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Mutagenic Activity in Diesel Exhaust Particulates

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Research on diesel exhaust particulates is necessitated by the expected increase in the production of diesel powered automobiles for better fuel economy. It is expected that 25% of 1985 model cars will have diesel engines. The discovery that diesel exhaust particulates were mutagenic in the Ames Salmonella bioassay prompted investigation of the organic phase of this aerosol. A unique property of diesel particulate extracts is the fact that mammalian enzymes are not required for activity. This is different from polynuclear aromatic hydrocarbons and some natural mutagens such as aflatoxin B_1 which are active only if S-9 Mix is present. Therefore, diesel exhaust particulates may contain a different class of mutagens.

The determination of the chemical identity of these mutagens is a difficult problem because of the complexity of the mixture. Extraction of particulates with a moderately polar solvent such as dichloromethane yields fractions with a higher mutagenic activity than either hexane or methanol extracts. On the basis of S-9 independent activity, one would predict that arene oxides or nitroarenes are present in the dichloromethane extracts. Although arene oxides have yet to be isolated from diesel exhaust, a weakly mutagenic oxygenated compound, cyclopenteno(c,d)pyrene anhydride has been detected (RAPPAPORT et al. 1980). A potent nitroarene mutagen, 1-nitropyrene has been tentatively identified in diesel exhaust particulates along with nitromethyl anthracene, hydroxynitrofluorene, and nitrodihydropyrene (SCHUETZLE et al. 1981). 2-Nitrofluorene has been detected in dichloromethane extracts of diesel exhaust particulates (XU et al. 1981). This is the first known carcinogen to be detected which is also a direct mutagen in the Ames test.

Another approach to the characterization of the diesel mutagen has been the use of nitro-reductase deficient Salmonella typhimurium bacteria. Because the Ames test bacteria can activate nitroarene to mutagens independently of S-9 Mix, these new tester

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strains would not be sensitive to nitroaromatics. Nitroarenes would then be indirect mutagens in these nitroreductase deficient Salmonella, allowing S-9 activation to be seen. The results obtained so far have been ambiguous. WANG (1980) found that 2-nitrofluorene was not active in a nitroreductase deficient strain of TA98 (TA98 nr). However, 1-nitropyrene activity in TA98 nr strain was only slightly less than in wild type TA98 and 4-nitroquinoline-N-oxide was equally potent in either TA98 and TA98 nr. Substrate specificity may determine which nitroaromatic is activated (ROSENKRANZ et al. 1980).

Nitro groups of aromatic compounds can be chemically reduced to the amine derivative without changing the carbon-carbon double bonds. HANAYA et al. (1979) have described a method for the quantitative reduction of nitroarenes using sodium borohydride (NBH) and a copper(II)-containing catalyst. If diesel exhaust particulates are composed of significant quantities of nitroarenes, then chemical reduction would convert these to aminoarenes. These reduced extracts would then be mutagenic only in the presence of S-9 Mix. If, on the other hand, alkylating agents or arene oxides are responsible for the indirect mutagenicity, then the reduction products would be non-mutagenic even in the presence of S-9 Mix.

MATERIALS AND METHODS

Diesel exhaust particulates were collected by EPA Testing Laboratory, Ann Arbor, MI, on pleated Dustfoe® filters (Mine Safety Appliance, Pittsburgh, PA). These samples were generously supplied at no charge by Dr. Thomas Baines of the Environmental Protection Agency. The filters were extracted for 24 hours using a Soxhlet apparatus containing 6 L of dichloromethane (HPLC grade, Burdick and Jackson, Muskegon, MI). Each filter yielded approximately 20 g residue which was concentrated by vacuum evaporation to a final volume of 120 mL. A column of BioSil A (100-200 mesh, BioRad Laboratories, Richmond, CA) 5 x 50 cm was prepared by making a slurry of silica in methanol which was successively washed with 1 L each of chloroform and hexane. The filter residue was then added and eluted from the column with 1 L aliquots of hexane, chloroform and methanol. The intermediate polarity fraction (chloroform) contained the highest specific activity and was used for subsequent experiments (480 TA98 net revertants/100 µg) (hereafter called the chloroform eluate).

2-Nitrofluorene (2NF) and 2-amino fluorene were obtained from Pfaltz and Bauer Company, Stamford, CT. Chemical reduction of 2NF or chloroform eluate was carried out using a modification of the method of HANAYA et al.(1979). The chloroform eluate (0.20 g/mL, 1 mL aliquot), copper (II) acetoacetonate catalyst (0.152 g), and NBH (0.228 g/11.4 mL ethanol) were placed in a liquid scintillation vial. The reaction mixture was purged with nitrogen, capped, and allowed to proceed to completion overnight. The reaction was stoped by the addition of 20 mL distilled water and the ethanol removed using a vacuum evaporator. Two volumes of chloroform were added to the aqueous residue and placed in a separatory

funnel. After shaking for two minutes, the phases were allowed to separate, the organic layer filtered through anhydrous sodium sulfate. The organic layer was pipetted into tared vials and the chloroform evaporated in a 45°C heating block under nitrogen. The residue was redissolved in dimethylsulfoxide to a final concentration of 10 mg/mL for use in the Ames Test.

S-9 Mix was prepared from Arochlor 1254 treated rats as previously described (AMES et al. 1975). TA98 tester strain was generously supplied by Dr. Bruce Ames of the Department of Biochemistry, University of California, Berkeley.

RESULTS AND DISCUSSION

Reduction of standard 2NF yielded 91% of the predicted amount of 2-aminofluorene as determined by ultraviolet spectroscopy and thin layer chromatography. Reduction of a chloroform solvent

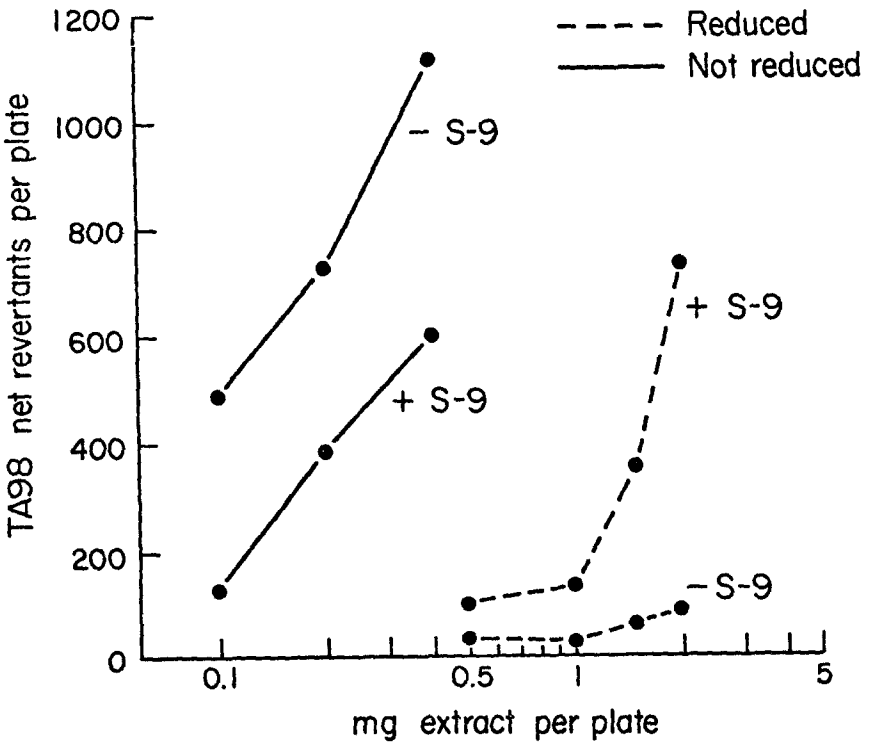

Figure 1. The ordinate is TA98 net his(+) revertants per plate, the abcissa is mg extract (chloroform eluate) per plate. The solid lines represent the unreduced chloroform eluate with and without S-9 Mix; the dotted line represents the reduced eluate, with and without 0.5 mL S-9 Mix. The spontaneous reversion rate was 30 colonies/plate and 1 µg 2-nitrofluorene standard produced approximately 90 revertant colonies/plate.

blank yielded a nonmutagenic sample with or without S-9 (data not shown). This blank also had no ultraviolet absorption at the characteristic wavelength of 2-amino fluorene.

Chloroform eluates of diesel exhaust particulate extracts were mutagenic in the Ames test. The mutagenic activity of approximately 480 revertants/100 μg of extract/plate did not require liver enzymes for its expression. The addition of liver enzymes decreased mutagenic activity, an effect which has previously been shown to be non-specific binding of the mutagen to liver proteins (WANG et al. 1981). After reduction of the diesel exhaust extract with sodium borohydride, the S-9 independent activity was greatly diminished. Addition of S-9 to the reduced sample now revealed the presence of S-9 dependent mutagenic activity.

These results, although not conclusive, provide additional evidence for the presence of nitroarene mutagens in diesel exhaust particulates. If, for example, the samples contained nitrofluorene, nitronaphthalene or nitropyrene, the reduction reaction would have formed aminofluorene, aminonaphthalene, and aminopyrene, compounds which are mutagens requiring liver enzyme activation in the Ames test. Our attempts to isolate the amine by acidic extraction were not successful, however, because of the weak mutagenic activity in the sample. In the future, the sodium borohydride method of HANAYA et al. may be utilized as a procedure for detecting nitroarene mutagens in complex environmental mixtures.

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