Mutation Research, 276 (1992) 125–132

MUTREV 00141

Application of a semi-automated SOS chromotest for measuring genotoxicities of complex environmental mixtures containing polycyclic aromatic hydrocarbons

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Keywords: SOS chromotest, Semi-automated; Polycyclic aromatic hydrocarbons

Summary

Soxhlet-extracted samples of standard reference materials (SRMs) 1649 (PAR1: urban dust/organics) and 1650 (PAR2: diesel particulate matter) from the U.S. Institute of Standards and Technology were tested for induction of SOS functions using a semi-automated version of the SOS chromotest with *Escherichia coli* PQ37. Concentrations of 10 polycyclic aromatic hydrocarbons in the extracts were determined using reversed-phase HPLC. Only the diesel particulate matter (PAR2) extracts expressed SOS induction activity, which decreased when metabolic activation was used. Mutagenic PAH compounds (e.g., chrysene) were found in higher concentrations in the PAR2 extracts than in the PAR1 extracts but this could not explain the genotoxicity while it was mainly exhibited without metabolic activation. The direct genotoxic activity of the diesel particulate matter sample PAR2 is probably caused by nitroaromatic compounds; this was also supported by parallel studies with the Ames/Salmonella assay.

The Salmonella/microsome assay (Ames et al., 1975; Maron and Ames, 1983) is to this day the most commonly used bacterial short-term mutagenicity test for screening of complex environmental mixtures. During the last decade another test type based on mutagen-inducible DNA repair (SOS repair) has also been used for screening purposes. This assay, the SOS chromotest, was first described by Quillardet et al. (1982) who introduced it for detection of genotoxic compounds. The advantages of using the SOS chromotest are that results can be obtained in one work day and that the test can be easily automated. An automated application of the SOS chromotest has been developed by Labsystems OY (Helsinki, Finland) using a Bioscreen analyzer system together with an interactive BioSOS software program.

Validation studies comparing the SOS chromotest with other types of bacterial short-term assays (mainly the Salmonella/microsome assay) have mostly been done using pure chemicals (Quillardet et al., 1985; Brams et al., 1987; Dayan et al., 1987; von der Hude et al., 1988), but studies with complex mixtures like foodstuffs (Poirier et al., 1989), airborne particulate matter (Schleibinger et al., 1989) and extracts of urban air particles (Courtois et al., 1988) have also been

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performed. A validation study with complex mixtures (Whong et al., 1986) has been performed using the closely related SOS/umu test (Oda et al., 1985).

The work reported here consists of optional studies performed at the Institute of Occupational Health (Helsinki, Finland) within the frame of the Collaborative Study on Complex Mixtures by the International Programme on Chemical Safety. The purpose of this work was to study the genotoxic effects of Soxhlet-extracted samples of two particulate materials consisting of urban dust/organics and diesel particulate matter using a semi-automated SOS chromotest. Attempts were made to find relationships between the SOS-inducing potencies, mutagenicities in the Salmonella/microsome assays and the concentrations of 10 polycyclic aromatic hydrocarbons in the samples.

Materials and methods

The samples

The test samples consisted of dichloromethane (DCM) solutions of the Soxhlet-extracted standard reference materials (SRMs) 1649 and 1650. During the study SRM 1649 and 1650 were referred to as samples PAR1 and PAR2. The aliquots from parallel extractions of PAR1 and PAR2, assayed in the semi-automated SOS chromotest, were labelled P1E1A7, P1E2A7, P2E1A7 and P2E2A7. The aliquots for chemical analysis of the extracted PAR1 and PAR2 samples were labeled P1E1A6, P1E2A6, P2E1A6 and P2E2A6.

Chemicals

o-Nitrophenyl-β-D-galactopyranoside (ONPG), *p*-nitrophenyl phosphate (PNPP) and 4-nitroquinoline-*N*-oxide (4-NQO) were purchased from Sigma (St. Louis, MO, U.S.A.). 2-Aminoanthracene (2-AA) came from Aldrich-Chemie (Steinheim, F.R.G.). Stock solutions of 4-NQO and 2-AA were prepared by dissolving 5 mg of the chemical in 1 ml in dimethyl sulfoxide (DMSO; E. Merck, Darmstadt, F.R.G.). Working solutions of the control mutagens were made by preparing 1:100 dilutions in DMSO. The molar concentrations of the working solutions were 263 nmole/ml for 4-NQO and 259 nmole/ml for 2-AA.

Escherichia coli PQ37 tester strain

The *Escherichia coli* PQ37 tester strain came from Labsystems OY, Finland, and had originally been supplied by M. Hofnung (Institut Pasteur, Paris, France). This tester strain has been characterized by Quillardet et al. (1982).

Preparation of media, buffers and reagents for the SOS chromotest

Preparation of media, buffers and reagents for the SOS chromotests were done as described in the Bioscreen application manual (Huttunen, 1987). These instructions are based on the ones published by Quillardet and Hofnung (1985), with modifications adopted to meet the special conditions of the semi-automated SOS chromotest.

Extraction of the particulate samples

The extraction thimbles (Macherey Nagel MN 649, 22 × 88 mm) to be used in the Soxhlet extractions of the particulate samples PAR1 and PAR2 were heated to incandescence at 450°C for 4 h, after which they were cooled down and placed in an exsiccator for 48 h. The thimbles were then weighed twice on separate days. Of the PAR1 sample, 1.4526 and 1.4522 g, and of the PAR2 sample, 96.4 and 94.8 mg were weighed for the first (E1) and second (E2) rounds of extraction. Dichloromethane (65 ml) was used as solvent in the Soxhlet extraction procedure. One extraction cycle lasted for 4 min and the total extraction time was 7 h, during which the samples were subjected to approximately 105 extraction cycles. Extractions were performed protected from light, under nitrogen pressure. The thimble volume of the extractions was 30 ml.

Procedure for the semi-automated SOS chromotest

The SOS chromotest was performed using a Bioscreen analyzer system together with a BioSOS program (Labsystems OY, Finland). The assay is semi-automated: the Bioscreen analyzer, controlled by the BioSOS dedicated software, performs the dilution and dispensing of samples on microtiter plates and uses a kinetic measurement principle for quantifying the enzymatic activities

of β-galactosidase and alkaline phosphatase (Huttunen, 1987; Janz et al., 1988).

The DCM extracts were evaporated and adjusted with fresh DCM to 10 ml. The solvent (DCM) was exchanged for dimethyl sulfoxide in the aliquots (P1E1A7, P1E2A7, P2E1A7 and P2E2A7) used for the BioSOS test. The amounts of DMSO were adjusted so that the PAR1 aliquots had a final concentration of 100 mg and the PAR2 aliquots 10 mg particles (starting material)/ml. The BioSOS tests of each sample were repeated within 1 week of the first round of tests.

The particulate samples were tested using a BioSOS protocol for testing with and without metabolic activation. In this protocol, an option for making 10 two-fold dilutions of each sample and the positive controls (4-NQO and 2-AA) in DMSO was used. The Bioscreen analyzer unit performed all dilutions and the dispensing of samples to microtiter cuvettes. Subsequently, the solutions of test bacteria and S9 mix were dispensed. The concentration of S9 in the incubation mixture (sample, bacteria and S9 mix) was 1.5%. The mixtures of samples, bacteria and S9 mix (or solvent) in microtiter cuvettes were incubated for 2 h at 37°C. The cuvette plates were intermittently shaken by the Bioscreen analyzer to ensure mixing and proper aeration. After the incubation period, the chromogenic substrates ONPG for β-galactosidase and PNPP for alkaline phosphatase were added. Kinetic measurements of absorbances at 420 nm in each cuvette were performed during a 30-min period, corresponding to 18 successive rounds of measurements. After the kinetic measurement period, the data were stored by the BioSOS program and subjected to data processing.

Data processing

The 'data processing' module of the BioSOS program starts by calculating the degree of β-galactosidase induction. First, the ratio R is calculated for each pair of cuvettes by dividing the amount of β-galactosidase (the slope of the kinetic measurements) by the amount of alkaline phosphatase in the accompanying cuvette:

$$R = \frac{\beta\text{-Gal (sample)}}{\text{Ap (sample)}}$$

This calculation yields an estimate of β-galactosidase activity corrected for toxicity.

Then the ratios R(s) obtained for each dilution of the sample are divided by the ratio R(c) in the solvent controls. This ratio indicates the degree of induction (induction factor) obtained at each different concentration of the tested sample:

$$I = \frac{R\text{s}}{R\text{c}}$$

or

$$I = \frac{\dfrac{\beta\text{-Gal (sample)}}{\text{Ap (sample)}}}{\dfrac{\beta\text{-Gal (control)}}{\text{Ap (control)}}}$$

When the induction factors are plotted against the concentrations of the sample in the cuvettes, a dose–response curve is obtained. The positive slope of this curve is used to obtain a figure called the SOS-inducing potency (SOSIP value), which is the slope of the curve expressed in mass units. A sample was considered positive if the SOS induction factor increased by over 1.5 (Quillardet et al., 1985). A positive sample also had to show a dose-dependent increase in β-galactosidase activities.

Based on the measurement data of alkaline phosphatase activities the BioSOS program also calculates inhibition factor values for each tested dose. The inhibition factor value expresses the toxicity or inhibitory effect on protein synthesis of each tested dose relative to the solvent control.

Chemical analysis of the samples

The solvent (1 ml dichloromethane) in the extracted PAR1 and PAR2 aliquots (P1E1A6, P1E2A6, P2E1A6 and P2E2A6) was exchanged to 1 ml dimethyl sulfoxide under nitrogen purge at room temperature. The samples (250 μl) were filtered through a 2-μm filter, diluted to 1 ml with acetonitrile and analyzed by reversed-phase HPLC (Perkin-Elmer Series 4) with fluorescence detection (Perkin-Elmer LS-4). A $30 + 100 \times 4$ mm HS-3 C_{18} (Perkin-Elmer) column was used with a mobile phase of acetonitrile in water. The

gradient ranged from 65 to 85% ACN (linear 1%/min), with a flow rate of 2 ml/min. Peak heights were used for quantitative calculations.

Results

The PAR2 extracts (P2E1A7 and P2E2A7) were the only ones causing induction of SOS functions in *E. coli* PQ37 (Fig. 1, Tables 1–3). The induction of SOS functions was stronger when the extracts were tested without metabolic activation. Increased induction factor values were also observed when the PAR1 extracts were tested with and without metabolic activation. There were, however, no dose-dependent increases in β-galactosidase activities without metabolic activation (Table 1). The increases in induction factor values apparently resulted from a specific inhibitory effect on the alkaline phosphatase enzyme. With metabolic activation, some doses of the PAR1 extracts showed induction factor values above 1.5, but these effects could not be repeated. The test results with the PAR1 extracts were therefore considered to be negative.

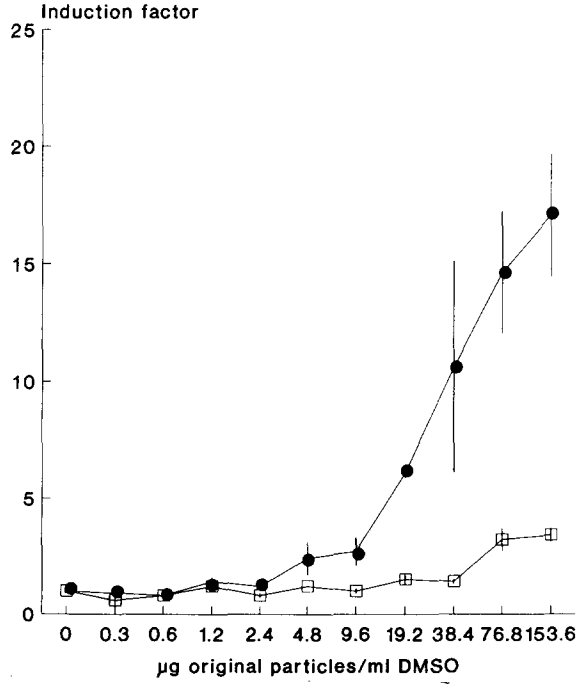

Fig. 1. SOS induction factor values (means of the repeated extractions and their parallel tests ± SD) of the diesel particulate sample SRM 1650 (PAR2), with S9 (□) and without S9 (●), using a semi-automated SOS chromotest.

The concentrations of 10 polycyclic aromatic hydrocarbons determined in aliquots P1E1A6, P1E2A6, P2E1A6 and P2E2A6 of the particulate extracts using reversed-phase HPLC are shown in Table 4. The concentrations of benzo[*a*]pyrene and benzo[*ghi*]perylene in PAR1 extracts were on average 10–20% lower than the certified values given for these compounds in SRM 1649 by the U.S. Institute of Standards and Technology. In PAR2 extracts, the concentrations of pyrene and benzo[*a*]pyrene were on average 10–15% lower than the certified values for these compounds in SRM 1650. The concentrations of benzo[*ghi*]perylene in PAR2 samples were actually about 36% higher than the certified value, but this was probably due to the use of an old standard solution giving too small standard peaks in the HPLC analysis.

Discussion

The results of testing the PAR1 and PAR2 (SRM 1649 and 1650) extracts using the Salmonella/microsome assay are presented in a paper by Claxton et al. (1992). The plate incorporation test results from the Institute of Occupational Health (Lab. 18) show that the diesel particulate matter extracts were mutagenic with both *S. typhimurium* strains used in the study (TA98 and TA100) and that the mutagenic activities decreased when metabolic activation was used. The urban air particulate matter extracts were mutagenic only with strain TA98. The SOS chromotest results show a better correlation with plate incorporation test results when strain TA100 was used. These results confirm the observation of von der Hude et al. (1988) who showed that substances which are mutagenic only in 'frameshift' strains (like TA98) do not induce SOS repair in *E. coli* PQ37.

The concentrations of pyrene, phenanthrene, anthracene and chrysene were higher in the PAR2 extracts than in the PAR1 extracts. Pyrene has given equivocal results in the Salmonella/mammalian microsome assay (Bridges et al., 1981). Phenanthrene is mutagenic in *S. typhimurium* strains TA1537 and TA100 only when

TABLE 1

INDUCTION OF SOS RESPONSES IN *E. coli* PQ37 BY SAMPLES (A7) FROM THE FIRST (E1) AND SECOND (E2) EXTRACTIONS OF THE URBAN AIR PARTICULATE MATERIAL SRM 1649 (P1)

Dose (μg/ml)	β-G	P-ase	β-G/P-ase	Ind.F.	Inh.F.	β-G	P-ase	β-G/P-ase	Ind.F.	Inh.F.
	P1E1A7 − S9 mix (1)					*P1E1A7 − S9 mix (2)*				
0	0.003	0.008	0.371	1.0	1.00	0.003	0.012	0.214	1.0	1.00
3	0.001	0.011	0.092	0.2	1.35	0.003	0.012	0.217	1.0	0.98
6	0.003	0.012	0.247	0.7	1.45	0.003	0.013	0.197	0.9	1.08
12	0.003	0.011	0.287	0.8	1.31	0.003	0.011	0.276	1.3	0.93
24	0.003	0.012	0.248	0.7	1.46	0.003	0.014	0.208	1.0	1.13
48	0.003	0.010	0.331	0.9	1.17	0.003	0.012	0.257	1.2	0.99
96	0.003	0.012	0.259	0.7	1.42	0.003	0.012	0.217	1.0	1.01
192	0.003	0.005	0.581	1.6	0.62	0.003	0.005	0.579	2.7	0.41
384	0.002	0.003	0.845	2.3	0.34	0.002	0.003	0.573	2.7	0.25
768	0.002	0.001	1.719	4.6	0.12	0.001	0.001	1.403	6.6	0.07
1536	0.000 [a]	0.000 [a]	0.975	2.6	0.06	0.000 [a]	0.001	0.309	1.4	0.05
	P1E2A7 − S9 mix (1)					*P1E2A7 − S9 mix (2)*				
0	0.001	0.003	0.341	1.0	1.00	0.002	0.013	0.192	1.0	1.00
3	0.000 [a]	0.002	0.088	0.3	0.83	0.001	0.014	0.073	0.4	1.09
6	0.001	0.004	0.190	0.6	1.22	0.003	0.015	0.173	0.9	1.16
12	0.001	0.003	0.385	1.1	0.96	0.003	0.012	0.240	1.2	0.92
24	0.001	0.004	0.188	0.6	1.34	0.003	0.016	0.169	0.9	1.28
48	0.001	0.003	0.445	1.3	0.92	0.003	0.012	0.248	1.3	0.95
96	0.001	0.003	0.297	0.9	0.98	0.003	0.015	0.183	1.0	1.21
192	0.001	0.001	0.599	1.8	0.48	0.002	0.006	0.346	1.8	0.50
384	0.001	0.001	0.629	1.8	0.28	0.002	0.005	0.354	1.8	0.41
768	0.000 [a]	0.000 [a]	0.968	2.8	0.09	0.001	0.001	1.523	7.9	0.08
1536	0.000 [a]	0.000 [a]	0.525	1.5	0.08	0.000 [a]	0.001	0.353	1.8	0.05
	P1E1A7 + S9 mix (1)					*P1E1A7 + S9 mix (2)*				
0	0.003	0.008	0.365	1.0	1.00	0.003	0.008	0.356	1.0	1.00
3	0.003	0.007	0.387	1.1	0.89	0.002	0.008	0.277	0.8	0.94
6	0.002	0.009	0.199	0.5	1.26	0.002	0.007	0.291	0.8	0.92
12	0.002	0.007	0.311	0.9	0.95	0.003	0.008	0.343	1.0	0.98
24	0.002	0.004	0.623	1.7	0.47	0.003	0.003	0.293	3.6	0.32
48	0.003	0.009	0.322	0.9	1.17	0.003	0.007	0.446	1.3	0.86
96	0.003	0.008	0.357	1.0	1.09	0.002	0.010	0.229	0.6	1.18
192	0.003	0.007	0.404	1.1	0.94	0.003	0.008	0.417	1.2	0.93
384	0.004	0.009	0.384	1.1	1.23	0.004	0.009	0.411	1.2	1.12
768	0.003	0.007	0.451	1.2	0.97	0.004	0.007	0.592	1.7	0.82
1536	0.004	0.009	0.495	1.4	1.19	0.005	0.008	0.594	1.7	0.95
	P1E2A7 + S9 mix (1)					*P1E2A7 + S9 mix (2)*				
0	0.001	0.002	0.318	1.0	1.00	0.003	0.008	0.326	1.0	1.00
3	0.001	0.002	0.323	1.0	0.90	0.003	0.007	0.396	1.2	0.87
6	0000 [a]	0.003	0.154	0.5	1.21	0.002	0.011	0.196	0.6	1.29
12	0000 [a]	0.002	0.181	0.6	1.00	0.003	0.008	0.320	1.0	0.99
24	0.001	0.002	0.265	0.8	0.86	0.003	0.010	0.323	1.0	1.23
48	0.001	0.002	0.305	1.0	0.98	0.002	0.008	0.328	1.0	0.93
96	0.001	0.003	0.270	0.9	1.22	0.004	0.010	0.340	1.0	1.26
192	0.001	0.002	0.336	1.1	0.94	0.003	0.007	0.388	1.2	0.85
384	0.001	0.003	0.254	0.8	1.29	0.003	0.010	0.333	1.0	1.28
768	0.001	0.002	0.475	1.5	0.77	0.003	0.008	0.405	1.2	0.92
1536	0.001	0.003	0.360	1.1	1.12	0.003	0.009	0.303	0.9	1.10

The doses are expressed as μg original particles per ml dimethyl sulfoxide. All enzyme activity values are given with three decimal places by the BioSOS program. More accurate values of enzyme activities are, however, used by the program for computing induction and inhibition factor values.

β-G, β-galactosidase activities; P-ase, alkaline phosphatase activities; Ind.F., Induction factor values; Inh.F., inhibition factor values; (1, 2), first and second round of testing.

[a] Enzyme activity values < 0.001.

TABLE 2

INDUCTION OF SOS RESPONSES IN *E. coli* PQ37 BY SAMPLES (A7) FROM THE FIRST (E1) AND SECOND (E2) EXTRACTIONS OF THE DIESEL PARTICULATE MATERIAL SRM 1650 (P2)

Dose (μg/ml)	β-G	P-ase	β-G/P-ase	Ind.F.	Inh.F.	β-G	P-ase	β-G/P-ase	Ind.F.	Inh.F.
	P2E1A7 − S9 mix (1)					*P2E1A7 − S9 mix (2)*				
0	0.003	0.008	0.371	1.0	1.00	0.003	0.012	0.214	1.0	1.00
0.3	0.003	0.011	0.243	0.7	1.25	0.002	0.014	0.162	0.8	1.12
0.6	0.003	0.013	0.219	0.6	1.55	0.003	0.014	0.218	1.0	1.12
1.2	0.004	0.011	0.356	1.0	1.36	0.004	0.012	0.331	1.5	1.01
2.4	0.004	0.013	0.249	0.9	1.53	0.005	0.015	0.307	1.4	1.21
4.8	0.006	0.010	0.596	1.6	1.23	0.007	0.012	0.569	2.7	1.01
9.6	0.009	0.014	0.666	1.8	1.65	0.010	0.017	0.581	2.7	1.36
19.2	0.016	0.009	1.850	5.0	1.06	0.013	0.012	1.029	4.8	1.02
38.4	0.024	0.012	1.974	5.3	1.42	0.024	0.008	2.869	13.4	0.70
76.8	0.030	0.007	4.301	11.6	0.83	0.025	0.009	2.932	13.7	0.71
153.6	0.029	0.006	5.045	13.6	0.68	0.032	0.007	4.381	20.5	0.60
	P2E2A7 − S9 mix (1)					*P2E2A7 − S9 mix (2)*				
0	0.001	0.003	0.341	1.0	1.00	0.002	0.013	0.192	1.0	1.00
0.3	0.001	0.003	0.333	1.0	0.91	0.003	0.013	0.204	1.1	1.00
0.6	0.001	0.004	0.267	0.8	1.23	0.003	0.017	0.159	0.8	1.34
1.2	0.001	0.003	0.497	1.5	0.97	0.004	0.012	0.310	1.6	0.98
2.4	0.001	0.004	0.349	1.0	1.37	0.004	0.017	0.243	1.3	1.36
4.8	0.003	0.003	1.135	3.3	0.94	0.005	0.014	0.392	2.0	1.09
9.6	0.004	0.004	1.158	3.4	1.28	0.009	0.017	0.553	2.9	1.36
19.2	0.009	0.003	3.252	9.5	0.92	0.012	0.012	1.006	5.2	0.95
38.4	0.009	0.003	3.554	10.4	0.87	0.021	0.015	1.421	7.4	1.17
76.8	0.011	0.002	6.408	18.8	0.62	0.027	0.010	2.727	14.2	0.78
153.6	0.008	0.001	5.398	15.8	0.51	0.036	0.010	3.499	18.2	0.82
	P2E1A7 + S9 mix (1)					*P2E1A7 + S9 mix (2)*				
0	0.003	0.008	0.365	1.0	1.00	0.003	0.008	0.356	1.0	1.00
0.3	0.002	0.007	0.256	0.7	0.91	0.002	0.007	0.318	0.9	0.98
0.6	0.003	0.008	0.389	1.1	0.94	0.003	0.009	0.294	0.8	1.22
1.2	0.003	0.008	0.425	1.2	0.96	0.003	0.008	0.346	0.9	1.05
2.4	0.002	0.009	0.272	0.8	1.11	0.003	0.010	0.295	0.8	1.28
4.8	0.003	0.007	0.420	1.2	0.92	0.003	0.006	0.456	1.2	0.84
9.6	0.003	0.009	0.368	1.0	1.12	0.003	0.010	0.343	0.9	1.32
19.2	0.004	0.008	0.583	1.6	0.93	0.004	0.007	0.552	1.5	0.94
38.4	0.004	0.009	0.465	1.3	1.12	0.005	0.010	0.466	1.3	1.30
76.8	0.007	0.007	0.973	2.7	0.89	0.009	0.007	1.246	3.4	0.95
153.6	0.011	0.009	1.213	3.4	1.07	0.011	0.009	1.201	3.3	1.24
	P2E2A7 + S9 mix (1)					*P2E2A7 + S9 mix (2)*				
0	0.001	0.002	0.318	1.0	1.00	0.003	0.008	0.326	1.0	1.00
0.3	0.000	0.002	0.000	0.0	0.67	0.002	0.008	0.198	0.6	1.01
0.6	0.001	0.003	0.216	0.7	1.20	0.002	0.010	0.244	0.7	1.19
1.2	0.001	0.002	0.460	1.4	0.90	0.003	0.007	0.472	1.4	0.84
2.4	0.001	0.003	0.234	0.7	1.31	0.003	0.011	0.235	0.7	1.34
4.8	0.001	0.002	0.400	1.3	0.99	0.003	0.008	0.379	1.2	0.97
9.6	0.001	0.003	0.244	0.8	1.44	0.003	0.009	0.345	1.1	1.15
19.2	0.001	0.002	0.573	1.8	1.03	0.003	0.008	0.401	1.2	0.96
38.4	0.002	0.003	0.469	1.5	1.34	0.005	0.010	0.480	1.5	1.18
76.8	0.003	0.002	1.229	3.9	0.88	0.007	0.008	0.888	2.7	0.94
153.6	0.004	0.003	1.234	3.9	1.23	0.009	0.009	0.965	3.0	1.16

The doses are expressed as μg original particles per ml dimethyl sulfoxide. All enzyme activity values are given with three decimal places by the BioSOS program. More accurate values of enzyme activities are, however, used by the program for computing induction and inhibition factor values.

β-G, β-galactosidase activities; P-ase, alkaline phosphatase activities; Ind.F., induction factor values; Inh.F., inhibition factor values; (1, 2), first and second round of testing.

TABLE 3

SOS INDUCTION POTENCY (SOSIP) VALUES OF THE ALIQUOTS (A7) FROM THE FIRST (E1) AND SECOND (E2) EXTRACTIONS OF THE PARTICULATE SAMPLES SRM 1649 (P1) AND SRM 1650 (P2)

Sample	SOSIP value
P1E1A7 −S9 mix	(−)
P1E1A7 +S9 mix	(−)
P1E2A7 −S9 mix	(−)
P1E2A7 +S9 mix	(−)
P2E1A7 −S9 mix	2080 ± 410/mg
P2E1A7 +S9 mix	208 ± 30/mg
P2E2A7 −S9 mix	1705 ± 15/mg
P2E2A7 +S9 mix	252 ± 70/mg
4-NQO −S9 mix	227 ± 49/nmole
2-AA +S9 mix	11 ± 5/nmole

SOSIP values are the means of 2 independent tests ± SD.
(−), negative result.
4-NQO, 4-nitroquinoline-*N*-oxide; 2-AA, 2-aminoanthracene.

large amounts of S9 are used (Oesch et al., 1981). Anthracene is generally considered to be non-mutagenic (Bridges et al., 1981). Chrysene is mutagenic using strain TA100 (McCann et al., 1975). Pyrene, phenanthrene and chrysene are all mutagenic only when tested with metabolic activation. They can therefore not explain the SOS chromotest results. Since SRM 1650 consists of diesel particulate matter and, according to the certificate issued by the U.S. National Institute of Standards and Technology, contains 19 μg 1-nitropyrene per gram particulate matter, it is most likely that the SOS chromotest result is caused by nitroarenes, which in most cases are mutagenic without metabolic activation (Rosenkranz and Mermelstein, 1983).

TABLE 4

CONCENTRATIONS OF 10 POLYCYCLIC AROMATIC HYDROCARBONS IN THE ALIQUOTS (A6) OF THE FIRST (E1) AND SECOND (E2) EXTRACTIONS OF THE PARTICULATE MATTER SAMPLES SRM 1649 (P1) AND SRM 1650 (P2) USING REVERSED-PHASE HPLC

Compound	Concentration (μg/g particles) in sample			
	P1E1A6	P1E2A6	P2E1A6	P2E2A6
Anthracene	0.37	0.38	1.74	1.73
Benzo[*k*]-fluoranthene	1.32	1.34	1.62	1.54
Benzo[*ghi*]perylene	4.10	4.10	3.18	3.36
Benzo[*a*]pyrene	2.31	2.31	1.05	1.01
Benzo[*e*]pyrene	4.68	4.88	6.15	5.42
Chrysene	2.22	2.39	9.52	8.51
Dibenzo[*ah*]-anthracene	0.79	0.79	1.08	1.00
Fluorene	0.17	0.16	0.35	0.34
Phenanthrene	2.95	2.84	43.5	38.7
Pyrene	3.84	3.92	44.4	41.9

The results of the work presented here indicate that the SOS chromotest, using *E. coli* strain PQ37, cannot be used as a single method for detecting genotoxic effects in complex environmental mixtures. The SOS chromotest may, however, because of its rapid execution, be used for prescreening of these kinds of samples. In case of a negative result the sample should be retested using another more thoroughly validated test system such as the Salmonella mutagenicity test.

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