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Detection of oxidative mutagens in an urban air-particulate extract: a preliminary study

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Summary

The Ames assays strains TA98 and TA100 have been useful in characterizing complex mixtures from organic solvent extracts of particles from diesel-powered vehicles, ambient air, and other sources. In this paper we report preliminary experiments using TA102, a bacterial strain that detects compounds that can oxidize DNA, to characterize the mutagenicity of an ambient air sample collected in Ann Arbor, MI. Four sets of ambient air filters were collected in duplicate over a period of several days. The mutagenicities of methylene chloride extracts of these filters were compared using strains TA98, TA100 and TA102. The concentration-mutagenicity data for TA98 and TA100 were linear over the concentration range 0–200 μg extract/plate. The mutagenicity of the extracts using TA102 was much lower than the other two strains and was non-linear over the concentration range tested. These results suggest that it would be difficult to use TA102 to identify the oxidative mutagens present in an ambient air particulate extract.

The bacterial strains used in the Ames assay have been useful in gaining insights into the type of compounds found in organic solvent extracts of particles from diesel powered vehicles (Ball et al., 1990; Jensen et al., 1985; Pederson and Siak, 1981; Salmeen et al., 1984) as well as ambient air samples (Arey et al., 1988; Siak et al., 1985). These extracts are extremely complex, containing thousands of chemicals (Tuominen et al., 1988).

Strains TA98 and TA98NR have been used extensively to identify and quantify the mutagenicity of extracts due to nitroarenes, dinitroarenes, hydroxynitroarenes and other nitroarene derivatives (Pederson et al., 1981; Salmeen et al., 1984). These nitro derivatives are also mutagenic in strain TA100, which complicates one of the properties of this strain of bacteria. Strain TA100 can detect alkylating compounds that react directly with DNA, without the need for endogenous or exogenous metabolic activation (Ball et al., 1984, 1987; McCann et al., 1975; Zieger et al., 1987). In theory then, TA100 could give us some information about alkylating agents present in these ex-

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tracts, although in practice the potent mutagenicity of nitroarenes overwhelms the response of TA100 to alkylating agents.

In the early 1980's, Ames and his coworkers developed a new strain of bacteria (TA102) that was selected to detect compounds that oxidize DNA (Levin et al., 1982). These compounds were not previously detected in the other Ames assay strains or were weakly mutagenic in these strains. One disadvantage of this bacterial strain is that it is difficult to get a reproducible response to a standard mutagen and the number of spontaneous revertants is high, on the order of 400 revertants/plate. While we have been able to obtain reproducible results with *tert.*-butyl hydroperoxide, we have not been able to reduce the number of spontaneous revertants.

Because this strain of bacteria detects compounds that oxidize DNA we wanted to see if these types of compounds were present in extracts of particles formed as the result of incomplete combustion. In the case of methylene chloride extracts of diesel particles, we found a distribution of TA102 mutagenic activity that could not be accounted for by the presence of nitroarenes (Ball and Young, 1992). We also wanted to investigate the feasibility of using strain TA102 to detect compounds that oxidize DNA (hereafter referred to as oxidative mutagens) in an ambient urban air-particulate sample. In this paper we report on a study of the comparative mutagenicity of an ambient air sample collected in Ann Arbor, MI.

Materials and methods

Ambient air sample

Ambient particulates were collected on the rooftop of the School of Public Health building on the campus of the University of Michigan in Ann Arbor. Ann Arbor is a small, midwestern city of population 110 000 with little manufacturing or industrial activity. Ambient particulate samples were collected using two high-volume samplers equipped with cascade impactor plates which eliminated particles greater than 2.5 μm . Fine fraction particulate matter less than 2.5 μm in size was collected downstream of the pre-filter onto Teflon-impregnated quartz filters (type

T60A20, Pallfex Co.). Filters were stored in fired aluminum foil at -40°C until analysis. Filters were extracted with dichloromethane overnight (16 h, dry nitrogen atmosphere) in a Soxhlet apparatus with one cycle taking 45 min. Dichloromethane was removed under rotary evaporation to a volume of less than 3 ml. The final mass of the extract was determined after drying to a constant mass by evaporating the dichloromethane under a stream of dry nitrogen.

Mutagenesis assays

Ames assays were carried out using *Salmonella typhimurium* strains TA98 and TA100 (Bruce Ames, Berkeley, CA) without the addition of rat-liver S9 extract, as described previously (Ball et al., 1984, 1989; Maron and Ames, 1983). Concentration-dependent mutagenicity curves for 2-nitrofluorene were determined as positive controls for Ames assays using strain TA98 (28 ± 5 rev/nmole, $n = 15$); concentration-dependent curves for methyl methanesulfonate were determined as positive controls for experiments using strain TA100 (147 ± 19 rev/ μmole , $n = 14$). The TA102 overnight culture was prepared using a slightly different procedure because it was difficult to obtain a reproducible level of mutagenicity with *tert.*-butyl hydroperoxide, a compound used as a reference mutagen for this strain (Levin et al., 1982). A 20- μl aliquot from a frozen culture was grown up overnight in Oxoid No. 2 broth. This culture was diluted with 0.02 M sodium phosphate pH 7.4 buffer and plated onto agar plates that contained Oxoid No. 2 broth and 10 $\mu\text{g}/\text{ml}$ tetracycline to obtain single colonies. The plate was allowed to incubate for 3 days and a large, single colony was used to inoculate 20 ml Oxoid No. 2 broth containing no tetracycline. This overnight culture (14 h) was used for mutation experiments. In our laboratories, the mutagenicity of *tert.*-butyl hydroperoxide was 2000 ± 200 rev/ μmole ($n = 6$) in good agreement with the literature value (2300 rev/ μmole , Levin et al., 1982). *tert.*-Butyl hydroperoxide (Aldrich Chemical Co.) was assayed for peroxide content using an iodometric titration and was 87% pure with the impurities being about equal amounts of water and *tert.*-butyl alcohol. The mutagenicity reported here for *tert.*-butyl hydroperoxide in

strain TA102 has been corrected for purity. Photomicrographs of the background lawn were taken to assess cell killing (Ball et al., 1990).

Results and discussion

The TA98, TA100 and TA102 mutagenicities of methylene chloride extracts of one sample of a duplicate set of filters from four different days are shown in Figs. 1–3, respectively. The mutagenicity of these extracts were linear over the concentration range from 0 to 200 μg extract/plate using strains TA98 and TA100. The specific mutagenicities, calculated as the slope of the concentration-mutagenicity data (revertants/ μg), of these extracts using TA98 and TA100 are summarized in Table 1. The mutagenicity of TA102 was non-linear over the concentration range studied (Fig. 3) and therefore, a specific mutagenicity was not calculated from this data. The strain that showed the highest mutagenicity over all 4 days was TA100 followed closely by

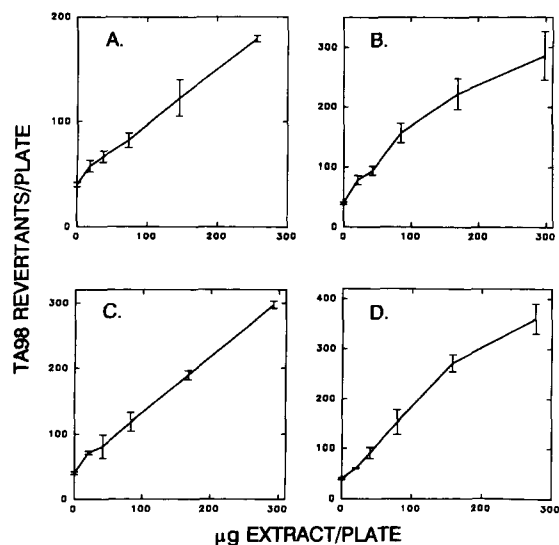


Fig. 1. TA98 mutagenicity of a methylene chloride extract of an urban air particulate sample. (A) Sample (a) collected August 26–27, 1991. (B) Sample (a) collected August 28–30, 1991. (C) Sample (a) collected between August 31–September 3, 1991. (D) Sample (a) collected September 11–13, 1991. Error bars represent the range of revertants from duplicate determinations. The line through the data is to help visualize the data and has no theoretical meaning.

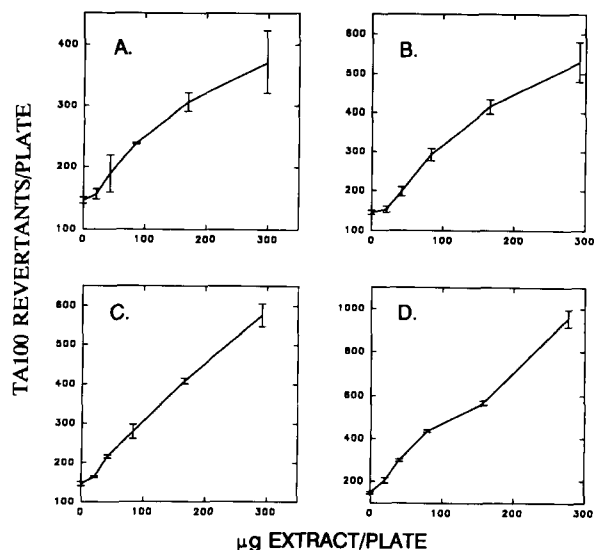


Fig. 2. TA100 mutagenicity of a methylene chloride extract of an urban air-particulate sample. (A) Sample (a) collected August 26–27, 1991. (B) Sample (a) collected August 28–30, 1991. (C) Sample (a) collected August 31–September 3, 1991. (D) Sample (a) collected September 11–13, 1991. Error bars represent the range of revertants from duplicate determinations. The line through the data is to help visualize the data and has no theoretical meaning.

TA98. The mutagenicity of TA102 was demonstrably weaker than these 2 strains of bacteria.

This preliminary study on the TA102 mutagenicity of an urban air-particulate extracts suggests that these samples contain compounds that can oxidize DNA. The shape of the concentration-mutagenicity data for strain TA102 shows a plateau at high concentrations of extract. Although we cannot explain the shape of this curve with our current understanding of the Ames assay, we have observed this same type of non-linear response using extracts from laboratory combustors (Salmeen et al., 1989). The non-linear response of TA102 towards an extract of an ambient air-particulate sample makes it difficult to compare this sample with other ambient air-particle samples or neat chemicals. For example, the TA102 mutagenicity of an extract from diesel particles was linear over a concentration range between 0 and 200 μg of extract (to be published elsewhere). The mutagenicity of a neat chemical, *tert*-butyl hydroperoxide, is also linear over 0–1400 $\mu\text{g}/\text{plate}$.

TABLE 1
 AMBIENT AIR FILTERS COLLECTED IN ANN ARBOR, MI

Date of filter collection	Total mass of particles (mg)	Mass of extract (mg)	Percent extractable mass (mg)	Volume of air filtered (m ³)	Particle mass per cubic meter of air (μg/m ³)	Revertants/μg extract		Revertants/cubic meter of air	
						TA98 ^a	TA100 ^a	TA98 ^a	TA100 ^a
26–27 Aug. 1991 (1)	nd ^b	15.60 ± 0.07	nd	3510	nd	0.53	0.98	1.2	4.4
26–27 Aug. 1991 (2)	nd	7.58 ± 0.04	nd	2203	nd	0.53	0.83	1.8	2.9
28–30 Aug. 1991 (1)	nd	13.85 ± 0.06	nd	3082	nd	0.73	1.7	3.3	7.6
28–30 Aug. 1991 (2)	nd	15.77 ± 0.04	nd	3969	nd	0.81	1.5	3.2	6.0
31 Aug.–3 Sept. 1991 (1)	153.11	14.26 ± 0.06	9.31	5403	28	0.86	1.5	2.3	4.0
31 Aug.–3 Sept. 1991 (2)	162.07	14.60 ± 0.05	9.01	5482	30	0.93	1.6	2.5	4.3
11–13 Sept. 1991 (1)	101.53	10.47 ± 0.08	10.3	3813	27	1.2	2.8	3.3	7.7
11–13 Sept. 1991 (2)	109.15	10.74 ± 0.09	9.84	3702	29	1.2	3.2	3.5	9.3

^a Calculated from the linear portion (0–200 μg/plate) of the concentration-mutagenicity curves.

^b nd, not determined.

The data suggest that fine fraction (< 2.5 μm) atmospheric particles contain organic compounds that can be extracted into methylene chloride and can cause oxidative damage to bacterial DNA.

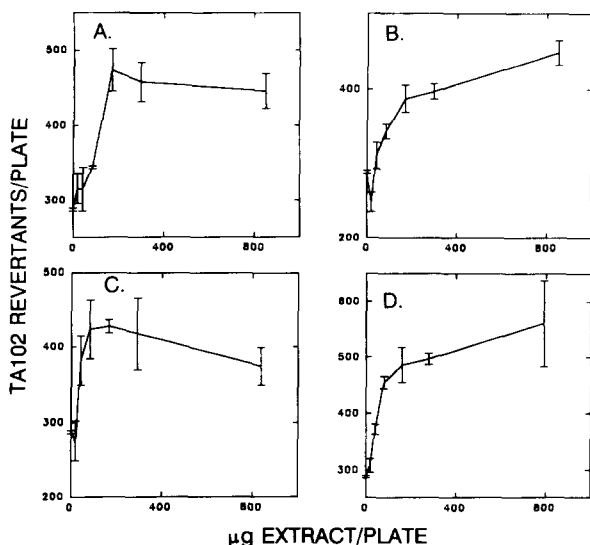


Fig. 3. TA102 mutagenicity of a methylene chloride extract of an urban air-particulate sample. (A) Sample (a) collected August 26–27, 1991. (B) Sample (a) collected August 28–30, 1991. (C) Sample (a) collected August 31–September 3, 1991. (D) Sample (a) collected September 11–13, 1991. Error bars represent the range of revertants from duplicate determinations. The line through the data is to help visualize the data and has no theoretical meaning.

However, the weak, non-linear concentration-mutagenicity response makes it difficult to use this strain of bacteria for further investigations into the identity of the compounds responsible for the oxidative mutagenicity observed by this strain.

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