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Development of an in situ microbial mutagenicity test system for airborne workplace mutagens: laboratory evaluation

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Summary

A simple on-site Salmonella mutagenicity test system for the detection of airborne mutagens in the workplace is being developed. The system permits entrapment of mutagenic airborne particles and vapors by impinging unfiltered ambient air into trapping medium containing bacterial tester cells. The trapping device consists mainly of a pump, an impinger and a cyclone. The impinging air flow generated by the pump is approximately 3 l/min. New *Salmonella typhimurium* testers which are resistant to streptomycin (Str) and 8-azaguanine (AG) were derived from the Ames testers TA98 and TA100 and the arabinose-resistant tester SV50, and were used as mutation indicators. Microbial contamination was sufficiently controlled by addition of ampicillin, Str, AG, and cycloheximide to the trapping and plating media. New tester strains retained a high mutagenic sensitivity from their parent strains. Laboratory studies with volatile mutagens (methyl methanesulfonate, ethyl methanesulfonate, and dimethylnitrosamine) showed that the vapor trapping of this system is promising. The study with suspended silica particles coated with a known mutagen (2,4,7-trinitro-9-fluorenone) indicated that the particle trapping of the system is satisfactory. Incorporation of metabolic activation into the trapping medium by confining S9 mix and tester cells in dialysis tubing enabled this system to detect promutagens. This in situ system may be useful for mutagenic monitoring in the workplace.

Mutagenic air pollutants can exist at the workplace in the form of particles, gases, or vapors. For the detection of mutagenic pollutants, laboratory mutagenesis analysis, where sample collection and extraction are prerequisite before mutagenesis testing, has been the primary method used. Filter and sorbent trappings are conventionally used to collect airborne particles and vapors (or gases), respectively (Chrisp and Fisher, 1980; Hughes et al., 1980). During the processes of collection, transport and extraction, it is possible that the short-lived mutagenic substances and intermediates are lost or that artifacts are generated. Therefore, a suitable system for workplace mutagenic monitoring is an in situ (on-site) mutagenicity assay, which allows a direct exposure of tester organisms to conditions that are much closer to the real environment. At the present time, only very few systems have been developed along this line for the open air or defined environments (Schairer et al., 1978; Lower et al., 1978; Politte et al., 1980; Ma, 1982; Plewa, 1982). Among these systems, the Tradescantia and the corn plant systems offer considerable promise as field monitors for gaseous mutagens (Schairer et al., 1978; Lower, 1981).

In the present study, we describe an in situ

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microbial mutagenicity test system which uses impingement to trap the mutagens and the Salmonella histidine (His) reversion and arabinose-resistant (Ara^r) tests as mutation detectors. In this system, both mutagenic airborne particles and vapors can be trapped simultaneously into trapping medium which contains tester cells. The results of laboratory validation of this system with known mutagens are presented.

Materials and methods

Test chemicals

Dimethylnitrosamine (DMN) was obtained from Sigma Chemical Co. (Saint Louis, MO). Methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), 2-aminoanthracene (2AA), and 2, 4, 7-trinitro-9-fluorenone (TNF) were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino] acridine · 2 HCl (ICR-191) was donated by Dr. H.J. Creech of the Institute for Cancer Research (Fox Chase, PA).

Tester strains

The three new tester TA98W, TA100W, and SV50W of *Salmonella typhimurium* derived from TA98, TA100 and SV50, respectively, were used throughout the investigation. These testers were obtained by selecting strains that are spontaneously resistant to streptomycin (St^r) and 8-azaguanine (AG^r). All the present strains carried an ampicillin-resistant (Amp^r) plasmid. TA98W and TA100W were used for the Ames His reversion assay (Ames et al., 1975), whereas SV50W was employed for the Ara^r forward mutation assay (Pueyo, 1978). The genetic characteristics of the parent strains have been described previously (Ames et al., 1975; Whong et al., 1981).

Sampling device

The apparatus designed for the in situ mutagenesis test system is diagrammed in Fig. 1. It consists of experimental and control sets. Each set has a pump (model P-4000, Du Pont), an impinger and a cyclone (Mine Safety Appliance Co., Murraysville, PA). The impinger contains 20 ml of trapping medium in a 100-ml glass flask (pear-shaped) and a fritted air bubbler. The fritted air bubbler

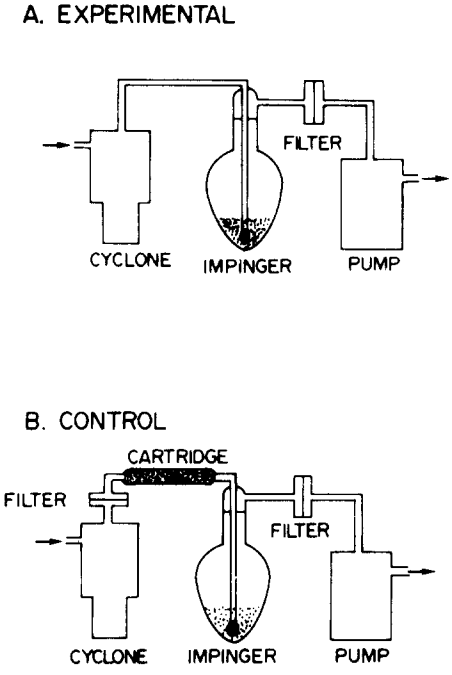

Fig. 1. Devices used for the in situ mutagenicity assay system.

contains many very small holes (> 80 μm in diameter). In the control set, a filter (47-mm diameter fiber glass filter) and a sorbent cartridge (5 ml each of charcoal, Tenax-GC, XAD-2 and metal silicates) were added between the cyclone and the impinger to remove respirable particles and vapors. The filter behind the impinger was used to prevent the release of aerosols containing tester cells into the environment.

Mutagenicity assay

Tester cultures were grown overnight in liquid synthetic medium. The liquid culture medium for tester TA98W and TA100W consisted of 2% glucose in water containing 25 μg histidine/ml, 0.1 μg biotin/ml and Vogel–Bonner medium E (Vogel and Bonner, 1956), whereas the liquid medium for SV50W was 0.2% glycerol in water containing 15 μg uracil/ml, 30 μg tryptophan/ml, 30 μg threonine/ml, 0.1 μg biotin/ml and M9 salt (Clowes and Hayes, 1968). 10 ml of the overnight culture diluted to 20 ml with the same medium were used as trapping medium in the impinging flask. The trapping medium was supplemented with 3% dimethyl sulfoxide (DMSO), 100 μg 8-azaguanine (AG)/ml, 10 μg cycloheximide (Cyc)/

ml, 100 μg streptomycin (Str)/ml, and 25 μg ampicillin (Amp)/ml.

During the experiment, air was drawn into the sampling device by the pump at a flow rate of 3 l/min. The air flow passed through the cyclone which removed non-respirable particles (> 10 μm).The respirable particles (≤ 10 μm) and vapors along with air were impinged into the trapping medium. Samples were taken from the trapping medium periodically to determine survival and mutation frequencies. For survival, 100, 500 and 2500 cells were plated separately onto 3 culture medium agar plates. For mutation frequency, samples (0.1 ml for TA98W and TA100W; 0.02 ml for SV50W) were plated onto Ames minimal agar plates (for TA98W and TA100W) or arabinose (60 mg/plate) agar plates (for SV50W). All 4 drugs (Amp, Str, Cyc and AG) were also incorporated into the agar plates. The addition of the drugs into the trapping and plating media was used to control microbial contamination. Survivors and Ara^r mutants or His^+ revertants were scored after 2 days (His reversion assay) or 3 days (Ara^r assay) incubation at 37°C.

Control studies with known mutagens

In the vapor-trapping study, a volatile mutagen was placed in a 125-ml glass bottle which was connected to the front opening of the sampling device. The vapor of mutagen was continuously generated as the air flow (produced by the pump) passed over the surface of the mutagen, and was impinged into the trapping medium containing tester cells. For testing the particle trapping, a known amount of mutagen was dissolved in acetone and then added to silica particles (≤ 10 μm). After the acetone was dried out, the silica particles coated with the mutagen were used for the study. The suspended mutagen-coated particles were generated in a bottle by air flow and were trapped into the trapping medium by impingement. Tester cells in the trapping medium were used to detect mutations.

For the study with promutagens, 5 ml S9 mix and 2.5 ml tester cells were added to dialysis tubing (0.25 in. in diameter, 6 in. in length) which was immersed into the trapping medium. Liver homogenate (male Wistar rats or Syrian golden hamsters pretreated with Aroclor 1254) and S9 mix were prepared according to Ames et al. (1975). After designated impingement times, cell samples from the dialysis tubing were plated to determine mutations. The activation ability of S9 mix at different temperatures inside the dialysis tubing with bubbling was also measured.

Results

Mutagenic sensitivities to 2AA and ICR-191 between the new tester strains and their parent strains were compared. Results are shown in Table 1. The mutagenic response of new testers was

TABLE 1

COMPARISON OF MUTAGENIC RESPONSE BETWEEN MULTI-DRUG-RESISTANT STRAINS AND THEIR PARENT STRAINS TO 2-AMINOANTHRACENE AND ICR-191 [a]

Mutagen	Conc. of mutagen (μg/plate)	His^+ revertants/plate				Ara^r mutants/plate	
		TA98	TA98W	TA100	TA100W	SV50	SV50W
2AA	0	37	42	103	139	208	198
	0.312	194	204	327	299	376	285
	0.625	376	351	568	675	886	796
	1.25	1094	843	1849	1736	1482	1648
	2.5	2374	2413	2614	2397	1964	2072
ICR-191	0	23	29	144	165	183	213
	1.25	186	204	251	218	310	387
	2.5	423	387	519	473	627	579
	5	607	586	842	813	847	866

[a] The plate incorporation test was employed for this study. S9 from rat liver was incorporated for 2AA test.

TABLE 2

MUTAGENIC RESPONSE OF TA100W TO THE VAPOR OF METHYL METHANESULFONATE AND ETHYL METHANESULFONATE WITH IMPINGEMENT [a]

Mutagen	Impinging time (h)	Control		Experimental	
		Survival (%)	His^+ revertants/10^7 survivors	Survival (%)	His^+ revertants/10^7 survivors
MMS	0.5	100	11.0	96.0	22.4
	1	100	16.8	92.0	64.7
	2	100	12.2	95.5	134.5
	4	100	17.0	51.0	1056.7
EMS	3	100	10.0	100.0	10.1
	6	100	11.4	78.8	80.3
	9	100	10.6	92.6	145.8
	12	100	9.3	98.4	218.9

[a] Mutagen vapor was generated from a bottle containing 0.3 ml of MMS or 0.5 ml of EMS by pulling air with a pump at a flow rate of 3 l/min and was impinged into the trapping medium containing TA100W.

similar to their corresponding parent strains indicating that the new testers possess mutagenic sensitivity comparable to their parent strains.

With the new testers, the developed in situ microbial system was evaluated using the volatile mutagens MMS and EMS. The vapor of MMS or EMS was generated and impinged into the trapping medium. Table 2 shows the mutagenic response of TA100W to the vapor of MMS and EMS. A clear mutagenic activity of MMS and EMS was demonstrated with this system. The mutagenicity increased as the impingement time increased. A positive mutagenic response was first observed after 0.5 h impingement for MMS, and 6 h impingement for EMS. The results of the Ara^r assay system with MMS and EMS under the same treatment condition are summarized in Table 3. With SV50W, the mutagenic activity of MMS and EMS vapors was detected after 1- and 4-h impingements for MMS and EMS, respectively. The mutagenicity also increased as a function of impingement time. For the study of trapping efficiency with the impingement, the percent trapping of MMS vapor in trapping medium was

TABLE 3

MUTAGENIC RESPONSE OF SV50W TO THE VAPOR OF METHYL METHANESULFONATE AND ETHYL METHANESULFONATE WITH IMPINGEMENT [a]

Mutagen	Impinging time (h)	Control		Experimental	
		Survival (%)	Ara^r mutants/10^6 survivors	Survival (%)	Ara^r mutants/10^6 survivors
MMS	0.5	100	55.0	123.0	70.2
	1	100	45.0	96.5	148.2
	2	100	45.0	77.3	283.0
	4	100	41.4	12.1	1102.8
EMS	2	100	49.4	100.0	59.6
	4	100	47.0	91.2	151.6
	8	100	36.3	90.0	401.7
	12	100	36.7	79.4	564.1

[a] Mutagen vapor was generated from a bottle containing 0.3 ml of MMS or 0.5 ml of EMS by pulling air with a pump at a flow rate of 3 l/min and was impinged into the trapping medium containing SV50W.

TABLE 4

METHYL METHANESULFONATE TRAPPING EFFICIENCY WITH IMPINGEMENT [a]

Trapping time (h)	Expt. No.	TA100W revertants/plate		MMS trapped (mg)	MMS recovery (%)
		Control	Experimental		
1	1	71	276	17.6	65.2
	2	80	232	14.4	62.6
	3	157	308	16.0	64.0
2	1	80	440	38.4	69.8
	2	84	388	32.0	66.7
	3	168	432	31.2	69.3
3	1	85	517	45.6	76.0
	2	79	538	51.2	73.1
	3	166	511	43.2	77.1

[a] The vapor of MMS was generated from 0.1 ml of MMS by drawing air with a pump at a flow rate of 3 l/min and was impinged into the trapping medium containing no tester cells. The mutagenicity of trapping vapors was determined with the plate incorporation test.

determined. By comparing the mutagenic activities obtained from trapping medium to a standard mutagenicity curve of known MMS concentration, recovery of MMS by impingement was found to be between 62 and 77% which was reproducible in 3 independent experiments (Table 4).

The respirable silica particles coated with TNF were used for the particle-trapping experiments. After 1 h trapping, the number of revertants per plate from the experimental set was 20 times higher than that from the control set (Table 5). Increase in impingement time resulted in increasing mutagenic activity.

TABLE 5

MUTAGENICITY OF TRINITROFLUORENONE COATED ON SILICA PARTICLES TRAPPED BY IMPINGEMENT [a]

Impingement time (h)	TA98W revertants/plate	
	Control	Experimental
1	13	277
2	19	1809
4	23	3070

[a] 1 ml of TNF (2 mg/ml) was spiked to 1 g of silica particles (<10 μm). 200 mg of silica particles were used for trapping.

TABLE 6

MUTAGENICITY OF 2-AMINOANTHRACENE ACTIVATED BY S9 MIX FROM DIALYSIS TUBING AFTER IMPINGEMENT [a]

Impingement time (h)	TA98W revertants/plate	
	Control	Experimental
0	23	1898
1	23	1922
2	22	2050
4	17	1850
6	18	1707

[a] 0.5 ml of S9 (rat liver) from dialysis tubing was sampled during impingement and was used to activate 2AA (2.5 μg/plate) with the plate incorporation test.

TABLE 7

ACTIVITY OF S9 MIX FOR 2-AMINOANTHRACENE MUTAGENESIS INSIDE DIALYSIS TUBING AT DIFFERENT TEMPERATURES [a]

Impingement time (h)	TA98W revertants/plate			
	25°C		32°C	
	Control	Experimental	Control	Experimental
0.25	26	135	17	195
0.5	21	214	20	326
1	18	259	27	523
2	30	542	33	567
4	32	863	37	900
6	32	1045	26	1000

[a] 25 μg 2AA was spiked into trapping medium outside the dialysis tubing. Mutagenic activity was determined by sampling tester cells from the dialysis tubing containing S9 (rat liver) and tester cells.

TABLE 8

MUTAGENICITY OF 2-AMINOANTHRACENE ACTIVATED BY S9 MIX INSIDE DIALYSIS TUBING USING *Salmonella typhimurium* SV50W [a]

Impingement time (h)	Ara^r mutants/plate	
	Control	Experimental
1	72	353
2	99	617
4	102	976
6	117	1193

[a] 25 μg of 2AA was spiked into trapping medium outside the dialysis tubing and the impingement was carried out at 25°C. Mutagenic activity was determined by sampling tester cells from the dialysis tubing containing S9 (rat liver) and tester cells.

TABLE 9

MUTAGENICITY OF DIMETHYLNITROSAMINE ACTIVATED BY S9 MIX INSIDE A DIALYSIS TUBING USING *Salmonella typhimurium* TA100W [a]

Impingement time (h)	His^+ revertants/plate		DMN evaporated (mg)
	Control	Experimental	
0.5	103	468	35.0
1	176	1743	76.2
2	151	2396	139.2
4	224	2509	243.6

[a] DMN vapor was generated and impinged at 25°C by drawing air with a pump. Mutagenic activity was determined by sampling tester cells from the dialysis tubing containing S9 (hamster liver) and tester cells.

Since S9 mix in a dialysis tubing was used in this system to detect promutagens, the stability of S9 mix inside the dialysis tubing was determined with the Ames assay. The results show that the activation ability of S9 remained unaffected even after 6 h impingement (Table 6). Table 7 shows the metabolic activity of S9 mix inside the dialysis tubing for 2AA mutagenesis in TA98W. The mutagenic activity of 2AA was detected with just 15 min impingement. A higher mutagenicity was observed as impingement time was increased. The mutagenic activities were similar between 32 and 25°C. A high mutagenicity of 2AA was also observed in SV50W using the dialysis tubing/impingement system (Table 8). With the same activation system, the mutagenicity of DMN vapors, which were trapped by impingement, was detected in TA100W after a 0.5-h impingement (Table 9). An extremely high mutagenicity of DMN vapors in SV50W was also found at the first-hour impingement (Table 10). The mutagenicity of DMN both in TA100W and SV50W was depended on the impingement time.

TABLE 10

MUTAGENICITY OF DIMETHYLNITROSAMINE ACTIVATED BY S9 MIX INSIDE A DIALYSIS TUBING USING *Salmonella typhimurium* SV50W [a]

Impingement time (h)	Ara^r mutants/plate		DMN evaporated (mg)
	Control	Experimental	
1	96	1029	43.6
2	79	1289	99.3
4	113	1451	224.6
6	110	1517	339.6

[a] DMN vapor was generated and impinged at 25°C by drawing air with a pump. Mutagenic activity was determined by sampling tester cells from the dialysis tubing containing S9 (hamster liver) and tester cells.

Discussion

Since the mutagenic air pollutants exist in the environment at relatively low quantities, a suitable in situ mutagenicity system must fulfill two important criteria: (1) highly sensitive tester strains which can detect mutagens at very low concentrations, and (2) efficient systems which can trap sufficient mutagenic substances. A comparison of mutagenic sensitivities between the new testers and their corresponding parent strains indicated that the new testers possess high sensitivity and, therefore, are suitable for use in the in situ system. Results of the mutagenic activity of MMS, EMS and DMN vapors trapped by the system demonstrate that the trapping efficiency of the mutagenic vapors by impingement is promising. The fritted air bubbler used in impingement can generate many small bubbles into the trapping media and certainly may help to increase the trapping efficiency for vapors. The size of the holes ($> 80\ \mu m$ in diameter) in the bubbler is much larger than that of respirable particles ($\leq 10\ \mu m$). Therefore, there is no difficulty for respirable particles to pass through the small holes in the bubbler. The results of the TNF-coated silica particles indicate that respirable particles can be efficiently trapped by impingement and mutagenic materials can readily release into the trapping medium from the particle. The uniqueness of this system is that both mutagenic airborne particles and vapors can be trapped simultaneously into one device. Furthermore, by the use of multi-drug resistant testers and presence of Str, Amp, Cyc, and AG in the trapping and plating media, the entrapment of airborne mutagens from unfiltered ambient air without microbial contamination becomes possible.

It appears that during the impingement the activation ability of S9 can be destroyed rapidly and a full flask of foam can be generated from the trapping medium if S9 is added directly into the

trapping medium. In our study, the use of dialysis tubing to confine S9 mix from trapping medium effectively avoided these technical problems. The finding of a relatively long stability of S9 activation inside the dialysis tubing is a promising indicator for the effectiveness of this system to detect promutagens. The dialysis tubing with a 12000 molecular weight cut-off may sufficiently retain microsomal enzymes inside the tubing and allow mutagenic molecules to diffuse in or out of the tubing. Satisfactory results of the activation of promutagens to mutagenic reactants by S9 in dialysis tubing were demonstrated with 2AA and DMN.

The efficiency of vapor trapping depends on the volatility of the substance being collected and can be increased by decreasing the temperature of trapping solution (Elkins et al., 1937; Gage, 1960). In addition, the temperature in most workplace environments is closer to 25°C than to 32°C. Therefore, it is desirable to lower the temperature of trapping medium without reducing the activity of S9. The observation of a full function of S9 at 25°C as compared to that at 32°C indicates that the in situ system can be effective at the low-temperature (25°C) environment.

Addition of DMSO (3%) in the trapping medium does not affect the viability of tester cells, whereas it may actually help to retain volatile vapors and to dissolve non-polar mutagens from trapped particles. In field studies, however, it is conceivable that the system described here may encounter problems such as insufficient entrapment of mutagenic gases and possible inability to detect mutagens which are strongly bound to particles. No doubt an improvement of the system is needed. Nevertheless, the results from laboratory studies suggest that this in situ microbial mutagenicity test system involving impingement may be useful for the on-site detection of airborne mutagens in the workplace. Further validation of the system in the laboratory and the workplace is in progress.

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