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NOTES

Mutagenicity of Teflon-Coated Glass Fiber Filters: A Potential Problem and Solutions

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■ Teflon-coated glass fiber filters, used in studies of airborne particulate matter, were tested for mutagenic activity with the *Salmonella*/mammalian-microsome (Ames) assay. Eight blank filters were simultaneously extracted with dichloromethane (DCM). Extracts were concentrated by rotary evaporation and a nitrogen purge and were solvent exchanged into dimethyl sulfoxide (Me_2SO) for bioassay. Mutagenicity testing was performed on strain TA98, with and without metabolic activation. Findings indicated that both direct- and indirect-acting frameshift mutagens were present on the blank filters. Preextracting the filters with DCM reduced the activity associated with the filters; however, forced-air baking increased the mutagenic response. Interference caused by filter mutagenicity was shown to be inversely proportional to extraction efficiency and particle load, suggesting that the effect of filter mutagenicity on typical urban samples will be negligible. However, when particle yield is low, as for samples collected at rural or background sites, interference from filter mutagenicity may be significant, and results should be interpreted with caution.

Introduction

Airborne organic pollutants are frequently characterized by testing particulate matter present in the air. This is accomplished by drawing air through a filter (which collects and concentrates the particulate matter), extracting the organics from the collected sample, and analyzing the extract. To determine potential mutagenic activity, the *Salmonella typhimurium* plate incorporation assay developed by Ames et al. (*1*) is commonly employed. Air samples collected at urban or industrial sites (*2–5*), from rural or nonindustrial areas (*5–8*), and from automotive and diesel exhausts (*9–12*) have been tested in the Salmonella assay, and results have revealed marked differences in mutagenic activities.

Proper interpretations of data from mutagenicity studies rely on the selection of appropriate controls. For many studies, the solvent blank represents the negative control, and its spontaneous activity is used to calculate the statistical significance of an observed response. However, for studies involving filter extracts we have found that the spontaneous activity of the solvent blank may not accurately represent the background response. Instead, values generated by blank filter extracts may more closely approximate the background activity of samples collected on filters. This claim is based upon our observation of mutagenic activity associated with blank Teflon-coated filters.

In an effort to determine whether the observed mutagenic response of the filters could be reduced effectively, two pretreatment methods were examined. Blank filters were either preextracted with dichloromethane (DCM) or baked in a forced-air oven. Preextracting the filters with DCM slightly reduced their mutagenic potential. In contrast, baking the filters at 175 °C resulted in an enhancement of mutagenic activity.

We also examined the potential effects of filter burden and percentage of extractable material on the expression of filter mutagenicity. We have demonstrated that filter mutagenicity is likely to influence results obtained from filters with light loadings of airborne particles and samples yielding low percentages of extractable organics.

Materials and Methods

Preparation of Filter Extracts for Bioassay. All filters were 8 in. by 10 in. Teflon-coated Pallflex T60A20 filters (Pallflex Products Corp., Putnam, CT) cut from production lot 427, rolls B and C. Eight clean, blank filters were collectively extracted to produce a single sample for bioassay. Each set of eight filters was extracted with 300 mL of DCM (Burdick and Jackson, spectrophotometric grade) for between 16 and 24 h. Extracts were filtered (0.2-μm fluoropore) and concentrated by rotary evaporation to approximately 8 mL. Two 1-mL aliquots were removed at this point for duplicate gravimetric determinations. Concentrated extracts were pipetted into a Kuderna–Danish apparatus and further concentrated by a nitrogen purge. The extracted material was then solvent exchanged into 3 mL of dimethyl sulfoxide (Me_2SO, Burdick and Jackson) for bioassay. Residual DCM (less than 0.1%) was verified by a gas chromatograph equipped with a flame ionization detector.

Samples were transferred to sterile glass vials with Teflon-lined screw caps and refrigerated (for no more than 24 h) until the mutagenicity assay could be performed. A concentrate of 300 mL of DCM was solvent exchanged into Me_2SO as described above and used as the solvent blank control. The residue recovered from the solvent blank had a mass of 0.023 mg/mL.

The two pretreatment methods (baking and preextraction) were performed as follows. Baked filters were heated in a forced-air oven at 175 °C for between 5 and 24 h and extracted the following day as previously described. Preextracted filters were Soxhlet extracted twice with DCM according to the procedures outlined above, and the second extract served as the bioassay sample.

Mutagenicity Assay. The *Salmonella* mutagenicity assay was performed as described by Ames et al. (*1*), except that minimal histidine and biotin were added to the plating medium rather than to the soft agar overlay (*13*). Because limited amounts of filter extracts were available for testing, samples were tested only with strain TA98. TA98 is reportedly the most sensitive tester strain for use on airborne particulate matter (*8*) and is cited extensively in the lit-

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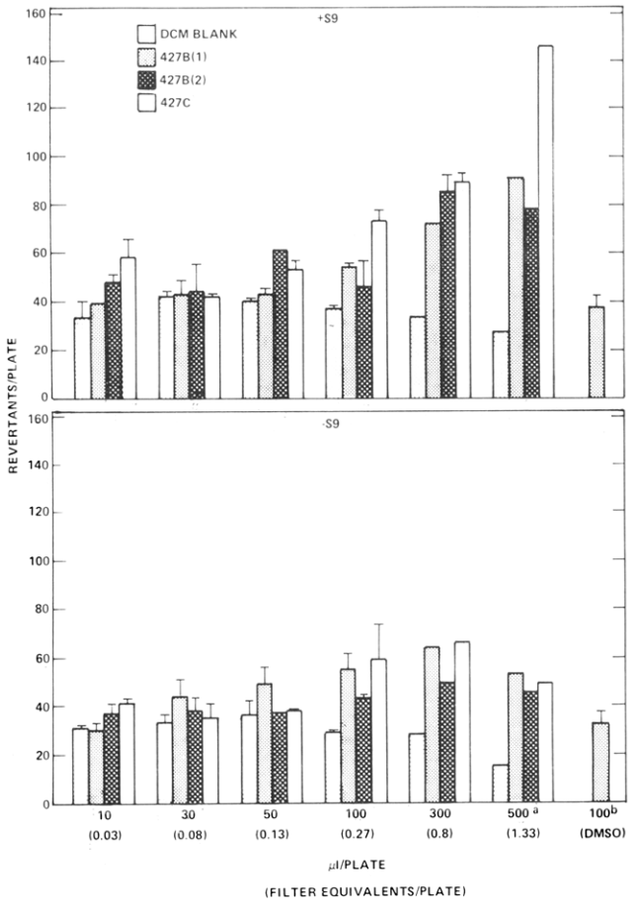

Figure 1. Mutagenic activity in *S. typhimurium* TA98 of the extracts obtained from rolls B and C of lot 427, Pallflex T60A20 filters. Total mass recovered from the extraction of the eight filters from lot 427B(1) was 0.43 mg/mL, from lot 427B(2) was 0.5 mg/mL, and from lot 427C was 0.43 mg/mL. Represented are the average revertants per plate and the standard deviation based on duplicate platings (except where noted). (a) Bar values represent results from a single plate. (b) Bar values represent the average result from plates tested in triplicate.

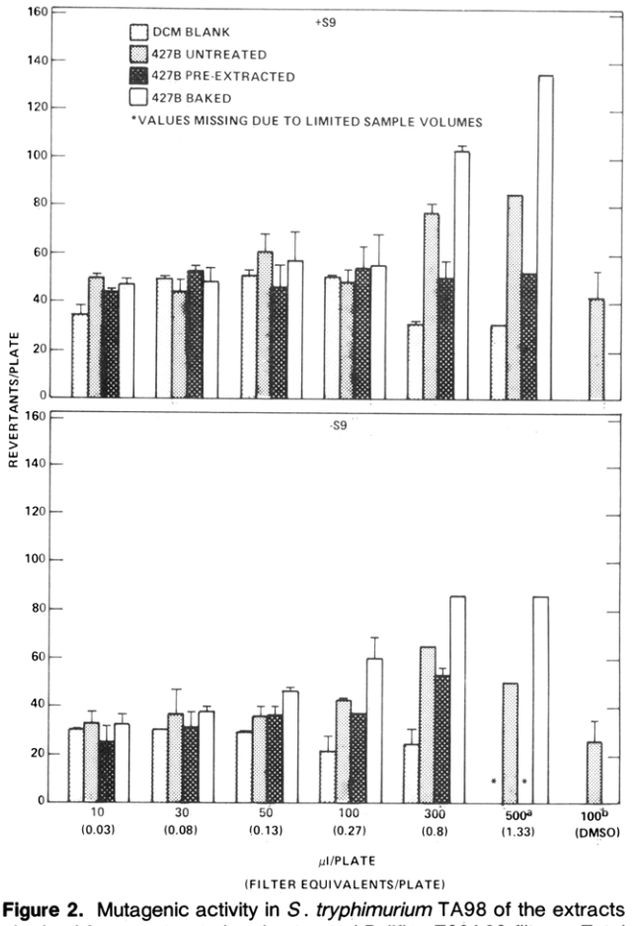

Figure 2. Mutagenic activity in *S. tryphimurium* TA98 of the extracts obtained from pretreated and untreated Pallflex T60A20 filters. Total mass recovered from the extraction of the eight untreated filters was 0.71 mg/mL, from the preextracted filters was 0.073 mg/mL, and from the baked filters was 0.44 mg/mL. Represented are the average revertants per plate and the standard deviation based on duplicate platings (except where noted). (a) Bar values represent results from a single plate. (b) Bar values represent the average result from plates tested in triplicate.

erature. Experiments were performed both with and without the addition of Aroclor 1254 induced male rat liver homogenate (S9). Filter extracts were tested in duplicate at five doses; a sixth dose—500 μL—was tested on single plates. Samples that were directly compared were assayed simultaneously, and testing was replicated when possible; however, the pretreated filter extracts were tested only once due to the limited amounts available for testing. Revertant colonies were counted with an automatic colony counter (Artek) following a 72-h incubation period at 37 °C. 2-Aminoanthracene (0.5 μg/plate with S9) and 2-nitrofluorene (3 μg/plate without S9) were used as positive controls to verify the reversion properties of the tester strain and to monitor the activity of the exogenous activation system.

Results

Graphic representations of the mutagenicity data are depicted in Figures 1 and 2. Each bar value represents an average based on duplicate platings at each dose, except as indicated. Because the data are based on a simultaneous extraction of eight filters, doses are expressed both as total volume and as filter equivalents per plate. Total gravimetric mass recovered from the filters is included in the legend to both figures. Spontaneous revertant values for the DCM and Me_2SO blanks are also included.

Figure 1 compares the mutagenic activities of two different filter rolls (B and C) obtained from the same filter lot (lot 427). Extracts obtained from roll 427B demonstrated more than a 3-fold increase in activity over the solvent blank values at high doses, whereas extracts from roll 427C demonstrated more than a 5-fold increase (with activation). Also illustrated are the activities of filters cut from different sections of the same roll [427B(1) and 427B(2)]. Section 427B(2) was noticeably darker in color than 427B(1), presumably due to a heavier coating of Teflon along that portion of the roll. There was, however, no significant difference between responses for the two sections.

Figure 2 compares the results obtained from untreated and pretreated filters cut from roll 427B. Whereas the extracts from the untreated filters yielded approximately 3 times the activity of the solvent blank at high doses, the response of the preextracted filters did not vary significantly from spontaneous (DCM) values. Baking, however, increased mutagenicity, yielding more than a 4-fold increase over spontaneous values at high doses. Metabolic activation only slightly increased mutagenic response.

On the basis of these observations of filter mutagenicity, we calculated the effect of sample mass on the activity expressed by a blank filter. For simplification, we examined the effect of mass irrespective of mutagenicity; in other words, we assumed that the hypothetical sample was not mutagenic. Figure 3 illustrates this relationship for both the preextracted and untreated filter sets. The X axis represents the mass of the hypothetical sample (milligrams

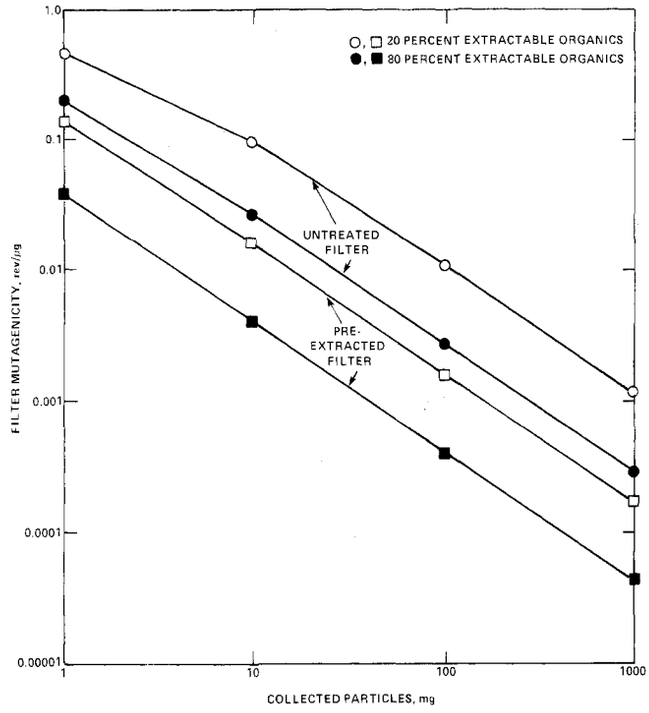

Figure 3. Influence of filter mutagenicity as a function of sample mass. Data are derived from mutagenicity slope values with methods described by Stead et al. (*14*) and the mass extracted from blank filters. (The mean extracted mass for single untreated and preextracted filters, determined experimentally and explained in the text, was 266.3 and 27.4 µg, respectively.) The number of revertants per blank filter was divided by the total mass extracted per filter (hypothetical particle mass extracted plus filter mass extracted) to yield the proportional activity contributed by the blank filter (revertants per microgram). Curves represent the calculated response for samples tested without metabolic activation using *S. typhimurium* TA98.

of particles). The *Y* axis represents the mutagenic activity contributed by the blank filter (expressed as revertants per microgram of material extracted from the blank filter). The mass extracted from the eight blank filters was determined gravimetrically, and a correction factor was applied so the influence of a single filter could be illustrated. As the hypothetical sample mass increases, the contribution of the coextracted filter mutagenicity decreases. For comparison, two organic extraction efficiencies are illustrated. Results show that interference from filter mutagenicity will be more pronounced for samples having low percentages of extractable organics.

The significance of these observations is illustrated in Table I, which shows that the number of revertants per plate due strictly to the mutagenic activity of the filter is inversely proportional to the particle mass collected on the filter and directly proportional to total mass applied to the bacterial plate. These numbers suggest that mutagenic activity related to particles collected on Teflon-coated glass fiber filters must be interpreted with caution. At very low particle loadings, an observed response may be partially or entirely related to filter mutagenicity and not to the collected particulate sample.

Discussion

This study has shown that blank Teflon-coated Pallflex T60A20 filters exhibit mutagenic activity. The response was due to extractable organic frameshift mutagens, some of which are direct-acting and some of which convert to their active form by exogenous activation. The similarity in response demonstrated by filters 427B(1) and 427B(2) suggests that filters cut from the same roll are comparable, despite color differences. It can thus be argued that the

Table I. Estimated Increase in Revertants Caused by Filter Mutagenicity, Expressed as the Number of Additional Revertants Expected per Plate (Revertants/Plate)[a]

particle mass collected on filter, mg	% extractable organics	total mass per plate, µg	filter mutagenicity, revertants/plate	
			untreated	preextracted
1	20	10	4.6	1.4
1	20	100	46	14
100	20	10	0.96	0.16
100	20	100	9.6	1.6

[a] Data are for plates tested without metabolic activation using *S. typhimurium* TA98. See text for full discussion.

Teflon coating per se is not responsible for the observed mutagenic response. However, the difference in mutagenic response between filter rolls (rolls B and C of lot 427) suggests that filters manufactured at the same time do not necessarily demonstrate similar properties when later tested. We conjecture that mutagenic organics present in the ambient environment adsorb to the filters during processing or storage and are later removed from the filter upon extraction. Adsorption of mutagens could thus produce sample-to-sample variation in bioassay results.

Baked filters produced extracts with the highest observed activity. This may be due to the transformation of artifacts on the filter to more mutagenic species upon heating, or alternatively, it is conceivable that mutagenic residual chemicals from the coating process are driven out during baking and subsequently extracted.

Preextracted filters rendered their mutagenic extractables to the first Soxhlet extraction; the first extract demonstrated more than twice the activity of the spontaneous control (results not shown) while the subsequent extract showed no significant increase in activity over background. This indicates that mutagenic organic extractables can be effectively removed from the filters.

Data supporting these findings have been presented by Alfheim and Lindskog (*15*), who compared the mutagencities (in TA98) of extracts from a variety of filters, including Gelman AE glass fiber filters, Stora Kopparberg glass fiber filters, and Pallflex Teflon-coated filters. The Pallflex filters were the only filters to demonstrate mutagenic activity. In addition, they showed that particulate samples collected on the Pallflex filters demonstrated greater mutagenic activity than when collected on ordinary glass fiber filters. Other investigations using ordinary glass fiber filters (*16–18*) and polystyrene and cellulose nitrate filters (*19*) have shown no evidence of filter mutagenicity.

The significance of filter mutagenicity has been shown to be dependent upon the amount of particles collected upon the filter (loading) and the organic extraction efficiency. Conditions that favor heavy loadings or high extraction efficiencies will yield results that are negligibly affected by filter mutagenicity. However, data from samples collected under conditions that typically yield small masses (such as collections at rural or background sites) must be interpreted carefully; interference from filter mutagenicity may play a significant role in the results of filters that are lightly loaded, especially if a series of filters are pooled.

To avoid spurious interpretations of data, we recommend that the spontaneous activity of blank filter extracts be included pro forma when evaluating the mutagenic potential of air samples. The spontaneous response of the blank filter extract may more closely characterize the background activity of samples collected on filters. Alternatively, a solvent wash could be adopted in an effort

to remove mutagenic artifacts on filters and thereby reduce concomitant interference.

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Determination of Mercury in River Water, Rain, and Snow

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■ For river water, even when a sample is not apparently polluted, addition of an oxidant and the heating process are confirmed to be necessary to determine the total Hg. Ten milliliters of concentrated H_2SO_4 and 10 mL of 0.5% $KMnO_4$ are added to a 1-L river water sample at sampling, and the solution is heated for 4 h at 100 °C. The Hg concentration in a river sample containing H_2SO_4 and $KMnO_4$ is unchanged for at least 10 days. For rain and snow, when acidification and the addition of a preserving agent were made after the collection of rain or the melting of snow, 20–90% of Hg in a rain or snow sample was lost during the collection of rain or the melting of snow, mainly owing to adsorption on the wall of a vessel. Therefore, to obtain a correct Hg value, H_2SO_4 and $KMnO_4$ must be added to the receiving vessel prior to the collection of rain or the melting of snow. At the end of the collection or melting, the concentrations of H_2SO_4 and $KMnO_4$ are adjusted to 0.2 M and 0.005%, respectively.

River Water

Natural water contains different kinds of species of Hg. Therefore, different concentration values of Hg may be obtained by different pretreatments of a natural water sample. Determinations of the so-called total Hg in river waters have been reported, e.g., with ultraviolet irradiation techniques (*1*, *2*), 1-month storage with addition of H_2SO_4 and NaCl (*3*), and digestion with strong acid–$K_2Cr_2O_7$, –$K_2S_2O_8$, or –$KMnO_4$ (*4*–*9*). Sometimes, such a pretreatment has been omitted by the addition of only HNO_3 or HCl when river water seems to be unpolluted.

Table I. Comparison of Various Pretreatments for a River Water Sample

method	pretreatment added to 1-L sample	no. of determinations	Hg found (mean ± SD), ng/L
A	10 mL of concd HNO_3, no heating	5	0.8 ± 0.3
B	10 mL of concd H_2SO_4, no heating	6	1.6 ± 0.3
C	10 mL of concd H_2SO_4 and 10 mL of 0.5% $KMnO_4$, no heating	6	3.7 ± 0.4
D	10 mL of concd H_2SO_4 and 10 mL of 0.5% $KMnO_4$, heating for 4 h	5	7.1 ± 0.8
E	25 mL of concd HNO_3, 20 mL of 5% $KMnO_4$, and 20 mL of 5% $K_2S_2O_8$, heating for 4 h	6	6.6 ± 0.6
F	10 mL of concd H_2SO_4 and 10 mL of concd HNO_3, heating for 4 h	6	6.9 ± 1.2

For an example, variations in Hg concentration in a river water sample determined by different chemical pretreatments and the stability of Hg in a treated sample are described in this paper. River samples were collected from the Akagawa River, which is so unpolluted that it is a source of city water.

Variation of Mercury Concentration in a River Sample by Different Pretreatments. Six different pretreatments described in Table I were each given to a

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