Development of Source Testing, Analytical, and Mutagenicity Bioassay Procedures for Evaluating Emissions from Municipal and Hospital Waste Combustors

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Inocerion is currently being used for disposal of about 10% of the solid waste generated in the United States, and this percentage will likely increase as land disposal declines. Siting new incinerators, however, is often controversial because of concerns related to the possibility of adverse health effects and environmental contamination from long-term exposure to stack emissions. Specific concerns relate to the adequacies of a) stack emission testing protocols, b) existing regulations, and c) compliance monitoring and enforcement of regulations. U.S. Environmental Protection Agency laboratories are cooperatively conducting research aimed at developing new testing equipment and procedures that will allow a more comprehensive assessment of the complex mixture of organics that is present in stack emissions. These efforts are directed specifically toward developing source testing equipment and procedures, analytical procedures, and bioassay procedures. The objectives of this study were to field test two types of high-volume source dilution samplers, collect stack samples for use in developing analytical and mutagenicity bioassay procedures, and determine mutagenicity of organics associated with emission particles from two municipal waste combustors and a hospital waste combustor. Data are presented for particle concentrations and emission rates, extractable organic concentrations and emission rates, and Salmonella (Ames) mutagenic potency and emission rates. The mutagenic emission rates and emission factors are compared to other incinerators and combustion sources.

Introduction

Solid waste management is increasingly becoming a public issue in many urban areas as city managers and engineers seek new ways of handling the nearly 250 million tons of trash that is generated in the United States each year. Approximately 160 million tons is produced by individual households and neighborhood businesses. Landfilling accounts for about 80% of solid waste disposal, and incineration and recycling each accounts for about 10% (1). Since 1979, however, 3600 landfills have been closed (2). Furthermore, the U.S. Environmental Protection Agency (EPA) projects that more than 30% of the existing landfills will close within 5 years. This will result in an overall yearly capacity loss of 56 million tons. At current construction rates, additional landfill space will be available for only 20 million tons, resulting in a significant shortfall of disposal capacity (1). Alternative solutions to these problems will include concentrating more effort into source reduction and recycling and also increased use of incineration through expansion of existing municipal waste combustion (MWC) units and construction of new ones. This incineration option is further reinforced in many areas because of problems that have been encountered with soil and groundwater contamination resulting from runoff and seepage from waste landfills.

The siting of new incinerators, however, is one of the most controversial environmental issues today. The adequacies of existing regulations, stack testing protocols, and compliance monitoring associated with incinerators

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are being challenged, especially in areas being considered as sites for new incinerator facilities. Of immediate concern to residents, of course, are stack emissions and their effect on air quality in nearby neighborhoods and areas downwind from the incinerator. The accompanying problems of disposal of bottom ash and precipitated fly ash in landfills are also a primary concern in many areas. The possibility of adverse health effects and environmental contamination that could result from long-term exposure to stack emissions has become an important issue. Research is needed to directly assess the potential health effects of incinerator emissions through biological studies of the complex mixtures that are emitted.

U.S. EPA health and engineering laboratories are cooperatively conducting research aimed at developing additional sampling and assay procedures for assessing possible health and environmental risks from MWC. This research includes developing MWC stack sampling equipment and analytical techniques that can be used to analyze the complex mixture of organics that is present in emissions. Stack emission samples are being analyzed by the Ames Salmonella typhimurium bacterial mutagenicity bioassay (3,4). These procedures represent simple, sensitive, rapid, and reliable screening tools for mutagenic substances. This approach, therefore, diverges from assessments that combine emission studies that determine individual compounds or a class of compounds (5-8) with toxicological data for those direct emissions. Analyses for specific organics (e.g., the chlorinated dibenzodioxins and dibenzofurans) will continue to be important in assessing potentially hazardous emissions. However, approaches are needed that analyze incinerator emissions as a whole complex mixture by using assays (e.g., mutagenicity bioassay) that are not compound specific but are targeted to specific health effects. Such assays are especially relevant for combustion emissions, which include products of incomplete combustion and pyrolysis products, because these emissions contain thousands of compounds. The potential hazards associated with such a complex mixture might be underestimated if only a few species were included in a risk assessment determination. Mutagenicity bioassay was also chosen for this study because chemicals and mixtures recognized as human carcinogens are generally mutagenic in short-term tests (9), and combustion emissions from other sources have been shown to be both mutagenic in this assay and carcinogenic in rodents. It has also been reported that chemicals that are rodent carcinogens across several species and organ sites (trans-species carcinogens) are generally also mutagenic in Salmonella typhimurium (10). Other biological studies were planned (e.g., rodent cancer studies) and will be reported elsewhere.

A study using the Ames Salmonella/microsomal mutagenicity bioassay to analyze MWC stack emissions was reported by Kamiya and Ose in 1987 (11). These investigators used a small sampling apparatus to collect fine particles (<7 μm) and gaseous organics in 10-15 m³ of stack gas emissions. They sampled a continuously operating modern incinerator with a complete combustion system and a discontinuous batch-type incinerator, where combustion was incomplete. Both incinerators were located in Aichi Prefecture, Japan. The authors found significant mutagenicity in the stack gas emissions from the latter MWC unit and, in fact, concluded "the emission gases from batch-type incinerators are mainly responsible for atmospheric pollution" (11). They also identified and quantified 13 polynuclear aromatic hydrocarbons (PAH) compounds in the same emission gases and found a high correlation between mutagenic activity and PAH concentrations.

Ahlborg and Victorin (12) analyzed emission samples from four MWC sites using mutagenicity bioassay. Results were reported in revertants per megajoule of fuel and were compared to other Swedish combustion sources. These authors also concluded that MWC can cause relatively high emission of organic mutagens and potentially carcinogenic compounds if well-controlled combustion conditions are not used.

More recent studies of incineration have also used the Ames mutagenicity bioassay to characterize emissions. Driver et al. (13) found that the mutagenic potencies of the stack fly ash from a medical pathological waste incinerator and from an adjacent industrial boiler were similar when both combustors were operated under "normal" conditions. This finding was unexpected considering the vastly different fuels for the medical waste incinerator and the industrial boiler burning no. 6 residual fuel oil. This assay was also able to determine significant increases in mutagenic emissions resulting from "upset" burn conditions caused by auxiliary burner failure. Linak et al. (15) and DeMarini et al. (15) determined mutagenicity emission factors for incineration experiments with the pesticide Dinoseb in a fuel oil/xylene solvent. Results with either air staging or air staging and reburning were similar to those measured for the burning of fuel oil for residential heating.

The primary goal of the work reported here was to begin development of procedures to assess mutagenicity of emissions from municipal or hazardous waste incinerators. Specific objectives were to a) field test two types of high-volume source-dilution samplers, b) collect stack samples from three incinerator units for use in developing analytical and mutagenicity bioassay techniques, c) determine the mutagenicity of organics associated with particle emissions, d) determine the stack emission rate for organic mutagens, and e) identify specific compounds/mutagens that are present in emission samples. This paper presents data on the mutagenic activity of stack gas emission samples and emission rates from a municipal waste incinerator, a municipal waste/hospital medical pathological waste incinerator, and an incinerator dedicated to hospital medical pathological waste combustion (HMPWIC). These data are compared to results from earlier MWC research and to other combustion sources (11-13,16). Details of the field sampling portion of this study will be described elsewhere (P.M. Lemieux, personal communication).

Experimental
Sample Collection

Two prototype source dilution samplers were field
tested at three sites in three U.S. states. These samplers, the 10 cfm (0.28 m³/min) source dilution sampler (SDS) (17) and the 100 cfm (2.88 m³/min) baghouse/dilution tunnel sampler (18), were designed to collect the large gram or even kilogram quantities of sample required for in vitro and in vivo toxicological studies including mutagenicity bioassay and animal carcinogenicity studies and for identification of principal organic components responsible for mutagenic/carcinogenic activity. Both samplers bring outdoor air into a mixing chamber to dilute emission gases in a 10:1 ratio; i.e., 100:10 for the SDS sampler and 1000:100 for the baghouse sampler. This dilution process simulates the flue gas quenching that occurs upon emission from the stack to the atmosphere and was incorporated into the design to give organic vapors a sufficient sampler residence time to condense on fine particles before being collected on a particle filter or trapped in the baghouse. Both units also have the possibility of collecting semivolatile organics on XAD-2 or other cartridge filters placed downstream from the particle filters; however, XAD-2 collections were not used in the present study.

In the SDS sampler, outdoor air was heated to approximately 75°F (24°C) and was filtered through HEPA (high-efficiency particulate air filter) and charcoal before entering the dilution chamber. Diluted particles were collected on round (approximately 26 in. [66 cm] diameter) Teflon-impregnated glass-fiber (TIGF) filters. A modified SASS cyclone used at the sampler inlet removed particles of > 2.5 μm before dilution and collection.

The baghouse/dilution tunnel sampler was also designed to filter heated outdoor air through HEPA and charcoal filters before mixing with the stack gases in a mixing chamber. Mixed gases passed into a baghouse unit containing a felt filter cartridge. This filter fabric consisted of a Gore-Tex membrane backed with 100% Nomex fiber. Periodically, inlet gas flow was interrupted long enough to use a reverse pulse of high pressure nitrogen to dislodge particles from the filter into a glass collection bulb at the bottom of the unit.

Source samples were collected by the SDS sampler in two field studies during September and November 1988 from a MWC unit (site A) and during December of 1988 from a MWC/HMPWC at site B. The baghouse sampler was field tested for the first time during the second sampling study at site A and then tested again during the sampling study at site B. The baghouse heater for the dilution air was not installed before sampling at site A. A third sampling study, which used only the baghouse sampler, was conducted during August 1990 at the HMPWC unit located at site C. The baghouse sampler at this site C, with the dilution air heater installed, was primarily used to gain further experience with operation of this modified sampler and to attempt to collect sufficient sample size for mutagenic and carcinogenic determinations. An experimental sampler was also installed upstream from the baghouse at site C to draw a small portion of the sample/dilution air mixture through a 142 mm TIGF particle filter. This small sampler flow was approximately 300% of isokinetic. Sampling was not conducted at isokinetic rates at any of the three sites.

The incinerator at site A had two refuse-fired boilers, each with a capacity of 100 tons/day. Each combustion unit had a reciprocating stoker, an economizer, and an electrostatic precipitator (ESP). Combustion emissions were vented into a common stack. The sampling probe was inserted into the emission gases from one boiler just before their entrance to the base of the stack. Stack gas temperature measured after the ESP unit was 425°F (218°C).

The incinerator at site B consisted of two 50 ton/day Consuntam starved-air combustors with a common ESP and stack. The unit burns primarily municipal waste and about 3–5 tons/day of hospital wastes. Stack gas temperature measured just after the ESP unit was 483°F (251°C). Sampling was conducted in the stack just downstream from the ESP outlet.

The HMPWC unit at site C consisted of a single 6.8 ton/day Consuntam starved-air combustion system. No air pollution control devices were used on this unit. Stack gases leave the secondary chamber and are vented to the atmosphere through the stack. The incinerator is operated for an 8-hr period and burns approximately 500–800 waste boxes/day, each weighing an average of 17.5 lb (7.9 kg). Sampling was performed with the baghouse sampler probe placed in the transfer duct between the secondary combustion chamber and the stack, where the gas temperature was approximately 1800°F (982°C). Stack emissions were sampled at a lower rate than used at sites A and B due to the requirement of limiting the dilution tunnel inlet temperature to a maximum of 450°F (232°C) so as to avoid decomposition of Teflon particles in the sampler. Site C emissions were also diluted with outdoor air to a greater extent than at the other sites due to the lower sampling rate. In addition to the baghouse samples, experimental particle samplers were fitted to the dilution tunnel before and after the baghouse. These samplers pulled dilution tunnel gases through a 142 mm TIGF filter at rates of approximately 2.6 ft³/min (4.4 m³/hr). The post-baghouse TIGF particle sampler pump failed, however, soon after start-up and did not collect a sample.

**Extraction and Fraction**

The SDS filters were cut into small pieces, placed in a glass or Teflon container along with 150 mL of dichloromethane (DCM) and sonicated (Branson 117 V 50/60 Hz Sonicator) for 10 min at 77°F (25°C). This process was repeated two additional times. Combined extracts were filtered in an all-glass apparatus through a 0.45 μm Teflon filter. Extracts were concentrated by rotary vacuum evaporation at 95°F (35°C) and transferred to volumetric flasks where volumes were adjusted to 10 mL. Aliquots were removed for gravimetric determination of extractable organic mass (EOM) and for solvent exchange to dimethyl sulfoxide (DMSO) before bioassay (19). Gravimetric determinations of EOM for each sample extract were performed using two or three 0.5-mL aliquots from the known volume of extract. Aliquots were placed in tared aluminum weigh pans, and solvent was evaporated in a hood. Sample pans were then equilibrated overnight in a desiccator before final weighing. Baghouse particles were Soxhlet extracted.
for 24 hr with 850 mL of DCM. Solvent cycle time was 13.5 min. Extracts were filtered, concentrated, volume adjusted, and aliquoted for gravimetric and bioassay determinations as above.

Aliquots representing specific amounts of EOM were solvent exchanged to DMSO and bioassayed whole or were fractionated on a nonaqueous ion-exchange column before DMSO solvent exchange and bioassay. The fractionation scheme used a quaternary ammonium styrene ion-exchange resin, Bio-Rad AGMP-1, to class fractionate DCM extracts of incineration samples. This solid phase extraction (SPE) procedure is described in more detail elsewhere (20) and is a modification of an earlier procedure (21). The modified procedure used 1 mL of washed and activated resin in a 1 cm i.d. column. Sample aliquots of <500 µL of DCM extracts were placed on the column and four 16-ml eluates were separately collected. The sequential elution solvents were a) DCM, b) methanol, c) methanol saturated with CO₂, and d) 10% trifluoroacetic acid in methanol. Gravimetric analyses on aliquots of the four fractions showed that fraction a contained approximately 70% of the organic mass placed on the column. Fraction a, used for determinations of mutagenic potency and emission rates, was concentrated and prepared for bioassay as previously described for whole sample extracts.

**Mutagenicity Testing**

The Ames *Salmonella typhimurium* histidine reversion assay (3) with strain TA98 was used for mutagenicity bioassay. This strain contains a -1 frameshift mutation. Thus, it permits the recovery only of additions and deletions of the appropriate type to produce a revertant. The limitations of this allele have been discussed recently (22). Samples having sufficient EOM to meet the minimum detectable limits of the assay were tested at a minimum of five doses using triplicate plates with and without Aroclor-induced rat liver S9 metabolic activation (+ S9 and - S9) at each dose. Duplicate plates were used for samples with limited quantities. The minimum amount of sample for testing was approximately 0.5 mg. Sample extraction/elution solvents were exchanged to DMSO to make bioassay stock solution concentrations of 1 mg/mL. Spontaneous counts for TA98 were 25–50 colonies per plate after a 72-hr incubation. Mutagenicities were confirmed by streaking revertant colonies onto minimal medium supplemented with biotin, but not histidine. A set of positive controls were incorporated in each experiment. These controls included 2-aminoanthracene (0.5 µg/plate) in the presence of S9 (average of 821 revertants/plate) and 2-nitrofluorene (3.0 µg/plate) in the absence of S9 (average of 487 revertants/plate). A negative bioassay control used in each experiment consisted of a DMSO blank. The range for spontaneous values (DMSO) were 32–61 for TA98 (+ S9) and 31–55 for TA98 (– S9). Controls, consisting of blank filters and/or laboratory blanks, were extracted and analyzed in parallel with actual samples.

Dose–response plots (revertants/plate versus microgram of EOM/plate) were constructed, and the mutagenic potency (slope of the linear regression of the dose–response curve) was calculated and expressed as revertants per microgram of EOM. At least three doses were used to construct each dose–response curve. By multiplying the mutagenic potency by the percent EOM obtained by DCM extraction, the number of revertants/milligram of particles was calculated. Stack gas flow rates and particle collection rates were also used to calculate revertants/cubic meter of emission gas, and stack emission rates in revertants/unit of time.

**Results and Discussion**

The mass burn municipal waste incinerator at site A was sampled in September 1988 with the SDS sampler and again in November of the same year with both the SDS and baghouse samplers. The SDS sampler collected five filter samples containing particle masses of 1.3–3.8 g during the first sampling study and one filter containing 3.4 g of particles during the second sampling trip. Collection periods were from approximately 1 to 4.5 hr. Table 1 lists the volumes collected during each period and also shows particle concentrations of 35–102 mg/m³ of stack gas. The percent EOM values ranged from 0.26 to 1.72% with a mean of 0.77%. The EOM concentrations in stack gases

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**Table 1. Particle and extractable organics in incinerator emissions.**

| Site A, MWC | Site B, MWC/HMPWC | Site C, HMPWC |
|-------------|--------------------|--------------|
| SDS filters | SDS filters        | Baghouse samples | TIGF |
| 1 | 2 | 3 | 4 | 5 | 3/2 | 7 | 21 | 23 | 1 | 2 | 3 | 4 | 5 | 1,3,4 |
| Standard m³ collected | 40.7 | 57.3 | 80.3 | 12.8 | 63.8 | 42.1 | 68.8 | 140.9 | 141.6 | 72.5 | 268.1 | 214.2 | 359.4 | 3.57 |
| Particles, mg/m³ stack gas | 93.4 | 34.9 | 41.1 | 101.6 | 36.1 | 78.9 | 78.5 | 52.5 | 33.2 | 33.1 | 16.2 | 62.8 | 8 | 59.7 |
| % EOM | 1.72 | 0.62 | 0.76 | 0.82 | 0.43 | 0.26 | 0.31 | 0.23 | 0.46 | 0.25 | 0.14 | 0.04 | 0.15 | 1.04 |
| EOM, mg/m³ stack gas | 1.61 | 0.22 | 0.31 | 0.83 | 0.16 | 0.21 | 0.24 | 0.12 | 0.15 | 0.08 | 0.02 | 0.03 | 0.01 | 0.02 |
| EOM, mg/min from stack | 1037 | 142 | 200 | 535 | 103 | 135 | 94 | 47 | 59 | 9.8 | 2.7 | 3.1 | 1.4 | 75 |
| Particles, g/min from stack | 60.1 | 22.5 | 26.5 | 65.4 | 23.2 | 50.8 | 30.9 | 20.6 | 13.0 | 4.0 | 2.0 | 7.6 | 1.0 | 7.2 |

**Abbreviations:** MWC, municipal waste combustion; HMPWC, hospital medical pathological waste combustion; TIGF, teflon-impregnated glass-fiber filter; EOM, extractable organic mass.

*Stack gas emission rates (wet basis) site A = 644 standard m³/min (flue gas temperature of 425°F); site B = 393 standard m³/min (flue gas temperature of 483°F); site C = 120.6 standard m³/min (flue gas temperature of 1400°F).*
were 0.16–1.61 mg/m³. Particle and EOM emission rates were calculated for the six SDS samples collected during the two sampling periods at site A. Average emission rates were 359 mg/m³ for EOM and 41.4 g/min for particles. These rates were calculated with a stack gas emission rate of 644 wet standard m³/min.

The weather conditions and consequently the nature of the municipal waste being burned were quite different for the two sampling studies at site A. Rainy weather during the second sampling study resulted in wet trash being fed to the two MWC units and, consequently, emission gases contained a higher percentage of water. This burn condition plus intake of water saturated dilution air (10:1 ratio of air to sample) led to condensation of very corrosive gases that damaged the stainless-steel transfer lines and sampler pumps. Condensation problems were also encountered inside the baghouse sampler, which caused collection of black liquid/particulate mixture in the sample jar. These liquid baghouse samples were deemed unusable for mutagenicity analysis.

The SDS samples collected during the same second study at site A were also different in character from those collected during the September sampling study. Only one SDS sample (identified as 3/2 in Table 1) was suitable for analysis. The DCM extract of this filter was corrosive, and mass determinations on aliquots of the extract resulted in aluminum weigh pans being corroded and mass determinations voided because of sample leakage. Polypropylene weigh pans, therefore, had to be used for these EOM analyses. A baghouse particle sample, which was obtained only after dismantling the filter housing assembly and physically rapping to dislodge particles, consisted of 102 g of wet filter cake. DCM extracts of this sample were also found to be corrosive. Mutagenicity assay of samples collected from both types of samplers failed because extracts proved to be too cytotoxic.

A sampling study at the site B MWC/HMPWC was initiated before analyses were completed for site A samples. Results for three SDS filters are reported in Table 1. Particle concentrations in stack emissions averaged 55 mg/m³. This value is a close comparison with the average particle concentration of 64 mg/m³ found at site A. The percent EOM and EOM concentrations (milligrams per cubic meter) in the stack emissions were also similar to those from site A. The smaller particle and EOM emission rates are partly due to the lower stack emission rate of 389 standard m³/min. These Table 1 values from sites A and B, however, are all remarkably similar. Corrosion problems were again encountered while sampling with the SDS sampler, and extracts of the SDS filters from site B were also found to be cytotoxic. The baghouse sampler at site B failed to collect a sufficient amount of particles for bioassay analysis.

A nonaqueous solid phase extraction (SPE) scheme (20), which was developed to separate acidic fractions from neutral organics, was applied to selected extracts from sites A and B. These highly acidic fractions are often too cytotoxic to measure any mutagenic activity. The first SPE column fraction, the DCM neutral fraction, was found to contain approximately 70% of the organic mass placed on the column. Moreover, this fraction did not exhibit cytotoxicity in the Ames bioassay.

Table 1 shows results for the HMPWC unit at site C. Four samples represent baghouse particles and one sample is a composite of three 142 mm TIGF filters collected by the experimental sampler with the intake positioned in the mixing chamber upstream from the baghouse sampler. The particle concentrations calculated for the baghouse samples at this site are approximately half of the amounts from sites A and B; however, the 59.7 mg/m³ particle concentration determined for the composited TIGF particle filters is very similar to the 64 mg/m³ average for site A and the 55 mg/m³ average for site B. The 0.15% average EOM for the baghouse samples are also about half of the average value for site B (0.33%) and one-fifth of the site A value (0.77%). The TIGF EOM of 1.04% is again similar to that observed from site A. Additional testing of the baghouse sampler is being planned to make further comparisons between the particles collected by the baghouse and those collected on a particle filter; e.g., the TIGF filter media used in the SDS sampler.

Table 2 shows mutagenic measurements for emissions expressed as revertant colonies (rev)/milligram of particles and rev/microgram of EOM, mutagenicity concentra-

| Site  | Sample no. | Fraction | /- S9 | mg Particles | µg EOM | m³ | Revertant colonies per |
|-------|------------|----------|-------|-------------|--------|-----|------------------------|
| A     | 1          | Whole    | +     | 458         | 26.6   | 42800 | 276 × 10⁶              |
| A     | 1          | Whole    | -     | 339         | 19.7   | 31700 | 204 × 10⁶              |
| A     | 2,3,4      | Whole    | +     | 6.9         | 0.95   | 304   | 1.96 × 10⁶             |
| A     | 2,3,4      | Whole    | -     | 25.4        | 3.48   | 1115  | 7.18 × 10⁵             |
| A     | 2,3,4      | Neutral  | +     | 353         | 48.3   | 15479 | 100 × 10⁵              |
| A     | 2,3,4      | Neutral  | -     | 351         | 48     | 15386 | 99 × 10⁵               |
| C     | BH 1       | Whole    | +     | 2.95        | 1.2    | 98    | 0.12 × 10⁵             |
| C     | BH 2       | Whole    | -     | 6.11        | 2.49   | 202   | 0.24 × 10⁵             |
| C     | BH 2       | Whole    | +     | 1.41        | 1.08   | 23    | 0.05 × 10⁵             |
| C     | BH 2       | Whole    | -     | 1.37        | 1      | 22    | 0.03 × 10⁵             |
| C     | BH 3,4     | Whole    | +     | 0.44        | 1.07   | 27    | 0.03 × 10⁵             |
| C     | BH 3,4     | Whole    | -     | 0.94        | 2.3    | 59    | 0.07 × 10⁵             |
| C     | BH 5       | Whole    | +     | 2.69        | 1.82   | 21    | 0.05 × 10⁵             |
| C     | BH 5       | Whole    | -     | 1.77        | 1.2    | 14    | 0.02 × 10⁵             |
| C     | TIGF 1,3,4 | Whole    | +     | 7.78        | 0.75   | 456   | 0.56 × 10⁶             |
| C     | TIGF 1,3,4 | Whole    | -     | 18.77       | 1.81   | 1221  | 1.36 × 10⁶             |

*Stack gas emission rates (standard cubic meters/minute): site A = 644; site C = 120.6.
tions (rev/cubic meter) and emission rates (rev/minute) for samples taken from incinerators at sites A and C. Results are shown for whole samples (unfractionated) and DCM neutral fractions (fraction 1) from SPE column separations. Three SDS filters (2, 3, and 4) from site A were composited before bioassay to provide sufficient sample size for further chemical characterization studies on high-performance liquid chromatography (HPLC) subfractions of the first elution (neutral fraction) from the SPE column. This bioassay directed fractionation and chemical characterization work is a continuing effort to identify specific compounds and/or compound classes that are responsible for observed mutagenicity. The small (142 mm) TIGF filters from site C were also combined to provide sufficient sample size for bioassay. The mutagenic potency data for the site A filters show divergent values for the whole sample extract. Filter 1 gave a + S9 value of 27 rev/μg compared to 0.95 rev/μg for the composite sample (filters 2, 3, and 4). The fractionated composite sample, however, shows a potent first (neutral) fraction from the SPE column, indicating the possibility that the 0.95 rev/μg value for the whole sample was depressed due to the presence of acidic cytotoxic components that inhibit the expression of mutagenicity. This type of bioassay requires that the Salmonella cells remain viable during incubation. A fractionation step, therefore, may be necessary for some or all incinerator samples to remove such interferences before the mutagenicity assay. Site B whole samples were also cytotoxic, and analyses of SPE fractions failed to demonstrate mutagenicity.

The emission factors in Table 3 were developed to make comparisons with previous MWC studies and other combustion sources. The mutagenicity per hour (rev/hr) values from site A closely match those reported for an incinerator in Aichi Prefecture, Japan (11). Better comparisons, however, can be made for emission results reported as revertants per kilogram of fuel. A study of four MWC units in Sweden reported approximately 10,000–100,000 revertants per megajoule (rev/MJ) of fuel (12). These values were converted to revertants per kilogram of fuel using the factor of 11.61 MJ/kg of municipal solid waste (MSW). These values ranged from 1 to 12 × 10^5 rev/kg, which compare with the 1.6 to 4.4 × 10^5 rev/kg values from the MWC at site A and 0.7 × 10^5 rev/kg from the HMPWC at site C. Data for comparison are also presented for other combustion sources, i.e., residential heating, industrial and utility boilers, and power plants burning various fuels, and for diesel- and gasoline-powered vehicles. The similarity of these emissions factors indicates that the mutagenic potency (revertants per microgram EOM) of emissions may not be greatly affected by the fuel source. The combustion condition or “completeness of combustion” combined with the effectiveness of pollution control equipment operating at the incinerator may be much more relevant factors to consider for controlling the mutagenicity of incinerator emissions.

The Table 3 emission factor for wood stoves of 6 × 10^6 rev/hr (23) is comparable to the 3.4 × 10^6 rev/hr found in

| Source | Revertants per fuela | M.Jb |
|--------|---------------------|------|
| MWC, site A | 1656 × 10^6 | 4.4 × 10^3 | 3.8 × 10^4 |
| MWC, site A neutral fraction | 600 × 10^6 | 1.6 × 10^3 | 1.4 × 10^4 |
| HMPWC, site C | 3.4 × 10^6 | 0.7 × 10^3 | 580 |
| MWC (11) | 1008 × 10^6 | 1–12 × 10^3 | 1–10 × 10^4 |
| Residential heating (16) | 50 × 10^5 | 2.5 × 10^3 |
| Industrial and utility boilers and power plants (16) | 40 × 10^5 | 230 |
| Automobiles and trucks (16) | 40 × 10^5 | 1000 |
| Diesel vehicles | 10 × 10^3 |
| Diesel trucks/buses | 10 × 10^3 |
| Gasoline noncatalyst | 1 × 10^2 |
| Gasoline catalyst | 1 × 10^2 |
| Wood stove (23) | 6 × 10^5 |
| Gasoline car (24) | 0.5 × 10^6 |
| Diesel car (24) | 6 × 10^6 |

Abbreviations: MWC, municipal waste combustion; HMPWC, hospital medical pathological waste combustion.
aWaste burn rates (kg/min): site A = 63; site C = 84.
bMegajoule: literature value or calculated for MWC using 11.614 MJ/kg of municipal solid waste.

cCalculated revertants/kg municipal solid waste using factor of 11.614 MJ/kg municipal solid waste.

The data reported here should be considered as pilot study information obtained during initial field testing of the SDS and baghouse/dilution tunnel samplers. Isokinetic sampling was not used at any of the incineration sites. Problems were encountered during each sampling event (e.g., condensation inside sampler housings and corrosion damage) which are currently being addressed. The analytical operations of sample extraction, gravimetric determinations, and bioassay also encountered problems related to the corrosive nature of extracts and to cytotoxic species that often prevented or otherwise affected mutagenic potency analyses on the whole or unfractionated extracts. A fractionation scheme had to be developed before bioassay could be successfully performed.

Engineering modifications have been made on the sampling equipment, and further field testing is needed to continue the development of incinerator emission sam-

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|-----------------|---------------------|------|
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| MWC, site A neutral fraction | 600 × 10^6 | 1.6 × 10^3 | 1.4 × 10^4 |
| HMPWC, site C   | 3.4 × 10^6          | 0.7 × 10^3 | 580 |
| MWC (11)        | 1008 × 10^6         | 1–12 × 10^3 | 1–10 × 10^4 |
| Residential heating (16) | 50 × 10^5 | 2.5 × 10^3 |
| Industrial and utility boilers and power plants (16) | 40 × 10^5 | 230 |
| Automobiles and trucks (16) | 40 × 10^5 | 1000 |
| Diesel vehicles | 10 × 10^3           |
| Diesel trucks/buses | 10 × 10^3 |
| Gasoline noncatalyst | 1 × 10^2 |
| Gasoline catalyst | 1 × 10^2 |
| Wood stove (23) | 6 × 10^5            |
| Gasoline car (24) | 0.5 × 10^6         |
| Diesel car (24) | 6 × 10^6            |

Abbreviations: MWC, municipal waste combustion; HMPWC, hospital medical pathological waste combustion.
aWaste burn rates (kg/min): site A = 63; site C = 84.
bMegajoule: literature value or calculated for MWC using 11.614 MJ/kg of municipal solid waste.

cCalculated revertants/kg municipal solid waste using factor of 11.614 MJ/kg municipal solid waste.

This study at the site C incinerator. Table 3 emission factors reported for gasoline and diesel vehicles range from 1 to 40 × 10^6 rev/kg of fuel (16). A comparison of the 0.5 × 10^6 rev/hr rate for a gasoline catalyst car (24) with 1000 × 10^6 rev/hr from a MWC unit (11) shows that MWC emission to be equivalent to approximately 2000 gasoline catalyst cars. However, it should be noted that comparisons between emission sources may be misleading. Automobile exhausts contain highly mutagenic, but not necessarily strongly carcinogenic, species, and MWC emissions may contain strongly carcinogenic species (i.e., chlorinated dibenzo-p-dioxins) that are not mutagenic (12).

The data reported here should be considered as pilot study information obtained during initial field testing of the SDS and baghouse/dilution tunnel samplers. Isokinetic sampling was not used at any of the incineration sites. Problems were encountered during each sampling event (e.g., condensation inside sampler housings and corrosion damage) which are currently being addressed. The analytical operations of sample extraction, gravimetric determinations, and bioassay also encountered problems related to the corrosive nature of extracts and to cytotoxic species that often prevented or otherwise affected mutagenic potency analyses on the whole or unfractionated extracts. A fractionation scheme had to be developed before bioassay could be successfully performed.

Engineering modifications have been made on the sampling equipment, and further field testing is needed to continue the development of incinerator emission sam-
plers. The quality assurance aspects of field sampling, sample handling, and analytical procedures all need additional study. The SPE fractionation method used and perhaps other similar methods need to be further examined for their suitability in removing cytotoxic species and separating organics into class fractions. Additional bioassay-directed fractionation procedures [e.g., HPLC coupled with microsuspension bioassay (25)] are being used to subfractionate the neutral fraction for further examination by mass spectroscopy to identify and quantify the principal organic mutagens. Further studies on the mutagenicity of emissions resulting from incineration of components of municipal waste, such as plastic, have also been performed (26).

Summary

Two types of incinerator stack samplers were field tested at three sites. Emission samples were collected from municipal waste and hospital medical pathological waste incinerators. Organics associated with emission particles were bioassayed for mutagenicity using the Ames plate incorporation assay. Stack emissions, which were characterized by particle concentration and percent extractable organic mass, were similar, although there were large differences in the nature of the fuel materials consumed at the various sites. Emissions, characterized by mutagenicity emission factors, also were similar for these same sites and were similar to those previously reported for a hospital medical pathological incinerator and for industrial and utility boilers burning coal, wood, and oil. The mutagenicity of incinerator emissions, therefore, may not be greatly affected by the fuel source. Burn conditions and pollution control devices are likely to be more important considerations for ensuring safest possible emissions.

Mutagenicity concentrations reported here for a MWC unit are also similar to those reported by other researchers. The mutagenicity of sample fractions from a MWC sample extract indicates the presence of potent organics mutagens associated with particle emissions. However, additional research is needed to identify such species and to determine their emission rates from various incinerators. Additional studies are also needed to further improve sampling equipment, sampling and sample handling procedures, analytical and sample preparation methods, and bioassay procedures.

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