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A simple in situ mutagenicity test system for detection of mutagenic air pollutants

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Air pollutants in the form of particles, gases, or vapors can exist in the occupational setting or in a defined environment. Such pollutants can be caused by the endproducts, by-products, or materials used in industrial processes. If any of the pollutants are mutagenic, they may pose a carcinogenic and genetic hazard to the exposed population. To determine whether workers are being exposed to mutagenic and potentially carcinogenic compounds, mutagenic monitoring of the workplace environment should be performed. Monitoring for the presence of mutagens in the workplace environment should be considered to be the first step for genotoxicity studies in the occupational setting. Based on the results of the environmental monitoring studies, human genetic monitoring and epidemiological studies may be performed, samples collected for industrial hygiene studies can be ranked for chemical analysis, and efforts can be made to reduce the exposure of workers to mutagenic compounds.

For the detection of mutagenic pollutants, laboratory analysis has been the primary method used, in which sample collection and extraction are prerequisites for mutagenesis testing. Filter and sorbent trappings are conventionally used to collect airborne particles and vapors (or gases), respectively. During the processes of collection, transport and extraction, short-lived mutagenic substances and intermediates may be lost or artifacts generated. Therefore, a desirable test system for detecting airborne mutagens in the workplace would be an in situ (on-site) mutagenicity assay which allow for direct exposure of test organisms to conditions that are much closer to the real environment. To date, very few systems have been developed along this line for the open air or defined environments. Among those systems, the Tradescantia and the corn plant assay systems show promise as field monitors for gaseous mutagens (Lower, 1981; Ma, 1982; Schairer et al., 1978). These systems, however, may not be suitable for the detection of mutagens associated with air particles or for use in most occupational (i.e. factory) environments.

Recently, we have developed an in situ microbial mutagenesis assay system that 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 (Fig. 1). The impinging air flow generated by the pump is approximately 3 l/min.

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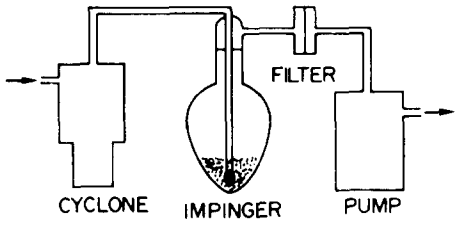

Fig. 1. Device used for the in situ mutagenicity assay system.

New *Salmonella typhimurium* strains (TA98W, TA100W and SV50W), which are resistant to streptomycin (Str) and 8-azaguanine (AG) were derived from the strains TA98 and TA100 (Ames et al., 1975) and the arabinose-resistant strain SV50 (Whong et al., 1981), were used as mutation indicators. Microbial contamination was sufficiently controlled by the addition of ampicillin (Amp), Str, AG and cycloheximide (Cyc) to the trapping and plating media. The new strains are as sensitive to mutagenesis as their parent strains (Whong et al., 1984). Laboratory studies with volatile mutagens and silica particles coated with a known mutagen indicated that this system could detect mutagenic activity of vapors and particles (Whong et al., 1984).

Efforts are being made to detect the mutagenic activity of air pollutants with this assay system. Cigarette smoke was selected for the study because it is a common air pollutant and because the smoke condensates as well as smoke per se are known to be mutagenic in bacterial assay systems (De Marini, 1983). Cultures of *S. typhimurium* TA98W were grown overnight in liquid synthetic medium, which consisted of 2% glucose, 25 μg

TABLE 1

MUTAGENICITY ASSAY OF CIGARETTE SMOKE BY THE IN SITU TEST SYSTEM[a]

Cigarette brand[b]	Treatment time (h)	Control set		Experimental set	
		Survival (%)	Revertant/10^8 survivors[c]	Survival (%)	Revertant/10^8 survivors[c]
A	0.5	100	10.9 (7)	99.0	40.4 (29)
	1	100	10.1 (9)	100.0	46.6 (42)
	2	100	6.7 (7)	100.0	52.7 (52)
	4	100	8.2 (7)	86.1	88.9 (78)
B	0.5	100	5.7 (9)	100.0	18.9 (32)
	1	100	6.0 (9)	100.0	32.4 (50)
	2	100	8.1 (8)	100.0	57.0 (59)
	4	100	10.3 (12)	70.8	86.9 (73)
C	0.5	100	5.4 (8)	76.2	31.6 (31)
	1	100	7.1 (8)	81.0	34.6 (34)
	2	100	4.5 (6)	83.3	58.8 (60)
	4	100	4.9 (5)	80.6	107.0 (97)
D	0.5	100	11.1 (7)	100.0	33.4 (24)
	1	100	12.1 (8)	100.0	44.3 (35)
	2	100	7.3 (4)	88.7	86.9 (59)
	4	100	5.6 (5)	79.9	117.3 (85)

[a]Results are average of 3 independent experiments. TA98W was tested with S9.

[b]Experiments for different cigarette brands were performed at different times. 4 different brands studied were common American cigarettes.

[c]Numbers in parentheses are revertant colonies per plate.

histidine/ml, 0.1 μg biotin/ml and Vogal medium E (Ames et al., 1975). 10 ml of the overnight culture diluted to 20 ml with the same medium were used as the trapping medium in the impinging flask. The trapping medium was supplemented with 3% dimethyl sulfoxide (DMSO), 100 μg AG/ml, 10 μg Cyc/ml, 100 μg Str/ml, and 25 μg Amp/ml. For in vitro activation, 5 ml of S9 mix and 2.5 ml of tester cells were added to dialysis tubing (1/4″ in diameter, 6″ length). The tubing was immersed into the trapping medium. S9 mix and liver homogenates (from male Wistar rats pretreated with Aroclor-1254) were prepared according to Ames and co-workers (1975).

During the experiment, a burning cigarette was placed on a cigarette holder that was directly connected to the trapping system. Smoke from the cigarette was pulled by the pump into the trapping medium that contained the in vitro microsomal activation system. The control set was run in the same manner as the experimental set except no burning cigarette was used. In another experiment, a burning cigarette was placed on a cigarette holder that was connected to the pump. Smoke pulled by the pump was released into the air inside a closed 9′ × 8.25′ × 7.2′ chamber. 5 min after 5 cigarettes were burned, smoke in the air, which represented the side-stream smoke, was impinged into the trapping medium. The Control Set inside the chamber was run in the same manner as the Experimental Set. However, clean air was circulated within the Control Set system. Samples were taken from the dialysis tubing periodically to determine survival and/or mutagenic activities. For the survival, 100, 500 and 2500 cells were plated separately onto 3 nutrient agar plates. For the mutagenic activity, 0.2 ml were plated onto minimal medium that contained all 4 drugs (Amp, Str, Cyc and AG). Sur-

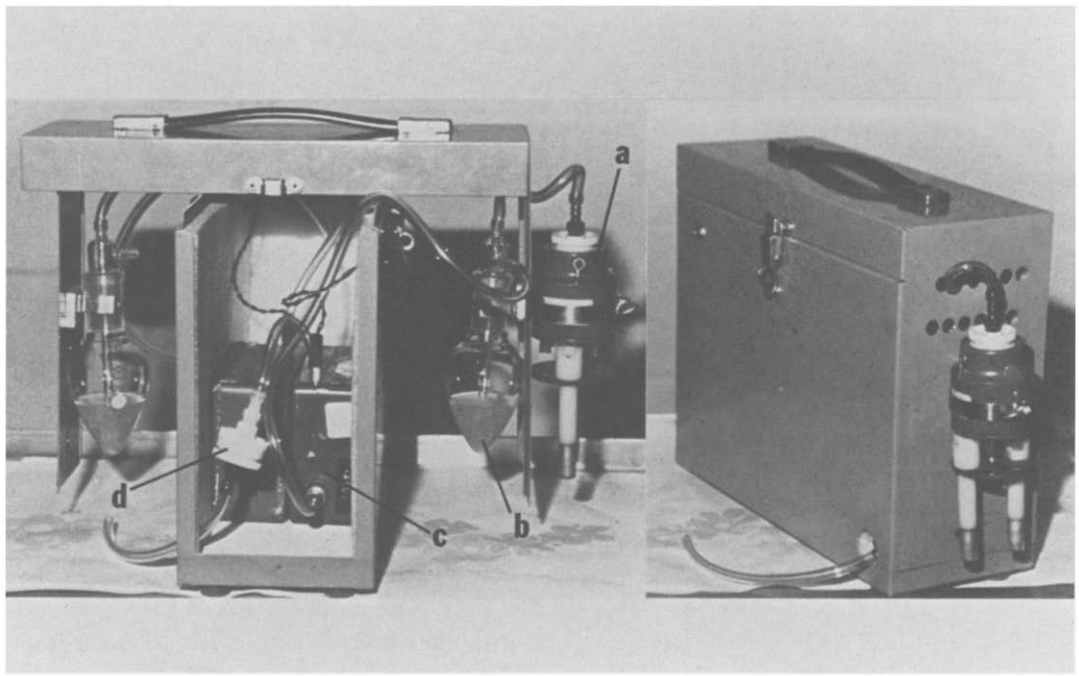

Fig. 2. The trapping device for the system can be packed and operated in a small 5 1/4″ × 14″ carry box. The components of the device are: (a) cyclone; (b) impinger; (c) pump; (d) filter. For the control set, clean air is recirculated in the system. The pump for pulling air through the system can be operated by batteries.

vivors and His^+ revertants were scored after 2 days incubation at 37°C.

The results of the direct trapping of cigarette smoke by the in situ assay system are summarized in Table 1. The concentration of cigarette smoke in each experiment was 0.036 cigarette per ml. These calculations are based on the assumption that all of the smoke from the cigarette was trapped by the medium (a total of 27.5 ml) and that chemicals from the smoke were evenly distributed outside and inside the dialysis tubing. In 30 min, the *his*$^+$ reversion frequencies or the number of revertants per plate increased approximately 3–5-fold in the experimental set over the control set. After 4 h the increase in mutation frequency was over 10-fold the average control value. A positive treatment-time related response was found in all experiments. The frequency of reversion and the number of revertants per plate appeared to be similar among the 4 different cigarette brands studied. These results seem to be in agreement with other studies (Sato et al., 1977) which showed that the mutagenic activity of cigarette smoke was independent of tar content.

TABLE 2

DETECTION OF THE MUTAGENIC ACTIVITY OF CIGARETTE SMOKE IN A CONTROLLED ENVIRONMENT

Sampling time (h)	His^+ revertants per plate[a]					
	Control set			Experimental set		
	Expt. 1	Expt. 2	$\bar{X}$	Expt. 1	Expt. 2	$\bar{X}$
2	7	7	7	10	10	10
4	6	3	5	16	22	19
6	4	4	4	30	22	26
8	3	3	3	44	28	36

[a]Each experiment was conducted in duplicate. TA98W was tested with S9.

As shown by the side-stream smoke experiment, the in situ system appears to detect the mutagenic activity of cigarette smoke at a low concentration in ambient air. After 4 h of sampling, the number of revertants increased from 5 in the control sets to 19 in the experimental sets (Table 2). These increases are statistically significant. Based on the chamber size (534.5 ft^3), number of cigarettes burned, the air-flow rate (3 l/min), and the sampling time (4 h), the concentration of cigarette smoke that could be detected for the mutagenic activity was 0.0065 cigarette per ml. Increasing the impinging time increased the number of revertants in a time-dependent manner.

To verify these results, cigarette smoke was trapped with the same apparatus in 20 ml trapping medium (without bacterial cells). The trapping medium was used immediately in the plate incorporation test (Ames et al., 1975). At 50 μl per plate, all 4 brands of cigarettes caused a positive mutagenic response (Table 3). Because only 1

TABLE 3

RESULTS OF THE PLATE INCORPORATION TEST FOR CIGARETTE SMOKE TRAPPED IN TRAPPING MEDIUM[a]

Cigarette brand	Amount of medium with trapped smoke tested (μl/plate)	His^+ revertants per plate
Negative control	0	7
Positive control (2AA)[b]		1425
A	25	14
	50	17
	100	32
	200	62
B	25	11
	50	18
	100	31
	200	55
C	25	11
	50	19
	100	30
	200	55
D	25	13
	50	23
	100	37
	200	65

[a]The results are average of 2 independent experiments. TA98W was tested with S9.
[b]2.5 μg 2-aminoanthracene/plate.

cigarette was used in each experiment, the smoke trapped in 1 ml trapping medium was from approximately 0.05 cigarette. Again, the mutagenic responses were similar among the different brands of cigarettes studied. Based on the number of revertants recovered, the in situ assay system is more effective in the detection of cigarette smoke mutagenicity than is the trapping of smoke in the medium and tested by the conventional plate incorporation test.

From the studies reported here, it appears that the in situ assay system we have developed may provide a simple, economical and sensitive method to detect and/or monitor ambient mutagens in the workplace or in other defined environments. However, further validation is needed before the system can be adopted for routine use.

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