in nitroreductase, an approximately 60% lower mutagenicity was found for both fuels, as compared to that of TA98−S9. Thus, 40% of the mutagenicity detected with strain TA98 in the absence of S9 could be attributed to nitro PAH.

The relative differences in mutagenicity between the fuels were higher with tester strain TA98. The EPEFE samples were approximately 8−10 times more mutagenic than the MK1 samples. Also with this strain, the mutagenicity was significantly higher in the absence of S9 indicating a high proportion of S9-independent mutagens in the samples.

In general, the crude extracts of the semi-volatile components derived from the EPEFE exhausts showed a higher mutagenicity than the corresponding samples from the MK1 fuel. In this case the EPEFE exhausts were 2.0 to 2.7 times more mutagenic than the MK1 samples. The addition of S9 to TA98 increased the mutagenicity 2.4 to 3.0-fold for MK1 and EPEFE, respectively. For strain TA100, a similar mutagenic effect was seen with and without S9 for the exhaust samples from both fuels. We have previously reported reduction as well as increased mutagenicity with the addition of S9 to semi-volatile raw extracts (*17, 21*).

Dioxin Receptor Affinity. The measure of activity with this assay is in negative relation with the metric use. Hence, a low *IC*50 value denotes a high biological effect. For that reason, the variable describing the activity is negatively correlated with the other activity variables. Accordingly, dioxin receptor affinity is negatively correlated with the PAH and general emissions of particulate exhausts. In earlier reports (*19*), however, we have observed a better correlation of dioxin receptor affinity with PAH than with other PAC species, indicating that the unmetabolized PAH species

TABLE 10. Dioxin Receptor Affinity Test Results (*IC*50 value mm/mL (mm driving distance per mL), $n = 4$, mean value $\pm$ standard deviation)

	MK1	EPEFE
particulate raw extract	310 $\pm$ 112	38 $\pm$ 23
semivolatile raw extract	214 $\pm$ 40	52 $\pm$ 12

interact with the dioxin receptor to a higher degree than most PAH derivatives.

In Table 10, the dioxin receptor affinity test *IC*50 data are presented. No significant difference was observed between binding affinities of particulate and semi-volatile phase from either MK1 or EPEFE fuels. A comparison of the fuels reveals that the emission from the EPEFE fuel is significantly more biologically active in both the particulate- and the semi-volatile-associated raw extract. The particulate-associated raw extract from EPEFE is approximately 8 times more biologically active than the one from MK1. For the semi-volatile crude extracts the corresponding activity ratio is approximately 4.

Aspects of Relative Cancer Risk. Cancer risks from urban air pollution – to a large extent originating from automotive engine exhausts – have mostly been estimated by inadequate methods. For this reason risk figures so far available in the literature possess considerable uncertainties. This concerns risk factors for individual compounds or classes of compounds and for the total mixed exposure where data for several, possibly harmful components are still lacking. Swedish risk figures, that have been presented despite the weak basis, were judged to be uncertain by a factor of 3 (*36*). These preliminary investigations had the main aim of showing whether the cancer risks associated with air pollution were, or were not, of a disquieting magnitude calling for risk-limiting measures. Since this question was answered in the affirmative (>500 cancer cases annually in the 8.5 million population of Sweden; *36*), with support from a recent epidemiological study (*37*), comparisons of fuels with respect to contents of carcinogens in exhausts became important.

At the low, to very low, concentrations of pollutants prevailing in ambient air, cancer, with mutation in tissue cells as the key event, is expected to be a predominant effect. This is because, at the present state of knowledge regarding dose–response (dose-risk), relationships should be considered to be linear without any threshold dose below which the risk is zero. In the following the two fuels are compared with regard to levels of certain high-risk components in the exhausts. Because of the linear interdependence of risk and dose, and assuming human exposure to be approximately proportional to emitted amounts, ratios of emission levels will be relevant to ratios of the risk contributions due to respective components.

According to cancer risk estimations of urban air pollution with special regard to compounds from automotive engine exhausts, the particulate organic matter, mainly PAH, has been judged to be a major risk factor (*36*, *38*). The difference in PAH contents of the respective exhausts, partly due to the PAH contents of the fuels (*20*; cf. Table 2) contributes to a considerable difference in cancer risk between the two fuels. This comparison (Tables 7 and 8) indicates that the PAH of EPEFE exhausts would give rise to a risk increment which is about 5 times larger than that of PAH in MK1 exhausts. This ratio has some support in the data for mutagenicity (after pooling data for particulate and gaseous compounds in Table 9). The comparison of the two fuels is very rough, however, considering differences in the relative abundancies of individual compounds as well as uncertainties in risk factors. The two diesel fuels are characterized by low levels of heavier PAHs and relatively high levels of light compounds such as phenanthrene and methylphenanthrenes. Some of the latter are rather efficient mutagens (*39*). The ratio of the two fuels with respect to the contents of fluoranthene, a compound that is possibly associated with a high risk (*41*, *42*), in the exhausts is relatively low, 3.5 times, evidently because of the relatively low content of fluoranthene in the EPEFE fuel.

Among volatile compounds certain unsaturated hydrocarbons, particularly butadiene and to some extent also ethene, have been judged to imply nonnegligible contributions to the cancer risk associated with engine exhausts (*36*). This also concerns benzene (which was not measured in the present study) and, among oxygenates, formaldehyde (*36*). The difference between the two fuels with respect to emission of these volatile compounds is small, if at all significant.

Butadiene has been judged to be an outstandingly strong carcinogen in polluted air (*36*, *38*). A correct determination of the level in exhausts of this compound and of its risk coefficient may therefore be important in a relative assessment of the total risk. It should therefore be remarked that the values given in Table 6 in the contents of butadiene are 5–20 times higher (relative to ethene) than those reported from studies using another analytical technique (*33*). This discrepancy calls for an evaluation of analytical methods for this compound. An improved determination of the risk coefficient (*36*) has to await measurement in butadiene-exposed humans of the most potent genotoxic metabolite, diepoxybutane (*40*).

By and large the bacterium tests for mutagenicity (Table 9) support the conclusions based on analytical data with respect to the difference in cancer risk associated with exhausts from the two compared fuels. In the tests of particulate raw extract the nitroreductase deficient strain, TA98NR, shows a strong reduction of the number of revertants, as compared to TA98. This indicates an important contribution of nitroarenes to the overall mutagenicity. In tests of the particulate extract (but not of the semivolatile extract), addition of the metabolising system, S9, leads to a strong net reduction in the number of revertants. The nature of this effect, often observed in similar experiments, is not fully clarified. A likely explanation is that the mutagenicity of nitroPAH is diminished by S9. However, the occurrence of, so far unknown, directly reactive mutagens that are eliminated in reactions with S9 components cannot be ruled out.

The dioxin receptor (i.e., Ah-receptor) affinity, mainly ascribed to certain unmetabolized PAHs, is an indication of the occurrence of compounds with the ability to induce enzymes (CYP 1A) that metabolize PAHs to reactive intermediates (epoxides and diolepoxides), often with mutagenic activity. Interaction with this receptor also leads to induction of growth-promoting conditions. Both these effects provoke high cancer incidence, particularly at high exposure levels (*42*).

Summing up, there seems to be advantages to using the Swedish environmental classified diesel fuel, MK1, with respect to environment and health, especially in densely populated areas. By selection of improved "green" fuels, the emissions from engines are expected to be reduced in the future; however, it is difficult to say how much the emission characteristics will be improved by further development of fuels and fuel-matched engines. Furthermore, exhaust emissions from low-sulfur fuels may be further reduced by using exhaust after-treatment devices such as oxidation catalysts.

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