A COMPARISON OF THE SKIN CARCINOGENICITY OF CONDENSED ROOFING ASPHALT AND COAL TAR PITCH FUMES

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Abstract

The relative tumorigenicity to mouse skin of condensed volatile components of roofing petroleum asphalts and coal tar pitches was investigated. The primary factorial experiment involved 32 groups of 50 mice each. The experimental variables were: two strains of male mice (CD-1 and C3H/HeJ); four initial roofing materials (asphalt, Types I and III; coal tar pitch, Types I and III); two temperatures of generation of volatile fume materials (232° and 316° C); and exposure to simulated sunlight vs. the absence thereof. Treatment with condensed volatiles and light was performed twice weekly for 18 months. Additional groups were a combination treatment (alternate weekly application of 316° C volatiles from Type III petroleum asphalt and Type I coal tar pitch); a positive control (benzo(a)pyrene, with and without sunlight); and negative controls for solvent and sunlight.

Analyses of the results have led to the following conclusions. Temperature used in preparation of the condensed fume material had a pronounced effect on tumorigenesis, the 316° C preparation being the more active. The C3H/HeJ strain was much more sensitive than the CD-1 strain to the tumorigenic activity of the fumes. Simulated sunlight, as used in this experiment, had an inhibitory effect on the rate of appearance of tumors and on the final tumor incidence. Ratios of tumor types, i.e., percent benign: malignant, were high for the C3H/HeJ groups (~ 30:60) and much lower for the CD-1 groups (~ 33:5). In comparison to benzo(a)pyrene (B(a)P) controls, the coal tar pitch fume materials showed effects that appeared to be consistent with their B(a)P content. The petroleum asphalt fume materials showed higher activity than would be expected based on their content of B(a)P or of total PAH, suggesting the presence of other factors contributing to the tumorigenic response.

Supplementary chemical analysis indicated that aliphatic hydrocarbons were major components of the petroleum asphalt fumes, while the coal tar pitch fume components were largely aromatic.

Introduction

Asphalt and coal tar pitch are bituminous materials which are similar in appearance and have been used interchangeably for roofing, paving and in other industrial applications[1,2]. Whether exposure to asphalt represents significant health risk has been debated for years. This debate is further clouded by the confusion in the definition and usage of the terms asphalt, pitch, coal tar, bitumen, petroleum pitch, and coal tar pitch. Bingham and coworkers,[2] in a critical review of the literature described petroleum technology and included a glossary of terms, which should serve as a guide in elucidating this sometimes perplexing terminology.

Coal tar pitch, a residue in the partial evaporation or fractional distillation of coal tar produced by the destructive distillation of bituminous coal, was among the first substances detected as a human carcinogen and successfully tested for its carcinogenic effects in animals.[3] Asphalt is a bitumen which occurs in nature or is obtained in the refining of crude petroleum. Several attempts[2,4,5,6,7,8,9,10,11,12] to produce tumors in animals exposed to raw, solvent diluted or aerosolized asphalt were reviewed by NIOSH which concluded that "reliable reports associating malignant tumors with exposure to asphalt fumes have not been found in the literature" and "although available information has not clearly demonstrated that a direct carcinogenic hazard is associated with asphalt fumes, NIOSH is concerned that future investigations may suggest a greater occupational hazard from asphalt fumes than is currently documented in the literature."[1]

Wallcave et al.[11] suggested that differences in bioassay results with coal tar pitches and asphalt were related to polynuclear aromatic hydrocarbon (PAH or PNA) content, reflecting their thermal histories. Asphalt production temperatures range from 350° to 400° C and those of coal tar pitches exceed 1000° C. PAH content of coal tar pitches may be several orders of magnitude greater than that of petroleum asphalts, with coal tar pitch containing more higher-molecular weight PAHs.[13] Wallcave's suggestion is useful for the raw products, but under field conditions thermal histories may be much different from those of the raw materials. For example, Thomas and Mukai[14] reported that heating in roofing kettle operations is poorly controlled and commonly the materials are heated as high as 638° C (1000° F), well above their recommended kettle temperatures of 204 to 273° C. This results in pyrolysis and increased production of PAHs. Some field operations such as the asphalt hot mix operation for road paving do maintain moderate temperatures, generally lower than 160° C.[15]

In a study of workers with at least 5 years and an average of 15.1 years exposure to asphalt in 25 oil refineries, Baylor and Weaver[16] found a few cases of bronchitis, asthma, and emphysema but no other significant health effects compared to a control population. They cited further information on workers employed in highway construction, roofing manufacture and truck driving over asphalt highways that exposure to petroleum asphalt constituted no health hazard. Zeglio in 1950 (cited by NIOSH[1]), reporting on Italian workers who used natural asphalt, possibly adulterated with coal tar pitch to insulate electrical cables and telegraph and telephone lines, concluded that characterizing bitumen vapors only as irritating did not recognize the

rhinitis, oropharyngitis, laryngitis, bronchitis, and X-ray and respiratory changes from rales to emphysema that they caused.

More recently Hammond et al.[17] found that occupational exposures of roofing workers to asphalt and coal tar pitch fumes for more than 20 years were associated with increased mortality from cancer and other pulmonary diseases including emphysema, chronic bronchitis and asthma. Leukemia and cancer of the lung, upper respiratory tract, stomach, bladder, and skin had excess mortality. Smoking habits were not considered, and the causative factors could not be identified because of mixed exposure to coal tar pitch and asphalt fumes.

Lawther[18] studied mean 8-hour shift exposures of roofing and gas retort workers to benzo(a)pyrene (B(a)P), a ubiquitous carcinogen often mentioned as an indicator of PAH exposure.[19,20] He found for roofers, exposures of less than 1 and 13 µg for asphalt and hot pitch, respectively and for gas retort workers exposures averaged 27 µg. Bingham et al.[2] noted that a single carcinogen, such as B(a)P, can serve as a guide to carcinogenic potency, but the presence or absence of B(a)P does not always account for the observed potency, and roles of other carcinogens, cocarcinogens and inhibitors of carcinogenesis must be considered. The International Agency for Research on Cancer[21] stated that predictions of human cancer risks cannot be made from simple knowledge of PAH levels.

In addition to the risks associated with exposure to the coal tar pitch and petroleum asphalt fumes in the roofers environment, the added risk of sunlight exposure in the out-of-doors environment must be considered. Various investigators working with experimental animals have found that ultraviolet and visible light augment the carcinogenicity of PAH exposure.[22,23]

NIOSH estimates that 12,000 roofing contractors employ over 116,000 workers in the U.S.[24] Since these workers have combined exposures to asphalt, coal tar pitch and sunlight, an experiment was designed to study the relative importance of each and combinations of them. The purposes were to assess: (1) the carcinogenic potentials of condensed volatiles from two commonly used roofing asphalts and two commonly used coal tar pitches collected from fumes generated at recommended application temperatures; (2) the carcinogenic potentials of the condensed volatiles collected from fumes generated at temperatures in excess of their recommended application temperatures; (3) the effects of simulated sunlight on the carcinogenic outcome of the above materials; and (4) the responses in pigmented and nonpigmented mice.

Materials and Methods

Sample Identity, Collection, and Characterization

There are four types of asphalt and three types of coal tar pitch used on roofs. Two types of each, chosen for this study on the basis of common use and extremes of classification, were Type I and Type III asphalt, referred to in the industry as "dead level" and "steep," respectively, and Type I and Type III coal tar pitch often referred to as "regular roofing" and "low fuming" or

"low burn" pitch, respectively. They are produced by several manufacturers to physical specifications recommended by the American Society of Testing and Materials.[25]

Approximately 270 kg of each of the materials were purchased: Type I and Type III asphalts, purchased from a distributor of Exxon, Inc., Roofing Products (Beacon Sales, Inc., Somerville, MA), were produced by distillation and air blowing of Arabian crude; Type I coal tar pitch, obtained from Reilly Tar and Chemical Corp. (Cleveland, OH), and Type III coal tar pitch, provided by Koppers Company, Inc. (Monroeville, PA). All materials were manufactured to ASTM specifications and were shipped from available inventory. Fumes were generated
from an easily controlled glass generation system and the condensed material was collected in a glass cryogenic system (Figure 1). Laboratory generation and collection was used instead of a roofing kettle to avoid problems of inadequate sample mixing, generation temperature extremes, exposure to sunlight and difficulty in collecting condensed field samples at a subambient temperature. The roofing material was made into small pieces, approximately 10L placed into a weighed 12L round bottom reaction flask, and warmed in a forced air oven at 150° C. When the the material was soft it was stirred with a stainless steel multifinned stirring rod at 250 to 300 rpm. The rod was driven by a compressed air motor and inserted through the neck of the flask lubricated with a teflon stirring gland. An electric heating mantle was used to attain the desired generation temperatures (± 5° C) of 232° C, the approximate recommended kettle temperature, and 316° C, just below the flashpoint of the materials.

The fume collection system consisted of 20 mm O.D. glass transfer tubes and 500 mL glass impingers placed in three cryotraps, one containing ice (0° C) and two dry ice/isopropanol (-77° C). An impinger containing a 50/50 mixture of cyclohexane/acetone was used after the cryotraps to provide additional collection through dissolution. Air, precleaned and dried using a high efficiency filter, silica gel and granular activated charcoal for removal of particulates, water, and organic vapors, respectively, and heated to 100° C was pulled through the system at a rate of 10 Lpm. The laboratory light was filtered through yellow cellulose acetate-butyrate filters to reduce ultraviolet light induced photooxidation and decomposition during generation and collection.

After the fumes were condensed and collected, the individual impingers and transfer lines were weighed and the material was quantitatively transferred from them with an excess of 50/50 cyclohexane/acetone solvent mixture to a large flask. The solvent was then removed at reduced pressure at 5° C, and analyzed by gas chromatography/mass spectrometry (GC/MS) to confirm that significant amounts of compounds of interest were not present. The collected condensates were weighed and dissolved in a 50/50 mixture of cyclohexane/acetone. One to two generations were needed to obtain the necessary amount of material with the coal tar pitches at either temperature; with the asphalts 34 to 59 generations were required at 232° C and 6 to 7 at 316° C. The materials collected from each generation were combined to produce about 4L each of eight skin painting solutions (4 materials x 2 temperatures).

The collections at 316° C compared to the collections at 232° C, yielded 9 to 16 times more volatile material from asphalts but only 2 to 7 times more from coal tar pitch.

For the final concentration of the skin painting solutions, the coal tar pitch condensates were diluted to attain a B(a)P concentration of 0.01 percent in order to produce an estimated 100 percent tumor incidence at approximately one year after the start of the experiment.[26] The final asphalt condensate solutions contained 50 percent total solids, which is a commonly used practice for assaying complex mixtures with low PAH concentrations. Concentrations of selected PAHs in skin painting solutions were determined by combined GC/MS (Finnigan Model 4023) analysis using a high resolution glass capillary column (25-m coated with SP-2250) for separation and quantitation of closely related isomers. A compound was considered as identified when the retention time and the mass spectrum of the PAH of interest matched those of a standard PAH. Table 1 shows the concentrations of 18 identified chemicals and total concentrations of condensed fumes in the 8 skin painting solutions.

Benzo(a)pyrene for the positive controls was obtained from the NCI Chemical Repository (IITRI, Chicago, IL). Cyclohexane and acetone were both HPLC grade (Fisher Scientific, Inc.). Cyclohexane/acetone was chosen as the solvent system for collection, rinsing and preparation of skin painting solutions because: (1) the mixture was a better solvent of the material than either alone; (2) each solvent had a low boiling point and was compatable with PAH stability; (3) the solvents did not absorb light in the simulated sunlight range; and (4) both solvents were inactive with respect to mouse skin bioassay.

Solar Simulator

The solar simulator (figure 2) used a 15-cm Atlas 6.5 kW Xenon arc, water cooled, quartz enveloped burner located about 53-cm above and midway between two turntables. Two additional turntables, completely shielded from light, were contained in the unit for sham irradiation. Wavelengths shorter than 290 nm were excluded by metal oxide coated, 3 mm Tempax glass, No. 114 Schott filters that also reflected infrared radiation (>790 nm) away from the mice. Light measurements were made to validate the geometric concept of the turntable arrangement using a silicon photodiodetector (EG&G) equipped with narrow-band optical interference filters (309 to 790 mm). An automatic integrating system adjusted exposure time for fluctuations and changes in intensity of the arc due to aging to maintain a constant exposure. The solar simulator was contained in an aluminum housing.

Stainless steel (3 x 3 mesh) cage units, specially designed so that two of them fit on each of the four round turntables, contained 50 4 x 9 x 3 cm cells to individually house all mice of a group for simultaneous exposure, and permitted simultaneous light exposure of four groups and sham exposure of four groups. During exposures the turntables were rotated at one rpm, so that on the average each mouse was exposed at the same distance from the source. Cooling air, was delivered to maintain the chamber temperature at about 24 to 28° C.

The goal of the experiment was to produce skin tumor onset in a light exposed control group at approximately 50 weeks. From Bingham and Nord[27] and Burns[28] it was estimated that the total UVB (280-320 nm) dose required was approximately 2 x 10^5 W s/m^2 (2 X 10^8 ergs/cm^2). Since the mice were theoretically to be exposed twice weekly for a total of 100 exposure sessions, and the wattage at the exposure site was 0.68 W/m^2 , the required duration of exposure per session was approximately 49 minutes. More accurate spectral data revealed that the original integrated irradiance between 280 and 320 nm was too high and thus the actual exposure per session was 1480 W s/m^2 with the desired total dose delivered in 135 sessions.

Skin Carcinogenesis Tests

Nonpigmented Swiss CD-1 (Charles River) and pigmented C3H/HeJ (Jackson Laboratories) male mice were used. Upon arrival, at six weeks of age, they were quarantined for 6 to 9 weeks. They were housed individually in suspended stainless steel cages and provided food and water ad libitum except during the chemical and light exposures. Each of the 48 experimental groups consisted of 50 randomized mice of one strain, and each mouse was individually identified. The 48 experimental groups were:

a. 32 groups for the primary factorial experiment, i.e., 2 strains x 4 materials x 2 generation temperatures x 2 light exposure conditions (presence or absence of simulated sunlight); each animal dosed twice weekly with 50 µL of the appropriate test material.

b. 4 groups for the solvent control, i.e., 2 strains x 2 light exposure conditions; each animal dosed twice weekly with 50 µL of the cyclohexane/acetone (1:1) vehicle.

c. 2 groups for cage control, i.e., 2 strains, dosed twice weekly with 50 µL solvent and not sham irradiated but always maintained in their individual cages.

d. 4 groups for the positive control, i.e., 2 strains x 2 light exposure conditions dosed twice weekly with 50 µL of the solution of 0.01 percent B(a)P in cyclohexane:acetone (1:1).

e. 4 groups for a combination treatment of asphalt and coal tar pitch fume condensate, i.e., 2 strains x 2 light exposure conditions; each animal dosed twice weekly with the high temperature condensate from Type III asphalt and Type I coal tar pitch, on alternate weeks.

f. 2 groups for light exposure twice weekly with no skin painting treatment i.e., 2 strains.

These groups are indicated in Table 2 where the effective number of mice are presented.

Cage racks were regularly rotated within the animal quarters, and to minimize effects of fluorescent lights, lamps were enclosed in clear filter tubes (Crown Plastics Corp.). The light cycle was 12 hours light and 12 hours

dark. Each mouse was weighed prior to each first weekly application for 6 weeks, and biweekly thereafter.

Hair was clipped as needed from the interscapular region, using a separate #40 clipper head for each test material, and the test material was applied with disposable-tip automatic pipettes in a ventilated hood. After chemical treatment, each mouse was placed in a cell of a clean solar exposure cage unit and kept under a hood.

At 30 minutes following the application to the last mouse in an experimental group, the group was exposed to the light, or sham exposed. To insure uniformity of exposure, the mice were rotated weekly into different cells of the solar cage unit by a formal procedure. The treatments continued for 78 weeks (18 months).

Mice were observed daily for systemic toxicity, and gross appearance of tumors. Gross diagnosis of a skin carcinoma was based on a lesion that upon palpation was attached to underlying tissues. Mice found dead were necropsied. Those that were moribund were killed and necropsied and when groups were terminated at 78 weeks, those remaining were necropsied. Tissues were examined and preserved in 10 percent buffered formalin. Skin tumors were excised and fixed in buffered 10 percent formalin and prepared for microscopic examination. The distribution of tumors between dermal sarcomas, papillomas and epidermal carcinomas was noted particularly.

Additional details of the experimental methods are given elsewhere.[29]

Statistical Methods

The survival curves were estimated using the product limit (Kaplan Meier) estimate[30] with day as the interval of time. The null hypothesis that the groups of interest had the same survival distributions was tested using the Breslow (generalized Wilcoxson) statistic. It should be noted that the Breslow test gives greater weight to the early events and is less sensitive to the events of interest that occur later in time when few animals remain in study. Animals sacrificed at termination of the study were considered censored, others as dying naturally. The mean, standard error of the mean, and 25, 50 and 75 quantiles were calculated. It should be noted that if the longest surviving animal is censored (as is usually the case in this study), the calculated mean value underestimates the true mean.

The time-to-tumor curves were calculated in the manner described previously for survival only the time of observation for the animal's first tumor (a tumor that was subsequently confirmed histopathologically) was used as the event rather then time of death.

To test for simple differences in the number of tumor bearing animals at risk, the Fisher-Irwin Exact Test was used. For tumor incidence, the heterogeneity of groups was tested, after allowing for differences in longevity, utilizing the "onset rate" analysis as suggested by Peto et al.[31] This method assumes that the appearance of skin tumors are mortality-independent events. Only animals that had tumors that were observed

and histopathologically confirmed were considered tumor bearing animals. The time period used in calculations was one day and the number of animals at risk for a given group on a given day are those animals alive and without any previously observed tumors. The number of tumor bearing animals were compared, although there may have been multiple tumors per animal.

For all analyses, the significance level selected was p=0.01. It was recognized that a given group was included in multiple comparisons and the probability level of a given test may have been greater than that calculated; therefore, p=0.01 level was used to insure conservative conclusions for the study.

The following group comparisons were made:

a. each group versus appropriate solvent group

b. each group versus appropriate benzo(a)pyrene group

c. light versus no light groups

d. high temperature versus low temperature groups

e. C_3H/HeJ species versus CD-1 species

The following comparisons were made for the groups:

a. survival distribution

b. time to tumor distribution

c. number of tumor bearing animals

d. onset rate analysis combining the above factors

Results

General Health and Survival

Body weights reflected no effect of the treatments. Weight gains occurred in the first 2 months; after that there were only minor oscillations. Killing moribund or cachectic mice was a factor in these oscillations.

Mean survival times in the treated C_3H/HeJ groups ranged from 44.3 to 68.7 weeks. Mean survival times (± standard error of the mean) for the solvent controls were 65.6 ± 3.0 and 73.9 ± 2.2 weeks for non-solar and solar groups, respectively. In the CD-1 groups, mean survival for the treated groups ranged from 52.6 to 67.8 weeks with the mean ± S.E. for solvent controls for non-solar and solar groups, 63.9 ± 2.6 and 67.8 ± 2.1 weeks, respectively. The control groups had expected survival rates, although there were more deaths in the CD-1 mice due to endemic urinary tract infections.

Tumor Incidence

Tumors resulted from administration of condensed fumes from both types of asphalt and coal tar pitches. Their corrected incidence and mean latent periods are summarized in figures 3 and 4, respectively. Cumulative tumor incidence corrected for number of animals at risk at the time of appearance of the first tumor are displayed in the appendix figures 1-10. The average latent period ranged from 39.5 to 56.1 weeks among the C3H/HeJ groups, and from 47.4 to 76.5 weeks among the CD-1 groups treated with the condensed fumes of the roofing materials.

The percentages of effective total mice bearing tumors are shown graphically in Figure 3 for benign (papillomas, kerato- acanthomas, fibromas and unclassified benign epitheliomas), malignant (squamous cell carcinoma and fibrosarcomas) and total tumors. Most of the benign tumors were papillomas and most malignant tumors were squamous cell carcinomas. The incidence of all malignant tumors was much lower in the CD-1 mice, about 5 percent versus about 60 in the C_3H/HeJ strain. Fibrosarcomas were seen more often in the C3H/HeJ mice than in the CD-1 mice.

Comparisons to Solvent Controls

The solvent control groups did not develop tumors with the exception of one benign tumor found in the C_3H/HeJ strain exposed to light. Therefore all groups had significantly more tumors than the comparable solvent control group.

All C_3H/HeJ groups with two exceptions had significantly different survival curves from the appropriate solvent control groups. The two exceptions were Type I asphalt (232^0 C) with and without light exposure. In all cases the treated mice died earlier than the solvent control groups. Survival of CD-1 groups differed significantly from the appropriate solvent control groups with the following six exceptions: both asphalt types at high and low temperature of generation without light exposure; Type I pitch (316^0 C) with light exposure; and Type III pitch (316^{0}C) without light exposure.

In the negative cage control groups only one CD-1 mouse developed a histologically confirmed benign tumor when treated with solvent only; the C_3H/HeJ strain remained tumor free. Exposure to the light source without solvent treatment caused development of one malignant tumor in the C_3H/HeJ strain but no tumors were observed in the CD-1 group. Regressed tumors not confirmed by histopathology, were observed in almost every group (Table 3).

Mouse Strain Comparisons

In comparing strains of mice, except for the solvent control groups, the survival time was in general shorter for the C_3H/HeJ strain. This difference was significant for: Type I asphalt (232^0 C) with light; Type III asphalt (316^0 C) without light; Type I pitch (232^0 C) with and without light; Type I pitch (316^0 C) without light; Type III pitch (232 and 316^0 C) without light; both combination exposure groups; and the C_3H/HeJ survived longer than CD-1 solvent control with light.

Comparing mouse strain differences in response to the various treatments, all C_3H/HeJ groups had a significantly higher incidence of tumor bearing animals (TBA). With two exceptions, i.e., Type I pitch (232^{o}) and B(a)P groups without light exposure, the time to tumor curves were significantly different with the C_3H/HeJ groups developing tumors earlier.

It should be emphasized that a significantly large number of grossly observed tumors regressed in the CD-1 strain as compared to the C_3H/HeJ strain (Table 3). These data were not incorporated into the current analyses since histopathological confirmation was not available.

Effects of Temperature

For both asphalts the mean latency period was reduced an average of 9.5 ± 2.5 weeks (mean ± S.D.) when C_3H/HeJ mice were treated with the higher temperature material compared to the response from the lower temperature condensed fumes (Table 4). Similarly, mean survival was reduced 10.0 ± 3.0 weeks which corresponds to the reduced latency period. Groups not exposed to light showed no differences in percent TBA and those exposed to light were found to have ~9.5 percent increased, but statistically insignificant, tumor incidence by Fisher's Exact Test. The onset rate analysis determined that all groups exposed to the higher temperature materials were significantly more susceptible.

Those C_3H/HeJ mice exposed to coal tar pitch fumes exhibited very little change in latency or survival due to increased temperature of generation, except for the Type III groups exposed to light, where an inhibitory effect was noted on time to tumor development, but there was no effect on tumor incidence. Onset rate analysis revealed a significant difference in the light exposed Type III pitch groups which appears to be an effect based solely on latency differences. The groups not exposed to light got more tumors (~9 percent) with increased temperature of generation but these increases were not significant. Even though the B(a)P concentrations were normalized for the pitch groups, changes were observed in the measured responses.

The CD-1 groups exposed to asphalts without light generally exhibited a non-significant increase in percent TBA with increasing temperature, but no changes in survival or latency were observed. The CD-1 groups exposed to condensed pitch fumes generally responded less to the higher temperature materials but no significant differences were noted for any individual measured parameters, or using the onset rate analysis.

Effects of Light

The C_3H/HeJ asphalt groups exposed to light generally responded by developing less tumors (Table 5). This was particularly noted with the low temperature groups. Latency and survival increased with this decreased tumor response. Onset rate analysis showed significant differences for both low temperature materials. The pitch groups of this mouse strain showed little if any change in percent TBA by altering light exposure. The most prominent change in TBA ($p<0.05$) was observed in the Type I pitch (232^{o} C). Increased

latency and survival were the trends. Onset rate analysis showed all four materials to affect mice differently in the presence of light. These results are almost entirely attributable to latency differences. Light inhibited ($p<0.05$) tumor formation in the B(a)P group with a corresponding increase in latency time and survival. Onset rate analysis showed a significant difference. The combination group responded in a fashion similar to the pitch group, with the overall effect being significantly different with light exposure.

The CD-1 mice exposed to asphalt fumes reacted erratically. The overall response in the higher temperature groups appeared to be decreased with light exposure, but these were not significant using the onset rate analysis method. There was an increased time to tumor noted in the higher temperature groups; however, these same groups showed decreased mean survival. The results of exposing the pitch fume groups to light were very dramatic; tumor development in all groups was inhibited by an average of 19.5 ± 5.8 percent. There was a corresponding significant increase in time to tumor development, an average of 11.7 ± 3.4 weeks. The survival time was not affected. Onset rate analysis showed significant differences in three of the four groups. As with the C_3/HeJ strain, tumor development caused by B(a)P in the CD-1 strain was severely inhibited by light exposure with a corresponding increase in latency, but there was no effect on survival. The combination group, as before, appeared to mimic the coal tar pitch fume effects. Both comparisons were statistically significant using an onset rate analysis.

Comparison to the B(a)P Response

Reviewing the responses in the C_3H/HeJ strain, fumes collected from Type I asphalt appeared to be more carcinogenic than the 0.01 percent B(a)P increasing the average percent TBA by approximately 11 ± 8 percent and decreasing the latency period by an average of 11.5 ± 8.5 weeks (Table 6). However, there was no consistent effect on survival; the largest decrease occurred with the Type 1 (316^0 C) receiving concommitant light exposure. The onset rate analysis showed significance for only the 316^0 C groups. The Type III asphalt fumes did not induce consistent change when compared with the corrected percent TBA from the B(a)P group; although, mean time to tumor was again significantly decreased an average of 12.8 ± 7.2 weeks and survival was generally decreased. As with the Type I asphalt, the Type III (316^0 C) group exposed to light showed the most change and only the 316^0 C groups were significantly different by the onset rate analysis.

Consistent trends were found in the C_3H/HeJ mice exposed to condensed pitch fumes as compared to B(a)P. The mice receiving the low temperature material without light showed no change in percent TBA but significant decreases in latency and survival. When the temperature of generation was increased, again with no light exposure, increases in percent TBA were observed with similar changes in latency and survival as noted with the low temperature material. Mice exposed to light and pitch fumes from both temperatures experienced significant increases in tumor development and decreases in latency when compared to the B(a)P group. Decreased mean survival times paralleled the time to tumor changes. All pitch groups were found to be significantly different by the onset rate method from the

corresponding B(a)P group. The groups receiving the combination treatment paralleled the effects noted above with the individual member component groups.

The CD-1 strain, in comparing their reaction to both types of condensed asphalt fumes and 0.01 percent B(a)P, had significantly decreased tumor incidences when exposed to the fumes and sham-irradiated. The average difference in reduced tumor response was 44.5 ± 9.9 percent TBA, with the largest reduction in the lower temperature groups. A corresponding significant increase in time to tumor was observed, i.e., approximately 14.5 ± 2.4 weeks with little reduction in survival as compared to the B(a)P group. All four groups were significantly different from the non-irradiated B(a)P group by the onset rate analysis method. The light exposed CD-1 mice as a subset of asphalt exposed groups showed a general slight but non-significant reduction in percent TBA, a significant decreased survival time and no change in mean latency time when compared to the B(a)P group. The overall effects analyzed by the onset rate method were not different from the B(a)P group.

The effects were reversed in the pitch exposed CD-1 groups. Slight reductions in percent TBA, significantly decreased survival and non-significant reductions in latency were observed in the groups not exposed to light as compared to the appropriate B(a)P group. These sham irradiated CD-1 pitch groups were not different from B(a)P by the onset rate method. Light exposed CD-1 mice, concurrently receiving condensed pitch fumes, had increased incidences of percent TBA, averaging 19.9 ± 6.0. Here as before with the non-solar asphalt fume groups, the lower temperature materials induced more dramatic results. Survival was significantly reduced an average of 8.9 ± 2.1 weeks in these groups compared to the B(a)P group and latency time was generally decreased (11.2 ± 2.3 weeks) to a significant degree. Onset rate analysis showed significant differences for all light exposed pitch groups of CD-1 mice. The combination group reacted as anticipated and appeared to be more affected by the pitch component.

Combination Treatment

In comparing the combination group to their respective controls, the C_3H/HeJ strain showed no differences in latency, but the combination group showed a significantly increased response by the onset rate analysis method as compared to the asphalt group (Type III--316° C) without light. The responses in CD-1 mice were similar in that the non-solar exposed combination group had an significantly increased tumor response by the onset rate method than the asphalt group. They also died earlier and developed tumors at a significantly earlier time than the asphalt group. When the combination groups were compared to the responses of the pitch groups, onset rate analysis showed that the sham irradiated pitch group (Type I--316° C) had a significantly greater tumor response than the combination group. No other differences were noted.

To summarize the results, it is obvious from an examination of the data that: the asphalt and coal tar pitch fumes were highly carcinogenic; the condensed coal tar pitch fumes had more carcinogenic activity than the asphalts; the two types of each material had very similar activities; the nonpigmented CD-1 strain was less responsive to the carcinogenic activity of

the materials than the pigmented C_3H/HeJ strain; increased temperature significantly increased the tumorigenic response of C_3H/HeJ mice to the condensed asphalt fumes. (The only pitch group to show a similar increased response was the Type III with concomitant exposure to light); no significant changes in the tumorigenic response of the CD-1 mice to increased temperature of fume generation were noted; simulated sunlight significantly inhibited tumorigenic response in C_3H/HeJ mice to both types of asphalt fumes collected at the lower temperature and no overall significant affects were noted in the CD-1 strain; both strains reacted similarly when exposed to condensed coal tar pitch fumes, B(a)P or the combination of pitch and asphalt in the presence of simulated sunlight, where the general response was a significant inhibition of tumorigenesis; in comparison to the C_3H/HeJ strain treated with 0.01 percent B(a)P, all coal tar pitch groups, the combination groups and those exposed the high temperature asphalt fumes showed an increased tumorigenic response; and, in the CD-1 strain, only non-solar asphalt fume exposed and solar exposed pitch fume groups showed this same significantly increased tumorigenic response when compared to the positive control.

Discussion

Condensed volatiles from heated asphalt roofing materials were strikingly more tumorigenic and carcinogenic to C3H/HeJ mice than was expected from previous studies[1,2]; their activity being nearly equal to that of the condensed volatiles from heated coal tar pitch roofing materials diluted to contain approximately 0.01 percent B(a)P. The CD-1 strain was more refractory to the asphalt fumes and the results were more in line with expectations based on historical observations, showing high tumorigenic activity with the coal tar pitch material and lower activity with the asphalts. These observations raise questions about the unique qualities of the two strains of mice used, e.g., levels of inducible microsomal enzymes and metabolic profile differences in handling PAHs. Pelkonen et al.[32], have found that additional genes besides the Ah locus may cause a particular mouse strain to be more sensitive or resistant to B(a)P initiated tumors than would be expected on the basis of metabolic induction capabilities. They found the C_3H inbred strain to be 5 to 15 times more responsive to subcutaneous fibrosarcoma initiation than other strains. They did not study the CD-1 strain.

It is interesting to note that Bingham et al.[2] reported very low or no activity with neat roofing asphalts in toluene (1:1 by weight) using the C3H mouse strain with essentially an identical protocol to the one used in the current study. Their results with solvent diluted roofing pitch derived from coal tar produced similar tumor incidences but with considerably shorter latent periods than observed in this study. The decreased latency is presumed to be related to the higher dosage of pitch used in the former investigation. This further supports the contention that the thermal history of a material is one of the prime determinants of its carcinogenic activity.

A significantly increased tumorigenic response was observed in the C_3H/HeJ strain using higher temperatures to generate the asphalt fumes. Most of this increased response was attributed to a decrease in time to tumor development rather than an increase in tumor bearing animals. This effect

with asphalt fumes was not observed in the CD-1 non-pigmented strain. The pigmented strain, but not the CD-1 mice, also exhibited a significantly increased tumor response to higher temperature Type III coal tar pitch fumes in the presence of simulated sunlight, in spite of concentration adjustments to standardize the B(a)P content. It is inferred that the higher temperature coal tar pitch fumes may have higher specific activity in both strains when undiluted.

There appeared to be virtually no differences in tumorigenic activity in the same animal strain as a result of exposure to condensates of the two different types of asphalt or coal tar pitch materials generated at the same temperature. Therefore, it can be concluded that there is not a large change in relative carcinogenic risk with the type of asphalt or coal tar pitch material used. The objective to show differences in carcinogenic activity of the different ASTM classifications of materials and hence relative safety of the material could not be demonstrated in this study. However, Lowe et al.[33], noting that many investigators found differences in efficacy using tar to treat dermatoses, demonstrated that pharmacologic action varied significantly among sublots of the same crude coal tar collection. There is no reason to expect that asphalt fume activity would not also vary with different sources of crude oil.

Simulated sunlight, as used in this investigation, clearly caused an inhibition of the tumorigenic response. This is somewhat surprising and quite the opposite effect of that expected. There is some evidence suggesting long wavelength ultraviolet (280-350 nm) may act in a cooperative way with chemicals to induce skin tumors in mice under certain laboratory conditions.[22,23] Further, Bingham and Nord[27] reported that exposure of mice to light at wavelengths above 350 nm coupled with exposure to normal alkanes results in tumor induction, whereas exposure to light alone or alkane alone yields few, if any, tumors. However, inhibition of the carcinogenic effects of UV light and dimethylbenzanthracene (DMBA) on the skin of Swiss mice was observed by Stenback and Shubik.[34] This was demonstrated when both UV light and DMBA were administered twice weekly for 4 weeks, with each UV treatment following DMBA treatment by 1 hour. Grossly observed tumors produced by DMBA alone numbered 36, whereas only 21 occurred with the combined treatments. In another schedule, a single UV exposure 1 hour before the first DMBA treatment, followed by 21 weeks of twice-weekly treatment of DMBA alone, resulted in an increased number of tumors as compared to the DMBA controls (52 vs. 30). Although a full comparison of these studies is not possible because of the different experimental conditions, i.e., strains of mice, materials and light source, it is clear that careful consideration must be given to the designs of future experiments involving interaction of light and chemicals. Attention must be given to similarities between the design and actual occupational exposure experience, such as with roofing workers.

Is this inhibition of carcinogenic response due to photooxidation/photodestruction of the carcinogens in these materials or to the modification of the skin, lessening its responsiveness, or to an actual cytotoxic effect on precancerous cells? Zepp and Schlotzhauer[35] as well as Mill et al.[36], determined half-lives of various photo-irradiated PAH in water to range from less than one hour to several hours. B(a)P and other

carcinogenic PAH were among those most easily photooxidized in less than one hour. Katz et al.[37] found the half-lives of many of these same chemicals in simulated sunlight atmospheric conditions to be about the same order of magnitude. The presence of 0.2 ppm ozone markedly decreased the observed half-lives. The relative contribution of this observation to the general inhibition phenomenon observed in the current study remains to be determined. Fisher and Kripke[38] using C_3H/HeN(MTV-) mice concluded that suppressor T lymphocytes induced in mice by UV radiation play a decisive role in skin carcinogenesis by reducing the latent period in photocarcinogenesis. This mechanism does not appear to apply to the current study where the general response was an inhibition of the carcinogenic process.

Preliminary chemical characterization according to EPA-RTP Level 1 procedures[39,40] of a direct extract of coal tar pitch used in these studies and of a pitch fume sample generated from a full-scale roofing kettle (which was not the identical fume material used in these carcinogenicity studies) suggests that heterocyclic sulfur and nitrogen compounds and organic nitriles are present in the pitch samples at levels of 1-10 percent of the PAH content (Table 7). El-Bayoumy and coworkers[41] tested tumor initiating activity of four nitro PNAs and their parent hydrocarbons and found that only nitroperylene induced higher activity than the parent compound; yet, this activity was still much less than that induced by B(a)P. Pelroy and coworkers[42,43] used microbial mutagenicity methods to assess the activity of sulfur heterocyclics. None of the compounds tested exhibited activity greater than B(a)P or the neutral fractions from which they were isolated. Thus, species of these types may contribute slightly to the carcinogenic effect of the roofing pitch fume materials; however, it remains clear based on the data in Table 8 and from a previous report[20] that B(a)P is a reasonable indicator substance of coal tar pitch fume exposure. From Table 8 it can be calculated that B(a)P accounts for approximately 75 percent of the total carcinogenic activity of the coal tar pitch fumes.

Since the PAH content of the asphalt volatiles is very low compared to that of the coal tar pitch preparations, what other chemical components do these asphalts fumes contain which contribute to the observed carcinogenicity? Are these promoters, cocarcinogens, or even other carcinogens not of the PAH chemical class? This point is further emphasized by the analysis presented in Table 8. The amount of total test material applied by the time of 50 percent tumor incidence (C3H mice) is very much larger for the asphalts compared to the coal tar pitch fume. Approximately 10 fold more asphalt fume is required to obtain the same biological effect. The amounts of total PAH analyzed or B(a)P found in asphalt fumes are 30 to 40 fold less than those amounts found to be effective in eliciting the same response with condensed coal tar pitch fumes. This suggests that the asphalt preparations contain other types of chemical components which may augment the activity of the very low concentrations of measured PAH found in this study. It is likely that alkylated PAH derivatives and/or aliphatic hydrocarbons may account for some of this activity. Although complete chemical characterization data of the asphalt and pitch fume samples are not available at this time, the PAH analyses have been supplemented by nuclear magnetic resonance (NMR) analysis[39]. The latter results imply that the asphalt fume material is <1 percent aromatic, >99 percent aliphatic, with straight-chain,

unbranched materials predominating. In contrast, NMR analysis of the pitch fume indicated >90 percent aromatic content. These analyses are reinforced by earlier work. Puzinauskas and Corbet[44] characterized the carbon types found in roofing materials. They found roofing pitch was composed of 79, 18 and 3 percent while roofing asphalt was characterized as 37, 23 and 40 percent aromatic, naphthene (cycloparaffins and cyclohexanes) and paraffinic carbon, respectively. The high aliphatic content of the asphalt fume sample is interesting in light of earlier observations[45,46,47] that some alkyl hydrocarbons exhibit cocarcinogenic activity. Further characterization, fractionation and testing of these materials are now underway which will emphasize the indentification of indicator compound(s) for asphalt fume occupational exposure.

In order to more fully judge the comparative risk of exposure to roofing material fumes, Figure 5 was compiled to illustrate relative emission rates, dosage of fumes to elicit an effective 50 percent tumor incidence (ED_{50}) and the number of minutes of generation needed to produce sufficient fumes for an ED_{50}. This was composed for asphalts and coal tar pitches at the suggested kettle operation temperature and at the temperature found during an overheat condition.

Theoretically, an asphalt kettle being maintained at the recommended temperature would emit approximately 37 and 11.5 times less fume than an overheated coal tar pitch or asphalt kettle, respectively. A similar comparison using a high temperature asphalt kettle could theoretically emit as much fume as a properly operated coal tar pitch kettle and approximately one third that of an overheated coal tar pitch kettle. When the time of generation needed to collect sufficient material to produce 50 percent tumor incidence in C3H/HeJ mice is calculated, the ratio of low temperature asphalt fumes to high temperature coal tar pitch fumes represents a greater than 500 fold difference; however, this difference is much less at the lower suggested temperatures of operation for the pitch. This analysis points out the need to maintain kettle temperatures as low as possible in order to reduce the carcinogenic risk.

Penalva and coworkers[48] studied mutagenic activity of aerosols and vapors emitted by road coating tar at various temperatures and found increasing activity as the temperature of the tar was increased from 250° to 550° C. Similar effects were noted by Mahlum[49] in his assessment of distillation cuts from coal liquids. This same trend was determined by Tye et al.[50] in assessing the carcinogenic potency of a catalytically cracked petroleum. He found that tumor initiating activity increased as the boiling point of the fraction increased. These results agree well with the observations from the current study, that higher temperature materials are more hazardous.

Table 9 lists theoretical air concentrations of total condensed fume, PAH and B(a)P from asphalt and coal tar pitch fumes when generated in the laboratory apparatus at 232 and 316° C. Also listed are field concentrations of PAH and B(a)P measured by Malaizandi and coworkers[51] at roofing sites using either asphalt or coal tar pitch materials. The absolute numbers are orders of magnitude higher for the laboratory data as compared to

field data, but this is expected since field concentrations should reflect significant dilution by ambient air. However, there is reasonable agreement among laboratory and field data when the ratios of PAH/B(a)P concentrations are calculated. The ratios for laboratory generation of asphalt fumes is 157 and 122 for low and high temperatures, respectively, and the field collected ratio is 330. For coal tar pitch fumes the laboratory ratio is 88 and 153 for low and high temperature generations, respectively, where the field collected ratio is 159. We conclude from these observations that the relative risk to fumes generated in the field should be similar to those demonstrated in the laboratory setting.

Conclusions

The data from these experiments vindicate the prior concern of NIOSH that there is a greater occupational hazard from exposure to asphalt fumes than was documented in the literature. This investigation clearly demonstrates that exposure to asphalt fumes significantly increases carcinogenic risk, and as expected, higher use temperatures further increase this risk.

The carcinogenic activity of roofing coal tar pitch fume materials may be understandable, to a first order approximation, in terms of their B(a)P content. This ubiquitous carcinogen is therefore a satisfactory indicator substance in estimating relative coal tar pitch fume exposures in roofing operations.

The carcinogenic activity of the asphalt fume materials, on the other hand, cannot even approximately be explained on the basis of their B(a)P content. It can be hypothesized, although it has not been demonstrated, that cocarcinogenic effects of aliphatic hydrocarbons, which may be major components of the asphalt fumes generated in this study, are probably responsible for this enhanced activity. It is evident that further research is necessary before the active components of asphalt fumes are identified. Evidence suggests that emphasis should be placed on the promoting/cocarcinogenic activity of asphalt fumes and that an approach different from that used in studying coal tar pitch must be devised. A protocol using PAH-free asphalt fume condensate administered simultaneously or following subcarcinogenic doses of B(a)P, similar to that used by Bingham and Falk[46], could provide useful information in the future characterization of this material.

In the interim reasonable measures should be taken to limit exposure as much as feasible to both coal tar pitch and asphalt fumes using available engineering and work practice controls. One major work practice that can be instituted immediately is the maintenance of proper heating conditions in the kettle thereby reducing risk possibly to a substantial degree, i.e., approximately a 7 to 14 fold theoretical decrease for coal tar pitch and asphalt fumes, respectively.

Even though it was not demonstrated in this study, it is quite possible that exposure to both asphalt and coal tar pitch fumes may be more hazardous than exposure to either alone. For if our suspicions prove to be correct, that the activity of asphalt fumes is primarily attributable to its

cocarcinogenic activity, then reduction of carcinogen levels to as low as one thousandth of the original concentration may still lead to carcinogenic expression above the threshold response[46]. This is partially supported in the simulated sunlight experiments where photooxidation probably destroys many carcinogens, but not the cocarcinogenic moieties. One must be very careful in assuming large reductions in risk due to relatively lower exposures to carcinogens and cocarcinogens. As Bingham and Falk[46] demonstrated, cocarcinogens of the aliphatic hydrocarbon type are most noticeable in their effect when the concentration of the carcinogen is low. They also demonstrated that low levels of cocarcinogens (less than 20 to 30 percent) appear to have the most marked effect on tumor outcome at lower levels of carcinogen exposure. This may be the condition most frequently encountered in the working population and therefore its importance cannot be overemphasized in attempts to determine the hazards to man.

It is interesting that sunlight may offer some protection against the carcinogenic activity of these materials; however, this will be extremely difficult to demonstrate in the working population. The future needs of extensive epidemiological studies, increased medical surveillance and detailed industrial hygiene surveillance in this hazardous work environment are quite evident.

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TABLE 1

Mean Concentration (μg/mL) of PAHs and Final Concentration of Condensed Fumes in Skin-Painting Solutions

PAH	Analytical GC/MS Ion	Asphalt				Pitch			
		Type I		Type III		Type I		Type III	
		232°C	316°C	232°C	316°C	232°C	316°C	232°C	316°C
Naphthalene	128	22	4	17	49	>1800	1770	288	620
Fluorene	166	36	22	39	28	--	740	--	--
Carbazole	167	20	1	6	--	1980	1450	540	1400
Anthracene/Phenanthrene	178	180	53	300	69	> 960	2960	>2580	>5200
Fluoranthene	202	86	10	97	7	>2940	2350	> 960	>2800
Pyrene	202	70	9	63	8	>2070	1790	> 720	>2300
Benz(a)anthracene	228	11	10	8	6	570	330	330	800
Chrysene/Tripnenylene	228	25	19	13	14	460	300	290	710
Benzofluoranthenes	252	2	4	5	--	230	230	250	250
Benzo(e)pyrene	252	6	8	4	1	42	51	46	46
Benzo(a)pyrene	252	2	2	3	--	96	85	102	90
Indeno(c,d)pyrene	276	3	3	2	--	33	2	11	7
Benzo(g,h,i)perylene	276	1	2	1	--	28	2	7	1
Dibenzanthracenes	278	2	--	2	--	12	--	4	--
Coronene	300	--	--	--	--	--	--	--	--
Dibenzopyrenes	302	--	--	--	--	--	--	--	--
Final concentration of condensed fumes (mg/mL)		500	500	500	500	78	55	84	30

Table 2

Effective Number of Mice Per Group and Week of First Papilloma*

Temperature of Generation	232°				316°			
Light Condition	Light		No Light		Light		No Light	
Mouse Strain	P	N	P	N	P	N	P	N
Asphalt I	49(33)*	10(56)	49(30)	46(24)	47(18)	10(50)	46(23)	45(24)
Asphalt III	44(34)	48(17)	47(21)	48(31)	49(16)	46(41)	46(24)	46(41)
Coal Tar Pitch I	46(37)	45(31)	47(33)	46(22)	45(37)	46(35)	47(26)	45(31)
Coal Tar Pitch III	48(17)	45(40)	43(34)	49(14)	42(39)	48(28)	46(28)	48(28)
Combination (Type III Asphalt and Type I Pitch)	--	--	--	--	44(21)	41(21)	48(25)	45(30)
No Temperature Variations								
B(a)P	45(40)	49(28)	42(38)	49(28)				
Solvent Controls	33(40)	-(-)	-(-)	-(-)				
Solvent Cage Controls	-(-)	-(-)	-(-)	48(41)				
Untreated Controls	21(80)	-(-)	--	--				

P = pigmented C3H/HeJ
N = nonpigmented CD-1
(-) = no tumors
-- = not tested
* = week of first grossly observed papilloma

TABLE 3
Percent of Original Mice with Regressed Tumors

Material	Temperature	Light Exposure	C3H/HeJ	CD1
Asphalt I	232	-	2	14
		+	6	28
	316	-	4	16
		+	0	20
Asphalt III	232	-	2	18
		+	8	14
	316	-	4	14
		+	0	12
Pitch I	232	-	10	26
		+	0	26
	316	-	2	28
		+	0	26
Pitch III	232	-	2	28
		+	4	20
	316	-	0	26
		+	4	34
Solvent		-	8	2
		+	12	4
Cage Control		-	2	2
B(a)P		-	6	14
		+	10	22
Combination		-	0	22
		+	0	26
Untreated		+	2	0

TABLE 4
The Effects of Increased Fume Generation Temperature on Net Change in Response

Type (± light)		C3H/HeJ Corrected Percent TBA	C3H/HeJ Mean Latency (Wks)	C3H/HeJ Mean Survival Time (Wks)	C3H/HeJ Onset Rate Analysis	CD-1 Corrected Percent TBA	CD-1 Mean Latency (Wks)	CD-1 Mean Survival Time (Wks)	CD-1 Onset Rate Analysis
Asphalt	I-	- 0.4	-10.9^{s}	-13.1^{s}	s	18.3*	-5.5	-3.5	ns
	I+	12.0	-11.3^{s}	-11.8^{s}	s	10.0	0.0	-0.8	ns
	III-	- 4.6	- 6.1	- 6.5	s	14.0	-1.7	4.7	ns
	III+	7.1	- 9.1^{s}	- 8.7^{s}	s	- 3.7	3.5	-2.1	ns
Pitch	I-	8.5	- 0.4	1.3	ns	- 3.0	2.6	-1.3	ns
	I+	- 2.2	0.9	0.0	ns	- 8.7	4.8	1.7	ns
	III-	9.4	0.4	4.4	ns	1.3	0.5	3.0	ns
	III+	- 1.2	8.7^{s}	3.5^{s}	s	-11.4	0.9	3.9	ns

* = significant at $p<0.05$ by Fisher-Irwin Exact Test
s = significant at $p<0.01$
ns = not significant

TABLE 5
The Effects of Light on Net Change in Response

Type	°C	C3H/HeJ Corrected Percent TBA	C3H/HeJ Mean Latency (Wks)	C3H/HeJ Mean Survival Time (Wks)	C3H/HeJ Onset Rate Analysis	CD-1 Corrected Percent TBA	CD-1 Mean Latency (Wks)	CD-1 Mean Survival Time (Wks)	CD-1 Onset Rate Analysis
Asphalt	I 232	-12.3	5.7	2.2	s	7.0	0.9	-9.2^{s}	ns
	I 316	0.1	4.8^{s}	3.5	ns	- 1.1	6.5^{s}	-6.5	ns
	III 232	-16.7*	6.5^{s}	2.2	s	- 6.2	0.9	0.8	ns
	III 316	- 5.0	3.5	0.0	ns	-23.9^{s}	6.1	-6.0	ns
Pitch	I 232	12.7*	2.2	5.2^{s}	s	-17.4	13.4^{s}	2.2	s
	I 316	- 2.0	3.5^{s}	3.9^{s}	s	-23.1*	15.5^{s}	5.2	s
	III 232	3.3	0.8	6.5^{s}	s	-12.3	8.7^{s}	-2.6	ns
	III 316	- 4.9	9.1^{s}	5.6^{s}	s	-25.0^{s}	9.1^{s}	-1.7	s
B(A)P		-17.0*	24.8^{s}	9.1	s	-44.9^{s}	16.5^{s}	2.2	s
Combination		1.5	3.4^{s}	1.3^{s}	s	-26.7^{s}	14.3^{s}	0.4	s

* = significant at $p<0.05$ by Fisher-Irwin Exact Test
s = significant at $p<0.01$
ns = not significantly different

TABLE 6
Group Comparisons to B(a)P Responses

Type (± light)	°C	C3H/HeJ Corrected Percent TBA	C3H/HeJ Mean Latency (Wks)	C3H/HeJ Mean Survival Time (Wks)	C3H/HeJ Onset Rate Analysis	CD-1 Corrected Percent TBA	CD-1 Mean Latency (Wks)	CD-1 Mean Survival Time (Wks)	CD-1 Onset Rate Analysis
Asphalt I-	232	5.8	- 1.3	8.7	ns	-56.4[s]	17.4[s]	- 3.0	s
	316	5.4	-12.2[s]	4.4	s	-38.3[s]	11.8[s]	- 6.5	s
I+	232	10.5	-10.4[s]	- 1.8	ns	- 4.5	1.8	-14.4[s]	ns
	316	22.5[s]	-22.2[s]	-10.0	s	5.5	1.8	-15.2[s]	ns
III-	232	- 0.9	- 4.8	1.7	ns	-48.6[s]	15.2[s]	- 4.3	s
	316	- 5.5	-10.9[s]	- 4.8	s	-34.6[s]	13.5[s]	0.4	s
III+	232	- 0.6	-13.1[s]	- 5.2	ns	- 9.9	- 0.4	- 5.7[s]	ns
	316	6.5	-22.2[s]	-13.9[s]	s	-13.6	3.1	- 7.8[s]	ns
Pitch I-	232	- 3.0	- 9.6[s]	- 9.1[s]	s	- 2.0	- 9.5	- 8.7[s]	ns
	316	5.5	-10.0[s]	- 7.8[s]	s	- 5.0	- 6.9	-10.0[s]	ns
I+	232	26.7[s]	-22.2[s]	-13.0[s]	s	25.5[s]	-12.6[s]	- 8.7[s]	s
	316	24.5[s]	-21.3[s]	-13.0[s]	s	16.8	- 7.8	- 7.0[s]	s
III-	232	0.3	-11.7[s]	-13.5[s]	s	- 8.2	- 4.8	- 6.9[s]	ns
	316	9.7	-11.3[s]	- 9.1[s]	s	- 6.9	- 4.3	- 3.9	ns
III+	232	20.6[s]	-25.7[s]	-16.1[s]	s	24.4[s]	-12.6[s]	-11.9[s]	s
	316	21.8[s]	-17.0[s]	-12.6[s]	s	13.0	-11.7[s]	- 7.8[s]	s
Combination -		3.6	-11.7[s]	- 7.4[s]	s	-16.1	0.5	- 8.2	ns
+		22.1[s]	-23.1[s]	-15.2[s]	s	2.3	- 1.7	-10.0[s]	ns

s = significant at $p<0.01$
ns = not significant
positive number indicates increased response compared to B(a)P

TABLE 7
Results of Level 1 Chemical Characterization of a Roofing Pitch and a Pitch Fume Sample*

Organic Compound Category	Approximate Concentration (mg/g) Pitch Extract	Pitch Fume
Aliphatic Hydrocarbons	---	8
Fused Aromatic Hydrocarbons	510	840
Heterocyclic Sulfur Compounds	31	83
Heterocyclic Nitrogen Compounds	15	180
Nitriles	16	10
Amines	8	---

Source: 39

TABLE 8
Total Dose of Test Material Applied up to Time of 50 Percent Tumor Incidence
(C3H Mice--Non Solar)

Test Material	Time to 50 percent T.I.* (weeks)	Amount of Material per application			Total Dose Applied for ED50**		
		Total Solids mg	PAH µg	B(a)P µg	Total Solids mg	PAH mg	B(a)P µg
Asphalts							
Type I - 232°	50.4	25	23.2	0.110	2520	2.34	11.1
Type I - 316°	39.5	25	7.4	0.095	1975	0.58	7.5
Type III - 232°	46.9	25	28.0	0.145	2345	2.63	13.6
Type III - 316°	40.8	25	9.1	<0.025	2040	0.74	<2.0
Coal Tar Pitches							
Type I - 232°	42.1	3.90	>560	4.80	328	>47.2	404
Type I - 316°	41.7	2.75	603	4.25	229	50.3	354
Type III - 232°	40.0	4.20	>306	5.10	336	>24.5	408
Type III - 316°	40.4	1.50	>711	4.50	121	>57.4	364
B(a)P	51.7	-	5.0	5.00	0.5	0.5	517

* = Time in weeks to produce 50 percent tumor incidence (histologically confirmed) where number of applications = weeks X 2.
** = No. of applications x amount per application = Effective Dose for 50 percent Tumor Incidence

TABLE 9
Asphalt and Coal Tar Pitch
Theoretical Laboratory[a] vs. Field Survey[b] Air Concentrations

	Total Condensed Fumes (g/m^3)	PAH ($\mu g/m^3$)	B(a)P ($\mu g/m^3$)	Ratio of PAH/B(a)P
Asphalt				
232°	4.3	3300	21	157
316°	51.3	15000	122	122
IH[b]	--	33.1(± 38.7)	0.119(±0.152)	330
Coal Tar Pitch				
232°	45.8	5.0×10^6	56.6×10^3	88
316°	161.7	56.1×10^6	366.7×10^3	153
IH[b]	--	476(± 260)	3.1(±4.3)	159

a = from this study based on emission rates at 10 Lpm
b = Malaizandi et al.[31] Field study mean (± standard deviation)

FIGURE 1

SCHEMATIC OF FUME COLLECTION SYSTEM

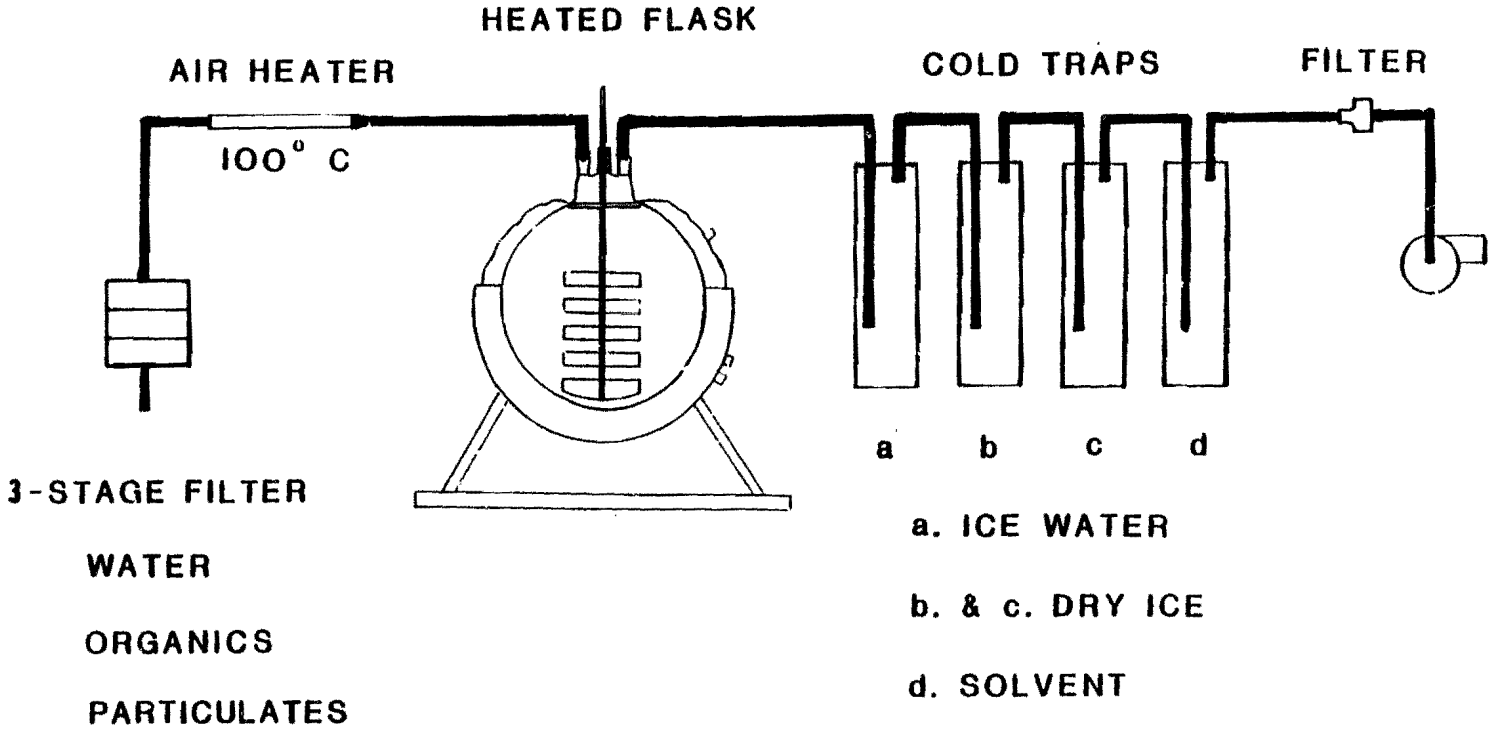
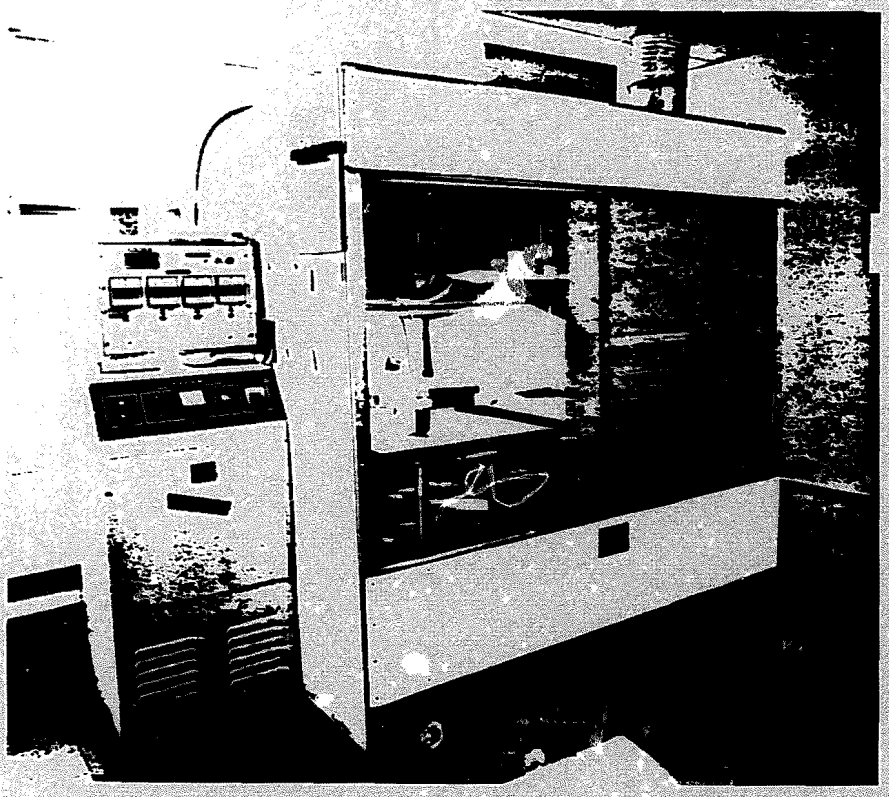


FIGURE 2
SOLAR SIMULATOR



The power source is on the lower left and immediately above it are the exposure counters. The large enclosure houses the light source in the upper center and four turntables, two with light exposure (upper level) and two without (lower level). Only two turntables are shown in this photograph. The exhaust port can be seen in the far upper right portion of the chamber.

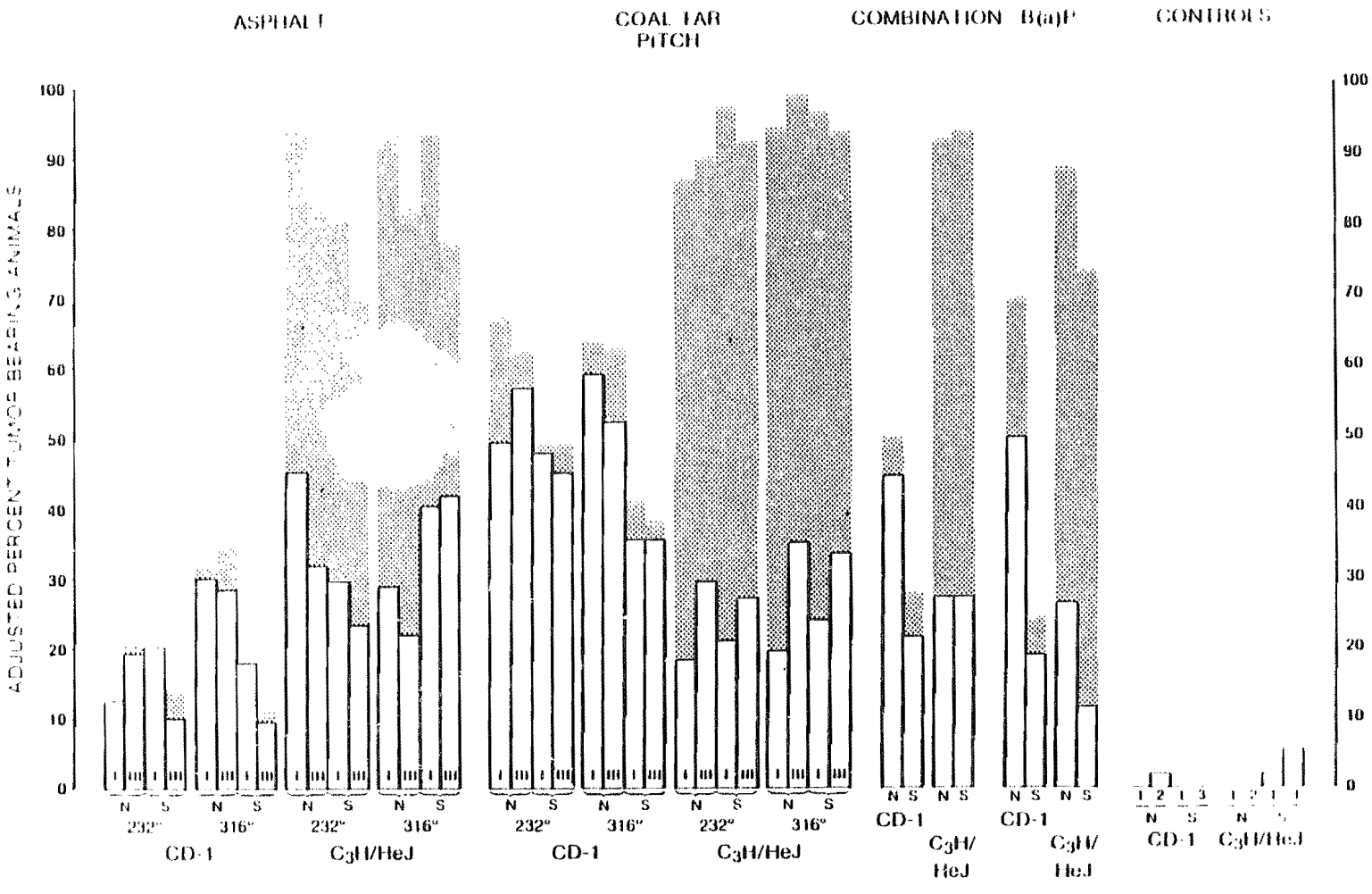


FIGURE 3--SKIN TUMOR INCIDENCE. Corrected for number at risk at the appearance of the first tumor. Open and hatched bars represent benign and malignant tumor proportions, respectively. Controls: 1 = solvent group ± light, 2 = cage control, and 3 = light exposed only. Types of materials are designated by I or III as noted in the text. N denotes no simulated sunlight exposure; S indicates a solar exposed group.

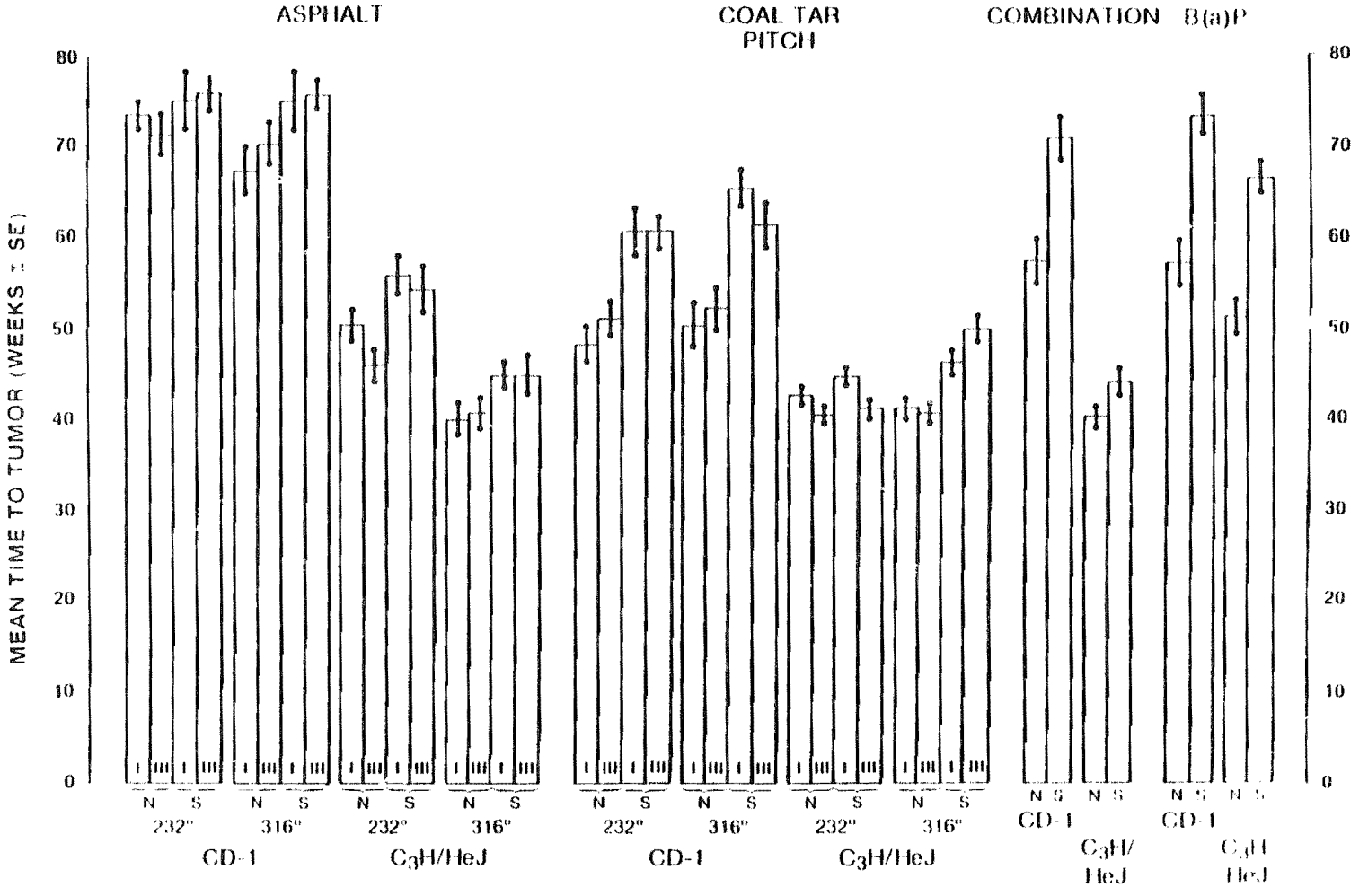
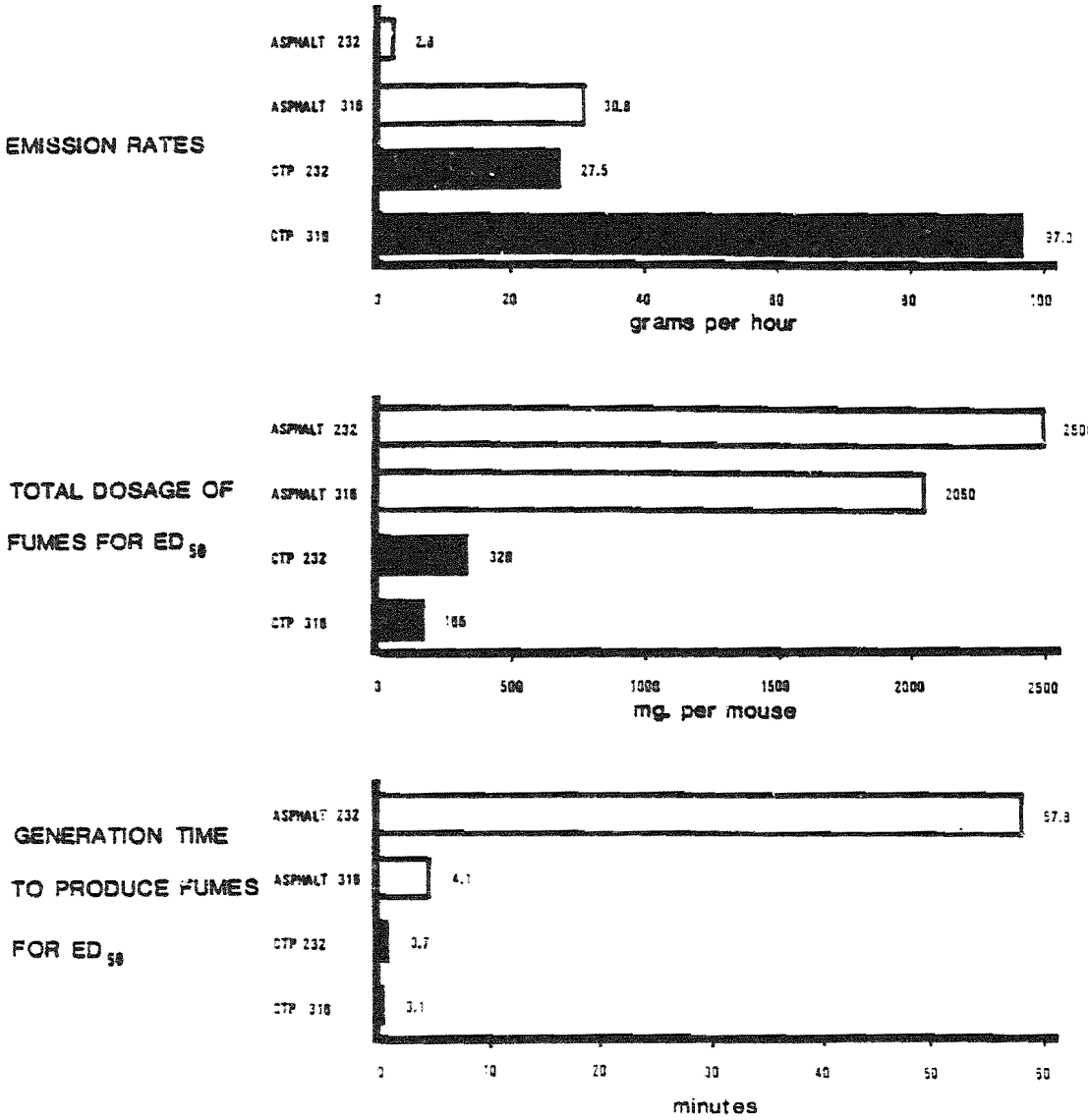


FIGURE 4--MEAN LATENCY TIME. Expressed in weeks ± standard error based on histopathologically confirmed tumors. Types of materials are designated as I or III. N denotes no simulated sunlight exposure; S indicates a solar exposed group.

FIGURE 5

Comparison of Generation Times Necessary to Produce Sufficient Roofing Material Fumes for an ED_{50} Tumorigenic Response in C3H/HeJ Mice



TUMOR INCIDENCE

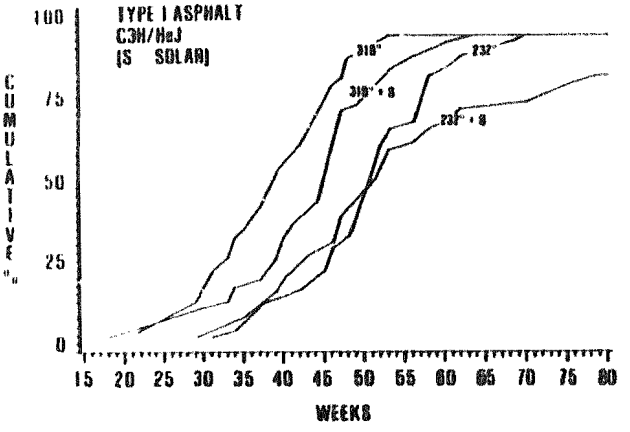


FIGURE A-1

TUMOR INCIDENCE

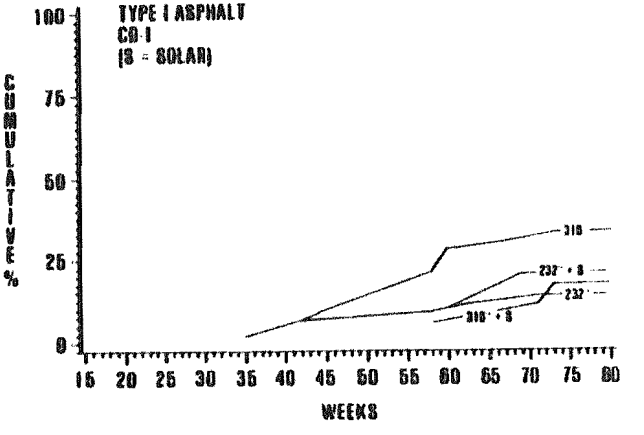


FIGURE A-2

TUMOR INCIDENCE

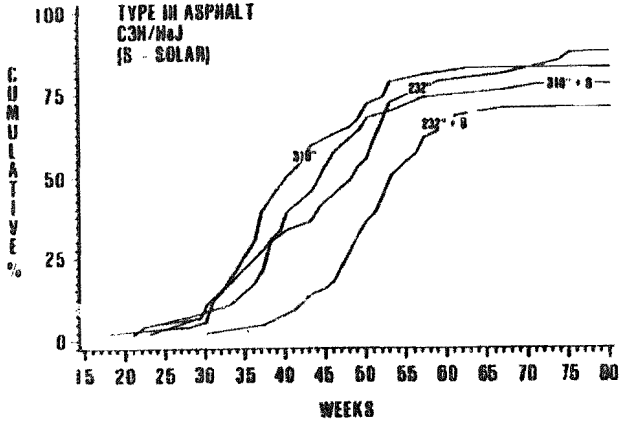


FIGURE A-3

TUMOR INCIDENCE

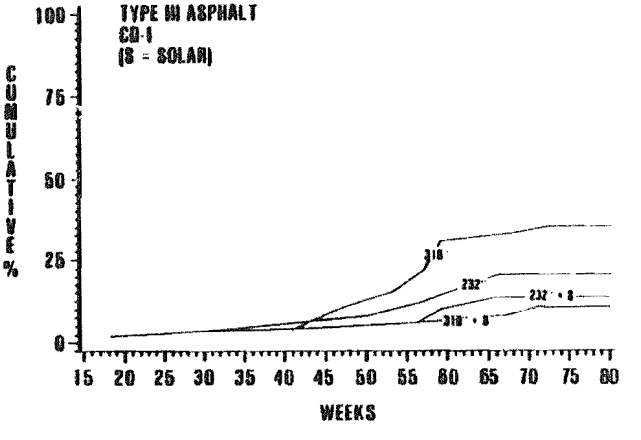


FIGURE A-4

TUMOR INCIDENCE

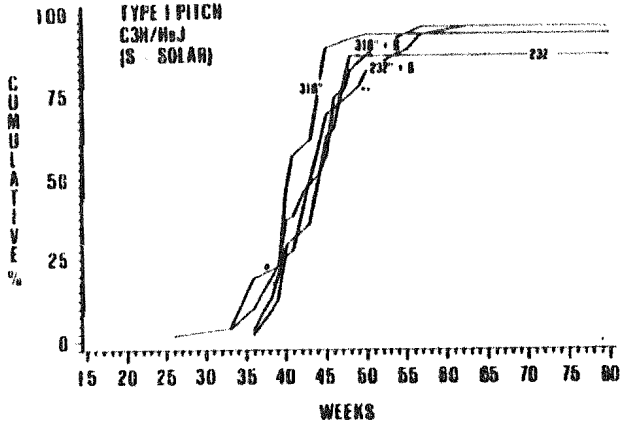


FIGURE A-5

TUMOR INCIDENCE

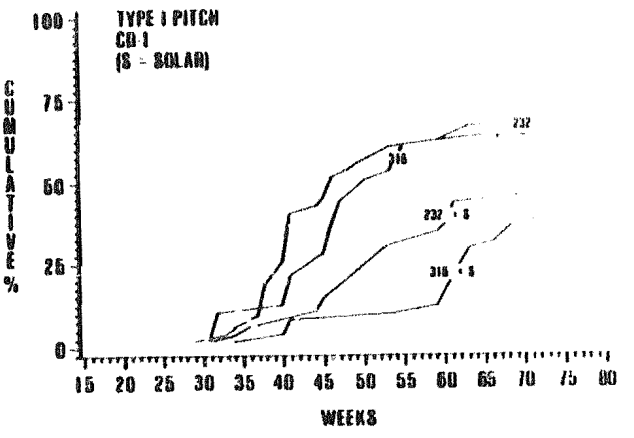


FIGURE A-6

TUMOR INCIDENCE

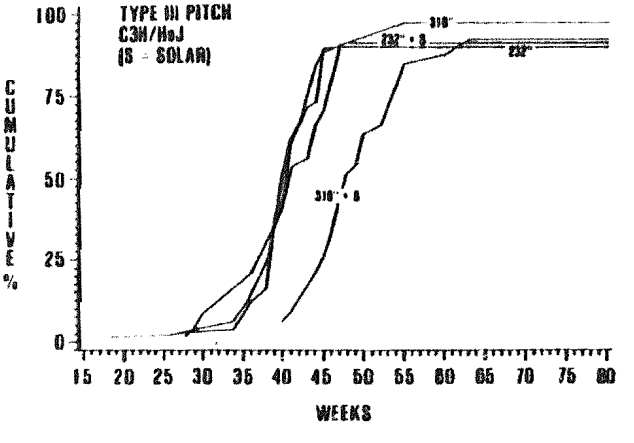


FIGURE A-7

TUMOR INCIDENCE

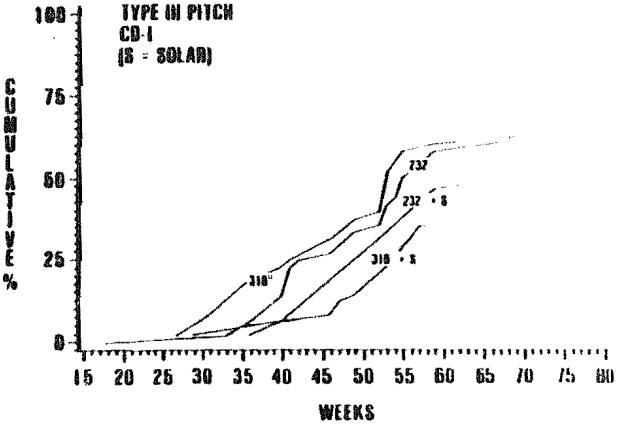


FIGURE A-8

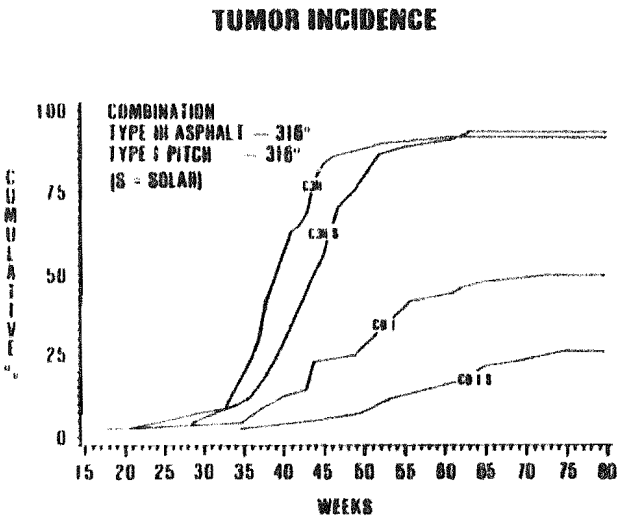


FIGURE A-9

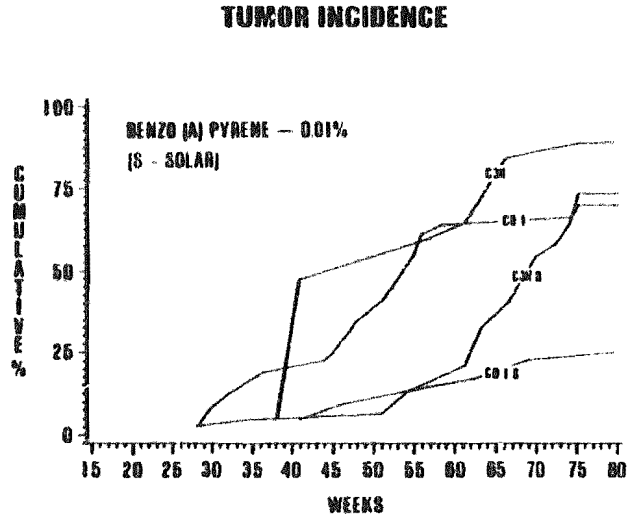


FIGURE A-10

Appendix Figures A-1 through A-10 represent the Cumulative Tumor Incidence expressed as percent of the number of mice at risk at the appearance of the first tumor. All tumors used in these analyses were histopathologically confirmed. -S represents the solar light exposed groups.