1984

Mutagenicity assays of particulate matter in Missoula Montana

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MUTAGENICITY ASSAYS OF PARTICULATE MATTER IN MISSOULA, MONTANA

by

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B.S., College of William and Mary, 1980

Presented in partial fulfillment of the requirements for the degree of

Master of Science

UNIVERSITY OF MONTANA

1984

Approved by:

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Chairman, Board of Examiners

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Dean, Graduate School

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Date

UMI Number: EP39946

UMI®

Dissertation Publishing

UMI EP39946

ProQuest®

Mutagenicity Assays of Particulate Matter in Missoula, MT.

Director: Dr. Walter L. Koostra $\mathcal{ULK}$

The purpose of this study was to supply new information on the mutagenicity of ambient air samples in Missoula, MT. Specifically, the study was designed 1) to create an index of mutagenicity based on the relationship between mutagenicity, weather, and air pollution data; 2) to help determine the sources of mutagens in Missoula's air; and 3) to evaluate the health impact of mutagenic particulate on Missoula's population.

High-volume samplers were used to collect ambient air samples at three Missoula locations between August 1982 and May 1983. Filter extracts were then assayed for mutagenicity using the Ames Assay. Statistical analyses were conducted to determine the relationship between mutagenicity, air pollution, and weather data. The health risk from mutagenic particulate was assessed by comparing results of the Ames Assay on Missoula's air and on cigarette smoke.

High-volume samples of particulate matter tested mutagenic, especially on cold, still days with temperature inversions and high respirable particulate. A highly significant negative correlation existed between mutagenicity and temperature and a highly significant positive correlation existed between mutagenicity and particulate concentration. Of all weather and air pollution data, RSP explained the greatest variability in mutagenicity ($r^2 = 0.31$). This low coefficient of determination indicated the great influence of the specific chemicals in the particulate matter on the mutagenicity of ambient air samples. Results of sampling did not implicate major sources of mutagenicity at two monitoring sites. But results showed motor vehicles contributed signficantly to mutagenicity at the other monitoring site where mutagenicity was highest year round. The mutagenicity levels found in this study were substantially lower than the levels found in a 1980 study, and the 1983 levels indicated that breathing mutagenic particulate increases the relative risk of lung cancer by 1.04 (most-probable) to 1.26 (worst-case) times.

Assaying from high-volume samples has several drawbacks that may have led to an underestimation of mutagenicity and consequently, the low health risk estimate obtained in this study. For future mutagenicity studies, dichotomous samplers should be used to sample respirable particulate for at most 12 hours.

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Missoula, Montana, lies in a mountain valley where narrow river canyons restrict horizontal air flow. Wintertime temperature inversions reduce the Missoula airshed volume, typically by 80% (Steffel, 1981), and cause air stagnation episodes. Pollutants accumulate and concentrate in the limited airshed during these episodes, turning the valley's air into an "impenetrable brown soup" (Missoula City-County Health Department, 1982). Because of this, Missoula has failed since 1970 to meet most federal and state air quality standards for total suspended particulate (TSP).

In 1977, the Montana legislature funded the Montana Air Pollution Study (MAPS) to investigate, among other things, the health effects of air pollution in the state. The study (Medvec, 1981) revealed evidence of health problems related to Missoula's high particulate levels. First, the lung function of Missoula children was compared to the lung function of children from other areas of Montana where TSP was 1/2 to 1/3 less than urban Missoula TSP levels. In almost all comparisons, Missoula children exhibited lower pulmonary function than children from other communities. Second, Missoula children exhibited decreased lung function as TSP levels increased. Third, among Missoula adults afflicted with chronic obstructive pulmonary disease (COPD) four of five morbidity symptoms increased as TSP increased.

In another MAPS investigation (Warren, 1981), Drs. Warren and Rogers of Montana State University used the Ames Assay, a convenient

microbial bio-assay for mutagens, on ambient air samples from ten Montana communities (Medvec, 1981).[1] Of the ten cities studied, Missoula's air exhibited the greatest mutagenicity with levels approaching those of New Jersey and Louisiana urban areas. Seasonal differences were seen: The highest levels occurred during winter months and the lowest levels occurred during summer months.

Residential wood-burning devices (RWDs) are suspected to be a major source of the mutagenic particulate in Missoula's winter air due to the large number of wood burners in Missoula and to the carcinogens identified in wood smoke. Roughly 50% of the households in Missoula burn wood. A 1980 source apportionment study by the Missoula City-County Health Department (CCHD) showed RWDs contributed to approximately 75% of Missoula's wintertime respirable particulate (Moyers et al., 1980). Studies by DeAngelis et al. (1980) and Lee et al. (1977) showed that smoke from wood and wood-burning stoves contains 14 known carcinogens and 4 known co-carcinogens.[2] Emission samples from wood-burning devices test mutagenic in the Ames Assay.

Mobile sources, including diesel engines, are another potential source of mutagens in Missoula. The 1980 source apportionment study showed mobile sources (i.e., exhaust emissions) contributed approximately 6% of wintertime TSP. Known mutagens have been identified in both diesel and gasoline engine exhaust samples, which also test mutagenic in the Ames Assay.

Rationale and Purpose

Since MAPS, no one has conducted further studies on the mutagenicity of Missoula's air. Recognizing the sparse data in this area, the Missoula 1981 Citizens Committee's Health Effects Task Force called for further biological, epidemiological and analytical monitoring of Missoula's air to determine the cancer risk from Missoula's air (Missoula City-County Health Department, 1981). With the expected increase in residential wood burning and the already unhealthy wintertime air, more information was needed on airshed pollutants, their levels, their sources and their health threat to Missoulians.

The purpose of this study was to supply additional information on the mutagenicity of Missoula's air. The following objectives were identified:

1. To analyze the mutagenicity of year-round samples of Missoula's ambient air using the Ames Assay;

2. To help determine the sources of mutagens in Missoula's air;

3. To create an index of mutagenicity based on the relationship between mutagenicity, weather, and air pollution data;

4. To coordinate this mutagenicity study with an in-progress study to identify the organic constituents in Missoula's air;

5. To evaluate the health impacts from mutagenic particulate on Missoula's population.

THE AMES ASSAY

Dr. Bruce Ames developed the Ames Assay in 1972. It uses mutant strains of bacterium Salmonella typhimurium, known as histidine auxotrophs, that cannot grow without added histidine. The assay, an in-vitro test, involves adding the test substance and bacteria to a solid medium lacking histidine in a petri plate. A mutagenic test substance can cause a back mutation (reversion) in some cells that restores the capacity for histidine synthesis. If this occurs, the revertant cells can grow and multiply into colonies. The reversion frequency, which is the number of colonies that grow on a test substance plate vs. the number of colonies that grow on a negative control plate, indicates the mutagenic potency of a test substance.

The mutant strains possess two additional mutations: 1) a deep rough mutation (rfa locus) which causes the loss of the lipopolysaccharide barrier that coats the surface of the bacteria and thus increases the permeability of large compounds through the cell wall; and, 2) a uvrB (ultraviolet radiation) mutation which causes loss of the excision repair system (uvrB locus) and thus prevents repair once the mutation occurs.

Ames further modified these strains by adding a plasmid, referred to as an R factor and designated pkM101. This plasmid carries ampillicin resistance markers as well as an error-prone DNA repair system that increases the sensitivity of the tester strains to mutagenesis by polycyclic compounds. The strains used in this study,

TA98 and TA100, contain this plasmid.

Strains TA98 and TA100 detect different types of mutations. Strain TA98 detects frameshift mutations--those that occur from shifts in pairing of repetitive sequences of DNA caused by addition or deletion of nucleotides. In frameshift mutations a ring-type compound usually intercalates with DNA. In addition to frameshift mutations, strain TA100 detects base pair substitutions--changes in the base pair sequence of DNA.

Many chemicals not carcinogenic and/or mutagenic in themselves may be metabolized in mammals to substances that are carcinogenic and/or mutagenic. These substances are known as promutagens or indirect mutagens. The Ames Assay mimics this metabolic activation in mammals by the addition of S-9 to the test substance and bacteria. S-9 is a homogenate of liver from rats that have been injected with a polychlorinated biphenyl mixture, Aroclor 1254.

The validity of mutagenicity testing to indicate carcinogenicity of substances rests on the somatic mutation theory of cancer. This theory holds that mutation in cellular nucleic acids causes cancer; therefore, all carcinogens should also be mutagens. Ames (1976a) outlines the major evidence supporting the somatic mutation theory.

A test on 300 chemicals showed that 90% of the known carcinogens (156/174) scored mutagenic in the Ames Assay and 87% of the noncarcinogens did not score mutagenic (McCann and Ames, 1978). In another evaluation of two complete subgroups of carcinogens and noncarcinogens, (120 compounds total), 91% of the carcinogens scored mutagenic and 93% of the noncarcinogens scored nonmutagenic (Purchase

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et al., 1976). These results clearly indicate a close association of mutagenesis with carcinogenesis.

Despite this success record in identifying carcinogens, the use of in vitro testing in bacteria to detect substances which are carcinogenic/mutagenic to humans remains a hotly debated issue. Opponents of Ames Assay use as a carcinogen screening test point to inconsistencies between animal and bacterial tests such as the weak mutagenicity in the Ames test of nitroso compounds, known to produce tumors in animals (Harnden, 1978; Whitmyre, 1980). Bridges (1976) notes the theoretical possibility of "false positives" due to the increased sensitivity of the strains to mutation events because of the pkM101 plasmid. Others question the equivalency of mutagenesis in bacterial systems with carcinogenesis in eukaryotic organisms (Sivak, 1976). The somatic mutation theory of cancer itself remains an unproven and contested hypothesis (Rubin, 1976; Sivak, 1976).

Mutagenic versus carcinogenic potency represents another area of controversy (Whitmyre, 1980). Coombs et al. (1976) found little quantitative correspondence between carcinogenic and mutagenic potency of 36 polycyclic aromatic hydrocarbons (PAHs). They suggest that:

> Each carcinogen has its own inherent specificity or different base sequences involved in these mutations, and hence it is unlikely that a quantitative relationship between mutagenicity and carcinogenicity would hold among a series of different compounds tested with a particular bacterial strain. Therefore, one would expect little quantitative correspondence between mutagenic and carcinogenic potency.

Ashby and Styles (1978) criticize the quantitative extrapolation of the Ames Assay to cancer risks in humans because the S-9 is not

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calibrated. This causes a variation of the basic activation and deactivation enzymes used in different S-9 preparations, which could dramatically alter mutagenic potency. They observed a variation of more than a factor of 100 with different preparations of S-9. Ames notes the correlation between mutagenicity and carcinogenicity for the assay is strictly qualitative, and not related to potency (Ames, 1976b).

However, other studies show a quantitative correlation between mutagenic and carcinogenic potency. Teranishi et al. (1975) found the relative mutagenic potential of PAHs determined by the Ames Assay equivalent to relative carcinogenic activities. In a study by Meselson & Russell (1977) on 14 carcinogenic and/or mutagenic compounds, carcinogenic potency in rodents was approximately equal to the mutagenic potency in Salmonella.

Despite criticisms, the Ames Assay is currently the simplest, quickest, and least expensive method used to qualitatively detect suspected carcinogens. Animal cancer tests typically cost at least $0.5 - 3 million per chemical, last at least 2 years, and require a large number of animals to detect a weak mutation. In contrast, the Ames Assay costs at least 100 times less per chemical, takes 3 days, and requires only a couple milliliters of bacteria stock culture to easily detect weak mutagens.

CHAPTER III

LITERATURE REVIEW

This section reviews previous work in the area of mutagenicity testing of ambient air samples. The findings of general studies of mutagenicity testing of ambient air and mutagenicity studies on air from Missoula and other wood-burning communities are reviewed. Next, since the suspected source of mutagens in Missoula's air are RWDs and motor vehicles, results of studies on emissions from these sources are presented. And finally, a brief review of various studies on cigarette smoke is provided as this information was used to estimate the health risk from breathing Missoula's air.

Variables in Mutagenicity of Air Pollution Extracts

Since 1975, when Dr. Ames introduced the Ames Assay, researchers have used it extensively to test the mutagenicity of air pollution extracts. Chrips and Fisher (1980), Huisingh (1981), and Hughes et al., (1980) have reviewed the studies on mutagenicity testing of air pollution extracts in detail. Studies specifically relevant to this investigation are summarized here.

In many studies, cascade impactors or other equipment were used to separate airborne particulate fractions by size. Results on the mutagenicity of the different particles all concur that mutagenic activity predominates in the smaller sized particles (<2 um) (Talcott and Harger, 1980; Preidecker, 1980; Commoner et al., 1978; Tokiwa et al., 1980; Pitts et al., 1977; Chrisp and Fisher, 1980; Lofroth, 1981a). In contrast to the larger particles, these smaller particles

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remain in the atmosphere for long periods of time (10^2 or 10^3 hours as opposed to 10 to 100 hours) (DeWeist, 1975) and easily penetrate the deep lung or alveoli (Huisingh, 1981). Studies showing the mutagenicity of air pollution extracts to be greater in the small than in the large fractions of particulate concur with studies showing a preponderance of organic (many carcinogenic) molecules in the small fraction (Towika et al., 1980; National Academy of Sciences, 1976; Natusch and Wallace, 1974)

Using a high-volume sampler to collect samples for mutagenicity testing presents some drawbacks. First, a preponderance of large particles may obscure the mutagenic effect of the small particles and thereby bias results towards less mutagenic activity than actually present (Chrisp and Fisher, 1980; Hoffman, 1982; Huisingh, 1981). Pitts et al.(1982) found mutagenicity of 3-hour samples to be considerably higher than corresponding 24-hour samples. Second, when large volumes of air are sampled, the more volatile organics can be lost by evaporation (Huisingh, 1981).

Third, potential surface reactions can take place on the filter. Pitts et al. (1978) first identified the problem of filter artifacts--reactions that occur between compounds on filters, but not in ambient air, that may alter the mutagenicity of samples. Filter samples containing benzo(a)pyrene (BaP), exposed to gaseous pollutants in smog exhibited direct mutagenicity, contrary to the expected indirect mutagenicity known to be caused by BaP. Further investigation showed that BaP reacted with O_3 and PAN to form nitro-derivates of BaP such as nitrobenzo(a)pyrene, which are direct

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mutagens. Such reactions may be catalyzed on the filters and may occur, to a lesser extent, in the ambient air (Cheremisihoff, 1981).

Various methodologies have been developed to detect the mutagenicity of ambient air samples fractionated into different chemical classes (e.g., neutral, basic, aromatic) (Kolber et al., 1981; Pellizzari et al., 1979; Tokiwa et al., 1980; Tokiwa et al., 1977; Teranishi et al., 1978; Hughes et al., 1980; Whong et al., 1981) The fractions and their constituents containing mutagenic activity can be identified. Some investigators in this area have found the sum of the mutagenic activity of the separate fractions to be greater than the mutagenic activity of the whole sample (Kolber et al., 1981; Hughes, 1980; Chrisp and Fisher, 1980). Thus, in complex mixtures masking of mutagenicity may occur, possibly due to toxic effects that can be reduced or removed upon fractionation.

However, the opposite effect can also occur in complex mixtures (Bridges, 1976). Teranishi et al. (1978) found less activity in the sum of fractions of urban air particulates than the reconstituted tar made by mixing the fractions. This demonstrated a synergism between substances in the complex mixture.

Studies have related mutagenicity of air pollution extracts to meteorological factors such as wind speed, wind direction, precipitation, sunlight, temperature of collected particles, and distribution of particulates by wind (Commoner et al., 1978; Moller and Alfheim, 1980; Chrisp and Fisher, 1980). Moller and Alfheim (1980) found a higher number of revertants per unit mass of particles on days with a low total concentration of particles in the air, mainly

days of rain and snow, than on days with a high total concentration of particles. Two reasons were suggested for this phenomenon (Chrisp and Fisher, 1980): 1) The larger particles were removed from the air so that the smaller, more mutagenic particles would remain for collection; and 2) under these conditions, collected particles may be acting as an absorbent for compounds in the gas phase. Alfheim (1982) also documented the long range transport of mutagenic particulate from Continental Europe or the British Isles to a coastal Norwegian town.

The location of the ambient air sampler may also influence mutagenicity testing. Moller and Lofroth (1982) showed that mutagenic activity may be several times higher at street level than at roof top level. Alfheim and Moller (1981) found that mutagenicity at street level but not roof level varied with traffic frequency. These results indicate some chemical transformation occurring while the pollutants are in the atmosphere.

Mutagenicity Studies of Wood-Burning Communities

Most studies point to PAHs as the basis of mutagenicity of wood smoke and consequently, mutagenicity of ambient air in wood-burning communities. Polcyclic organic matter (POM) encompasses all organic matter with two or more benzene rings and PAHs are POMs that contain only carbon and hydrogen. PAH's are ubiquituous in the atmosphere, produced by the combustion of compounds containing carbon and hydrogen (e.g., auto and diesel exhaust, heat and power generation, coke production, refuse burning, forest fires) and also synthesized by plants and microorganisms.

PAHs can exist in the atmosphere in three forms: 1) adsorbed on foreign particulate matter; 2) condensed in suspension with air (aerosol form); and 3) in the vapor phase. Because of their high melting and boiling points, the bulk of PAHs, including most of the carcinogenic ones (Choudhury and Bush, 1980), are believed to be associated with respirable particulate. Adsorption of PAHs to particulate may increase the carcinogenic effect of the PAHs (Whitmyre, 1980). Many factors affect the stability of PAH's in particulate form, namely available light, temperature, presence of oxidizing agents, particle size, porosity of the particles, and adsorption factors (Perera and Karim, 1978).

More POMs exist in the vapor state under high combustion temperatures than under low combustion temperatures. Cautreels and Van Cauwenberghe (1978) conducted an experiment on the distribution of organic pollutants between airborne particulate matter and the corresponding gas phase. They found low molecular weight compounds predominantly in the gas phase but high molecular weight compounds were preferentially retained on filter material. Vapor phase organic compounds may react with particulates by chemical reaction with aerosols, dissolution with aerosols, liquefaction in the interstices of aerosols, and reactions with a compound already dissolved in or attached to an aerosol (National Academy of Sciences, 1976).

Many PAHs are known or suspected carcinogens; most require metabolic activation to be carcinogenic. In the Ames Assay, PAHs require addition of S-9 to cause frameshift and base pair substitution mutations in the bacteria (Ames et al., 1972).

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A) Past Missoula Studies

Only two previous studies investigated the mutagenicity of compounds in Missoula's airshed.

First, Drs. Warren and Rogers conducted the Ames Assay on 12 high-volume filters taken at a residential monitoring station between April 1979 and March 1980 (Medvec, 1981). CCHD staff selected one filter during the middle weeks of each month that most closely approximated the mean monthly particulate level for that month. The highest levels of mutagens occurred during the fall and winter months with S-9 activation (Figure 1A, Appendix A). As stated before, these levels reflect the levels of mutagenicity found in urban New Jersey and Louisiana. Of the ten Montana cities studied, Missoula's air showed the greatest mutagenicity.

Second, researchers at Dartmouth University measured ambient BaP, a known carcinogen, levels in Missoula in February and March of 1980. Because BaP is the most studied and most common of all PAHs, ambient BaP levels are typically used to indicate PAH levels and the carcinogenic potential of ambient air (Missoula City-County Health Department, 1981). Levels of BaP in February and March 1980 measured 1.45 ng/m^3 on fiberglass high-volume filters and 2.74 ng/m^3 on membrane filters (probably the more meaningful method). In comparison, BaP levels average 0.45 ng/m^3 in rural New England and 2.6 ng/m^3 in Denver in the wintertime (Missoula City-County Health Department, 1981).

Source apportionment studies conducted in 1980 by four independent laboratories indicated a possible source of mutagenic activity during

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the wintertime months in Missoula. X-ray diffraction on high-volume filter samples showed wood combustion contributed 53% of the total suspended particulate in Missoula's ambient air during the winter months (Davis, 1980). Three other studies (optical microscopy, x-ray fluorescence and chemical mass balance, and chemical analysis) linked 76%, 68%, and 75%, respectively, of Missoula's winter respirable particulate to residential wood combustion (Hedstrom, 1980; Cooper and DeCesar, 1980; Moyers et al., 1980).

Drs. Walter Koostra and Kit Johnson used data from the above studies to estimate the carcinogenic potential of wood smoke in Missoula (Missoula City-County Health Department, 1981). Given the available data, their findings indicate that Missoula's air causes a very low increased risk of respiratory cancer to citizens. Both researchers acknowledged the tenousness of the assumptions used in their estimates.

Dr. Koostra used three different methods--1) estimates of BaP levels in ambient air versus cigarette smoking; 2) BaP levels in ambient air versus EPA estimates of lung cancer caused by BaP; and 3) MAPS mutagenesis data--to arrive at an estimate of 5-8 additional lung cancers per year (worst scenario). However, using the Dartmouth study on ambient BaP concentrations during February and March 1980, Koostra reduced the estimate to 0.5 to 0.8 additional lung cancers per year.

Using the 1980 source apportionment study and data from Cooper (1980) on the proportion of wood particles that is carcinogenic, Johnson estimated for humans at rest the daily ventilatory exposure wood smoke-derived carcinogens in ambient air during February and

March, 1980. He found this figure to be roughly equivalent to 1/1000 of the exposure of smoking one pack of cigarettes per day, which would represent an increased relative risk of cancer on the order of 0.01, given constant levels of exposure for 20 years.

B) Other Communities

Literature on mutagenicity testing simply does not exist for communities facing pollution problems similar to Missoula's. The Montana cities studied in MAPS, including Missoula, seem to be the only wood-burning communities where researchers have used the Ames Assay on filter extracts. However, several reseachers have conducted studies on the PAH levels in wood-burning communities.

Murphy et al. (1981) measured the wintertime concentrations of BaP in Telluride, CO, a community with a wood-burning pollution problem. They used Soxhlet extraction and High Performance Liquid Chromatography (HPLC) to analyze BaP concentrations. At one site, they found the mean concentration of BaP to be 7.4 ng/m^3, relatively high for a mountain community and five to six times higher than the levels found in most urban areas. They suspected wood-burning devices as the BaP source.

Using HPLC, Hornig et al. (1981) analyzed yearly POM levels from high-volume filter samples of ambient air in Hanover, NH, a small town where wood is an important supplementary fuel, and in Lyme Center, NH, a semi-rural village where wood is the predominant heating fuel. Sixty percent of the homes in Lyme Center were heated partly with wood. The authors attributed a weak correlation between POM concentrations and TSP values to the lack of precipitation and a high

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contribution of windblown dust to TSP. The fingerprint, the relative amounts of each POM, of Lyme Center and Hanover were very similar, with the highest POM levels at Lyme Center (on clear, cold, windless days with a marked inversion) similar to the average POM levels from 13 urban Hanover samples (Table 1A and Figure 2A, Appendix A). In addition, the fingerprints of Lyme Center and two rural sites were almost identical (Figure 3A, Appendix A). The authors attributed this similarity to the large scale mixing of air over the entire Connecticut River Valley area. The highest value of BaP was 1.2 ng/m^3 at Lyme Center. On average, however, the POM concentrations in Lyme Center reflected the lower POM values in the surrounding rural, not urban, areas.

In a study on the air quality in Petersville, Alabama, Imhoff et al. (1982) quantified the contribution of RWDs to ambient levels of PAH and other organic compounds. The neighborhood studied had a density of wood stoves and fireplaces of about 60 units per km^2. Chemical mass balance studies showed that, on the average, RWDs emitted 80% of the fine particles collected. They found: 1) inefficiency of the Anderson impacter filter in retaining PAHs, 2) very high concentrations of important mammalian carcinogens, such as BaP and benzo(a)anthracene, 3) higher than normal levels of high molecular weight PAHs, and 4) high levels of low molecular weight volatile organics, such as benzene, phenol, napthalene, fluorene, and phenantrene (Tables 2A and 3A, Appendix A).

Imhoff, et al. (1982) compared the ambient levels of PAH species with those of modeled concentrations from two previous studies. The

ambient levels of PAHs were at least three times higher than those the two models predicted (Table 4A, Appendix A).

The studies by Imhoff et al. (1982) and Hornig et al. (1981) both demonstrate the uncertainty associated with measuring ambient concentrations of PAHs. Many experiments have demonstrated the inefficiency of glass fiber filters to collect PAHs, particularly the highly-volatile low molecular weight species (Thrane and Mikalson, 1981; Cautreels and VanCauwenberghe, 1978; Imhoff et al., 1982; Hornig et al., 1981; Chrisp and Fisher, 1980).

Although most of the PAHs with a high vapor pressure are not carcinogenic, they may influence the potential activity of other PAHs (Thrane and Mikalsen, 1981). One study showed that more than 50% of some POMs may be on particles too small to be collected on glass-fiber filters (Hornig et al., 1981). Other problems in the analysis of PAHs include: chemical degradation of POMs during collection and analysis, incomplete extraction of PAHs, volatization of PAH during collection and the concomitant loss from particulate matter, and potential reactions catalyzed on the filters but not in the ambient air (Cheremisimoff, 1981). While methods are being developed to remedy these problems, the concentrations of PAHs measured in ambient air must be seen as approximations.

The BaP concentrations in the communities reviewed in this section range from a low of 0.48 ng/m^3 in Lyme Center to a high of 68 ng/m^3 in Petersville, Alabama—a considerable range of concentrations. Excluding the data on Petersville, the range changes to 0.48 to 7.4 ng/m^3, considerably less variable. The study on Petersville (Imhoff,

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et al., 1981) differed from those on the other cities because its authors used tandem filters and XAD-2 resin sampling with subsequent extraction to measure BaP concentrations. Unlike other studies, they found a good portion of BaP in the gas phase. Further study is needed to determine whether the different sampling method used in this study could account for the unusually high concentrations of BaP found.

Mutagenicity of Wood Stove and Motor Vehicle Emissions

The amount and type of substances emitted from motor vehicles and wood stoves varies with operating conditions. The complexity of motor vehicle and RWD emission samples makes identification of compounds in the emissions difficult. PAHs are suspected as the mutagens present in both emissions. Appendix B contains detailed information on the PAH emissions from RWDs. Only those studies where the Ames Assay was used to investigate the mutagenicity of motor vehicle and RWD emissions are reviewed here.

A) Wood Stove Emissions

In the Monsanto study (DeAngelis et al., 1980), 24 emission samples obtained by a source assessment sampling train (SASS) from baffled and nonbaffled wood stoves and a fireplace fueled by different wood all showed mutagenic activity and clonal toxicity (Table 5A, Appendix A). The four fly ash samples showed no mutagenic activity.

Rudling et al. (1981) measured the mutagenicity of emissions from wood and wood chip combustion in a small central heating furnace and from wood combustion in a closed fireplace. The measured mutagenicity, expressed as revertants per unit amount of fuel, was

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very high, exceeding that of conventional gasoline-powered motor vehicles and of the same magnitude as that produced by diesel engines (Figure 4A, Appendix A). Emissions from dried wood in closed fireplace ovens exhibited mutagenicity in both strains TA98 and TA100. Addition of S-9 sometimes enhanced and sometimes decreased the mutagenic response for different samples (Table 6A, Appendix A). Organic compounds and mutagenic substances from RWDs measured 10 to 1000 times higher than those from the burning of wood in industrial boilers or in a prechamber oven.

Lewtas (1981) assayed the condensed organic matter emitted by wood stoves and other energy sources. The wood emissions were the most activation-dependent emissions tested, suggesting to Lewtas that PAH's and/or aromatic amines primarily cause the observed mutagenicity. Compared on a revertants per ug of organics basis, automobile emissions and oil heaters produce more revertants than wood stoves (Figure 5A, Appendix A). However, wood stoves have a significantly greater mutagenic activity when compared on a fuel basis (Table 7A, Appendix A).

Lofroth (1978) used the Ames test on emissions from a hot water boiler fueled by wood. Mutagenicity occurred in bacteria that detect frameshift mutations and not in bacteria that detect base-pair substitution mutations. This suggested that the mutagenic compounds in wood stove emissions are polycylic compounds, which cause frameshift mutations. In addition, he found a substantial part of the mutagenicity to be associated with gases. Lofroth believed this might have resulted from incomplete condensation of the compounds on

particles in hot flue gases or from evaporative losses from the filter-collected particles during sampling.

Dasch (1982) measured the mutagenicity of particulate emissions from three fireplaces using different fuels. Litton Bionetics conducted the Ames test on extracts from glass-fiber filters that were taken to dryness and dissolved in dimethylsulphoxide (DMSO). A correlation between BaP levels and the number of revertants existed. With the exception of the synthetic log, higher mutagenicity occurred with S-9 than without S-9 (Figure 6A and Table 8A, Appendix A).

B) Motor Vehicle Emissions

The increased use of light-duty diesel automobiles has spurred concern over and study of the mutagenicity of exhaust extracts. Ninety percent of the diesel emission particulates are in the respirable range, 2.5 um, and serve as deposition sites for high molecular weight organic compounds (Huisingh, 1981). These organic compounds extracted from diluted particulate emissions have been tested for mutagenicity with various bioassays, in particular, the Ames Assay.

Diesel exhaust extracts exhibit direct mutagenicity in the Ames Assay--addition of S-9 results in decreased mutagenic activity of the samples (Henderson et al., 1980a; Salmun et al., 1982, Lofroth, 1981a; Lofroth, 1981b; Lofroth and Lazardis, 1983; Claxton, 1980; Tokiwa et al., 1978). Thus, the exhaust contains compounds that are direct-acting mutagens or are converted to ultimate mutagens by bacterial metabolism. Typically, activity occurs only in strains that

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detect frameshift mutation (Claxton, 1980; Claxton and Kohan, 1981; and Henderson et al., 1980b).

Various PAHs, including some promutagens, have been identified in diesel particulate and are believed to be major contributors to the mutagenic activity of organic extracts of diesel particulates (Whitmyre, 1980; Lofroth, 1981b; Henderson et al., 1980a; Salmun et al., 1982; Hanson et al., 1979). Many investigators believe that diesel fuel aromatic hydrocarbons react with NO_2 to form nitro-derivative direct-acting mutagens (Henderson et al., 1980a; Henderson et al., 1980b; Salmun et al., 1982; Clark et al., 1981b; Hanson et al., 1979; Clark et al., 1981a, Lewtas, 1983). Henderson et al. (1980b) demonstrated that nitro-derivatives of most of the diesel fuel aromatics, two- to three-ring PAHs, were produced upon reaction with NO_2. After reacting, the products tested mutagenic without activation. It is suspected that nitrated PAH may contribute to the mutagenicity of particle extracts for gasoline engines as well as diesel engines (Clark et al., 1981a; Claxton, 1980, Wang et al., 1978).

Lofroth and Lazardis (1983) and Clark et al. (1981b) further investigated this hypothesis by comparing assays of extracts from diesel exhaust using regular mutant strains of bacteria (TA98 and TA100) and nitroreductase deficient strains. Nitroreductase deficient strains of bacteria cannot metabolize the nitrogen components of a chemical. A lowered mutagenic response occurred in the nitroreductase strains than the regular strains, indicating the presence of mutagenic nitro compounds in the organic extracts. The researchers suggested

that the nitroreductase strains' ability to convert nitro-PAHs to corresponding electrophilic metabolites (mutagenic) may cause the decreased response in these strains. The role of nitroreductase enzymes in human toxicology is poorly understood (Clark et al., 1981b).

Other hypotheses concerning the decreased mutagenic activity of diesel exhaust upon activation include: 1) the protein in the S-9 may block transport of the mutagens to the DNA (Wang and Wei, 1981); 2) the mutagens may bind to the protein of S-9 and thus reduce mutagenicity (Wang and Wei, 1981); 3) detoxification of direct-acting mutagens partially masks activation of promutagenic PAHs (Clark and Vigil, 1979; Choudhury and Bush, 1980); and 4) unsubstituted PAHs are not readily detected in the Ames Assay because their concentrations in diesel exhaust particulate are less than their detection threshold in the Ames Assay under standard conditions (Wei and Rappaport, 1980).

The mutagenicity of extracts from gasoline engines exceeds that of diesel engines on a per weight basis (Claxton and Kohan, 1981). But on a per mile basis, diesel engines produce 10 to 100 times greater mutagenic combustion products than gasoline engines because they produce greater amounts of particles (Clark et al., 1981a; Lofroth, 1981a; Onishi et al., 1980; Pierson et al., 1983).

In contrast to diesel exhaust, mutagenicity of gasoline engine exhaust increases upon activation with S-9 (Lofroth, 1981a; Claxton, 1980, Lewtas, 1983). Lofroth (1981a) assayed particulate and condensate from both gasoline and diesel engine exhaust. Except for the particulate matter from the gasoline exhaust, the highest

mutagenic response occurred without activation. Lofroth (1981a) suggested that, since in the Ames Assay the detection of indirect mutagens depends on the concentration of direct mutagens, only particulate matter from gasoline exhaust had a composition favorable to the detection of indirect mutagens.

Cigarette Smoke

An immense volume of literature exists on the health risk from cigarette smoking. Because of this large information base and the similarity between tobacco and wood smoke, these studies provide the best means of estimating the cancer risk from breathing Missoula's air. Three types of studies were referred to in making this study's estimation: 1) studies identifying the components of cigarette smoke; 2) studies determining the relative cancer risk from cigarette smoke; and 3) studies using the Ames Assay to test the mutagenicity of cigarette smoke.

Wynder and Hoffman (1967) provide an excellent review of studies identifying the components of cigarette smoke. Tables 9A and 10A, (Appendix A) list the carcinogens, co-carcinogens, and tumor promoters in the particulate phase of tobacco smoke.[2] Substances listed in these tables and also identified in wood smoke include: benzo(ghi)perylene, benzo(a)pyrene, dibenz(a,h)anthracene, fluoranthene, pyrene, methyl fluoranthene/pyrenes, benzofluoranthenes, dibenzopyrenes, indenopyrene, benz(a)anthracene, chrysene, methyl chrysenes, dibenzocarbozoles, and benzo(c)phenanthrene.

The 1979 Surgeon General's Report on Smoking and Health (U.S.

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Department of Health, Education and Welfare, 1979) reviewed studies on the increased cancer risk from smoking. Although studies attribute smoking to the cause of cancers other than respiratory cancers, such as kidney, bladder, mouth and larnyx cancer, only respiratory cancers will be considered here.

In eight epidemiologic studies, the mortality ratios for lung and bronchus cancers ranged from 3.64 to 15.9[3]. Many factors influenced estimates, such as age when first started smoking, occupation, lifestyle, degree of inhalation, use of filters, duration of smoking, cigarettes smoked per day. The Report concludes that cigarette smokers, on the average, are 10 times more likely to develop lung cancer than nonsmokers. A two-pack-a-day smoker is likely to increase his risk of cancer by 20 times.

In estimating the risk of cancer from breathing Missoula's air, Drs. Koostra and Johnson used the statistics of a one-pack-a-day habit which: 1) induces lung cancer at a rate of about 1 per 1000 smokers per year; and 2) increases the risk of all forms of respiratory cancer about 10 to 15 times. (Missoula City-County Health Department, 1981).

The Ames Assay has been used to investigate the mutagenic activity of cigarette smoke (Yamasaki and Ames, 1977; Hutton and Hackney, 1975; Kier et al., 1974; Lewtas, 1983; Mizusaki et al. 1977a and 1977b; Lofroth et al., 1983). However, in only one of these studies, Lofroth et al. (1983), was mutagenicity determined on the basis of revertants per meter-cubed (m^3) of air sampled, the variable used to measure mutagenicity in this study. Lofroth et al. (1983) sampled

particulate matter from cigarette sidestream smoke by smoldering 100 cigarettes of common brands in a funnel-shaped hood and collecting particulates on a glass-fiber filter from the top of the hood with a high-volume sampler run on reduced air flow. The filters were Soxhlet extracted with acetone and converted into a DMSO solution for the Ames Assay. They observed a response of 15,000 and 1,000 TA98 revertants per cigarette, with and without activation, respectively. The smoke from the cigarettes, spread in a volume of 300 m^3, resulted in a mutagenic activity of 50 TA98 revertants per m^3-cigarette with S-9 activation.

CHAPTER IV

MATERIALS AND METHODS

This chapter describes the methods used to determine the mutagenicity of ambient air sampled at three sites in Missoula, MT. Detailed in this chapter are: 1) the methods used to collect particulate samples and prepare the samples for assaying; 2) the conventional Ames Assay procedures; 3) the study's sampling design and statistical methodology; 4) the system used to assay constituents of Tenax columns; and 5) the methodology used to assess the health risk from breathing mutagenic particulate.

Sample Collection

Airborne particulate matter was collected on 8" X 10" glass-fiber filters using General Metals high-volume samplers at various locations in Missoula from midnight to midnight. The following morning the CCHD staff removed the filters from the samplers, transported them to the Department and measured the wet weight. They then placed the filters in a dessicator for 18 to 72 hours before measuring the dry weight. To prevent exposure to light and loss of volatile material, the filters were then wrapped in aluminum foil and placed in the freezer until extraction. Because of heavy workloads, lack of personnel, etc., the time between the sampling date and placement of the filters in the freezer varied. Usually, the filters were extracted a week after the sampling date.

Extraction of Filters

Descending chromatography with DMSO as a solvent was used to

extract soluble matter from a 1" by 6" strip taken from a glass-fiber filter. Warren (1981) found this method best for analyzing air filters. A volume of 6.8 ml of reagent grade DMSO (FW 78.1) ran down from caps through each strip for at least 4.5 hours, collecting approximately 3 ml of sample in a beaker below. The extracted liquids were then frozen in vials until the day of the assay.

To prevent contamination from airborne microorganisms on the filters, 1 drop of chloroform was added to and N_2 bubbled through the samples at $37^\circ C$ for 1/2 hour prior to the assay.

The Assay

Preparation of the media and culture: The staff in Dr. Warren's lab prepared Noble top agar and petri plates as described by Ames et al. (1975). They also routinely verified the identity of the strains by checking for the presence of known characteristics: spontaneous reversion frequency, sensitivity to ultraviolet and crystal violet, and sensitivity to ampicillin.

Preparation of the bacteria: On the morning of the assay test, the test cultures were prepared by adding 9 ml of Difco nutrient broth to 1 ml of stock culture and then placing the mixture in a shaker bath at $37^\circ C$ for two and a half hours. A spectrophotometer and standard curve were used to assure a viable count of 1×10^8 bacteria before beginning the assay.

S-9 Preparation: 1 ml of S-9 mix consisted of:

    0.1 ml Lot #084 Aroclor induced S-9 enzyme extract
    0.1 ml NADGP-6-P
    0.02 ml 1.65 M KCL
    0.02 ml 0.4 M MgCl$_2$
    0.26 ml sterile H$_2$0
    0.50 ml 0.2 N sodium phosphate buffer (pH 7.4)

The S-9 mix was prepared fresh on each assay day and kept on ice

throughout the test.

Using winter samples from 1981, Dr. Warren's staff determined the

optimum amount of S-9 mix to add per plate. A volume of 0.2 ml was

the minimum amount of S-9 mix that produced the highest number of

mutagenic colonies.

Preparing the assay plate: The procedure detailed by Ames (1975)

for combining the bacteria, test substance, and S-9 into a pour plate

was followed. For each filter, four types of mixtures to be added to

the bottom agar layer were prepared:

    1. 0.1 ml sample + 2 ml Noble top agar + 0.1 ml TA98 bacteria
    2. 0.1 ml sample + 2 ml Noble top agar + 0.1 ml TA98 bacteria +
        0.2 ml S-9 mix
    3. 0.1 ml sample + 2 ml Noble top agar + 0.1 ml TA100 bacteria
    4. 0.1 ml sample + 2 ml Noble top agar + 0.1 ml TA100 bacteria +
        0.2 ml S-9 mix

On each day of the test, positive and negative control plates were

also prepared. The positive controls included known carcinogens or

mutagens:

    1. 0.1 ml TA98 + 2 ml Noble top agar + 40 ul 5 mg/ml Dexon
    2. 0.1 ml   TA100 + 2 ml Noble top agar + 15 ul 1 mg/ml
       Nitrosoguanidine
    3. 0.1 ml TA98 + 2 ml Noble top agar + 0.2 ml S-9 mix
       + 50 ul 2.5 mg/l 2-Anthramine
    4. 0.1 ml TA100 + 2 ml Noble top agar + 0.2 ml S-9 mix
       + 50 ul 2.5 mg/l 2-Anthramine

The negative controls consisted of the bacteria, with and without S-9

mix, and 2 ml of top agar.

Assay of activity: After incubation at 37°C for three days, the number of revertant colonies were counted by hand or with the aid of a Biotran II automatic colony counter. If the spontaneous revertants (negative controls) numbered between 20-60 for TA98 and between 110-180 for TA100, the test was considered valid. Generally, a background lawn of microcolonies resulting from the trace of histidine added to the top agar grew on the plate. Absence of this lawn indicated that the test substance was toxic. In a valid test, the counts of the triplicate plates per sample were averaged. If the standard deviation of the triplicate plates exceeded 10 the experiment was repeated only once due to financial limitations. Subtracting the spontaneous revertants for each test from the test plate revertants gave the number of revertants per 0.1 ml of sample extract. A sample tested mutagenic if the number of induced revertants exceeded twice the number of spontaneous revertants.

Sampling Design

Samples were obtained from three sites in Missoula, shown in Figure 1, between August 1982 and May 1983.

Rose Park: Rose Park serves as the primary site for Missoula's particulate sampling program and the most "representative" air monitoring station in the Missoula airshed. It lies in the geographic population center of the city, at the intersection of Franklin and Blaine. Because of the busy street (Brooks) and residential sections near the park, trees, vehicles, dusty roads, and RWDs all contribute to particulate matter collected at the site. In a 1982 field survey,

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Legend:
1 Rose Park
2 Malfunction Junction
3 Boyd Park

Missoula
Missoula County
Montana
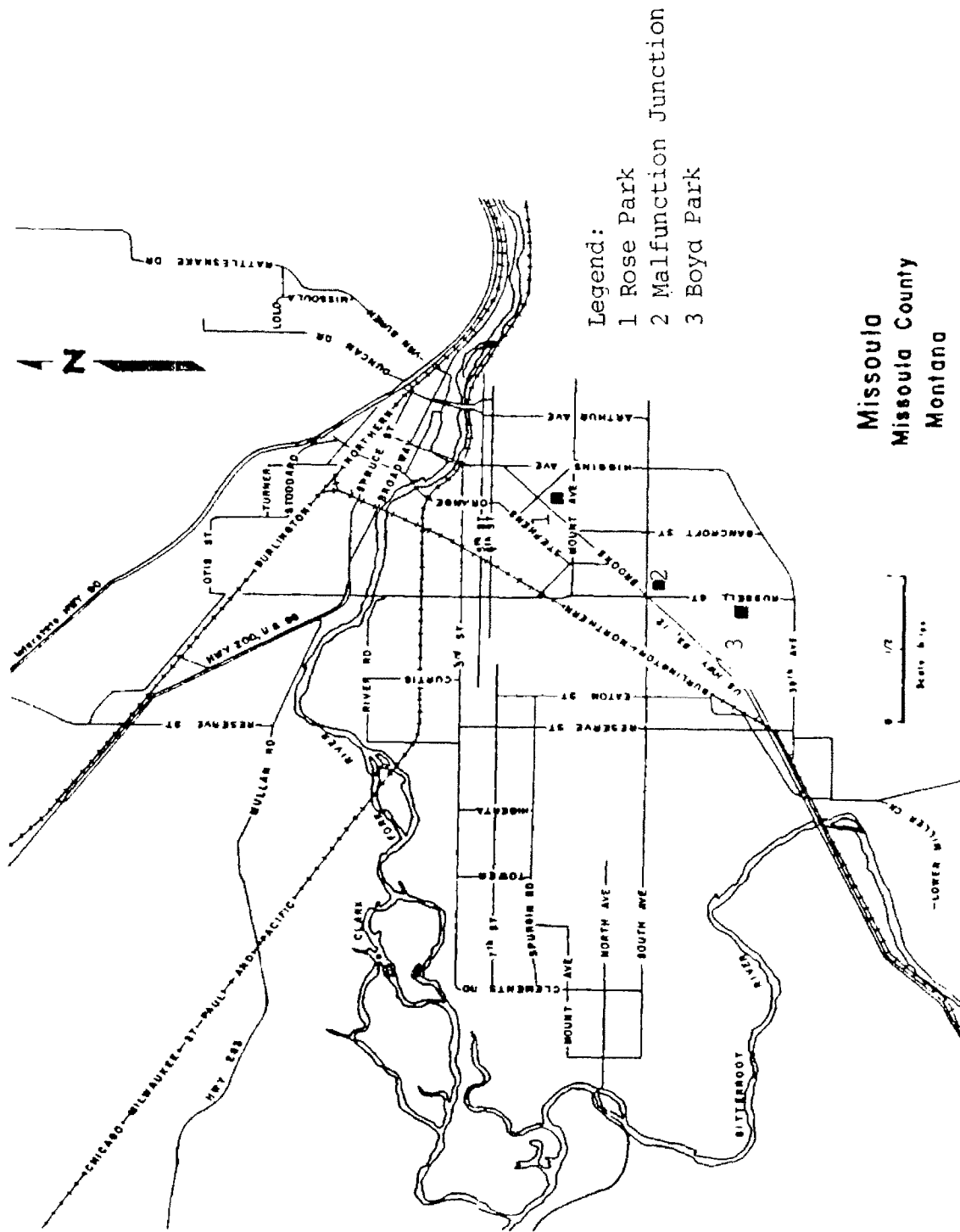
Figure 1.
Location of sampling sites.

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the Air Quality Unit of the CCHD found that approximately 66% of the houses in a 1/8-mile radius of Rose Park had chimneys suitable for use with RWDs (Missoula City-County Health Department, 1982). A 1979 survey showed that 40% of the homes within a one-mile radius of the site actually used their RWDs regularly during the winter months (Missoula City-County Health Department, 1982).

Because the majority of Missoula's air pollution data comes from Rose Park, most of the samples were collected there. Samples collected every Wednesday between August 1982 and May 1983 and every 3 days during the months of December, January, and February were assayed in this study.

Malfunction Junction: Situated at the busy intersection of South, Brooks, and Russell, the Malfunction Junction station was sited to monitor pollution primarily from motor vehicles. Approximately 44,000 vehicles pass through the intersection per day (Kress, 1983). The number of RWDs near the intersection is unknown.

Although filters taken every three days at Malfunction Junction between August 1982 and May 1983 were extracted, only two samples a month were assayed: the one with the highest TSP and the one with the TSP closest to the monthly geometric mean.

Boyd Park: Boyd Park monitoring station lies in a residential area on the south side of town one block west of Russell at the intersection of Washburn and Ernest. Like Malfunction Junction, the number of RWDs in the area is unknown. No major transportation route lies near the site. The same sampling system was used at Boyd Park as at Malfunction Junction.

Mutagenicity of Tenax Column Extracts

Jon Beihoffer, a chemistry graduate student at the University of Montana, used Tenax columns to collect the organic constituents (primarily gases) of Missoula's air at the Rose Park monitoring station between January and May 1983. He used gas chromatography and mass spectrometry to identify the constituents that were able to volatilize from the columns at 300°C. Extracts from six of Beihoffer's duplicate columns, taken on days of varying TSP conditions, were assayed. A gas extraction system similar to Beihoffer's was designed for use in this study. A furnace with a thermocouple was used to heat the columns to 300°C for 15 minutes. Nitrogen gas flowed through the columns at 20 ml/minute. The gases condensed on the sides of the glass collection tube, kept frozen by a mixture of dry ice and acetone. After warming the collection tube to room temperature in a water bath, the sample was dissolved in 1 ml of DMSO. The Tenax samples were tested for mutagenicity using the Ames Assay.

Health Assessment Methodology

To assess the cancer risk from breathing Missoula's air, the value of 50 revertants per m^3 per cigarette obtained by Lofroth et al. (1983) was compared to the revertants per m^3 of ambient air samples[4]. Two scenarios were developed--"worst-case and "most-probable". For the worst-case scenario, it was assumed that Missoulians breathe the highest monthly mutagenicity observed in this study year-round. For the most-probable scenario, the average monthly mutagenicity levels at Rose Park were used. For this comparison, the estimate that a

one-pack-a-day smoker increases his risk of developing cancer by 13 times was used.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) and the conversational version of SPSS (SCSS) were used for all statistical analyses. All statistical inferences were made at a 0.10 significance level. The SPSS programs Breakdown, Oneway, Anova, Scattergram, Condescriptive, Frequencies, T-Test, and New Regression were used to investigate the relationship between mutagenicity, air quality, and weather data and to examine site differences. Air quality data consisted of 1) TSP, total suspended particulate, ug/m^3, collected on the sample filter; and 2) RSP, respirable suspended particulate, ug/m^3 of particles less than 3.0 microns in diameters. RSP was measured by a GCA, a beta-attenuated dichotomous sampler at Rose Park[5]. Weather data on the sampling dates included in statistical analyses were:[6] TEMP, average daily temperature, ^oF; 2) PREC, total daily precipitation, inches; 3) WIND, average wind speed, miles per hour (mph); and, 4) DIRECT, average wind direction. Variables used to indicate mutagenicity were 1) TA98, number of revertant colonies per plate, without S-9 activation; TA98S, number of revertant colonies per plate, with S-9 activation; TA98M3, number of revertant colonies per m^3, without activation[7]; and TA98SM3, number of revertant colonies per m^3, with activation[7].

RESULTS

This chapter details the results of ambient air mutagenicity testing. The first section provides trends analysis using first, Rose Park data, and second, Malfunction Junction and Boyd Park data. Included in this section are results of correlating mutagenicity, weather, and air pollution data. The second section shows results of mutagenicity testing on Tenax column constituents. In the third section ambient air mutagenicity is compared to cigarette smoke mutagenicity to derive a health risk estimate. The results of this study are compared to the results of the 1979-80 MAPS mutagenicity study in section four.

Ambient Air Samples: Trends Analysis

Table 1C, Appendix C lists the results of the triplicate assays on the samples and air quality and weather data for the sample date. After approximately one-half of the samples were assayed, it was decided to continue testing with TA98 bacteria only, due to financial limitations and little evidence of sample mutagenicity with TA100 bacteria. Therefore, all data analyses pertain only to TA98 bacteria.

Part A) Rose Park: Since Boyd Park and Malfunction Junction were sampled nonrandomly twice monthly, the following trend analyses do not include data from those sites. Using the Rose Park data, several statistical analyses were conducted to determine how the mutagenicity of Missoula's air changes over time, and how it correlates with weather and air pollution data. Some analyses consider data from winter months only, Nov. 1982 to Feb. 1983, or data from the "other"

months only, Aug. - Oct. 1982 and March - May, 1983.

Figures 2a and 2b depict the change in mutagenicity of nonactivated and activated samples with time. The number of revertants per m^3 tended to be higher during the wintertime, although great variability occurred from one sample day to the next. Table 1 contains summary statistics on mutagenicity, TSP, and RSP for the 10 sampling months and Figures 3a and 3b depict mutagenicity by month. With the exception of April, the winter months exhibited higher values for these variables than the other months. One-way analysis of variance showed a difference in mutagenicity for both nonactivated and activated samples between months (F prob. = 0.01).

TABLE 1

General Statistics: Mutagenicity and air quality data for Rose Park by month.

Month	TA98M3 [1] [5] Mean S.E.		TA98SM3 [2] Mean S.E.		N [6]	TSP [3] mean	RSP [4] mean
August 1982	1.55	0.56	0.23	0.58	4	51.70	----
September 1982	1.89	0.58	0.91	0.25	5	44.84	15.67
October 1982	3.60	0.45	3.17	0.64	4	52.13	----
November 1982	4.79	1.20	5.32	1.03	3	101.77	50.00
December 1982	5.72	0.64	4.65	0.53	13	52.85	49.15
January 1983	5.80	0.71	5.00	0.48	14	186.16	83.57
February 1983	6.36	0.37	5.94	0.40	12	118.26	45.58
March 1983	3.00	0.58	2.55	0.34	5	58.70	19.00
April 1983	5.01	0.59	4.17	1.21	3	60.83	----
May 1983	2.09	0.64	0.93	0.27	4	52.42	----

[1] TA98 revertants per m^3, without activation
[2] TA98 revertants per m^3, with activation
[3] total suspended particulate, ug/m^3
[4] respirable particulate ug/m^3
[5] standard error
[6] number of samples
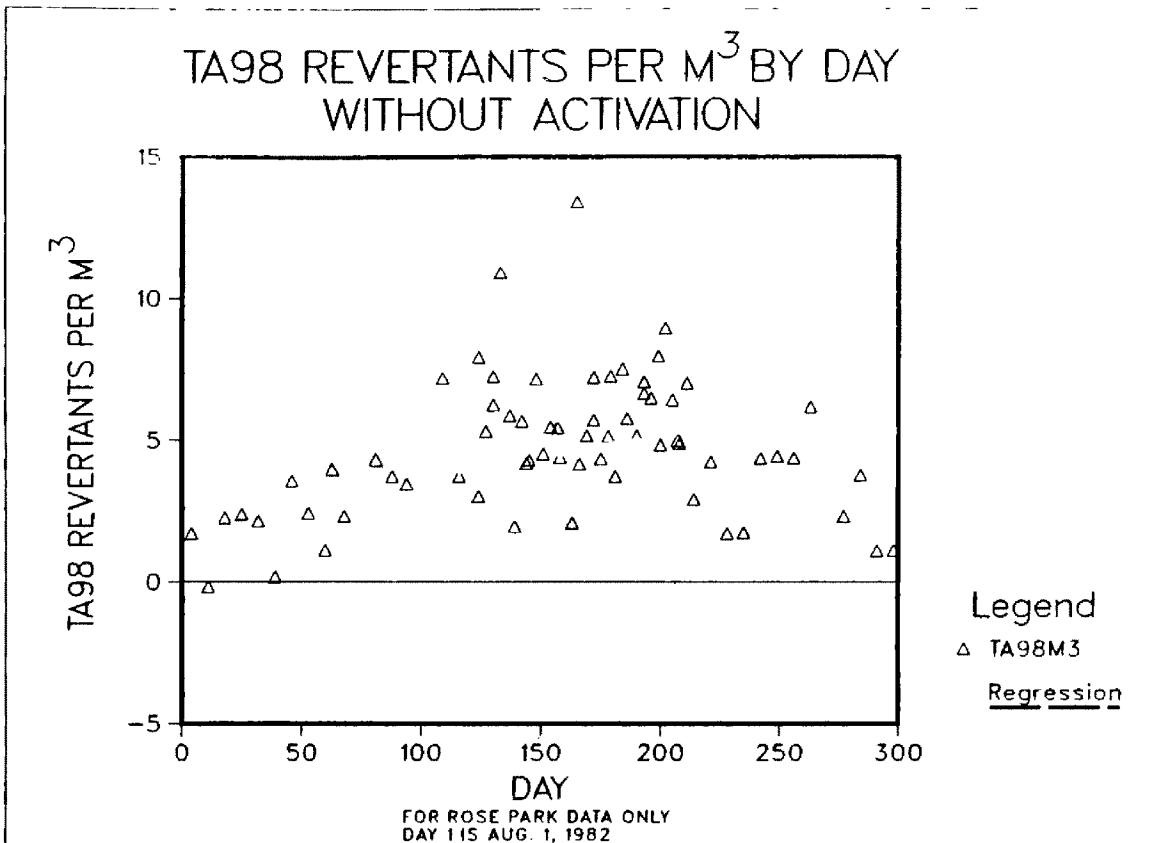
Figure 2a
Scatterplot: Mutagenicity by day.
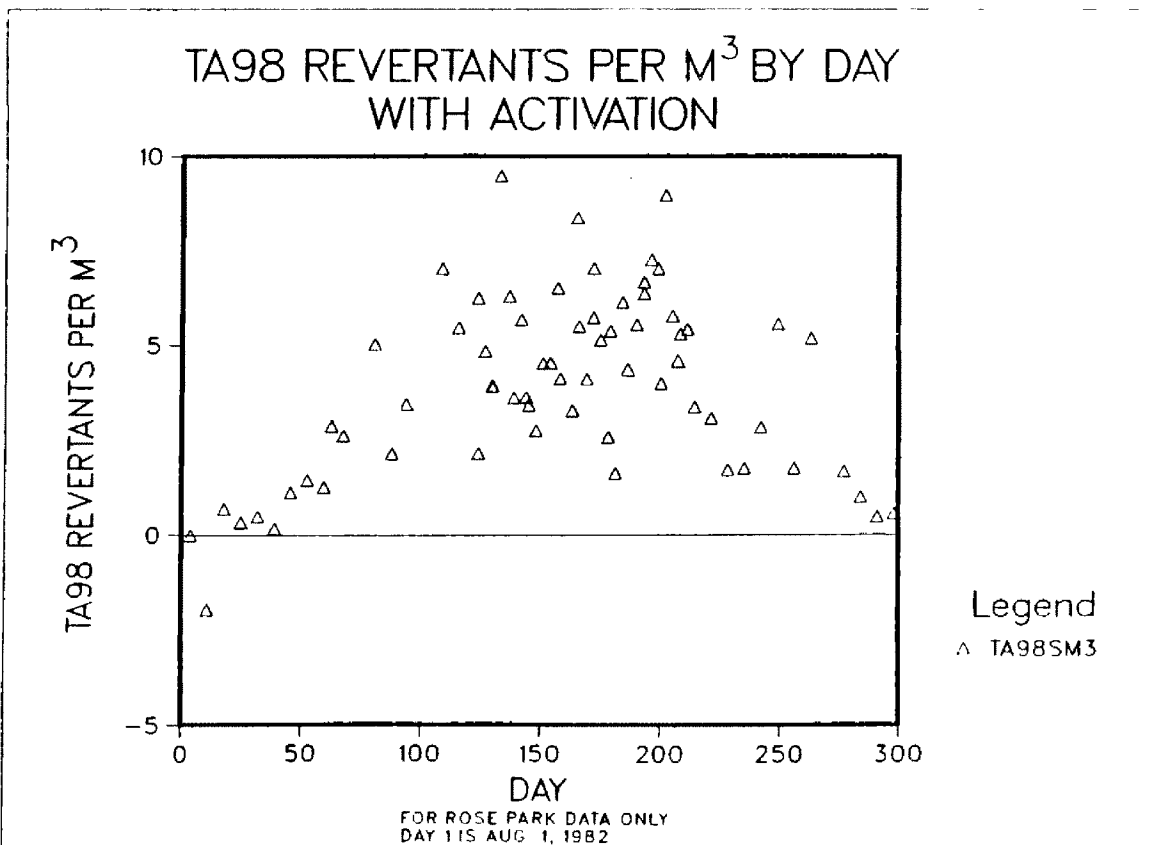
Figure 2b
Scatterplot: Mutagenicity by day.
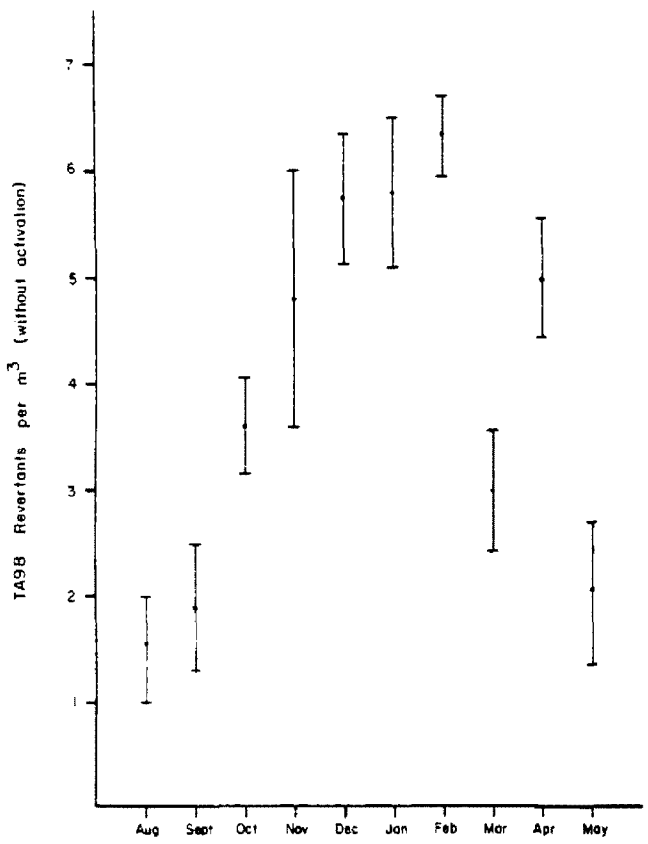
Sampling Months

Figure 3a
Mutagenicity by month at Rose Park.
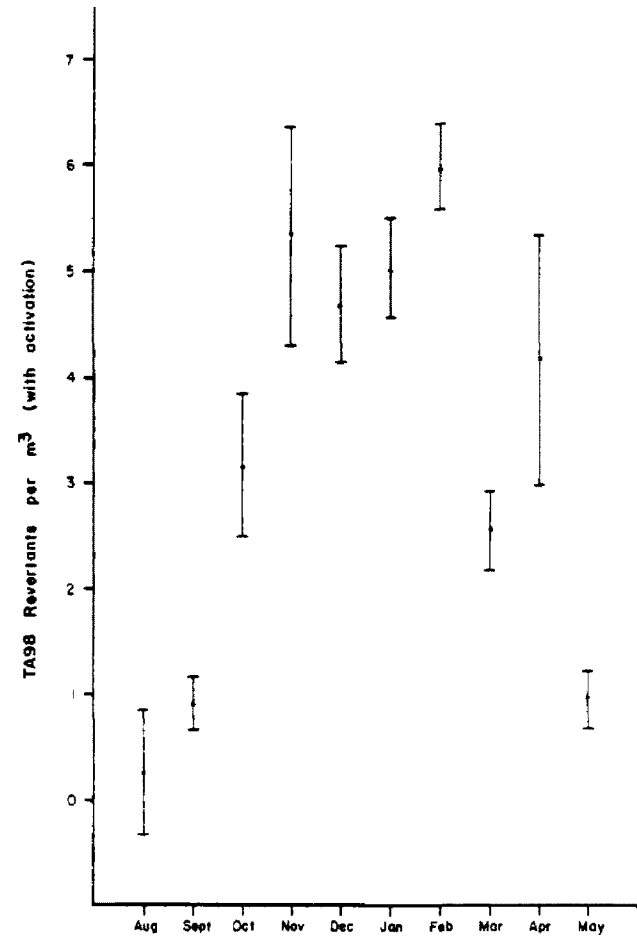
Sampling Months

Figure 3b
Mutagenicity by month at Rose Park.

Table 2 shows the results of grouping the number of revertants into discrete categories. A sample tested mutagenic if the number of induced revertants exceeded twice the background (i.e., number of spontaneous revertants). Using Wednesday samples, for nonactivated and activated samples in 62% and 52% of all samples, respectively, the number of revertants exceeded approximately twice the background. During the winter months, for nonactivated and activated samples, in 100% and 95% of the samples, respectively, the number of revertants exceeded approximately twice the background. During the other months, for nonactivated and activated samples, in 32% and 20% of the samples, respectively, the number of revertants exceeded approximately twice the background.

A paired t-test on all Rose Park data showed TA98 revertants per m^3 without activation to be higher than TA98 revertants per m^3 with activation ($p < 0.001$). Similar results were obtained for winter months ($p = 0.004$) and other months ($p < 0.001$) (Table 3).

Concurring with a seasonal trend was a high negative correlation between mutagenicity and temperature ($r = -.60$, $p < .001$, TA98M3; $r = -64$, $p < .001$, TA98SM3). The cooler the ambient temperature, the higher the mutagenicity.

TABLE 2

Number and frequency of weekly samples in various mutagen
level classes for various time periods at Rose Park.

Time Periods	Variable	Number of Revertants	N[1]	Relative Frequency
All Months	TA98[2]	0-19	17	38.6
		20-39	17	38.6
		40	10	22.7
	TA98S[3]	0-19	21	47.7
		20-39	15	34.1
		40	8	18.2
Winter Months (Nov-Dec)	TA98	0-19	0	0.0
		20-39	10	52.6
		40	9	47.4
	TA98S	0-19	1	5.3
		20-39	11	57.9
		40	7	36.8
Other Months (Aug.-Oct and March-May)	TA98	0-19	17	68.0
		20-39	7	28.0
		40	1	4.0
	TA98S	0-19	20	80.0
		20-39	4	16.0
		40	1	4.0

[1] number of samples
[2] TA98 revertants, without activation
[3] TA98 revertants, with activation

Table 3

T-Test: Revertants per m^3 with activation vs. revertants per m^3 without activation.

Sampling Period	Variables Compared	Mean	S.D.[1]	T.	Two-tail T-Prob.	N[2]
All Months	TA98M3[3]	4.70	2.46	4.78	0.001	67
	TA98SM3[4]	3.93	2.39			
Nov.-Feb.	TA98M3	5.86	2.14	3.02	0.004	42
	TA98SM3	5.18	1.72			
Aug.-Oct.	TA98M3	2.74	1.54	4.34	0.001	25
March-May	TA98SM3	1.82	1.74			

[1] standard deviation
[2] number of samples
[3] TA98 revertants per m^3, without activation
[4] TA98 revertants per m^3, with activation

Other than temperature, weather variables did not correlate highly with mutagenicity (Table 4). No difference existed between days of precipitation ≤ 0.02 and precipitation > 0.02 (F prob. = 0.43, TA98M3; F prob. = 0.48, TA98SM3). Analysis of variance revealed a difference in mean mutagenicity between days of different average wind directions (F prob. = 0.07, TA98M3; F prob. = 0.03, TA98SM3). Mutagenicity measured highest in samples representing air masses coming from the east (TA98SM3) and northeast (TA98M3). The days of average wind speed above 3 mph had lower mean mutagenicity for nonactivated but not for activated samples than the days of average wind speed below 3 mph (F prob. = 0.05, TA98M3; F prob. = 0.29, TA98SM3).

A difference existed in mutagenicity between different weather

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categories defined by Steffel (1983a) (F prob. = 0.005, TA98M3, F prob. = 0.0012, TA98SM3) (Table 5). Samples from cold, dry days with low wind speeds and wet roads/frozen soil conditions had the highest mutagenicity. Sample mutagenicity on these days measured higher than the sample mutagenicity on warm, dry days with slow wind speeds and dry road/soil conditions (Scheffe Range Test, $p < .05$). Further analysis revealed that samples from days of wet and frozen road/soil conditions exhibited higher mutagenicity than days of dry road/soil conditions (p = 0.034, TA98M3 and p = 0.014, TA98SM3)

TABLE 4
Correlation of air quality and weather variables with mutagenicity.

		TA98M3 [1]	TA98SM3 [2]	N [3]
RSP [4]	r [9]	0.556	0.470	45
	p [10]	0.001	0.001	
	r^2 [11]	0.309	0.221	
TSP [5]	r	0.611	0.517	67
	p	0.001	0.001	
	r^2	0.373	0.267	
TEMP [6]	r	-0.605	-0.641	67
	p	0.001	0.001	
	r^2	0.366	0.411	
WIND [7]	r	-0.111	-0.013	67
	p	0.184	0.380	
	r^2	0.012	0.002	
PREC [8]	r	-0.085	0.097	67
	p	0.246	0.218	
	r^2	0.007	0.009	

[1] TA98 revertants per m^3, without activation
[2] TA98 revertants per m^3, with activation
[3] number of samples
[4] respirable particulate, ug/m^3
[5] total suspended particulate, ug/m^3
[6] average daily temperature, F
[7] average wind speed, mph
[8] average precipitation, inches
[9] coefficient of correlation
[10] significance of correlation
[11] coefficient of determination

Table 5
Mutagenicity arranged by weather groups[*]

Mean TA98M3[2]	Mean TA98SM3[3]	Weather Conditions[1]			Road/Soil Conditions	Number of Samples	Relative Frequency
		Temp.	Prec.	Wind Speed			
6.44[a]	5.19[a]	Cold	Dry	Slow	Wet/Frozen	16	23.9
5.18[ab]	4.83[ab]	Cold	Dry	Slow	Dry/Frozen	2	3.0
5.39[ab]	5.34[ab]	Cold	Wet	Slow	Wet/Frozen	7	10.4
5.78[ab]	4.66[ab]	Warm	Dry	Slow	Wet/Frozen	10	14.9
3.17[b]	2.13[b]	Warm	Dry	Slow	Dry	20	29.9
3.45[ab]	3.70[ab]	Warm	Wet	Slow	Wet	11	16.4
4.37[ab]	2.84[ab]	Warm	Wet	Fast	Wet	1	1.5

[1]Weather Conditions defined by Steffel (1983b):

Temp. - Cold when temperature $\leq$ 34 F, warm when temperature >34 F

Prec. - Dry when precipitation $\leq$.02 inches, wet when precipitation >34 F

Wind Speed - Slow when $\leq$ 5 mph, fast when >5 mph

[2]TA98 revertants per m^3, without activation

[3]TA98 revertants per m^3, with activation

[*]All weather data gathered at Rose Park

a,b - groups with different subscripts are groups whose
means differ at the 5% significance level (Scheffe Range Test)

The relationship between mutagenicity, TSP and RSP was investigated through regression analysis and analysis of variance. Figures 4a-5b show the regression of mutagenicity on TSP and on RSP. In general, as TSP and RSP increased mutagenicity increased.

TSP was recoded to 1982 alert levels and RSP was recoded to examine the differences in mutagenicity at the various levels. The highest mutagenicity occurred at the highest level of TSP and RSP, the lowest mutagenicity occurred for the lowest level of RSP and TSP (Tables 6a and 6b).

Tables 6a and 6b

Mutagenicity with and without activation at various total suspended particulate and respirable particulate levels.

RSP[1]	TA98M3[2] mean	TA98SM3[3] mean	N[4]
Low to 50	4.81^a	3.99^a	23
51 to 80	5.80^a	5.64^{ab}	14
81 to 110	5.51^a	4.58^{ab}	4
111 to 140	5.46^a	5.52^{ab}	2
141 to high	12.17^b	8.93^b	2

TSP[5]			
Low to 75	3.54^a	2.75^a	33
76 to 100	5.26^{ab}	4.58^{ab}	12
101 to 150	4.81^{ab}	4.44^{ab}	9
151 to 300	6.50^b	5.71^b	12
301 to high	13.42^c	8.37^{ab}	1

[1] respirable particulate, ug/m^3
[2] TA98 revertants per m^3, without activation
[3] TA98 revertants per m^3, with activation
[4] number of samples
[5] total suspended particulate, ug/m^3
[a,b] groups with different subscripts are groups whose mean differ at the 5% significance level (Scheffe Range Test)

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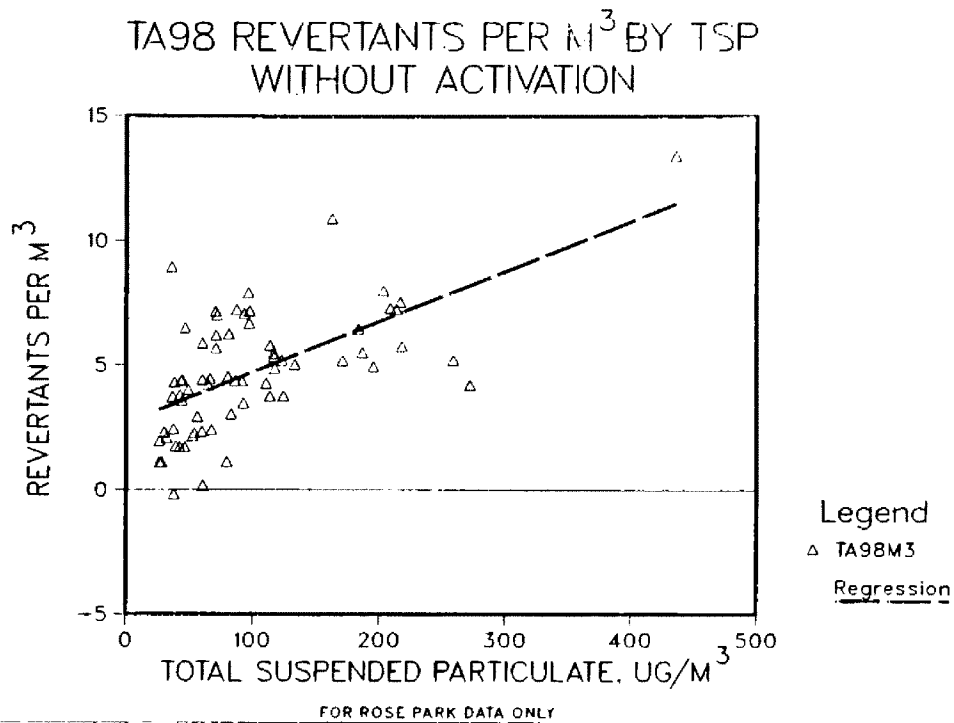
Figure 4a
Least squares regression of TA98 revertants per m^3, without
activation, by TSP, uq/m3 (r^2 = .37, p $\leq$ .01).
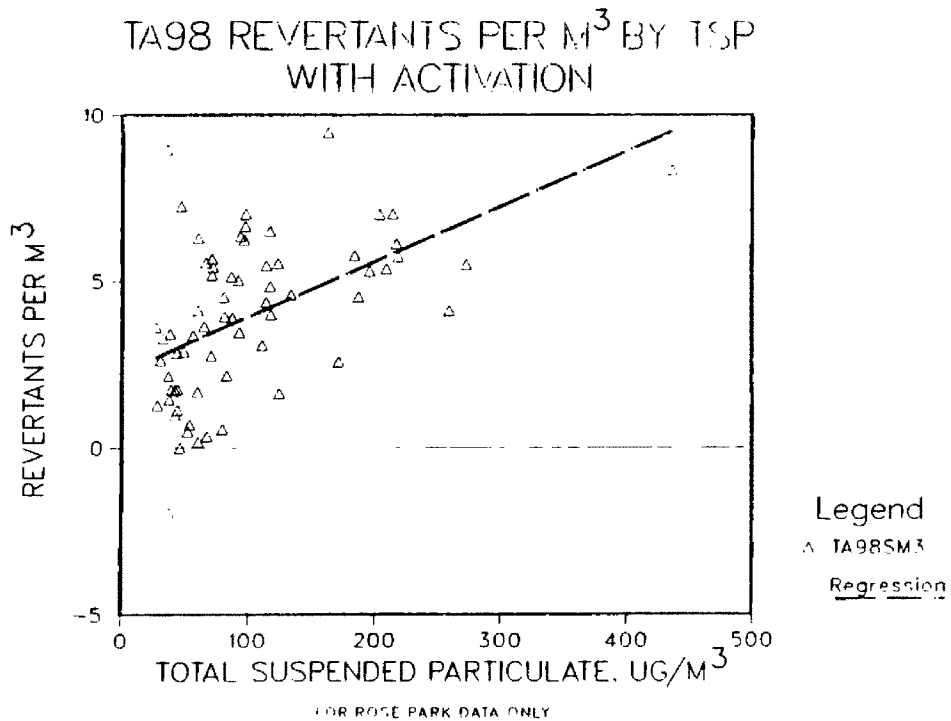
Figure 4b
Least squares regression of TA98 revertants per m^3, with
activation, by TSP, uq/m3 (r^2 = .27, p $\leq$ .01).

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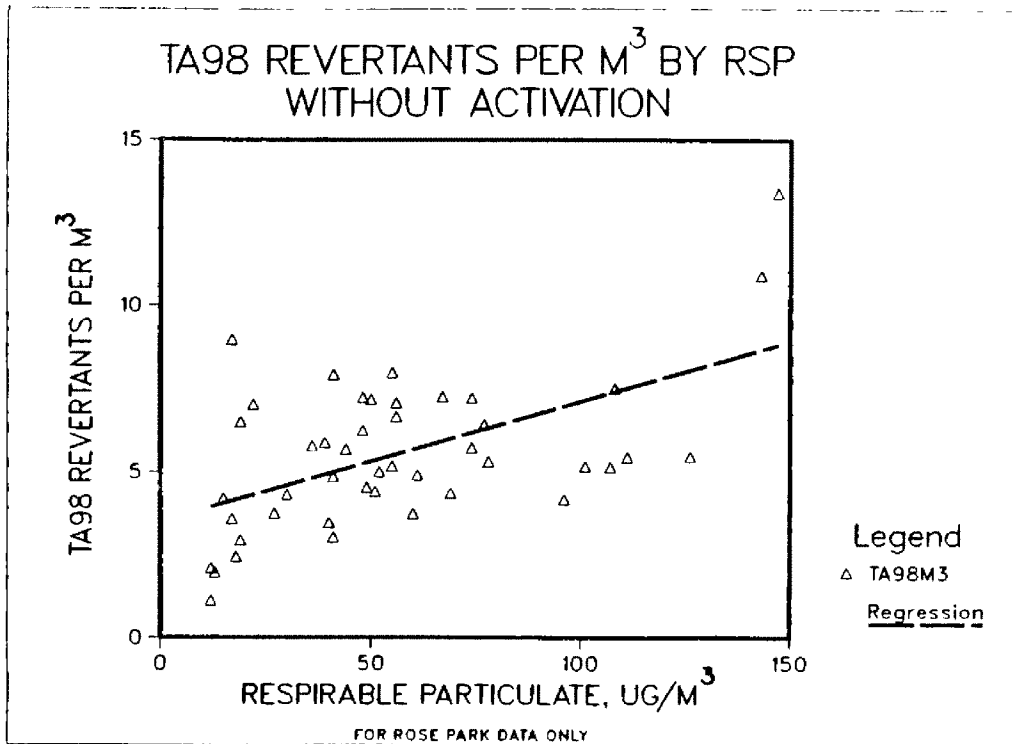
Figure 5a
Least squares regression of TA98 revertants per m³ without activation by RSP, ug/m³ (r² = .31, p = .001).
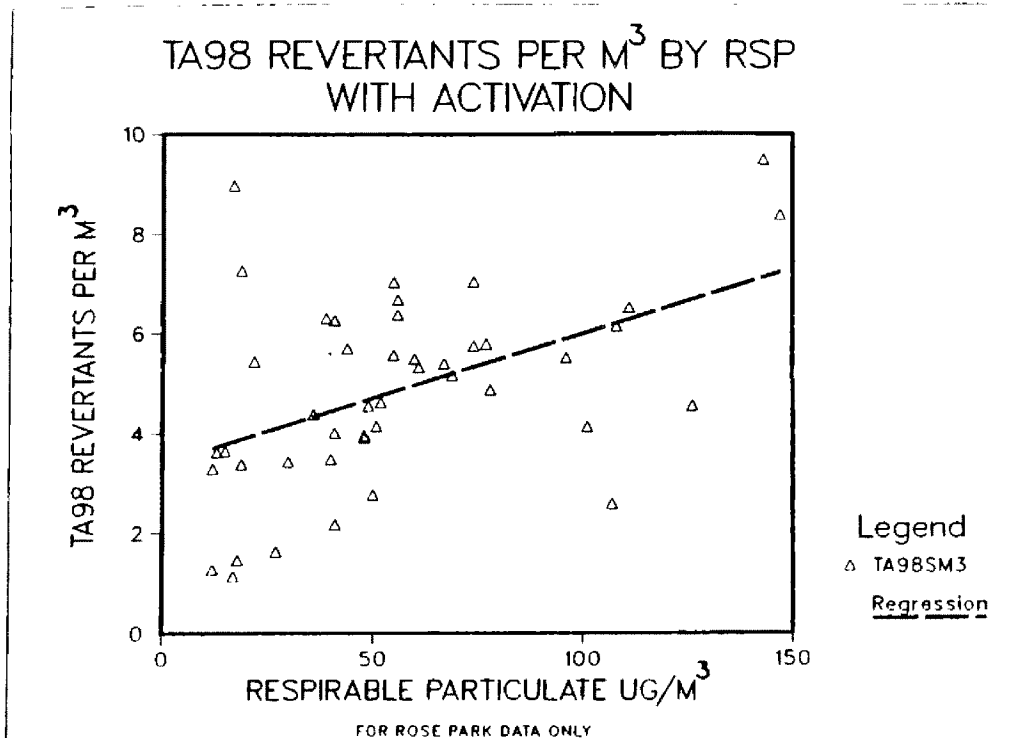
Figure 5b
Least squares regression of TA98 revertants per m³ with activation by RSP, ug/m³ (r² = .22, p = .001).

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Analysis of covariance was used to determine whether air quality data explained some of the same variation in mutagenicity explained by weather data. Tables 2C and 3C (Appendix C) present results of analysis of covariance on mutagenicity by recoded TSP and RSP groups with temperature as a covariate. With the effect of temperature on mutagenicity removed, there remained a difference in mutagenicity between the recoded RSP groups (F prob. = 0.001, TA98SM3; F prob. = 0.004, TA98SM3) and recoded TSP groups (F prob. < 0.001, TA98M3; F prob. = 0.086, TA98SM3). Tables 4C and 5C (Appendix C) present results of analysis of covariance on mutagenicity by recoded temperature groups with TSP and on mutagenicity by recoded temperature groups with RSP. With the effects of RSP on mutagenicity removed, mutagenicity was not different between the temperature group (F prob. = 0.235, TA98M3; F prob. = 0.560, TA98SM3). With the effect of TSP on mutagenicity removed, mutagenicity was different between the temperature groups (F prob. < 0.001, TA98M3; F prob. < 0.001, TA98SM3).

The relationship between weather groups and air quality data was further explored by conducting a analysis of covariance on mutagenicity by weather groups (defined in Table 5) with RSP and on mutagenicity by weather groups with TSP. With the effects of RSP on mutagenicity removed, no difference existed in mutagenicity between the weather groups (F prob. = 0.564, TA98M3; F prob. = 0.594, TA98SM3) (Table 6C, Appendix C). With the effect of TSP on mutagenicity removed, mutagenicity was different between the weather groups (F prob. = 0.011, TA98M3; F prob. = 0.002, TA98SM3) (Table 6C, Appendix C).

Using only those sample days with RSP data, regression analyses were performed to determine the air quality and weather variables that best explained (linearly) variability in mutagenicity. For nonactivated and activated samples, the best prediction equation of mutagenicity based on the highest r^2, the lowest standard error (SEE), the significance of F change, and normal residual characteristics were:

TA98M3 = 0.036(RSP) + 3.5; r^2 = 0.31, F = 19.23, F Prob.$<$0.001, SEE = .92

TA98SM3 = 0.026(RSP) + 3.38; r^2 = 0.22, F = 12.20 F prob.$<$0.001, SEE = 1.73

Part B) Malfunction and Boyd Park:

Tables 7a and 7b contain the information on mutagenicity and air quality data at Malfunction Junction and Boyd Park, respectively, for those samples chosen as representative of average air quality conditions for each month. Figures 6a and 6b graphically display this information. At Boyd Park, an increase in mutagenicity during the winter months occurred, with January mutagenicity exceeding all other months by at least three-fold. At Malfunction Junction, although the winter months exceeded most of the other months in mutagenicity, a wintertime increase is less evident.

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Table 7a

General Statistics: Mutagenicity and air quality data for Malfunction Junction by month.**

Month	TA98M3[1]	TA98SM3[2]	TSP[3]
August 1982	6.31*	5.23	104.6
September 1982	3.55	1.88	67.8
October 1982	3.85*	4.66	104.6
November 1982	10.78*	7.95*	172.4
December 1982	7.17*	3.94	133.0
January 1983	7.53*	6.10*	148.9
February 1983	7.26*	5.50*	154.8
March 1983	4.70*	3.92	91.2
April 1983	5.03*	6.28*	84.9
May 1983	10.32*	3.94	69.1

Table 7b

General Statistics: Mutagenicity and air quality data for Boyd Park by month.**

Month	TA98M3	TA98SM3	TSP
August 1982	2.52	-0.84	74.5
September 1982	0.79	1.97	87.0
October 1982	2.69	5.08	118.7
November 1982	6.85*	5.48	184.2
December 1982	5.98*	3.99*	166.8
January 1982	22.34*	17.61*	194.0
February 1982	3.29	3.29	92.2
March 1983	3.25	2.84	87.3
April 1983	5.69	3.71	88.8

** Results from the one sample assayed per month with TSP closest to the monthly TSP geometric mean
[1] TA98 revertants per m^3, without activation
[2] TA98 revertants per m^3, with activation
[3] Total suspended particulate, ug/m^3
* Sample tested mutagenic

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MUTAGENICITY BY MONTH AT MALFUNCTION JUNCTION
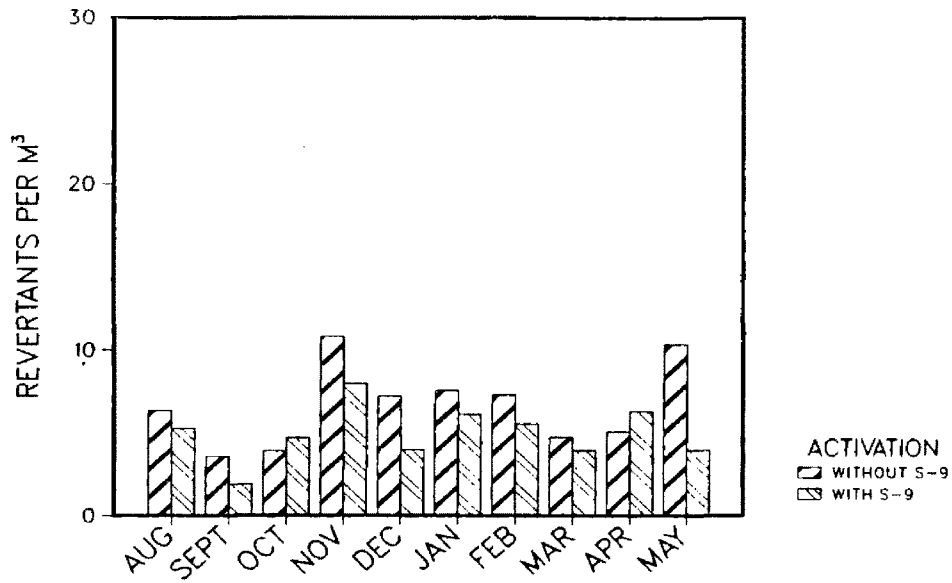
Figure 6a
Mutagenicity by month at Malfunction Junction.

MUTAGENICITY BY MONTH AT BOYD PARK
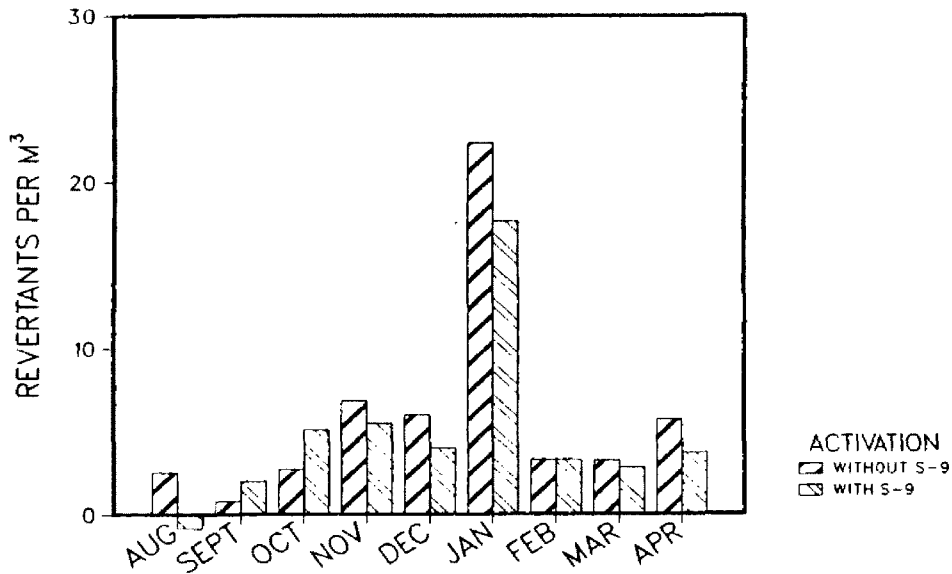
Figure 6b
Mutagenicity by month at Boyd Park.

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Tables 8a and 8b shows the relative and absolute frequencies of revertants grouped into approximately one, two, and three times the background. At Malfunction Junction, the majority of the samples measured twice the background. At Boyd Park, the majority of the activated samples measured less than twice the background and the majority of nonactivated samples measured more than twice the background.

Tables 8a and 8b

Revertants per plate (with and without activation) stratified into groups for Malfunction Junction and Boyd Park data.

Malfunction Junction

Number of Revertants	N^3	TA98[1] Rel. Freq.[4]	N	TA98S[2] Rel. Freq.
0-19	0	0.0	0	0.0
20-39	3	42.9	6	85.7
40-HI	4	57.1	1	14.3

Boyd Park

0-19	1	12.5	4	50.0
20-39	5	62.5	2	25.0
40-HI	2	25.0	2	25.0

[1] TA98 revertants, without activation
[2] TA98 revertants, with activation
[3] number of samples
[4] relative frequency

At Malfunction Junction, mutagenicity of nonactivated samples was higher than mutagenicity of activated samples (p = 0.002). At Boyd Park, mutagenicity of nonactivated samples was higher than mutagenicity of activated samples (p = 0.004) (Table 9).

Table 9

T-Test: Comparing activated to nonactivated samples at Boyd Park and at Malfunction Junction for various time periods.

Malfunction Junction

Variable	Mean	S.D.[1]	T.	T Prob.	N[2]
TA98M3[3]	6.64	2.11			
			3.46	0.003	19
TA98SM3[4]	4.97	1.76			

Boyd Park

TA98M3	6.88	6.24			
			3.04	0.007	19
TA98SM3	5.31	5.09			

[1] standard deviation
[2] number of samples
[3] TA98 revertants per m^3, without activation
[4] TA98 revertants per m^3, with activation

At Malfunction Junction, a paired t-test showed mean mutagenicity of the samples representing the monthly TSP high were not different than those samples representing the monthly TSP average (p = 0.98, TA98M3, p = 0.95, TA98SM3, at Malfunction Junction; p = 0.54, TA98M3, p = 0.69, TA98SM3, at Boyd Park) (Tables 10a and 10b).

Paired t-tests were conducted to compare mutagenicity at Boyd and Malfunction Junction. For all samples, for high samples only, and for average samples only, mean mutagenicity between the two sites did not differ (p = 0.87, TA98M3, p = 0.78, TA98SM3 for all samples; p = 0.61,

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TA98M3, p = 0.68, TA98SM3 for high samples; p = 0.75, TA98M3 and p = 0.93, TA98SM3 for average samples.).

Tables 10a and 10b

T-Test: Comparing the mutagenicity with and without activation between monthly high TSP and monthly average TSP samples at Malfunction Junction and Boyd Park.

Malfunction Junction

Variable	TSP[1]	mean	S.D.[2]	T	T-prob.	N[3]
TA98M3[4]	High	6.62	1.73			9
				-0.03	0.98	
	Average	6.65	2.49			10
TA98SM3[5]	High	4.99	1.97			9
				0.06	0.95	
	Average	4.94	1.67			10

Boyd Park

TA98M3	High	7.74	6.28			10
				0.62	0.54	
	Average	5.93	6.45			9
TA98SM3	High	5.77	5.26			10
				0.41	0.69	
	Average	4.79	5.15			9

[1] Total suspended particulate, ug/m^3
[2] standard deviation
[3] number of samples
[4] TA98 revertants per m^3, without activation
[5] TA98 revertants per m^3, with activation

At Malfunction Junction, no correlation existed between TSP and mutagenicity (p = 0.17, TA98M3; p = 0.18, TA98SM3). At Boyd Park however, a correlation did exist between TSP and mutagenicity--r^2 =

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0.28 for TA98M3 (p = 0.01) and r^2 = 0.21 for TA98SM3 (p = 0.02). An

increase in TSP is associated with an increase in mutagenicity at Boyd

Park, but not at Malfunction Junction.

Mutagenicity of Tenax Column Extracts

This test served as a preliminary investigation of the

mutagenicity of compounds collected by Tenax columns at Rose Park.

Table 11 shows the results of assays on Tenax samples. For

comparative purposes, results of the assay on high-volume samples

taken over the same time period are included in the table.

As shown in Table 11 , all the Tenax samples tested nonmutagenic,

with at most seven colonies above the background. No clear

distinctions existed in mutagenicity among the different samples and

between metabolically activated and nonactivated samples.

Table 11

Results of Ames Assay test on Tenax column extracts.

Sampling Date	TSP[1]	RSP[2]	Airflow, ml/min	Tenax[3] TA98[5]	TA98S[6]	Particulate[4] TA98	TA98S
1/19/83[7]	218.5	77	205	-3	-7	41*	41*
1/19/83[7]	218.5	77	205	6	-5	41*	41*
2/9/83	97.6	61	207	1	5	41*	41*
2/16/83	118.0	39	106	-2	-5	34*	38*
2/23/83	133.8	55	114	-2	5	37*	34*
3/9/83	111.2	--	114	2	-1	29*	21
3/23/83	39.5	--	100	0	-1	10	10

[1] total suspended particulate, ug/m^3
[2] respirable particulate, ug/m^3
[3] Tenax Column Extracts
[4] high-volume filter extracts
[5] TA98 revertants per plate, without activation
[6] TA98 revertants per plate, with activation
[7] molecular seive extract
*sample tested mutagenic

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Health Assessment

The highest monthly mutagenicity level (22 revertants per m^3) occurred in January at Boyd Park. Inhaling this level of mutagens year-round is roughly equivalent to smoking eight packs of cigarettes per year (Eqs. 1-3). Since this is 2% of the number of cigarettes inhaled by one-pack-a-day smoker per year, breathing the highest level of mutagenicity observed in this study year-round increases a nonsmoker's risk of developing lung cancer by 1.26 times. For the most-probable scenario, using monthly averages for revertants per m^3 at Rose Park, this relative risk reduces to 1.04 (Eqs. 4-5).

Worst-Case Scenario:

1) Revertants/m^3-year

   22 revertants/m^3-day X 365 days = 8030 revertants/m^3-year

2) Equivalent packs per year smoked

$$\frac{8030 \text{ revertants/}m^3\text{- year}}{50 \text{ revertants/}m^3\text{-cigarette}*} = 160 \text{ cigarettes/year}$$

$$= 8.03 \text{ packs/year}$$

3) Risk from 8 packs a year compared to one-pack-a-day smoker[8]

$$\frac{8.03 \text{ packs/year}}{365 \text{ packs/year}} \quad \frac{x}{13}$$

   x = 0.26; Relative risk = 1.26

Most-Probable Scenario

4) $\frac{1219.24 \text{ revertants/}m^3\text{-year}}{50 \text{ revertants/}m^3\text{-cigarette}} = 24.38 \text{ cigarettes/year}$

$$= 1.22 \text{ packs/year}$$

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5) Risk from 1.22 packs a year compared to one-pack-a-day smoker

$$\frac{1.22}{365} \qquad \frac{x}{13}$$

x = 0.04; Relative risk = 1.04

*from Lofroth et al., 1983

Comparison with 1979-80 MAPS data

Table 12 compares the monthly averages of revertants per m^3 for MAPS and this study. For every month, the 1979-80 mutagenicity exceeded (up to seven times) the 1982-83 mutagenicity.

Table 12

Comparison of monthly mutagenicity between 1979-80 and 1982-83 samples.

Month	1979-80[1] mutagenicity[2]	1982-83 mutagencity
April	8.93	5.01
May	15.47	2.09
June	6.85	----
July	----	----
August	----	----
September	9.23	1.89
October	16.52	3.60
November	17.23	5.32
December	13.99	5.72
January	13.02	5.80
February	10.41	6.36
March	9.19	3.00

[1] Warren, 1981
[2] TA98 revertants per m^3

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CHAPTER VI

DISCUSSION

This chapter discusses the trends in mutagenicity observed at the three sampling sites in terms of two study objectives. Then the results from assays on Tenax column extracts and the health assessment are interpreted. And finally, the results of comparing the 1979-80 MAPS mutagenesis study (Warren, 1981) with this study are discussed.

Ambient Air Trends

Two objectives were to 1) create an index of mutagenicity based on the relationship between mutagenicity, weather, and particulate data; and 2) help determine the sources of mutagens in Missoula's air.

Part 1: Index of Mutagenicity: In general, samples taken on cold, calm days of inversions exhibited the highest mutagenicity. Statistical analyses showed that mutagenicity had a highly significant negative correlation with temperature (r = -0.61, TA98M3, $p <$ 0.001, r = -0.64, TA98SM3, $p <$ 0.001). Except for temperature, none of the weather variables correlated highly with mutagenicity (Table 4).[9] Moller and Alfheim (1980) found that mutagenicity increased with increasing precipitation, but this Missoula study indicates no correlation between mutagenicity and precipitation. Samples taken on days with wet and frozen road/soil conditions exhibited higher mutagenicity than those taken on days of dry road/soil conditions. However, since the dry roads occurred primarily during the warm sampling months, temperature differences, not precipitation, may

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explain this.

Differences in temperature may also explain why mutagenicity was highest on days of easterly wind directions. Air masses from the east probably represent the cold air drainages from Hellgate Canyon. On sample days when the average wind direction was east and northeast, mutagenicity was highest and temperatures were lowest, thus supporting this hypothesis.

In a controlled experiment, a researcher seeks correlations of 0.8 to 0.99 to demonstrate a strong relationship between two variables. But in a field experiment with many uncontrolled variables, such as in air pollution studies, such high correlations are not usually observed (Arkley and Glauser, 1980; Strojan and Turner, 1978; Phillips et al., 1977a and 1977b, Thompson, 1981). Factors that could have affected this study's results include instrument operation, weather, particulate constituents, and site location. In light of the many uncontrolled factors in this study, the correlations of mutagenicity with TSP ($r = 0.61$, TA98M3; $r = 0.52$, TA98SM3) and with RSP ($r = 0.56$, TA98M3 and $r = 0.47$, TA98SM3) seem reasonable and indicate a strong positive relationship between mutagenicity and particulate concentrations.

Since TSP can consist of a variety of materials such as soil particles, pollen and plant fibers, soot, dust, PAHs, etc., the correlations of 0.61 and 0.52 for nonactivated and activated samples, respectively, were even higher was expected. However, given the literature finding the highest mutagenicity with the smallest particles (Talcott and Harger, 1980; Preidecker, 1980; Commoner et

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al., 1978; Tokiwa et al., 1980; Pitts et al., 1977; Chrisp and Fischer, 1980; Lofroth, 1981a), a higher correlation between mutagenicity and RSP than observed than expected. Perhaps this results follows from using high-volume samplers to collect samples for assays, but using the GCA to calculate RSP. The respirable particulate actually collected could have been very different from the respirable particulate detected by the GCA. RSP can also consist of a variety of materials. The sanding material used on icy streets during the wintertime can measure in the respirable range (Hedstrom, 1984). On several days during the sampling winter sanding material accounted for most of the RSP. Additionally, the GCA used by the Missoula City-County Health Department often malfunctions, measuring higher RSP than TSP levels and negative particulate concentrations, resulting in incorrect records.

Finally, masking of smaller mutagenic particles by large nonmutagenic particles particles might have occurred. Comparing individual data neither confirms nor precludes this occurrence of masking.[10] One interesting observation was results from assaying a 13.5 hour sample at Malfunction Junction. This sample, taken on 12/11/82, had the highest mutagenicity of all the samples assayed--81 nonactivated TA98 revertants and 118 activated TA98 revertants. Other 24-hour samples containing greater amounts of total and respirable particulate did not exhibit higher mutagenicity than this sample, including a sample taken on that day at Rose Park. This observation appears consistent with the observation of Pitts et al. (1982) that 3-hour samples had higher mutagenicity than corresponding 24-hour

samples and indicates the possibility of masking. To determine whether this sample was higher than the 24-hour samples because of masking, an experiment designed like that of Pitts et al. (1982) would be needed.

Using only those sampling dates with RSP data, air quality and weather data were both entered into variance, covariance, and regression analyses with hopes of explaining greater variability in mutagenicity. Analysis of variance and covariance showed RSP and temperature explained similar variation in mutagenicity.[11] The best equations used RSP to predict mutagenicity.

A satisfactory index of mutagenicity was not developed because the coefficients of determination for the best predictive equations, only explained 31 and 22% of the variability in mutagenicity of nonactivated and activated samples, respectively. It appears that the constituents of particulate matter, not necessarily the quantity of particulate matter greatly influence the mutagenicity of ambient air samples. It can be stated that mutagenicity was greatest on cold, windless days having temperature inversions and high RSP.

Part 2) Sources of mutagenicity: Since air quality in Missoula deteriorates during wintertime, an increase in the mutagenicity of air samples was expected. The highly significant correlation between ambient temperature and mutagenicity, the greater percentage of winter vs. other months having more than twice the revertants per plate than the background, and the higher mean mutagenicity of winter than summer months observed at Rose Park demonstrate this.

There are three possible explanations for this. First,

temperature inversions common in winter may trap and concentrate air pollutants which would otherwise escape the airshed. This could cause the apparent seasonal problem even if the same air pollutants are present year-round. Second, a source of emissions associated with wintertime, such as RWDs, could cause the increase. And finally, the increase in mutagenicity could result from a combination of the above two factors.

Using all the data, the correlation between mutagenicity and temperature was the highest of any correlation with mutagenicity observed in this study. The observations of higher mutagenicity on days with wet/frozen road/soil conditions than on days with dry road/soil conditions and of greater mutagenicity associated with easterly winds seem to demonstrate the effect of temperature on mutagenicity. What appears to be happening is not only do wintertime inversions trap pollutants, but also greater condensation of mutagens into particle form and/or adsorption onto particulate matter occurs at cold temperatures.

But are the higher levels in wintertime due to weather phenomena alone or does data indicate an additional source of mutagenicity?

Results of sampling at Malfunction Junction indicate a source of mutagenicity not specifically associated with a wintertime increase in mutagenicity. Unlike samples at Rose Park and Boyd Park, samples from Malfunction Junction tested mutagenic year-round, suggesting a year-round source unique to that site. In addition, the mutagenicity at Malfunction Junction exceeded levels from the two other sites. Since the heaviest concentration of traffic in Missoula occurs at

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Malfunction Junction and remains relatively the same all year (Kress, 1983), automobile traffic could explain these observations. Finally, the nonsignificant correlation between mutagenicity and TSP could reflect the large amount of suspended road dust caused by the 44,000 motor vehicles that pass through the intersection daily. Added up, these observations implicate motor vehicles as the likeliest major source of mutagenic particulate at Malfunction Junction.

The 1980 source apportionment study found 75% of wintertime RSP load attributable to RWDs (Missoula City-County Health Department, 1981). Since RWDs emit mutagens and are a wintertime source of particulate matter that tested mutagenic, they seem the likeliest source of wintertime mutagenicity observed in this study. However, RSP only explained 30% of the variation in mutagenicity; thus it cannot be inferred that since RWD's are the major source of wintertime particulates they are also a major source of wintertime mutagencity. In addition, the source apportionment data is from 1979-80, not 1982-83, when sampling for this study occurred.

Since monitoring began at Boyd Park one year ago, it has been observed that the wintertime TSP levels at Boyd Park usually exceed the wintertime levels at the other monitoring sites. It is suspected that the higher levels are due to the greater input of particulate from RWDs near Boyd Park. If this is in fact the case, a dramatic increase in winter mutagen levels at Boyd Park relative to the other sites would tend to indicate that RWDs are emitting the mutagenic particulate.

A dramatic increase in mutagenicity was observed only for the Boyd

Park January sample. On this day, 1/4/83, falling snow removed all the coarser airborne particles but not the respirable particles. Comparing results from this day between Rose Park and Boyd Park suggests an additional source of mutagenicity at Boyd Park over Rose Park:

Boyd Park: TSP = 194; TA98M3 = 22, TA98SM3 = 18

Rose Park: TSP = 117; TA98M3 = 5; TA98SM3 = 7

During the same sampling period, the particulate on the Boyd Park filter was 77 ug/m^3 greater, the mutagenicity without activation 4.4 times greater, and the mutagenicity with activation 2.6 times greater than on the Rose Park filter. However, these results come from one sampling day and seem anomalous for mutagenicity data on the other days when both Boyd and Rose Park were sampled do not show this great a difference.

The effect of activation on the samples and changes in that effect can indicate sources of mutagenicity. For example, Wang et al. (1978) observed that the ambient air samples from residential urban areas of Buffalo, N.Y. exhibited higher mutagenicity without activation than with activation. They suspected automobile exhaust as the likely source of direct mutagenicity and precluded many mutagenic compounds that require activation to be mutagenic as constituents of the air samples.

The observation that mutagenicity of nonactivated samples exceeded the mutagenicity of activated samples at all sampling sites suggests an input of pollutants from sources having emissions that exhibit higher mutagenicity without activation than with activation. By

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comparison with the study of Wang et al. (1978), the fact that the Malfunction Junction samples exhibited the highest mutagenicity without activation during all sampling months indicates the likely contribution of mutagens from transportation sources at that site.

However, interpretations of these results is difficult because a review of the literature reveals no clear difference in the type of mutagenicity exhibited by the two suspected sources of mutagenicity--motor vehicles and wood stoves. An additionally complicating factor is that while diesel engines exhibit decreased mutagenicity with S-9 activation, gasoline engines do not (Lofroth, 1981a; Claxton, 1980; Lewtas, 1983). Further, these three investigators found that the gasoline engine emissions have higher mutagenicity with activation, which is in direct contrast to Wang et al. (1978) who found the emissions have higher mutagenicity without activation. Obviously, interpretations may differ, but given that the Malfunction Junction site is probably most affected by transportation-related exhaust, the study's findings that particulates from this site for all months exhibit greatest mutagencity without activation would seem to support the findings of Wang et al. (1978).

Concerning this variability in the activation effect, Lofroth (1981a) commented that the concentration of direct mutagens can influence the detection of indirect mutagens in the Ames Assay. If it is true that mutagenicity of emissions from RWDs increase upon activation with S-9 as observed by Lewtas (1981), Dasch(1983) and Lofroth (1978), it is possible that the presence of a direct mutagen (i.e., auto exhaust) masked the presence of an indirect mutagen.

To further complicate interpretation, Rudling et al. (1981) found

S-9 sometimes enhanced and sometimes decreased the mutagenicity of RWD

emissions (Table 6A), offering the following discussion concerning

their studies:

> The relative mutagenic response in the absence and presence of
> mammalian activation systems depends on several factors such as
> the level of compounds which require mammalian activation, the
> level of compounds which do not require this activation and the
> behavior of these compounds, i.e., extent of deactivation, in the
> presence of the activation system. A high level of mutagenic
> response in the absence of mammalian activation caused by
> compounds which are inactivated by the mammalian system can
> obviously conceal a substantial level of compounds requiring
> mammalian activation.

The same explanation can be offered for the results here: the various

combinations of compounds in the ambient air samples can influence the

effect of mammalian activation.

Another possible explanation of the greater direct mutagenicity

than indirect mutagenicity is the likelihood of chemical reactions

occurring on the sampling filters and resulting filter artifacts

(Pitts et al., 1978). In assaying samples of Los Angeles air, Pitts

found the the promutagen BaP reacted with O_3 and PAN to form direct

mutagens. It seems unlikely that this would occur in Missoula where

concentrations of ozone and nitrogen oxides fall well below federal

and MT standards and measure considerably lower than Los Angeles

levels (Gelhaus, 1981). However, it is not known which chemical

concentrations are the rate limiting for these reactions. If only

small concentrations of O_3 and PAN are needed to react with BaP, then

filter artifacts could have occurred.

In sum, while the Rose Park data indicated a trend of increased

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mutagenicity in the wintertime, it cannot be determined from this study whether this increase was due either to an additional source of mutagens in the wintertime, or to inversions, or to both. Previous studies showed that RWD emissions contain mutagens and test mutagenic in the Ames Assay and that RWDs are the major source of wintertime RSP (See Literature Review). Therefore, it seems likely that RWDs are a major source of wintertime particulate in residential areas such as Rose Park and Boyd Park. But results of this study offer no proof that RWDs are a major source of mutagenic particulate at any of the sites. In contrast, results of sampling at Malfunction Junction indicate that motor vehicles contribute to mutagenic particulate at the site.

Mutagenicity of Tenax Column Extracts

Ames Assay results indicate that the seven Tenax samples did not contain mutagens, even on days when the corresponding particulate samples tested mutagenic. These results concurred wih Beihoffer's chemical analysis that revealed no carcinogens or mutagens present at significant levels in the duplicate samples or in any of his samples (Beihoffer, unpublished). In contrast, on many of these sampling dates, the corresponding high-volume samples tested mutagenic. It appears that mutagens in Missoula's air exist primarily in the particulate, not gaseous form.[12]

Assuming that PAHs cause the mutagenicity of the particulate matter, these findings concur with the findings of Cautreels and Van Cauwenberghe (1978). They found that low molecular weight compounds exist primarily in the gaseous state, but high molecular weight

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compounds, which include most of the carcinogenic PAHs, are associated with particulate matter.

Health Assessment

The most-possible estimate of the relative risk of developing lung cancer from breathing Missoula's of 1.04 is consistent with the low estimates of 1.02 and 1.01 generated by Drs. Koostra and Johnson, respectively (Missoula City-County Health Department, 1981), with the low mutagenicity levels obtained during the sampling period, and with a number of epidemiological studies investigating air pollution as a risk factor in lung cancer (Vena, 1982; Speizer, 1982; Eisenbud, 1978). These studies showed that, after controlling for smoking and occupation, air pollution increased the risk factor minimally.

The worst-case estimate of 1.26 indicates a significant increase in the risk of developing lung cancer from breathing mutagenic particulate. This is probably is an overestimate. It assumes a year-round mutagenicity level of 22 revertants/m^3, the highest monthly mutagen level obtained in this study, which is an unlikely assumption given the much lower levels during the other sampling months. In addition, this high level was observed on a day when the CCHD noted that falling snow removed all the coarse particles, with the TSP consisting almost entirely of RSP, an unusual occurrence in Missoula.

In contrast, the monthly levels used to predict the most-probable estimate appear more representative of the average mutagenicity levels on a year-round basis. As shown in Table 13, the monthly TSP averages and the TSP averages for the sampling days are similar. Even so, the most-probable health risk scenario probably underestimates the health

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risk because the samples were extracted from high-volume sampler filters on which masking of the mutagenicity of RSP by TSP may have occurred. Data analyses support the possibility that masking occurred. Had only the RSP been assayed, then the estimates would be more accurate, as studies on the mutagenicity of ambient air samples show mutagenicity predominates in the small, not large particles.

TABLE 13

Comparing monthly TSP averages at Rose Park for sampling dates and all dates.

Month	TSP[1] ug/m^3	
	All Dates	Sampling Dates
August '82	51.3	51.7
September '82	40.6	44.8
October '82	57.3	52.1
November '82	91.1	101.8
December '82	91.2	52.8
January '82	183.9	186.2
February '83	115.3	118.3
March '83	58.4	58.7
April '83	49.0	60.8
May '83	43.1	52.4

[1] total suspended particulate, ug/m^3

Several provisos in the evaluation of these findings are in order. First, this method assumes the constituents of Missoula's air and cigarette smoke are the same. While cigarette and wood smoke contain similar carcinogens/mutagens, it was not established that the particulate consisted primarily of wood combustion particles. The eventual results from the 1982-83 source apportionment study conducted by the Missoula City-County Health Department and the U.S. Environmental Protection Agency should show whether the major constituents of the particulate are characteristic of wood smoke.

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Second, the value of 50 revertants/m^3 per cigarette was obtained from sampling of sidestream cigarette smoke, but the figure used to calculate the risk comes from epidemiological data on smokers. The constituents of sidestream smoke differ from smoke inhaled by a smoker, and sidestream smoke can be more harmful (Stock, 1980). Third, this risk assessment involved extrapolating results from a bacterial mutagenesis assay to human carcinogenesis. This assumes the somatic mutation theory of cancer and that the relative potency of carcinogens in the Ames Assay to bacteria is directly proportional to the relative potency in humans. These assumptions are heavily criticized.

Provisos aside, the best estimate of the relative risk of lung cancer from breathing mutagenic substances in Missoula'a air lies between 1.04 and 1.26. This estimate would be higher for high-risk groups such as children, smokers, pregnant women, and those suffering from respiratory ailments. Additionally, this assessment addresses only the risk associated with mutagenicity and in no way considers the other risks of inhaling the fine respirable particulates present in Missoula's air. While the risk from mutagenic particulate is low compared to that from smoking, cigarette smokers make a conscious choice to smoke. All Missoulians must breathe the air.

Comparison with 1979-80 MAPS Data

Several plausible explanations exist for the observation of higher monthly mutagenicity of Missoula's air in 1979-80 than in 1982-83. First, the 1982-83 winter was milder than the winter of 1979-80. The 1979-80 winter was colder than normal, with 39 greater heating degree

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days; 1982-83 winter was warmer than normal, with 358 fewer heating degree days. Second, with the exception of one month, TSP levels for the 1979-80 samples exceeded TSP levels for the 1982-83 samples. Third, the 1979-80 samples came from Lions Park, whereas the 1982-83 samples came from Rose Park. The Lions Park monitoring station, situated at the intersection of Bancroft and Kent, was improperly sited too close to the road to fulfill EPA siting criteria. As shown in this study, mutagenicity varies at different sampling sites. It is therefore possible that the Lions Park monitor received a greater input of particulate from transporation sources which might have increased mutagenicity.

CHAPTER VII

CONCLUSIONS

In summary, analysis of mutagenicity of year-round samples of Missoula's ambient air using the Ames Assay has yielded the following observations:

1) Particulate matter collected by high-volume samplers tested mutagenic. Mutagenicity was greatest on cold, windless days with temperature inversions and high RSP.

2) Mutagenicity had a highly significant negative correlation with temperature ($r = -0.61$, p 0.001, TA98M3; $r = -0.64$, $p < 0.001$, TA98SM3) and a highly significant positive correlation with TSP ($r = 0.61$, $p < 0.001$, TA98M3; $r = 0.52$, $p < 0.001$, TA98SM3) and with RSP ($r = 0.56$, $p < 0.001$, TA98M3; $r = 0.47$, $p < 0.001$, TA98SM3).

3) RSP was the best predictor of mutagenicity, but only explained 30% of the variation in mutagenicity. Therefore, an index of mutagenicity was not determined. It appears that the constituents of particulate matter, not necessarily the quantity of particulate matter greatly influence the mutagenicity of ambient air samples.

4) To assay ambient air samples for mutagenicity, the samples should be collected by dichotomous samplers, not by high-volume samplers. This would eliminate the problem of masking of mutagenicity that seemed to occur with some of the samples.

5) While previous studies suggest RWDs as the likely major source of a wintertime increase in mutagenicity observed with the Rose Park

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data, this hypothesis cannot be proved or disproved with the results of this study. However, study results do implicate motor vehicles as a major source of mutagenic particulate at Malfunction Junction where mutagenicity was highest on a year-round basis.

6) From assaying Tenax columns, it appears that mutagens in Missoula's air exist primarily in the particulate, not gaseous state.

7) Breathing mutagenic substances in Missoula's air increases the risk of developing lung cancer between 1.04 (most-probable) and 1.26 (worst-case) times.

7) Mutagenicity of the 1982-83 sampling period was higher than that of the 1979-80 sampling period, corresponding with the observation that the 1982-83 winter was milder and of better air quality than the 1979-80 winter.

CHAPTER VIII

RECOMMENDATIONS FOR FURTHER STUDY

1) Between September 1982 and September 1983 the CCHD conducted an ambient air and source sampling program to perform a source apportionment analysis of the particulate contribution of sources in the Missoula airshed. To complete this analysis, Enviromental Protection Agency and CCHD researchers used two methods: optical microscopy and x-ray fluorescence in conjunction with chemical mass balance. To compare mutagenicity and source apportionment data, high-volume samples collected at Rose Park on the same days as the source apportionment study during the months of December, January, and February (every three days) were assayed. Unfortunately, results of this effort were not available for consideration here. When the study is completed, the relationship between mutagenicity and sources of particulate can be explored to determine which sources, if any, are associated with high mutagenicity. Also, the methodology of Johnson (Missoula City-County Health Department, 1981) can be used to assess the health risk from carcinogens in the air.

2) For the health risk assessment, a value from the literature on mutagenicity of cigarette smoke was used. A more accurate comparison could be made by employing this study's methodology to sample cigarette smoke.

3) Assaying from high-volume samplers has several drawbacks that may have caused an underestimation of mutagenicity of particulate samples and consequently the health risk estimate in this study.

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Ideally, only respirable particulate should be sampled. This would necessitate setting up dichotomous samplers at the monitoring stations. To prevent volatilization the dichotomous samplers should be run with unheated heads for a short time and assayed immediately. Dr. Warren has successfully used sonication to extract particulate matter from the dichotomous filters. She has observed higher mutagenicity from the dichotomous filters than corresponding high-volume filters (Warren, 1983).

4) Assaying during different times of the day like that of Pitts et al. (1978) will provide information concerning major sources of mutagenicity. For example, is mutagenicity highest during rush hour periods or just after?

5) The observation that the 13.5 hour sample exhibited the highest mutagenicity of all samples warrants further investigation. Does assaying 24-hour instead of 12-hour samples lead to an underestimation of the mutagenicity of the air? This investigation should be coordinated with suggestion number four.

6) The constituents of particulate matter, their concentration, and their biological activity need to be known to accurately assess a health risk. While the source apportionment data might provide some of this information, ideally the complex air samples should be separated into fractions and then assayed for mutagenicity. Various methodologies could be employed to detect the mutagenicity of fractionated samples of ambient air (Kolber et al., 1981; Pellizzari et al., 1979; Tokiwa et al., 1980, Tokiwa et al., 1977; Teranishi et al., 1975, Teranishi et al, 1978; Hughes et al.

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1980).

FOOTNOTES

1) A mutation is defined by Webster's Ninth New Collegiate Dictionary as a relatively permanent change in hereditary material involving a biochemical change in the codons that make up genes. A mutagen increases the frequency or extent of mutation. Most mutagens are known carcinogens.

2) "Chemicals which can induce the first steps of malignant transformation are known as carcinogens or tumor initiators. Tumor promoters are compounds which continue the process of tumor formation when they are applied to tissue following initial treatment with a chemical carcinogen. Compounds known as co-carcinogens exert their effects when administered simultaneously with carcinogens or tumor initiators. Compounds which act as co-carcinogens do not necessarily have tumor-promoting properties." (U.S. Dept. of Health, Education, and Welfare, 1979).

3) A mortality ratio is "obtained by dividing the death rate for a classification of smokers by the death rate of a comparable group of nonsmokers" (U.S. Dept. of Health, Education, and Welfare, 1979).

4) It was attempted to compare the Ames test results on ambient samples and on BaP to generate a health risk assessment. The validity of this method rests on the assumption that BaP is a major constituent of Missoula's air. Recent improvements in quantitative analytical methods have shown BaP levels do not correlate well with the total PAH and carcinogenic PAH levels in ambient air (Lewtas, 1983). A method used by Flessel et al. (1981) was followed to determine the amount of mutagenic activity in March, 1980 that could be explained by the March, 1980 BaP levels reported by Dartmouth researchers (Missoula City-County Health Department, 1981):

$$\frac{2.74 \ ng/m^3 \ BaP}{252 \ ng/nmol \ BaP} \quad = \quad .01 \ nmol/m^3 \ BaP$$

$$0.01 \ nmol/m^3 \ BaP \quad X \ 121 \ revertants/nmol \ BaP*$$

$$= \ 1.21 \ revertants/m^3$$

*Flessel et al., 1981

$$\frac{1.21 \ revertants/m^3}{9.19 \ revertants/m^3 **} \quad = 13\%$$

**Data from Warren, 1981

Thus, the BaP levels measured in March 1980 could only account for 13% of the mutagenic activity observed for that month. Because of this low percentage and because BaP levels were not measured during this study, this methodology was not used.

5) The GCA recorded hourly RSP readings at Rose Park. Typically, the high-volume TSP and GCA TSP readings differed. Since the RSP collected on the high-volume sampler was not measured, it was approximated by the following method:

$$\frac{TSP \ (on \ high\text{-}volume \ filter)}{TSP \ (on \ GCA)} = \frac{RSP \ (on \ high\text{-}volume \ filter)}{RSP \ (on \ GCA)}$$

6) The City-County Health Department provided the meteorological data collected at Rose Park and used in comparison with Rose Park data. The National Weather Service collected the meteorological data at Johnson Bell field which was used with Boyd Park and Malfunction Junction data.

7) The mutagenicity of airborne samples is commonly expressed in terms of revertants per m^3 of air sampled. Revertants per m^3 was calculated based upon the number of revertant colonies per sample plate, with and without activation and the volume of air sampled:

$$\frac{Revertants \ per \ plate}{0.1 \ ml \ extract/plate} \ X \ \frac{3 \ ml \ extract}{6 \ in^2 \ area} \ X \ \frac{63 \ in^2 \ total \ area}{m^3 \ of \ air \ sampled}$$

$$equals \quad \frac{315 \ X \ revertants/plate}{m^3 \ of \ air \ sampled}$$

This method assumes 100% extraction of mutagens and no masking of mutagenicity.

8) This comparison assumes a simplistic linear relationship between the number of cigarettes smoked and the risk of cancer.

9) It was expected that mutagenicity would have a high negative correlation with inversion height, but this relationship cannot be analyzed because the Weather Service did not measure inversion height during the sampling period. They only calculate inversion heights during the burning season.

10) By comparing days of similar RSP but different TSP, masking can be investigated:
A) Rose Park
12/11: TSP = 163, RSP = 143, TA98M3 = 10.9, TA98SM3 = 9.5;
1/12 : TSP = 435, RSP = 147, TA98M3 = 13.4, TA98SM3 = 8.36

B) Boyd Park (RSP levels from GCA at Rose Park)

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1/4:   TSP = 194, RSP = 112; TA98M3 = 22.34, TA98SM3 = 17.60;
1/13: TSP = 366, RSP = 92, TA98M3 = 5.1 , TA98SM3 = 3.1

1/4:   TSP = 194, RSP = 112, TA98M3 = 22.34, TA98SM3 = 17.60;
11/26: TSP = 322, RSP = 110, TA98M3 = 6.76, TA98SM3 = 10.39

If masking occurred due to large TSP levels, then the sample with
the higher TSP should exhibit less mutagenicity than the sample
with the lower TSP. This occurred for the samples shown above at
Boyd Park but not at Rose Park. However, it cannot be determined
from these comparisons if differences are due to masking and or
due to the variety of constituents of particulate matter from day
to day.

11) Using the full data set, TSP and temperature had higher
    correlations than RSP had with mutagenicity (Table 4). However,
    all the sample days could not be used in regression analysis
    because RSP measurements were only taken during winter months.

12) This statement is qualified because the Tenax columns collected
    both particulate matter and gases, because of the sample number of
    Tenax samples assayed, and because Beihoffer's technique was not
    designed to detect PAHs.

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APPENDIX A

LITERATURE CITED TABLES

REVERTANTS PER M^3

MUTAGENICITY BY MONTH AT LIONS PARK
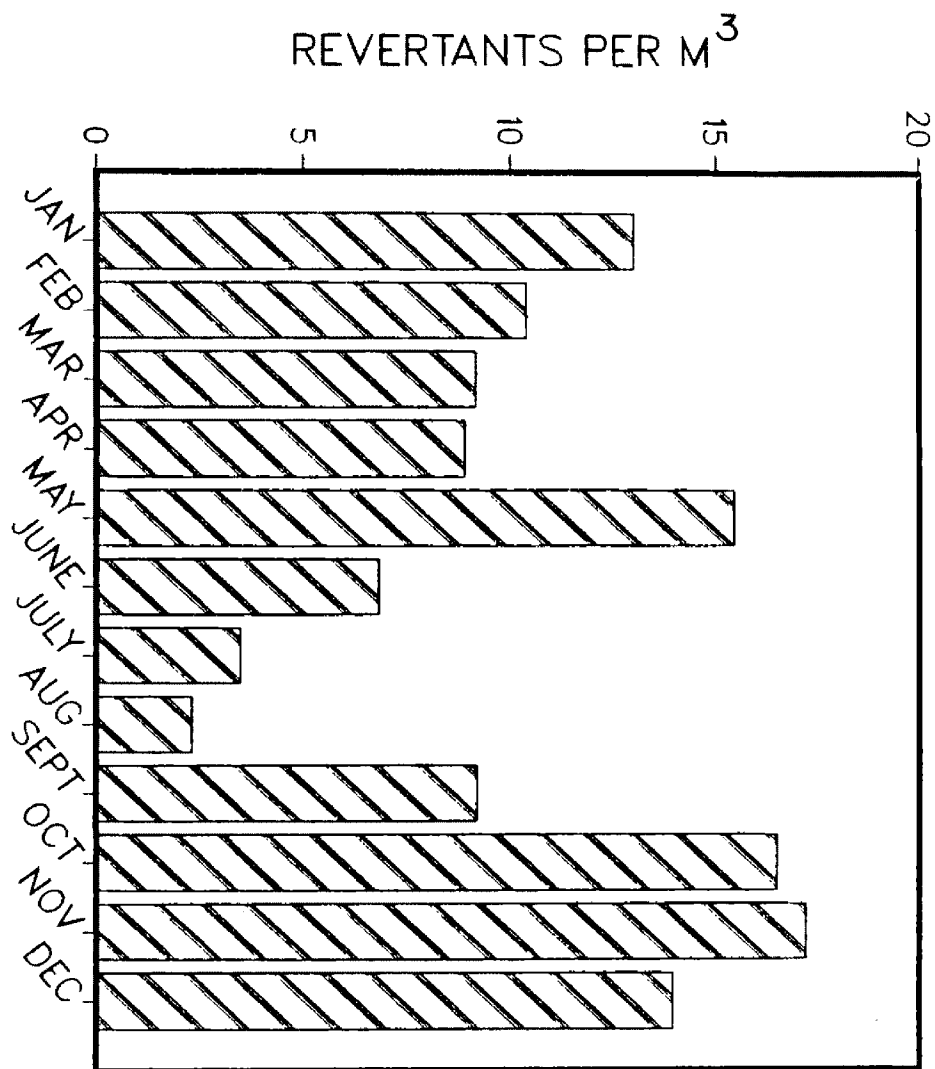
DATA FROM WARREN, 1981

Figure 1A

Monthly mutagenicity for 1979-80 MAPS study.

Table 1A

Averaged PAH concentrations at indicated locations (ng/m^3).
(Hornig et al. 1981, p. 509)

	Lyme Center mean (range)	Urban Hanover mean (range)	Moose Mountain New Hampshire mean (range)	Brigham Hill Vermont mean (range)
Phenanthrene	0.05 (0.01-0.90)	0.60 (0.04-2.01)	0.18 (0.14-0.22)	0.13 (0.04-0.18)
Anthracene	0.04 (0.00-0.60)	0.09 (0.00-0.26)	0.02 (0.02-0.02)	0.02 (0.01-0.04)
Fluoranthene	0.53 (0.00-2.61)	2.59 (0.43-6.93)	0.41 (0.28-0.56)	0.51 (0.20-1.04)
Pyrene	0.58 (0.00-2.70)	2.92 (0.85-9.77)	0.43 (0.34-0.51)	0.82 (0.29-1.66)
Triphenylene	0.11 (0.06-0.14)	0.56 (0.16-1.14)	0.17 (0.12-0.22)	0.15 (0.04-0.27)
Benz(a)anthracene	0.43 (0.14-1.43)	1.50 (0.26-2.76)	0.33 (0.24-0.39)	0.25 (0.05-0.51)
Chrysene	0.63 (0.26-2.07)	1.80 (0.48-2.94)	0.61 (0.38-0.83)	0.41 (0.10-0.65)
Benzo(e)pyrene	0.97 (0.53-2.18)	2.81 (1.16-5.07)	0.87 (0.73-1.01)	0.52 (0.15-0.96)
Benzo(b)fluoranthene	0.83 (0.21-2.91)	1.72 (0.48-2.53)	0.88 (0.45-0.79)	0.54 (0.12-0.97)
Benzo(a)pyrene	0.48 (0.18-1.23)	1.62 (0.40-2.97)	0.37 (0.30-0.44)	0.40 (0.08-0.73)
Dibenz(ah)anthracene[a]	1.95 (0.80-6.71)	4.59 (1.36-7.73)	1.71 (1.40-2.01)	1.28 (0.83-1.82)
Benzo(ghi)perylene	0.66 (0.20-2.06)	1.53 (0.42-2.32)	0.55 (0.42-0.68)	0.36 (0.05-0.58)
TSP (µg/m^3)	34.5 (11-100)	78.7 (41.9-115)	19.3 (16.1-22.5)	18.4 (10.5-25.2)
No. of Filters	12	13	2	5

[a]Data suspect due to probable interference.
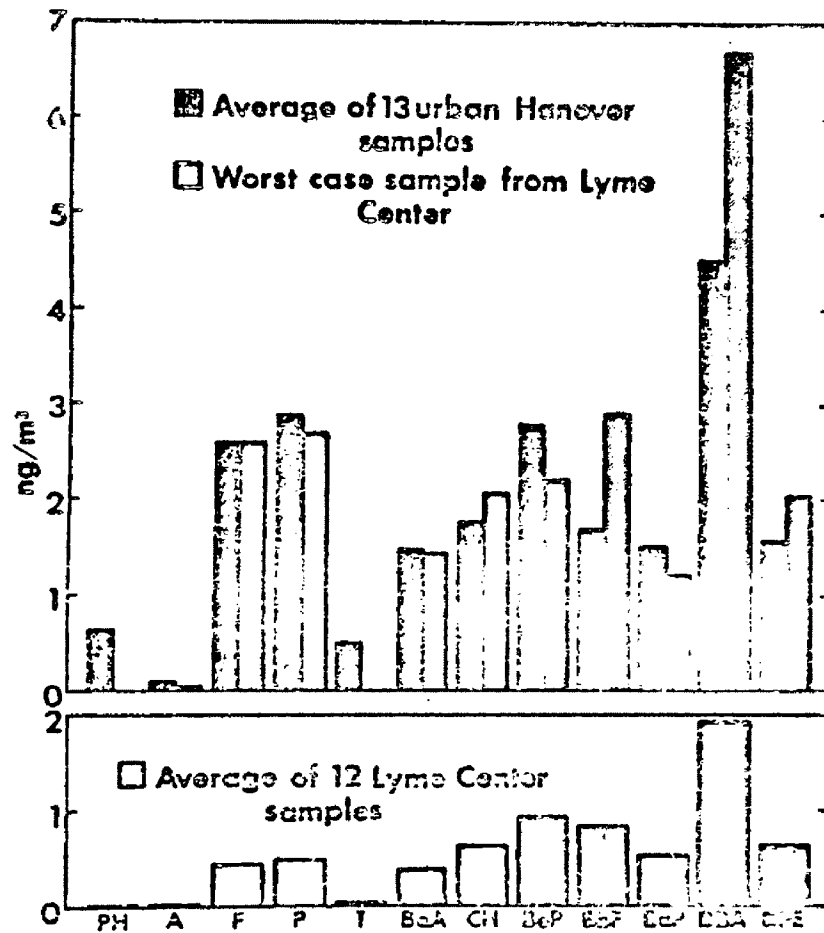
Figure 2A

COMPARISON OF THE AVERAGE LYME CENTER "FINGERPRINT"
WITH THE WORST CASE LYME CENTER SAMPLE AND WITH THE
AVERAGE URBAN HANOVER RESULTS

PH=phenanthrene, A=anthracene, F=fluoranthene, P=pyrene
T=triphenylene, BaA=benz(a)anthracene, CH=chrysene,
BeP=benzo(e)pyrene, BbF=benzo(b)fluoranthene, BaP=benzo(a)pyrene
DBA=dibenzo(ah)anthracene, BPE=benzo(ghi)perylene

(Hornig et al., 1981, p. 512)
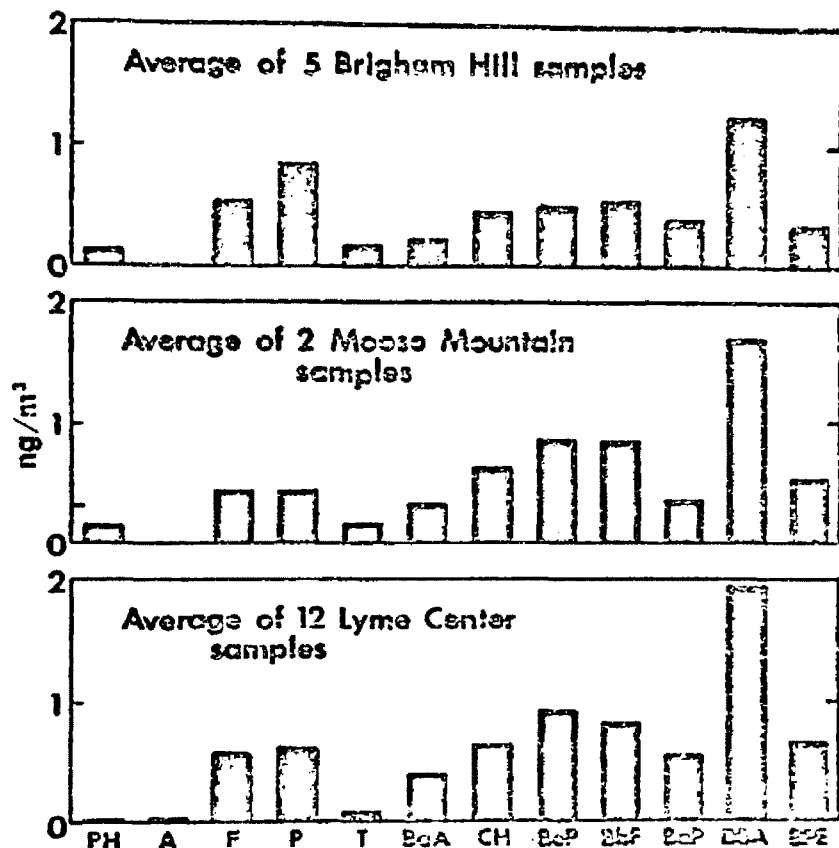
Figure 3A

COMPARISON OF AVERAGE LYME CENTER "FINGERPRINT" WITH
RURAL SAMPLES FROM MOOSE MOUNTAIN, NH, AND BRIGHAM
HILL, VT

PH=phenanthrene, A=anthracene, F=fluoranthene, P=pyrene
T=triphenylene, BaA=benzo(a)anthracene, CH=chrysene,
BeP=benzo(e)pyrene, BbF=benzo(b)fluoranthene, BaP=benzo(a)pyrene
DBA=dibenzo(ah)anthracene, BPE=benzo(ghi)perylene

(Hornig et al., 1981, p.513)

Summary of ambient semivolatile polynuclear aromatic hydrocarbons
concentrations (ng/m^3). (Imhoff et al., 1982, p.170)

Sample Date	Feb. 2	Feb. 6	Feb. 6	Feb. 8	Feb. 8	Feb. 8	Feb. 8	Feb. 14	Feb. 14
Sampling Location	Down	Bgnd	Mobile	Bgnd	Bgnd	Mobile	Mobile	Mobile	Mobile
Phase	Total	Total	Total	Part.	Gas	Part.	Gas	Part.	Gas
Compound									
Acenaphthene	2	ND	140	0.2	ND	9	320	ND	58
Acenaphthylene	18	52	35	15	22	19	75	14	27
Fluorene	6	1200	210	0.8	11	15	1200	ND	20
Phenanthrene	2	1800	2200	16	13	47	1900	12	15
Anthracene	ND	60	78	2	ND	34	86	ND	15
Fluoranthene	7	70	110	4	15	51	110	5	17
Pyrene	5	160	260	4	17	52	110	4	11
Benzo(a)anthracene	ND	7	80	4	ND	66	79	4	ND
Chrysene	2	27	65	5	18	76	64	5	18
Benzo(k)fluoranthene	ND	14	93	6	17	86	85	4	ND
Benzo(e)pyrene	10	160	210	7	9	85	240	2	25
Benzo(a)pyrene	7	7	39	5	29	68	45	3	ND
Perylene	5	97	140	6	19	79	170	0.7	ND
Indeno(1,2,3-cd)pyrene	ND	ND	53	ND	1	150	85	1	2
Benzo(g,h,i)perylene	ND	2	51	6	1	130	85	ND	ND
Coronene	ND	ND	ND	ND	ND	150	2	ND	ND

Down - primary van stationary at a site downwind from Petersville; Bgnd - second van stationary at site
outside of Petersville; Mobile - primary van driven through Petersville; ND - not detected.

Gas phase concentrations of volatile organic
species (ug/m^3). (Imhoff et al., 1982, p.171)

Date Sampled (February)	2	2	6	6	8	8	14	14
Tenax Sample Number	6B	5D	4B	11M	14B	13M	12B	18M
Benzene	22	24	24	34	11	9.4	3.0	17
Phenol	2.8	9.8	5.4	16	ND	1.1	ND	0.3
O-Chlorophenol	ND	ND	0.1	0.2	ND	ND	ND	ND
O-Cresol	ND	0.2	0.05	ND	ND	ND	ND	ND
M/p-Cresol	ND	ND	ND	ND	ND	ND	ND	ND
Xylenols	ND	0.15	ND	ND	ND	ND	ND	ND

B - background.
D - downwind.
M - mobile.
ND - not detected.

Table 4A

PAH emission rates from residential wood stoves from Battelle and
Monsanto studies and measured and modeled concentrations.
(Imhoff et al., 1982, p.180)

PAH Species	Average Measured (ng/m^3)	Battelle[a]		Monsanto[b]	
		Emission Factor (mg/kg)	Modeled Concentration (ng/m^3)	Emission Factor (mg/kg)	Modeled Concentration (ng/m^3)
Phenanthrene	1040	2.10	11.6	74.5	411
Anthracene	53	1.49	2.70		
Fluoranthene	98	0.99	5.46	18.0	99.3
Pyrene	146	0.75	4.14	-	-
Benzo(a)anthracene	76	1.40	7.72	-	-
Benzofluoranthenes	89	1.90	10.5	12.8	70.6
Benzo(e)pyrene	187	0.44	2.43	-	-
Benzo(a)pyrene	52	1.40	7.72	-	-
Benzo(g,h,i)pyrene	89	0.46	2.54	4.5	24.8
Coronene	51	0.11	0.61	-	-
Total PAH	3260	16.82	92.8	212	1,170

[a] Manning, et al., 1981

[b] DeAngelis, et al., 1980

Table 5A

Results of bioassays performed on SASS and
combustion residue samples.
(DeAngelis et al., 1980, p.64)

Sample code[a]	Combustion equipment	Wood type	Ames mutagenicity[b]	CHO clonal toxicity[c]
A-1 (1)	Fireplace	Seasoned oak	+	H
A-1 (2)	Fireplace	Seasoned oak	+	H
A-1 (3)	Fireplace	Seasoned oak	-	
A-2 (1)	Fireplace	Green oak	+	H
A-2 (2)	Fireplace	Green oak	+	H
A-2 (3)	Fireplace	Green oak		
A-3 (1)	Fireplace	Seasoned pine	+	H/M
A-3 (2)	Fireplace	Seasoned pine	+	H
A-3 (3)	Fireplace	Seasoned pine		
A-4 (1)	Fireplace	Green pine	+	H
A-4 (2)	Fireplace	Green pine	+	H
B-1 (1)	Baffled stove	Seasoned oak	+	M
B-1 (2)	Baffled stove	Seasoned oak	+	H
B-2 (1)	Baffled stove	Green oak	+	H
B-2 (2)	Baffled stove	Green oak	+	H
B-2 (3)	Baffled stove	Green oak	-	
B-3 (1)	Baffled stove	Seasoned pine	+	H
B-3 (2)	Baffled stove	Seasoned pine	+	H
B-3 (3)	Baffled stove	Seasoned pine	-	
B-4 (1)	Baffled stove	Green pine	+	H/M
B-4 (2)	Baffled stove	Green pine	+	H
C-1 (1)	Nonbaffled stove	Seasoned oak	+	H
C-1 (2)	Nonbaffled stove	Seasoned oak	+	H
C-2 (1)	Nonbaffled stove	Green oak	+	H
C-2 (2)	Nonbaffled stove	Green oak	+	H
C-2 (3)	Nonbaffled stove	Green oak		
C-3 (1)	Nonbaffled stove	Seasoned pine	+	H
C-3 (2)	Nonbaffled stove	Seasoned pine	+	H
C-3 (3)	Nonbaffled stove	Seasoned pine		
C-4 (1)	Nonbaffled stove	Green pine	+	H
C-4 (2)	Nonbaffled stove	Green pine	+	H
C-4 (3)	Nonbaffled stove	Green pine	-	

[a]Corresponds to the following sample fractions: (1) particulate
catch extract, supplied to LBI as methylene chloride solutions;
(2) combined organic module rinse and XAD-2 extract, supplied to
LBI as dimethylsulfoxide solutions; (3) combustion residue (bottom
ash), supplied to LBI as dry particulates.

[b]"+" designates mutagenic activity "-" designates no mutagenic
activity.
[c]Clonal toxicity measured by the reduction in colony forming
ability of cultured Chinese hamster (CHO-Kl cell line) cells after
24-hour exposure to the test material. ND, no detectable toxicity;
NT, not tested; L, low toxicity; M, moderate toxicity; H, high
toxicity.

-94-
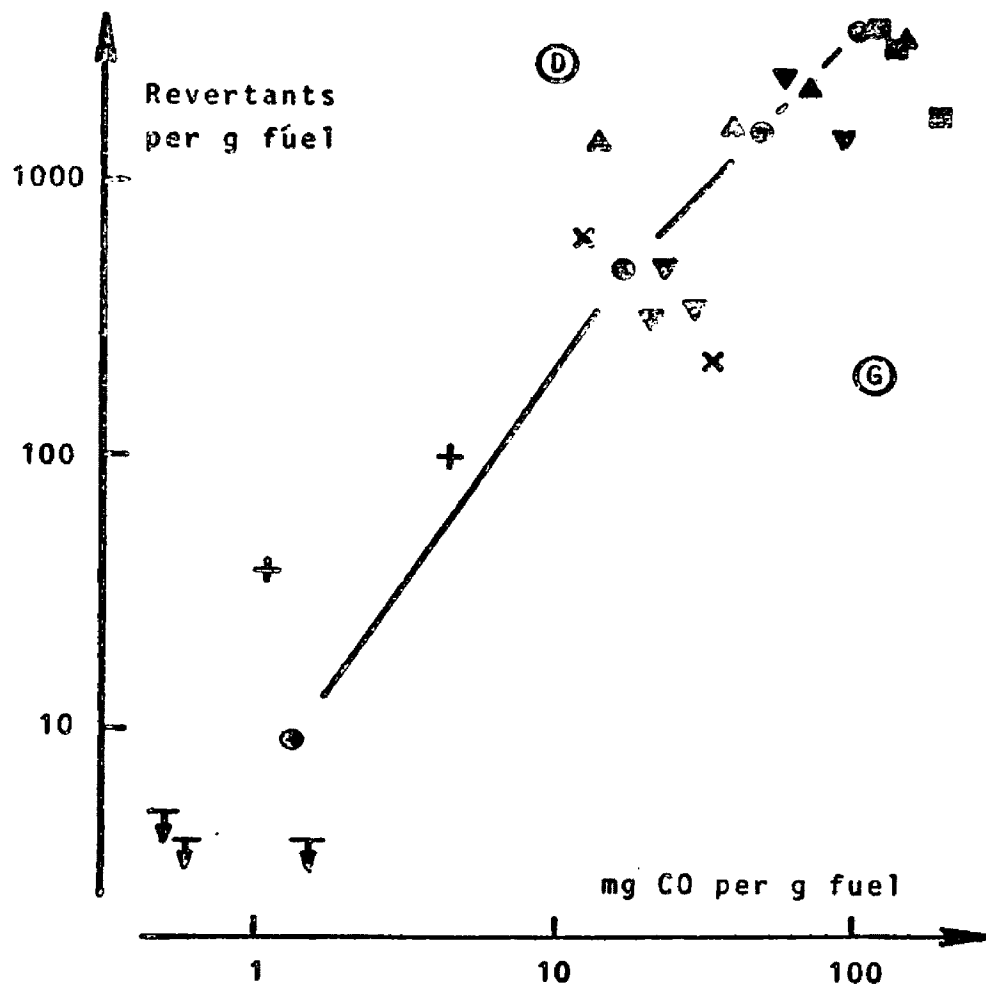
Figure 4A

Mutagenic response versus carbon monoxide emission.
(Rudling et al., 1981, p.48)

● — ● , wood-chips in the prechamber furnace, sample 1-4

■ , wood in the furnace, sample 5-7

▲ , wood in the modified fireplace, sample 11-14

▼ , wood in the airtight fireplace stove, sample 15-19

D and G , diesel and gasoline motor vehicles (ref. 8)

+ , wood-chips in a 30 MW industrial furnace (ref. 11)

✕ , wood-chips in a 3 MW industrial furnace (ref. 11)

⊤ , oil, coal and peat in >100 MW power plants (ref. 11)

Table 6A

Summary of combustion data and results. (Rudling et al., 1981, p. 43)

1. Sample no.	1	2	3	4	5	6	7	11	12	13	14	15	16	17	18	19
2. Unit	Furnace with prechamber	Furnace with prechamber	Furnace with prechamber	Furnace with prechamber	Furnace	Furnace	Furnace	Modified fireplace	Modified fireplace	Modified fireplace	Modified fireplace	Airtight stove	Airtight stove	Airtight stove	Airtight stove	Airtight stove
3. Fuel	Wood-chips	Wood-chips	Wood-chips	Wood-chips	Wood	Wood	Wood	Wood	Wood	Wood	Wood	Wood	Wood	Wood	Wood	Wood
4. Fuel load, kg/h	4.2	7	7	7	7	7	13	5	4	4	4	4	4	4	4	2
5. Fuel moisture content, %	21	48	48	48	14	14	36	19	19	19	41	16	16	16	41	16
6. Damper	-	-	-	-	-	-	-	50 % open	25 % open	25 % open	25 % open	open	open	open	open	closed
7. Flue gas flow, dry m^3/h	61	68	68	124	74	113	212	59	41	33	46	32	35	34	36	15
8. Excess of air	3.5	3.4	3.3	5.8	2.8	4.1	4.6	4.1	2.5	2.0	5.0	2.5	2.6	2.9	3.4	2.7
9. Flue gas temp. at sampling, °C	153	190	190	180	200	200	190	440	575	650	400	450	500	350	500	450
10. Carbon dioxide, % (range)	6.5 (5 - 8)	6.7 (6 - 8)	7.0 (6 - 10)	4.0	8.0 (6 - 10)	5.7	5.0 (3 - 8)	5.7 (4 - 9)	9.0 (1 - 12)	11.1 (5 - 15)	4.7	9.1 (7-13)	8.6 (4-13)	8.0 (5-12)	6.8 (2-9)	8.3 (6-11)
11. Carbon monoxide, ppm (range)	60 (10 - 100)	700 (100 - 600)	2100 (200-3500)	2500 (200-4500)	5000 (350-10000)	6000 (4000-9000)	6500 (3500-8000)	2300 (10 - 4000)	4400 (100 - 7000)	1100 (100 - 7000)	6000 (800 - 7000)	1000 (200-5000)	2200 (400-6000)	1600 (190-5100)	4800 (1200-8000)	5000 (1300-15000)
12. Nitrogen oxides, ppm	75	50	15	-	25	18	20	8	13	30	27	17	21	19	22	9
13. Hydrocarbons, ppm CH_4 (range)	30 (15 - 150)	50 (10 - 300)	110 (30-300)	400 (100-6000)	-	-	-	(100 - 700)	-	(300 - 3000)	(1000 - 6000)	(10 - 800)	(100-3000)	(60 - 1200)	(300-8000)	(140-4000)
	Emissions per kg dry fuel															
14. Particulate matter, mg	670	910	1000	31000	3600	1300	3300	560	260	110	3200	750	320	570	1500	700
15. Ethene, mg	-	-	1500	-	22000	830	-	3800	180	15	2400	520	-	-	-	-
16. Benzene, mg	< 0.07	32	100	-	5200	180	1500	1200	30	89	160	630	-	-	-	-
17. Formaldehyde, mg	3	46	100	-	-	230	470	56	78	320	230	190	-	63	30	110
18. Acetaldehyde, mg	1	12	72	-	-	67	620	110	90	70	710	28	-	8.6	130	69
19. Extractable matter, mg	5	77	240	10000	6000	1700	6400	3200	810	950	11000	340	540	720	7000	1300
20. Chlorobenzenes, µg	3	3	2	13	22	6	9	36	45	41	32	24	44	30	41	180
21. Sum of PAH, µg	< 80	530	2900	31000	160000	25000	36000	8200	9600	5900	320000	5700	5100	6000	83000	[illegible]
22. Phenanthrene, µg	< 6	63	320	4000	19000	2600	2800	1100	1200	650	42000	700	700	600	[illegible]	19000
23. Pyrene, µg	< 6	50	220	3100	21000	2200	2800	1000	1100	750	35000	500	500	600	6900	15000
24. Benzo e pyrene, µg	< 6	< 2	< 13	420	1900	90	100	100	130	90	5100	80	9	110	[illegible]	2000
25. Benzo(a)pyrene, µg	< 6	< 2	< 13	600	3700	180	220	180	230	150	8100	100	27	190	1900	2000
26. Mutagenicity, revertants (highest response in strain and + or -S9)	9200 TA 100 +S9	5×10^5 TA 100 ±S9	16×10^5 TA 100 -S9	37×10^5 TA 100 ±S9	38×10^5 TA 100 +S9	29×10^5 TA 100 -S9	18×10^5 TA 100 +S9	16×10^5 TA 100 +S9	22×10^5 TA 100 -S9	15×10^5 TA 100 -S9	34×10^5 TA 100 +S9	33×10^4 TA 98 -S9	36×10^4 TA 100 -S9	52×10^4 TA 100 -S9	15×10^5 TA 98 +S9	25×10^5 TA 100 +S9

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Figure 5A

A comparison of the mutagenicity (with and without exogenous activation) for stationary and mobile sources.
(Lewtas, 1981, p.611)

Table 7A

Particle bound organics mutagenicity.
(Lewtas, 1981, p. 614)

Source	Fuel	mg/kg Fuel	ng/J	TA98 + S9	
				Rev x 10^5/kg Fuel	Rev x 10^{-3}/J
Woodstove	Pine	8_0940	508	158	900
Woodstove	Oak	3,096	187	29	169
Residential Furnace #1	No. 2 Fuel Oil	21	0.5	0.4	1.0
Residential Furnace #2	No. 2 Fuel Oil	70	1.5	3.6	7.6
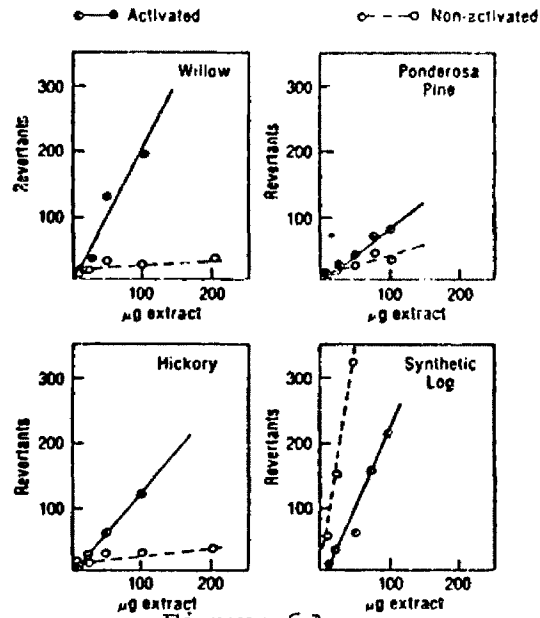
Figure 6A
Results of Ames testing on
four types of fireplace
particulate extract.
(Dasch, 1982, p.642)

Table 8A

Results of Ames Test
(Dasch, 1982, p.643)

wood	extracted, % [a]	nonactivated revertants, μg [b]	activated revertants, μg	BaP, μg/g
Ponderosa Pine	46	0.14	0.38	24
Willow	74	0.067	1.5	105
Hickory	87	0.10	1.1	120
Hickory (impinger)	100	0.16	0.056	
synthetic log	10	0.67	0.24	40

[a] benzene-ethanol extractable fraction of filter.

[b] revertants per ug of extracted material.

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Table 9A
Carcinogenic agents in the particulate phase of tobacco smoke. (U.S. Department of Health, 1979, p.5-56)

Smoke compounds		Amount in smoke of one cigarette
Tumor Initiators[2]	Biol. Act.[2]	
Benzo(a)pyrene	(+ + +)	10 50ng
5-Methylchrysene	(+ + +)	0.6ng
Dibenz(a,h)anthracene	(+ +)	40ng
Benzo(b)fluoranthene	(+ +)	30ng
Benzo(j)fluoranthene	(+ +)	60ng
Dibenzo(a,h)pyrene	(+ +)	present
Dibenzo(a,i)pyrene	(+ +)	present
Dibenz(a,j)acridine	(+ +)	3 10ng
Indeno(1,2,3-cd)pyrene	(+)	4ng
Benz(a)anthracene	(+)	40 70ng
Chrysene	(+)	40 60ng
Methylchrysenes	(+)	18ng
Methylfluoranthenes	(+)	50ng
Dibenz(a,c)anthracene	(+)	present
Dibenz(a,h)acridine	(+)	0 1ng
Dibenzo(c,g)carbazole	(+)	0 7ng
Benzo(c)phenanthrene	(+)	present
Organ specific carcinogens[1]		
A Esophagus		
N' Nitrosonornicotine		140ng
Nitrosopiperidine		0 9ng
Nitrosopyrrolidine		1 110ng
Unknown Nitrosamines		?
B Lung		
Polonium-210		0 03 13pCi[4]
Nickel compounds		0 600ng
Cadmium compounds		9 70ng
Unknowns		?
C Pancreas		
Nitrosamines		?
Unknowns		?
D Kidney and Bladder		
β-Naphthylamine		22ng
x Aminofluorene		present
x Aminostilbene		present
o-Toluidine		present
Unknown Aromatic Amines		?
o-Nitrotoluene		21µg
Unknown Nitro compounds		?
Di-n-butylnitrosamine		0 3ng
Unknown nitrosamines		?

[1]So far with certainty identified
[2]Biol. Act. = Relative carcinogenic activity on mouse skin + + + highly active, + + moderately active, + weakly active
[3]These carcinogens also may act on other target organs
[4]pCi = picoCurie, 10⁻¹²Curie

Table 10A
Tumor promoters and co-carcinogens in the particulate phase of tobacco smoke. (U.S. Department of Health, 1979, p.5-57)

Smoke compounds	Amount in smoke of one cigarette
Tumor promoters	
Volatile phenols	150 500µg
Unknown weakly acidic compounds	?
Unknown neutral compounds	?
Co-carcinogens	
Pyrene	50 200ng
Methylpyrenes	30 300ng
Fluoranthene	100 260ng
Methylfluoranthenes	180ng
Benzo(ghi)perylene	60ng
Benzo(e)pyrene	30ng
Other PAH	?
Napthalenes	0.3 6.3µg
1-Methylindoles	0.83µg
9-Methylcarbazoles	0.14µg
4,4'-Dichlorostilbene	1.5µg[2]
Other neutral compounds	?
Catechol	200 500µg
4-Alkylcatechol	10µg
Other acidic compounds	?

[1] So far with certainty identified.
[2] Values are decreasing because of lesser use of DDT and DDD for tobacco cultivation

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APPENDIX B

WOOD STOVE EMISSIONS

Cooper (1980b) described how PAHs are produced during pyrolysis, the thermal degradation of organic material in wood burning:

> Wood burns with a diffusion flame, that is, oxygen must diffuse from outside the flame into the combustion zone in contrast to a gas flame where the fuel and air are mixed before combustion. As oxygen is consumed, the region inside the flame becomes oxygen starved. Both free carbon atoms and hydrocarbon fragments are formed in this oxygen depleted zone. If these species escape the high temperature combustion region before they are oxidized, they will form soot and hydrocarbons commonly called creosote and tars consisting of a variety of aliphatic, olefinic, and polynuclear aromatics. It is clear that a myriad of inital pryrolysis products formed during partial combustion are mixed in a sea of chemical reactivity including pyrolysis, oxidation, and reduction. These reactions and the resulting compounds are influenced by the chemical and physical state of the reacting species which can serve as catalysts, as well as ions, electrons, free radicals, and free atoms, which form molecules that combine and decompose.

Operating conditions greatly influence the type and quantity of PAHs emitted from RWDs, as detailed in the following studies. Therefore, in examining the emission tests, the reader must be cautious in comparing results of different studies using different operating procedures.

Butcher and Sorenson (1979) and Butcher and Buckley (1977) measured particulate emissions from the Jotul #602 and Riteway #2000 stove fueled with wet or dry pine and oak under different burning conditions. They measured the percentage of benzene extractable material (containing POM) and found it decreased with increased burning time and decreased moisture content of fuel. In a similar experiment, Butcher and Ellenbacker (1982) mixed the stove effluent with four volumes of outside air and sampled two minutes after mixing. The particulate emissions were lower than in the two previous studies. The authors suggested that such a difference may be due to a higher

proportion of vapor phase organics in the dilute sample than in the undiluted sample.

In a pioneering study, DeAngelis et al. (1980) analyzed emissions from a fireplace, a baffled stove, and a nonbaffled stove fueled with seasoned oak and pine. They used gas chromatography/mass spectrometry to identify and quantify POMs (Table 1B). Over 50% of organic material was nonchromatographable. Baffling and wood type did not influence POM emissions. Wood stoves produced significantly higher POMs than fireplaces on a per unit of fuel basis. However, on a delivered BTU basis, wood stoves produced lower POMs than fireplaces due to a fireplace's tremendous inefficiency for heating. Using this study by DeAngelis et al. (1980) and another on fireplaces by Lee et al. (1977), Cooper (1980a) summarized the known and suspected PAH carcinogens and cocarcinogens identified in wood stove and fireplace effluent (Table 2B).

Truesdale and Cleland (1982) compared emissions of a wood stove during the combustion of eight different fuels--compressed wood logs, with and without binders, cardboard logs, bituminuous coal, newspaper logs, treated lumber, peat and wood. They measured the PAH concentrations of emissions using gas chromatography. The highest PAH emissions occurred with compressed wood logs without binders, and with treated lumber (Table 3B). The concentration of the PAH compounds emitted in this study differed by as much as an order of magnitude from the concentrations found by DeAngelis et al. (1980) (Table 4B). The authors attributed this difference to the difference in stove type and combustion conditions in the two studies.

Rudling et al. (1981) reported on the emissions from combustion of: 1) wood chips in a 35 kW central heating furnace equipped with a prechamber oven; 2) of wood in the same furnace with the prechamber oven disconnected; 3) of wood in a fireplace stove with glass shutters; and, 4) of wood in an air-tight stove equipped with a fan and heat exchanger. They analyzed flue gases for PAHs. The combustion of dried wood chips in the furnace with the prechamber oven produced the least amount of organic pollutants, 10 to 100 times less than the other three combustion regimes.

Dasch (1982) measured the BaP concentration of particulate emissions from the combustion of various wood types in three wood-burning fireplaces (Table 5B). In comparison to other fuels, the BaP emissions from wood were higher on a ug/kg basis, with the exception of residential coal burning (Table 5B).

In a study of emissions from air-tight wood-burning stoves fueled with seasoned red-oak logs, Hubble et al. (1981) found that faster burn rates with smaller logs produced more POM emissions than slower burn rates with large logs. Fractionation of condensible organics into aliphatic, polar and aromatic fractions revealed a shift from single-ring alkylated benzene with the low-burn rate conditions to multi-ring organic compounds with high-burn rate conditions. The authors explained the presence of higher molecular weight species in the high-burn as opposed to the low-burn rate experiments by the increase in condensible organics with increasing temperatures, even though the total concentration of organics decreased as temperature increased. They detected POM compounds DeAngelis et al. (1980) (the

Monsanto study) did not report and did not detect other compounds DeAngelis et al. (1980) identified. Some of the POM concentrations differed by two orders of magnitude from the Monsanto study, possibly due to the disparate burn-rate ranges associated with the two studies or to differences in the fractionation scheme employed in obtaining these emission factors (Table 6B).

Cooke et al. (1981) tested the stack gas composition of three commercially available wood stoves operated under different burn modes and with different fuels. Their results concurred with those of Hubble et al. (1981), showing that low temperature combustion (280°C to 600°C) yields high organic emissions with small quantities of high molecular weight PAHs. PAH emissions increase until the temperature reaches 800°C, beyond which both total organic and PAH emissions decrease (Figure 1B). Table 7B shows the PAH measurements obtained under the different operating conditions. The emissions decreased with secondary combustion. Down-draft burning showed significantly lower concentrations of most high molecular weight five to seven ring PAHs than either the side- or up-draft burning.

These studies all showed the presence of carcinogenic PAHs in emissions from wood-burning devices. Many researchers compared their results on PAH emissions from RWDs with the results of the pioneering Monsanto study (DeAngelis et al., 1980). As much as an order of magnitude difference occurred in the various concentrations of PAH compounds reported. These differences are attributed to differences in stove type, combustion conditions, fuel type, burn rates, and the analytical schemes the researchers employed.

Table 1B

POM emissions (g/kg fuel) from wood combustion.(DeAngelis et al., 1980, p.60)

	Fireplace			Baffled stove		Nonbaffled stove		
	Seasoned oak		Green pine	Seasoned oak	Seasoned pine	Seasoned oak	Green pine	
POM compounds	POM train[d]	SASS train[e]	POM train	POM train	POM train	POM train	POM train	SASS train
Anthracene/phenanthrene	0.0082	0.0114	0.0069	0.0745	0.1463	0.0618	0.1034	0.0104
Methyl-anthracenes/-phenanthrenes	0.0027	0.0034	0.0083	0.0211	0.0510	0.0167	0.0513	0.0028
C₂-alkyl-anthracenes/-phenanthrenes	<0.0014[a]	0.0011	0.0014	0.0040	0.0070	0.0045	0.0094	0.0008
Cyclopenta-anthracenes/-phenanthrenes	<0.0014[d]	0.0004	0.0014	0.0032	0.0086	0.0030	0.0047	0.0002
Fluoranthene	<0.0014[d]	0.0026	0.0016	0.0180	0.0316	0.0208	0.0188	0.0012
Pyrene	<0.0014[d]	0.0026	0.0016	0.0156	0.0240	0.0169	0.0188	0.0013
Methyl-fluoranthenes/-pyrenes	<0.0014[d]	0.0021	0.0016	0.0128	0.0167	0.0103	0.0142	0.0016
Benzo(ghi)fluoranthene	[b]	0.0009	0.0014	0.0048	0.0067	0.0047	0.0047	0.0004
Cyclopenta(cd)pyrene	<0.0014[d]	0.0010	0.0014	0.0048	0.0089	0.0051	0.0138	0.0005
Benzo(c)phenanthrene		0.0004	0.0013	0.0016	0.0023	0.0016	0.0046	0.0002
Benz(a)anthracene/chrysene	<0.0014[d]	0.0020	0.0014	0.0125	0.0138	0.0076	0.0371	0.0013
Methyl-benzanthracenes-benzphenanthrenes/-chrysenes	<0.0014[d]	0.0013	0.0016	0.0062	0.0104	0.0062	0.0040	0.0009
C₂-alkyl-benzanthracenes/-benzophenanthrenes/-chrysenes			0.0014	0.0055	0.0044	0.0037	0.0047	0.0005
Benzofluoranthenes	<0.0014[a]	0.0022	0.0016	0.0129	0.0159	0.0112	0.0141	0.0015
Benzopyrenes/perylene	<0.0014[d]	0.0017	0.0014	0.0083	0.0116	0.0084	0.0094	0.0011
Methyl cholanthrene				0.00007				
Indeno(1,2,3-cd)pyrene								
Benzo(ghi)perylene	0.0013	0.0013	0.0015	0.0045	0.0099	0.0043	0.0048	0.0011
Anthanthrene			0.00005				0.00005	
Dibenzanthracenes/-phenanthrenes	0.0003	0.0003		0.0007	0.0014	0.0010	0.00002	0.0002
Dibenzocarbazoles								
Dibenzopyrenes	0.0007	0.0007	0.0001	0.0011	0.0010	0.0007	0.00002	0.0005
Total[c]	0.0249	0.0365	0.0360	0.2121	0.3715	0.1985	0.3187	0.0265

[a]Compound was identified but not quantified because of the detection limits of the analytical method.

[b]Blanks indicate POM compound not detected.

[c]The detection limit was taken as the emission factor for compounds that were identified but not quantified.

[d]EPA method 5 equipment supplemented with XAD-2 Resin trap used to obtain samples of particulate, POM, and other organic emissions.

[e]Source assessment sampling system designed to collect and size classify organic species, POM, and trace elements.

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Table 2B

Carcinogenic compounds observed in smoke from residential wood combustion sources.[a,b] (Cooper, 1980a, p.857)

Compound	Carcinogenic Activity[c]	Reference Observed	Emission Factor (g/kg)[d]	
			Stove	Fireplace
Dimethylbenzanthracene	++++	a		
Benz(a)anthracene	+	a,b	.0177	.0019
Dibenzanthracene		a	.0010	.00018
Dibenz[a,h]anthracene	+++	b		
Dibenz[a,c]anthracene	+	b		
Benzo[c]phenanthrene	+++	a	.0025	.008
Benzofluoranthenes		a	.0135	.0019
Benzo[b]fluoranthene	++	e	e	e
Benzo[j]fluoranthene	++	e	e	e
Methylcholanthens		a		
3-methylcholanthene	++++	e	e	e
Benzopyrenes		a	.009	.0015
Benzo(a)pyrene	+++	b	.0025[g]	.00073[g]
Indeno(1,2,3-ed)pyrene	+	a,b		
Chrysene	±	a,b	f	f
Dibenzopyrenes		a	.0007	.0004
Dibenzo[a,l]pyrene	high	e	e	e
Dibenzo[a,h]pyrene	+++	e	e	e
Dibenzo[a,e]pyrene	+++	e	e	e
Dibenzocarbazoles		a		
Dibenzo[a,g]carbazole	±	e	e	e
Dibenzo[c,g]carbazole	+++	e	e	e
Dibenzo[a,i]carbazole	±	a	e	e
TOTAL			.038[h]	.0059[h]

[a] DeAngelis, et al. (grams emissions per kilogram of wood)

[b] Lee, et al.

[c] Based on classification in Reference 29; ± uncertain or weakly carcinogenic; + carcinogenic; ++, +++, ++++ strongly carcinogenic.

[d] Average of results from Tables 20 and 22 of Reference 21 excluding SASS train results for green pine in stove.

[e] These compounds were not specifically identified except as a group.

[f] Included in the benz(a)anthracene number.

[g] See footnote c, Table I.

[h] Total will be upper limit because of the inclusion of some noncarcinogenic isomers included in the general classes measured. The benzopyrene class value was not used, only the benzo(a)pyrene value.

Table 3B
PAH emission factors, (mg/kg fuel consumed).
(Truesdale and Cleland, 1982, p.124)

Compound	W	CW	CWB	C	N	TW	P	BC
Naphthalene	36.7	40.7	297.8	89.6	103.5	206.9	59.7	47.0
Biphenyl	11.4	5.1	23.0	8.5	27.1	33.0	13.3	7.8
Acenaphthene	8.0*	1.8	1.4	2.9	7.6*	8.7	3.6*	0.08
Fluorene	4.7	4.1	6.1	6.1	11.8	18.9	10.4	6.2
Phenanthrene	9.8	15.7	45.7	20.9	22.6	33.4	13.1	16.1
Anthracene	6.0	5.3	3.5	2.2	8.6	20.2	8.6	4.1
Carbazole	ND	0.05	2.2	0.1	ND	1.1	0.04*	0.5*
1-Methylphenanthrene	14.7	3.7	20.3	4.2	4.8	10.7	9.1	7.3
9-Methylanthracene	2.2	3.0	10.0	3.2	ND	0.5	6.8	ND
Fluoranthene	3.7	11.1	20.6	12.4	9.2	12.6	10.1	5.7
Pyrene	2.6	7.8	13.0	6.2	1.3	10.0	6.0	4.4
Benz(a)anthracene	0.07	3.5	0.9	2.4	1.0	3.4	3.2	2.0*
Chrysene	1.6	1.9	3.2	2.0	2.5	1.5	4.3	1.4
12-Methylbenz(a)-anthracene	0.07*	ND	ND	0.02	ND	ND	ND	ND
6-Methylchrysene 7-Methylbenz(a)-anthracene	0.5*	1.1	2.0	0.8	0.1*	0.4	0.3*	0.2*
7,12-Dimethylbenz(a)-anthracene	1.5	4.1	4.1	2.9	0.4	5.2	0.04	1.2
Benzo(b)fluoranthene Benzo(k)fluoranthene	ND	ND	0.5	ND	0.1	ND	0.1	ND
Benzo(e)pyrene	0.5	2.3	1.8	2.1	0.4	2.7	2.0	0.8
Benzo(a)pyrene	0.6	2.3	3.2	1.5	0.3	2.4	3.0	0.5
Perylene	0.8	1.0	7.6	0.6	0.09	0.3	4.7	1.7
3-Methylcholanthene	0.3	0.8	0.3	0.5	0.9*	6.8	0.04	0.1
Dibenz(a,h)anthracene	ND	NA	NA	NA	ND	NA	ND	ND
Benzo(g,h,i)perylene	0.04	1.5	0.007	0.9	ND	0.3	0.7	ND
Coronene	ND	0.5	0.6	ND	ND	0.6	ND	ND

*Results from single test.
NA = Not analyzed.
ND = Not detected.
W-wood; CW-compressed wood logs; CWB-compressed wood logs
with binders; C-cardboard logs; N-newspaper logs; TW-treated
lumber; P-peat; BC-bituminous coal

Table 4B

Comparison of emission factors of PAHs (g/kg).
(Truesdale and Cleland, 1982, p. 125)

	Oak, This Study	Oak, Baffled[a] Stove[b]
Naphthalene	0.0367	0.2729
Biphenyl	0.0114	0.0228
Fluorene	0.0047	0.0224
Anthracene/phenanthrene	0.0158	0.0745
Fluoranthene	0.0037	0.0180
Pyrene	0.0026	0.0156
Chrysene/benz(a)anthracene	0.0017	0.0125
7,12-Dimethylbenz(a)-anthracene	0.0015	--
Benzopyrenes and perylene	0.002	0.0083
3-Methylcholanthrene	0.0003	0.00007

[a]Truesdale and Cleland, 1982

[b]DeAngelis et al., 1980

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Table 5B
Benzo(a)pyrene content of filterable
fireplace emissions. (Dasch, 1982, p.642)

wood	ppm[a]	μg of BaP/kg of wood
Ponderosa Pine	24	50
Willow	105	700
Willow	141	1900
White Ash	3	5
White Ash	7	17
Sugar Maple	11	45
Hickory	120	130
synthetic log	18	58
synthetic log	40	400
average		370
median		58
coal, power plant[b]		2
coal, residential[b]		25000
auto, noncatalyst[c]		16
auto, catalyst[c]		0.4

[a] ug of BaP/g of filterable particulate

[b] Nagda, N.L.; Pelton, D.J.; Swift, J.L.
presented at the 72nd Annual Meeting of
the Air Pollution Control Association,
Cincinnati, OH, 1979

[c] Williams, R.L.; Swarin, S.J., presented
at the Society of Automotive Engineers
Meeting, Detroit, MI 1979

Table 6B

POM emission factors (mg/kg)
(Hubble et al., 1981, p. 96)

POM Compounds	Monsanto, Seasoned Oak, Baffled Stove[c]	0.12 m Logs, 0.82 kg/hr Burn Rate	0.06 m Logs, 7.73 kg/hr Burn Rate
Phenanthrene/ Anthracene	74.5	0.88	2.30
C_1- Phenanthrenes/ Anthracenes	21.1	0.42	0.18
C_2- Phenanthrenes/ Anthracenes	4.0	0.11	0.04
Cyclopenta- Phenanthrenes/ Anthracenes	3.2	ND[a]	ND
Pyrenes	15.6	0.33	1.39
Fluoranthene	18.0	0.25	0.10
Benz(a)fluorene	NR[b]	0.26	0.26
Unidentified POMs	—	0.70	1.31
C_1-Fluoranthenes/ Pyrenes	12.8	0.10	ND
Benz(ghi)fluorene	NR	0.04	0.10
9- Phenylanthracene	NR	0.04	0.20
C_3-Phenanthrenes/ Anthracenes	NR	0.20	0.03
Benzo(ghi)fluoranthene	4.8	ND	ND
C_2-pyrenes	NR	0.08	ND
Cyclopenta(ed)pyrene	4.8	ND	ND
Benzo(c)phenanthrene	1.6	ND	ND
Benz(a)anthracene/ Chrysene	12.5	0.44	1.35
Higher-molecular-weight POMs	39.2	0.25	1.26
Total	212.2	4.10	8.52

[a] ND - Not Detected

[b] NR - Not Reported

[c] DeAngelis et al, 1980

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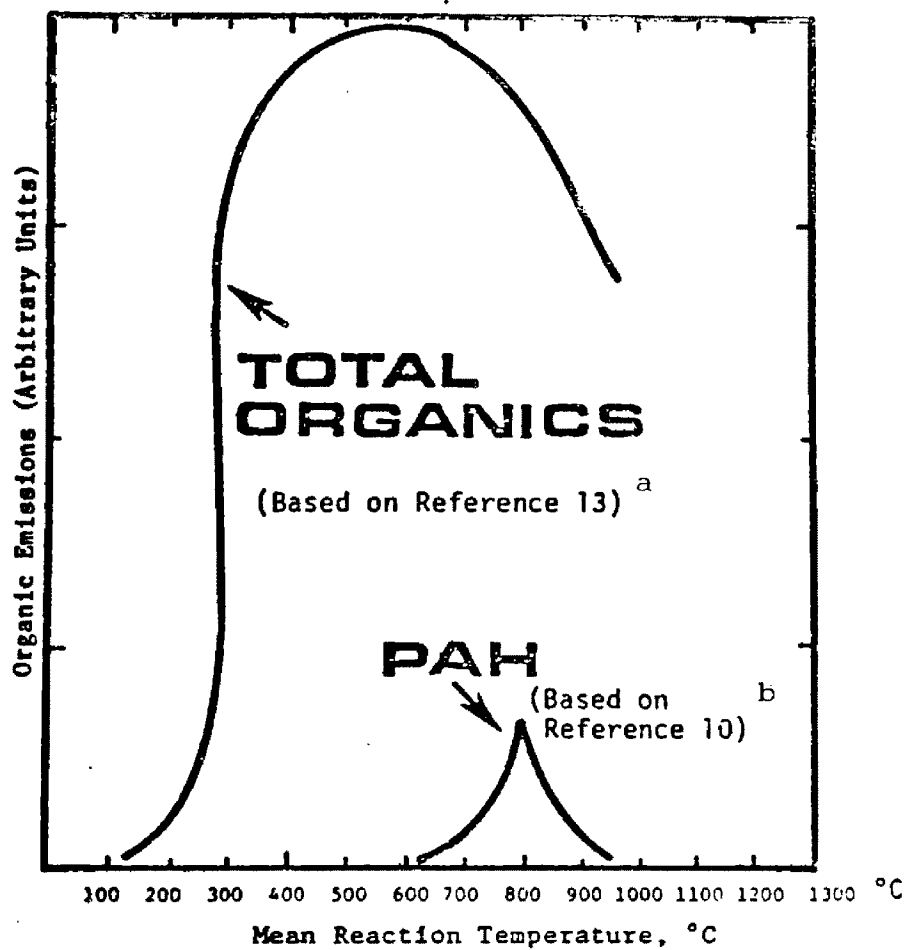
Figure 1B
Representation of total organic
and PAH emissions as a function
of mean combustion temperature.
(Cooke et al., 1981, p.145)

[a]Juon, 1907

[b]Commins, 1969

Table 7B

Total PAH(ng) measured in wood stove emissions.
(Cooke et al., 1981, p.159)

Burn Mode Fuel	Side Draft Oak-Seasoned		Side Draft Pine-Green		Up Draft Oak-Seasoned		Down Draft Oak-Seasoned		High Turbulence Oak-Seasoned	
Species Measured	Particulate	Gaseous	Particulate	Gaseous	Particulate	Gaseous	Particulate	Gaseous	Particulate	Gaseous
Naphthalene	1.2	45	0.22	80	0.16	31	0.07	32	0.36	62
Acenapthene	0.12	58	0.27	89	0.10	18	0.12	31	0.60	31
Acenaphthylene	0.06	26	0.06	7.3	0.04	6.4	0.05	4.9	0.27	1.9
Fluorene	0.08	19	0.12	26	0.07	8.2	0.09	3.4	0.46	2.6
Phenanthrene	0.24	62	2.2	100	0.35	20	0.17	96	0.99	40
Anthracene	0.09	18	0.35	19	0.09	4.6	0.08	11	0.43	2.2
Methylanthracenes/ Methylphenanthrenes	0.07	3.2	0.13	7.6	0.07	1.4	-	2.0	0.33	0.99
Fluoranthene	2.6	18	2.9	11	0.20	9.1	0.12	68	0.73	15
Pyrene	2.8	8.6	3.1	6.1	0.28	5.8	0.07	38	0.46	9.6
Methylpyrenes/Methyl- fluoranthenes	0.79	0.53	0.63	0.82	0.16	1.9	-	2.6	0.30	1.5
Benzo(a)anthracene	5.9	1.0	3.0	0.22	0.39	13	0.16	6.1	0.96	1.8
Chrysene	5.6	1.1	3.2	0.21	0.37	8.2	0.11	2.5	0.73	1.9
Methylchrysenes	0.56	0.14	0.54	0.12	0.16	1.0	-	0.26	-	0.60
Dimethylbenzo(a)anthracene	0.29	-	0.26	-	0.09	0.25	-	0.13	-	-
Benzofluoranthenes	14	0.96	21	0.15	1.3	17	0.11	3.7	0.93	1.8
Benzo(e)pyrene	3.5	0.24	7.1	0.08	0.42	3.8	0.07	0.48	0.50	0.43
Benzo(a)pyrene	5.0	0.27	13	0.08	4.3	6.5	0.07	0.20	0.50	0.40
Perylene	0.51	0.10	1.8	0.09	0.14	0.65	0.09	0.09	0.50	0.46
Indeno(1,2,3-cd)pyrene	15	0.29	38	-	1.3	3.9	0.17	0.15	1.1	0.80
Benzo(g,h,i)perylene	22	0.20	30	-	1.2	3.2	0.13	0.09	0.93	0.50
Coronene	10	0.11	16	-	0.51	0.55	0.17	-	0.86	0.63
Dibenzo(a,h)pyrene	4.2	-	9.3	-	0.39	0.23	0.12	-	-	-
Total	95	263	153	348	14	146	2	369	12	175
Total Emission	338		502		160		351		137	

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APPENDIX C
RESULTS TABLES

Table 1C
Mutagenicity, air quality, and weather data.

SI	DATE	WTR	AIRFLOW	TSP	RSP	TA98					TA98S				
						1	2	3	RV	S.D.	1	2	3	RV	S.D.
RP	7/21	9	1428.58	60.4	-	23	35	30	4	6.03	29	22	20	-6	4.73
RP	8/4	9	1842.32	46.6	-	36	36	30	10	3.46	31	30	21	0	5.51
RP	8/11	9	1775.97	38.0	-	27	21	20	-1	3.79	17	18	19	-11	1.00
RP	8/18	9	1801.62	54.2	-	34	35	31	13	2.08	26	33	30	4	3.51
RP	8/25	9	1842.32	68.0	-	44	35	23	14	10.54	25	29	30	2	2.65
RP	9/1	9	1904.77	52.9	-	36	40	39	13	2.08	36	32	22	3	7.21
RP	9/8	9	1795.48	61.1	-	20	19	19	1	.58	19	29	29	1	5.77
RP	9/15	9	1942.56	44.5	17	41	44	41	22	1.73	31	28	40	7	6.24
RP	9/22	9	1943.18	37.6	17	27	45	34	15	9.07	33	37	36	9	2.08
RP	9/29	11	1982.37	28.1	12	32	36	27	7	4.51	34	41	38	8	3.51
RP	10/2	9	1966.82	49.4	-	41	50	57	25	8.02	44	50	40	18	5.03
RP	10/7	11	2037.60	30.2	-	34	43	42	15	4.93	40	47	49	17	4.73
RP	10/20	7	2251.88	92.0	-	58	46	49	31	6.24	70	54	57	36	8.50
RP	10/27	7	2197.32	36.9	-	42	53	42	26	6.35	38	47	38	15	5.20
RP	11/2	1	2002.66	93.2	40	44	44	43	22	.58	40	53	52	22	7.23
RP	11/17	7	2151.49	97.9	62	75	64	69	49	5.51	77	64	76	48	7.23
RP	11/24	1	2361.05	114.2	60	53	57	50	28	3.51	71	69	66	41	2.52
RP	12/2	1	2187.65	83.5	41	48	35	40	21	6.56	39	36	43	15	3.51
RP	12/2	1	1512.05	96.8	41	62	62	60	38	1.15	49	62	72	30	11.53
RP	12/5	1	2014.73	117.4	78	59	61	58	34	8.02	66	57	50	31	1.53
RP	12/8	1	1697.59	87.7	48	67	74	44	39	15.70	40	59	52	21	9.61
RP	12/8	1	2320.18	81.6	48	67	75	70	46	4.04	68	50	59	29	9.00
RP	12/11	1	2191.51	163.0	143	97	87	114	76	13.65	102	102	86	66	9.24
RP	12/15	1	2251.99	60.7	39	63	69	70	42	3.79	76	67	77	45	5.51
RP	12/17	11	2091.13	26.6	13	32	37	44	13	6.03	48	48	58	24	5.77
RP	12/20	5	1608.94	71.4	44	60	54	49	29	5.51	57	60	50	29	5.13
RP	12/22	5	2251.99	65.4	15	44	57	50	30	10.02	61	46	42	26	6.51
RP	12/23	5	2119.28	38.4	30	49	50	56	29	3.79	59	54	49	23	5.03
RP	12/26	1	1714.58	71.0	49	76	64	47	39	14.57	42	46	50	15	4.00
RP	12/29	1	2365.15	80.9	49	49	50	64	34	.58	58	58	57	34	8.39
RP	1/1	1	1668.54	187.1	126	60	45	45	29	8.66	58	46	42	24	8.33
RP	1/4	5	2080.24	117.1	111	51	54	67	36	10.21	77	74	50	43	8.50
RP	1/5	11	2215.79	60.5	74	59	64	46	31	9.29	45	58	68	29	11.53
RP	1/10	11	2111.24	32.2	12	32	37	47	14	7.64	50	41	55	22	7.09
RP	1/12	1	2183.50	435.9	147	110	95	143	93	24.55	99	83	84	58	8.96
RP	1/13	9	1660.84	272.4	96	44	45	53	22	4.93	54	52	62	29	5.29
RP	1/16	3	2068.05	259.3	101	66	66	45	34	12.12	69	48	44	27	12.53
RP	1/19	1	2254.46	218.5	75	60	66	73	41	6.51	66	58	83	41	12.77
RP	1/19	1	1657.69	214.0	75	69	65	55	38	8.54	73	63	56	37	7.21
RP	1/22	5	2022.72	86.6	69	49	52	47	28	2.52	59	54	60	33	3.21
RP	1/25	7	1588.45	171.6	107	48	49	56	26	4.36	46	38	45	13	4.36
RP	1/26	7	2167.54	209.1	69	68	68	73	50	2.89	66	53	63	37	6.81
RP	1/28	7	1938.32	124.8	23	46	54	43	23	5.69	44	41	34	10	5.13
RP	1/31	9	1592.48	217.1	108	66	64	60	38	9.00	67	58	49	31	3.06
RP	2/2	1	2234.64	75.0	36	65	58	68	41	5.13	57	62	66	31	4.51
RP	2/6	3	1704.06	123.5	55	45	56	46	28	6.08	52	53	59	30	3.79
RP	2/9	5	2227.86	93.7	56	77	61	72	50	8.19	75	73	59	45	8.72
RP	2/9	5	1938.32	97.6	56	68	58	71	41	6.81	86	58	60	41	15.62
RP	2/12	11	1648.78	46.8	19	54	59	64	34	5.00	62	69	72	38	5.13
RP	2/15	7	1974.51	203.6	55	72	75	71	50	2.08	63	76	86	44	11.53
RP	2/16	11	2209.26	118.0	41	52	64	47	34	8.74	40	75	42	28	19.66
RP	2/18	11	1616.15	35.9	17	71	72	65	46	3.79	75	81	75	46	3.46
RP	2/21	7	1910.71	183.9	77	65	55	65	39	5.77	61	73	65	35	6.11
RP	2/23	7	2328.68	133.8	52	49	62	60	37	4.51	63	58	54	34	7.00
RP	2/24	9	1604.20	195.8	61	57	43	51	25	7.02	54	67	51	27	8.58
RP	2/27	7	1974.52	72.3	22	72	64	64	44	4.62	65	65	66	34	.58
RP	3/2	11	2149.45	56.6	19	51	45	39	20	6.00	47	55	50	23	4.04
RP	3/9	9	2149.45	111.2	-	49	46	52	29	3.00	46	39	51	21	6.03
RP	3/16	9	1648.78	42.5	-	36	32	34	9	3.21	34	35	40	9	2.00
RP	3/23	11	1795.28	39.5	-	28	36	40	10	6.11	41	41	32	10	5.20
RP	3/30	12	1440.50	43.7	-	39	42	42	20	1.73	36	41	36	13	2.89
RP	4/6	9	1982.36	66.6	-	53	51	48	28	2.52	69	57	72	35	7.94
RP	4/13	9	1793.55	44.4	-	--	55	45	25	7.07	34	37	39	10	2.52
RP	4/20	9	2491.05	71.5	-	72	67	76	49	4.51	74	73	70	41	2.08
RP	5/4	9	1886.83	60.4	-	40	40	38	14	1.15	32	39	39	10	4.04
RP	5/11	9	1576.34	42.6	-	44	47	36	19	5.69	31	33	43	5	6.43
RP	5/18	11	1982.37	26.5	-	26	34	37	7	5.69	26	30	34	3	13.30
RP	5/25	9	1686.19	80.1	-	33	28	32	6	2.65	36	28	27	3	4.93

SI	DATE	WTR	AIRFLOW	TSP										
BP	8/5	9	1498.84	74.5	-	35	35	34	12	0.58	27	25	30	-4	2.52
BP	8/25	9	1584.71	115.2	-	45	39	40	16	3.21	34	36	36	8	1.16
BP	9/3	10	1584.63	122.0	-	42	38	46	17	4.00	30	24	22	-5	4.16
BP	9/18	10	1600.18	87.0	-	28	28	28	4	0.00	32	41	--	10	6.36
BP	10/9	9	1054.82	118.7	-	44	24	31	9	10.15	48	39	50	17	5.86
BP	10/15	9	1005.04	162.3	-	75	72	56	43	2.52	68	73	70	43	10.21
BP	11/2	1	1150.07	184.2	-	51	46	47	25	2.65	48	45	59	20	7.37
BP	11/26	1	1121.32	322.2	-	--	57	53	37	8.14	45	58	43	24	2.83
BP	12/2	1	1105.83	166.8	-	44	32	41	21	4.93	41	33	42	14	6.24
BP	12/14	1	1121.32	298.0	-	103	106	105	80	1.53	76	90	93	56	9.07
BP	1/4	5	1198.52	194.0	-	117	105	108	85	6.24	96	91	94	67	2.52
BP	1/13	10	1423.50	366.4	-	44	41	45	23	1.00	38	37	39	14	2.08
BP	2/21	7	1572.28	246.8	-	52	54	59	34	3.61	65	54	65	36	6.35
BP	2/27	8	1628.59	92.2	-	40	38	32	17	5.29	47	39	37	17	11.40
BP	3/5	10	1552.38	87.3	-	39	45	40	16	7.00	42	49	35	14	3.21
BP	3/8	7	1612.09	108.4	-	49	40	42	19	4.73	48	51	43	19	4.04
BP	4/7	10	1273.81	88.8	-	44	50	50	23	6.43	45	35	47	15	3.46
BP	4/19	9	1114.58	179.2	-	58	50	42	24	9.87	49	48	47	17	1.00
BP	5/25	10	1190.48	114.8	-	32	23	44	8	10.54	40	32	36	9	4.00
MJ	8/26	9	1346.55	461.4	-	48	54	66	33	9.7	47	47	42	14	2.89
MJ	8/31	10	1497.61	104.6	-	58	58	43	30	8.7	43	57	67	25	12.06
MJ	9/3	10	1529.99	112.4	-	65	56	62	36	4.58	48	53	44	18	4.51
MJ	9/21	10	1506.57	67.8	-	30	39	36	17	5.77	31	31	41	9	4.58
MJ	10/13	9	1552.89	104.6	-	39	37	34	19	2.52	52	44	--	23	5.66
MJ	10/20	7	1632.61	130.1	-	48	53	63	30	7.64	42	49	67	23	12.90
MJ	11/17	7	1664.78	172.4	-	--	71	79	57	5.66	54	68	78	42	7.21
MJ	11/26	1	1742.93	236.7	-	57	62	59	41	2.52	54	58	52	30	3.66
MJ	12/2	1	1757.27	133.0	-	45	53	60	40	7.57	44	42	56	22	7.51
MJ	12/11	1	980.58	237.0	-	145	137	148	118	5.69	105	107	120	81	8.14
MJ	1/13	10	1608.48	566.7	-	80	66	58	43	11.14	50	56	55	26	3.21
MJ	1/22	5	1757.27	148.9	-	72	64	64	42	4.62	64	60	62	34	2.00
MJ	2/21	7	1385.20	366.7	-	56	49	59	30	5.13	60	77	55	37	11.50
MJ	2/27	8	1431.55	154.8	-	57	54	49	33	4.04	54	53	41	25	6.03
MJ	3/11	12	1520.53	302.0	-	50	42	44	20	7.57	60	62	48	29	4.16
MJ	3/20	3	1608.48	91.2	-	42	45	44	24	1.53	44	50	37	20	6.51
MJ	4/19	9	1679.01	131.6	-	67	65	70	44	2.52	86	54	62	36	16.65
MJ	4/25	10	1755.38	84.9	-	50	52	58	28	4.16	75	52	59	35	11.79
MJ	5/7	10	1679.09	69.1	-	78	83	74	55	4.51	51	59	45	21	7.02
MJ	5/25	10	1721.32	126.2	-	49	46	39	20	5.13	43	34	33	10	5.51

Table Guide:
SI - Sampling Site: RP - Rose Park; BP - Boyd Park; M. - Malfunction Junction;
DATE - Sampling Date; WTR - Weather Groups as defined by Steffel (1983) and
shown in Table 20; AIRFLOW - air volume sampled, m³; TSP - Total Suspended
Particulate, ug/m³; RSP - Respirable Suspended Particulate, ug/m³; TA98 -
TA98 colonies per plate, without activation; TA98S - TA98 colonies per
plate, with activation; RV - Revertants per plate; S.D. - Standard Deviation.

Table 2C

Analysis of covariance: effect of total suspended particulate on mutagenicity with temperature as a covariate.

Source of Variation	Sum of Squares	df	Mean Square	F	Significance of F
A. Without Activation					
Main Effects					
NEWTSP	80.752	4	20.188	7.173	≤0.001
Covariates					
TEMP	145.822	1	145.822	51.813	≤0.001
Residual	171.678	61	2.814		
B. With Activation					
Main Effects					
NEWTSP	27.348	4	6.837	2.144	0.086
Covariates					
TEMP	154.791	1	154.791	48.549	≤0.001
Residual	194.487	61	3.188		

Table 3C

Analysis of covariance: effect of respirable particulate on mutagenicity with temperature as a covariate.

Source of Variation	Sum of Squares	df	Mean Square	F	Significance of F
A. Without Activation					
Main Effects					
NEWRSP	79.302	4	19.826	6.314	0.001
Covariates					
TEMP	28.417	1	28.417	9.050	0.005
Residual	122.461	39	3.140		
B. With Activation					
Main Effects					
NEWRSP	48.248	4	12.062	4.564	0.004
Covariates					
TEMP	14.226	1	14.226	5.382	0.026
Residual	103.081	39	2.643		

Table 4C

Analysis of covariance: effect of temperature on mutagenicity with respirable particulate as a covariate.

Source of Variation	Sum of Squares	df	Mean Square	F	Significance of F
A. Without activation					
Main Effects					
NEWTEMP	5.304	1	5.304	1.449	0.235
Covariates					
RSP	71.124	1	71.123	19.429	≤0.001
Residual	153.752	42	3.661		
B. With activation		2			
Main Effects		2			
NEWTEMP	1.053	1	1.053	0.346	0.560
Covariates					
RSP	36.611	1	36.611	12.023	0.001
Residual	127.891	42	3.045		

Table 5C

Analysis of covariance: effect of temperature on mutagenicity with total suspended particulate as a covariate.

Source of Variation	Sum of Squares	df	Mean Square	F	Significance of F
A. Without activation					
Main effects					
NEWTEMP	67.374	2	33.687	11.639	≤0.001
Covariates					
TSP	148.542	1	148.542	51.324	≤0.001
Residual	182.335	63	2.894		
B. With activation					
Main effects					
NEWTEMP	91.954	2	45.977	15.739	≤0.001
Covariates					
TSP	100.635	1	100.635	34.450	≤0.001
Residual	184.036	63	2.921		

Table 6C

Analysis of covariance: effect of weather on
mutagenicity with total suspended particulate
as a covariate.

Source of Variation	Sum of Squares	df	Mean Square	F	Significance of F
A. Without Activation					
Main Effects					
WTHR	59.320	6	9.887	3.064	0.011
Covariates					
TSP	148.542	1	148.542	46.032	≤ 0.001
Residual	190.389	59	3.226		
B. With Activation					
Main Effects					
WTHR	78.904	6	13.151	3.937	0.002
Covariates					
TSP	100.635	1	100.635	30.126	≤ 0.001
Residual	197.087	59			

Table 7C

Analysis of covariance: effect of weather on
mutagenicity with respirable particulate as a
covariate.

Source of Variation	Sum of Squares	df	Mean Square	F	Significance of F
A. Without Activation					
Main Effects					
WTHR	14.975	5	2.995	0.790	0.564
Covariates					
RSP	71.124	1	71.124	18.758	≤ 0.001
Residual	144.081	38	3.792		
B. With Activation					
Main Effects					
WTHR	11.522	5	2.304	0.746	0.594
Covariates					
RSP	36.611	1	36.611	11.848	0.001
Residual	117.422	38	3.090		