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Evaluation of Motor Vehicle and Other Combustion Emissions Using Short-Term Genetic Bioassays

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Research Triangle Park, NC

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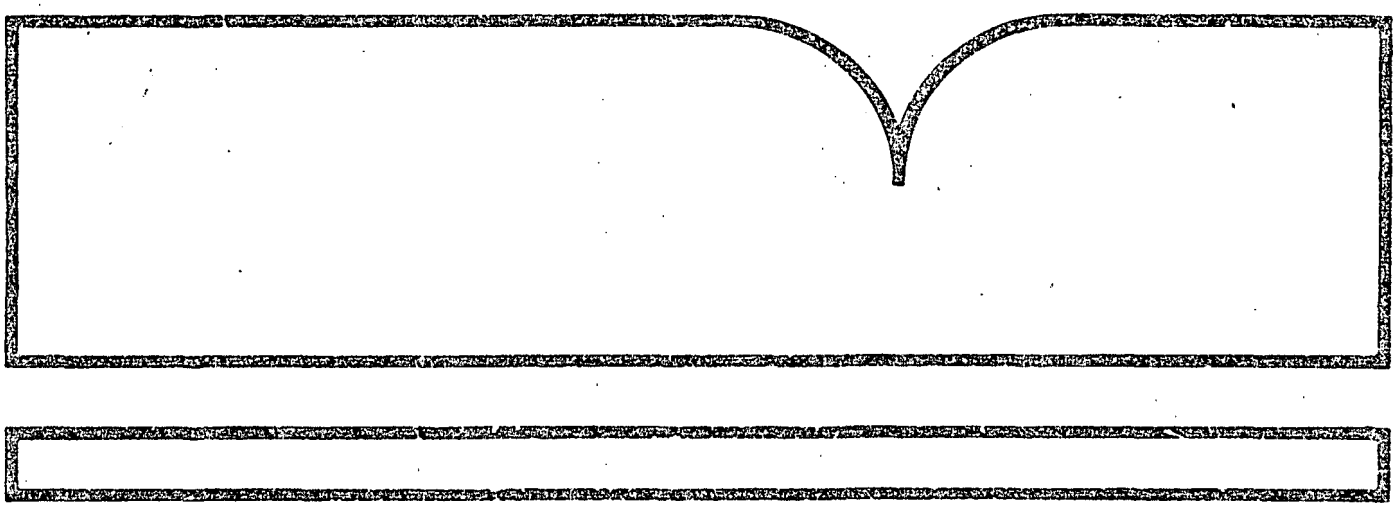

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EVALUATION OF MOTOR VEHICLE AND OTHER COMBUSTION EMISSIONS USING SHORT-TERM GENETIC BIOASSAYS

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ABSTRACT

Short-term genetic bioassays have been useful in evaluating unregulated organic combustion emissions from motor vehicles. Identification of mutagens and carcinogens in complex exhaust emissions has been greatly facilitated by the use of bioassay-directed fractionation and characterization methods. It has also been possible to evaluate the effect of fuels, engine types, and control technologies on the rates of mutagenic emissions from motor vehicles. Greater differences in the rate of mutagenic emissions have been observed between different engines (e.g., diesel *vs.* gasoline) and control technologies (e.g., with and without catalyst) than between different fuels. A comparative evaluation of various combustion sources indicates that motor-vehicle emissions make a major contribution to the mutagenicity observed in ambient air.

INTRODUCTION

Combustion emissions from both motor vehicles and stationary sources contain a complex mixture of organics. Chemical characterization of these organics shows that they contain carcinogenic polycyclic aromatic hydrocarbons (PAH), such as benzo(a)pyrene. Recently, chemical characterization studies of motor-vehicle emissions have identified the presence of methylated PAHs (e.g., methylphenanthrenes) (1), nitrated PAHs (e.g., nitropyrene) (2), oxidized PAHs (e.g., 4 oxapyrene-5-one)

(3), and a variety of other polycyclic organic compounds not yet evaluated in animal cancer bioassays.

The development of short-term genetic bioassays has provided relatively simple, sensitive, and rapid bioassays for mutagenic and potential carcinogenic activity. Short-term genetic bioassays have been particularly useful in evaluating organic combustion emissions. This paper summarizes the results of studies where short-term genetic bioassays have been used in the following areas:

(1) identification of mutagens and carcinogens in complex exhaust emissions,

(2) evaluation of the effect of fuels, engine types, and control technologies on the mutagenic activity of the emissions, and

(3) comparative assessment of mutagenicity and carcinogenicity of various combustion sources and their contributions to the mutagenic activity of ambient air.

IDENTIFICATION OF MUTAGENS AND CARCINOGENS IN COMPLEX EXHAUST EMISSIONS

Bioassay-directed fractionation and characterization closely coupled to chemical characterization has been shown to be the most efficient and effective approach to identifying the specific chemical compounds in a complex mixture that exhibit a particular biological activity (4). This approach has been used to identify tumor initiators and tumor promoters in cigarette-smoke condensates (5), automotive exhaust emissions (6), and urban-air particles (7). More recently, this approach has been coupled with short-term genetic bioassays, including both microbial and mammalian-cell mutation assays, to identify mutagens and potential carcinogens in complex mixtures (8). We first employed this method to identify the chemical classes and specific components associated with diesel particulate emissions that were mutagenic in the Ames Salmonella typhimurium mutagenesis assay (9).

Diesel particles collected by the dilution-tunnel method (10) were Soxhlet-extracted with dichloromethane and solvent-partitioned into organic acids, bases, and neutral components. The neutral components were further fractionated into paraffins (hexane), aromatics (1% ether/hexane), transitional compounds (1% ether/hexane, yellow fluorescence), and oxygenated compounds (50% acetone/methanol). The mutagenic activity of each fraction was determined using the Ames Salmonella typhimurium/microsome assay in TA1535, TA1537, TA1538, TA98, and TA100 (9). The

distributions of the mass of each fraction and of its mutagenic activity in TA98 are shown in Figure 1 and Table 1 for the four-stroke V-8 Caterpillar 3208 engine used in urban service vehicles. The moderately and highly polar neutral compounds in the transitional (TRN) and oxygenated (OXY) fractions account for 89-94% of the mutagenic activity of the extractable organics and only 32% of the mass. Conventional gas chromatography/mass spectroscopy identified many nonmutagenic fluorenones and methylated fluorenones as major constituents of these fractions. None of these or other identified constituents account for the direct-acting frameshift mutagenic activity observed. Mutagenic

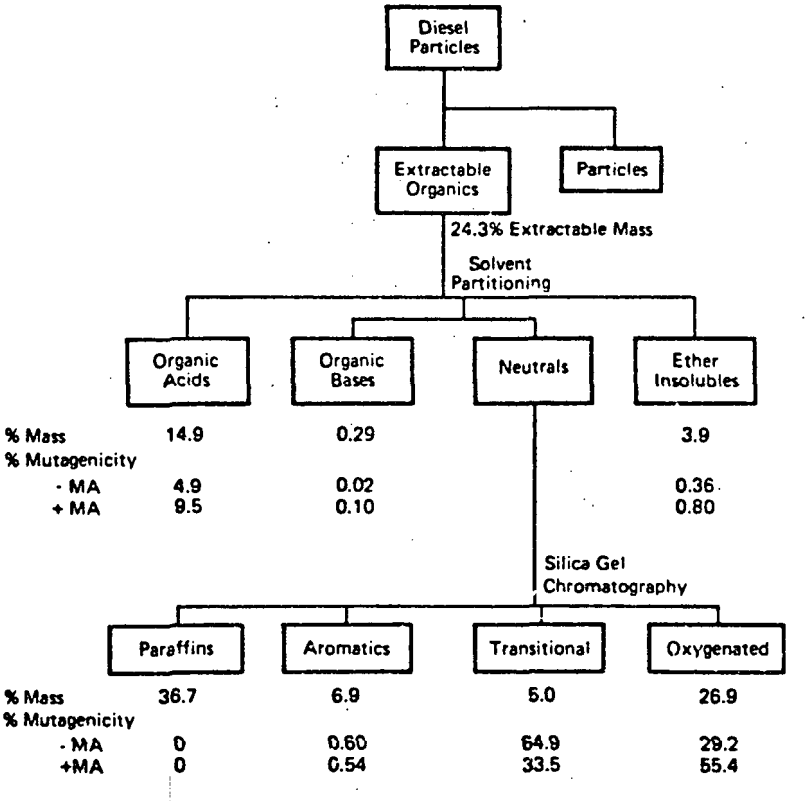

Figure 1. Distribution from diesel particles of mass and mutagenic activity in Salmonella typhimurium TA98.

Table 1. Distribution of the mass and mutagenic activity of fractionated diesel particle organics[a]

Fraction	Mass (%)	Mutagenic Activity[b] (rev/mg)		Weighted Mutagenic Activity[c] (rev/mg)		Distribution of Mutagenic Activity (%)	
		-MA	+MA	-MA	+MA	-MA	+MA
Organic acids	14.9	193	248	28.8	37.0	4.9	9.5
Organic bases	0.3	43.8	132	0.13	0.40	0.02	0.10
Ether insolubles	3.9	53.9	80.9	2.1	3.2	0.36	0.80
Paraffins	36.7	Neg.	Neg.	0.0	0.0	0.0	0.0
Aromatics	6.9	49.5	30.1	3.42	2.1	0.60	0.54
Transitionals	5.0	7520	2620	376	131	64.9	33.5
Oxygenates	26.9	629	798	169	215	29.2	55.4

[a]-MA = without metabolic activation; +MA = with metabolic activation.
[b]Slope determined from linear regression analysis of the initial portion of the dose-reponse curve.
[c]Determined by multiplying the mutagenic activity by the percent mass.

activity is significantly less in nitroreductase-deficient strains of Salmonella typhimurium, suggesting that nitrated polycyclic compounds are present (11). Nitrated polycyclic aromatic hydrocarbons (NO_2-PAHs) are potent direct-acting frameshift mutagens detected in xerographic toners (12). Identification and quantification of a series of NO_2-PAHs in diesel extracts allow us to estimate their contribution to the mutagenic activity of diesel particulate emissions (13). Particulate emissions from catalyst-equipped gasoline-engine vehicles using unleaded fuel contain significantly less of these NO_2-PAHs. The mutagenic activity of both leaded- and unleaded-gasoline emissions is substantially increased with the addition of an exogenous metabolic activation (MA) system, suggesting that the classical PAHs may play a more important role than do NO_2-PAHs in the mutagenicity and carcinogenicity of gasoline emissions (14).

EVALUATION OF THE EFFECTS OF VARIOUS FUELS, ENGINES, AND CONTROL TECHNOLOGIES

Short-term bioassays have proven useful in evaluating the effects of various engines, fuels, and control technologies on the mutagenicity of emissions. To draw meaningful conclusions from such comparisons, however, Claxton and Kohan (15) studied the normal variations in the emissions and in bioassay results for emissions due to sampling, preparation, and storage for one engine under standard conditions. For this study, an Oldsmobile 350 diesel vehicle was run on repeated Highway Fuel Economy Test (HWFET) cycles during one day and on separate days. The coefficients of variation (CV) for these parameters, as shown in Table 2, ranged from 0.07 to 0.11. A computerized statistical method recently developed by Stead et al. (16) for analysis of dose-response data from the Ames Salmonella typhimurium bioassay greatly facilitated these comparisons. An example of this

Table 2. Coefficients of variation of assay parameters for standard operation of one diesel vehicle

Parameters	All Days (CV)	Range of Separate Days (CV)
Particle emission rate	0.07	0.02 ~ 0.05
% Organic extractables	0.09	0.07 - 0.08
Mutagenicity slope	0.11	0.01 - 0.11

analysis is shown in Figures 2a and 2b. The average non-linear-model slope, shown with 95% confidence limits, is then used in the comparisons.

To compare the mutagenicity of particle emissions from different sources or fuels, the percent of organics extractable from the particles and the vehicle's particle emission rate must also be included in the analysis. The final rate of mutagenic emissions is determined from all three parameters (Table 3). The significantly lower rate of mutagenic emissions from the catalyst-equipped, gasoline-engine vehicle is due primarily to its much lower particle emission rate (0.0033 g/km), compared with the leaded gasoline and diesel emissions. The significantly lower rate of mutagenic emissions from the GM bus (Table 3) than from the diesel truck and car, however, is due to the substantially lower mutagenic activity of the organics.

The mutagenic and carcinogenic activities of the extractable organics from a series of diesel and gasoline particle emissions have been compared in a battery of short-term bioassays (14). The bioassays that provided the best quantitative and reproducible dose-response data were (1) the Ames Salmonella typhimurium mutagenesis assay, (2) the mouse lymphoma mutagenesis assay, and (3) the Chinese hamster ovary-cell sister-chromatid-exchange assay. The results of short-term genetic bioassays were compared with those of a skin-tumorigenesis assay in SENCAR mice (17). Within the diesel and gasoline vehicle emission samples examined, a very high correlation was observed among the results of these three genetic bioassays and also with the mouse skin tumor initiation assay (14).

The influence of fuel on the mutagenicity of emissions was initially examined for five fuels, including four No. 2 diesel fuels and one No. 1 jet fuel, in the two light-duty diesel vehicles (a Volkswagen Diesel Rabbit and a Mercedes 240D) (9). In the VW Rabbit, the emissions from the minimum-quality fuel, with a higher aromatic and nitrogen content, were significantly (approximately 5 times) more mutagenic per milligram particulate emission than were the other fuels. These fuels did not differ significantly in mutagenicity when they were burned in the Mercedes (9).

Similar studies were recently conducted (Table 4) for five fuels in the three heavy-duty vehicles:

(1) GM city bus with a Detroit DD8V-71 engine;

(2) Mack truck with a Mack ENDT 676 engine; and

Table 3. Comparison of the rates of mutagenic emissions from motor vehicles

Source	Mutagenicity of Organics (rev/µg)[a]	Extractable Organics (%)	Particle Emission Rate (g/km)	Mutagenic Emission Rate (rev/km)
Diesel fuel				
Car (Mercedes)[b]	12.0	8	0.24	240,000
Truck (Mack)[c]	2.3	11	1.3	320,000
Bus (GM)[c]	0.1	17	2.1	37,000
Gasoline fuel				
Non-catalyst (Ford Van)[d]	32.0	19	0.03	180,000
Catalyst (Mustang II)[e]	3.5	43	0.0033	5,000

[a] Salmonella typhimurium TA98 with metabolic activation; non-linear-model slope analyzed by the method of Stead et al. (16).

[b] Mercedes 300D, 1977 model, operated on the HWFET cycle using No. 2 diesel fuel obtained from Union 76.

[c] Mack ENDT 676 diesel engine in a dual-drive tandem-axle truck and GM bus with a Detroit diesel DD8V-71 engine were operated with the same average No. 2 diesel fuel (EM-239-F) on the 1983 transient heavy-duty cycle.

[d] Ford Van, 1970, in-line 6-cylinder engine, operated with leaded gasoline (Premium A) on the HWFET.

[e] Ford Mustang II-302, 1977, operated with unleaded gasoline on the HWFET.

SAMPLE ID: MSER-81-0015 LAB: NSNC ACTIVATION: -
STRAIN: TA98 DATE: 06/10/81 TECHNICIAN: BCM

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	17	19		18.00	1.41
3.00*	UGS	289	308	302	299.67	9.71
5.00	UGS	112	108	81	100.33	16.86
10.00	UGS	208	191	175	191.33	16.50
30.00	UGS	532	459	522	504.33	39.58
50.00	UGS	735	677	725	712.33	31.01
100.00	UGS	1220	1219	1191	1210.00	16.46
300.00	UGS	1748	1769	1580	1699.00	103.59

	B(0)	B(1)	B(2)	B(3)
ESTS.	17.513	2.9114	.9851	.00365

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	30.62	13	.0038	-92.5840
ADEQUACY	5.66	3	.1294	-95.4141
TOXICITY	382.76	1	.0000	-286.7941
MUTAGENICITY	14924.67	2	.0000	-7557.7505

AVERAGE SLOPE (NONLIN. MODEL) = 16.880
95% CONF. LIMITS = (14.056, 20.272)

AVERAGE SLOPE (LINEAR REGR.) = 5.316
95% CONF. LIMITS = (4.255, 6.378)

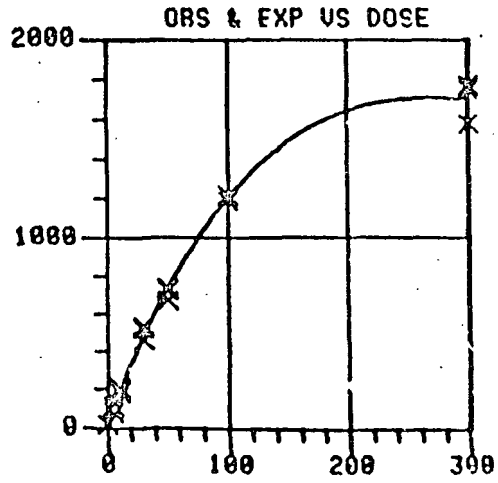

Figure 2a. Example of a computerized statistical method for the analysis of dose-response data from Ames Salmonella typhimurium without metabolic activation.

SAMPLE ID: MSER-81-0015 LAB: NSNC ACTIVATION: +
STRAIN: TA98 DATE: 06/10/81 TECHNICIAN: BCM

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	25	26	26	25.67	.58
.50*	UGS	539	509	526	524.67	15.04
5.00	UGS	81	76	65	74.00	8.19
10.00	UGS	124	111	126	120.33	8.14
30.00	UGS	440	483	469	464.00	21.93
50.00	UGS	697	657	716	690.00	30.12
100.00	UGS	1301	1326	1220	1282.33	55.41
300.00	UGS	1792	1890	1999	1893.67	103.55

	B(0)	B(1)	B(2)	B(3)
ESTS.	24.497	1.9236	1.2412	.00487

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	23.75	14	.0490	-90.7998
ADEQUACY	19.06	3	.0003	-100.3287
TOXICITY	616.38	1	.0000	-408.5193
MUTAGENICITY	20855.64	2	.0000	-10528.1465

AVERAGE SLOPE (NONLIN. MODEL) = 27.090
95% CONF. LIMITS = (23.835, 30.790)

AVERAGE SLOPE (LINEAR REGR.) = 6.196
95% CONF. LIMITS = (5.138, 7.254)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE

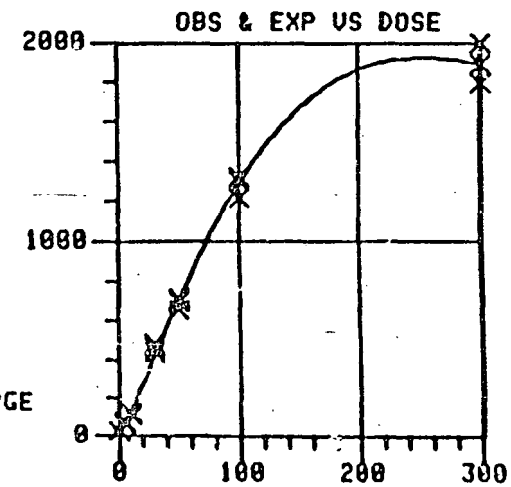

Figure 2b. Example of a computerized statistical method for the analysis of dose-response data from Ames Salmonella typhimurium with metabolic activation.

Table 4. Effect of fuel quality on rate of mutagenic emissions

Fuel	Fuel Characteristics			Mutagenic Activity[a]/km x 10^5		
	% Aromatics	% Nitrogen	Cetane No.	Ford/Cat	Mack Truck	GM Bus
Jet	2.7	0.025	56.0	-	-	0.54
Diesel #1	10.5	0.006	49.0	-	-	0.88
Diesel #2						
Premium	16.9	0.046	52.1	7.3	4.2	0.22
Average	21.3	0.040	48.0	6.8	5.6	0.79
Minimum	35.8	0.61	42.0	14.0	3.7	1.1

[a]Salmonella typhimurium TA98 without metabolic activation.

(3) Ford Bob Tail Van (LN 7000) with a Caterpillar 3208 engine.

The five fuels ranged in aromatic content from 2.7% to 35.8% and in nitrogen content from 0.006% to 0.61%. The minimum-quality fuel produced a higher rate of mutagenic emissions in the Ford/Cat Van and the GM bus than did the higher-quality fuels. This difference was not observed for the Mack Truck (Table 4). The fuels with the lowest aromatic and nitrogen content, jet and diesel No. 1, did not produce less mutagenic emissions in the GM bus than did the diesel No. 2 premium fuel. However, the mutagenic activity (revertants per microgram) for the extractable organics from the bus particle emissions was much lower than that for the emissions from the other two vehicles.

These studies suggest that although poorer-quality fuel, with relatively high aromatic and nitrogen content, can increase the rate of mutagenic emissions in certain vehicles, such differences are not observed in all vehicles. In general, the changes in mutagenic activity of emissions as a function of fuel quality are much smaller than the differences between different types of engines (e.g., diesel vs. gasoline) and control technologies (catalyst-equipped vs. non-catalyst-equipped vehicles).

COMPARATIVE ASSESSMENT OF VARIOUS COMBUSTION SOURCES

The extractable organics from diluted and cooled combustion particle emissions from both stationary and mobile sources were tested in a battery of mutagenesis and carcinogenesis bioassays (Table 5). The mutagenic or carcinogenic activity per microgram of extractable organics was generally within two orders of magnitude (10^2). The emission rates for the particle-bound organics differed in some cases by as much as 5×10^5. To compare the mutagenicity of emissions from various sources, it is critical to compare the rates of mutagenic emissions on the same basis (e.g., fuel consumption, distance driven, energy consumption, or yield). To compare rates of mutagenic emissions of mobile sources, we have compared the mutagenic activity per kilometer, as shown previously in Table 3. The rates of mutagenic emissions from stationary sources are compared on the basis of fuel consumption or energy consumption (Table 6). Although stationary and mobile sources could be directly compared on a fuel-consumption basis, the distribution of fuel is fixed by consumption demands based on the relative needs for transporation, home heating, or energy.

The relative contributions of these various combustion sources to the mutagenicity observed in ambient-air particles can be estimated using emission inventories for a particular geographic region or source-apportionment data from ambient-air samples. Such studies, now in progress, suggest that combustion emissions from motor vehicles contribute nearly half of the

Table 5. Comparative mutagenicity and carcinogenicity of extractable organics

Source	Mouse Skin Tumor Initiation[a] (papillomas/ mouse/mg)	Mutation in L5178Y Mouse Lymphoma Cells (TK mutants/ 10^6 surviving cells/µg/ml)		SCE in CHO Cells[b] (SCE/cell/µg/ml)		Ames Salmonella TA98[c] (rev/µg)	
		-MA	+MA	-MA	+MA	-MA	+MA
Diesel							
Mercedes	0.37	0.03	1.5	0.09	0.16	10.0	12.0
Nissan	0.59	4.2	2.9	0.30	0.071	11.0	13.0
Volkswagen Rabbit	0.24	0.98	0.72	0.075	0.030	3.8	3.0
Oldsmobile	0.31	1.2	1.3	Neg.	0.017	2.2	1.5
Caterpillar	Neg.	0.25	0.063	0.011	Neg.	0.38	0.31
Gasoline catalyst							
Mustang II	0.17	0.38	1.1	0.076	-	1.6	3.5
Gasoline non-catalyst							
Chevrolet 366	-	1.2	4.9	0.72	0.22	2.9	6.2
Ford Van	-	2.1	5.7	0.62	0.47	17.0	32.0

(continued)

[a]Based on papilloma multiplicity data in SENCAR mice (17).
[b]-MA was 21.5 h exposure and +MA was a 2 h exposure.
[c]Determined from simple linear regression analysis.
[d]IP = In progress.

Table 5, continued

Source	Mouse Skin Tumor Initiation[a] (papillomas/ mouse/mg)	Mutation in L5178Y Mouse Lymphoma Cells (TK mutants/ 10^6 surviving cells/µg/ml)		SCE in CHO Cells[b] (SCE/cell/µg/ml)		Ames Salmonella TA98[c] (rev/µg)	
		-MA	+MA	-MA	+MA	-MA	+MA
Residential heaters							
Oil	0.12	1.2	2.6	0.06	0.04	1.3	2.1
Wood	-	-	-	-	-	0.15	0.93
Coal	-	-	-	-	-	IP[d]	IP
Utility power plants							
Coal, conventional	-	IP[d]	IP	IP	IP	3.1	-
Coal, FBC	-	IP	IP	IP	IP	9.4	5.2
Oil	-	-	-	-	-	IP	IP

[a]Based on papilloma multiplicity data in SENCAR mice (17).
[b]-MA was 21.5 h exposure and +MA was a 2 h exposure.
[c]Determined from simple linear regression analysis.
[d]IP = In progress.

Table 6. Rate of mutagenic emissions from stationary sources

Source	Fuel	Mutagenicity[a] of Organics (rev/μg)	Organic Emission Rate (mg/kg fuel)	Organic Emission Rate (ng/J)	Mutagenic Emission Rate (rev/kg fuel)	Mutagenic Emission Rate (rev x 10^{-3}/J)
Residential heaters						
Woodstove	Pine	1.3	8940	508.0	12,000,000	660.0
	Oak	0.9	3096	187.0	2,800,000	168.0
Residential oil furnace #1	No. 2 fuel oil	2.0	21	0.5	40,000	1.0
furnace #2	No. 2 fuel oil	5.1	70	1.5	360,000	7.6
Utility power plants (coal)		3.1	-	0.01	-	0.031

[a] *Salmonella typhimurium* TA98 with metabolic activation.

mutagenic activity observed from respirable air particles collected in urban and suburban street-level locations. The relative contributions from diesel, leaded-gasoline, and unleaded-gasoline vehicles depends on their distribution at the particular location.

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