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65 A carcinogenesis bioassay of condensed pyrolysis effluents from iron foundry casting operations using hamsters was conducted. Syrian-golden-hamsters were administered effluents from shell sand, green sand, furan (110009) or urethane (51796) casting molds intratracheally weekly for 18 weeks. Concentrations were 5 or 10 milligrams (mg) per 0.2 milliliter distilled water except for effluents from the urethane mold which were administered in amounts of 2.5 or 5.0mg. Positive controls were given 3.0mg benzo(a)pyrene (50328) plus 3.0mg ferric-oxide (1309371) or 3.0mg ferric-oxide. The animals were maintained until 98 percent mortality occurred or up to 103 weeks in the case of females or 117 weeks in the case of males. None of the effluents induced a significant carcinogenic response. Comparable numbers of respiratory tract tumors occurred in effluent treated and vehicle controls. Malignant lung tumors occurred with an incidence of 54 to 64 percent in the positive controls. Alveolar and bronchiolar cell hyperplasia was round in all effluent treated and control animals. Nonrespiratory tract tumors occurred sporadically in all treated and control animals. The authors conclude that foundry mold effluents are not carcinogenic in hamsters at the concentrations used. The doses may have been too low, however, to induce a carcinogenic response.

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PREFACE

Recent epidemiological studies suggest that certain foundry workers have a higher than normal risk of lung cancer. The present study was conducted as part of an effort to determine whether the increased risk is due to the presence of carcinogens in mold emissions. The work was supported by the National Institute for Occupational Safety and Health under Contract No. 210-78-0033 with Borriston Laboratories, Inc., Temple Hills, Maryland.

The project was initiated on September 28, 1978. The first contract year was devoted to the selection of the foundries and the development of the methodology for sample collection. During the foundry selection process, surveys were made in order to identify pouring lines which met the criteria for the study, and extensive negotiations were conducted to ensure the participation of the foundries. Since established sample collection methods suitable for this study could not be identified, a custom-designed sampler was developed which will provide technology for future investigations. Sample collection for the first phase of the bioassay, the acute range-finding study, was conducted during September, 1979 through March, 1980. The acute studies were begun in October, 1979 and were completed in May, 1980. The collection and analysis of emission samples for the second phase of the bioassay, the chronic carcinogenicity study, were performed during March, 1980 through January, 1981. The chronic study was initiated in April, 1981 and was completed in July, 1983.

Dr. W. G. Palmer served as Principal Investigator throughout the sample collection and analysis phases and the acute range-finding studies. Dr. L. T. Mulligan assumed the role of Principal Investigator for the chronic bioassay. Mr. William Moorman served as the NIOSH Project Officer throughout the contract. Dr. T. E. Shellenberger functioned as the Principal Investigator during the final report preparation. Key technical personnel responsible for the conduct of the bioassays were Mr. Jess Rowland, Technical Supervisor, and Ms. Ruth Ramseur, Laboratory Technician. Gross and microscopic pathologic evaluations were performed by Dr. F. M. Garner. The present report was prepared by Dr. W. G. Palmer (sample collection and analysis) and Ms. Linda Plankenhorn (acute range-finding and chronic carcinogenicity studies).

We gratefully acknowledge Robert Scholz and Calvin Bruce of Rexnord, Inc. for their dedicated technical support in the development of the sampling method and the collection of the foundry samples.

CARCINOGENIC POTENTIAL OF CONDENSED PYROLYSIS EFFLUENTS
FROM IRON FOUNDRY CASTING OPERATIONS
BORRISTON PROJECT NO. 0102

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I. ABSTRACT

A study designed to determine the carcinogenic potential of condensed pyrolysis effluents obtained from iron foundry casting operations was performed by Borriston Laboratories, Inc. Emissions from four types of casting molds (shell sand, green sand, furan and urethane) were collected at the foundries, analyzed and prepared for tests of their ability to induce cancer when administered repeatedly to the respiratory tracts of hamsters by intratracheal instillation.

A 5-week range-finding study was conducted to determine doses to be used for the chronic carcinogenicity study. Based on the results of this preliminary study, two dose levels (the maximum tolerated dose and one-half the maximum tolerated dose) were recommended for each test material. Dose levels of 5.0 and 10.0 mg were selected for the shell sand, green sand and furan samples. Dose levels of 2.5 and 5.0 mg were chosen for the urethane sample due to acute toxic responses observed in animals treated with this material.

The chronic bioassay was initiated on April 20, 1981. Each effluent sample was administered intratracheally to hamsters at the specified dose levels in 0.20 ml distilled water. A vehicle control group received intratracheal doses of 0.20 ml distilled water. A positive control (3.0 mg BaP + 3.0 mg Fe_2O_3) and a ferric oxide control group (3.0 mg Fe_2O_3) were also used. Each test group consisted of 50 male and 50 female Syrian golden hamsters. Intratracheal instillations were performed once weekly for 18 consecutive weeks. After the dosing phase, hamsters were held for observation until 98% mortality was attained.

Intratracheal administration of effluents from shell sand, green sand, furan and urethane casting molds at the dose levels used in this study did not induce a carcinogenic response in male or female hamsters. Respiratory tract tumors were diagnosed with low and approximately equal incidence in the effluent-treated and vehicle control groups.

Alveolar/bronchiolar cell hyperplasia was present to some degree in all groups, including the vehicle control. However, the increased incidences observed in several effluent-treated groups (males and females treated with shell sand and furan effluents at both dose levels, males treated with green

sand effluent at both dose levels, and females treated with green sand effluent at the high-dose level) were attributed to the test materials. This hyperplastic response is common in the hamster, even with relatively mild insult to the lungs, and is not necessarily preneoplastic. The reaction was rarely extensive enough to be classified as adenomatosis.

Non-respiratory tract tumors were observed with sporadic incidence in all treated and control groups and were of the types that spontaneously occur in this species. The non-neoplastic pathologic alterations observed in other organ systems were random changes unrelated to treatment. The most consistently noted changes (amyloidosis, heart thrombosis, chronic nephropathy and hepatic cysts) occur spontaneously in hamsters and are related to the aging process.

Under the conditions of this study, the foundry mold effluents failed to induce a carcinogenic response in the hamster respiratory tract. However, these negative results do not prove that foundry casting emission products are not carcinogenic. The effluents are complex mixtures of a wide range of chemical and mineral components with known acute and chronic toxic properties. Dose levels used in this study were limited by the high acute toxicity of the effluent samples, and this dose limitation may account for the low tumor yields.

II. SAMPLE COLLECTION AND ANALYSIS

A. SUMMARY

Recent epidemiologic studies in the United States and abroad indicate that certain foundry workers, particularly molders and metal pourers, have a higher than normal risk of lung cancer. The current study was conducted as part of an effort to determine whether the increased risk is due to the presence of carcinogens in mold emissions. Emissions from molds composed of four types of binders, including furan (urea-formaldehyde-furfuryl alcohol), green sand with sea coal (pulverized bituminous coal), urethane (phenolic-isocyanate), and phenol-formaldehyde resins in shell molds were collected, analyzed, and prepared for tests of their ability to induce cancer when administered repeatedly to the respiratory tracts of hamsters by intratracheal instillation.

Source samples were collected from molds shortly after they were poured. Emissions from shell and green sand molds were collected from dense smokes in cooling tunnels serving fully automated pouring lines. Samples from urethane molds were collected with the use of a shroud placed over moving molds as they passed through a cooling hood serving a manually operated pouring line. Emissions from furan molds were collected from vent holes on the top of large individual molds which were poured and cooled on the open foundry floor.

Emissions were collected with a wet scrubber sampler. The sampler was designed specifically for use in this study to efficiently collect those components of the emissions that could be administered to hamsters by intratracheal instillation. These components consisted of respirable particles between 0.5 to 10 μm in diameter and water-soluble vapors and gases. Water-insoluble organic chemicals were not collected because they cannot be administered to the lungs by intratracheal instillation. The sampling device consisted of three units: a cyclone precleaner which eliminated particles larger than 10 μm; a venturi scrubber in which respirable particles and water soluble vapors and gases were trapped in water droplets; and an entrainment separator in which the water vehicle and its entrapped components settled into a collection sump.

The bioassays were performed in two stages; the first was a 5-week range-finding study which determined the doses to be used for the second segment, the chronic carcinogenicity bioassay. The dose used for the latter test was the maximum tolerated dose (the maximum dose that the animals can tolerate without developing clinical disorders, other than cancer, that can shorten their lifespan).

Samples were collected twice at each foundry, once for each stage of the bioassay. The amount of each sample (7.5 grams) to be collected for the range-finding study was established by doses that were selected on the basis of the published literature. The quantity of sample required for the chronic bioassay was determined by the results of the range-finding study. Approximate totals of 36, 45, 26, and 35 grams of the green sand, furan, urethane, and shell emissions, respectively, were collected for the chronic bioassays and the attendant chemical analyses.

The collected samples consisted of a water-soluble phase and water-insoluble particulates. The portion of the dry weight of the samples represented by particulates varied from 1-2% for the urethane sample to 50% for the shell sample. Sixteen metals, eight polycyclic aromatic hydrocarbons, and five phenols were quantified in the collected emissions from each type of mold. The levels of polycyclic aromatic hydrocarbons were lowest in the urethane and furan samples. The levels of phenols were highest in the urethane samples. These data are characteristic of the source emissions that were collected and do not necessarily reflect the characteristics of mold emissions in the breathing zone of exposed workers.

B. BACKGROUND

1. The Sand Mold

Castings are made by pouring molten metal into molds. Sand is the basic ingredient of most molds used in iron foundries. Binders are added to hold the sand particles together and other additives are frequently included in the molding aggregates to impart desired characteristics to the surface of the casting. Various natural organic substances as well as synthetic chemical polymer systems may be used as sand binders. The synthetic chemical binders

have come into use during the past several decades. Until that time, green sand molds were the most common type.

The additives used in green sand molds include clay, coal dust, wood flour, and other natural organic materials which are added in amounts up to 8 percent of the total weight of the molds. Green sand molds are prepared by packing the molding aggregates around a pattern and compressing the sand particles into the mold under pressure.

Molds using synthetic chemical polymers as binders can be prepared by several different processes. Some of these employ heat to cure the binder systems (hot box molds) while others are composed of polymer systems that are catalyzed in the absence of applied heat (no-bake resins). The shell molding process, which was introduced in 1948, consists of coating each grain of sand with a phenolic resin. The coated sand is dumped on a metal pattern heated to 450° F. In 15-30 seconds, a shell of sand held together with fused resin is formed next to the pattern. The excess sand mix is removed by inverting the pattern, and the pattern with its adhering sand shell is cured 1 to 2 minutes in an oven at 800 to 1000° F. The cured mold is then stripped from the pattern. These molds are used for high precision casting and have the advantage of allowing the casting to cool rapidly.

Many molds contain cores which are used to form internal cavities in the castings. Cores are usually made with the newer synthetic chemical polymers or with oil sand systems.

2. Literature Survey

Epidemiologic investigations of the mortality rates of foundrymen have indicated that workers in certain areas of the foundries may experience an elevated risk of lung cancer. The first reports of elevated lung cancer mortality among foundrymen were issued in England and were based on census population figures and official death records. In 1938, Turner and Grace reported that foundry workers in Sheffield, England had the highest lung cancer rates among the 12 occupations examined.[1] In 1939, the Registrar General of Great Britain reported that molders and metal pourers ranked fourth among occupations with high lung cancer rates in London and Wales.[2] Milham

later reported similar findings for foundry workers in the state of Washington.[3] In a recent study, based on the death records from the International Molders and Allied Workers Union's death program, Egan and co-workers reported that there is a 1.5-fold excess of lung cancer cases among foundrymen.[4]

The epidemiological studies which are perhaps best known were those performed in Finland by Koskela et al.[5] and in Canada by Gibson and co-workers.[6] Both studies found that molders, casters and cleaning room operators were at a two- to threefold higher than normal risk of developing lung cancer. The Canadian group also found that crane operators are at an elevated risk of lung cancer, but this was not found to be the case in a study of crane operators in steel foundries performed by Lerer et al.[7] Finally, in a recent report of the lung cancer rates in workers in a U.S. foundry, Decoufle and Wood reported that there was a twofold excess of lung cancer in workers with more than 5 years exposure in the foundries.[8]

Several of these studies indicated that molders and casters are the foundry workers at highest risk of dying from lung cancer. In most cases these data reflect conditions which were present in the foundries at least 30 years ago. At that time, molders were pouring metal into the molds that they produced. Today, it is unusual for molders to pour metal into their own molds and a new group of workers, referred to as casters or pour-off men, has assumed this responsibility. Thus, the epidemiological data concerning the molders reflect exposures to cooling molds as well as to materials released during mold preparation. Other changes which have occurred in the foundries during the last 30 years include changes in control technologies that limit the exposure of workers to dust and fumes and the introduction of new manufacturing procedures which have altered the types of chemicals and dusts that workers are exposed to. Most notable, perhaps, is the use of new molding materials composed of synthetic chemical binders. The epidemiological studies do not reflect the exposure of workers to fumes from molds composed of these new synthetic chemical binders and it is only recently that the chemical and physical properties of emissions from these and from the more traditional green sand molds have been studied.

Benzo(a)pyrene (BaP) has been found in association with dusts and fumes in numerous locations in the foundries.[9] BaP and other polycyclic aromatic hydrocarbons (PAH) have been identified in condensates on particles emitted from molds using both traditional binders and the modern synthetic chemical binders. The concentrations of BaP liberated from dry sand and green sand molds were substantially higher than those liberated from molds composed of the newer synthetic chemical binders.[10] Several reports indicate that the addition of sea coal to green sand molding aggregates does not contribute to the formation of PAH but that coal tar pitch may be a major source of the PAH liberated from green sand molds.[9,11,12] *In vitro* bioassays performed with organic extracts of dusts collected from a green sand foundry indicate that the mutagenic activity of samples is greatest when the foundry is operating at full capacity. At such times, compounds other than PAH make the greatest contribution to the mutagenic activity of the extracts of the dust samples.[13]

C. OBJECTIVES

The purpose of this project was to collect emissions from green sand molds and from molds composed of some widely used synthetic chemical binders in preparation for bioassays of their carcinogenicity in hamsters. Emissions were collected from molds composed of four types of binders, including furan (urea-formaldehyde-furfuryl alcohol), green sand with sea coal (pulverized bituminous coal), urethane (phenolic-isocyanate), and phenol-formaldehyde resins in shell molds. The emissions were collected, analyzed, and prepared for tests of their ability to induce cancer when administered repeatedly to the respiratory tracts of hamsters by intratracheal instillation.

The results of the bioassays will be reported separately. However, since the route and frequency of administration of the samples to experimental animals established the quantities of mold emissions that would be needed, as well as the method to be used for sample collection, the design and scope of the bioassays are outlined below.

1. Design and Requirements of the Bioassays

For the bioassays, samples were administered to hamsters by intratracheal instillation. With this technique, a blunt-tipped needle is inserted into the

opening of the larynx and through most of the length of the trachea. A small volume of fluid containing the test sample is pushed from the syringe, through the needle and into the lower respiratory tract. The sample is injected as the animal inhales so that it is drawn into the peripheral lung.

The bioassays of the foundry samples were divided into two phases. Phase I, the acute range-finding study was conducted to establish dose levels for the chronic carcinogenicity study (Phase II). The dose of each foundry sample selected for the chronic bioassay was the maximum tolerated dose, or the highest dose that could be given to animals without shortening their life spans through causes other than cancer. This dose was established by examining the toxicological effects of a range of four different dose levels of each foundry sample.

In the acute range-finding study, four different doses of the foundry samples were administered to groups of 20 animals (10 males and 10 females) once a week for five weeks. The doses used for this study were 20, 10, 5 and 2.5 mg per instillation. Each dose was administered in a total volume of 0.20 ml per instillation. Throughout the study the animals were routinely examined for signs of ill health or clinical symptoms of toxicity such as moribundity, mortality, failure to gain weight, lethargy, diarrhea, blood in the excreta or other signs of obvious distress. One week after the fifth and final dose was administered, the animals were killed and the external and internal organs inspected for signs of gross abnormalities. These pathological and clinical observations served as the basis for determining doses for the chronic bioassays.

For the chronic bioassays, the maximum tolerated dose and one-half the maximum tolerated dose of each foundry sample were administered to two groups of 100 animals (50 males and 50 females per group). The doses for the green sand, furan and shell mold emissions were 10 and 5 mg per instillation; those for the urethane emissions were 5 and 2.5 mg per instillation. The animals were held until 98% had died. At that time, the remaining animals were sacrificed and examined by a board-certified veterinary pathologist who evaluated the tissues of the respiratory tract and the other major internal organs for the presence of tumors, the tumor type and the frequency of occurrence of each type of tumor.

Samples were collected twice at each foundry, once for each stage of the bioassay. Sufficient material was collected for the bioassays and the necessary chemical and physical analyses of the samples. Approximately 7.5 grams of emissions from each type of mold were collected for the acute range-finding study. For the chronic bioassays, approximately 45, 36, 26 and 36.5 grams of emissions were collected from furan, green sand, urethane and shell molds, respectively. Differences in the quantities required for each sample were due to 1) differences in doses; and 2) differences in the amount of particulates in each sample. The latter factor affected the amounts required for the PAH analyses since the quantities of PAH were determined separately in the aqueous phase and in the water-insoluble particulates and more materials were required for samples having low quantities of particulates. (The PAH were quantified only in the samples collected for the chronic bioassays.)

D. SAMPLE COLLECTION APPARATUS

1. Rationale For Design

The large quantities of concentrated materials required for the bioassays necessitated the use of a high volume sampler from which the samples could be retrieved in a concentrated form. Since the samples had to be administered to animals in an aqueous medium, a sampler which could trap mold emissions in water was desired.

Other requirements of the sampling method were that it efficiently collect particles between 0.5 and 10 μm in diameter and exclude particles larger than 10 μm in diameter; that collected particles be maintained as a fine dispersion rather than as agglomerates or a condensed tar; the sampler had to be portable and compact to facilitate its use in the foundries; it had to maintain the physical and chemical integrity of collected mold emissions; and organic solvents could not be used to retrieve the sample from the matrix of the collection device. The latter criterion was established because several studies have indicated that the potency of carcinogenic PAH may be influenced by their adsorption or condensation on the surface of respirable particles.[14,15] Some carcinogenic PAH and mutagenic compounds are known to be present in mold emissions as condensates on the surface of fine

particles.[13,10] The use of organic solvents could remove these compounds from the surface of particles and thereby alter the toxicity of the samples.

Established sampling methods that were considered included cold traps, solvent impingers, cyclones, electrostatic precipitators and porous membrane filters. Of these, the first three were rejected since they cannot efficiently collect particles less than 1 μm in diameter. A high volume electrostatic precipitator was tested and rejected because ozone, which is known to interact with PAH and other organic compounds,[16] was generated during its operation. Methods which trap particles on filters were also excluded since recovery of the particles from the matrix of the filters could be difficult.

Since established methods for the collection of large quantities of airborne materials which met the criteria for this study could not be identified, a sampler based on the principle of the venturi scrubber used in industrial stacks was designed. The sampler consisted of a train of three components. The first was a cyclone precleaner which removed particles greater than 10 μm in aerodynamic diameter, the second a venturi scrubber which trapped respirable particles in water droplets and the third an entrainment separator in which trapped particles were removed from the airstream.

This system afforded a means for collecting particles in the desired size range (0.5 to 10 μm) as well as water-soluble gases and vapors. Samples could be rapidly collected (up to 1 g per hour could be collected under the sampling conditions used in some foundries) and could be retrieved from the sampler in a highly concentrated form. Furthermore, since the airborne materials were collected directly into water, samples were virtually ready for administration to animals as soon as they were removed from the collection device. This represented a considerable advantage both in terms of convenience and from the standpoint that virtually nothing was known about the stability of the materials being collected and it was therefore essential to minimize the handling of samples after collection to reduce the possibility of denaturation.

2. Specifications

The wet scrubber sampler was manufactured by Fisher Klosterman, Inc. (Louisville, KY) in accordance with the following specifications:

1. As high an efficiency as possible in the particle size range 0.5 - 10 μm while satisfying the constraints listed below.

2. Collection of the sample in a minimum volume of fluid.

3. Airflow rate of 100 ft ^{3}/min in the temperature range of 21 to 74° C.

4. Removal of particulate greater than 10 μm before scrubbing.

5. Simple, non-fouling contact chamber free of internal adjustable mechanisms or intricate baffles.

6. Use of a peristaltic pump instead of an impeller pump for fluid transport.

7. Absence of fluid manifolds; fluid transported in replaceable tubing.

8. Provisions for quick disassembly of the unit to provide complete accessibility for cleaning and inspection.

9. Rated for continuous operation.

10. Inert lining on wetted contact chamber surfaces and tubing connectors.

11. Minimum amount of interconnecting ductwork between the sampling chambers.

3. Construction

The wet scrubber sampling system is composed of three cylindrical chambers:

1. A cyclone precleaner to remove particles larger than 10 μm (internal chamber dimensions - 5 in. diameter x 20 in. long).

2. A contactor (venturi scrubber) in which aerosol particles less than 10 μm contact finely divided water droplets (internal chamber dimensions - 4 in. diameter x 16 in. long).

3. An eliminator (entrainment separator) which removes the particle/droplet agglomerates from the airstream (internal chamber dimensions - 6 in. diameter x 26 in. long).

The three chambers were securely mounted on a steel cart. The assembled sampler (without the blower) was 25" L x 19" W x 35" H.

The chambers of the three sampling units were constructed from mild steel; the inner surfaces were lined with Teflon (Du Pont, Wilmington, DE).

The scrubber components were small in size so that size and weight would not hinder their maneuverability in the foundries. These air cleaning components of the wet scrubber sampler could be readily disassembled with non-removable hand-turned screws. The access flanges were located in a position that provided good accessibility for visual inspection and cleaning of internal surfaces. The venturi scrubber was fitted with a removable cover so that the sample collection fluid (deionized, filtered water) could be readily replenished during use. To satisfy the requirements for portability as well as high volume, a light weight, high-suction blower was used. This unit, the Paxton Series BD Centrifugal Compressor, has a rated capacity of 35 in. water suction at 100 ft^3/min (under standard conditions). The compressor part of this high speed unit (13,500 RPM, belt-driven) is constructed with die cast aluminum housing and impellers. Complete with its integral 2 hp motor, this blower weighs only 50 lbs and measures 10" W x 18" L x 12.5" H.

Fluids were recycled through the collection system by a Masterflex peristaltic pump (model 7535-00; Chicago, IL). The fluid lines consisted of Tygon Special tubing (Norton Co., Worcester, MA) with an inside diameter of 0.313 inches.

The blower and peristaltic pump were the only parts of the wet scrubber system that used electricity. The total electrical draw for the system was less than 20 amps (1 phase, 110 volts).

4. Basic Sampling Scheme

A schematic drawing of the wet scrubber sampler is shown in Figure 1. The air being sampled enters the cyclone precleaner through a tangential inlet at a rate of 100 ft^3/min. As the airstream spirals downward, particles greater than 10 μm are forced against the outer wall of the chamber by cyclonic action. Once in contact with the wall, the particles move downward and are deposited in a sealed catch basin below where they are stored for disposal at the end of the sampling run.

The airstream exits through a vortex finder tube, exhausts at the top of the cyclone precleaner, and immediately enters the venturi scrubber through a tangential, restricted throat in which the velocity is increased to about

10,000 ft/min. A stream of water is injected into the high velocity airstream which disperses the fluid into very small droplets that become entrained in the airstream. The airstream carries the fluid droplets in a downward spiraling path through the scrubber chamber where the intimate contact between water droplets and particles causes particles to be trapped inside the water droplets.

The airstream, now laden with fluid droplets, exits from the bottom of the venturi scrubber and tangentially enters the entrainment separator at a point 4 in. above its collection sump.

In the entrainment separator, the water/particle agglomerates are removed from the airstream by cyclonic action. A small quantity of water is injected tangentially through a Teflon nozzle into the top of the entrainment separator to wet the walls and prevent droplets from "skipping" up the wall and escaping. The particle-laden fluids which collect on the walls of the entrainment separator flow downward into a collection sump which has a maximum capacity of about 2 liters.

The fluid from the sump of the entrainment separator is recycled by a peristaltic pump at a flow rate of 2.3 liters/min. Most of the recirculated fluid is directed through a Teflon nozzle into the venturi scrubber where it again serves as the sample collection fluid; the remainder of the fluid is directed back into the entrainment separator to wet the walls of the unit. Thus, the fluid in the system is continuously recycled, allowing the concentration of collected materials to build up with time. Fresh water is added to the system only as required by losses through evaporation.

The air leaving the entrainment separator is discharged through a suction blower. The flow through the system is regulated by a damper, and is monitored by a flow meter calibrated in terms of the differential pressure across the cyclone precleaner.

5. Sampler Operation

A photograph of the sampler in operation is shown in Figure 2. The arrangement of the three chambers in the photograph coincides with the sketch in Figure 1. The peristaltic pump appears in the lower forefront. The blower

is not shown. The sampler fluid tubes are covered with aluminum foil to shield out light to protect any photosensitive materials which may be present.

The short covered tube on the right side of the entrainment separator sump is used to monitor fluid levels visually. The unshielded tubes (upper) are the flow meter leads. The large hose (upper right) leads to the blower which is separate from the unit.

6. Operating Temperatures

The scrubber was operated with approximately 2 liters of distilled water. When collecting hot inlet gases with temperatures ranging between 60-74°C, the rate of evaporation was about 1 liter per hour. Sample collection was periodically interrupted so that more fluid could be added to the sampler to compensate for the loss by evaporation.

The temperature of the emissions collected from cooling molds generally ranged between 60-74°C at the inlet to the sampler. The heat lost by evaporation effectively cooled the scrubber fluids and the walls of both the scrubber and the entrainment separator. The outer walls of the cyclone precleaner, which operates in the absence of liquids, remained hot to the touch throughout the runs. The temperature of the remainder of the sampling train (venturi scrubber and entrainment separator) did not exceed 30°C. This operating temperature was maintained as long as the inlet air temperature was less than 74°C. With an inlet air temperature of 82°C, the temperature of the scrubber fluid was approximately 35°C. Since, in some cases, the collected materials remained in the sampler for as long as 6 to 8 hours during the collection period, it was desirable to maintain the sampler fluids at the lower temperatures. Therefore, in all situations, sampling conditions were selected in which the inlet air temperature was at a maximum of 74°C, maintaining the sampling fluids at less than 30°C.

7. Particle Collection Efficiency

The efficiency at which the sampler removed particles from inlet air was determined with a modified EPA Method 5 sampling train (Figure 3) while mold emissions were being collected by the sampler from a shell mold line under the

standard collection conditions. The particle size distribution was determined in the airstreams at the inlet and outlet to the sampler using a Sierra® Model 228 cascade impactor. This instrument has eight impactor stages with aerodynamic particle size cut-offs ranging from 0.22 to 15 μm in diameter followed by a built-in filter to collect particles less than 0.22 μm in diameter. The impactor was operated at a flow rate of 10 liters per minute.

To prevent condensation, the impactor and probe were heated. Temperatures throughout the sampling train were monitored with thermocouples connected to a pyrometer located in the control case. Both inlet and outlet samples were collected at isokinetic sampling rates, which were within ± 10% of the duct velocities, as specified by EPA Method 5 guidelines.

Particulate material was collected on 0.47 mm slotted glass fiber collection substrates. Mass concentrations were determined by dividing the weight of particles collected on each of the collection substrates by the volume of air sampled (corrected to standard conditions). Fractional removal efficiencies for each of the eight particle size ranges were calculated for the inlet and exhaust airstreams of the scrubber. Particles deposited in the probe were not included in these calculations because their particle size distributions were unknown and the catches on the inlet were mainly composed of particles greater than 10 μm in diameter.

Particle sizing tests were performed both at the beginning of the sampling cycle when the recirculated water was clean and toward the end of the cycle after the sampler water had accumulated particles for 8 hours. Inlet particulate concentrations for these tests in the size range less than 10 μm averaged over 40 mg/M^3.

Aerodynamic particle size distributions in both the inlet and the outlet airstreams of the scrubber sampler are presented in Figure 4. The shell mold emissions used for these tests had a large percentage of small submicron particles; over half by weight of the particulate in the inlet airstream was less than 0.5 μm in diameter. It can be seen in Figure 4 that the mean diameter of the particles in the outlet airstream was smaller than that of particles in the inlet airstream. This is because the efficiency of the sampler increased with the aerodynamic diameter of the particles (Figure 5). The fractional

removal efficiencies increased with particle size from a low of about 30 percent for particles less than 0.2 μm to about 80 percent for particles with a diameter of 15 μm. Figure 6 compares the average fractional removal efficiencies during operation of the sampler with clean and particle-laden ("dirty") water. In these tests, the particle-laden water contained about 2 mg/ml contaminants. The fractional removal efficiency was not significantly affected by this concentration of contaminants in the sampler fluids.

E. SAMPLING CONDITIONS

1. Criteria for Selection of Foundries

Samples were collected at foundries at which gray iron was cast. In identifying pouring lines for use in this study, care was taken to select lines where samples could be collected without significant cross-contamination with airborne emissions from other processes, such as melting or shakeout. To help accomplish this, samples were collected in isolated areas, such as cooling tunnels, wherever possible. However, even an enclosed and ventilated cooling tunnel draws its air from the air in the plant and may carry with it contaminants from other processes. Thus, foundries were sought in which cross-contamination was further minimized either by appropriate local ventilation of nearby processes or by the absence of other fume- or dust-emitting processes in the vicinity of the mold cooling area.

Another important criterion in the selection of foundries was that there could be no emissions from sand binders other than the particular binder being studied. Therefore, for those molds which contained cores, the molds and cores had to be composed of the same chemical binders. Also, reclaimed sand could only be taken from pouring lines using the same chemical binders as the molds being sampled in order to avoid contamination from other resins remaining associated with the recycled sand.

The four pouring lines selected for use in this study met these criteria. Emissions were collected from each mold type soon after the metal was poured. The mold emissions were transported from the molds to the nearby sampler through a short section of ductwork using as few elbows as possible to minimize deposition losses.

Each pouring line presented special conditions which had to be met during the assembly of the sampling equipment. The conditions under which samples were collected at each pouring line are given below.

2. Sample Collection - Furan Molds

Furan samples were collected from large molds that were poured in an open floor area (Figure 7). The size of a typical mold from which samples were collected was 4'L x 4'W x 4'H. The molds were enclosed in flasks and most of the visible effluents escaped from vent holes, approximately 4 inches in diameter, in the top surface of the mold. The gases escaping from the vent holes usually burned continuously for approximately 2 hours after the molds were poured. The sample was collected during this period.

Since the molds were not cooled in a cooling tunnel or in a confined area, there was no central location from which concentrated effluents could be collected. Therefore, a device was constructed which permitted the capture of effluents directly from the vent holes (Figure 7). A 4-inch flexible aluminum duct extended from the sample collector to the vicinity of the mold. At the end of the duct work was placed a manifold from which four ducts, 3 inches in diameter, extended. A funnel, whose mouth was 10 inches in diameter, was at the terminus of the 3 inch ducts. The manifold was supported by a 5'L x 3'H x 2'W aluminum frame.

The air currents in the foundry caused the gases to diffuse rapidly as they escaped from the vent holes. Therefore, cylindrical baffles were placed over the vent holes to direct the effluents towards the outlet of the sampling manifold. The baffles were constructed from galvanized stove pipe and were 18 inches in diameter and 24 inches high. The baffles sat on 1 inch legs which allowed air to enter into the bottom of the chimney and permitted combustion to occur as it did under normal mold cooling conditions. The sampler was operated at a flow rate of 115 to 123 cfm. The air temperature at the inlet to the sampler ranged from 39 to 94°C. Mold emissions were collected at a rate of approximately 1 gram/hour.

The composition of the molding aggregate and a typical elemental analysis of the poured metal are given on the following page.

Production Data* - Furan Foundry

Typical Sand Mold Make-up

1,000 pounds washed silica sand
15.5 pounds resin (furfuryl alcohol-urea-formaldehyde)
2.4 pounds catalyst (toluenesulfonic acid)
Sand to melt ratio - approximately 3:1.

Binder Specifications (Volatiles)

1.7 - 2.3% N
3.0 - 4.0% water
0.5% free formaldehyde
<0.5% free phenol
0.1 - 0.15% silane
remainder furfuryl alcohol

Catalyst Specifications

65% solution of 94% toluenesulfonic acid in water.

Foundry Production Rate

Approximately 35 tons/day

Metallurgical Data

Typical Metal Analysis

Metal	% Concentration
Carbon	3.2
Phosphorus	0.04
Copper	0.20
Silicon	2.00
Nickel	0.05
Manganese	0.80
Aluminum	<0.01
Sulfur	0.06
Molybdenum	0.03
Chromium	0.16
Titanium	0.02
Iron	remainder

*Typical foundry production rate on dates of sample collection.

3. Sample Collection - Green Sand Molds

The green sand molds were approximately 7'L x 3'W x 3'H. The molds were poured on an automated conveyor line and carried within 30 seconds after pouring into a close-fitting cooling tunnel.

Since the space between the molds and the walls of the tunnel was limited, precluding the use of a capture hood, it was necessary to take the sample from an existing exhaust duct which conveyed effluents from the tunnel. The duct selected was 10 inches in diameter and was located approximately 2 minutes downstream of the casting area. This exhaust point was located immediately above the center of the mold during the dwell period. To obtain maximum collection rates, samples were collected in an area where the molds rested when the line was stationary. Mold effluents were drawn from the exhaust duct through a presized fitting which was placed in the wall of the duct. The presized fitting, designed to enable the isokinetic withdrawal of particles, was connected to the sampler by approximately 20 feet of 4 inch diameter galvanized duct (Figure 8).

The air temperature at the inlet to the sampler ranged from 48 to 80°C. The sampler was operated at a flow rate of 114 to 123 cfm. Approximately 85 molds were poured per hour. The collection rate was approximately 500 mg/hour.

The composition of the molding aggregate and a typical elemental analysis of the poured metal are given on the following page.

Production Data* - Green Sand Foundry

Metal Cast/Shift - 190.67 tons
Pounds Metal/Casting - 550

Sand - Additive Data

Pounds sand/mold - 6610
Pounds additive/pounds of sand -
New sand (facing sand): 100 lbs additives/1000 lbs sand
Old sand (recycled sand) - 21 lbs additives/mold

Sand Additives

Regular Sand: 59% sea coal (pulverized bituminous coal)
30% cob flour
11% trolumen (derived from petroleum)

Facing Sand: 59% sea coal (pulverized bituminous coal)
30% cob flour
11% trolumen (derived from petroleum)
- bentonite (trace compound)

Metallurgical Data

Typical Metal Analysis

Metal	% Concentration
Carbon	3.4
Sulfur	0.1
Phosphorus	0.3
Silicon	2.9
Manganese	0.45
Chromium	0.08
Nickel	0.05
Tin	0.01
Aluminum	0.01
Copper	0.20
Titanium	0.03
Boron	0.006
Molybdenum	0.02
Iron	remainder

*Typical foundry production rate on dates of sample collection.

4. Sample Collection - Urethane Molds

The urethane molds from which emissions were collected were approximately 2'W x 3'L x 1'H. Molds were poured under a side draft hood adjacent to a partially enclosed, ventilated cooling hood (Figure 9). They were pushed into the cooling hood shortly after the metal was poured. They remained in the cooling hood for approximately one hour before being removed to the shakeout area. Approximately 10 molds were poured per hour.

Visible emissions from the cooling molds were low, and became rapidly diluted within the cooling hood. Therefore, effluents from the cooling molds were collected with the use of two 20'L x 2'W aluminum sheet metal canopy hoods. The hoods were suspended approximately 24 inches above the top of the molds. Each hood covered five molds. The canopies acted as receiving hoods by capturing the effluents rising in the thermal draft off the top of the molds. The mold stayed under the sampling canopy for approximately 45 minutes.

The sampler was located outside the cooling area. It was connected to the canopy hoods with 4 inch diameter galvanized duct work. The sampler was operated at a flow rate of 115 to 123 cfm. The air temperature at the inlet to the sampler ranged from 27°C to 55°C. Effluents were collected at an average rate of 110 mg/hr.

The composition of the molding aggregate and a typical elemental analysis of the poured metal are given on the following page.

Production Data* - Urethane Foundry

Molds Poured/Shift	- 70
Metal Cast/Day	- 8.5 tons
Pounds Metal/Mold	- 245
Pounds Sand/Mold	- 1250
Pounds Resin/Mold	- 12.75

Sand Resin Mixing Data

Sand mixing rate (Ribbon flow mixer) - 550 lb/min.
Pep Set - 1505 (phenol formaldehyde resin in an aromatic hydrocarbon) 48 oz/min.
Pep Set - 2590 (polymeric MDI) - 40 oz/min.
Pep Set - 3500 (4-propylpyridine) - 52-55 ml/min.

Metallurgical Data

Typical Metal Analysis

Metal	% Concentration
Carbon	3.3 - 3.4
Silicon	1.4 - 1.6
Manganese	0.4 - 0.9
Chromium	0.55 - 0.65
Sulfur	0.2 max
Phosphorus	0.2 max
Copper	0.1 max
Nickel	0.1 max
Molybdenum	0.35
Iron	remainder

*Typical foundry production rate on dates of sample collection.

5. Sample Collection - Shell Molds

The shell molds were approximately 2'L x 1'W x 6'H. Molds were poured on a continuous monorail conveyor system which carried them into a narrow cooling tunnel within 2 to 5 minutes after pouring (Figure 10). The effluents from the poured molds were collected at a point in the cooling tunnel approximately 3 feet above the top of the molds. The molds reached the point of sample collection between 2 and 10 minutes after the metal was poured.

The sample was collected through a 4 inch diameter galvanized duct which was attached at one end to the inlet of the sampler and extended through a small opening in the wall of the cooling tunnel. The sampling probe extended approximately 6 inches into the cooling tunnel. The length of this ductwork was approximately 6 feet.

The air temperature at the inlet to the sampler ranged from 54° to 65°C. The sampler was operated at a flow rate of 115 to 123 cfm. The collection rate was approximately 550 mg/hour.

The composition of the molding aggregate and a typical elemental analysis are given on the following page.

Production Data* - Shell Foundry

Metal Cast/Shift - 36.8 tons

Sand - Resin Mix Data

Hexamethylenetetramine - 0.025 lbs/lb sand
Resin - 0.035 lbs/lb sand
Vinsol (wax) - 0.003 lbs/lb sand

Metallurgical Data

Typical Metal Analysis

Metal	% Concentration
Silicon	2.28
Manganese	0.66
Carbon	3.39
Phosphorus	0.0692
Sulfur	0.098
Chromium	0.11
Molybdenum	0.03
Copper	2.85
Magnesium	0.54
Aluminum	<0.01
Vanadium	0.01
Titanium	0.03
Iron	remainder

*Typical foundry production rate on the dates of sample collection.

6. Collection Rates

The collection rates varied with the mold type, the activity of the pouring line, production rates, and the dilution of the emissions at the point at which samples were collected. Typically 3 to 4 grams of shell and green sand emissions could be collected per shift. About one gram per shift could be collected from the individually sampled furan molds. The urethane line, which was a low production line and also offered no location from which heavily concentrated emissions could be sampled, required the most time and allowed the collection of about 0.8 gram per shift. The total time required to collect emissions at each foundry during the chronic sampling phase is shown in Table 1.

F. PREPARATION OF SAMPLES FOR THE BIOASSAYS

1. pH Adjustment

The pH of the samples varied with the mold type (Table 2). Furan and green sand emissions were acidic and had a pH of 2 and 1.8, respectively. The pH of collected urethane mold emissions was about 7, and shell mold samples had a pH of about 8. Since pH extremes can damage lung tissues, all samples were adjusted to pH 7 before administration to hamsters. Initial adjustments were made in the foundries by titrating acidic samples, with the use of pH indicator paper, to about pH 6 with 0.2 N NaOH and the shell sample to about pH 7 with 0.1 N HCl. Final adjustments to pH 7 were made in the laboratory by electrometric titration before samples were administered to animals.

2. Sample Concentration

At the end of each sample collection shift, the materials were withdrawn from the sampler and aliquots set aside for weight determinations and metal analyses. The remainder was reserved for the biological assays and chemical analyses. The pH of the collected samples was adjusted to near neutrality and the bulk of the sample, which had a volume of about 500 to 600 ml fluid, was stored frozen until the required quantity of emissions was collected from the mold line being sampled. At the end of the collection period, the samples were combined and the volume reduced to that required for the bioassay by evaporation within the wet scrubber sampler. Samples were concentrated by

allowing them to circulate through the wet scrubber sampler with the blower running at 100 ft^3/min without replenishing the water supply as the fluid evaporated. The minimum level to which the fluid in the scrubber could be reduced, while still having fluid circulation, was 150 ml. The samples were concentrated at the same location and under the same conditions in the foundries under which they had originally been collected. In this way, the heat required to rapidly evaporate the samples was provided and it was not necessary to take precautions against the collection of extraneous materials during the concentration phase.

G. CHARACTERISTICS OF COLLECTED MOLD EMISSIONS

1. Particulates

The collected samples contained components that were soluble in water as well as a particulate fraction that was water-insoluble. Table 3 shows the percentage of the total dry weight of each sample that was present as water-insoluble particulates. These values were obtained by measuring the portion of the total dry weight of a known volume of sample retained by a preweighed Millipore filter (0.22 μm pore size). Dry weights of the sample and its fractions were obtained by drying samples in tared vessels at 60°C in a circulating hot air oven (Lipshaw Laboratory Oven, Model 218, Detroit, MI) and then desiccating overnight before weighing.

The shell sample, with 50% particulates, contained the most water-insoluble material, followed by green sand, composed of 25% particulates, and furan with 10% particulates. The urethane sample had less than 2% particulate, which was the lowest value observed.

Most of the PAH and other organic compounds which are associated with the water-insoluble fraction can be extracted from the particulates with cyclohexane. The portion of the particulate fraction soluble in cyclohexane varied from 16-36% between mold types; the values from urethane and furan molds were lower than those from shell and green sand molds (Table 3, percent particulate soluble in cyclohexane). The cyclohexane-soluble components from the urethane and furan samples were 16 and 19% of the total particulate fraction, respectively, while those from shell and green sand were approximately 35%. Since most of the PAH are known to be associated with particulates, and since the

total quantity of particulates varies between foundry samples, it is important to consider the quantity of cyclohexane-soluble materials in terms of the whole sample (Table 3, percent total sample soluble in cyclohexane). For example, about 35% of the water-insoluble particulates in the shell samples are soluble in cyclohexane. Since the particulate fraction represents 50% of the whole sample, the cyclohexane-soluble fraction is actually about 18% of the total dry weight of the sample. This value is 7 to 9% for green sand, 2 to 3% for furan, and 0.2 to 0.3% for urethane.

To compare the concentrations of elemental carbon, the water-insoluble fraction of each sample was collected on filters, washed thoroughly with water to remove adhering water-soluble chemicals, and Soxhlet-extracted to remove associated organic materials. The carbon and hydrogen contents were determined by combustion analysis at Galbraith Laboratories in Knoxville, TN. Table 4 shows the percent of carbon and hydrogen in the particulate fraction from each of the foundry samples. The shell sample contained considerably more carbon than the particulates from the other three samples. Carbon represented 85% of the total dry weight of the washed shell particles, 45% of the green sand, 31% of the urethane and only 23% of the furan particulates.

Table 5 shows the ratio of the moles of carbon atoms to the moles of hydrogen atoms in the particulate fraction of each foundry sample. The ratios of 0.4:1 and 0.6:1 in the furan and urethane samples, respectively, indicate that some of the carbon may be present in residual organic compounds that were not extracted with cyclohexane. However, as the ratio of carbon to hydrogen approaches one, as in the green sand sample, it becomes more probable that the carbon is present in the elemental form. In the shell sample, the ratio of almost 4:1 definitely indicates that most of the carbon is present as elemental carbon. These values may be pertinent to interpretations of the health effects of foundry samples that are based on their physiochemical composition since the adsorption of the toxins to carbon particles may affect their bioavailability to surrounding tissues.[17, 18]

2. Elemental Analyses

The elemental analyses of the mold emissions from each foundry sample are shown in Tables 6-9. Sixteen elements were determined by atomic absorption.

(Tin, silicon, silica and arsenic were analyzed by Trace Elements Inc., Park Ridge, IL.; the remainder were analyzed by Rexnord, Inc.) Silica was determined colorimetrically by Trace Elements Inc. A comparison between the metal analyses of the emissions from the four types of mold binders indicates that the mold binder had no apparent effect on the metal content of the mold effluents. In all of the mold emissions, the concentrations of certain metals were consistently lower than might be expected in the emissions on the basis of the metallurgical content of the poured iron. Mercury was not detectable in any of the samples. The concentrations of iron and chromium were consistently lower and those of certain other elements, such as nickel, aluminum, lead and copper, were present in much higher quantities than might be expected on the basis of their concentrations in the poured metal. These data show that the metals that are most readily released into the mold emissions are those with the highest vapor pressure at the pouring temperature of the iron.

3. PAH Determinations

The concentrations of eight PAH and related compounds in the aqueous and particulate phases of the mold emissions were determined by Ruby James at Southern Research Institute, Birmingham, AL.

Particulates were removed from the aqueous phase by filtration and washed thoroughly with distilled water to remove residual water-soluble materials. They were dried overnight in a desiccator, weighed and the PAH were extracted by sonication in cyclohexane.

Each of the cyclohexane extracts was concentrated by evaporation to a final volume of 2 ml. Half of the concentrated cyclohexane extract was analyzed by gas chromatography/mass spectroscopy (GC/MS) while the remaining half was subjected to cleanup on a silica gel column, concentrated, solvent exchanged to acetonitrile and analyzed by HPLC/UV coupled to a fluorescence detector.

The GC/MS samples were analyzed on a 6-ft. glass column (0.25 in. OD by 2 mm ID) packed with 1% SP-2250 coated on 100/120-mesh Supelcoport. The column oven was temperature-programmed from 50 to 270°C at 15°C/min. The gas

chromatograph/mass spectrometer employed was the Hewlett-Packard 5985A. The mass spectrometer scanned from 45 to 450 atomic mass units (amu) in 3 seconds.

The high-pressure liquid chromatography (HPLC) samples were analyzed on a reverse phase column, HC-ODS Sil-X, 250 mm long by 2.6 mm ID (Perkin-Elmer Part No. 089-0716). Isocratic elution was employed for 5 minutes with 40% acetonitrile/60% water; then gradient elution to 100% acetonitrile was employed over 35 minutes. The solvent flow rate was 0.5 ml/min. The HPLC injection was 25 µl.

Figure 11 a-d shows reconstructed total ion current chromatograms of the cyclohexane extracts of the particulates in each of the four samples. The components of the major peaks are identified in the chromatograph of the green sand sample. The largest peak in the green sand sample (Figure 11a) contains phenanthrene and anthracene, two closely related compounds which were not separated by the GC/MS system used. Some of the other PAH in this sample are pyrene, fluoranthene, chrysene and benzo(a)anthracene. The last two isomers both elute in the same position and were not differentiated by GC/MS. Benzo(a)pyrene is present in concentrations that are too low to cause a distinguishable peak.

Of these PAH, BaP is a strong carcinogen, chrysene and benzo(a)anthracene are both very weak carcinogens, and phenanthrene and anthracene are not carcinogenic.

The other peaks present in the part of the spectrum which follows phenanthrene represent the methylated and dimethylated derivatives of the PAH. The addition of methyl groups to the PAH can potentially alter their biological activity. For example, chrysene itself is only slightly carcinogenic while two of the eleven possible methyl derivatives (4- and 6-methylchrysene) may be slightly more carcinogenic than chrysene, and 5-methyl chrysene may be as strong a carcinogen as BaP. The methyl derivatives of chrysene were not resolved in this chromatogram. Which of the methylchrysene isomers are present and whether or not those isomers that are present in this sample have any carcinogenic activity is not known.

The concentrations of the PAH in the cyclohexane extracts of particulates from the four foundry samples are shown in Table 10. The data are presented

in terms of μg PAH per gram particulate. Since two of the GC/MS peaks each contained two PAH, the analyses were also performed by HPLC. Although this technique tends to be more subject to interferences for PAH than is GC/MS, it can differentiate between certain combinations of compounds, such as benzo(a)anthracene and chrysene, which were not distinguished by GC/MS. Table 10 shows the combined concentrations, as determined by GC/MS, of benzo(a)anthracene and chrysene. Using HPLC (data not shown), it was found that chrysene represented a considerably greater portion of the total benzo(a)anthracene/chrysene GC/MS peak than did benzo(a)anthracene in three of the four foundry samples. Of these two compounds, only chrysene was detected in the furan sample and there was approximately four times as much chrysene as benzo(a)anthracene in the urethane and shell samples. The reverse situation occurred in the green sand sample which had about five times as much benzo(a)anthracene as chrysene. There were no detectable quantities of either acridine, carbazole or dibenz(a,h)-anthracene in any of the samples. Naphthalene was found in only the urethane sample, and phenanthrene was present in all four samples. Measurable quantities of BaP were only present in particulates from the shell and green sand samples.

For the analyses of PAH and related compounds in the aqueous phase, the base-neutral fraction, which contains these compounds, was extracted with methylene chloride after adjustment to pH 11. The residual aqueous phase was saved for the extraction of phenols which is described later in this report. One ml of the base-neutral concentrate was analyzed by GC/MS while the remaining 1 ml was subjected to cleanup on a silica gel column, concentrated, solvent-exchanged to acetonitrile, and analyzed by HPLC. The GC/MS and HPLC operating parameters were the same as those employed for the analyses of the cyclohexane extracts of the water-insoluble particulates.

The chromatograms of the methylene chloride extracts of the aqueous phase from each of the foundry samples are shown in Figure 12 a-d. In contrast to the chromatographic profiles of the extracts from the particulates, the aqueous phase had little material which eluted in the PAH region of the chromatograph.

Table 11 shows the concentrations of PAH and related compounds in the aqueous phase of all four samples. Of the compounds analyzed, the only com-

pounds detected were acridine in the furan, green sand and urethane samples, and phenanthrene in the shell and green sand samples.

Table 12 presents a summary of the PAH data. These values are presented in terms of µg/g whole sample (water-soluble and insoluble materials combined) so that the data for the total quantities of PAH in the samples are directly comparable. This table clearly shows that the PAH concentrations in the urethane and furan samples are much lower than those in the shell and green sand samples.

4. Phenols

The last series of compounds that were analyzed were the phenols. These compounds are water-soluble and hence only the aqueous phase was examined. To extract the phenols, the aqueous phase from each sample that had been held in reserve after extraction of the base-neutral fraction was adjusted to pH 2 and was again extracted with methylene chloride to remove acid compounds. The extracts were concentrated to a final volume of 2 ml and analyzed for phenols by GC/MS . The procedure was based on a 6-ft glass column (0.25 in. OD by 2 mm ID) packed with 1% SP-1240 DA on 100/120-mesh Supelcoport. The column oven was temperature-programmed from 70 to 190°C at 8°C/min. The mass spectrometer scanned from 45 to 350 amu in 3 sec. The results are presented in Table 13.

Of the compounds examined, phenol itself was present in the highest concentration and was found in all four foundry samples. 4-Nitrophenol, the second most prevalent phenol, was present in all but the green sand sample. 2-Nitrophenol was found in the shell sample only and 2,4-dimethylphenol was found in the shell and urethane samples.

H. DISCUSSION

This report presents a partial description of the composition of emissions collected from four types of molds in common use in iron foundry casting operations. The results of these analyses will serve as background information for carcinogenicity bioassays of the mold emissions. The materials analyzed may represent only a small portion of the total quantity of material collected from each mold type. In practice, it was not possible to more fully

characterize the composition of the emissions. Therefore, substances with known toxic properties, or which are chemically related to acute or chronic toxicants and are likely to be associated with them in emissions from combustion processes, were selected for study.

These analyses showed that more PAH are present in the emissions collected from shell and green sand molds than in those from urethane and furan molds. Phenols, which were present in the emissions from all four mold types, were most prevalent in those from urethane molds. Certain toxic or biologically active metals, such as lead and zinc, although present in only trace or undetectable amounts in the poured metal, were present in readily detectable quantities in some mold emissions as a result of their high vapor pressures.

The data presented give the concentrations of metals, particulates and organic compounds found in that portion of the emissions collected for use in the carcinogenicity bioassays, and do not necessarily reflect the absolute concentrations of these components in the airborne emissions as they evolve from the molds. This was so because the sample collection apparatus was designed to collect those components of the emissions that could be administered to animals by intratracheal instillation. For example, non-particulate water-immiscible chemicals cannot be administered by this technique and were not collected by the sampler. Also, the efficiency at which the various components of the emissions were collected may have varied. This is not known because only the efficiency at which particles were collected was determined.

The total quantities of particulates and BaP which evolve from twelve different types of molds were recently compared by W. Scott and R. James at Southern Research Institute (SoRI).[10] They found that the total quantity of particulates and BaP in the mold effluents was lowest for urethane, followed by the furan and shell molds. The highest quantity of particulates was evolved from green sand molds. In our study, the portion of particulates and BaP in the total sample was higher for shell than for green sand molds. An important difference between the two studies is that the SoRI study was performed in a laboratory setting under which parameters such as sand-to-binder and sand-to-metal ratios could be carefully controlled. This was not feasible in our study because mold effluents were collected in commercial foundries during normal operation. In our study, the sand-to-metal ratio in

the green sand molds was considerably higher than that in the other mold types. The large bulk of sand surrounding the iron casting could have trapped much of the particulates and prevented their escape. Since BaP in mold effluents is associated with particulate material, this may account for the differences in the relative quantities of particulates and BaP found between green sand and shell molds in the two studies.

Ideally, urethane, furan and green sand molds with the same sand-to-metal ratios should have been used for this study. However, a green sand pouring line which met the criteria of the study and also poured gray iron into molds with a lower sand-to-metal ratio could not be found. A suitable green sand line was the most difficult to identify because of the criterion that cores and molds had to be composed of the same binder. Since green sand is not used for making cores, the number of available casting lines was severely limited.

To obtain the large quantities of emissions required for the bioassay, it was necessary to collect emissions at the most concentrated source available. Therefore, samples were either collected at a point close to the surface of the molds (furan and urethane) or in enclosed cooling tunnels (shell and green sand). Thus, this report describes the compositon of mold emissions collected from concentrated sources specifically for use in hamster carcinogenicity bioassays. The characteristics of these samples do not necessarily reflect those of emissions in the breathing zone of exposed workers.

III. ACUTE RANGE-FINDING STUDY

A. METHODOLOGY

1. Objective

The acute range-finding study was conducted to determine the maximum tolerated dose of pyrolysis effluents from green sand, shell sand, urethane, and furan molds when administered intratracheally to hamsters.

2. Test Articles

Effluents from green sand, shell sand, urethane, and furan molds were collected and concentrated at the foundries by Rexnord, Inc. (Milwaukee, Wisconsin) and were sent to Borriston Laboratories for use in the acute range-finding study. Information pertaining to the receipt and identification of the test materials is shown below:

TEST ARTICLE	BRL NUMBER	DATE OF RECEIPT
Shell Sand	186	09-27-79
Furan	195	11-14-79
Urethane	231	02-11-80
Green Sand	243	03-19-80

The samples were stored frozen in the dark until used. The total storage time for the samples (from collection through instillation) was approximately 12 weeks.

3. Test Animals

Prior to initiation of the acute study, two separate murine virus antibody determinations were conducted on hamsters obtained from four suppliers as a preliminary assessment of animal quality. On both occasions, hamsters obtained from Charles River Breeding Laboratories were found to be negative for the five viruses tested (PVM, Reo_3, Sendai, SV_5, and LCM). Based on these results, Charles River was chosen as the test animal supplier for this project.

Weanling golden Syrian hamsters were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts). Prior to placement on study, all animals were quarantined for a period of two weeks. All animals were

individually housed in solid polycarbonate cages with sterile shredded aspen bedding (American Excelsior, Annapolis Junction, Maryland), and the cages were suspended from stainless steel racks equipped with non-woven filter sheets. Animals were kept under optimum hygienic conditions in a temperature-controlled (22 ± 3° C) room. A manually controlled 12-hour light/dark cycle was maintained. The relative humidity of the room generally ranged from 30% to 70%, although it was not controlled. Room air was exchanged at a rate of 10 to 15 changes per hour and was not recirculated. Animals received Purina Laboratory Rodent Chow® #5001 and water ad libitum. Cages were washed weekly, and fresh bedding and water bottles were provided weekly.

4. Treatment Groups

At the end of the quarantine period, healthy animals were assigned to treatment groups using a random numbers table. Each animal was given a unique identification number. The study design is summarized below:

GROUP	TEST ARTICLE	DOSE LEVEL (mg/0.20 ml distilled water)
1	Green Sand	2.5
2	Green Sand	5.0
3	Green Sand	10.0
4	Green Sand	20.0
5	Shell Sand	2.5
6	Shell Sand	5.0
7	Shell Sand	10.0
8	Shell Sand	20.0
9	Control (Distilled H_2O)	-
10	Urethane	2.5
11	Urethane	5.0
12	Urethane	10.0
13	Urethane	20.0
14	Furan	2.5
15	Furan	5.0
16	Furan	10.0
17	Furan	20.0
18	Control (Distilled H_2O)	-

Each group consisted of ten male and ten female hamsters.

5. Preparation of Suspensions

Stock solutions of the condensed pyrolysis effluents were sonicated on an

ultrasonic vibrator for 2 or 3 minutes to ensure homogeneity of the solutions. After sonication, appropriate dilutions were made, using distilled water as the vehicle, to reach the required dose levels. Suspensions were kept frozen and in the dark until used. All suspensions were kept homogeneous by stirring on a magnetic stirrer during the course of the instillation.

6. Intratracheal Administration

Hamsters were intratracheally instilled with the appropriate test or control article at the specified dose levels in distilled water once weekly for 5 consecutive weeks. An instillation volume of 0.20 ml was used. Control hamsters (Groups 9 and 18) received 0.20 ml distilled water only. Before each treatment, the animals were anesthetized with an intraperitoneal injection of 0.40 ml of 1% Brevital® Sodium solution (Eli Lilly and Co., Indianapolis, Indiana). The dosing suspensions were administered through a 0.25 ml tuberculin syringe fitted with a blunt 19-gauge needle. The needle was 60 mm long and bent at a 135° angle 45 mm from the tip. Separate syringes and needles were used for each test group. The dosing apparatus was sterilized prior to use. Before withdrawing each dosing volume from the flask, the syringe was flushed 3 or 4 times to ensure homogeneity of the suspension. The needle was gently inserted under the epiglottis into the tracheal lumen, the suspension injected, and the needle withdrawn. The animals were kept on the restraining board, and the pharynx was inspected for a short period to ensure that no suspension was regurgitated.

Hamsters that exhibited severe respiratory distress after release of the suspension into the lungs were revived by artificial resuscitation methods. This technique consisted of external heart massage, clearing mucus from the oral cavity and placing the animal on a heating pad for 5 to 15 minutes.

7. Observations

All animals were examined twice daily for mortality and moribundity. Clinical signs and body weights were recorded at initiation and once weekly for the duration of the study. All surviving animals were sacrificed during the sixth week of study.

8. Necropsy

A complete necropsy was performed on each animal at the time of death or sacrifice. All tissues and organs were examined in situ, dissected from the carcass, and fixed in 10% neutral buffered formalin. All gross lesions were recorded in narrative, descriptive terms including location, size, number, shape, color, texture and consistency.

9. Histopathology

After fixation and processing, a histopathologic examination of tissues from the following animals were conducted:

- All animals that died or were sacrificed moribund prior to scheduled sacrifice.
- All animals in Group 9 (Control).
- All animals from the 10.0 mg group for the green sand, shell sand, and furan samples and from the 5.0 mg group for the urethane sample.[a]

The following organs and tissues were examined:

Mandibular Lymph Node	Heart
Salivary Gland	Esophagus
Sternebrae, Femur, or Vertebrae (including marrow)	Stomach
	Ovaries
Thyroid	Uterus
Parathyroids	Cerebrum
Small Intestine	Cerebellum
Large Intestine	Thymus
Liver	Nasal Cavity
Gallbladder	Trachea
Prostate	Larynx
Testes	Pancreas
Lungs (section from each lobe)	Spleen
Mainstem Bronchi	Kidneys
Skin	Adrenals
Mammary Gland	Urinary Bladder
Eyes (if grossly abnormal)	Pituitary
Spinal Cord (if neurologic signs are present)	Gross Lesions, Tissue Masses and Regional Lymph Nodes

[a] Protocol originally specified that histopathologic examinations be conducted on all animals in the highest dose group with survivors at the time of terminal sacrifice. However, the higher dose levels (20.0 mg for the green sand, shell sand, and furan samples; 10.0 and 20.0 mg for the urethane sample) could not be considered for the chronic study due to the severe acute toxic responses observed in these groups. Therefore, histopathologic examinations were not conducted on those hamsters which survived until terminal sacrifice from the higher dose groups.

B. RESULTS

1. Green Sand

a. Mortality and Clinical Signs - Cumulative mortality data are presented in Table 14A. One male from the 2.5 mg dose group was paralyzed as the result of an accidental injury and was sacrificed moribund during Week 2. One female from the 2.5 mg group was found dead during Week 2; the cause of death was not ascertained. Two males and three females from the 20.0 mg dose group died during the course of the study. These deaths were considered treatment-related.

All animals treated with green sand effluent at a dose of 20.0 mg exhibited severe respiratory difficulties such as choking, severe dyspnea, polypnea, regurgitation of the compound through the nasal cavity, and collapsed lungs immediately after the suspension was released into the repiratory tract. These signs were not observed at the lower dose levels.

b. Body Weights - Mean body weights are presented in Table 15A. No treatment-related effects on weight gain were apparent.

c. Gross and Microscopic Pathology - Grayish-white foci, red foci, and mottling of the lungs were the most frequently noted gross findings. The number of affected animals ranged from 8/10 to 10/10 in all green sand male and female groups, except for the low-dose (2.5 mg) males, where 4/10 animals had these lesions. Similar findings were noted in 2/10 female control (Group 9) hamsters. Enlarged bronchial lymph nodes were seen in the treated groups only. The highest incidence (4/10) occurred in the 20.0 mg females; one or two animals from all other treated groups were affected.

Histopathological findings are summarized in Table 16. Intraluminal alveolar and/or bronchial pigment and histiocytosis were observed in all hamsters examined from the green sand groups. Alveolar/bronchiolar hyperplasia was present in all hamsters examined from the 10.0 and 20.0 mg dose groups and was most severe at the 20.0 mg level. No microscopic changes were noted in the lungs of the control hamsters.

d. Recommended MTD - Treatment-related mortality occurred only in the 20.0 mg dose group. Although one female from the 2.5 mg dose group died

during the study, it is doubtful that this death was treatment-related since no deaths occurred in hamsters treated with this sample at dose levels of 5.0 and 10.0 mg. On the basis of these observations, an MTD of 10.0 mg was recommended for the chronic study.

2. Shell Sand

a. Mortality and Clinical Signs - Cumulative mortality data are presented in Table 14B. One male from the 10.0 mg dose group died during Week 1. Treatment-related mortality was observed in nine males and seven females from the high-dose (20.0 mg) group; these deaths occurred during the first two weeks of the study.

Hamsters receiving the shell sand sample at a dose level of 20.0 mg exhibited severe respiratory difficulties (choking, dyspnea, polypnea, regurgitation of the compound through the nasal cavity, and collapsed lungs) immediately after instillation. These signs were not noted at the lower dose levels.

b. Body Weights - Mean body weights are presented in Table 15B. No treatment-related effects on body weight were noted.

c. Gross and Microscopic Pathology - The lungs of all hamsters from the shell sand groups were mottled with grayish-black foci or dust deposits at the time of necropsy. Similar pigmentation was noted in the tracheobronchial lymph nodes of several animals from the 2.5 mg (males, 2/10; females, 5/10), 5.0 mg (males, 4/10; females 7/10) and 10.0 mg (males, 5/10; females, 5/10) dose groups. This finding was seen in only one 20.0 mg animal (a female) since most hamsters at the high-dose group died after the first or second treatment.

Histopathologic findings are summarized in Table 16. Intraluminal alveolar/bronchiolar pigment and alveolar histiocytic hyperplasia were present in all hamsters from the 10.0 mg group. All hamsters at the 20.0 mg level showed diffuse dust deposits throughout the lungs, adenomatous hyperplasia associated with the pigment, acute congestion, pneumonitis, and bronchiolar pneumonia. Tissues from animals in the lower dose groups were not examined. No microscopic findings were seen in the lungs of the control animals.

d. Recommended MTD - No deaths occurred at dose levels of 2.5 and 5.0 mg. No significant histopathological findings were observed for the one male from the 10.0 mg dose group which died; however, the body weight of this animal was noted to be lower than any other animal in this group. Based on the results of this study, it was recommended that an MTD of 10.0 mg be used for the chronic study.

3. Urethane

a. Mortality and Clinical Signs - Cumulative mortality is presented in Table 14C. Two males from the 2.5 mg dose group died during the first two weeks of study. Two males and two females from the 10.0 mg group died during Week 1. Nine males and ten females from the 20.0 mg group died during Week 1; the tenth male in this group died during Week 2. All deaths were attributed to instillation of the test material.

All animals instilled at a dose level of 20.0 mg exhibited severe respiratory distress immediately after the first instillation; the signs included choking, severe dyspnea, polypnea, regurgitation of the material through the nose, and collapsed lungs. Only one male responded to resuscitation attempts; this animal died after the second instillation.

Similar respiratory problems were observed in hamsters from the 10.0 mg group after the first instillation. However, all but two animals (one male and one female) were revived by artificial resuscitation methods (heart massage, clearing mucus from the oral cavity, and placing the animal on a heating pad for 10 to 15 minutes). An additional male and female from this group died on the day following the first instillation. Survivors continued to show severe respiratory distress after each subsequent instillation.

Respiratory difficulties were also observed at the 5.0 mg dose level beginning with the third instillation; however, the severity of the signs was less than that observed at the higher dose levels. The response worsened after the fourth and fifth instillations at which time all animals had to be revived following the treatment. Resuscitation techniques were successful, and no deaths occurred in this group. Animals treated at a dose of 2.5 mg exhibited no respiratory problems.

b. Body Weights - Mean body weights are presented in Table 15C. No treatment-related effects on weight gain were observed in those animals that survived the treatment period.

c. Gross and Microscopic Pathology - Tinctorial changes (stippling, mottling, and/or foci) were frequently noted in lungs of hamsters treated with urethane effluent. These findings were seen in 9/10 or 10/10 animals of each sex from all dose groups. Foci were present in one male and one female from the control group (Group 18).

Histopathological findings are summarized in Table 16. Intraluminal alveolar/bronchiolar pigment and alveolar/bronchiolar hyperplasia were observed in all hamsters examined microscopically from the urethane groups. Fibrinopurulent pneumonia, associated with the aspiration of pigment, was also seen in two males and two females examined from the 10.0 mg dose group and in all (10/10) males and females from the 20.0 mg dose group.

d. Recommended MTD - Urethane was the most acutely toxic of the four samples studied. As described earlier, all hamsters receiving this material at doses of 5.0, 10.0 and 20.0 mg exhibited severe respiratory difficulty and had to be resuscitated after instillation. Because of the extreme reaction of the hamsters, even at the 5.0 mg level, it was recommended that 5.0 mg be selected as the MTD for the chronic study. However, since the respiratory difficulties observed at this dose level worsened with time, it was anticipated that animals might encounter problems during the 20-week dosing period of the chronic study.

4. Furan

a. Mortality and Clinical Signs - Cumulative mortality data are presented in Table 14D. Death occurred only in the high-dose (20.0 mg) group; nine males and ten females at this dose level succumbed during the first three weeks of study.

All animals in the 20.0 mg dose group exhibited severe respiratory problems (choking, dyspnea, polypnea, regurgitation of the compound through the nose, and collapsed lungs) immediately after instillation. These signs were not seen in animals from the lower dose groups.

b. Body Weights - Mean body weights are presented in Table 15D. No treatment-related differences in body weight were noted between the treated and control groups.

c. Gross and Microscopic Pathology - Dark red-brown mottling, grayish stippling, and/or dark focal areas were frequently observed in the lungs of all hamsters treated with furan effluent. These findings were present in all (10/10) hamsters from the following treated groups: 5.0 mg females; 10.0 mg males and females; and 20.0 mg males and females. They also occurred in 7/10 males and 8/10 females from the 2.5 mg group and in 9/10 males from the 5.0 mg group. Red foci were reported in the lungs of one male and one female from the control group (Group 18).

Histopathologic findings are summarized in Table 16. Intraluminal alveolar/bronchiolar pigment and alveolar/bronchiolar hyperplasia were observed in all animals examined from the 10.0 (10/10 males; 10/10 females) and 20.0 mg (9/9 males; 10/10 females) groups. All hamsters from the 20.0 mg group also showed diffuse necrotizing pneumonia.

d. Recommended MTD - Since no mortality occurred in animals receiving doses less than 20.0 mg, an MTD of 10.0 mg was recommended for the chronic study.

C. CONCLUSIONS

1. Recommended Dose Levels for the Chronic Bioassay

On the basis of the data presented in this report (observation of animals during instillation, mortality, body weight, and histopathology), it was recommended that two dose levels (MTD and $\frac{1}{2}$ MTD) be administered in the chronic bioassay. Doses of 10.0 and 5.0 mg were recommended for the green sand, shell sand, and furan samples. Due to the acute toxic responses observed in animals instilled with the urethane sample, dose levels of 5.0 and 2.5 mg were recommended.

2. Justification for a Single Positive Control Group (BaP + Fe_2O_3)

The purpose of a known carcinogen treatment group (positive control) in chronic carcinogenicity studies is to demonstrate that the test animals

respond in a predictable manner to a known carcinogen, as well as to show the effectiveness of the model system.

A mixture of 3 mg BaP + Fe_2O_3 has been shown to be effective in inducing respiratory tract tumors in hamsters by intratracheal instillation. A positive dose response with BaP and Fe_2O_3 has also been demonstrated in earlier studies, and an adequate amount of experimental data is available in the literature with regard to the tumor types and incidences of BaP and Fe_2O_3 induced lung tumors in hamsters.[14, 19, 20, 21, 22, 23, 24, 25] Therefore, it was recommended that a single positive control group (3 mg BaP + 3 mg Fe_2O_3) be used as the positive control group for the chronic bioassay. This is in compliance with the NCI guidelines "Guidelines for Carcinogen Bioassay in Small Rodents," DHEW Publication No. 76.801.

IV. CHRONIC CARCINOGENCITY STUDY

A. METHODOLOGY

1. Objective

This study was designed to determine the carcinogenic potential of condensed pyrolysis effluents obtained from iron foundry casting operations. Emissions from four types of casting molds (shell sand, green sand, furan, and urethane) were collected at the foundries and tested for their ability to induce cancer when administered repeatedly to the respiratory tracts of hamsters by intratracheal instillation. The study was initiated on April 20, 1981 and was concluded with the terminal sacrifice of all surviving animals on April 6, 1983 (females) and July 15, 1983 (males).

2. Test Articles

Effluents from shell sand, green sand, furan, and urethane molds were collected and concentrated at the foundries by Rexnord, Inc. (Milwaukee, Wisconsin) and were sent to Borriston for use in the chronic carcinogenicity study. The test article samples were stored frozen in the dark until used. The total storage time for the samples (from collection through instillation) was approximately 12 months. A mixture of benzo(a)pyrene (BaP) and ferric oxide (Fe_2O_3) was used as the positive control material. Benzo(a)pyrene (99%, Gold Label) was obtained from Aldrich Chemical Company (Milwaukee, Wisconsin) and was stored in the dark at room temperature. Ferric oxide was obtained from Fisher Scientific Company (Silver Spring, Maryland).

Information pertaining to the receipt and identification of the test and control articles is shown below:

TEST OR CONTROL ARTICLE	LOT NUMBER	BRL NUMBER	RECEIPT DATE	PHYSICAL DESCRIPTION
Shell Sand	-	186A	04-30-80	Brown Liquid
Green Sand	-	243B,C	02-05-81	Black Liquid
Furan	-	195A	06-04-80	Black Liquid
Urethane	-	231A	02-05-81	Black Liquid
Benzo(a)pyrene	794294	172B	03-27-81	Yellow Powder
Ferric Oxide	LD020897	232B	03-23-81	Red Powder

3. Test Animals and Animal Maintenance

Syrian golden hamsters (700 of each sex) were purchased from Charles River Breeding Laboratories (Newfield, New Jersey) and were received on April 1, 1981. Males were 46 days old (date of birth - February 15, 1981), and females were 52 days old (date of birth - February 9, 1981) at the time of receipt. Upon arrival at Borriston, the animals were examined for general physical appearance, and five hamsters from each shipping crate were weighed to determine a weight range (males, 70 to 97 g; females, 75 to 101 g). All animals were assigned temporary identification numbers and were placed in quarantine for a 20-day period prior to study initiation.

The Syrian golden hamster was selected as it is considered an appropriate experimental animal for intratracheal instillation studies. The species is refractory to respiratory infection and rarely develops spontaneous lung tumors. The hamster respiratory tract is morphologically similar to that of humans, and therefore provides a reasonable basis for extrapolation to human exposure. Historical data also indicate that the types of lung tumors induced in the hamster model, as well as their location and progression, are similar to those reported for humans.

Throughout the quarantine and testing periods, the test animals were individually housed in suspended solid-bottom polycarbonate cages (Allentown Caging and Equipment, Allentown, New Jersey) with San-i-cel® bedding (Paxton Processing Company, Paxton, Illinois). Cages were washed and fresh bedding was supplied weekly. Purina® Rodent Laboratory Chow® #5001 (Ralston Purina Company, St. Louis, Missouri) was available ad libitum in food hoppers, and water was provided in water bottles which were filled as necessary. Hoppers and water bottles were sanitized weekly. The animals were housed in an air-conditioned room, the average temperature of which was 22 ± 3° C. Room air was exchanged at a rate of 10 to 15 changes per hour and was not recirculated. A manually controlled 12-hour light/dark cycle was maintained. The relative humidity of the room was not controlled, but generally ranged from 30 to 70%.

During quarantine (April 10, 1981), random fecal samples were collected and examined for intestinal parasites. The eggs of Aspiculuris tetrapetra

(pinworms) were present, and all hamsters were treated with Piperazine 34% Liquid Wormer (Med Tech Inc., Elwood, Kansas) in drinking water (3 to 5 mg/ml) for a 10-day period. Fecal samples were again collected and examined on May 12, 1981; all samples were negative for pinworms.

4. Treatment Groups

At the end of the quarantine period, 1100 hamsters, (550 of each sex) in apparent good health were assigned to treatment groups and were given unique permanent identification numbers using a computer randomization program. Each group was initiated with 50 male and 50 female hamsters. However, animals found to be mis-sexed, animals that escaped from their cages, and those for which tissues were inadvertently lost during processing were subsequently eliminated from pathologic evaluations and statistical analyses (survival, body weight, and tumor incidences). Therefore, the adjusted number of hamsters was less than 50 for some sex groups. Treatment group assignments, dose levels, and the adjusted number of animals per group are shown below:

GROUP	TREATMENT	DOSE LEVEL (mg/0.20 ml distilled water)	ADJUSTED NUMBER OF ANIMALS	
			MALES	FEMALES
1	Vehicle Control	-	48	48
2	Fe_2O_3 + Vehicle	3.0	50	50
3	BaP + Fe_2O_3	3.0 + 3.0	48	50
4	Shell Sand	5.0	50	49
5	Shell Sand	10.0	50	50
6	Green Sand	5.0	50	48
7	Green Sand	10.0	48	50
8	Furan	5.0	50	48
9	Furan	10.0	49	47
10	Urethane	2.5	50	47
11	Urethane	5.0	49	46

Hamsters were identified with unique animal numbers by cage card; cage cards were color coded for treatment group identification.

5. Preparation of Dosing Suspensions

a. Ferric Oxide Control - The Fe_2O_3/distilled water suspension was prepared fresh weekly on the day of dosing. The correct amount of Fe_2O_3 was weighed on a Mettler H33AR pan balance and placed into a flask. Distilled

water was added to obtain the final concentration. The resulting admixture was mixed until a homogeneous suspension was achieved.

b. BaP + Fe_2O_3 (Positive Control) - Prior to preparation of the dosing suspensions, the total amount of BaP needed for the study was ground (approximately 1 gram at a time) for eight hours in a Fisher Mullite Mortar Grinder and collected into a foil-wrapped glass vial. The amount of ground BaP required for each weekly dosing suspension (330 mg each) was weighed out on a Mettler H33AR pan balance and placed in separate glass vials (one vial per dosing session). An equal amount of Fe_2O_3 (330 mg) was weighed out and added to each vial. The vials were wrapped in foil and stored in the dark until used. One vial was removed each week (on the day of dosing), and distilled water (22 ml) was added to obtain the final concentration. The mixture was sonicated on a Heat Systems Ultrasonic (Model W220-F) Sonicator for five minutes to obtain a homogeneous suspension. The suspension was continuously stirred on a Corning Magnetic Stirrer during dosing.

c. Condensed Pyrolysis Effluents - In preparation for concentration determination, the shell sand, green sand, furan and urethane samples were removed from the freezer and allowed to come to room temperature. The test articles were stirred, and approximately 10 ml of each was poured into a beaker. Each sample was hand-swirled, and 100 µl was pipetted into a pre-weighed foil weigh boat. The weigh boats were covered and placed in a Lipshaw laboratory oven for a 4-hour period. After drying, the weigh boats were placed in a desiccation jar under vacuum overnight. On the following day, the weigh boats were weighed on a Cahn/Ventron Electro balance, and the initial concentration (mg/ml) of each test article was calculated. The test articles were then diluted with the appropriate amount of distilled water to achieve the desired final concentrations for each dose level. The admixtures were mixed to obtain homogeneous suspensions. Sufficient amounts of the test article/distilled water suspensions were prepared to complete the projected 20-week dosing period.[b] The suspensions were aliquoted into glass vials, each

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[b] Protocol originally specified that instillations be continued for 20 consecutive weeks. However, due to the difficulty encountered in reviving the animals following dosing, the instillations were discontinued after 18 weeks.

containing sufficient material for one instillation session. The glass vials were sealed and stored frozen until used. Prior to dosing, the required vials were removed from the freezer and allowed to come to room temperature. The suspensions were continuously stirred on a Corning Magnetic Stirrer during dosing.

6. Intratracheal Administration

Hamsters were intratracheally instilled with the appropriate test or control article at the specified dose levels in distilled water once weekly for 18 consecutive weeks. An instillation volume of 0.20 ml was used. Hamsters in Group 1 (vehicle control) received 0.20 ml distilled water only. Before each treatment, the animals were anesthetized with an intraperitoneal injection of 0.40 ml of 1% Brevital® Sodium solution (Eli Lilly and Co., Indianapolis, Indiana). The dosing suspensions were administered through a 0.25 ml tuberculin syringe fitted with a blunt 19-gauge needle. The needle was 60 mm long and bent at a 135° angle 45 mm from the tip. Separate syringes and needles were used for each test group. The dosing apparatus was sterilized prior to use. Before withdrawing each dosing volume from the vial, the syringe was flushed 3 or 4 times to ensure homogeneity of the suspension. The needle was gently inserted under the epiglottis into the tracheal lumen, the suspension injected, and the needle withdrawn. The animals were kept on the restraining board, and the pharynx was inspected for a short period to ensure that no suspension was regurgitated.

Hamsters that exhibited severe respiratory distress after release of the suspension into the lungs were revived by artificial resuscitation methods. This technique consisted of external heart massage, clearing mucus from the oral cavity and placing the animal on a heating pad for 5 to 15 minutes.

Intratracheal administration was selected for this study as it is a sensitive, reliable and reproducible route for the testing of materials in the respiratory tract of the hamster.

7. Observations

Animals were observed twice daily for mortality and moribundity throughout the study. Observations for pharmacotoxic signs were conducted once weekly, and all findings were recorded. Body weights were measured once

weekly, prior to each instillation session, during Weeks 1[c] through 18. During the post-dosing phase, body weights were measured at monthly intervals.

8. Termination and Gross Necropsy

During the course of the study, hamsters in moribund condition were sacrificed with an intraperitoneal injection of Brevital® Sodium. After the initial dosing period, animals were held until a 98% mortality rate was attained in animals of a given sex in any one group.[d] At that time, all survivors of the same sex were sacrificed with an intraperitoneal injection of Brevital ® Sodium. Female groups were terminated during Week 103, and male groups were terminated during Week 117.

A complete gross necropsy was performed on each animal at the time of death or sacrifice. Necropsy included external examination of the following organs and tissues:

Gross Lesions
Mandibular Lymph Nodes
Salivary Gland
Tracheobronchial Lymph Nodes
Sternebrae (including marrow)
Costochondral Junction and Rib
Thymus
Larynx
Trachea
Bronchi
Lungs
Spleen
Adrenals
Kidneys
Urinary Bladder
Prostate
Testes
Seminal Vesicles
Skin
Heart
Thyroid
Parathyroids
Esophagus
Stomach
Duodenum
Ileum
Colon
Cecum
Rectum
Mesenteric Lymph Nodes
Liver
Gallbladder
Pancreas
Uterus
Ovaries
Brain
Pituitary
Tissue Masses or Suspect Tumors and Regional Lymph Nodes

[c] Since animals were weighed prior to instillation, the Week 1 values were the initial body weights.

[d] The positive control group was excluded from the criterion for study termination. All positive control animals were dead as of Week 81 (males) and Week 91 (females).

All tissues and organs were examined *in situ*, dissected from the carcass and fixed in 10% neutral formalin. The trachea and lungs were removed *en masse*, infused with formalin to normal inspiratory volume, then submerged in formalin for fixation.

9. Histopathology

The following tissues from all hamsters were histologically processed and examined microscopically:

Gross Lesions
Thymus
Larynx
Trachea
Bronchi
Lungs (section from each lobe)
Bronchial Lymph Node
Spleen
Kidneys
Adrenals
Thyroid
Parathyroids
Esophagus
Pancreas
Pituitary
Tissue Masses or Suspect Tumors and Regional Lymph Nodes

Additional histopathology was conducted on 10 males and 10 females selected at random from each treatment group. The following tissues from these animals were processed and examined:

Skin/Mammary Gland
Mandibular Lymph Node
Salivary Gland
Sternum/Rib Junction (marrow)
Femur (marrow)
Urinary Bladder
Seminal Vesicles
Prostate
Testes
Ovaries
Uterus
Nasal Cavity
Thigh Muscle
Heart
Stomach
Duodenum
Jejunum
Ileum
Colon
Cecum
Rectum
Mesenteric Lymph Node
Liver
Gallbladder
Brain
Sciatic Nerve

10. Data Interpretation and Statistical Analysis

a. Survival - Survival time (in study weeks) was calculated for each animal that died during the course of the study. The Kaplan-Meier product-limit method[26] was used to estimate the survival distribution for each test group (each sex separately), and the resulting survival curves were plotted. Survival curves were compared using Breslow's version of the generalized

Wilcoxon test[27] and the Mantel-Cox (generalized Savage) test[28] in order to determine whether differences in survival distribution between test groups were statistically significant. The following group comparisons were conducted:

- Comparison between the vehicle control group (Group 1) and each test group (Groups 2 through 11) by sex.
- Comparison between the low-dose and high-dose groups for each of the four test materials by sex (Group 4 vs Group 5; Group 6 vs Group 7; Group 8 vs Group 9; Group 10 vs Group 11).
- Comparison between males and females within each test group.
- Comparison among groups receiving the four test materials at the $\frac{1}{2}$ MTD level (Groups 4, 6, 8 and 10) by sex.
- Comparison among groups receiving the four test materials at the MTD level (Groups 5, 7, 9 and 11) by sex.

b. Body Weights - The similarity of body weights among all same-sexed test groups at the initial interval was assured by a one-way analysis of variance.[29] At each subsequent body weight interval, the similarity of the changes in body weight (from the initial weight) among all same-sexed groups was tested using ANOVA. Additionally, t-tests were conducted between the vehicle control group (Group 1) and each test group (Groups 2 through 11) at each interval. Since a single control group was used for all comparisons, a multiple comparison procedure [30] was used to adjust for the multiplicity of tests.

c. Tumor Incidence - Statistical comparisons of the number of tumor-bearing animals (as determined by histopathological examination) were conducted between groups using a two-tailed Fisher's exact test.[31] Tumor incidences in the following organs or organ systems were analyzed: all tissues, lungs, total respiratory tract (combining larynx, trachea, mainstem bronchi, and lungs), thyroid, and adrenals. For each of these organs or organ systems, the following group comparisons were conducted:

- Comparison between the vehicle control group (Group 1) and each test group (Groups 2 through 11) by sex.
- Comparison between the low-dose and high-dose groups for each of the four test materials by sex (Group 4 vs Group 5; Group 6 vs Group 7; Group 8 vs Group 9; and Group 10 vs Group 11).

- Comparison between males and females within each test group.
- Comparisons between groups treated with the four test materials at the $\frac{1}{2}$ MTD level (Groups 4, 6, 8 and 10) by sex.
- Comparisons between groups treated with the four test materials at the MTD level (Groups 5, 7, 9 and 11) by sex.

In conducting the Fisher's analyses, a ratio of the number of tumor-bearing animals (numerator) to the effective number of animals at risk (denominator) was formed for each of the two groups involved in a given comparison. The number at risk was determined as the number of survivors in each of the two groups at the time of death of the first animal in either of the two groups which was found (upon subsequent microscopic examination) to have a tumor in the organ or organ system being considered. This procedure was used since the diagnosed tumors were nonobservable during the in-life phase of the study and therefore, the actual time to tumor occurrence could not be determined. Data was analyzed by Fisher's exact test at the 5% level of probability; therefore, values of $p \leq 0.05$ were considered to be statistically significant.

B. RESULTS

1. Vehicle Control, Ferric Oxide Control and Positive Control

This section discusses comparisons between the vehicle control (Group 1) and ferric oxide (Group 2; Fe_2O_3 + Vehicle) or positive control (Group 3; BaP + Fe_2O_3) hamsters by sex and between the sexes within each of these groups.

a. Survival - Individual mortality data are presented in Appendix 1; cumulative mortality is summarized in Table 17. Analysis of survival data (Kaplan-Meier product-limit method) is presented in Appendix 2. Graphic illustration of survival and the results of statistical comparisons of the survival curves are presented in Figures 13A (vehicle control vs ferric oxide or positive control) and 13C (males vs females).

No significant differences in survival distribution were observed for the male or female ferric oxide control hamsters when compared to their same-sexed vehicle control counterpart.

Male hamsters from both the vehicle control and ferric oxide control groups showed increased survival as compared to their respective female counterparts. Study duration, which was based on mortality, differed for males and females. After the initial dosing period, the animals remained on study until a 98% mortality rate was observed in any one sex group (excluding the positive control group). At that time, all remaining hamsters of the same sex were sacrificed. This mortality criterion was met earlier by the female test groups than by the males; terminal sacrifice of the females occurred during Week 103 while the males continued on study until Week 117. Comparison of the survival curves of the vehicle control males and females indicated a significantly higher survival rate for the males at the 5% probability level ($p = 0.0340$) as determined by the Mantel-Cox test. These groups were not significantly different when analyzed using Breslow's generalized Wilcoxon test. The male ferric oxide control group showed significantly greater survival than the female companion group by both methods of comparison (Breslow, $p = 0.0072$; Mantel-Cox, $p = 0.0084$).

Male and female positive control hamsters showed decreased survival during the study compared with the vehicle control group. Survival for the positive control males reached 0% by the end of Week 80; in contrast, 50% of the vehicle control males were alive at that time. The difference in survival rates between the positive control and vehicle control females was less extreme. The positive control females reached 0% survival during Week 91 as compared to 16% survival for the vehicle control females at that time. The differences in survival distribution between the positive control and vehicle control groups were highly significant ($p \leq 0.0001$) for both sexes.

Significantly decreased survival was observed for the positive control males when compared to the positive control females using the Mantel-Cox test ($p = 0.0187$). The difference in the survival patterns of these two groups approached a significant level ($p = 0.0542$) when compared using Breslow's statistic.

b. Pharmacotoxic Signs - The incidence of pharmacotoxic signs observed at each weekly interval throughout the study period is summarized in Tables 18A (males) and 18B (females).

Signs of respiratory distress (wheezing and/or dyspnea) became apparent in one or two ferric oxide control and positive control hamsters during Weeks 6 through 10 of the dosing phase. Wheezing and/or dyspnea were observed with increasing frequency in the ferric oxide and positive control groups during Weeks 16, 17, and 18 of the dosing phase; these signs also were evident in the vehicle control group at this time. Intratracheal instillations were discontinued after 18 weekly treatments due to the respiratory distress exhibited by the hamsters and the difficulty encountered in reviving the animals following instillation.

Hamsters in the vehicle control, ferric oxide control, and positive control groups continued to exhibit wheezing and/or dyspnea throughout most of the study period; however, these signs were noted sporadically after Week 95. Polypnea was also reported in all three groups, but was observed less consistently and with lower incidence than wheezing and dyspnea. No treatment-related patterns were identified with respect to the incidences of these signs.

Other clinical findings observed during the course of this study were those which are normally associated with laboratory animals and included soft feces, alopecia, enlarged abdomen, sores, thin appearance, hunched posture, lethargy, ataxia, and squinted eyes. These signs occurred with similar frequency in the vehicle control, ferric oxide control, and positive control groups.

c. Body Weights - Individual body weights are presented in Appendix 3; mean weights are summarized in Table 19. The results of statistical analyses of changes in body weight (weight at each interval minus the initial weight) between the vehicle control group and the ferric oxide and positive control groups are presented in Appendix 4.

The similarity of the initial body weights among the three control groups was assured (each sex separately) by an analysis of variance.

The body weight changes observed for the male ferric oxide control group were comparable to those of the male vehicle control group at all intervals during the study. The ferric oxide control females showed significantly lower

than vehicle control weight changes at a few scattered intervals (Weeks 5, 7, 8, 10, 11, 22, 38, and 42).

Body weight changes of the positive control males and females were consistently and significantly lower than those of the vehicle control animals throughout the dosing period. During the post-dosing phase, weight changes of the positive control females remained significantly lower than those of the vehicle controls through Week 30 but were similar to the controls for the remainder of the study. Weight changes of the positive control males were significantly lower than the vehicle controls through Week 50.

d. Gross Pathology - Gross pathology observations are summarized in Table 20A (males) and 20B (females). A description of respiratory tract tissue masses observed at necropsy is presented in Table 21.

High incidences of masses and nodules were observed in the respiratory tracts of hamsters treated with the positive control material. Lung masses were present in 46% of the males from this group. A slightly lower incidence (30%) was seen in the positive control females. The incidence of lung nodules followed a similar pattern, occurring in 21% and 8% of the positive control males and females, respectively. Lung masses or nodules were seen in three males (6%) from the ferric oxide control group and one female (2%) from the vehicle control group. Tracheal masses were observed in 8% of the males and 8% of the females from the positive control group but did not occur in the vehicle control or ferric oxide control groups.

Emphysematous rupture of the alveoli and interlobular or pleural adhesions were seen in the lungs of the positive control hamsters and were related to the administration of the BaP/Fe_2O_3 suspension. Alveolar rupture and adhesions both occured with higher incidence in the positive control males (23% and 15%, respectively) than in the female companion group (8% and 4%, respectively). These lesions did not appear in the vehicle control or ferric oxide control groups.

Enlarged bronchial lymph nodes were seen in the positive control hamsters (males, 15%; females, 8%). This finding was considered to be related to the administration of the positive control material.

A variety of other gross changes were seen in the non-respiratory tract organs and tissues of these animals. The most frequently observed findings included: atrial enlargement and thrombosis in the heart; adrenal masses or enlargement of the adrenals; thickening, congestion, or discoloration of the gastrointestinal mucosa; pitted, granular and/or pale kidneys; cystic livers; enlarged and/or reddened lymph nodes; reduced spleens; small or atrophied testes; subcutaneous edema (anasarca); and accumulation of clear or blood-tinged fluid in the thoracic or abdominal cavity. These types of changes are commonly associated with hamsters and are either spontaneous in nature or related to the aging process. Most of these lesions occurred with the expected frequencies and with comparable incidences in all three control groups. However, several age-related lesions (liver cysts; pitted, granular and/or pale kidneys; and testicular atrophy) appeared with lower incidence in the positive control group due to the decreased survival of these animals.

e. Microscopic Pathology - Individual histopathology findings are presented in Appendix 5 and are summarized in Table 22. An individual animal tumor inventory is presented in Appendix 6. The results of Fisher's analysis of tumor incidences, including the number of tumor-bearing animals and the corresponding number at risk used in each statistical comparison, are presented in Tables 23A (vehicle control vs ferric oxide or positive control) and 23C (males vs females).

Respiratory Tract Pathology - Lung carcinomas occurred with high incidence, as expected, in the positive control males (54%) and females (64%). These tumors were also present in two males (4%) and one female (2%) from the ferric oxide control group. Other tumors appeared with low incidence in all control groups. Adenomas were seen with only a slight increase in the positive control males (6%) and females (8%) when compared to the vehicle control hamsters of both sexes (2%). Adenomas were seen in two males (4%) from the ferric oxide control group; no adenomas were present in the female ferric oxide group. One malignant lymphoma and one plasmacytoma occurred in the positive control and ferric oxide control females, respectively.

Carcinomas occurred in the mainstem bronchi of the positive controls with similar incidences in the males (15%) and females (13%). Hyperplastic changes in this tissue were seen with higher incidence in the positive control males

(22%) than in the females (9%). A carcinoma occurred in the mainstem bronchi of one ferric oxide control male.

Malignant tumors and hyperplastic changes of the trachea appeared almost exclusively in the positive control group. Carcinomas were diagnosed in 21% of the males and 26% of the females from this group. A carcinoma occurred in one male from the ferric oxide control group. Hyperplasia was observed with incidences of 17% and 10% in the positive control males and females, respectively.

Laryngeal neoplasia, ranging from focal cellular atypia to frank carcinoma, was present in the positive control hamsters. Carcinomas were diagnosed with much higher frequency in the males (26%) than in the females (8%). Epithelial changes such as mucosal atypia and metaplasia occurred at an incidence of about 10% in the males only. Hyperplasia was seen in comparable numbers of males (22%) and females (20%) from this group. A single carcinoma was present in a ferric oxide control male. Papillomas were seen in one vehicle control and one ferric oxide control male.

A highly significant increase ($p < 0.00001$) in the number of hamsters with lung tumors occurred in the positive control males (30 tumor-bearing animals/44 at risk) and females (33/48) as compared to the vehicle control group (males 1/48; females 1/48). The incidences of total respiratory tract tumors were similarly increased for the positive control males (37/48) and females (36/48) when compared to the same-sexed vehicle control group (males, 2/48; females, 1/48). Comparable numbers of respiratory tract and lung tumors occurred in the vehicle and ferric oxide control groups.

Alveolar/bronchiolar cell hyperplasia was present in the lungs of hamsters from all three control groups but was more prevalent in the ferric oxide and positive control groups. This lesion occurred more frequently in the ferric oxide controls (males, 74%; females, 64%) than in the positive controls (males, 46%; females, 54%). The lowest incidence appeared in the vehicle control males (33%) and females (19%).

Pigment deposition was observed with similarly high incidence (90% to 94%) in the lungs of the ferric oxide and positive control males and females and was related to the administration of ferric oxide. This treatment-related

deposition of pigment was also common in the tracheobronchial lymph nodes of the positive and ferric oxide control hamsters. It appeared with incidences of approximately 90% and 80% in the positive and ferric oxide controls, respectively. A pigment resembling hemosiderin was seen in the lungs of four (8%) males and in the tracheobronchial lymph nodes of 6 (21%) males from the vehicle control group.

Non-Respiratory Tract Pathology - The non-respiratory tract tumors observed in the control groups were of the types that occur spontaneously in this species.

The highest incidence of spontaneous tumors occurred in the adrenals. Most adrenal tumors were diagnosed as cortical adenomas; one carcinoma in a positive control male and pheochromocytomas in two ferric oxide control males were also noted. Fewer adrenal tumors were present in the positive control males (3 tumor-bearing animals/29 at risk) than in the vehicle control males (9/45), but the difference between these two groups was not statistically significant ($p = 0.52114$). The decreased tumor incidence in the positive control males is probably related to the decreased survival. In the females, these tumors were seen with higher incidence in the ferric oxide controls (8/32) than in the vehicle controls (2/32); the difference between these groups was significant ($p = 0.04137$). A significantly increased ($p = 0.02259$) number of adrenal tumor-bearing animals was present in the vehicle control males (9/32) when compared to the vehicle control females (2/33). This sex-related increase in adrenal tumors is common in male hamsters.

Adrenal cortical cell hyperplasia was seen with a pattern quite similar to that noted for adrenal tumors. The higher incidences observed in the ferric oxide (47%) and vehicle control (37%) males as compared to the positive control males (17%) were related to longevity. This lesion occurred less frequently in all female groups (12% to 18%) than in the vehicle control or ferric oxide control males.

A much higher incidence of parathyroid adenomas (19%) was observed in the vehicle control female group than in all other control groups of either sex. One adenoma occurred in a vehicle control male and a ferric oxide control female. This tumor is frequently seen in hamsters.

No significant neoplasia occurred in the any of the organs examined.

A variety of non-neoplastic changes were diagnosed in the other organs of these hamsters. The specific types of lesions seen and the incidences in each group are shown in Table 22.

The majority of the non-neoplastic changes observed were those which are normally associated with aging hamsters. The most frequent age-related findings included the following: amyloidosis involving multiple organs; atrial thrombosis and its attendant sequelae (passive congestion in the liver or pulmonary histiocytosis); liver cysts; and glomerular nephrosis. These lesions occurred with the expected frequencies in the vehicle control and ferric oxide control groups. They were present in substantially fewer positive control hamsters due to decreased survival.

2. Shell Sand

This section discusses the results observed for hamsters treated with the shell sand sample at dose levels of 5.0 mg (low-dose; Group 4) and 10.0 mg (high-dose; Group 5) and compares these results to those seen in the vehicle control (Group 1) animals. Comparisons between the low- and high-dose shell sand groups by sex and between males and females within each of these groups are also addressed.

a. Survival - Individual mortality data are presented in Appendix 1; cumulative mortality is summarized in Table 17. Analysis of survival data (Kaplan-Meier product-limit method) is presented in Appendix 2. Graphic illustration of survival and the results of the statistical comparisons of the survival curves are presented in Figures 13A (vehicle control vs treated groups), 13B (low-dose vs high-dose) and 13C (males vs females).

Males treated with shell sand effluent at a dose level of 5.0 mg had slightly increased survival as compared to the vehicle control males. Mean survival times for the shell sand-treated and vehicle control male groups were 87 weeks and 77 weeks, respectively. Statistical evaluation indicated that the difference in survival patterns of the two groups was statistically significant (Breslow, $p = 0.0461$; Mantel-Cox, 0.0382). The survival curve of males treated with shell sand effluent at a dose of 10.0 mg was statistically com-

parable to that of the vehicle control males. No difference in survival distribution was observed between the low-dose and the high-dose shell sand male groups.

Survival distributions of female hamsters treated with shell sand effluent at both dose levels were comparable to that of the vehicle control females. Statistical analysis of survival patterns for the low-dose and high-dose shell sand female groups revealed no significant difference between these two groups.

Increased survival was observed for male hamsters instilled with shell sand effluent at both dose levels as compared to their respective female counterparts. The difference in the survival distributions of the male and female groups was found to be statistically significant at both the 5.0 mg (Breslow, $p = 0.0344$; Mantel-Cox, $p = 0.0381$) and 10.0 mg (Breslow, $p = 0.0104$; Mantel-Cox, $p = 0.0187$) levels. This pattern of increased survival for males as compared to females was evident within all test groups during this study, except for the positive control. Female test groups were terminally sacrificed at an earlier time (Week 103) than the males (Week 117).

b. Pharmacotoxic Signs - The incidence of pharmacotoxic signs observed at each weekly interval during the study are summarized in Tables 18A (males) and 18B (females).

Wheezing and/or dyspnea were the most frequently observed clinical signs in these test groups. These signs first became evident in the shell sand and vehicle control groups during the latter part of the dosing phase of the study (Week 16 through 18). Intratracheal instillations, originally scheduled to occur once weekly for 20 consecutive weeks, were discontinued after 18 treatments due to the respiratory signs observed in the hamsters and the difficulty encountered in reviving the animals following treatment.

Hamsters in the vehicle control and shell sand groups continued to exhibit wheezing and/or dyspnea throughout most of the post-dosing phase. Polypnea was also seen in all groups at most intervals but this sign occurred with lower incidence than either wheezing or dyspnea. No specific treatment-related trends were evident. The incidence of these signs declined rapidly after Week 90 in the shell sand and vehicle control groups.

Other clinical signs observed in these groups included alopecia, soft feces, sores, enlarged abdomen, lethargy, ataxia, squinted eyes, and hunched and/or thin appearance. These findings are commonly seen in laboratory animals and occurred with approximately equal frequency in control and treated hamsters.

c. Body Weights - Individual body weights are presented in Appendix 3; mean weights are summarized in Table 19. The results of statistical analyses of changes in body weight (weight at each interval minus the initial weight) between the vehicle control group and each shell sand group are presented in Appendix 4.

The similarity of initial body weights among the vehicle control and shell sand groups of the same sex was assured by an analysis of variance.

The body weight changes of the high-dose shell sand males were significantly lower than the vehicle control values at all available intervals during the instillation phase. During the post-dosing phase, the weight changes of the high-dose males remained significantly lower than controls only at the first three intervals (Weeks 22, 26, and 30).

The effect on weight gain during the instillation phase was less severe in the low-dose shell sand male group than in its high-dose counterpart. The weight changes of the low-dose males were significantly lower than the vehicle control values at Weeks 4 through 11 and at Weeks 14 and 15. The treated group values were similar to those of the control group at the last three intervals of the dosing phase (Weeks 16 through 18) and throughout the post-dosing phase.

The body weight changes of the low- and high-dose female groups were consistently and significantly lower than those of the vehicle control animals throughout the dosing phase. During the post-dosing phase, the weight changes of the high-dose females were significantly lower than the control values at a few scattered intervals (Weeks 22, 30, 38, 42, and 62). The weight changes of the low-dose females were similar to the controls throughout the post-dosing phase.

d. Gross Pathology - Gross pathology observations are summarized in Tables 20A (males) and 20B (females). A description of respiratory tract tissue masses observed at necropsy is presented in Table 21.

Black foci, dark spots, or dark areas were frequently observed in the lungs of hamsters from both shell sand groups and were considered treatment related. The incidences in the treated groups ranged from 54% to 76%; similar findings were noted less frequently in the vehicle control males (15%) and females (2%).

No respiratory tract masses were seen in the shell sand groups. One lung mass was present in a vehicle control female.

Gross changes described in the other organs and tissues were not related to the instillation of the test material. A wide range of spontaneous disease lesions and age-related changes common to this species occurred in the expected number of animals; the incidences of these finding are shown in Table 20. The most consistently noted observations included the following: atrial enlargement and thrombus formation in the heart; adrenal masses or enlargement; pitted, granular, and/or pale kidneys; cystic livers; reddened and/or enlarged lymph nodes; small spleens; testicular atrophy; subcutaneous edema; and clear or red-tinged fluid in the thoracic or abdominal cavities. These changes were seen with approximately equal frequency in the treated and control groups.

e. Microscopic Pathology - Individual histopathology findings are presented in Appendix 5 and are summarized in Table 22. An individual animal tumor inventory is presented in Appendix 6. The results of Fisher's analysis of tumor incidences, including the number of tumor-bearing animals and the corresponding number at risk used in each statistical comparison, are presented in Tables 23A (vehicle control vs treated groups), 23B (low-dose vs high-dose) and 23C (males vs females).

Respiratory Tract Pathology - Pulmonary neoplasia was limited to the vehicle control group; an alveolar/bronchiolar cell adenoma and a cystadenoma were diagnosed in one male and one female, respectively. No tumors were seen in hamsters treated with shell sand at either dose level.

Alveolar/bronchiolar cell hyperplasia was diagnosed in all groups but occurred with much higher incidences in the low-dose and high-dose hamsters (males, 88% and 94%, respectively; females, 76% at both dose levels) than in the controls (males, 33%; females, 19%). The increased incidence of this lesion in the treated hamsters was attributed to the administration of shell sand.

Foci of clumped black material, usually around the airways, were prominent in the lungs of most of the treated animals. This pigment was usually free in the tissues and often produced a strong, local hyperplastic response in the alveolar/bronchiolar tissue. Inflammatory response to the pigment was seen infrequently. The tracheobronchial lymph nodes of the treated hamsters contained intercellular aggregates of the same pigment seen in the lungs. A pigment described in four vehicle control males appeared to be hemosiderin.

Other non-neoplastic lung lesions, including adenomatosis, eosinophilic deposits (pulmonary alveolar proteinosis), and histiocytosis (accumulations of alveolar macrophages) were seen with low incidences and did not indicate any signficant treatment effects.

No treatment-related changes occurred in the larynx or trachea. Laryngeal papillomas were diagnosed in one vehicle control male and one low-dose shell sand male.

Non-Respiratory Tract Pathology - Tumors diagnosed in other organs appeared to be spontaneous and not related to treatment. Adrenal cortical adenomas and parathyroid adenomas were the most frequently observed neoplasms and are discussed below.

Adrenal cortical adenomas were present in all groups and occurred with higher incidence in the males; this sex-related pattern is common in hamsters. This tumor was seen with similar frequency (18% to 22%) in the treated and control males. Two additional tumors (one medullary adenoma and one carcinoma) were observed in the low-dose male group. Slightly higher incidences of cortical adenomas were present in the treated female groups (approximately 14%) than in the vehicle control females (4%). Fisher's analysis failed to indicate a statistically significant difference between any of these groups with respect to the incidence of adrenal tumors. Adrenal

hyperplasia occurred with higher incidence in all male groups (approximately 40%) than in the females (approximately 20%). For a given sex, however, similar incidences of adrenal hyperplasia were present in the treated and control groups.

Parathyroid adenomas were observed in 19% and 12% of the vehicle control and high-dose shell sand females, respectively. This tumor occurred with lower incidences (2% to 4%) in the low-dose female group and all male groups.

Insignificant numbers of tumors were diagnosed in the thyroid, pancreas, liver, kidneys, uterus, and spleen. They were noted with comparable incidences in the treated and control groups. The types and incidences of these tumors are shown in Table 22 (Summary of Histopathology Findings) and Appendix 6 (Tumor Inventory).

Many non-neoplastic changes were seen in other organs of the shell sand hamsters. However, no treatment-related changes were identified. The types of lesions seen and the numbers of animals affected are shown in Table 22. The most frequently observed findings were characterized as amyloidosis (a naturally occurring degenerative disease of hamsters) which was particularly evident in the adrenals, thyroid, liver, and kidneys; glomerular nephrosis (degenerative changes in the kidneys); atrial thrombosis (an age-related cardiac lesion); chronic passive hepatic congestion often accompaned by centrilobular necrosis (usually secondary to atrial thrombosis or associated with an agonal process); and hepatic cysts. All of these findings are anticipated in aging hamsters and occurred with expected frequencies in the treated and control groups. The incidences of amyloidosis, atrial thrombosis, and hepatic cysts tended to be higher in the females, whereas glomerular nephrosis was more common in the males. These sex-related patterns are typically seen in hamsters.

Clear cell foci (clusters or foci of slightly enlarged hepatocytes with clear cytoplasm) occurred more frequently in the livers of the treated females (11% and 13%) than in the controls of either sex (2%); no clear cell foci were diagnosed in the treated males. The significance of the increased incidence of this change in the livers of the treated females is not clear.

3. Green Sand

This section discusses the results observed for hamsters treated with the green sand sample at dose levels of 5.0 mg (low-dose; Group 6) and 10.0 mg (high-dose; Group 7) and compares the treated group results to those seen in the vehicle control (Group 1) animals. Comparisons between the low- and high-dose green sand groups by sex and between males and females within each of these groups are also addressed.

a. Survival - Individual mortality data are presented in Appendix 1; cumulative mortality is summarized in Table 17. Analysis of survival data (Kaplan-Meier Product-Limit Method) is presented in Appendix 2. Graphic illustration of survival and the results of the statistical comparisons of the survival curves are presented in Figures 13A (vehicle control vs treated groups), 13B (low-dose vs high-dose) and 13C (males vs females).

The survival curves of males and females treated with green sand effluent at both dose levels were similar to those of the same-sexed vehicle control group. Additionally, no significant differences in survival patterns were observed between the low- and high-dose green sand groups for either males or females.

Comparison of survival curves for the low-dose males and females indicated that the difference between these groups was significant when analyzed by the Mantel-Cox test ($p = 0.0247$). In the high-dose groups, the difference in survival between males and females was also significant ($p = 0.0445$) when compared using the Mantel-Cox statistic. No significant differences were noted between males and females at either dose level when compared using Breslow's generalized Wilcoxon test (low-dose, $p = 0.2192$; high-dose, $p = 0.2204$).

b. Pharmacotoxic Signs - The incidence of pharmacotoxic signs observed at each weekly interval during the study are summarized in Tables 18A (males) and 18B (females).

Treatment-related clinical signs observed in these groups included wheezing, dyspnea, and polypnea. These signs became apparent in the green sand and vehicle control groups during the latter part of the dosing phase (Weeks 16 through 18). Intratracheal instillations, originally scheduled to

occur once weekly for 20 consecutive weeks, were discontinued after 18 treatments due to the respiratory distress exhibited by the hamsters and the difficulty encountered in reviving the animals following treatment.

Hamsters in the vehicle control and green sand groups continued to show signs of respiratory distress throughout the post-dosing phase, although the signs occurred sporadically after Week 90. Wheezing and dyspnea were reported with higher incidence than polypnea at all intervals. Comparison between the incidences of these findings in the vehicle and green sand groups or between the low- and high-dose treated groups failed to reveal any specific treatment- or dose-related patterns.

Other clinical signs observed during the course of this study occurred with low and approximately equal frequency in the vehicle control and green sand groups. The signs noted were those which are commonly seen in laboratory animals and included alopecia, soft feces, sores, enlarged abdomen, ataxia, lethargy, squinted eyes, and hunched and/or thin appearance.

c. Body Weights - Individual body weights are presented in Appendix 3; mean weights are summarized in Table 19. The results of statistical evaluation of body weight changes (weight at each interval minus the initial weight) between the vehicle control group and each green sand group are presented in Appendix 4.

The initial body weights of the vehicle control and green sand-treated groups of the same sex were similar as indicated by an analysis of variance.

The weight changes of the high-dose males were significantly lower than those of the vehicle control animals at all available intervals during dosing except Week 3. Significant differences between the low-dose and control groups were noted less consistently, occurring at Weeks 2, 4 through 10, 13, 15, and 18. During the post-dosing phase, the weight changes of both treated male groups were similar to the control values.

The weight changes of the high-dose females were significantly lower than those of the vehicle control animals at all instillation intervals except Week 3. During the post-dosing phase, the high-dose weight changes were significantly lower only at Weeks 22 and 54. Body weight changes of the low-dose females were significantly lower than the control values at Weeks 2, 7 through 10, 12 through 15, and 17 during the dosing phase but were similar to the controls for the remainder of the study.

d. Gross Pathology - Gross pathology observations are summarized in Tables 20A (males) and 20B (females). Descriptions of all respiratory tract masses seen at necropsy are presented in Table 21.

No treatment-related gross lesions were seen in the respiratory tracts of hamsters instilled with green sand effluent at either dose level. Tinctorial changes in the lungs appeared with similar incidences in the treated and control groups. Lung masses or nodules were present in insignificant numbers, occurring in one high-dose male, one high-dose female, and one vehicle control female.

Other gross changes observed in the green sand groups were considered to be spontaneous in nature or related to the aging process. The specific types and incidences of these lesions are shown in Table 20. Age-related changes (including enlarged atria; pitted, granular, and/or pale kidneys; and reduced testes) were present in the expected numbers of animals and with approximately equal frequency in the treated and control groups. Cystic livers, also common in older hamsters, were seen with slightly higher incidence in the vehicle control females (31%) than in any of the other groups (13% to 20%). Other spontaneous changes (enlarged adrenals; enlarged and/or reddened lymph nodes; thickened, roughened, or discolored gastrointestinal mucosa; intestinal nodules of an inflammatory nature; reduced spleens; and subcutaneous edema) were seen with scattered incidence in the treated and control groups.

Accumulation of clear or red-tinged fluid in the abdominal or thoracic cavities was seen with higher incidence in the high-dose females (18%) than in any of the other groups (6% to 8%). This finding, which is usually secondary to atrial thrombosis or associated with an agonal process, is not related to treatment.

e. Microscopic Pathology - Individual histopathology findings are presented in Appendix 5 and are summarized in Table 22. An individual animal tumor inventory is presented in Appendix 6. The results of Fisher's analysis of tumor incidences, including the number of tumor-bearing animals and the corresponding number at risk used in each statistical comparison, are presented in Tables 23A (vehicle control vs treated groups) 23B (low-dose vs high-dose) and 23C (males vs females).

Respiratory Tract Pathology - No primary lung neoplasms were diagnosed in hamsters treated with green sand effluent. An alveolar/bronchiolar cell adenoma and a cystadenoma occurred in the vehicle control group. A metastatic carcinoma (of adrenal orgin) and a lymphoma were noted in the high-dose male group.

Alveolar/bronchiolar cell hyperplasia was the only treatment-related finding observed in the respiratory tracts of hamsters treated with green sand effluent. In the males, this lesion was observed in 50% and 60% of the low-dose and high-dose hamsters, respectively; it occurred in approximately 30% of the vehicle control males. Alveolar/bronchiolar cell hyperplasia occurred with similar frequency in control and low-dose females (19% and 17%) but occurred in approximately twice as many high-dose females (36%).

The incidence of alveolar macrophages (histiocytosis) was about the same within the treated and control groups of the same sex. A two- to threefold increase was noted for the females (21% to 30%) as compared to the males (10% to 12%).

Pigment was observed infrequently in the lungs of the treated hamsters but was present in small amounts in the tracheobronchial lymph nodes of a few animals from each treated male and female group; the incidences ranged from 10% to 30%. The pigment was usually gray-black, but occasionally was golden brown, resembling hemosiderin. A 21% incidence of pigment, presumably hemosiderin, was observed in the lymph nodes of the vehicle control males; this finding was not noted in the vehicle control females.

No treatment-related changes were seen in the larynx or trachea of hamsters instilled with green sand effluent. A papilloma was diagnosed in the larynx of one vehicle control male.

Non-Respiratory Tract Pathology - An increased incidence of proliferative lesions (neoplasia and hyperplasia) of the thyroid gland occurred in the high-dose females. Six adenomas were diagnosed in this group; one papilloma and two follicular cell hyperplasias were also noted. One adenoma and one neoplasm not otherwise stated (N.O.S.) due to advanced autolysis were present in the high-dose male group. Males and females treated with the low-dose also showed proliferative changes in the thyroid. Two adenomas and one hyperplasia

were present in the males, and three adenomas were present in the females treated at the lower dose level. One follicular cell adenoma was found in the female vehicle control group; no proliferative lesions were present in the thyroids of the vehicle control males. Although the increased incidence of proliferative thyroid lesions in the treated groups, particularly the high-dose females, suggested a treatment-related effect, no statistically significant differences in the numbers of tumor-bearing animals were noted between the treated and control groups.

Parathyroid adenomas occurred most frequently in the vehicle control female group where 7 (19%) of these tumors were observed. This was two to three times the number seen in the treated female groups (5% and 7%). Adenomas were present in one vehicle control and two low-dose males. Hyperplastic lesions were seen with much higher incidence (21%) in the high-dose males than in the other male groups (3% and 7%), yet no tumors appeared in this group. Proliferative lesions of the parathyroid gland are fairly common in hamsters, but tend to occur with variable incidence.

Cortical cell adenomas of the adrenals occurred with slightly higher incidence in the vehicle control males (22%) than in either treated group (11% to 14%). Adrenal hyperplasia occurred at approximately the same level in all male groups (32% to 40%). A single cortical cell carcinoma was present in each treated male group. Cortical cell adenomas were observed in similar numbers of treated and control females (4% to 8%). The incidence of adrenal hyperplasia was noted to be slightly higher in the treated females (32% and 29%) than in the female controls (18%). Proliferative adrenal lesions are common in hamsters, and the incidences in these groups did not differ from those seen in previous studies.

Neoplastic changes in other organs were noted sporadically and with similar incidences in the treated and control groups of both sexes. The tumors observed in each animal are shown in Appendix 6 (Tumor Inventory) and are summarized in Table 22 (Summary of Histopathology Findings).

A wide range of non-neoplastic lesions were seen in these groups of hamsters. The incidences of these findings are summarized in Table 22. The most frequently observed findings included amyloidosis (affecting multiple

organs) which occurred with highest frequency in females; glomerular nephrosis which occurred with highest frequency in males; atrial thrombosis; chronic passive hepatic congestion, with or without centrilobular necrosis; hepatic cysts; and proliferative enteritis (inflammation) of the cecum. These changes occur spontaneously in aging hamsters and were encountered in the expected numbers of treated and control animals in this study.

Clear cell foci occurred slightly more frequently in the livers of the treated females (12% and 7% in the low-dose and high dose groups, respectively) than in the controls (2%). The significance of the increased incidence in the treated females is not known.

4. Furan

This section discusses the results for hamsters treated with the furan sample at dose levels of 5.0 mg (low-dose; Group 8) and 10.0 mg (high-dose; Group 9) and compares the treated group results to those seen in the vehicle control (Group 1) animals. Comparisons between the low- and high-dose furan groups by sex and between males and females within each of these groups are also discussed.

a. Survival - Individual mortality data are presented in Appendix 1; cumulative mortality is summarized in Table 17. Analysis of survival data (Kaplan-Meier product-limit method) is presented in Appendix 2. Graphic illustration of survival and the results of the statistical analysis of the survival curves are presented in Figures 13A (vehicle control vs treated groups), 13B (low-dose vs high-dose) and 13C (males vs females).

No significant differences in survival distribution were observed for males or females treated with furan effluent at either dose level when compared to the vehicle control group of the same sex. Comparison between the low-dose and high-dose furan groups revealed no significant differences in survival patterns for either males or females.

Decreased survival was observed for females treated with the furan sample as compared to males treated with this material at the same dose level. The difference in survival distribution between males and females was significant at both the low-dose (Breslow, $p = 0.0179$; Mantel-Cox, $p = 0.0031$) and high-dose (Breslow, $p = 0.0031$; Mantel-Cox, $p = 0.0013$) levels.

b. Pharmacotoxic Signs - The incidence of pharmacotoxic signs observed at each weekly interval during the study are summarized in Tables 18A (males) and 18B (females).

Signs of respiratory distress (wheezing, dyspnea and/or polypnea) were frequent observations in the vehicle control and furan-treated groups. These signs first appeared during the latter part of the dosing phase (Weeks 16 through 18). Instillations were discontinued after 18 (rather than 20) weekly treatments due to the severity of these signs. Hamsters continued to exhibit these signs throughout the post-dosing phase, although a scattered incidence was seen after Week 95. Wheezing and dyspnea were observed more frequently than polypnea. No definite treatment- or dose-related patterns were observed, although higher incidences of wheezing and dyspnea were noted in males from the high-dose furan group as compared to either the low-dose furan or vehicle control male groups at a few later intervals (Weeks 85, 90, and 94).

No other noteworthy pharmacotoxic signs were seen in these groups. Signs noted were those which are normally associated with laboratory animals and included soft feces, alopecia, enlarged abdomen, sores, thin appearance, hunched posture, and lethargy. These findings occurred with similar incidence in the treated and vehicle control groups.

c. Body Weights - Individual body weight data are presented in Appendix 3; mean values are summarized in Table 19. The results of statistical evaluation of body weight changes (weight at each interval minus the initial weight) between the furan-treated and vehicle control groups are presented in Appendix 4.

The similarity of body weights among the groups at study initiation was assured by an analysis of variance.

The weight changes of the high-dose furan males were significantly lower than the control values at all intervals during the instillation period with the exception of Week 12. The weight changes of the high-dose males remained significantly lower than those of the control animals at six of the first eight intervals during the post-dosing phase (Weeks 22, 26, 38, 42, 46, and 50). No differences were seen between these two groups after Week 50. Significantly decreased weight changes were noted less consistently for the

low-dose males during dosing and occurred at Weeks 2 through 5, 8 through 13, and 15. During the post-dosing phase, the weight changes of the low-dose males were significantly lower than the control values only at Weeks 38, 42, and 46.

The body weight changes of the high-dose females were significantly lower than the control values at most intervals during the dosing phase (Weeks 2 through 10, 15, and 16). During the post-dosing phase, the high-dose weight changes were lower than the control values at Week 22, 30, 38, 42, and 54 but were similar to the controls after Week 54. The low-dose females were noted to have significantly lower weight changes only at Weeks 8, 10, and 15 during the instillation phase. During the post-dosing phase, the weight changes of the low-dose females were significantly lower than the control values at Weeks 22, 26, 30, 38, and 42 but were similar to the controls after Week 42.

d. Gross Pathology - Gross pathology findings for hamsters that died or were sacrificed during the study are summarized in Tables 20A (males) and 20B (females). A description of all respiratory tract tissue masses observed at necropsy is presented in Table 21.

Gray-black foci or dark spots in the lungs were the only treatment-related gross lesions observed in the respiratory tracts of hamsters treated with the furan sample. The incidences in the treated groups ranged from 30% to 59%, while that in the vehicle control group was 15% for the males and 2% for the females. Masses or nodules were present in the lungs or bronchi of only one high-dose male and one vehicle control female. A tracheal mass was observed in one high-dose female

Many gross lesions were described in the other organs and tissue of these hamsters, and the incidences are shown in Table 20. Most of these changes were considered to be spontaneous or age related; none were attributed to treatment with the furan sample. The most consistently noted gross observations included the following: atrial enlargement and thrombosis; enlarged adrenals; thickened, reddened or roughened gastrointestinal mucosa; pitted, granular and/or pale kidneys; cystic livers; enlarged and/or reddened lymph nodes; testicular atrophy; enlarged or small spleens; subcutaneous edema; and accumulation of fluid in the abdominal or thoracic cavities. These

changes were seen in the expected numbers of animals and with about equal incidence in the treated and control groups.

e. Microscopic Pathology - Individual histopathology findings are presented in Appendix 5 and are summarized in Table 22. An individual animal tumor inventory is presented in Appendix 6. The results of Fisher's analysis of tumor incidences, including the number of tumor-bearing animals and the corresponding number at risk used in each statistical comparison, are presented in Table 23A (vehicle control vs treated groups), 23B (low-dose vs high-dose) and 23C (males vs females).

Respiratory Tract Pathology - An undifferentiated sarcoma in a low-dose female and an alveolar/bronchiolar adenoma in a low-dose male were the only primary neoplasms present in the lungs of the treated hamsters. Adenomas occurred in one male and one female from the vehicle control group. The sarcoma in a high-dose male and the carcinoma in a low-dose female were metastases from other sites.

Alveolar/bronchiolar cell hyperplasia occurred with much higher incidence in the male and female treated groups (approximately 90%) than in the vehicle control (males, 33%; females 19%) and was considered to be enhanced by treatment. In the treated groups, the hyperplasia was associated with clumps of brownish-black pigment, which are closely associated with the airways. This hyperplastic response was extensive enough to be classified as adenomatosis in four (8%) low-dose and three (6%) high-dose females. Adenomatosis was not seen in the treated male groups.

Eosinophilic deposits (alveolar proteinosis) occurred with higher incidence in the treated groups. A definite dose-related pattern was observed in the low- and high-dose males (12% and 29%, respectively) and females (6% and 64%, respectively). The incidences of this lesion in the vehicle control males and females were 4% and 2%, respectively. The eosinophilic deposits are aggregates of phospholipid material secreted by the Type II alveolar macrophages, usually in response to some irritant. The material may occur in a large aggregates involving several alveoli or in small clumps within a single alveolus. In the latter instance, inflammatory cells and macrophages are usually present. The material is eventually removed by the macrophages.

No significant tracheal lesions occurred in any of the groups. A papilloma was recognized in the larynx of a vehicle control male. A focus of epithelial hyperplasia occurred in the larynx of a high-dose male.

The tracheobronchial lymph nodes of the treated hamsters frequently contained intercellular aggregates of the same blackish-brown pigment seen in the lungs.

Non-Respiratory Tract Pathology - The tumors diagnosed in other organs appeared to be spontaneous and not related to treatment. The most frequently observed neoplasms occurred in the endocrine system (adrenal, thyroid, and parathyroid glands) and are discussed below.

Adrenal tumors occurred more frequently in the control males (9 tumor-bearing animals/32 at risk) than in either treated male group (low-dose 4/36; high-dose 6/42). Most of the adrenal tumors were cortical cell adenomas; a single cortical cell carcinoma was present in a high-dose male. No significant differences in the numbers of animals with adrenal tumors were noted between the treated and control male groups. Cortical cell adenomas were observed with lower incidence in all female groups, occurring in two vehicle control and two low-dose treated females. Statistical evaluation indicated a significant increase ($p = 0.03156$) in the number of high-dose males with adrenal tumors (6/42) when compared to their female companion group (0/33). A similar significant increase ($p = 0.02259$) in adrenal tumors was noted between the vehicle control males (9/32) and females (2/33). The incidence of adrenal cortical cell hyperplasia in all male groups also exceeded that seen in the females. The increase in proliferative adrenal lesions is common for male hamsters.

Proliferative lesions (neoplasia and hyperplasia) of the thyroid, although present at insignificant levels in all groups, had a slightly higher incidence in the treated hamsters than in the controls. Adenomas were diagnosed in one male and one female from the low-dose group and in two males and two females from the high-dose group. A carcinoma was present in one low-dose male. Thyroid hyperplasia was observed in three low-dose males, one high-dose male, and one low-dose female. A single proliferative lesion (an adenoma) occurred in the female control group.

Adenomas of the parathyroid occurred more frequently in the female control group than in all other male or female groups. These tumors were diagnosed in seven (19%) of the vehicle control females. In the other groups, adenomas were observed in two high-dose females, one control male and one low-dose male. Hyperplasia of the parathyroid, however, occurred with slightly higher frequency in both treated male groups (9% and 7%) than in the male control (3%) and in the high-dose female group (14%) than in the female controls (3%).

Insignificant numbers of tumors were diagnosed in the liver, spleen, kidneys, stomach, ovaries, and uterus. These neoplasms were seen with comparable incidences in the treated and control groups. The specific types of tumors observed in each animal are shown in Appendix 6 (Tumor Inventory) and are summarized in Table 22 (Summary of Histopathology Findings).

Non-neoplastic lesions observed in other organs are summarized in Table 22. No treatment-related findings were identified in hamsters instilled with furan at either dose level.

The majority of the non-neoplastic changes were directly related to the aging phenomenon in hamsters. The most frequently observed findings were characterized as amyloidosis involving several organs; atrial thrombosis; glomerular nephrosis; chronic passive hepatic congestion with or without centrilobular necrosis; hepatic cysts; and inflammatory lesions associated with proliferative enteritis in the large or small intestine. These findings occcurred with the usual incidences in the treated and control groups. Amyloidosis, atrial thrombosis, and hepatic cysts were seen with higher incidences in females, while glomerular nephrosis was seen with higher incidence in males. These sex-related patterns are typical of hamsters.

Clear cell foci in the liver showed only a slightly higher incidence in all treated groups (4% to 6%) than in the controls (2%). This liver lesion was not considered treatment related.

5. Urethane

This section discusses the results for hamsters treated with the urethane sample at dose levels of 2.5 mg (low-dose, Group 10) and 5.0 mg (high-dose;

Group 11) and compares these results to those seen in the vehicle control (Group 1) animals. Comparisons between the low- and high-dose urethane groups by sex and between males and females within each of these groups are also discussed.

a. Survival - Individual mortality data are presented in Appendix 1; cumulative mortality is summarized in Table 17. Analysis of survival data (Kaplan-Meier product-limit method) is presented in Appendix 2. Graphic illustration of survival and the results of the statistical analyses of the survival curves are presented in Figures 13A (vehicle control vs treated groups), 13B (low-dose vs high-dose) and 13C (males vs females).

No significant differences in survival distribution were seen for males or females instilled with urethane effluent at either dose level when compared to the same-sexed vehicle control group. Comparable survival patterns were also evident for the low- and high-dose urethane groups of both sexes.

Survival of the urethane-treated males was slightly increased compared with that of the females. At the low-dose level, the difference in survival distribution between males and females was significant as determined by the Mantel-Cox statistic ($p = 0.0055$). The difference in survival between males and females at the high-dose level was significant by both methods of analysis (Breslow, $p = 0.0131$, Mantel-Cox $p = 0.0004$).

b. Pharmacotoxic Signs - The incidence of pharmacotoxic signs observed at each weekly interval during the study is summarized in Tables 18A (males) and 18B (females).

Treatment-related clinical signs consisted of wheezing, dyspnea, and polypnea and occurred in both treated and vehicle control hamsters. These signs were first seen during the latter part of the dosing phase (Weeks 16 through 18). Instillations, originally scheduled to continue for 20 consecutive weeks, were stopped after 18 treatments, due to these signs of respiratory distress.

Hamsters continued to exhibit these signs throughout the post-dosing phase. Wheezing and dyspnea occurred with higher incidence than polypnea. No treatment-related trends were apparent.

Other clinical signs observed during this study were those which are commonly seen in laboratory animals. The most frequently noted signs were alopecia, soft feces, squinted eyes, enlarged abdomen, thin and/or hunched appearance, and lethargy. These occurred with similar incidence in the urethane and vehicle control groups.

c. Body Weights - Individual body weight data are presented in Appendix 3; mean values are summarized in Table 19. The results of statistical comparisons between treated and control group body weight changes (weight at each interval minus the initial weight) are presented in Appendix 4.

The similarity of the initial body weights among the groups was demonstrated (each sex separately) by an analysis of variance.

The weight changes of the high-dose males were significantly lower than the vehicle control values at only six intervals during the instillation phase (Weeks 3, 5, 6, 8, 9, and 13). During the post-dosing phase, the weight changes of the high-dose males were significantly lower than the controls only at Weeks 38, 42 and 46. Body weight changes of the low-dose males were similar to those of the vehicle control males at all intervals during the study.

The body weight changes of the high-dose females were significantly lower than those of the vehicle controls at Weeks 4, 5, 7, 8, 9, and 10 during the instillations. During the post-dosing phase, weight changes of the high-dose females were significantly lower at Weeks 38, 42, 46, and 54 but were similar to the controls for the remainder of the study. A significantly lower than control weight change was noted for the low-dose females at Week 8 during dosing. During the post-dosing phase, the weight changes of the low-dose females were significantly lower than those of the controls at Weeks 22, 26, 30, 38, and 42 but were similar to the controls for the remainder of the study.

d. Gross Pathology - Gross pathology findings are summarized in Tables 20A (males) and 20B (females). A description of all respiratory tract tissue masses observed at necropsy is presented in Table 21.

No treatment-related lesions were seen in the respiratory tracts of hamsters treated with urethane effluent. Congestion and various tinctorial

changes occurred with similar incidence in the treated and vehicle control groups. Masses were observed in one low-dose male and one vehicle control female.

A variety of spontaneous disease lesions and age-related changes commonly associated with hamsters were present in these groups; the incidences of these findings are shown in Table 20. The most consistently noted observations included the following: atrial enlargement and thrombosis; enlarged adrenals; congested, thickened, enlarged or discolored gastrointestinal mucosa; intestinal nodules of an inflammatory nature; pitted, granular and/or pale kidneys; cystic livers; reddened and/or enlarged lymph nodes; reduced or enlarged spleens; testicular atrophy; and subcutaneous edema. These lesions were described in the usual numbers of control and treated animals.

Fluid in the thoracic cavity was seen with higher incidence in the high-dose males (18%) than in the controls (6%) but occurred with comparable frequency in the treated and control females. This finding is often indicative of an agonal process and is often secondary to atrial thrombosis.

e. Microscopic Pathology - Individual histopathology findings are presented in Appendix 5 and are summarized in Table 22. An individual animal tumor inventory is presented in Appendix 6. The results of Fisher's analysis of tumor incidences, including the number of tumor-bearing animals and the corresponding number at risk used in each statistical comparison, are presented in Tables 23A (vehicle control vs treated groups), 23B (low-dose vs high-dose) and 23C (males vs females).

Respiratory Tract Pathology - Pulmonary neoplasms were seen with very low incidence in these groups. A carcinoma occurred in a low-dose male and was the only primary lung tumor seen in the treated hamsters. Malignant lymphoma was diagnosed in the lungs of one low-dose male and one high-dose female. This neoplasm does not originate from lung tissue and, therefore, is not considered to be a primary lung tumor. It is a fairly common tumor in the hamster. Lung adenomas were seen in one male and one female from the vehicle control group.

Alveolar/bronchiolar cell hyperplasia was the most common non-neoplastic lesion in the lungs. The males in all groups tended to have slightly higher

incidences than their female counterparts. The incidence of this lesion did not appear to be enhanced by treatment with urethane. Within each sex, alveolar/ bronchiolar cell hyperplasia occurred with comparable frequency in the vehicle control and high-dose urethane groups (males, 33% and 37%, respectively; females, 19% and 20%, respectively). Fewer low-dose hamsters were affected (males, 18%; females, 13%).

Alveolar macrophages (histiocytosis) were seen more frequently in the high-dose males (22%) than in the other male groups (10% and 12%). The increase in the high-dose males is probably secondary to atrial thrombosis (an age-related cardiac lesion common in hamsters) which was also seen with slightly higher incidence in this particular group. In the females, however, the lowest incidence occurred in the high-dose group (15%) as compared to the low-dose and vehicle control groups (21%).

No significant pathology occurred in the larynx or trachea of the urethane-treated groups. A papilloma occurred in one vehicle control male.

Non-Respiratory Tract Pathology - The most frequently diagnosed non-respiratory tract tumors in these groups occurred in the endocrine system (adrenals, thyroid and parathyroids). These tumors occur spontaneously in hamsters and were not related to treatment with urethane effluent.

The number of adrenal tumors (primarily cortical cell adenomas) diagnosed in each male group far exceeded that seen in the respective female companion group. Statistical analysis between the low-dose groups revealed a significant increase ($p = 0.01104$) in the number of males with adrenal tumors (11 tumor-bearing animals/49 at risk) as compared to the females (2/45). A similar significant increase ($p = 0.02259$) occurred in the vehicle control male group (9/32) as compared to its female companion group (2/33). Within each sex, the incidence of adrenal tumors observed in each of the treated groups was statistically comparable to that of the same-sexed vehicle control group. A similar pattern (i.e., higher incidence occurring in the males than in the females, but comparable incidences between treated and control groups of the same sex) was noted for cortical cell hyperplasia. Proliferative adrenal lesions commonly occur with higher incidence in male hamsters than in females.

Follicular cell adenomas of the thyroid occurred with low incidence in the females with a slight and insignificant increases in the treated females (low-dose, 2/24; high-dose, 3/33) compared with the vehicle control females (1/35). A slight increase in thyroid tumors was also noted in the low-dose male group (4/25) compared with the vehicle control males (0/19); the difference in tumor incidence between these two groups was not significant ($p = 0.12174$). One adenoma occurred in the high-dose male group.

Parathyroid adenomas were seen with slightly higher incidence in the high-dose males (10%) than in the controls (3%). The combined incidence of adenomas plus hyperplasia in the high-dose males (12%) was similarly increased compared to the control (5%). In the females, however, parathyroid adenomas were seen with higher incidence in the controls (19%) than in either treated group (5%). Proliferative lesions of the parathyroid are fairly common in hamsters and tend to occur with varying incidences.

Neoplasms were seen with very low incidence in all other organs and occurred with similar frequency in the treated and control groups. The types of tumors observed in each animal are presented in Appendix 6 (Tumor Inventory) and are summarized in Table 22 (Summary of Histopathology Findings).

The majority of non-neoplastic lesions observed in these hamsters were spontaneous in nature and directly related to the aging process. The incidences of these findings are summarized in Table 22. The most frequently noted changes were characterized as amyloidosis which was especially common in the adrenals, thyroid, liver, and kidneys; glomerular nephrosis or chronic nephropathy; atrial thrombosis; chronic passive hepatic congestion with or without centrilobular necrosis; and hepatic cysts.

Amyloidosis, atrial thrombosis, and glomerular nephrosis were encountered in the usual numbers of treated and control hamsters and with the expected sex-related patterns (i.e., higher incidences of amyloidosis and atrial thrombosis in females, and a higher incidence of glomerular nephrosis in males). Hepatic cysts were more frequent in the treated males (18% and 16%) than in the control males (4%) but appeared in more control (31%) than treated females (26% and 15%). Passive hepatic congestion was seen more frequently in

the treated males (low-dose, 22%; high-dose, 31%) and females (low-dose, 13%, high-dose, 15%) than in the controls of either sex (4%). This type of hepatic congestion is a non-specific finding which is often secondary to atrial thrombosis and was unrelated to treatment.

6. Comparisons Between Test Materials

This section will discuss comparisons between the groups treated with the four test materials at the $1/2$ MTD (low-dose) level (Groups 4, 6, 8, and 10) and between groups treated with the materials at the MTD (high-dose) level (Groups 5, 7, 9, and 11).

a. Survival - Individual mortality data are presented in Appendix 1; cumulative mortality is summarized in Table 17. Analysis of survival data (Kaplan-Meier product limit method) is presented in Appendix 2. Graphic illustration of survival and the results of the statistical comparisons between groups receiving the test material at either the $1/2$ MTD or MTD level are presented in Figure 13D.

All same-sexed groups instilled with the four test materials at either the $1/2$ MTD or MTD level showed comparable survival patterns.

b. Pharmacotoxic Signs - The incidence of pharmacotoxic signs are presented in Tables 18A (males) and 18B (females).

Treatment-related clincial signs were limited to wheezing, dyspnea and polypnea. These signs of respiratory distress were present in all treated groups during the last three weeks of instillation and throughout the post-dosing phase.

c. Body Weights - Individual body weights are presented in Appendix 3; mean body weights are summarized in Table 19. The results of statistical evaluation of body weight changes (weight of each interval minus the initial weight) between the vehicle control group and each of the effluent-treated groups are presented in Appendix 4.

The similarity of initial body weights among all groups was assured by an analysis of variance.

Males and females treated with the shell sand, green sand, and furan samples showed lower than control body weight gains during the instillation phase of the study. The weight changes of the high-dose effluent-treated groups were significantly lower than the control values at most intervals during the instillation period. Significant differences between the low-dose effluent-treated and control groups were also present at many intervals, although the differences were noted less frequently than in the high-dose groups. The urethane-treated males and females at both dose levels showed significantly lower than control weight changes at noticeably fewer intervals during the instillation phase than did the other effluent-treated groups. During the post-dosing phase, the weight changes of the shell sand, green sand and urethane groups were significantly lower than the control group at only a few scattered intervals. The furan groups, particularly the high-dose males and the low- and high-dose females, showed significantly lower than control weight changes at more intervals during the first part of the post-dosing phase as compared to the other effluent-treated groups. The weight changes of all furan groups were similar to the control values after Week 50 (males) and Week 54 (females).

d. Gross Pathology - Gross pathology observations are summarized in Tables 20A (males) and 20B (females). A description of the respiratory tract tissue masses observed at necropsy is presented in Table 21.

Respiratory tract masses or nodules were observed with very low frequency in the effluent-treated groups, occurring in one male and one female from the high-dose green sand and furan groups and in one low-dose urethane male. Dark foci were frequently noted in all shell sand and furan groups and were considered to be treatment-related. This observation was seen infrequently in the green sand and urethane groups. No other significant gross pathology was seen in the respiratory tracts of these animals.

Gross lesions described in the other organs and tissues were those which are commonly seen in this species. The most consistently observed findings included the following: atrial enlargement and thrombosis in the heart; pale, pitted, and/or granular kidneys; liver cysts; enlarged and/or reddened lymph nodes; reduced spleens; testicular atrophy; and accumulation of clear or red-tinged fluid in the abdominal and/or thoracic cavities. These lesions are

considered to be spontaneous or age related and were seen with approximately equal incidences in all effluent-treated groups.

e. Microscopic Pathology - Individual histopathology findings are presented in Appendix 5 and are summarized in Table 22. An individual animal tumor inventory is presented in Appendix 6. The results of Fisher's analysis of tumor incidences, including the number of tumor-bearing animals and the corresponding number at risk used in each statistical comparison, are presented in Table 23D (comparisons between test materials).

Respiratory tract tumors occurred with very low incidence in all effluent-treated groups. Alveolar/bronchiolar cell hyperplasia was the most frequently observed lesion in the lungs of the effluent-treated groups and was treatment-related. It occurred with much higher incidence in the shell sand and furan males and females (approximately 80% to 90%) than in the urethane males and females (13% to 37%). Incidences of 50% and 60% were present in the low- and high-dose green sand male groups; this lesion appeared in 19% and 36% of the low- and high-dose green sand female groups, respectively.

Eosinophilic deposits occurred with higher incidence in the lungs of hamsters treated with the furan sample. A dose-related pattern was observed in the low- and high-dose males (12% and 29%, respectively) and females (6% and 64%, respectively). All other treated groups showed incidences of 0% to 4%.

A significantly decreased ($p = 0.02109$) number of total tumor-bearing animals was observed for the high-dose furan females (5 tumor-bearing animals/30 at risk) as compared to the high-dose shell sand females (13/27) and apparently was due to a significant difference ($p = 0.01454$) between the numbers of adrenal tumor-bearing animals in these two groups (shell sand, 6/27; furan, 0/25). No other significant differences in tumor incidences were observed between hamsters treated with the four test materials at either the $\frac{1}{2}$ MTD or MTD levels.

A variety of non-neoplastic microscopic lesions were present in the other organs and tissues of the effluent-treated hamsters. The most frequently described lesions were those which occur spontaneously in hamsters as part of the aging process and included amyloidosis involving multiple organs; atrial

thrombosis and its sequelae (histiocytosis in the lungs and chronic passive congestion in the liver); glomerular nephrosis; and hepatic cysts. These findings were encountered in the expected numbers of animals in all groups and were unrelated to treatment with the effluent samples.

C. DISCUSSION AND CONCLUSIONS

The development of the intratracheal instillation technique by Saffiotti and co-workers [14] has provided an appropriate animal model in which bronchogenic carcinoma is experimentally produced by a treatment that closely approximates the conditions of the human route of exposure to respiratory carcinogens. Benzo(a)pyrene (BaP), when instilled in combination with the carrier agent ferric oxide (Fe_2O_3), is effective in inducing a high incidence of respiratory tract tumors. The Syrian golden hamster is frequently selected for studies of this type. The species rarely develops spontaneous lung tumors and is refractory to chronic pulmonary infections which might interfere with the interpretation of results. Additionally, the hamster respiratory tract is morphologically similar to that of humans. A wide range of studies investigating the complex factors involved in the induction of lung neoplasia have been made possible by the establishment of this model system.

This particular study was designed to evaluate the potential carcinogenicity of four types of foundry mold effluents. A mixture of BaP + Fe_2O_3 was used as the positive reference control.

The survival rate of hamsters in this study was unaffected by treatment with the foundry effluents. The survival pattern in each male and female test group was comparable to that seen in the vehicle control group of the same sex, with the exception of the low-dose shell sand males which showed significantly increased survival as compared to the controls. The male hamsters outlived the females; study duration (based on 98% mortality) was 117 weeks for males and 103 weeks for females. Decreased survival was seen in the positive control group due to neoplastic involvement of the respiratory tract.

Wheezing, dyspnea, and polypnea were observed in all treated and control groups throughout the study. No specific treatment- or dose-related patterns were noted, and these clinical signs did not appear to correlate with survival or pathologic findings.

Males and females treated with the shell sand, green sand, and furan samples showed lower-than-control body weight gains during the 18-week instillation period. This effect was more consistently noted in the high-dose effluent-treated groups than in their low-dose counterparts. The urethane-

treated males and females showed lower-than-control weight changes at noticeably fewer intervals during the instillation phase than did the other effluent-treated groups. At the completion of the instillation phase, the animals began regaining weight, and significantly lower-than-control weight changes were noted at only a few scattered intervals. The lower body weight gains noted for the treated groups during the instillation period did not have any apparent effect on survival rates.

The foundry mold effluents did not induce a carcinogenic response in the respiratory tract of the hamster. Respiratory tract tumors were diagnosed with very low incidence and occurred with similar frequency in the vehicle control and effluent-treated groups. A high incidence of respiratory tract neoplasia was induced with the positive control material.

Alveolar/bronchiolar cell hyperplasia was present to some degree in all groups, including the vehicle control. However, the increased incidences observed in several effluent-treated groups (males and females treated with shell sand and furan effluents at both dose levels, males treated with green sand effluent at both dose levels, and females treated with green sand effluent at the high-dose level) were attributed to the test materials. The incidences of hyperplasia in the low-dose green sand females and all urethane-treated groups were comparable to those present in the vehicle control group of the same sex. This hyperplastic response is common in the hamster, even with relatively mild insult to the lungs, and is not necessarily preneoplastic. The reaction was rarely extensive enough to be classified as adenomatosis.

Eosinophilic deposits occurred more frequently in the lungs of hamsters instilled with the high-dose of furan effluent, particularly the females, and were presumably enhanced by this compound. These aggregates of phospholipid material are secreted by alveolar macrophages, usually in response to an irritant. The material is eventually removed by the macrophages.

Most of the neoplasms observed in this study were of endocrine origin. Tumors of the adrenal cortex were seen most frequently and appeared with higher incidence, as expected in the males. The numbers of tumors observed in the vehicle control and treated male groups were generally comparable. Higher incidences of adrenal tumors were noted in both shell sand-treated female

groups than in the female control group. Thyroid tumors were also present in most groups, but a slightly higher incidence was noted for the high-dose green sand females. Parathyroid tumors were diagnosed sporadically in all groups with the highest incidences occurring in the vehicle control female, high-dose shell sand female, and high-dose urethane male groups. Endocrine gland tumors are the most common spontaneous neoplasms in hamsters, and variable incidences have been reported in the literature.[32,33] One explanation cited for this variation is that it is difficult to detect these often microscopic tumors in a single random tissue section. The incidences observed in the present study were consistent with those reported in the literature, and the variation noted between groups did not appear to be related to treatment.

A variety of non-neoplastic changes were described in all groups. Amyloidosis, a common degenerative disease of aging hamsters, was encountered in many organs and was more prevalent in the females than in the males. This sex-related pattern is typical of this condition in hamsters. Glomerular nephrosis, a term used to denote senescent changes in the kidneys, was more common in the males as a result of longevity. Atrial thrombosis, with its associated effects in the liver (chronic passive congestion) and lungs (alveolar histiocytosis), and hepatic cysts were also frequently noted. These findings are also anticipated in aging hamsters. All age-related changes occurred with the expected frequencies and did not appear to be influenced by treatment. Clear cell foci were encountered slightly more frequently in the livers of females treated with the shell sand and green sand samples. The significance of this finding is not clear, but it was not thought to be related to treatment.

Under the conditions of this study, the foundry mold effluents failed to induce a carcinogenic response in the hamster respiratory tract. However, these negative results do not prove that foundry casting emission products are not carcinogenic. Foundry mold effluents are complex mixtures of a wide range of chemical and mineral components. The partial analyses performed on the bioassay samples quantified several components with known toxic properties and components which are chemically related to acute or chronic toxicants. Eight polycyclic hydrocarbons were quantified in the water-soluble and insoluble (particulate) fractions, sixteen metals in the whole sample, and five phenols

in the water-soluble fraction. The present bioassay was conducted as an exploratory investigation of the potential carcinogenicity of the entire foundry mold effluent, not individual components; therefore, each test material was dosed using the total emission sample. Dose levels were limited because of the high acute toxicity of the emissions. This dose limitation may explain the low tumor incidence observed in this study. Since most of the polycyclic aromatic hydrocarbons are known to be associated with particulates, administration of the particulate fraction alone conceivably might induce different responses in the test system. However, this approach would not have taken into consideration the possible effects of the complex mixture of components present in the whole sample which might include compounds that can act as tumor promoters or cocarcinogens.

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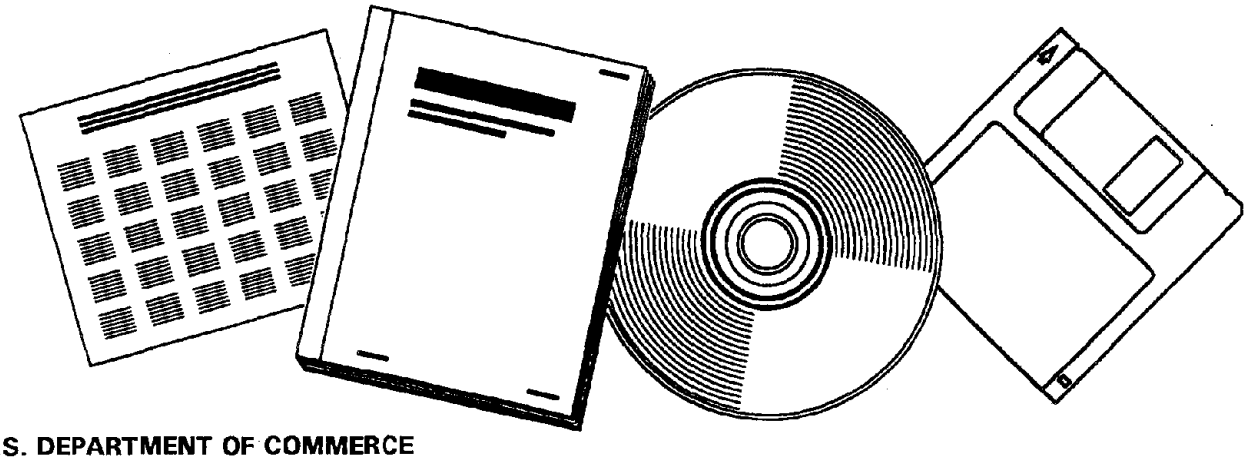

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