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ROOFING ASPHALTS, PITCH AND UVL CARCINOGENESIS

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The tumorigenicity to mouse skin of volatile components of roofing asphalts and coal tar pitches was investigated. Male CD-1 and C3H/H3J-mice were exposed to 25 milligrams per application of asphalt volatiles or 1.5 to 4.2 milligrams per application of pitch preparations. Treatment was twice weekly for an 18 month period with two temperatures of generation of volatile materials and exposure or lack of exposure to simulated sunlight. The C3H strain of mice was more sensitive to the tumorigenic activity, while simulated sunlight had an inhibitory effect on the rate of appearance of tumors but not on the final tumor incidence. The higher temperature of preparation of the asphalt volatiles resulted in greater tumorigenic activity. The authors conclude that the asphalt volatiles were almost as tumorigenic and carcinogenic to C3H-mice as the pitch volatiles.

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ABSTRACT

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

Preparations of asphalt volatiles were applied as 50% solutions in cyclohexane/acetone, providing 25 mg per mouse per application of 50 µL. Pitch preparations were diluted to produce a solution containing 0.01% benzo(a)pyrene which resulted in total solids concentrations of 3.0 to 8.4% in the various preparations, and application of 1.5 to 4.2 mg per mouse per application. All preparations were analyzed by GC/MS for selected PAH compounds.

The solar simulator consisted of an Atlas 6.5 kW xenon arc, appropriately filtered and mounted to provide one solar equivalent to the mice at the nearest point on two rotating turntables, enclosed in an air-cooled chamber.

The following conclusions are made:

- The male C3H mouse is more sensitive than the male CD-1 mouse to the tumorigenic activity of the asphalt volatiles, in particular, but also of the pitches.
- Simulated sunlight, as used in this experiment, has an inhibitory effect more on the rate of appearance of tumors than on the final tumor incidence. In some cases, there is little inhibition, but no enhancement.
- With the asphalt volatiles, there is a pronounced effect of temperature of preparation, the 316° preparations being the more active.
- The pitches do not show this effect, but since the 316° preparations were applied at lower concentrations and each shows tumorigenicity equivalent to that of the corresponding 232° preparation, it is inferred that the former have a higher specific activity.
- The combination treatment groups show effects as might be expected from the individual activities of the two 316° preparations used, without evidence of additivity or synergism.

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CONTENTS

FIGURES

TABLES

TABLES (continued)

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INTRODUCTION

BACKGROUND

Exposure to polynuclear aromatic hycrocarbons (PAH) in humans has been associated with cancer occurrence for over two hundred years. The environmental exposures that have been implicated include occupations such as chimney sweeps (1), coke oven workers (2,3,4,5) and roofing workers (6). Moreover, it is likely that the effects on health of cigarette smoking, particularly pulmonary neoplasia, are due in considerable measure to exposure to PAH (7). Experimental laboratory studies have revealed two important factors with respect to the biological effects of PAH. The first is that the effects of this chemical class of carcinogens exhibit a range of organ sensitivities depending on the route of administration (8), and that mouse skin painting is an efficacious assay to predict carcinogenicity (9). The second factor is that PAH can have multiple effects as enzyme inducers, cocarcinogens and inhibitors of carcinogenesis (10,11,12). This multifaceted response pattern must be taken into consideration in the evaluation of experimental results with exposure to materials as complex as the condensed volatiles from roofing asphalts and pitches. Previous work with cigarette smoke condensate suggests that this material may act predominantly as a tumor-promoter or cocarcinogen (13,14) and suggests that the roofing material volatiles may also fall into this category as modifiers of tumor response in addition to any activity they may have as primary carcinogens. Moreover, the evidence from both laboratory and epidemiological studies shows that the carcinogenic process is generally a sequential, multifactorial one regardless of target organ site (15).

In addition to the body of information that points to mixtures of chemicals acting in concert to produce tumors, there is some evidence suggesting that physical stimuli, specifically the long wavelength ultraviolet (280-350 nm) component of solar light reaching the earth, may act in a cooperative way with chemicals to induce skin tumors in mice under laboratory conditions (16, 17). Further, there is a report by Bingham and Nord that exposure of mice to light at wavelengths above 350 nm coupled with exposure to n-decane or n-dodecane results in tumor induction, whereas exposure to light alone or alkane alone yields few, if any, tumors (18).

The importance of the spectral range of exposure for the biological response of tumor induction with chemicals is demonstrated by several studies. Bingham and Falk found that exposure of mouse skin to fluorescent whitening agents along with ultraviolet light at 254 nm peak intensity produced tumors (19). In contrast, Forbes and Urbach showed that exposure of mice to ultraviolet light in a spectral range available at the surface of the earth (290-320 nm), or to sunlight did not enhance tumor formation with fluorescent whitening agent exposure (20,21). Inhibition of the carcinogenic effects of UV light and dimethylbenzanthracene (DMBA) on the skin of Swiss mice was observed by Stenbäck and Shubik (22). This was manifested when both UV and PAH treatments

were administered twice weekly for four weeks, with each UV treatment following DMBA treatment by one hour. Grossly observed tumors produced by DMBA alone numbered 36, whereas only 21 occurred with the combined treatments. On another schedule, a single UV treatment one hour before the first DMBA treatment, followed by 21 weeks of twice-weekly treatment with DMBA alone, resulted in an increased number of tumors over the DMBA controls (52 vs. 30). Although a full comparison of these studies is not possible because of the differences in strains of mice used, the results indicate the necessity for considering spectral range in the design of cocarcinogenesis experiments involving chemicals and electromagnetic radiation.

The potential carcinogenicity of petroleum hydrocarbons has been extensively reviewed by Bingham et al. (23), including many studies involving skin-painting on mice. No experiments appear to have been done with the volatile fractions of roofing asphalts (or coal-tar pitches), as used in the present study. Numerous studies generally showed a low degree of tumorigenicity of asphalts, when tested on mice (several strains), rats and guinea pigs, and by several routes (sub-cutaneous, skin-painting, inhalation and intramuscular). Bingham et al. (24) made a direct comparison of three coal-tar derived roofing pitches with a petroleum asphalt roofing pitch, each applied to the skin of C3H mice twice a week at 50 mg per application. No tumors were elicited by the asphalt, whereas each of the pitches produced a high incidence of tumor-bearing animals (84 to 96% of the original number), with a preponderance of malignant tumors.

SCOPE OF WORK

This study was conducted by Arthur D. Little, Inc., under Contract 210-78-0035 with NIOSH. It had three stated objectives:

1. To assess, in the animal model, the carcinogenic potential of condensed volatiles collected from roofing asphalt and coal tar pitch fumes generated at the recommended application temperatures.

2. To assess the carcinogenic potential of the condensed volatiles collected from fumes generated at temperatures in excess of their recommended application temperatures. Since the heating process in the roofing kettle operations is usually not controlled, it is common for the materials to be heated above their recommended application temperatures. The excessive heating is known to result in pyrolysis of the materials, leading to an increased production of polynuclear aromatic hydrocarbons.

3. To assess the effects of concomitant exposure to simulated sunlight on the potential carcinogenic outcome of exposure to the above materials.

EXPERIMENTAL DESIGN

The tumorigenicity to mouse skin of volatile components of roofing asphalts and coal tar pitches has been investigated in an 18-month experimental program. The main factorial experiment involved 32 groups of 50 mice each. The experimental variables were: male mice of two strains (CD-1 and C3H/HeJ); four roofing materials (asphalt, Types I and III; coal tar pitch, Types I and III); two temperatures of generation of volatile materials (232° and 316°C); and exposure to simulated sunlight vs. the absence thereof. Treatment with

TABLE 1

Experimental Variables: Materials, Code Designations, and Number of Treatments

Name Used in This Study	Synonyms and CAS No.	Volatiles: Temp. of Preparation (C°)	Volatiles: Code Designation	Volatiles: Solids (% W/V)	Animal Treatments: CD-1 Mice: Solar: Experimental Group	Animal Treatments: CD-1 Mice: Solar: No. of Treatments	Animal Treatments: CD-1 Mice: Non-Solar: Experimental Group	Animal Treatments: CD-1 Mice: Non-Solar: No. of Treatments	Animal Treatments: C3H Mice: Solar: Experimental Group	Animal Treatments: C3H Mice: Solar: No. of Treatments	Animal Treatments: C3H Mice: Non-Solar: Experimental Group	Animal Treatments: C3H Mice: Non-Solar: No. of Treatments
Experimental Materials												
Type I Asphalt	Dead level asphalt (64742-93-4)	232	A	50	3	154	1	152	4	153	2	153
		316	B	50	5	154	7	156	6	139	8	138
Type III Asphalt	Steep asphalt (64742-93-4)	232	C	50	11	150	9	156	12	155	10	155
		316	D	50	13	156	15	156	14	138	16	137
Type I Coal Tar Pitch	Regular roofing cool tar bitumen, pitch type A (65996-93-2)	232	E	7.8	19	152	17	138	20	122	18	118
		316	F	5.5	21	152	23	137	22	124	24	126
Type III Coal Tar Pitch	"No burn" or "low-burn" roofing coal tar bitumen (pitch) (68187-57-5)	232	G	8.4	27	144	25	146	28	122	26	118
		316	H	3.0	29	152	31	146	30	126	32	118
Combination (D and F in Alternate Weeks)	- - - - - -	-	-	-	45	153	43	142	46	129	44	125
Control Groups												
I - Solvent (in chamber)					35	158	33	158	36	157	34	157
M - Solvent (returned to cage)							37	158			38	157
J - 0.01% benzo(a)pyrene					41	153	39	156	42	157	40	149
L - No solvent, light only					47	158			48	157		

condensed volatiles and light was done twice weekly. Additional groups included: a combination treatment (alternate weekly application of 316° - volatiles from Type III asphalt and Type I coal tar pitch); a positive control (benzo(a)pyrene), with and without sunlight); and negative solvent and sunlight controls. These groups are all identified in Table 1, with the laboratory code designation for each test material, and the experimental group numbers.

Asphalt preparations were applied as 50% solutions in 1:1 cyclohexane/acetone, providing 25 mg per mouse per application of 50 µL. Pitch preparations were diluted to produce a solution containing 0.01% benzo(a)pyrene which resulted in total solids concentrations ranging from 3.0 to 8.4% in the various preparations, and applications of 1.5 to 4.2 mg per mouse per application of 50 µL.

The solar simulator consisted of an Atlas 6.5 kW Xenon arc, appropriately filtered and mounted to provide one solar equivalent to the mice at the nearest point on two rotating turntables, enclosed in an air-cooled chamber.

Also given in Table 1 are the maximum numbers of treatments received by any mice of each experimental group. These vary because of differences in survival. They are particularly low in a number of C3H groups, in which a high tumor incidence resulted in early killing of animals.

METHODOLOGY

Although there are four types of Asphalt, Types I through IV, and three types of coal tar pitch (bitumen), Types I through III, available for roof damp-proofing and waterproofing, two types of each are commonly used in the roofing industry (personal communication, J. Weideman, Technical Director, Building Materials Division, Koppers Company, Inc.). These are specifically 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" pitch, respectively. As indicated by their common names, the two asphalts have different flow properties appropriate for use on roofs of differing slope. In the case of the two pitches, flow properties are similar but the "low fuming" type emits less fumes during normal heating and application. These four materials are produced by several manufacturers according to physical specifications recommended by the American Society of Testing and Material (ASTM) (25). The specific requirements for the asphalts (ASTM D-312-71) and the pitches (ASTM D-450-78) are shown in Tables 2 and 3.

PREPARATION OF COAL TAR PITCH AND ASPHALT VOLATILES SAMPLES

Approximately 270 kg of each of the four commonly used materials were purchased to permit generation of sufficient condensed fumes to provide the necessary volume of skin painting solutions for the mouse bioassay. Type I and Type III asphalts were purchased from a local distributor of Exxon, Inc., Roofing Products (Beacon Sales, Inc., Somerville, MA.) These asphalts were produced by distillation and air blowing of Arabian crude and meet ASTM specifications (personal communication, R. Dennis, Exxon, Inc., Everett, MA). Type I pitch was provided by Reilly Tar and Chemical Corporation, Cleveland, OH. Type III pitch was provided by Koppers Company, Inc., (Organic Materials Group, Building Materials Division, Monroeville, PA). Both materials were manufactured to ASTM specifications and were shipped from available inventory.

Material Characterization

To characterize and to determine independently the amount of volatiles which might be emitted from each type of roofing material during fume generation, Thermo-Gravimetric Analysis (TGA) was conducted. Small portions (about 5-20 mg) of each material were placed in a DuPont 950/990 TGA system, heated rapidly (120°C/min) from ambient temperature to 220°C, run isothermally for an additional 30 minutes. The results indicate that under conditions of high surface-to-volume ratio (e.g., 20 cm^2/mL), the following percent of mass may be volatilized during isothermal heating:

TABLE 2

Physical Requirements of Asphalt for Constructing Built-Up Roof Coverings

	Type of Roofing			
	Type I		Type III	
	Min	Max	Min	Max
Softening Point (ring- and ball-method) °C	57	66	82	93
Flash point (Cleveland open cup), °C	225	---	225	---
Penetration:				
0°C (32°F) 200 g, 60 s	3	---	6	---
25°C (77°F) 100 g, 5 s	18	60	15	35
46°C (115°F) 50 g, 5 s	90	180	---	90
Ductility at 25°C (77°F) (5 cm/min), cm	10	---	3	---
Loss on heating at 163°C (325°F) 50 g, 5 h, percent	---	1	---	1
Penetration of residue, percent of original	60	---	60	---
Total bitumen (soluble in CS_2), percent:				
Mineral stabilized asphalt	65	---	65	---
Asphalt without mineral stabilizer	99	---	99	---
Proportion of bitumen soluble in CCl_4, percent	99.5	---	99.5	---
Ash, percent:				
Mineral stabilized asphalt	10	35	10	35
Asphalt without mineral stabilizer	---	1	---	1
Coarse particles retained on 200-mesh sieve as percentage of matter insoluble in CS_2, percent[a]	---	12	---	12

[a]This limit applies only on mineral-stabilized or native asphalt.

SOURCE: Ref. 25, ANSI/ASTM D 312-71.

TABLE 3

Physical Requirements of Coal-Tar Bitumen for Built-Up Roofing, Dampproofing, and Waterproofing

	Type I	Type III
Water, Max, %	0	0
Specific gravity, 25/25°C	1.22 to 1.34	1.22 to 1.34
Softening point (ring-and-ball)[a]: °C (°F)	52 to 60 (126 to 140)	56 to 64 (133 to 147)
Flash point, Cleveland open cup, °C (°F)	120 (248)	120 (248)
Total bitumen soluble in carbon disulfide, %	72 to 85	72 to 85
Ash, max, %	0.5	0.5
Total distillate:		
0 to 300°C (32 to 572°F), max, %	10	0
0 to 315°C (32 to 599°F), max, %	--	0
0 to 360°C (32 to 680°F), max, %	--	5
Specific gravity of distillate from 0 to 300°C (32 to 572°F), min, 38/15.5°C	1.03	--
Softening point (ring-and-ball) of residue from distillation to 300°C (572°F), max, °C (°F)	80 (176)	--

[a]These values are 2°C higher if Method D 2398 (ring-and-ball in ethylene glycol) is used in place of the specified Method D 36 (ring-and-ball in water).

SOURCE: Ref. 25, ANSI/ASTM D 450-78.

Material	Percent Loss of Mass	
	220°C	310°C
Type I Asphalt	2.5	13.5
Type III Asphalt	3.0	11.0
Type I Pitch	17.0	35.0
Type III Pitch	18.1	34.0

Although it is expected that less fumes may be emitted from the laboratory generation system due to a lower surface/volume ratio, e.g., 10^{-2} cm^2/mL, the TGA values indicate the maximum expected mass emission.

Fume Generation

The collection of fumes from a roofing material kettle in the field would be awkward at best due to the difficulty in controlling sample mixing, temperature, exposure to sunlight and in collecting a condensed sample at a subambient temperature. Therefore, laboratory generation of fumes from an easily controlled glass generation system and collection of condensed material in a glass cryogenic system was utilized for production of required amounts of fumes. A 12 L spherical reaction flask was used to contain about 10 L of roofing material during individual fume generations. A stainless steel stirring rod operated by an air pump was inserted through a three-hole head with a Teflon stirrer gland to permit uniform mixing. An electric heating mantle capable of reaching 450°C was used to heat the roofing material to the desired generation temperatures; mantle temperatures were controlled with a Variac and Honeywell controller. This system is shown in Figure 1. The specifications for all components are given in Table 4.

In order to confirm that the roofing material could be maintained within 5°C of the desired generation temperature without temperature gradients inside the hot melt, nine iron-constantan thermocouples (A. Richards, Inc., Newton, MA) were arrayed as shown in Figure 2, and temperatures were monitored during a profile run. This profile run permitted optimization of the scheme for filling and preparing the test flasks and of generation conditions. The scheme used was as follows: roofing material was broken up with a chisel and/or warm knife and placed in the 12 L flask. The flask was then placed in a forced air oven (Figure 3) at 150°C (302°F) and as the material melted, additional pieces were added until approximately 10 L of the material was present. The flask and hot contents were placed in the lower preheated mantle, thermocouples were installed and temperatures were monitored. Between the softening point and 30°C above it, the stirrer was installed.

Mixing was difficult at this point because of the viscosity and during the profile run, the three thermocouples inside the melt indicated a temperature differential of greater than 5°C. At greater than 30°C above the softening point, uniform mixing could be maintained at a stirring rate of about 250-300 rpm. Airborne particles were not physically produced by stirring at this speed as indicated by the absence of visible scattering of a narrow beam of light. The temperature differential inside the melt is then less than 2°C and this was true even when the melt temperature was increasing at greater than 3°C/minute. The temperature controller permitted unattended operation for up to

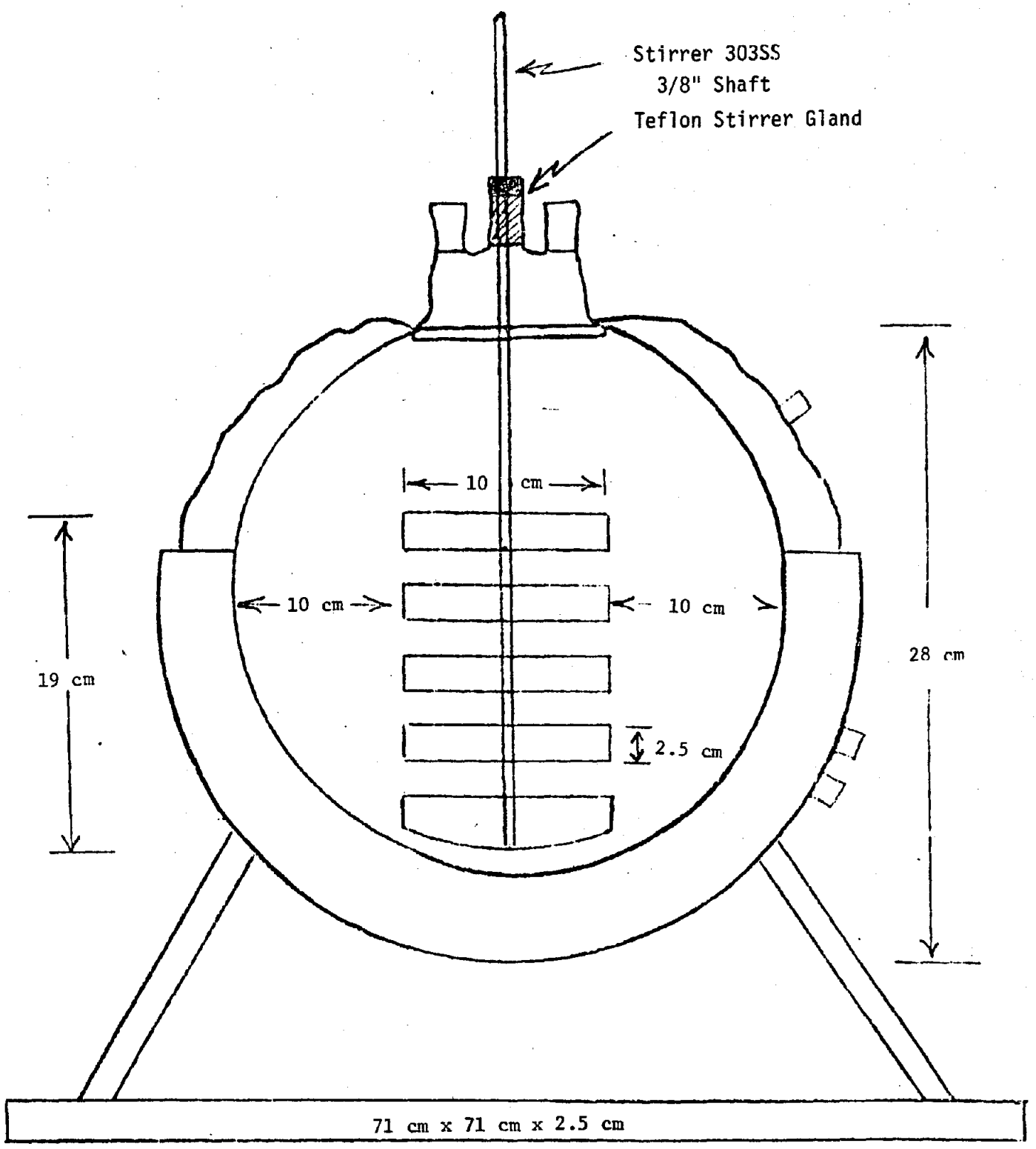


FIGURE 1. Generator Dimensions

TABLE 4

Equipment Specifications

Temperature Controller:	Honeywell, Type 4, Model R7168
Stirrer Motor:	Gast Model 1AM
Stirrer Oiler:	Gast Model AH102L
Stirrer:	303 SS, fabricated by contractor
Stirrer Gland:	Teflon, fabricated by contractor
Upper Mantle:	GLAS-COL, Model MO-116-3, 590 watts
Lower Mantle:	GLAS-COL, Model M-116, 1200 watts
Reaction Flask:	Ace Glass, Catalog No. 6479, 12 Liter
Oven:	Blue M, Inc., Model OV

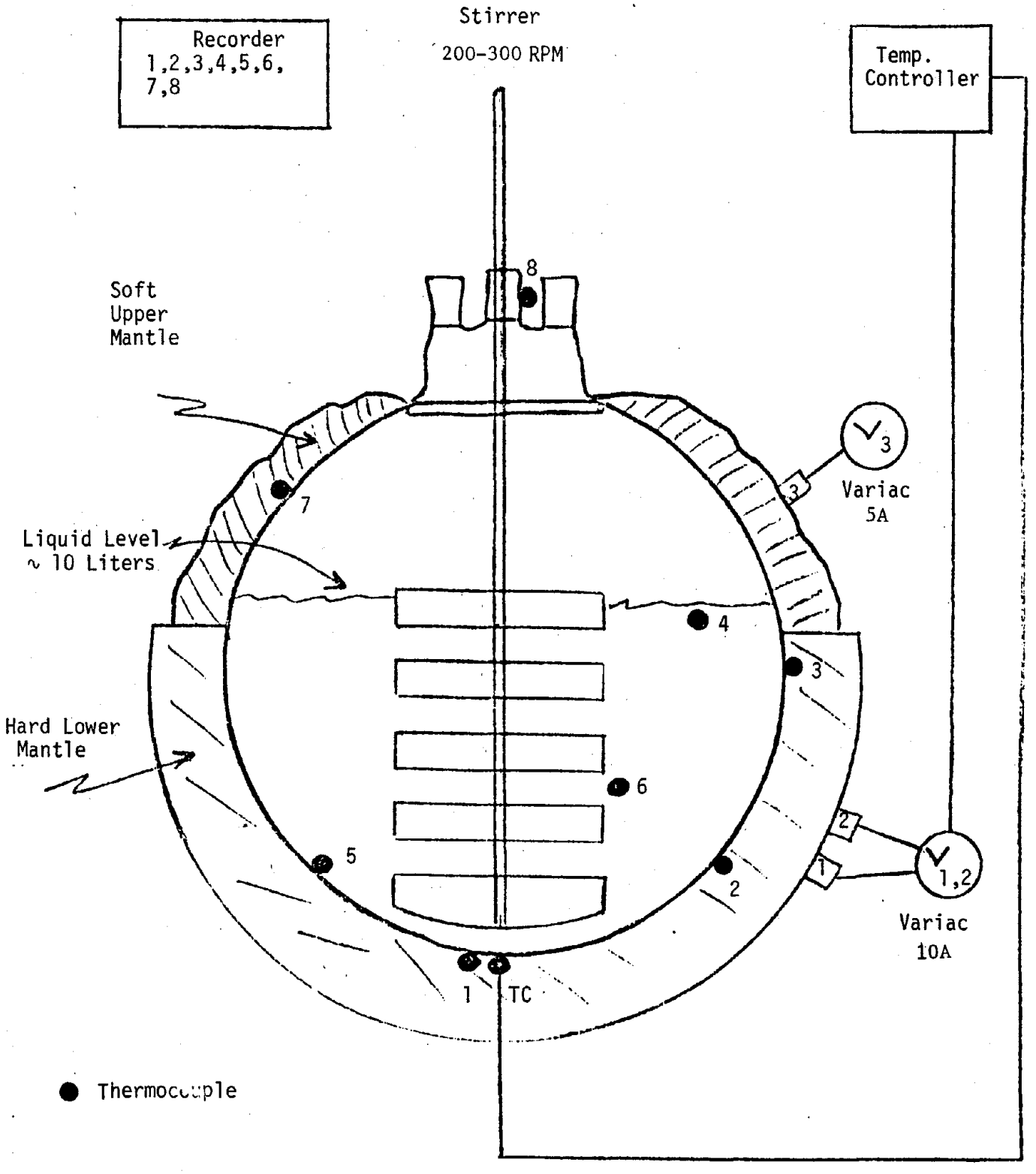


FIGURE 2. Temperature Monitors

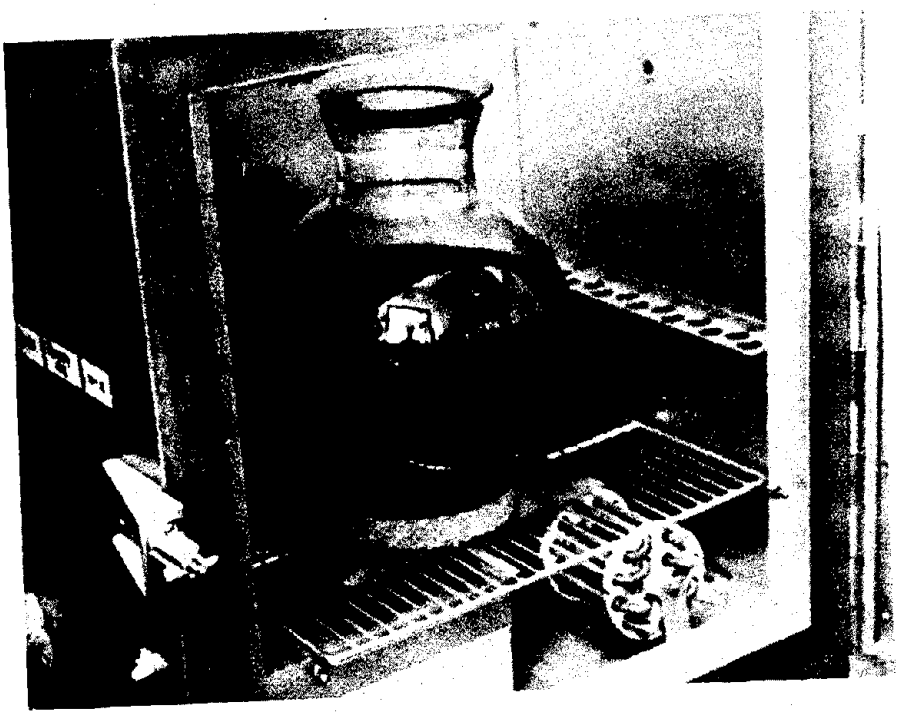


FIGURE 3. Preparing Material (Heating)

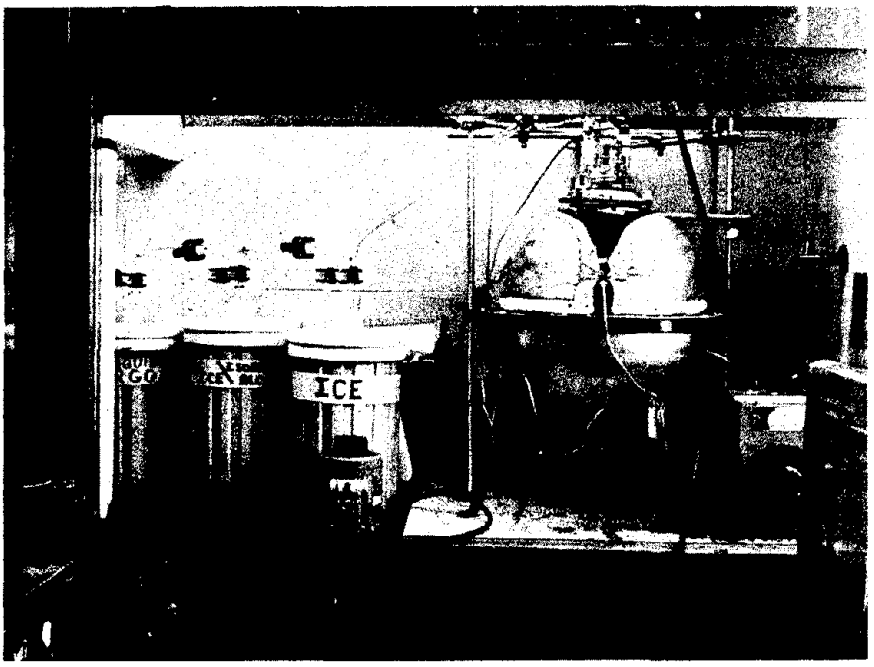


FIGURE 4. Generator/Collection System

four hours. Since considerable care had been required with multiple thermocouples in the profile run to prevent entanglement in the stirrer, only one thermocouple was kept in the melt during actual generations.

The fume collection system consisted of glass transfer tubes (20mm OD) and 500 mL glass impingers (Ace Glass, Inc.) placed in three individual cryotraps containing successively: ice (0°C), dry ice/isporopanol (-77°C), liquid argon (-186°C) (Figure 4). An additional impinger containing a 50/50 mixture of cyclohexane/acetone was used after the cryotraps to provide additional collection through dissolution. After the first few generations, a second dry ice/isopropanol bath was used to replace liquid argon because of cost considerations. The impact of this change was determined for Type I asphalt and Type I pitch at 232°C and 316°C. No change in breakthrough was observed. The cryotraps were replenished every four hours. A glass fiber filter was occasionally placed downstream of the impingers to permit an indication of breakthrough of particulates. The small amounts collected on the filter were not added to the fume samples.

Clean, dry air was pulled through the system at 10 LPM with a vacuum pump (Gast, Inc.) and needle valve restrictor (Hoke, Inc.) to sweep fumes from the reaction flask into the cryotraps. To clean this air it was passed through a high efficiency filter (Filterite 0.45 μm Microflow cartridge), silica gel and granular charcoal for removal of particulates, water and organic vapors prior to entering a rotameter for flow monitoring and then a tube furnace to preheat the air to about 100°C. The preheating prevents premature condensation of fumes on the reaction flask wall. The air was then passed through the reaction flask where it entrained volatiles and thence into the cryotraps. The generation/collection system was contained in a laboratory which received only yellow light filtered through cellulose acetate-butyrate filters to reduce exposure to ultraviolet light. Fumes collected in the system were stored in brown glass bottles at 4°C.

Fumes were generated at approximate field kettle temperatures for each material and high overheat temperature which may also occur in the field (26,27,28). Temperatures observed in the field for each material are given in Table 5. Note that the normal kettle temperature may be up to 28°C (50°F) higher than the temperature desired at the point of application (i.e., roof). The normal generation temperature for all materials was 232°C (450°F) which represents a kettle temperature frequently observed in the field. This temperature is somewhat low for steep asphalt but makes the temperature difference between the laboratory simulated kettle temperature and overheat temperature (316°C) equivalent for all materials. It is not apparent that one type of material will be overheated to a greater extent than another in the field.

Solution Preparation

After the fumes were condensed and collected in the sampling train and individual impingers and transfer lines were weighed, all material was quantitatively transferred to a large flask with an excess of cyclohexane/acetone solvent mixture used to assist complete transfer. Sufficient solvent was added to ensure complete solution of the collected fumes. If a water phase existed, this phase was removed by transferring the entire solution to a separatory funnel. The organic phase was transferred to a rotary evaporator (Buechler,

TABLE 5

Typical Roofing Material Temperatures °C (°F)

Material		ASTM Softening Point[1]	Typical Point of Application Temperature[2]	Typical Kettle Temperature[3]
Asphalt	Type I	57 - 66 (135 - 151°F)	177 - 204 (350 - 400°F)	204 - 246 (400 - 475°F)
	Type III	85 - 96 (185 - 205°F)	199 - 246 (390 - 475°F)	227 - 288 (440 - 550°F)
Pitch	Type I	52 - 60 (126 - 140°F)	174 - 190 (345 - 375°F)	190 - 232 (375 - 450°F)
	Type III	56 - 64 (133 - 147°F)	174 - 190 (345 - 375°F)	190 - 232 (375 - 450°F)

SOURCES: [1]Ref. 25
[2]Ref. 25, 26, 27
[3]Ref. 26, 27, 28

Inc.) and the solvent removed at reduced pressure with a water aspirator at a temperature of 50°C. The solvent was discarded after gas chromatographic/mass spectrometric (GC/MS) confirmation that no significant amounts of compounds of interest were present. The aqueous phase, (when present) was transferred to an evaporating dish and the water removed in a vacuum oven at 300 mm Hg and 50°C. The materials (fumes) remaining after removal of the solvents (water and cyclohexane/acetone) were weighed and dissolved in a 50/50 (v/v) cyclohexane/acetone mixture and combined. The cyclohexane and acetone were both HPLC grade (>99% purity) (Fischer Scientific, Fairlawn, NJ).

Once completely transferred, the condensed fumes dissolved further in the solvent mixture. Portions of the solution were then transferred to a separatory funnel to permit separation of the aqueous phase and organic solvent phase which existed in all cases. The water phase was removed and taken to dryness as noted. The mass of fumes found in the water phase was generally less than one percent of the total mass of volatiles collected. For the PAHs of interest, including carbazole and acridine, the octanol/water partition coefficients are greater than 10^2. Therefore, a preferred partitioning of the PAHs of interest into the moderately polar cyclohexane/acetone phase occurs. For this reason, and due to the small mass of material in the aqueous phase, the negligible loss of PAHs during drying of this phase was ignored. The organic phase was transferred to a rotary evaporator and the solvent removed. In order to confirm that no significant amount of roofing material volatiles were lost during evaporation of solvent, the solvent removed in this process was analyzed by GC/MS for the PAHs of interest. The results indicate that some of the lower boiling PAHs, e.g., phenanthrene/anthracene, are lost during solvent removal. However, the mass lost typically amounts to a small percent of these compounds in the remaining fume material (Table 6). For compounds of a molecular weight greater than 202, the loss is not measurable.

Due to the small loss of PAHs during sample processing, this method of preparation of pure roofing material fumes was utilized throughout the study.

A solution containing 0.01% B(a)P was desired for all four roofing materials, as long as the total solids concentration did not exceed 50% w/v. In the case of the coal tar pitch fumes, sufficient B(a)P was present to require dilution to prepare a solution containing 0.01% B(a)P. The resulting level of solids present in the coal tar pitch fume solutions was less than 10% (Table 1). However, in the case of the asphalt fumes, less B(a)P was present, and all asphalt solutions were prepared at 50% w/v. Also indicated in Table 1 are the designations used as test codes in the bioassay laboratory. These solutions were stored in brown glass bottles (Amber-Glas, Rockaway Glass Company, Inc., via PGC Scientifics, Rockville, MD) at 4°C prior to use for skin painting. For analysis, one mL of each solution (often resulting from the combination of several generations) was diluted to 10 mL with 50/50 cyclohexane/acetone and the resulting solution analyzed by GC/MS for quantitation of selected polynuclear aromatic hydrocarbons.

Generation of Fumes

During this study, a total of one hundred forty-four daily generations were conducted to provide sufficient consensed material from the heating of the four different roofing materials (i.e., Type I and Type III asphalt and Type I

TABLE 6

Fraction of PAHs Lost in Processing
(Rotary Evaporator)
(Volatiles from Type I Asphalt, 316°C Generation)

PAH	Mass in Solvent Removed (µg)	Mass in Sample (µg)	% Lost
Naphthalene	<5	<471	-
Phenanthrene/anthracene	554	16,000	3.5
Fluoranthene	119	13,000	0.9
Pyrene	79	12,246	0.6
Benz(a)anthracene	<5	13,000	<0.04
Chrysene	<5	26,000	<0.02
Benzofluoranthenes	<5	11,000	<0.04
Benzo(e)pyrene	<5	17,000	<0.03
Benzo(a)pyrene	<5	<471	-

and Type III coal tar pitch) at two different temperatures (i.e., 232°C and 316°C) to permit the preparation of about four liters each of eight skin painting solutions. While only one or two generations were necessary to provide enough pitch at each temperature, 59 generations of Type I asphalt at 232°C were required. Details of each generation used for preparation of skin painting solutions are reported in Table 7. The generations are listed in chronological order and information is included concerning type of material, temperature, duration of generation and mass of volatiles collected. Also reported is the batch code of skin painting solutions in which the volatiles from individual generations were pooled. When possible, several generations were combined into a single batch. Although it was desirable to use only one batch of each solution, the slow generation of asphalt volatiles required preparation of many individual batches over the duration of the skin painting experiments. Several generations were not utilized for several reasons (Table 8). Most frequent was a significant temperature excursion during low temperature generations.

Generation/Preparation of Skin Painting Solutions

The analytical confirmation of proper generation and preparation of skin painting solutions required four separate quality control procedures:

1. Determination of the collection efficiency of the cryogenic traps;
2. Comparison of the mass of fumes emitted and collected in the generation system;
3. Assessment of loss of material during preparation of skin painting solutions; and
4. Analysis of concentration of selected polynuclear aromatic hydrocarbons (PAH) in skin painting solutions.

The results of these procedures are described in the following paragraphs.

Sample Collection--
As described above, fumes generated in the 12 L reaction flask were drawn through the cryogenic collection system at about 10 Lpm. The fumes were successively condensed in transfer lines and three impingers (cryotraps) and dissolved in a fourth impinger containing cyclohexane/acetone and, in some cases, trapped on a glass fiber filter. The mass of fumes collected in the transfer lines and impingers was determined for each generation carried out in this program. In general, the mass of fumes trapped in successive impingers decreased rapidly. The percent of fumes found in each impinger typically decreased as shown in Table 9. The percent of particulates which passed through the solvent trap and collected on the glass filter were also reported. The data indicate that the breakthrough of condensed matter in the system was generally less than 0.5%. Since the temperature of the gas in the last impinger during a high temperature generation was about -3°C, little breakthrough of high molecular weight PAHs was expected. Of course, this does not account for any gases or low molecular weight vapors emitted from the flask but not condensed in the collection system. In order to determine if such vapors were significant, on several occasions the mass of material emitted from the generation flask was compared with the total mass of fumes collected. The agreement between these masses was generally consistent with the measured collection efficiency, that is, the ratio of mass collected to that emitted

TABLE 7

Yields of Volatiles from Pitches and Asphalts

Material	Temp. (°C)	Run No.*	Time (hrs)	Volatiles (gm)	Pool Label (Material:Batch)
Type I Asphalt	232	4	4.7	17.1	A:A
		5	5.7	16.2	A:A
		6	6.3	15.0	A:A
		19	6.0	21.1	A:A
		20	7.3	11.4	A:A
		21	6.0	14.4	A:A
		28	5.5	23.7	A:B
		29	4.7	28.9	A:B
		30	5.4	32.2	A:B
		34	6.3	12.0	A:B
		35(49)	7.7	32.0	A:C
		44	4.7	15.2	A:D
		45	6.5	4.7	A:D
		46	7.0	4.2	A:D
		47	7.0	5.2	A:E
		48	6.5	10.0	A:E
		49	6.5	12.1	A:E
		51	6.5	15.4	A:F
		52(65)	6.2	18.3	A:F
		53(67)	5.0	15.4	A:F
		61	5.0	6.7	A:G
		62	5.2	4.8	A:G
		63	6.0	5.7	A:G
		65	6.7	5.0	A:H
		67	5.7	5.4	A:I
		69	6.0	10.1	A:J
		71	6.5	6.7	A:K
		73	6.7	9.7	A:L
		75	6.5	13.5	A:L
		77	6.0	5.9	A:L
		79	11.5	20.1	A:M
		81	12.7	21.2	A:N
		83	14.5	29.7	A:N
		85	11.0	12.7	A:N
		87	11.5	28.3	A:N
		89	12.0	15.8	A:O
		91	13.5	20.6	A:O
		93	10.5	10.5	A:O
		95	9.5	24.2	A:O
		97	11.5	60.9	A:O
		99	6.2	11.2	A:O
		101	11.7	14.1	A:O
		103	10.2	13.7	A:O

TABLE 7 (continued)

Yields of Volatiles from Pitches and Asphalts

Material	Temp. (°C)	Run No.	Time (hrs)	Volatiles (gm)	Pool Label (Material:Batch)
Type I Asphalt (cont.)	232	118	11.0	26.7	A:P
		124	12.0	12.3	A:P
		125	10.5	23.8	A:P
		126	11.2	18.4	A:P
		127	11.7	12.3	A:P
		128	9.5	24.4	A:P
		130	12.2	18.3	A:P
		131	8.0	14.0	A:P
		132(134)	11.7	19.9	A:P
		133(135)	14.5	24.9	A:P
		134	10.5	19.7	A:P
		135	9.5	15.1	A:P
		136	9.7	36.6	A:P
		137	5.0	15.1	A:P
		138	9.2	15.0	A:P
		139	7.2	18.4	A:P
Type I Asphalt	316	14	6.3	234	B:A
		16	6.0	237	B:A
		23	7.1	320	B:B
		107	10.0	118	B:C
		109	8.0	118	B:C
		110	5.5	23.5	B:C
		114	4.2	209	B:C
Type III Asphalt	232	7	6.2	22	C:A
		8 (37)	6.4	17	C:A
		17	5.5	20.2	C:A
		22	7.1	13.6	C:B
		24	6.2	11.2	C:A
		31	4.1	11.1	C:B
		32	7.1	17.7	C:B
		36 (38)	6.5	65.6	C:C
		37	5.8	71.5	C:C
		38	5.7	46.2	C:D
		41	5.7	27.3	C:D
		42	5.6	20.3	C:D
		56	6.7	15.4	C:E
		57	6.7	16.7	C:F
		58	6.2	19.2	C:F
		59	6.5	14.3	C:G
		60	6.5	10.1	C:G

TABLE 7 (continued)

Yields of Volatiles from Pitches and Asphalts

Material	Temp. (°C)	Run No.	Time (hrs)	Volatiles (gm)	Pool Label (Material:Batch)
Type III Asphalt (cont.)	232	64	5.5	4.3	C:H
		66	6.5	6.0	C:I
		68	6.3	9.0	C:I
		70	5.5	4.6	C:J
		72	6.2	8.1	C:K
		74	6.0	4.7	C:L
		76	6.0	8.1	C:L
		78	6.2	10.2	C:L
		80	6.0	8.6	C:M
		84	12.25	47.3	C:N
		86	12.25	57.6	C:O
		90	12.0	48.2	C:O
		96	8.0	31.1	C:O
		98	6.0	12.1	C:O
		100	10.5	40.6	C:O
		102	6.0	5.1	C:O
		106	5.25	15.0	C:P
Type III Asphalt	316	2	6.0	68	D:A
		3	6.2	174	D:A
		25	7.0	294	D:B
		26 - 27	4.8, 6.3	438	D:B
		115	7.0	261	D:C
		143	5.75	61.5	D:C
		144	5.0	89.8	D:C
Type I Pitch	232	10	6.0	275	E:A
	232	18	6.0	187	E:A
	316	12	6.0	476	F:A
Type III Pitch	232	9	6.0	154	G:A
	232	13	5.0	43.0	G:A
	316	11	5.2	596	H:A

Solvent not removed from pitch samples since additional dilution was required. Final volatile mass was calculated from dry weight and volume of final pool.

*Successive runs made from a single flask of material are indicated either by a vertical bracket or by a number in parentheses which is the other run number.

TABLE 8

Generations Not Used for Skin-Painting

Material	Temp. (°C)	Run No.	Reason
Type I Asphalt	232	50	Equilibrium temperature outside desired range (± 5°C)
		105	
		119	
		120	
		121	
		122	
		123	
		129	
		140	Confirmation of collection efficiency
Type I Asphalt	316	15	Suspected contamination of flask; very high yield
		108	
Type III Asphalt	232	1	First generation - test only
		33	Equilibrium temperature outside desired range (± 5°C)
		39	
		40	
		43	
		54	
		55	
		82	
		88	
		92	
		94	
		104	
		113	
		116	
		117	
		141	Confirmation of collection efficiency
Type III Asphalt	316	111	Suspected contamination of flask; very high yield
		112	
Type I Pitch	232	142	Confirmation of collection efficiency

TABLE 9

Typical Distribution of Fumes in Collection System
(Grams Collected)

Material		Impinger				Filter	Total
		Ice	Dry Ice/ Isopropanol	Dry Ice/Iso-propanol (second)	Cyclohexane/Ace-tone Solution		
Type I Asphalt (232°C)	gm	32	25	3	1	0.2	61.2
	%	52	41	5	1.6	0.3	
Type III Asphalt (316°C)	gm	138	53	17	3	0.1	211.1
	%	65	25	8	1.4	0.05	
Type I Pitch (232°C)	gm	410	86	32	5	<0.1	533
	%	77	16	6	0.9	<0.02	
Type III Pitch (316°C)	gm	720	28	2	1	<0.1	751
	%	96	3.7	0.3	0.1	<0.01	

is near one (Table 10). The mean of the mass ratios is, in fact, slightly higher than one in all but one case. However, the precision is poor enough to preclude placing any significance on this fact. The poor precision is attributed to the difficulty of accurately determining the relatively small change in mass of the 12 L flask as a result of the generation.

Both the distribution of fumes in the collection system and the good agreement of mass collected and emitted tend to confirm that most fumes were trapped efficiently in the cryotraps utilized in this study.

Generation Yield

The total mass of volatiles emitted during heating of roofing materials may differ significantly over a temperature range from a typical kettle temperature to a high overheat temperature and also between the different types of asphalts and pitches. Since both the dose and the specific carcinogenicity of a material are generally important in determining the health hazard each presents, the mass emission rate of volatiles during laboratory generations may be of interest as a simulation of field emission rates. The emission rate during each generation was simply calculated from the mass of volatiles collected in a given time period. The data summarized in Table 11 indicate the marked increase in the mean mass emission rate at the elevated temperature. The emission rate for asphalts is generally about ten times higher at 316°C than at 232°C. In the case of coal tar pitches, the emission rate is about two to seven times greater at the higher temperature. The emission rate is much greater for the coal tar pitches than the asphalts. This observation corroborates the greater percentage of volatiles indicated by the TGA analysis. The fact that the emission rate is not proportional to the percent volatiles emitted during the TGA analysis may indicate that the physical transfer of volatile species across the interface may be the controlling factor in fume generation rather than the mass available. The TGA analysis is performed with a small sample of high surface area whereas the bulk generation system utilized a $\sim$10 L volume with a surface area of $\sim$700 cm^2, and a depth of 10-15 cm.

The variability in mass emission rate for individual generations of each type of asphalt is quite large, i.e., relative standard deviations are as high as 80%. Since careful control of generator temperature and efficient fume collection were confirmed, other variables apparently influenced yield. Some batches of the asphalts were used for more than one generation. A comparison of yield data (Table 7) indicates there were no consistent differences between yield of the first and subsequent generations. Stirring rate was also observed to slightly affect emission rate over the typical operating range of 200-350 rpm. Air flow rate occasionally varied from 10 Lpm to about 3 Lpm due to partial plugging of the impingers. This variation apparently affected transfer of fumes from the generation flask to the cryotraps and thus the apparent mass emission rate. As noted, asphalt from six different containers (45 kG each) was used to generate fumes during the skin-painting experiments. Variation of yield is apparently influenced to a large extent by differences between these batches.

TABLE 10

Mass of Volatiles Emitted and Collected

Material	Temperature (°C)	Mean[1] (Mass Collected / Mass Emitted)	Standard Deviation
Type I Asphalt	232	1.19	0.69
Type I Asphalt	316	1.33	0.19
Type III Asphalt	232	1.52	1.12
Type III Asphalt	316	1.15	0.23
Type I Pitch	232	0.91	0.16
Type I Pitch	316	1.11	- (1 generation)
Type III Pitch	232	1.84	- (1 generation)
Type III Pitch	316	1.19	- (1 generation)

[1]Mean of 2-6 generations

TABLE 11

Mass Emission Rate

Material	Temperature (°C)	Mean[1] Emission Rate (g/hr)	Std. Deviation
Type I Asphalt	232	2.1	1.2
Type I Asphalt	316	33	16
Type III Asphalt	232	3.1	2.6
Type III Asphalt	316	27	13
Type I Pitch	232	38	10
Type I Pitch	316	79[2]	--
Type III Pitch	232	17	12
Type III Pitch	316	115[2]	--

[1]Mean of all generations listed on Table 7.

[2]Calculated on the basis of one generation.

GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC (GC/MS) ANALYSIS

By gas chromatographic separation of individual PAHs in the complex fume matrix and specific and sensitive mass spectrometric detection, a wide range of PAHs from naphthalene to dibenzopyrenes was quantified. A glass capillary column capable of high resolution was generally employed to permit separation and quantitation of the selected PAHs which included closely related isomers such as benzo(e)pyrene and benzo(a)pyrene. The GC/MS analytical conditions are listed in Table 12. The mass spectrometer used was a Finnigan Model 4023. Compounds were considered to be identified when the retention time for the PAH of interest relative to the retention time of an added internal standard, i.e., d_{10}-anthracene, 9-phenylanthracene or 9,10-diphenylanthracene, matched the relative retention time observed in a calibration standard and the mass spectrum of the PAH of interest matched that obtained from a calibration standard. These identification criteria apply to all of the analytical results.

A second GC/MS technique was occasionally used to yield benzo(a)pyrene concentrations for independent analysis. In this second technique, a nematic liquid crystal phase coated onto an appropriate support was used as the GC column packing. This column isolated and separated benzo(a)pyrene and its isomers from the remainder of the sample constituents, as well as from each other. The mass spectrometer detection system was adjusted to detect only the characteristic ions. Specific GC/MS analytical conditions for this method are also tabulated in Table 12.

Analysis of Polynuclear Aromatic Hydrocarbons (PAH)

The concentration of benzo(a)pyrene [B(a)P] and other selected PAHs in skin painting solutions was measured after preparation of pooled batches ready for application. Such a batch of skin painting solution identified by a two-letter pool label (see Table 7) generally included material from more than one generation. The selected list of PAHs included several compounds identified by NIOSH and EPA as suspect carcinogens. B(a)P was selected as a marker compound in the skin painting solutions due to correlation of B(a)P concentration and carcinogenicity.

Due to the chemical complexity of the skin painting solutions, gas chromatography/mass spectrometry was selected for analysis of the PAHs of interest. During initial preparation and analysis of skin painting solutions, the two GC/MS methods were used to provide independent confirmation of B(a)P concentrations. The comparison between the two methods is shown in Table 13. B(a)P concentrations agree within ±42%.

For routine analysis of all selected PAHs, the capillary column GC/MS method was used. The concentrations of PAHs in all pooled skin painting solutions are reported in Tables 14 through 18. The mean concentration of each PAH in the pooled skin painting solutions of each material and temperature combination, for example, solutions AA through AP which represent asphalt Type I, low temperature generations, are summarized in Tables 18 and 19. The usefulness of the mean concentration as an indicator of total PAH dose is suspect for low temperature, Type I and III asphalt volatiles due to the large

TABLE 12

GC/MS Conditions for the Analysis of Fume Volatiles

	PAH Analysis	BaP Analysis
a) GC Conditions		
column	25-m glass capillary coated with SP-2250	1.8-m SP-301 packed column
injection/volume	Grob injection, 2 μL	2 μL
temperature program	30°C isothermal for 2 min. 30 - 175°C at 20°C/min. 175 - 285°C at 3°C/min. 285°C isothermal for 30 min.	260°C isothermal
b) MS Condition (Finnigan 4023)		
mode	full mass scan	limited mass scan
mass range (amu)	100 - 350	248 - 256, 328 - 332
scan rate	2 sec/scan	.140 sec/amu
electron energy	50eV	50eV
filament emission	45ma	45ma
electron multiplier voltage	-1900v	-1900v

TABLE 13

Comparison of Benzo(a)pyrene Concentrations by Specific Method (Liquid Crystal) and General Method (Capillary)

Material:Batch Sample (Pool Label)	Concentration (µg/mL)[1] B(a)P (Liquid Crystal) Concentration	Range	B(a)P (Capillary)[2] Concentration	Range
E:A	260	230 - 310	320	240 - 480
G:A	120	110 - 140	170	130 - 260
H:A	100	90 - 120	90	68 - 140
A:E	16	-	13	-
A:N	0.9	-	0.7	-
A:J	0.3	-	0.3	-

[1]Values in µg/mL as analyzed in sample: for E:A, G:A, and H:A prior to final dilution to 100 µg/mL.

[2]Values reported may include a slight amount of benzo(e)pyrene GC peak tail.

TABLE 14

Concentration (μg/mL) of PAHs in Skin-Painting Solutions
(Type I Asphalt, 232°C)

	Analytical Ion	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	Mean
Naphthalene	128	22	4.5	--	3.8	9.1	20	17	98	12	13	22	16	17	42	10	38	22
Fluorene	166	X	X	X	31	10	23	25	24	15	36	49	34	57	65	21	72	36
Carbazole	167	--	--	--	--	58	--	23	18	16	--	--	--	--	--	--	--	20
Anthracene/Phenanthrene	178	280	120	95	156	720	220	220	93	110	190	170	170	103	46	50	165	180
Fluoranthene	202	25	14	13	142	870	35	110	46	87	2.2	--	0.6	3.9	7.1	9.7	3	86
Pyrene	202	19	18	23	102	650	42	86	72	72	4.6	--	3.1	4.4	9.2	15	7	70
Benz(a)anthracene	228	14	15	15	45	62	--	63	1	13	--	--	--	--	--	--	--	11
Chrysene/Triphenylene	228	28	32	38	24	120	--	32	35	43	--	--	--	--	20	27	--	25
Benzofluoranthenes	252	--	8.1	11	7.1	--	--	--	--	--	--	--	--	--	1.1	1.1	--	1.8
Benzo(e)pyrene	252	7.7	12	18	1.0	45	--	--	--	--	--	--	--	--	4.7	--	--	5.5
Benzo(a)pyrene	252	7.2	5.5	6.6	1.3	13	--	--	--	--	--	--	--	--	0.7	0.7	--	2.2
Indeno(c,d)pyrene	276	15	--	5.8	--	--	--	--	--	--	--	--	--	--	22	--	--	2.7
Benzo(g,h,i)perylene	276	5.5	--	0.8	--	--	--	--	--	--	--	--	--	--	6.7	--	--	0.8
Dibenzanthracenes	278	9.6	--	6.5	--	--	--	--	--	--	--	--	--	--	10	--	--	1.6
Coronene	300	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Dibenzopyrenes	302	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

X = Not measured

-- = None detected (<0.5).

TABLE 15

Concentration (μg/mL) of PAHs in Skin-Painting Solutions
(Type I Asphalt, 316°C)

	Analytical Ion	BA	BB	BC	Mean
Naphthalene	128	5.2	--	8.0	4.4
Fluorene	166	X	X	22	22
Carbazole	167	<0.5	4.3	--	1.4
Anthracene/Phenanthrene	178	120	17	21	53
Fluoranthene	202	16	14	1.0	10
Pyrene	202	13	13	1.0	9.0
Benz(a)anthracene	228	17	14	--	10
Chrysene/Triphenylene	228	27	28	3.0	19
Benzofluoranthenes	252	--	12	--	4.0
Benzo(e)pyrene	252	6.8	18	--	8.3
Benzo(a)pyrene	252	5.8	--	--	1.9
Indeno(c,d)pyrene	276	--	9.4	--	3.1
Benzo(g,h,i)perylene	276	--	4.5	--	1.5
Dibenzanthracenes	278	--	--	--	--
Coronene	300	--	--	--	--
Dibenzopyrenes	302	--	--	--	--

X = Not measured

-- = None detected (<0.5)

TABLE 16

Concentration (ug/ML) of PAHs in Skin-Painting Solutions
(Type III Asphalt, 232°C)

	Analytical Ion	CA	CB	CC	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM	CN	CO	CP	Mean
Naphthalene	128	47	0.1	0.19	0.07	18	25	24	23	54	26	9.0	26	6.2	2.9	6.8	4.0	17
Fluorene	166	11	X	X	X	20	31	36	32	59	75	85	75	27	17	27	13	39
Carbazole	167	--	14	15	4.5	--	8.3	20	--	39	--	--	--	--	--	--	--	6.3
Anthracene/Phenanthrene	178	680	280	160	46	710	510	240	246	360	840	250	360	88	19	39	12	300
Fluoranthene	202	190	71	98	68	220	200	130	58	190	250	20	47	4.4	11	--	1.0	97
Pyrene	202	130	48	64	50	180	130	49	31	120	140	19	28	7.1	14	--	1.0	63
Benz(a)anthracene	228	6.5	21	18	21	--	8.6	2.9	36	5.2	36	2.8	--	--	--	--	--	7.6
Chrysene/Triphenylene	228	19	37	20	22	--	28	15	--	19	24	14	1.9	1.9	--	5.2	5.0	13
Benzofluoranthenes	252	--	20	18	30	--	--	--	--	--	13	1.9	--	--	--	--	--	5.2
Benzo(e)pyrene	252	--	19	8.2	9.2	--	--	--	--	--	11	6.3	4.2	--	--	--	--	3.6
Benzo(a)pyrene	252	--	15	11	12	--	--	--	--	--	5.7	1.2	1.0	--	--	--	--	2.9
Indeno(c,d)pyrene	276	--	22	3.1	3.1	--	--	--	--	--	1.7	1.8	4.2	--	--	--	--	2.2
Benzo(g,h,i)perylene	276	--	6.8	1.6	2.2	--	--	--	--	--	0.7	0.3	1.4	--	--	--	--	0.8
Dibenzanthracenes	278	--	22	1.8	1.5	--	--	--	--	--	0.5	0.6	2.7	--	--	--	--	1.8
Coronene	300	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Dibenzopyrenes	302	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

X = Not measured
-- = None detected (<0.5)

TABLE 17

Concentration (μg/mL) of PAHs in Skin-Painting Solutions
(Type III Asphalt, 316°C)

	Analytical Ion	DA	DB	DC	Mean
Naphthalene	128	15	52	80	49
Fluorene	166	X	41	14	28
Carbazole	167	--	--	--	--
Anthracene/Phenanthrene	178	110	81	16	69
Fluoranthene	202	20	--	2.0	7.3
Pyrene	202	19	1	3.0	7.7
Benz(a)anthracene	228	17	--	--	5.7
Chrysene/Triphenylene	228	36	--	5.0	14
Benzofluoranthenes	252	--	--	--	--
Benzo(e)pyrene	252	4.3	--	--	1.4
Benzo(a)pyrene	252	--	--	--	--
Indeno(c,d)pyrene	276	--	--	--	--
Benzo(g,h,i)perylene	276	--	--	--	--
Dibenzanthracenes	278	--	--	--	--
Coronene	300	--	--	--	--
Dibenzopyrenes	302	--	--	--	--

X = Not measured

-- = None detected (<0.5)

TABLE 18

Concentration (μg/mL) of PAHs in Skin-Painting Solutions (Pitches)

	Analytical Ion	Type I 232°C E	Type I 316°C F	Type III 232°C G	Type III 316°C H
Naphthalene	128	>1800	1770	288	>620
Fluorene	166	X	740	X	X
Carbazole	167	1980	1450	540	1400
Anthracene/Phenanthrene	178	>960	2960	>2580	>5200
Fluoranthene	202	>2940	2350	>960	>2800
Pyrene	202	>2070	1790	>720	>2300
Benz(a)anthracene	228	570	330	330	800
Chrysene/Triphenylene	228	460	300	290	710
Benzofluoranthenes	252	230	230	250	250
Benzo(e)pyrene	252	42	51	45	46
Benzo(a)pyrene	252	96	85	102	90
Indeno(c,d)pyrene	276	33	1.7	11	6.8
Benzo(g,h,i)perylene	276	28	2.0	7.2	0.7
Dibenzanthracenes	278	12	-	4.1	-
Coronene	300	-	-	-	-
Dibenzopyrenes	302	-	-	-	-
Total analytes (μg/mL)		>11221	12060	>6127	>14224
Total solids (Table 1) (mg/mL)		78	55	84	30
% Analytes/total solids		>14.4	21.9	>7.3	>47.4

X = Not measured

- = None detected (<0.5)

TABLE 19

Concentration (μg/mL) of PAHs in Skin-Painting Solutions — Summary (Asphalts)

	Analytical Ion	Type I 232°C A	Type I 316°C B	Type III 232°C C	Type III 316°C D
Naphthalene	128	22	4.4	17	49
Fluorene	166	36	22	39	28
Carbazole	167	20	1.4	6.3	--
Anthracene/Phenanthrene	178	180	53	300	69
Fluoranthene	202	86	10	97	7.3
Pyrene	202	70	9.0	63	7.7
Benz(a)anthracene	228	11	10	7.6	5.7
Chrysene/Triphenylene	228	25	19	13	14
Benzofluoranthenes	252	1.8	4.0	5.2	--
Benzo(e)pyrene	252	5.5	8.3	3.6	1.4
Benzo(a)pyrene	252	2.2	1.9	2.9	--
Indeno(c,d)pyrene	276	2.7	3.1	2.2	--
Benzo(g,h,i)perylene	276	0.8	1.5	0.8	--
Dibenzanthracenes	278	1.6	--	1.8	--
Coronene	300	--	--	--	--
Dibenzopyrenes	302	--	--	--	--
Total analytes (μg/mL)		464.6	147.6	559.4	182.1
Total solids (Table 1) (mg/mL)		500	500	500	500
% Analytes/total solids		0.093	0.030	0.11	0.036

-- = None detected (<0.5)

variability in concentration among pooled batches of solution. The variability in PAH concentrations for asphalt volatiles generated at 232°C may be due to differences in the PAH content of bulk materials which were purchased from a local distributor's inventory in six containers of 45 Kg each. It does not appear, however, that total volatiles content of bulk materials varies by such an amount (Table 7). A comparison of replicate analyses of individual samples of both pitch and asphalt solutions indicates a mean relative standard deviation (coefficient of variation) of 12% or less for all PAHs and as high as 41% for individual PAHs (Table 20).

Inspection of total PAH concentrations of the eight skin painting solutions indicates that the pitch solutions as used contain higher levels of PAHs than asphalt solutions. Also, a solution prepared by generation at the high temperature does not necessarily contain higher concentrations of individual (or total) PAHs than that generated from the same starting material at typical kettle temperature.

To monitor the fate of PAHs as a result of long-term storage of skin painting solutions during the mouse bioassay experiments, replicate analyses were planned at eight to ten month intervals. Since small batches of asphalt solutions were prepared during the course of the mouse bioassay, they were consumed in short periods of time. As a result, the planned replicate analysis of these solutions was not undertaken (with few exceptions). In the case of pitch solutions, the concentration of PAHs in the single pooled batches was measured on two occasions after the initial preparative analysis. The results shown in Table 21 indicate that although an expected loss of some PAHs is observed, there is no consistent degradation during the eighteen months of use.

LIGHT SOURCE

Spectrum

In the laboratory, artificial sources generally are used to simulate solar irradiance. The most simple and reliable light source is the incandescent lamp but it has a comparatively low color temperature, limited by the maximum tolerable evaporation rate of the tungsten filament. The result is a color temperature of, at most, 3400°C and, correspondingly, an ultraviolet output which is many orders of magnitude too low for solar simulation.

For broadband simulation of sunlight, the high-pressure Xenon arc is more suitable. It is a very high pressure, very high temperature plasma source. A strong continuum underlies a line spectrum that has been very much broadened by the high pressure. The result is a spectrum of very high intensity resembling that of daylight to a much better approximation than either tungsten or other gas discharge lamps. Typical spectra of Xenon arcs are shown in Figures 5 and 7. A (nominal) 6.5 kW ATLAS arc was chosen for this study because it is available in a well-proven, highly reliable, and cost-effective system including a power supply, cooling water circulation system, lamp cooling, and mounting hardware. Its spectrum, according to ATLAS, is shown in Figure 5. Figure 7 (based on an OSRAM Xenon arc, which has basically the same spectrum as the ATLAS) shows the modifications that are necessary in order to make the spectrum of the Xenon arc approach sunlight. In particular, the Xenon arc emits several strong lines and some continuum between 800 and

TABLE 20

Precision of GC/MS Analysis of PAHs in Skin-Painting Solutions
(Relative Standard Deviation - %)

Compound	Solution Batch		
	AP[1]	DB[2]	HA[2]
Naphthalene	10.2	0.7	4.7
Fluorene	2.9	4.1	8.7
Carbazole	-	-	3.4
Phenanthrene	2.0	2.3	2.9
Anthracene	13.2	3.5	-[3]
Fluoranthene	30.1	11.0	4.0
Pyrene	1.9	7.8	5.0
Chrysene & Isomers	-	15.1	2.3
Benzofluoranthenes	-	-	6.4
Dibenzothiophene	24.0	6.0	2.3
Benz(a)anthracene	-	-	10.1
Dibenz(a,h)anthracene	-	-	40.9
Mean % Std. Dev.	12.0	6.3	8.2

[1]Two replicates

[2]Three replicates

[3]Due to large size of peaks, anthracene value included with phenanthrene

TABLE 21

Effect of Storage[1] on PAH Concentrations (μg/mL) in Pitch Solutions

	Analytical Ion	Initial E	10 mo. E	19 mo. E	Initial F	10 mo. F	19 mo. F	Initial G	10 mo. G	19 mo. G	Initial H	10 mo. H	19 mo. H
Naphthalene	128	>1800	>1390	>1320	1770	>1440	>1850	288	> 4400	> 850	> 620	>1250	> 380
Fluorene	166	X	>1610	>1830	740	>2000	>2380	X	> 4600	>1940	X	>1680	> 840
Carbazole	167	1980	>1060	1630	1450	>1420	1840	540	> 3740	2190	1400	>1240	1000
Anthracene/ Phenanthrene	178	> 960	>5500	>5500	2960	>700	>7400	>2580	>15000	>7000	>5200	>7000	>2700
Fluoranthene	202	>2940	>3060	>3500	2350	>4260	>5100	> 960	> 7400	>4900	>2800	>3530	>2500
Pyrene	202	>2070	>2600	>2600	1790	>3570	>3400	> 720	> 6400	>3800	>2300	>2920	>1900
Benz(a)anthracene	228	570	440	370	330	770	800	330	702	1400	800	363	610
Chrysene/ Triphenylene	228	460	400	240	300	660	550	290	541	1000	710	342	420
Benzofluoranthenes	252	230	120	89	230	204	280	250	1200	540	250	103	270
Benzo(e)pyrene	252	42	160	5	51	37	62	45	60	120	46	25	57
Benzo(a)pyrene	252	96	23	15	85	63	86	102	68	166	90	37	76
Indeno(c,d)pyrene	276	33	25	--	1.7	8	--	11	1	--	6.8	12	--
Benzo(g,h,i)perylene	276	28	7	--	2	7	--	7.2	1	--	0.7	--	--
Dibenzanthracenes	278	12	4	--	--	2	--	4.1	--	--	--	--	--
Coronene	300	--	--	--	--	--	--	--	--	--	--	--	--
Dibenzopyrenes	302	--	--	--	--	--	--	--	--	--	--	--	--

X = Not measured

-- = None detected (<0.5)

[1]Initial analysis on 4/30/79; 10 month storage on 2/21/80; 19 month storage on 11/19/80.

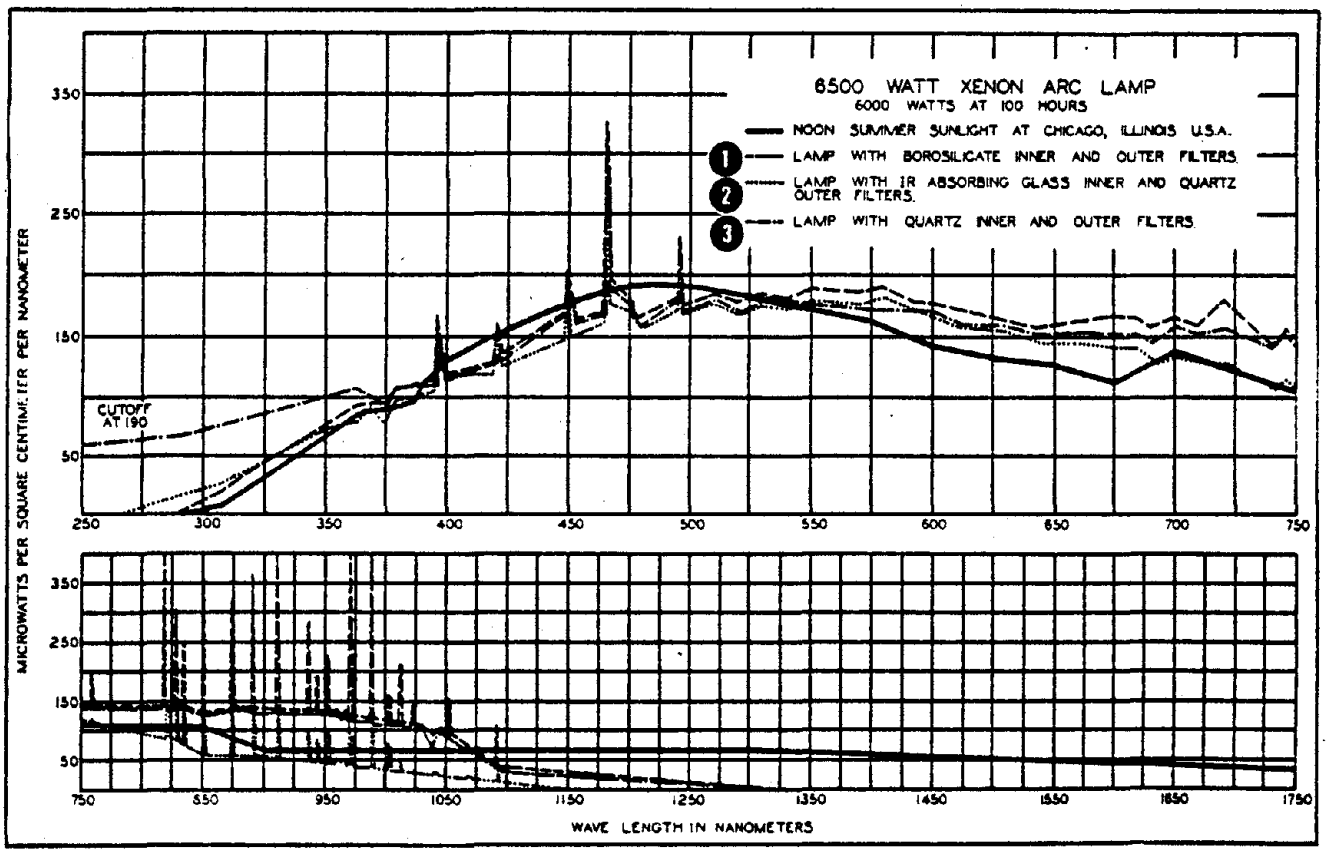


FIGURE 5. Atlas 6.5 kW Xenon Arc Spectrum (provided by Atlas)

Type	Main Characteristics or Applications	Spectral Range of T_{min} (R_{max}) [nm]	Substrate	Maximum Size [mm]
114	IR-reflection with extended UV-transmission	800 . . . 850	Tempax® fused silica	600×500

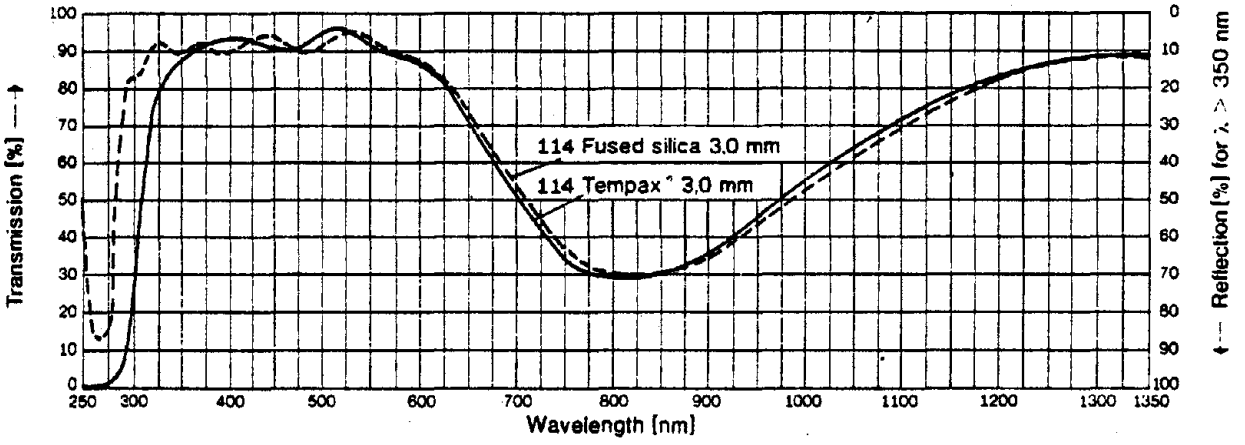


FIGURE 6. Schott 114 Filter

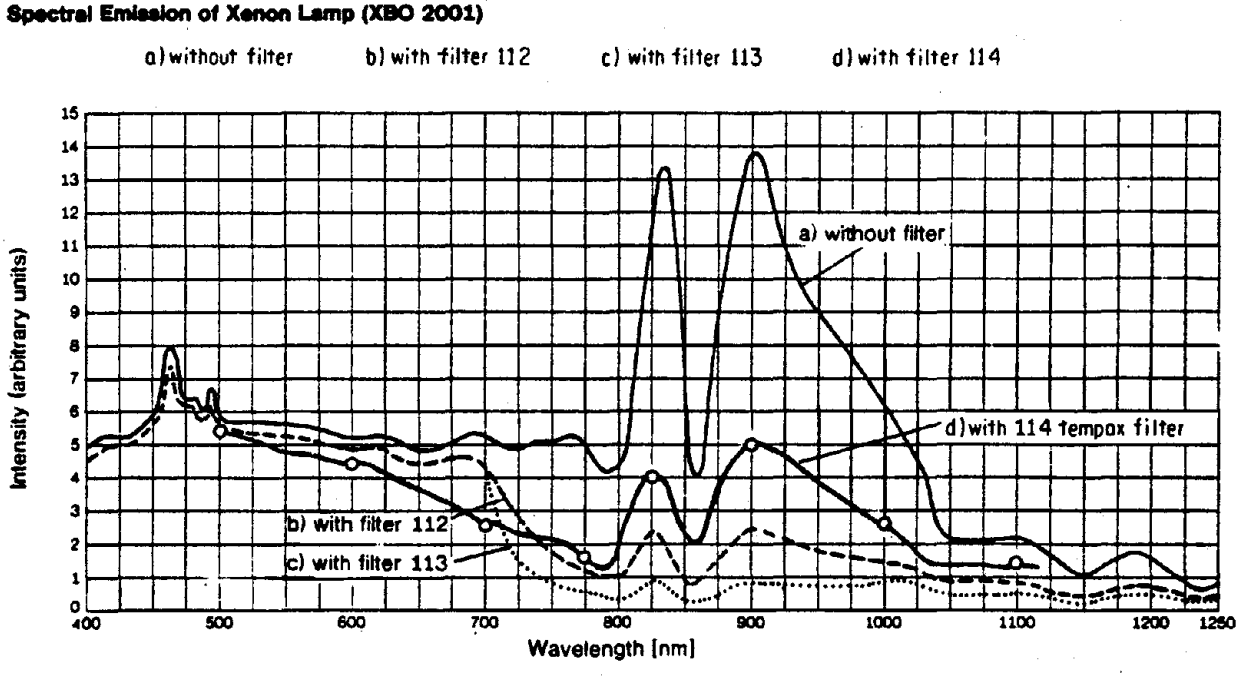


FIGURE 7. Xenon Arc Spectrum with Infrared Reflecting Filters

1000 nm that must be attenuated. Specifically designed filters for this purpose are available from Schott Optical Glass, Inc., Duryea, PA. Their effect is shown in Figure 7. Figure 6 specifically shows the filter (No. 114) chosen. It is an IR-reflecting, metal oxide coated, Pyrex-like glass ("TEMPAX"), 3 mm thick, with extended UV transmission. It was obtained in 150 mm squares from Schott in Mainz, W. Germany. Filters 112 and 113 from Schott have better longwave rejection, but over-attenuate the ultraviolet.

Uniformity of Exposure

Perhaps the most difficult practical problem in building a solar simulator for large animal populations is to achieve uniform irradiance over a large horizontal surface. Any other arrangement (amphitheater, cylinder, sphere) is likely to lead to difficulties with designing cages that are practical for handling the animals, that assure correct attitude, and protect the animals from injury in handling and during the exposure. A very large share of the effort and cost of such experiments goes to handling the animals and correct cage design is crucial to avoid the risk of injury or serious discomfort to the animals.

The sun, of course, delivers a uniform irradiance over large areas, because it is a quasi-point source very far away. In theory, a high-efficiency reflecting collector could be put around a Xenon arc to produce the equivalent of such a far-away source. A reflector suitable for this purpose was not commercially available. Even if it had been, uniformity of irradiance would have been very hard to achieve that way, according to past experience with other solar simulator set-ups. The angular emission characteristics of high-pressure arc sources, including their high strength quartz envelopes and cooling systems, are ordinarily not very uniform, and this shows up, after focusing by the reflector, as local variations in irradiance. Making the reflector diffuse or partly diffuse will help, but at a large loss in irradiance. Recently, optical mixing (kaleidoscope) systems have been developed to solve these problems, some on the basis of fiber optic bundles.

None of these elegant solutions, however, were seen to be within the scope of the present program. Instead, a geometric arrangement was chosen that takes advantage of the inverse square law (irradiance $\alpha \frac{1}{\text{distance}^2}$) and produces a uniform average exposure over a comparatively large horizontal surface. It is shown in Figure 8. It was originally proposed by John Strong (29) for uniform vacuum deposition of metals. His paper, reproduced in the appendix, shows that uniform exposure is obtained on the surface of a turntable from a point source that has been placed with respect to the turntable as shown in Figure 8. The arrangement was modified slightly to use two turntables symmetrically disposed under one lamp, and a line source of about 15 cm in length; i.e., the ATLAS 6.5 kW Xenon arc burner.

The enclosure containing the light source and turntables for exposure of the animals was constructed of aluminum framing covered with sheet masonite. Overall dimensions of the enclosed space were 2.1 m wide x 1.2 m deep x 1.5 m high. Air was exhausted through the top at approximately 8.5 m^3/min (300 cfm), with the incoming air led from the open bottom, through baffles placed to maximize ventilation of the test cubicle arrays. Air temperature (measured

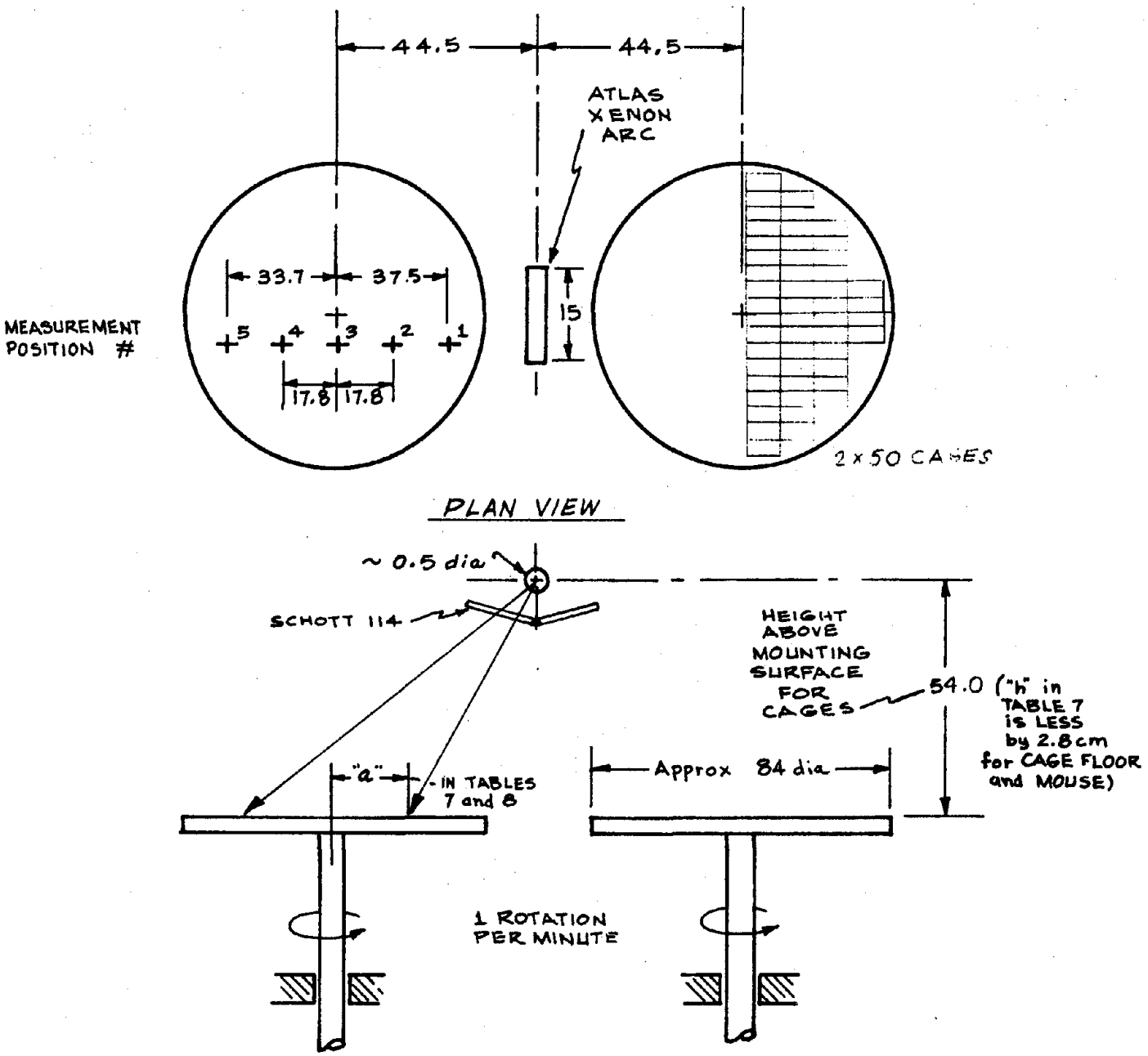


FIGURE 8

Turntables and Xenon Arc Geometry

daily) in the box was thus kept in a range between 24 and 28°C (75-80°F). There was no olfactory evidence of the production of ozone (Figure 9).

Animals were confined individually in one cell (4 x 9 x 3 cm) of a 50-cell exposure unit, constructed of 3 x 3 stainless steel mesh. The openings (0.7 cm^2) provide for free passage of air through the cubicle and minimal obstruction of the light. Two 50-cell units could be placed on each turntable, thus four "solar" groups could be irradiated at once in the two upper turntables and another four "non-solar" groups could be "exposed" on the non-illuminated lower turntables.

The average exposure calculated for such an arrangement with a point source is shown in Table 22, excerpted from Table IV (29). The exposures are calculated on the turntable at radius "a" from its center of rotation. The source stands on radius "r" ("r" = 1) and a height "h" above the edge of the turntable, as illustrated in Figure 8.

By direct measurement, the uniformity shown in Table 23 was found. The measurements were at first complicated by directional effects in the detector and its mount. After experimentation to eliminate these confusing effects, good data were obtained by use of an opal glass diffuser (3mm thick, A. Jaegers, Lynbrook, NY) over the silicon detector (UV-215B, EG&G, Salem, MA). The data were taken by mounting the detector and diffuser exactly horizontally on the turntable at the average expected level of the back of the mouse above the cage support table. The cages themselves were removed for the experiment. The turntable was then rotated slowly, and the detector output was integrated for four quarter-revolutions. The silicon photodiode operated in virtual short circuit into an op-amp circuit (LM208A, National Semiconductor Corporation, Santa Clara, CA) which converted the diode output current into a voltage. That voltage was amplified, fed to a voltage-to-frequency converter, and from there into a counter. The counter thus indicates the time integral of the photodiode current. The system was carefully checked to have it operate in its linear range and for the signal to be large compared to zero drift and noise.

Table 23 shows the results for full revolutions at four different radii. The integral of irradiance is measured in arbitrary units because a combination of a diffuser and a wideband neutral density filter was found necessary to provide an optical signal independent of angle of incidence. The purpose of this measurement was only to validate the geometric concept of the turntable arrangement. The narrow band spectral filters with which spectral irradiance is measured would not have functioned correctly in this set-up. With such interference filters, there would have been a strong dependence of spectral transmission on the angle of incidence and thus a diffuser would have been required. With diffuser and narrow band filter, not enough signal would have been available for accurate exposure data. Table 23 shows the numerical results of the exposure uniformity measurements. The variations from the mean are between -4 and +4%. The uniformity is as good as, or better than, indicated by the predictions of Table 22. The final configuration has a lamp height of approximately h = 1.1a, and the outermost radius of cage location is 38 cm, corresponding to a = 0.85. The measured results are thus seen to be in very good agreement with predictions.

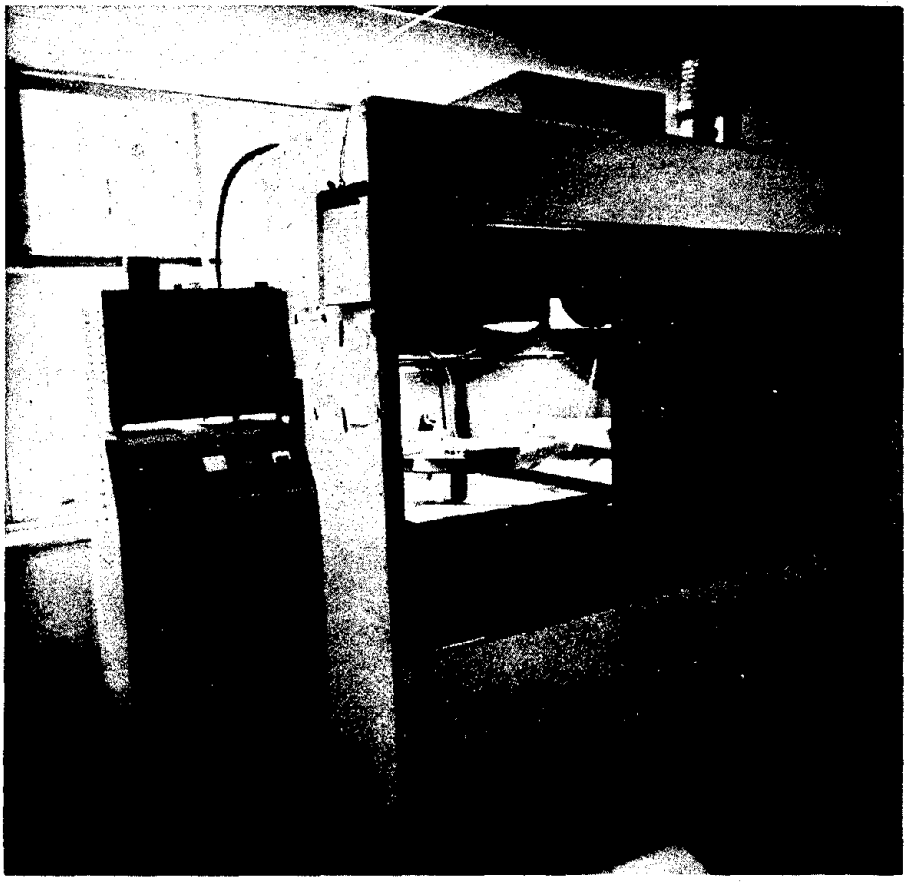


FIGURE 9. Light Treatment Apparatus

TABLE 22

Effect of Source Height on Exposure Distribution on the Turntable
(see text, Appendix, and Figure 8)

Relative Radius on Turntable	Calculated Relative Exposure at Radius a (source at height h and radius r)		
a	h = r	h = 1.1r	h = 1.2r
0.00r	2.22	1.91	1.65
0.25r	2.24	1.93	1.65
0.50r	2.29	1.93	1.63
0.75r	2.28	1.89	1.57
0.80r	2.27	--	--
0.90r	2.22	--	--
1.00r	2.11	1.74	1.45

TABLE 23

Measured Exposure Distribution on the Turntable
(see text and Figure 8)

Radius a (cm)	(Counts per revolution, see text)	Variation from Mean (%)
0 (center)	66400	-2
13	69600	+3
25	70300	+4
36	64900	-4
Mean	67800	

Spectral Irradiance

Figure 8 also shows the dimensions of the apparatus. For the scheme described in the last section to work correctly and provide uniform exposure, the relative spectral distribution of irradiance must be the same at all locations on the turntable. The air path is too short to affect absorption significantly in the range from 280 to 700 nm and the lamp is rotationally symmetrical about its axis. However, as the heat-rejecting filters No. 114 were only available in flat panes, the design of the filter holders had to involve a compromise, and the filters are not used throughout at normal (perpendicular) incidence. Also, flat aluminum reflectors were placed above the lamp in order to make use of the energy the lamp radiates in the upper hemisphere and to reduce the heat load on the lamp house.

Measurements were taken to determine the degree to which the geometry just described made the spectral distribution of the irradiance a function of slant angle to the turntable surface. Spectral irradiance measurements were taken at five diametral positions on the turntable, as shown in Figure 8. A special silicon photodiode (EG&G Type UV-215B, Serial No. 7835-01, spectrally calibrated by EG&G from 200 to 1100 nm) was mounted in a holder that would accept one of five calibrated narrowband optical interference filters. (At the time these measurements were taken, the 309 nm filter was not yet available.) The photodiode in its holder was moved from Position 1 step-wise to Position 5 (see Figure 8), and data were taken with each of the five filters at each position. For every measurement, the filter-detector holder was carefully aligned to be perpendicular to the line towards the lamp. That is necessary for the interference filters to perform correctly. Operating them substantially off-axis shifts their passband and makes them grossly sensitive to polarization.

Table 24 shows the result of these spectral irradiance distribution measurements. Since the slant ranges, i.e., the distances from the light source, are exactly known for each position (see bottom row), the measured irradiance data can be checked for consistency and for systematic effects of slant range of slant angle. In each position, an apparent lamp spectral intensity I can be calculated from the measured spectral irradiance H, according to:

$$I = H \times R^2 \qquad \text{(W/steradian nm, i.e., spectral power intensity per unit solid angle)}$$

where R is the slant range, i.e., the distance from the light source.

If the measurements are error-free, and if there are no systematic effects of slant angle or slant range, one would calculate the exact same apparent lamp intensity from each position. In fact, this was found to be true with a coefficient of variation of 2 to 6 percent of mean intensity at 4 wavelengths from 600 to 340 nm. At 790 nm, a larger inconsistency was found (C.V. 28%), presumably due to the substantial effect of angle on the long-wave attenuation of the Schott 114 filters. That effect is very small in the visible, but becomes particularly noticeable in the infrared.

The data for Position 1 constitute the maximum irradiance on the turntable. The animals on the outermost radius are exposed to it once per revolution, and

TABLE 24

Spectral Irradiance at Five Positions Along a Diameter of the Turntable

Nominal filter wavelength (nm)	Irradiance H ($\mu W/cm^2$ nm) on Surface Facing the Lamp (normal incidence)				
	Pos. #1 below lamp	2	3 (center)	4	5
340	43.8	37.3	28.6	22.3	15.4
410	110	95.6	72.3	53.5	37.3
520	144	122	91.8	65.7	49.8
600	129	109	79.2	58.7	42.5
790	76	52.2	29.7	17.8	13.7
Distance from Light Source (cm)	54.1	60.0	69.7	82.2	94.8

they experience the largest fluctuation. An animal at the center would receive a constant irradiance, as measured at Position 3. That irradiance at the center is, at the same time, the average irradiance for all radii, as demonstrated in the previous section on uniformity. The irradiance levels were adjusted so that the peak irradiance would correspond to solar irradiance through air mass 1, according to Thekaekara's data (30).

In conclusion, the spectrum of irradiance was found to be substantially the same everywhere on the turntable. The only substantial deviation occurred at the position nearest the lamp as noted above.

Constancy of Irradiance

Table 25 shows spectral irradiance data taken during the setting-up and the operation of the solar simulator. Measurements were taken at the center of the turntable, where the average irradiance prevails, at 17.8 cm from the center toward the lamp, i.e., at Position 2 (see Figure 8) and at the cage position closest to the lamp, in Position 1. For comparison, the last column gives solar irradiance data taken from Thekaekara (30). The experiment was begun with five optical filters to monitor the spectrum of the solar simulator, 340, 410, 520, 600, and 790 nm. In May 1979, shortly after the beginning of the experiment, a sixth one, at 309 nm, was added to be able to better characterize the ultraviolet spectrum. At this wavelength, the low sensitivity of the silicon photodiode and the very low irradiance level together make measurement somewhat difficult. Still, it added to confidence in the measurements.

The last row shows monitor readings. These refer to the output of the automatic exposure control detector. It is a second, initially uncalibrated silicon photodiode that is illuminated directly through a 340 nm optical bandpass filter, and is located at a point to the side and slightly above the turntables. Its output is fed into the virtual null input of an op-amp that converts the diode current into a voltage (see also paragraph 2 of this section). A voltage-to-frequency converter, an electronic divider, and a mechanical counter then give the integral of the photodiode current; i.e., a measure of accumulated exposure. This integrating system eliminates the possible effects of fluctuations or slow changes in intensity of the arc and allows substantial tolerance in the instantaneous lamp output. It automatically maintains a constant exposure by adjusting, if necessary, the exposure time to make up for instantaneous lamp intensity. The monitor readings themselves are the instantaneous output of the monitor photodiode. Initially, with a new lamp and perfectly clean filters, 5.5 kW input to the Xenon arc produced approximately 1600 mV at the output of the op-amp; i.e., as a "Monitor" reading.

Table 25 shows that the irradiance level has remained substantially constant during the life of the experiment and that the irradiance level at Position 1; i.e., under the lamp, corresponds quite accurately to the actually measured solar spectrum at air mass 1.

Exposure

The experiment was planned to produce tumor onset from UVB in mice in less than 50 weeks. Based on the experience of Bingham and Nord (18) and Burns (F. Burns, NYU Medical Center, personal communication, 1978), we concluded that the total

TABLE 25

Spectral Irradiance

Nominal Filter (nm)	At Center (Pos #3)				Toward lamp 18 cm from Center (Pos #2)				Under LAMP (Pos #1)	SOLAR (Thekaekara)
	$\mu W/cm^2$ nm									
	3/10/79	7/17/79	7/30/80	7/30/80	3/10/79	5/15/79	7/30/80	7/30/80	3/10/79	
309	---	2.73	1.98	2.02		3.19	2.12	1.99	∿3	3
340	28.6	27.9	24.8	---	37.3	40.2	34.2	---	43.8	43.1
410	72.3	69.8	75.8	69.4	95.6	110	104	95.5	110	107
520	91.8	84.8	102	90.7	122	136.7	144	128	144	139
600	79.2	74.3	84.4	75.4	109	116	121	106.5	129	132
790	29.7	31.7	35.1	32.1	52.2	54.6	59.1	55.3	76	100
Monitor (mV)	≈1600	1545	1280 lamp replaced	1435 new lamp	≈1600	1525	1280 lamp replaced	1435 new lamp	≈1600	

UVB dose (280 to 320 nm) that is required to produce the onset of tumors without chemical treatment in less than 50 weeks will be of the order of $E \sim 2 \times 10^5$ Wsec/m^2. As shown in the previous paragraph, the peak irradiance level in the apparatus was set to correspond to the actual solar spectral irradiance at the wavelengths of our filters.

The ATLAS arc, when operating at 5.5 kW input, delivers, according to our measurements (Tables 24 and 25), a spectral irradiance of 28.6 μW/cm^2 nm at 340 nm. The measurement at 309 nm is nuch less certain but indicates a spectral irradiance of 2.7 μW/cm^2 nm. From the known slope of the Xenon arc's spectrum in the range below 230 nm, and from the measured transmission curves of the Schott 114 filters, there is obtained an estimated plot of irradiance in the UVB range. Table 26 shows the procedure, and Figure 10 shows a graph of the results. The second column is the measured spectral irradiance from Tables 24 and 25 at the center of the turntable. The third column contains ATLAS catalog data for a distance of 48 cm from the burner. The fourth column is the measured spectral transmittance of the Schott 114 filters. As will be discussed below, it turned out to be lower in the short ultraviolet than was assumed from Schott's data and original inquiries. The last column, then, is the product of the ATLAS irradiance data converted by the inverse square law to the 69.7 cm distance from the lamp to the center of the turntable and then multiplied by the transmission of the Schott filter. These values are plotted in the graph of Figure 9, in addition to the measured data from the second column (at 309 and 340nm) and Thekaekara's solar irradiance data. The latter will, of course, be higher than the irradiance at the center of the turntable, because the solar simulator is adjusted so that solar irradiance corresponds to peak irradiance, not the average. Graphic integration of the curve between 280 and 320 nm yields an average irradiance at the turntable center between 280 and 320 nm of

$$H = 68\ \mu W/cm^2$$
$$= 0.68\ W/m^2$$

The goal was to accumulate a dose of 2×10^5 Wsec/m^2 in 50 weeks and thus 100 sessions each of:

$$E = 2000\ Wsec/m^2$$

The required duration of each session is then found to be:

$$T = 49\ min.$$

From the data in Table 24, it is known that this irradiance is achieved at approximately 1550 mV on the monitor. From the known electrical characteristics of the monitor voltage-to-frequency conversion and counting circuit, there can be computed the setting of the counter necessary to achieve 49 min. exposure when the monitor operates at 1550 mW. At lower monitor outputs, the counter will run accordingly slower and increase the exposure time. It will thus provide a true automatic exposure control for each session, independent of the actual lamp intensity.

The exposure setting employed for the experiment was determined from an original estimate of irradiance based on the Schott data of the UV transmission

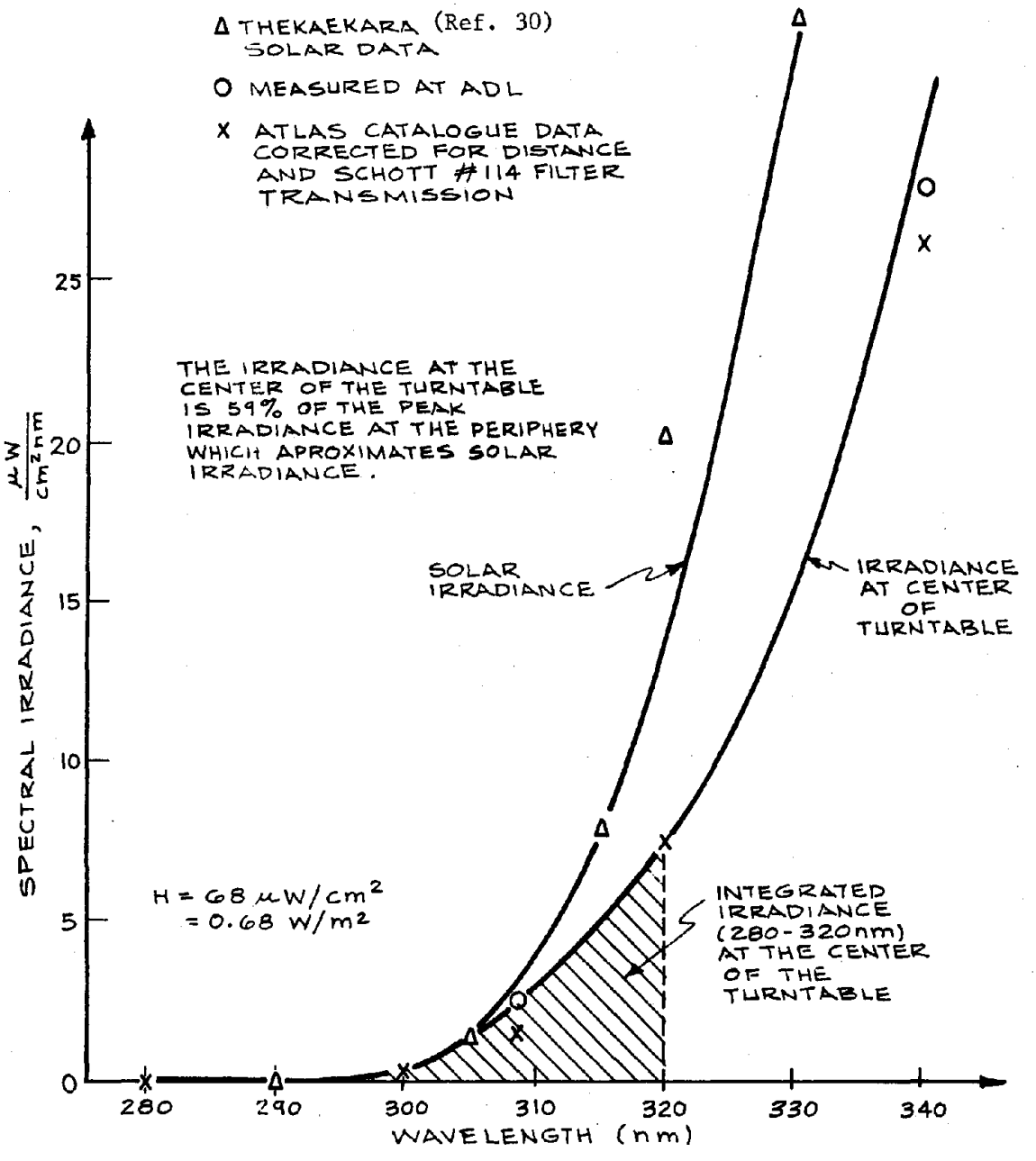


FIGURE 10

Integrated UV Irradiance

TABLE 26

Exposure Data

(nm)	Lamp Meas. (at center)*	Atlas data (at 48 cm)**	Schott 114 (meas.)	Atlas Data Converted to 69.7 cm and Schott Transmission
280		∿0	0.01	∿0
290				
300		26	0.02	0.25
309	2.73	36	0.10	1.7
320		50	0.33	7.8
330				
340	27.9	71	0.78	26

*Calibrated filters were available only at 309 and 340 nm (see text).

**Atlas data were available for two relevant cases: quartz inner and outer filters and borosilicate inner and outer filters. Their geometric mean was taken to represent the effect of the quartz inner and borosilicate outer filters, used in the experiment.

of the 114 filter. These filters were later checked to see whether their transmission had been affected by the long operation, and they were found to have remained entirely stable by comparison to an unused filter that had been kept as a reference and spare. However, their transmission was found to decrease faster towards the ultraviolet than Schott's data and original inquiries had indicated. The original estimate of integrated irradiance between 280 and 320 nm was thus too high, and actual exposure has thus been lower than intended. With our present, more accurate spectral data, we estimate the actual exposure per session to have been

$$E \sim 1480 \text{ Wsec/m}^2$$

over the range from 280 to 320 nm. The total dose desired was thus delivered in 135 sessions.

Detector and Filter Calibration

Spectral irradiance during the experiment was monitored with a set of interference filters and a calibrated silicon photodiode. Recently (31,32), some problems that originally had plagued silicon photodiodes in the UV have been solved. As a secondary standard and as a monitor diode, two EG&G UV-grade silicon photodiodes of this type were used. The diodes are operated into a virtual short-circuit; i.e., into a transimpedance amplifier. This assures perfect linearity of the diode. According to EG&G, the photodiode calibration is traceable to NBS and can be expected to hold for at least two or three years. The calibrated detector has been stored in the dark for most of the time, and can therefore with confidence be expected to have retained its calibration. The circuit diagram is shown in Figure 11.

The second element to affect spectral irradiance calibration is the interference filters. On recommendation from ATLAS, interference filters made by Ditric Optics, Inc., Hudson, MA, were chosen. According to ATLAS, these are particularly stable against environmental effects and ultraviolet irradiance. Interference filters are frequently very sensitive to moisture and other atmospheric agents creeping from the edges between the layers of the filter. Ditric mounts their filters in a well-fitted metal holder and appears to be able to seal them successfully in this fashion. No changes have been observed so far in any filters.

A transmission curve was run for each filter and compared it to Ditric's data originally supplied. These curves were then graphically integrated and an equivalent 100% transmission bandwidth was calculated for each filter. Multiplication of this number by the spectral sensitivity of the silicon detector from EG&G's spectral calibration table yielded the calibration factor for each filter-detector combination. These factors are used to convert the measured photodiode current into spectral irradiance data (see Figure 12).

The ATLAS Xenon arc is operated from 60 Hz AC. Its output is, therefore, deeply modulated at 120 Hz. It is, therefore, averaged in the monitor circuit by the time constant (τ = 10ΩM x 0.8 μF) of the transimpedance amplifier over 0.8 seconds. For irradiance calibration and checking, an entirely independent system consisting of a digital voltmeter with high 60 and 120 Hz rejection is used, preceded by a special assembly of terminating resistors and filtering

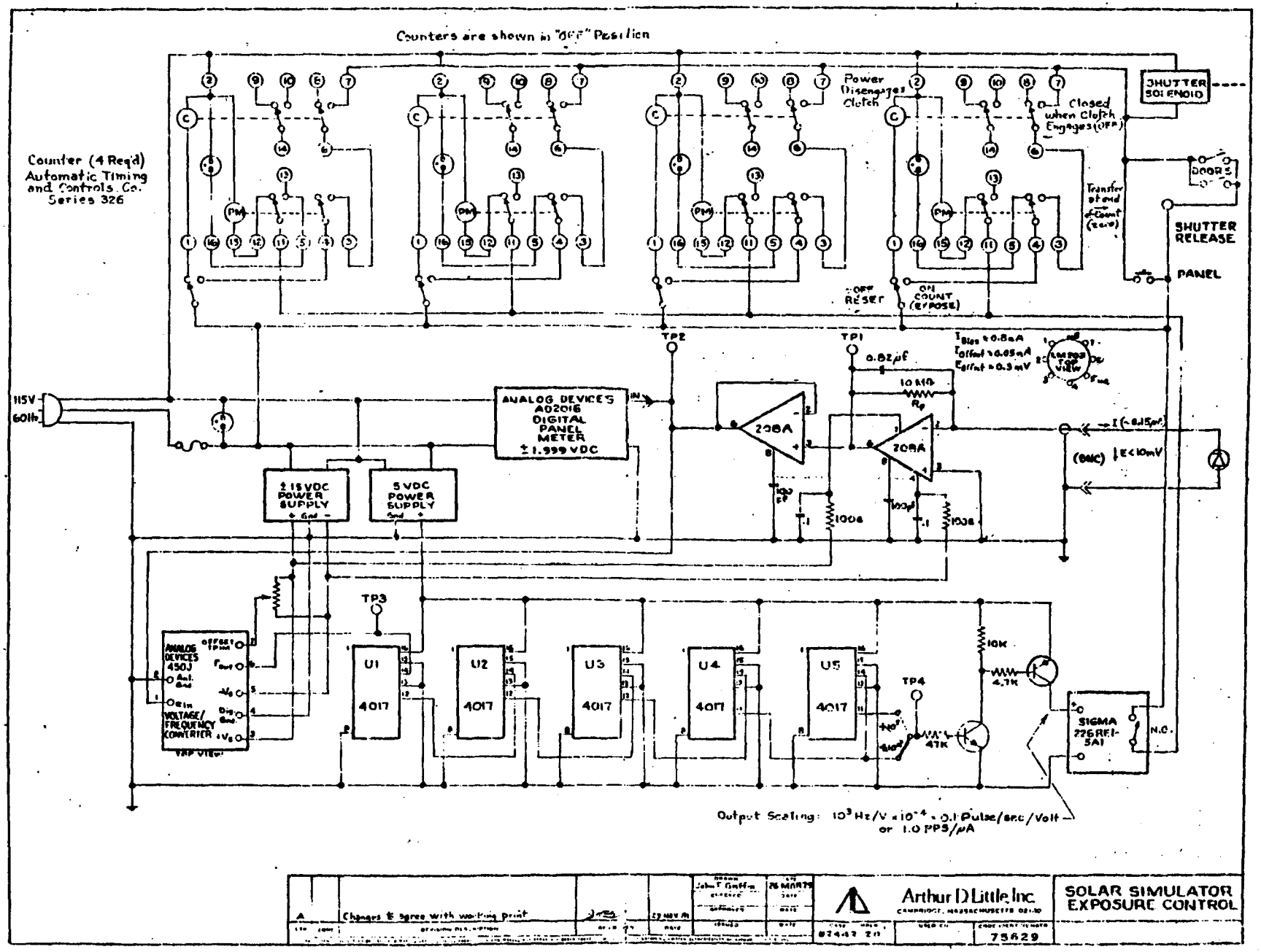
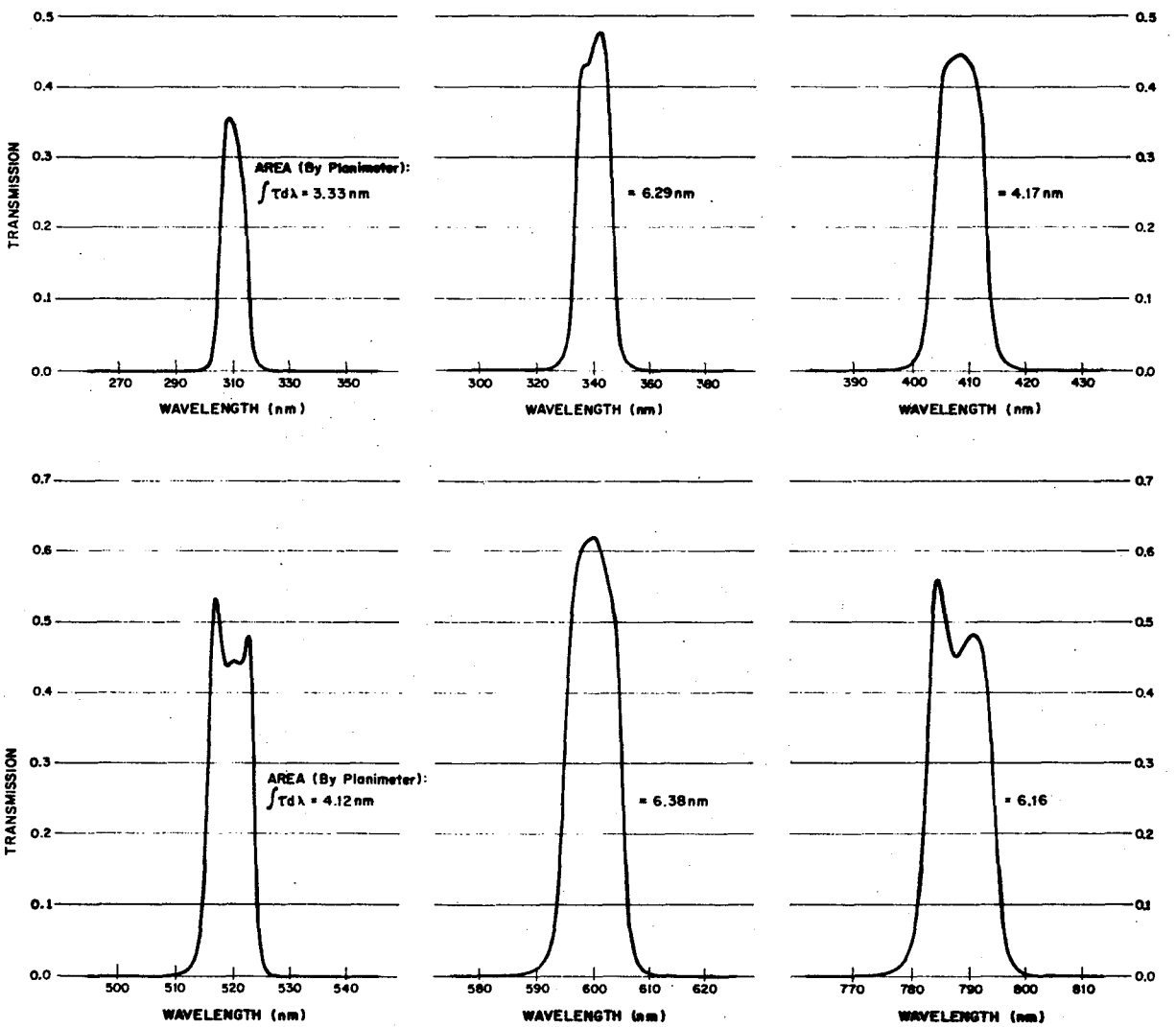


FIGURE 11. Circuit diagram

FIGURE 12

CURVES TAKEN AT ADL ON 4/17/79 AND 4/15/79



Filter Calibration (ADL Measurements)

capacitors. In this case the diode is not operating into a perfect short circuit, but rather into the 100 mV range of the DVM, and thus, for each measurement, the termination resistor keeps the diode voltage below about 50 mV. According to EG&G's data, this will maintain linearity to better than 1 percent for the small photocurrents in this application.

BIOASSAY

Test Animals

The male mice used in these bioassays were of two strains: CD-1 (albino "Swiss") and C3H/HeJ. The CD-1 mice (weight, 15-20 gms; age, 3-5 weeks) were received in two shipments of 675 each from Charles River Breeding Laboratories, Wilmington, MA. The C3H/HeJ mice (weight, 15-20 gms; age, 3-5 weeks) were received in four shipments from Jackson Laboratories, Bar Harbor, ME. The mice were quarantined and kept for 6-9 weeks before the testing period began. They were housed in stainless steel stock cages (8" x 11" x 6"), 25 to a cage, with Formulab Chow® 5008 food pellets (Ralston Purina Company, St. Louis, MO) fed throughout from suspended stainless steel containers. Both food and water were provided ad libitum.

The experiment consisted of 48 groups, test and control (Table 1), 24 of each strain. Each test group consisted of 50 formally randomized mice, housed individually in metal cages suspended over the excretory materials (Fenco Model 60 CU, single width stainless steel unit, with paper roll hardware and stainless steel feeder attachments), when not being treated by test material application and/or irradiation. Each mouse was individually identified within a group by cage number and by toe-clipping. An ear punch system was used to identify mice by experimental group. A cage tag color was designated for each test material. The C3H/HeJ mice were all in even-numbered experimental groups, the CD-1's in odd-number groups. The home cages for the two strains were kept in separate rooms in which the racks were regularly rotated. To minimize the possible effects of fluorescent room lighting and its associated UV component, room lamps were enclosed in filter tubes (FR213W, Crown Plastics Corporation, Boston, MA). The light cycle was automatically set for 12 hours ON, 12 hours OFF. Target conditions for animal rooms were 75°F and 50% relative humidity. The actual temperatures were recorded daily, and ranged from 72-78°F. Relative humidity was not regularly recorded.

Each mouse was weighed before the experiment began, prior to each first weekly application for six weeks, and bi-weekly thereafter.

At the completion of the experiment (18 months), the maximum number of treatments (light and/or test solutions) received by any group was 158 (Table 1). Some groups received fewer treatments, because of termination for reasons of carcinoma incidence or morbidity. An experimental group was terminated when survival fell below 15%, with the exception of groups 3 and 5, in which a laboratory accident had caused excess mortality from thermal stress (see Results).

Test Procedure

The general procedure involved the clipping of hair from the interscapular area of each animal prior to test painting as needed using an Oster Company (Racine, WI) Model A-2 small animal clipper with a size 40 blade. A separate clipper head was used for each test material. Special care was exercised to minimize skin abrasions and other evidences of irritation. Fifty µl of each test material was applied twice (2x) weekly to each mouse of the respective test groups. Initially, all treatments were administered using a Zinger™ Precision Micro-Dispenser Model Z-500 µl (PGC Scientifics Corporation, Rockville, MD) set to dispense 50 µl per application. Hand pipettes with disposable tips (Precision Pipetting System, Medical Laboratory Automation, Mt. Vernon NY), were adopted after 6 months, because of difficulties in keeping the Zingers operable.

Each animal in the negative [1:1 cyclohexane/acetone] control group (I) received 50 µl of the vehicle twice weekly. The positive control group animals (J) received 50 µl each of 0.01% benzo(a)pyrene (5 µg of benzo(a)pyrene) in 1:1 cyclohexane-acetone twice weekly. Benzo(a)pyrene (of high but undefined purity) was obtained from the NCI Chemical Repository, IITRI, Chicago, IL.

The combination groups (K) were treated in alternate weeks with the high temperature condensates from the Type III asphalt (D) and the Type I pitch (F).

Prior to each test application, the individual animal was weighed (when scheduled), and hair clipped as needed, then confined in a individual cell of the multi-cell exposure unit. This confinement procedure was also used for the non-irradiated animal groups. To insure the long-term uniformity of exposure to the light source, the animals were rotated weekly into different cubicle locations on the unit by a formal procedure.

Initially, the Zinger system was used to deliver 50 µl of test material to each application site by dropping through the wire mesh. After four months, it was decided necessary to apply the material to the animals before confining them, to insure proper dose control. Test material handling and administration were done in a ventilated hood. At least 30 minutes and not longer than 45 minutes following the application of the appropriate test material to the last animal in the array, the animals were exposed to the simulated sunlight. The duration of exposure was determined by the monitoring system as described above. It usually ranged from 40-60 minutes, depending on the age of the lamp in use.

Observations of mice were made recorded daily for mortality, evidence of systemic toxicity and gross appearance of tumors. The mice of a specific group were treated and observed until 85% of the group had died or until 18 months had elapsed. Mice found dead were necropsied and those that were moribund were killed and necropsied. When a group was terminated at 18 months, the remaining animals were necropsied. Necropsies were conducted in accordance with the NCI Guidelines (33). Organs and tissues examined included the NCI list (Table 27). Organs and tissues with gross lesions were fixed in formalin. All tumors and suspect masses on the skin of dead or killed mice were fixed in 10% formalin and submitted for histopathological examination.

To prepare a tumor-bearing segment for fixation, it was stretched over an appropriately sized piece of cardboard (file card weight) to which it adhered firmly because of the accompanying fluids. On the reverse side, the location of any tumors was indicated with indelible ink to aid the histologist in sectioning.

The terms used in describing outgrowths grossly are given in Table 28. It should be emphasized that gross diagnosis of carcinoma was based on a lesion that upon palpation was found to be attached to underlying tissues, which generally indicates invasion of connective tissue or muscle layers.

Certain conventions were adopted in the counting of positive tumors (papillomas). Those which regressed to a lesser outgrowth status or disappeared entirely after being reported only once, were eliminated from the tumor count, in accordance with the practice of the NCI Carcinogenesis Bioassay program. Those which regressed after being positive at two or more successful observations were counted. Papillomas diagnosed as positive only by histopathological analysis were considered to have occurred on the date of the animal's death. Tumors occurring clearly outside the area of application of the test material, of which there were very few (less than 10, in CD-1 mice only) were included in the tumor count only in solar-treated groups. This was adopted in view of the negligible incidence of tumors in the negative controls (both solar and non-solar).

TABLE 27

Tissues and Organs Examined at Gross Necropsy

Gross lesions
Tissue masses or suspect tumors and regional lymph nodes
Skin
Mandibular lymph node
Mammary gland
Salivary gland
Larynx
Trachea
Cecum
Colon
Rectum
Mesenteric lymph node
Liver
Thigh muscle
Sciatic nerve
Sternebra, vertebrae, or femur (plus marrow)
Costochondral junction, rib
Thymus
Gallbladder
Pancreas
Spinal Cord
Lungs and bronchi
Heart
Thyroids
Parathyroids
Esophagus
Stomach
Duodenum
Jejunum
Ileum
Spleen
Kidneys
Adrenals
Bladder
Seminal vesicles
Prostate
Testes
Ovaries
Uterus
Nasal cavity
Brain
Pituitary
Eyes

TABLE 28

Glossary of Terms used for the Description of Skin Appearance

Depilation	Loss of hair caused by treatment with test substances.
Pared epidermis	Adventitious removal of sections of epidermis often caused by scratching.
Lesion	Wound, injury, or pathological change.
Abraded lesion	A scraping away of a portion of the surface or of a previous lesion often caused by clipping.
Atypical healing	Pseudo-bulbous development, i.e., prominent peripheral area(s) of healing surrounding abraded site. May be representative of carcinogenic activity.
Suspicious area	Any site suspect for possible outgrowth development.
Thickened epidermis	Focal or diffuse epidermal thickening which may be suggestive of carcinogenic activity.
Spicule	Focal hyperkeratotic outgrowths.
Horny outgrowth	Similar to the wart-like outgrowth with the exception of no pinpoint hemorrhagic areas. Texture is smooth.
Suspicious Wart-like	Hard scaly outgrowth, one or more pinpoint lesions on surface, not flexible, no thickened base.
Typical Wart-like*	Hard scaly outgrowth, single or multiple lesions on surface, flexible, narrow but not thickened base.
Atypical Wart-like*	Wart-like outgrowth but lacking one or more of the characteristics for a typical wart-like outgrowth.
Suspicious bulbous*	Bulbous type development though less definitive with regression possible.
Typical bulbous*	Outgrowth with a thickened base, extending under the epidermis, and a crater-like center.
Possible carcinoma	Bulbous type growth with minimal lateral and/or ventral extension: regression possible.
Probable carcinoma	Bulbous type growth with lateral and/or ventral extensions: regressions unlikely.

*Rated as "papilloma" if larger than 2 x 2 mm.

RESULTS

SURVIVAL

Survival of treated and control groups was high and comparable through 11 months for CD-1 mice, and 10 months for C3H mice. After these periods, the experimental groups showed sharper declines because of killing of animals to ensure preservation of tumors for histopathological examination. The negative control groups of both strains showed survival after those times approximately as expected. There were excess deaths in the CD-1 controls, apparently related to an endemic incidence of urinary tract infection. Detailed life tables are presented in the Appendix (Tables A-1 and A-2).

Two groups of CD-1 mice (numbers 3 and 5) suffered major losses of animals when the ventilation system in the solar simulator failed temporarily on the 95th exposure (11 months) and the temperature within the chamber rose to over 40°C.

ANIMAL HEALTH AND GROSS PATHOLOGY

Weights

Animal weights (Appendix Tables A-3 and A-4) show very little effect of the treatments on weight gain. The major weight gains occurred in the first two months, and after that there were only minor oscillations. The effect of sacrificing moribund or cachectic animals is a factor in these oscillations. A statistical analysis of the mean weights at 12 months has been performed. Since the weights for the two strains differed considerably, they were analyzed within strain only. To prepare for the analysis, the data for the three negative control groups receiving solvents were pooled, and the data for each experimental group compared to them by Student's t-test. Two sets of data were so analyzed: the mean weights at 12 months, and the mean weight change from the time of initiation (t_0) to 12 months.

The results of these analyses are summarized below by showing the number of experimental groups differing (or not differing) from the pooled controls, with the experimental group numbers given in parentheses for the least numerous classes.

Table 29. Significant Weight Differences

Difference from Controls ($P \leq 0.05$)	12 Month Mean Weight		12 Month Weight Increment	
	CD-1	C3H	CD-1	C3H
Higher	1 (Gp. 47)	19	1 (Gp. 47)	19
No Difference	3 (Gps. 1,3,5)	1 (Gp. 30)	1 (Gp. 5)	1 (Gp. 30)
Lower	17	1 (Gp. 42)	19	1 (Gp. 42)

With the CD-1 mice, most of the 12-month weights and increments are lower than pooled controls, as expected since the solvent treatment is considered a milder one than treatment with the test materials, or benzo(a)pyrene. The one group, No. 47, which had both a higher mean weight and increment, was the solar control group, which received no solvent. The situation with the C3H/HeJ mice is quite different, in that 19 of 21 experimental groups had both higher mean weights and greater increments than the averaged controls. This must be attributed to a poorer state of health of the control groups, although no gross observations were made which otherwise support this.

Erythema and Eschar

In the early months of the experiment, a large number of the CD-1 (white) mice were noted to have a high incidence of erythema following treatment, with many of them showing progression to eschar formation. A formal confirmation of these incidental observations was conducted approximately 3 months after the testing began. The results are presented in Table 30. No systematic examination was made of the C3H mice since incidence of both abnormalities was only sporadic in both treated and control groups. The results with the CD-1 mice suggest that simulated sunlight (usually considered a cause of erythema) has an effect only in combination with the pitches, and there more on eschar formation than on erythema. The effects of the asphalts (all four of which were applied at the same concentration) appear to be the same with or without simulated sunlight.

Correlation coefficients were calculated from data for all CD-1 groups for correlation between: (1) incidence of eschar vs. final number of tumor-bearing animals (TBA) and (2) incidence of erythema vs. TBA. When only the 16 groups receiving pitch or asphalt volatiles were considered a moderate negative correlation of TBA with eschar was found ($r = -0.46$), but none with erythema ($r = 0.11$). Eschar and erythema were positively correlated ($r = 0.49$).

Gross Pathology

Gross pathological examination of necropsied animals revealed appreciable incidences of abnormal conditions in liver, spleen, and lung (Table 31, details by group in Appendix Tables A-5 and A-6). A series of χ^2 tests were used to compare test material effects to negative controls and the positive control values were compared between strains. In CD-1 mice, some appear to be associated with aging since they occurred in negative control groups as

TABLE 30

Incidence of Skin Irritation — CD-1 Mice

(3 months on test)

Test Material	Eschar		Erythema	
	Solar	Non-Solar	Solar	Non-Solar
Asphalt				
A	20/50	24/49	13/50	21/49
B	22/47	20/49	5/47	4/49
C	12/50	18/50	3/50	9/50
D	19/48	23/50	5/48	4/50
Total	73/195 (37%)	85/198 (43%)	26/195 (13%)	38/198 (19%)
Pitch				
E	23/48	7/48*	1/48	1/48
F	29/50	4/49*	12/50	0/49*
G	26/50	15/50*	33/50	21/50*
H	21/50	14/48	11/50	4/48
Total	99/198 (50%)	40/195* (21%)	57/198 (29%)	26/195* (13%)
Combination (D,F)	8/48	14/48	6/48	9/48
Benzo(a)pyrene	4/49	14/48	1/49	0/49
Solvent	4/49	2/49	1/49	1/49
Solvent (cage)	—	8/50	—	1/50
Solar only	1/49	—	0/49	—

*Solar group has significantly higher incidence than non-solar group (χ^2 test, $P < 0.05$).

TABLE 31

Summary of Gross Pathology

		Liver						Spleen				Lung	
CD-1 Mice	No. of Animals	Enlarged	Significance	Pale	Significance	O.G.*	Significance	Enlarged	Significance	Mottled	Significance	O.G.*	Significance
Asphalts	400	68	a, c, d^+	191	b	28		88	a, b	50		42	
Pitches	400	61	a	180		17		114	a, d^-	71	a, d^-	42	
Combination	100	13	a, c	40		5		27	a	16		13	
Benzo(a)-pyrene	100	15	a	36		4		35	a	20	a	12	
Controls	200	12		85	c	12		30	c	19	c	21	
C3H Mice													
Asphalts	400	44	a	187	a	122	c	201	a, c	239	a, c	102	a, c
Pitches	400	53	a, d^-	244	a, b, c	75	a, b, c	204	a, c	272	a, b, c	120	a, b, c
Combination	100	5		52	a	18	a, b, c	45	a, c	64	a, c	34	a, c
Benzo (a)-pyrene	100	7		45	a	30	c, d^+	46	a	52	a, c	17	
Controls	200	11		58	d^+	61	c	6		8		23	

*O.G. = "outgrowth", or presumptive tumor
Significance (by χ^2 test, $p \leq 0.05$):
a - Significantly different from strain controls
b - Significantly different from benzo(a)pyrene group
c - Value for strain significantly greater than corresponding value for other strain
d - Significant difference between solar and non-solar sub-groups (not shown)
d^+ = S > NS; d^- = NS > S

much as in groups treated with pitch or asphalt volatiles. These include pale livers, and apparent liver and lung outgrowths. Other pathology, although different from strain controls, was not significantly different between the benzo(a)pyrene controls and volatile-treated groups, and thus may be attributable to a generalized effect of PAH's. Varying with strain and test material (footnoted "a", but not "b"), these include: enlarged livers, and enlarged and mottled spleens. Five organ pathologies (footnoted both "a" and "b") have significantly higher incidence in asphalt (1) or pitch (4) level volatile groups compared to both benzo(a)pyrene and negative controls, but none of them are significant in both strains of mice, nor with both types of test material. Firm conclusions are difficult in the face of such varying patterns of significance.

TUMOR OBSERVATIONS

Effective Totals

The number of animals at risk for tumor, i.e., the effective total was recorded as the number of animals alive in a group (original number, 50 per group) when the first positive tumor (papilloma) arose in that group. They are closely comparable for most groups, ranging from 43 to 50 to the CD-1 mice and 42 to 49 for the C3H mice (Table 32). The exceptions are: group 48 (ET = 21), the C3H untreated control, in which a tumor appeared at the end of the experiment, and the CD-1 groups (3 and 5) which were involved in the over-heating of the light source, reducing their effective totals to 17 and 38, respectively. No effective totals are presented for groups in which no tumors occurred.

Tumor Incidence

Tumor incidence, both as observed grossly during the course of the experiment and histopathologically is presented in detail in Tables 33 (C3H) and 34 (CD-1). Certain derived summaries are presented in later tables, i.e., for tumor-bearing animals as a percent of the effective total, mean latent period, distribution of multiple tumors, direct comparison of the combination groups with those for the two components of the combination, and statistical analysis.

Although a variety of tumor types was seen, the preponderance of the benign tumors were classified as papillomas and of the malignant tumors, as squamous cell carcinomas. A much lower incidence of malignant fibrosarcomas was seen, more in C3H mice than in CD-1's. Other benign tumors included keratoacanthoma (or "keratoacanthoma-like"), fibromas and unclassifiable benign epitheliomas.

The keratoacanthoma-like has not been reported by the Contractor's pathologists in 25 years experience with skin-painting of mice with cigarette smoke condensates, fractions thereof, and individual polycyclic hydrocarbons. The strains of mice involved were of various "Swiss" albino types, including CAF_1, Millerton Swiss, and Ha/ICR. Slides for the last five years of the tobacco program were read by the same pathologist who read the slides of the presently reported study. The pathologist's description of this tumor is presented.

The keratoacanthoma (as seen in the canine) is characterized by a downward growth of epidermis forming a crypt which often becomes a multiloculated cyst crowded with keratin. Folding of this epithelium produces laminated masses

TABLE 32

Effective Total

Surviving Animals (ET) — As of First Positive Papilloma in Each Group

(Numbers in parentheses are weeks on test at first papilloma)

Test Material	CD-1						C3H/HeJ					
	Solar			Non-Solar			Solar			Non-Solar		
	Group No.	ET	(Wk)	Group No.	ET	(Wk)	Group No.	ET	(Wk)	Group No.	ET	(Wk)
A	3	17	(56)	1	45	(24)	4	48	(33)	2	49	(30)
B	5	38	(50)	7	48	(24)	6	47	(18)	8	47	(23)
C	11	50	(17)	9	48	(31)	12	42	(34)	10	47	(21)
D	13	45	(41)	15	46	(41)	14	48	(16)	16	45	(24)
E	19	44	(31)	17	47	(22)	20	45	(37)	18	46	(33)
F	21	45	(35)	23	45	(31)	22	44	(37)	24	46	(26)
G	27	43	(40)	25	50	(14)	28	47	(17)	26	42	(34)
H	29	48	(29)	31	48	(28)	30	42	(39)	32	46	(28)
Solvent	35	--	--	33	--	--	36	44	(40)	34	--	--
Solvent, cage				37	48	(41)				38	--	--
B(a)P	41	49	(28)	39	49	(28)	42	45	(40)	40	42	(38)
Combination (D, F)	45	46	(21)	43	45	(30)	46	44	(21)	44	48	(25)
None	47	--	--				48	21	(80)			

TABLE 33

Skin Tumor Incidence

C3H/HeJ Mice

Expt. No.	Test Material		Solar	Gross Observations[a] TBA	CBA	Total Tumors	ET[b]	Final Histopathology: Tumor-Bearing Animals[c] Total	Benign[d]	Malignant[e]	Tumors[f] PAP	KA	CA	FS	Other	Total
2	A - Type I Asphalt	232°	-	47	20	118	49	47	24(10)+1	22(11)	34	10	25	4	3	76
4	" "	"	+	43	17	79	48	43	14(8)+2	27(9)	22	10	25	5	0	62
8	B "	316°	-	45	27	128	47	45	13(8)+1	31(13)	27	7	31	9	4	78
6	" "	"	+	44	24	116	47	44	18(8)	26(8)	36	6	26	4	1	73
10	C - Type III Asphalt	232°	-	42	19	94	47	42	15(7)+2	25(10)	32	3	19	9	3	66
12	" "	"	+	33	18	57+1	42	34	11(7)+3	20(7)	14	8	19	2	1	54
16	D "	316°	-	40	28	126	45	40	12(8)	28(19)	24	6	36	9	7	82
14	" "	"	+	36	22	80+2	48	38	20(10)	18(10)	34	7	20	2	2	65
18	E - Type I Pitch	232°	-	42	38	122	46	42	11(6)	31(20)	34	6	38	2	3	83
20	" "	"	+	45	32	121	45	45	12(9)	33(18)	41	5	39	2	8	95
24	F "	316°	-	45	42	127	46	45	11(10)	34(21)	43	8	40	2	3	96
22	" "	"	+	42	28	94+1	44	43	13(10)	30(10)	28	7	33	1	2	71
26	G - Type III Pitch	232°	-	38	30	115+1	42	39	12(4)	27(18)	27	8	28	2	5	70
28	" "	"	+	43	28	114+1	47	44	12(9)	32(20)	39	8	39	4	5	95
32	H "	316°	-	44	37	117+1	46	45	14(6)	31(21)	28	7	39	7	10	91
30	" "	"	+	38	21	77+3	42	40	15(11)+1	24(13)	28	9	24	4	8	73
44	K - Combination (D,F)		-	42	30	171+2	48	44	14(11)	30(15)	40	7	35	1	8	91
46	" "		+	41	23	114	44	41	11(4)	30(17)	24	6	34	4	7	75
40	J - Benzo(a)pyrene		-	38	24	60	42	38	11(3)	27(13)	12	8	29	2	2	53
42	" "		+	34	21	68+1	45	35	7(3)+1	27(4)	11	5	22	5	0	43
34	I - Solvent		-	0												0
36	" "		+	1	1	2	44	1	1(1)	0	2					2
38	M - Solvent (cage)		-	0												0
48	L - Untreated		+	1	1	1	21	1	0	1	0	0	1	0	0	1

[a] TBA = Tumor-bearing animals; CBA = animals bearing one (or more) presumptive carcinomas; based on "positive" tumors observed while the mice were alive. "Total tumors" includes all seen during lifetime, with additional ones added in histopathology shown as "+..."

[b] ET = Effective total: number of mice alive in a group at the time of the first positive tumor in that group.

[c] "Total" does not necessarily equal TBA for gross observations, since some animals were positively diagnosed as tumor-bearing only in histopathology.

[d] Number in parentheses is number of animals with ≥2 benign tumors. "+" indicates regressed gross positive animals, counted only if reported for two or more consecutive observations.

[e] Number in parentheses is number of animals with at least one malignant tumor plus one or more other tumors, whether malignant or benign.

[f] Total will not necessarily equal total grossly observed, due to fusion and regression. Kerato-acanthomas often covered a large area, masking other once discrete tumors. Many papillomas regressed on animals which were still tumor-bearing. Tumor types: PAP = papilloma (benign), CA = squamous cell carcinoma (malignant), FS = fibrosarcoma (malignant), KA = kerato-acanthoma (benign), OTHER includes fibromas and unclassified benign epitheliomas.

TABLE 34

Skin Tumor Incidence

CD-1 Mice

Expt. No.	Test Material		Solar	Gross Observations[a] TBA	CBA	Total Tumors	ET[b]	Final Histopathology: Tumor-Bearing Animals[c] Total	Benign[d]	Malignant[e]	Tumors[f] PAP	KA	CA	FS	Other	Total
1	A - Type I Asphalt	232°	-	7	1	14+2	45	8	6(4)+2	0	12					12
3	" "	"	+	4	0	5	17	4	2(1)+2	0	3					3
7	B "	316°	-	21	2	37	48	21	13(4)+7	1	18	0	0	1		19
5	" "	"	+	5	0	8+2	38	7	3 +4	0	3	0	0			3
9	C - Type III Asphalt	232°	-	12	2	20+2	48	14	9(1)+4	1	11	1	1			13
11	" "	"	+	10	0	14+1	50	11	5 +4	2	5	0	1	1		7
15	D "	316°	-	17	1	27+3	46	20	13(3)+4	3(1)	17	0	1	2		20
13	" "	"	+	4	0	6+2	45	6	4(1)+1	1	5	0	1			6
17	E - Type I Pitch	232°	-	36	10	102+2	47	38	22(10)+7	9(5)	39	7	9	1	2	58
19	" "	"	+	27	1	52+2	45	29	20(11)+8	1(1)	31	5	1			37
23	F "	316°	-	36	11	100+2	45	38	27(13)+8	3(2)	49	3	2	1		55
21	" "	"	+	19	2	34+4	45	23	17(7)+4	2(2)	24	0	1	1	2	28
25	G - Type III Pitch	232°	-	33	9	83+4	50	37	26(10)+7	4(2)	42	1	4			47
27	" "	"	+	20	2	45+3	43	23	20(8)+2	1	29	1	1	0	3	34
31	H "	316°	-	38	7	99+3	48	41	26(11)+10	5(2)	40	3	5	0	3	51
29	" "	"	+	27	2	43+1	48	28	19(3)+9	0	21	1	0	0	1	23
43	K - Combination (D,F)		-	30	7	62+3	45	33	22(9)+8	3(1)	36	3	3			42
45	" "		+	11	2	11+4	46	15	9(1)+5	1	10	0	0	1		11
39	J - Benzo(a)pyrene		-	38	17	101+2	49	40	24(8)+5	11(6)	43	3	10	3		59
41	" "		+	12	3	24+1	49	13	9(5)+1	3	11	4	1	2		18
33	I - Solvent		-	0												0
35	" "		+	0												0
37	M - Solvent (cage)		-	0		0+1	48	1			1					1
47	L - Untreated		+	0												

For footnotes, see Table 33.

of keratin surrounded by a squamous epithelium whose cells maintain their usual polarity and orderly arrangement. Many of the mouse skins in this study have structures with this general morphologic appearance. To separate these tumors from typical papillomas they are referred to as keratoacanthoma-like. In many, the epithelium lacks normal polarity and is composed of anaplastic pleomorphic cells with numerous mitotic figures. Although many of these tumors have many features of malignancy, they are not classified as such unless they show evidence of invasion of underlying tissues. The majority of these keratoacanthoma-like tumors may be the precursors of squamous cell carcinomas since many of them have some of the same general features.

Tumor incidence with time is presented in the "Removal of Animals" tables in the Appendix (Tables A-1 and A-2) which indicate when animals became tumor-bearing, or died tumor-free. Cumulative incidences with time, i.e., number of tumor-bearing animals expressed as percentage of effective total, are presented graphically in Appendix Figures A-1 to A-10.

Several clear-cut features of the results can be stated even before dealing with statistical considerations:

1. The male C3H mouse was much more sensitive than the male CD-1 to the tumorigenic activity (all tumors) of the asphalts in particular, but also of the pitches, in that the rate of appearance of tumors was lower in the CD-1 groups. The final incidences were nearly comparable for the two strains, in the absence of simulated sunlight, for the pitches.

2. The incidence of malignant tumors was much lower in the CD-1 mice, e.g., in all 16 groups of each strain with the test materials (pitches and asphalts) there were only 34 malignant tumors on 33 animals, compared to 549 such tumors on 439 animals in the corresponding C3H groups.

3. Simulated sunlight by itself as used in this experiment, did not produce the expected modest tumor incidence. In the CD-1 mice, there were no tumors observed in control groups 35 and 47. With the C3H mice, there were 2 benign tumors on one mouse in group 36 (solvent plus light) and one malignant tumor on a mouse in group 48 (light only).

4. Simulated sunlight had an inhibitory effect on both rate of appearance of tumors (Appendix Figures) and on final tumor incidence, more notably in the CD-1 groups than the C3H groups. The final incidence data are dealt with in detail in the statistical analysis, but a simple overall summary based on the 32 tar and asphalt groups shows the inhibitory effect (without taking into account the starting material or temperature of preparation):

TABLE 35. Pooled Tumor Incidences — Light

	Tumor-bearing Animals (Final)		Malignant Tumors (Histopath.)		Total Tumors (Histopath.)	
	CD-1	C3H	CD-1	C3H	CD-1	C3H
4 Asphalts - non-solar	63	174	5	142	64	302
4 Asphalts - solar	28	159	3	103	19	254
4 Pitches - non-solar	154	171	22	158	211	340
4 Pitches - solar	103	172	4	146	122	334

Similar patterns of inhibition are seen with the combination groups and, notably, the benzo(a)pyrene controls. It is suggested that the slower rate of tumor development, i.e., lower sensitivity to the tumorigenic materials, in the CD-1 mice, allows this apparent inhibition to become more evident whatever its mechanism.

5. With the asphalt volatiles, all of which were applied at the 50% (w/v) concentration, there are differences in tumor incidence related to temperature of preparation, the 316° preparation being the more tumorigenic according to 5 of the 6 comparisons. As for the 6th, tumor-bearing animals in C3H groups are greater than 90% of the effective totals. The pitches, which were applied at constant benzo(a)pyrene concentration, but varying concentrations of the total solids (Table 1) also show a degree of temperature dependence for all three criteria with the C3H mice, but not with the CD-1 mice. These observations are demonstrated by pooled figures for each strain, omitting the solar-treated groups, and combining results for both asphalts and both pitches:

TABLE 36. Pooled Tumor Incidences — Temperature

	Tumor-bearing Animals (Final)		Malignant Tumors (Histopath.)		Total Tumors (Histopath.)	
	CD-1	C3H	CD-1	C3H	CD-1	C3H
Asphalts - 232°	22	89	1	57	25	142
Asphalts - 316°	41	85	4	75	39	160
Pitches - 232°	75	81	14	70	105	153
Pitches - 316°	79	90	8	88	106	187

The higher or equivalent activity of the 316° pitch preparations compared to the 232° preparations for all measures of tumorigenicity (except CD-1 malignant tumors), indicates a higher specific activity of the 316° preparations. That is, with each pitch the 316° preparations were applied at lower amounts per application (Type I, 2.75 mg vs. 3.9 mg; Type III, 1.5 mg vs. 4.2 mg), producing a higher tumor yield per unit weight applied.

6. The combination groups, receiving different treatments in alternate weeks, show incidences of tumor-bearing animals approximating the averaged activity of the 316° asphalt and pitch preparations used, i.e., D and F. Statistical analysis of many of the conclusions stated is presented below.

Latent Period

The time incidence data have been utilized for calculation of latent period for each group, by averaging the times of appearance of the first positive tumor in each tumor-bearing animal of the group (Table 37). Latent periods for all groups of CD-1 mice are longer than for the corresponding C3H groups, except for the benzo(a)pyrene controls. The mean increment (CD-1 minus C3H), excluding benzo(a)pyrene groups, is 2.2 ± 1.1 months. The increment is less than a month for only two groups (test materials A and E, non-solar). The inhibitory effect of the simulated sunlight is manifested in these data, since in every pair but one (test material D on CD-1 mice) the latent period for the solar group is longer than for the corresponding non-solar group. This is pronounced with the CD-1 mice for which the mean increase (excluding D) with solar treatment is 2.1 ± 0.7 months, compared to 1.0 ± 0.6 months for the C3H's.

The incidences of tumor-bearing animals expressed as percentage of effective total, both for all tumors and malignant tumors (carcinomas or fibrosarcomas) are given in Tables 38 and 39. Also included are the "conversion ratios", or percentage of tumor-bearing animals which developed a malignant tumor. The CD-1 mice (Table 38) showed a moderately high total tumor incidence, but a low incidence of malignant tumors, and hence low conversion ratios. Although the asphalts produced a much lower tumor incidence, the conversion ratios were comparable for these and the pitches. With the C3H mice (Table 39), values were quite high for all three measures of effect. No pronounced effects of temperature of preparation are evident in these data. These data show, again, the high activity of pitches on both strains of mice and of asphalts on the C3H mice only.

Statistical Analysis

Two major analyses of variance have been performed, the first on the "balanced subset," i.e., the 32 experimental groups involving only the separate pitch and asphalt preparations. The second involved the whole experiment, i.e., including the positive control and the combination groups, excluding only the negative controls which because of their negligible (or zero) tumor incidence would tend to distort the analysis.

To provide for an estimate of random error, each experiment was divided into two parts (odd-numbered vs. even-numbered animals) and all independent variables measured separately for each. Although not in the original design, this was considered to be acceptable since the animals were originally assigned numbers at random.

Balanced Subset--
In this analysis of variance, there were four independent variables (starting material, temperature of generation, solar treatment, and strain of mouse), analyzed as a 4×2^3 full factorial experimental design with two replications

TABLE 37

Mean Time to Tumor

(months ± SD)

Test Material	Solar	CD-1 Group No.	CD-1 $\bar{X}$	CD-1 SD	CD-1 Δ_s*	C3H/HeJ Group No.	C3H/HeJ $\bar{X}$	C3H/HeJ SD	C3H/HeJ Δ_s*
Asphalts									
A	-	1	13.8	2.9		2	11.7	2.6	
	+	3	15.0	2.4	1.2	4	12.2	2.4	0.5
B	-	7	12.2	3.5		8	9.2	2.1	
	+	5	14.1	2.8	1.9	6	10.0	2.1	0.8
C	-	9	12.0	3.0		10	10.7	2.9	
	+	11	13.4	4.3	1.4	12	11.7	1.8	1.0
D	-	15	12.8	2.4		16	9.4	1.9	
	+	13	12.2	3.2	-0.6	14	10.2	2.7	0.8
Pitches									
E	-	17	9.7	3.7		18	9.5	1.2	
	+	19	12.1	3.0	2.4	20	10.0	1.1	0.5
F	-	23	10.2	2.1		24	9.0	1.0	
	+	21	13.7	2.8	3.5	22	10.1	1.6	1.1
G	-	25	10.7	2.3		26	8.9	0.9	
	+	27	12.2	1.5	1.5	28	9.3	1.5	0.4
H	-	31	10.8	2.8		32	9.0	1.3	
	+	29	12.4	2.2	1.6	30	11.2	1.7	2.2
Combination	-	43	11.4	2.6		44	8.9	1.5	
	+	45	13.0	3.6	1.6	46	10.0	2.1	1.1
Benzo(a)pyrene	-	39	11.4	2.9		40	13.0	2.4	
	+	41	13.2	3.2	1.8	42	14.7	2.4	1.7

*Δ_s= Increment due to effect of simulated sunlight.

Controls not included; only one tumor-bearing animal in CD-1 strain, and two in C3H/HeJ.

TABLE 38

Total and Malignant Tumor-Bearing Animals as Percent of Effective Total — CD-1

Material	Solar	ET	Total Tumor-Bearing[a]		Malignant Tumor-Bearing[b]		Conversion (%)[c]
			No.	%	No.	%	
A	-	45	8	17.8	0	0	0
	+	17	4	23.5	0	0	0
B	-	48	21	43.8	1	2.1	4.8
	+	38	7	18.4	0	0	0
C	-	48	14	29.2	1	2.1	7.1
	+	50	11	22.0	2	4.0	18.2
D	-	46	20	43.5	3	6.5	15.0
	+	45	6	13.3	1	2.2	16.7
E	-	47	38	80.9	9	19.1	23.7
	+	45	29	64.4	1	2.3	3.4
F	-	45	38	84.4	3	6.7	7.9
	+	45	23	51.1	2	4.4	8.7
G	-	50	37	74.0	4	8.0	10.8
	+	43	23	53.5	1	2.3	4.3
H	-	48	41	85.4	5	10.4	12.2
	+	48	28	58.3	0	0	0
Combination	-	45	33	73.3	3	6.7	9.1
	+	46	15	32.6	1	2.2	6.7
B(a)P	-	49	40	81.6	11	22.4	27.5
	+	49	13	26.5	3	6.1	23.1
Controls	-	--	0	0	0	0	--
Solvent	+	--	0	0	0	0	--
Solvent (Cage)	-	48	1	2.1	0	0	--
Untreated	+	--	0	0	0	0	--

ET = Effective Total; see Table 32.

[a]Total tumor-bearing: all animals which had a positive tumor whether observed histopathologically or not.

[b]Malignant tumor-bearing: all animals which had a histopathologically confirmed malignant tumor.

[c]Number of malignant tumor-bearing animals as percentage of total bearing tumors.

TABLE 39

Total and Malignant Tumor-Bearing Animals as Percent of Effective Total — C3H/HeJ

Material	Solar	ET	Total Tumor-Bearing[a]		Malignant Tumor-Bearing[b]		Conversion (%)[c]
			No.	%	No.	%	
A	-	49	47	95.9	22	44.9	46.8
	+	48	43	89.6	27	56.2	62.8
B	-	47	45	95.7	31	66.0	68.9
	+	47	44	93.6	26	55.3	59.1
C	-	47	42	89.4	25	53.2	59.5
	+	42	34	81.0	20	47.6	58.8
D	-	45	40	88.9	28	62.2	70.0
	+	48	38	79.2	18	37.5	47.4
E	-	46	42	91.3	31	67.4	73.8
	+	45	45	100.0	33	73.3	73.3
F	-	46	45	97.8	34	73.9	75.6
	+	44	43	97.7	30	68.2	69.8
G	-	42	39	92.9	27	64.3	69.2
	+	47	44	93.6	32	68.1	72.7
H	-	46	45	97.8	31	67.4	68.9
	+	42	40	95.2	24	57.1	60.0
Combination	-	48	44	91.7	30	62.5	68.2
	+	44	41	93.2	30	68.2	73.2
B(a)P	-	42	38	90.5	27	64.3	71.1
	+	45	35	77.8	27	60.0	77.1
Controls	-	--	0	0	0	0	--
Solvent	+	44	1	2.3	0	0	--
Solvent (cage)	-	--	0	0	0	0	--
Untreated	+	21	1	4.8	1	4.8	100.0

ET = Effective Total: see Table 32.

[a]Total tumor-bearing: all animals which had a positive tumor whether observed histopathologically or not.

[b]Malignant tumor-bearing: all animals which had a histopathologically confirmed malignant tumor.

[c]Number of malignant tumor-bearing animals as percentage of total bearing tumors.

per cell (the odd- and even-numbered animals). The five dependent variables analyzed were as presented in Table 40.

The actual calculations were carried out at the Arthur D. Little, Inc., Computation Center using the GLM program from the S.A.S. statistical software package.

The results show that the single factors of starting material, light and mouse strain each contribute significantly to overall variance for all dependent variables. Temperature of volatiles generation shows significance only for latent period. The data of Table 37 show that latent periods for the asphalts are, in general, shorter for the high temperature asphalts compared to the corresponding low temperature preparations, but that the converse is true for the pitches. The significance of the material-temperature interaction for latent period is consistent with this. None of the two-factor interactions is significant for any of the dependent variables. The malignant tumor variables (2 and 4) show no two-factor interactions at all. Latent period is related to all single factors, and all two-factor interactions except one (temperature-light). The higher order interactions appear not to have any biological significance, nor do they occur in any consistent manner. They add nothing to the interpretation of the data.

A Duncan's multiple range test (calculated by part of the same computer program) ranks each of the independent variables for each dependent variable, and calculates if each group is significantly different from the others (Table 41). By this procedure, the pitches do not differ from each other by any measure of effect. Nor do the asphalts, except for variable 1 (total tumors per mouse at risk). The pitches together are different (more active) from the asphalts by all measures, except variable 2 (malignant tumors), in which some overlap occurs. The results for temperature, light, and strain are as expected from the analysis of variance. For all three of them, the relative position of the two groups are in reverse order for latent period.

Full Data Set--
In this analysis of variance (and Duncan's multiple range test) the data were grouped differently to provide for inclusion of the combination and benzo(a)-pyrene groups, which could not be included in an analysis in which temperature of preparation was an independent variable. The appropriate set of independent variables is shown in Table 42. The dependent variables remained the same as in the previous analysis.

As would be expected from the previous analysis, all three independent variables (treatment, light and mouse strain) show significant differences among the test groups for all 5 dependent variables (Table 42). Again, no interactions were significant for the two malignant tumor measures. The other three measures show all two-factor interactions as significant (except for latent period vs. treatment-light). The results of the Duncan's multiple range test (Table 43) are quite similar to the previous ones as regards the simple pitch and asphalt preparations, but allow an assessment of their individual rankings, and a comparison with benzo(a)pyrene and the combination treatment. (The latter comparison is made more specifically in a separate table (Table 46) derived from Table 43.)

TABLE 40

Analysis of Variance
Balanced Subset — All Asphalts and Pitches

Independent Variables

	Levels	Variables
M	4	Asphalts I and III, Pitches I and III
T	2	232°; 316°
L	2	Light (-, +)
S	2	C3H/HeJ, CD-1
Rep	2	

Dependent Variables

1. Number of total tumors (gross observations) per mouse at risk.
2. Number of malignant tumors (histopathologically confirmed) per mouse at risk.
3. Percent of mice at risk bearing one or more tumors.
4. Percent of mice at risk bearing one or more malignant tumors.
5. Average latent period for first tumor.

Significant Differences Found
Independent Variables and Interactions

Dependent Variable	M	T	L	S	MT	ML	MS	TL	TS	LS	MTL	MTS	MLS	TLS
1	*		*	*	*		*	*				*	*	
2	*		*	*										*
3	*		*	*			*	*		*				*
4	*		*	*										
5	*	*	*	*	*	*	*		*	*	*	*	*	

*F value significant at $P \leq 0.05$

TABLE 41

Duncan's Multiple Range Tests

Balanced Subset — All Asphalts and Pitches

Independent Variables	Dependent Variable 1	2	3	4	5
Materials (in descending order)	P-I P-III A-I A-III	P-I P-III A-III A-I	P-I P-III A-I A-III	P-I P-III A-I A-III	A-I A-III P-I P-III
Temperature	316 232	316 232	316 232	316 232	232 316
Light	- +	- +	- +	- +	+ -
Mouse Strain	C3H CD-1	C3H CD-1	C3H CD-1	C3H CD-1	CD-1 C3H
Replicates	NS	NS	NS	NS	NS

TABLE 42

Analysis of Variance
Full Data Set (Excluding Negative Controls)

Independent Variables

	Levels	Variables
T	10	Each asphalt and pitch at each temperature (8); positive control [B(a)P]; combination (D,F)
L	2	Light (-, +)
S	2	C3H/HeJ; CD-1
Rep	2	Odd-, even-numbered animals

Dependent Variables

1	Number of total tumors (gross observations) per mouse at risk.
2	Number of malignant tumors (histopathologically confirmed) per mouse at risk.
3	Percent of mice at risk bearing one or more tumors.
4	Percent of mice at risk bearing one or more malignant tumors.
5	Average latent period for first tumor.

Significant Differences Found
Independent Variables and Interactions

Dependent Variable	T	L	S	TL	TS	LS	TLS
1	*	*	*	*	*	*	*
2	*	*	*				
3	*	*	*	*	*	*	*
4	*	*	*				
5	*	*	*		*	*	

* = F value significant at $P \leq 0.05$

TABLE 43

Duncan's Multiple Range Test
Full Data Set (Excluding Negative Controls)

Arranged in descending order of numerical value of mean. Brackets enclose groups in which the values do not differ significantly one from the other at P ≤0.05.

A. Independent Variable: Treatment*

Dependent Variable	Treatment	Mean
1. Number of tumors per animal at risk	E	2.211
	G	2.055
	F	2.009
	Combination	2.006
	H	1.885
	B	1.571
	B(a)P	1.410
	D	1.390
	A	1.221
	C	1.038
2. Number of malignant tumors per animal at risk	E	0.512
	F	0.451
	G	0.441
	Combination	0.433
	B(a)P	0.419
	H	0.416
	D	0.402
	B	0.379
	A	0.308
	C	0.290

TABLE 43 (continued)

A. Independent Variable: Treatment* (continued)

Dependent Variable	Treatment	Mean
3. Percent of mice at risk bearing one or more tumors	E	85.1
	H	84.8
	F	82.7
	G	79.9
	Combination	73.4
	B(a)P	69.9
	B	63.0
	A	60.7
	D	59.1
	C	55.6
4. Percent of mice at risk bearing one or more malignant tumors	E	41.2
	B(a)P	38.4
	F	38.3
	G	36.3
	Combination	35.3
	H	33.8
	B	30.9
	D	28.4
	C	26.8
	A	25.5
5. Latent period	A	13.28
	B(a)P	13.26
	C	11.91
	B	11.38
	D	11.11
	Combination	10.95
	H	10.85
	F	10.71
	G	10.30
	E	10.29

TABLE 43 (continued)

B. Independent Variable: light (- = non solar, + = solar)

Dependent Variable	Light	Mean
1.	-	2.012
	+	1.347
2.	-	0.445
	+	0.364
3.	-	78.1
	+	64.8
4.	-	35.9
	+	31.1
5.	+	12.10
	-	10.70

C. Independent Variable: strain of mice

Dependent Variable	Strain	Mean
1.	C3H	2.337
	CD-1	1.022
2.	C3H	0.752
	CD-1	0.058
3.	C3H	92.6
	CD-1	50.2
4.	C3H	61.5
	CD-1	5.5
5.	CD-1	12.37
	C3H	10.44

The treatment section of Table 43 (A) is partucilarly useful, since the sections for light (B) and strain (C) merely show the relation of the two groups. For each dependent variable, the asphalts (E, F, G, and H) are not different from each other, except for total tumors per animal in which H falls slightly below the other three. Furthermore, the asphalts as a group are significantly more active than the pitches (A, B, C, and D) for both total tumors per animal at risk and percent of mice at risk bearing one or more tumors. Varying degrees of overlap are shown for the other measures.

Tumor Multiplicity

Data on tumor multiplicity (of grossly observed tumors) another measure of activity, are presented in summary form in Tables 44 and 45. In the C3H mice, all treatments, except B(a)P, produced an average of more than 2 tumors per tumor-bearing animal, and the overall average was 2.4 (1976/749). The CD-1's averaged 2.1 (850/404), with only the non-solar pitches and B(a)P groups being at or above 2.0. Treatment with simulated solar light decreased the degree of multiplicity, as might be inferred from the decreased total incidence, and more so in CD-1 mice.

Combination Treatment

The combination treatment was designed to see if there was any interaction in tumorigenicity between a high temperature asphalt preparation (D, Type III, 316°) and a high temperature pitch preparation (F, Type I, 316°) when they were applied in alternate weeks. The interaction could be an enhancement or inhibition of activity of one by the other, or simple additivity.

The pertinent data from the larger Duncan's multiple range test (Table 43) have been summarized more simply for D, F and the combination (Table 46). These results, of course, represent the pooled data for both strains and both solar conditions. The three test materials do not differ from each other for three measures: 2, malignant tumors per mouse at risk; 4, percent of animals at risk with malignant tumor(s); and 5, latent period. The total tumor criteria, 1 and 3, show patterns of significant differences. All placements, however, suggest that the tumorigenic effects of the combination are most like an averaging of the effects of the two components.

Another comparison was made using the multiplicity data of Tables 44 and 45, testing (by χ^2 test) whether the distribution pattern of tumors for the combination groups in each strain, separately analyzed for solar and non-solar treatments, was significantly different from the patterns for either of the two components and from the average distribution of the two (Table 47).

With C3H mice, the distribution of multiple tumors is significantly different from all three distributions used for comparison, with a higher multiplicity. This is true for both non-solar and solar groups. On the other hand, analysis of the CD-1 results shows mixed relationships. With the non-solar groups, the distribution for the combination is different from (and higher than) that for D, different from (and lower than) that for F, and not different from the averaged results. With the CD-1 solar groups, the distribution for the combination is different from (and lower than) all three reference distributions. No clear-cut pattern of interaction is present, but the C3H results suggest a slight enhancement of activity.

TABLE 44

Distribution of Multiple Tumors — CD-1 Mice

(Entries are number of animals with given number of grossly diagnosed tumors)

Test Material	Solar	Effective Total	No. of Tumor-Bearing Animals	Number of Tumors in Animals 0	1	2	3	4	5	>5	Total Tumors	Mean Tumors per Tumor-Bearing Animals
Asphalts												
A	-	45	8	39	3	3	1	1			16	2.0
	+	17	4	13	3	1					5	1.2
B	-	48	21	27	13	4	2		2		37	1.8
	+	38	7	31	5	1	1				10	1.4
C	-	48	14	34	8	3	1	1			21	1.5
	+	50	11	39	8	2	1				15	1.4
D	-	46	20	26	12	6	2				30	1.5
	+	45	6	39	4	2					8	1.3
Pitches												
E	-	47	38	9	8	13	7	3	5	2	104	2.7
	+	45	29	15	12	12	3	1	1		54	1.9
F	-	45	38	7	10	9	11	5	1	2	102	2.7
	+	45	23	22	13	7	1	2			38	1.7
G	-	50	37	13	12	9	10	4	1	1	87	2.4
	+	43	23	20	10	6	4	2		1	48	2.1
H	-	48	41	7	13	11	9	5		3	102	2.5
	+	48	28	20	17	8	2		1		44	1.6
Combination	-	45	33	12	13	11	6	3			65	2.0
	+	46	15	29	15						15	1.0
Benzo(a)-pyrene	-	49	40	8	16	6	7	5	3	3	103	2.6
	+	49	13	36	4	5	4				26	2.0
Controls Solvent	-	--	0								0	-
	+	--	0								0	-
Solvent (cage)	-	48	1	47	1						1	1.0
Untreated	+	--	0								0	-

TABLE 45

Distribution of Multiple Tumors — C3H/HeJ Mice

(Entries are number of animals with given number of grossly diagnosed tumors)

Test Material	Solar	Effective Total	No. of Tumor-Bearing Animals	Number of Tumors in Animals							Total Tumors	Mean Tumors per Tumor-Bearing Animals
				0	1	2	3	4	5	>5		
Asphalts												
A	-	49	47	2	10	18	11	3	3	2	118	2.5
	+	48	43	5	22	10	7	4			79	1.8
B	-	47	45	2	9	7	18	6	3	2	129	2.9
	+	47	44	3	13	7	10	10	3	1	118	2.7
C	-	47	42	5	14	17	4	5		2	94	2.2
	+	42	34	8	17	10	7				58	1.7
D	-	45	40	5	5	9	13	7	2	4	126	3.2
	+	48	38	10	12	14	8	2	2		82	2.2
Pitches												
E	-	46	42	4	5	14	15	2	3	3	122	2.9
	+	45	45	0	7	16	9	10	3		121	2.7
F	-	46	45	1	4	13	20	5	1	2	127	2.8
	+	44	43	1	11	16	13	2	1		95	2.2
G	-	42	39	3	5	11	10	8	4	1	116	3.0
	+	47	44	3	9	12	14	5	4		115	2.6
H	-	46	45	1	12	6	17	7	3		118	2.6
	+	42	40	2	18	9	11	1		1	79	2.0
Combination	-	48	44	4	5	6	5	8	13	7	173	3.9
	+	44	41	3	6	17	9	1	5	3	114	2.8
Benzo(a)-pyrene	-	42	38	4	21	13	3	1			60	1.6
	+	45	35	10	15	10	7	2	1		69	2.0
Controls	-	--	0								0	-
Solvent	+	44	1	42	0	1					2	2.0
Solvent (cage) - Untreated	+	21	1	20	1						1	1.0

TABLE 46

Duncan's Multiple Range Test

Combination relative to its components (D and F)

= Not significantly different from
> Significantly higher than

Dependent Variable	
1	Tumors per animal at risk (F = combination) > D
2	Malignant tumors per animal at risk F = combination = D
3	Percent of mice at risk bearing one or more tumors F > combination > D
4	Percent of mice at risk bearing one or more malignant tumors F = combination = D
5	Latent period D = combination = F

See Table 43 for details and other treatments.

TABLE 47

Comparison of the Frequency Distribution of Tumors in D, F and the Combination*

	Solar	Effective Total	Tumor Bearing-Animals	Number of Tumors 0	1	2	3	4	5	5	ΣT	Tumors per Tumor-Bearing Animal	Significance**
C3H/HeJ													
D	-	45	40	5	5	9	13	7	2	4	126	3.2	*
F	-	46	45	1	4	13	20	5	1	2	127	2.8	*
1/2 (D + F)	-		42.5	3	4.5	11	16.5	6	1.5	3	126.5	3.0	*
Comb.	-	48	44	4	5	6	5	8	13	7	173	3.9	
D	+	48	38	10	12	14	8	2	2		82	2.2	*
F	+	44	43	1	11	16	13	2	1		95	2.2	*
1/2 (D + F)	+		40.5	5.5	11.5	15	10.5	2	1.5		88.5	2.2	*
Comb.	+	44	41	3	6	17	9	1	5	3	114	2.8	
CD-1													
D	-	46	20	26	12	6	2				30	1.5	*
F	-	45	38	7	10	9	11	5	1	2	102	2.7	*
1/2 (D + F)	-		29	16.5	11	7.5	6.5	2.5	0.5	1	66	2.3	
Comb.	-	45	33	12	13	11	6	3			65	2.0	
D	+	45	6	39	4	2					8	1.3	*
F	+	45	23	22	13	7	1	2			38	1.7	*
1/2 (D + F)	+		14.5	30.5	8.5	4.5	0.5	1			23	1.6	*
Comb.	+	46	15	29	15						15	1.0	

*D = Type III Asphalt - 316°

F = Type I Pitch - 316°

Combination = Alternate weekly treatments with D and F.

**Frequency distribution of combination group different from that for given distribution (same strain, same solar condition) by χ^2 test at $p \leq 0.05$.

DISCUSSION

The asphalt volatiles were strikingly more tumorigenic and carcinogenic to the C3H mice than was expected from previous studies, their activity being nearly equivalent to that of the pitch volatiles. The CD-1 mice gave results more in line with expectation, showing quite high activity of the pitches, and low activity of the asphalts. These differences raise several related questions:

- Is there something unique about the C3H mouse? It is known to have higher levels of inducible arylhydrocarbon hydroxylase (AHH) than several other inbred (and hybrid) strains (34) but has apparently never been compared to random-breds deriving from ICR or Swiss strains, like the CD-1. ICR mice are moderately inducible (35).

- 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 have which contribute to the observed carcinogenicity, as promoters, cocarcinogens, or even other carcinogens not of the PAH chemical class?

- Linking these two, is there some way in which the C3H mouse responds differently to some unique components of the asphalt materials, either in some cocarcinogenic or promoting sensitivity and/or sensitivity to the carcinogenic activity of non-PAH compounds?

- Why did the simulated sunlight inhibit the rate of appearance of tumors? This may be related to the lack of tumor induction to any significant extent by the artificial sunlight alone. Although the total dose delivered reached the desired level, the time required to reach it was somewhat longer than originally planned for. Enhancement was expected because of the known carcinogenicity of UV light to mouse skin, although a few scattered reports in the literature indicate that inhibition has been observed under certain schedules of illumination and application of a PAH carcinogen (22). Is the inhibition due to photo-destruction of carcinogens or to modification of the skin, somehow lessening its responsiveness? Stenbäck and Shubik discuss both of these alternative explanations, without strongly favoring either (22).

- Why did the CD-1 mice respond more rapidly than the C3H mice, i.e., with shorter latent periods, to the positive control carcinogen, benzo(a)-pyrene, when their response to the complex mixtures was slower? (See Table 38 and Appendix Figure A-10.)

The second point, regarding the differences in composition between pitch and asphalt volatiles, is further emphasized in the analysis presented in Table 48. The amount of total test material (solids) applied by the time of 50%

tumor incidence (C3H mice) is very much larger for the asphalts, by factors of 6-22 over the pitches yet the amounts of B(a)P and total PAH applied are much smaller, by factors of 0.01 - 0.11 (PAH) and 0.005 to 0.04 (B(a)P.) This points up the suggestion that the asphalt preparations contain other materials, which are unidentified carcinogens or which augment the activity of the PAH. In short, with the use of mixtures as complex as the test materials of this study, tumorigenic activity as manifested on mouse skin is an integrated response to a number of factors, which may include inhibitory factors, as well as enhancing ones.

To what extent the carcinogenic activities observed in the present study are derived from pyrolysis products vs. pre-existing components of the starting materials is not known since the latter were not analyzed. Asphalts in general are expected to have much lower levels of B(a)P (and PAH) than coal-tar pitches (23).

TABLE 48

Total Dose of Test Material Applied up to Time of 50% Tumor Incidence — C3H Mice
(non-solar groups)

Preparation	Time to 50% (mo.)	No. of Applications*	Amount of Material per Application			Total Dose Applied**		
			Total Solids mg	PAH µg	B(a)P µg	Total Solids mg	PAH mg	B(a)P µg
Asphalts								
A Type I - 232°	12	104	25	23.2	0.11	2600	2.4	11.4
B Type I - 316°	9.3	81	25	7.4	0.095	2025	0.60	7.7
C Type III - 232°	11	96	25	28.0	0.145	2400	2.7	13.9
D Type III - 316°	9.5	83	25	9.1	<0.025	2075	0.76	<2.1
Pitches								
E Type I - 232°	9.7	84	3.9	>560	4.8	328	>47.0	403
F Type I - 316°	9	78	2.75	603	4.25	214	47.0	332
G Type III - 232°	9	78	4.2	>306	5.1	328	>23.9	398
H Type III - 316°	8.8	77	1.5	>711	4.5	116	54.7	346
B(a)P	13.7	119	-	-	5.0	-	-	595
Combination (D, F)	8.9	D 39				975	0.35	<0.98
		F 38				104	22.9	161.5
					Total D, F	1079	23.25	161.5+

*Months x 8.7 (average no. of applications per month at 104/year)

**Number of applications x amount per application

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APPENDIX

This reproduces the relevant pages from Reference 29 which show a circular line source located above the edge of a round table at a height comparable to the table's radius. This arrangement, when correctly dimensioned, as shown in Table IV of Reference 29, will produce an approximately uniform irradiance over the entire surface of the round table.

order to obtain maximum tenacity between the aluminum film and the glass. Also, this procedure yields harder films.

Uniform films. In order to obtain a uniform coat on large mirrors, aluminum is evaporated from several tungsten sources suitably arranged, rather than from one movable source.

The evaporation of polonium in a high vacuum from a point source has been investigated by Bonét-Maury.[27] This metal was chosen on account of its radioactivity. He found that the condensation on a plane surface is proportional to the inverse square of the distance from the source, and to the cosine of the angle between the normal to the surface and the line connecting the surface with the source. We may assume that the same is true of other metals which have a low vapor pressure at room temperature.

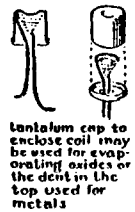

Fig. 20.

Starting with this assumption, we may consider the distribution of the film thickness τ produced by various experimental arrangements. In the case of evaporation to the inside surface of a sphere of radius ρ from a point source of vapor at its center, the situation is very simple. We get a uniform film of which the thickness τ_0 is

$$\tau_0 = \frac{m}{4\pi\partial\rho^2}. \tag{1}$$

Here m is the mass of metal evaporated and ∂ is its density.

The film thickness at P on a plane surface at the normal distance ρ from a point source of evaporation is

$$\tau_P = \frac{m}{4\pi\partial r^2}\cos\theta = \tau_0\left(\frac{\rho}{r}\right)^3. \tag{2}$$

Here τ_0 is the thickness at P, r is the distance from the source to P, and θ is the inclination of the surface P to the molecular rays emitted by the source which impinge on it there.

[27] Bonét-Maury, P., *Ann. de Physique*, *11*, 253 (1929).

The film thickness produced on a plane surface by a circular array of vapor sources can be determined by applying the above formula to each of the sources. (See Fig. 21.) If there are N coils spaced uniformly around a circle at a distance ρ from the surface to be coated, the film thickness on the surface at P, which is at a distance a from the intersection of the axis of the circle with the face of the mirror, is given by the expression

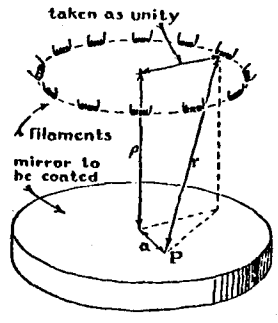

Fig. 21.

$$\tau_P = \frac{M\rho}{4\pi\partial N}\sum_i^N \frac{1}{r_i^3}. \tag{3}$$

Here M is the total mass of metal evaporated, and r is the distance from P to the coil represented by the summation index i.

Dr. Edward M. Thorndike made the same calculation, assuming a continuous circular source. The thickness is given in this case by

$$\tau_P = \frac{M\rho}{8\pi^2\partial}\int_0^{2\pi}\frac{d\theta}{r^3}. \tag{4}$$

Here the point source at distance r from the point P is replaced by a line source represented by the angle element $d\theta$ at distance r, as before. This calculation involves the integration

$$\int_0^{2\pi}\frac{d\theta}{r^3} = \int_0^{2\pi}\frac{d\theta}{(1+a^2+\rho^2-2a\cos\theta)^{3/2}} = \frac{4}{[(a-1)^2+\rho^2]\sqrt{(a+1)^2+\rho^2}}\,E\left(\frac{2\sqrt{a}}{\sqrt{(a+1)^2+\rho^2}}\right), \tag{5}$$

in which E represents the elliptic function.[28] Values of this integral calculated by Thorndike are given in Table IV.

[28] Bierens de Haan, David, *Nouvelles tables d'integrales definies*, Table 67, Eq. 3, page 102. Leyden: P. Engels, 1867.

TABLE IV

VALUES OF $\int_0^{2\pi}\frac{d\theta}{r^3}$ FOR VARIOUS PARAMETERS

a	$\rho = \frac{1}{2}$	$\rho = 1$	$\rho = 1.1$	$\rho = 1.2$	$\rho = 2$	$\rho = 4$
0.00	4.50	2.22	1.91	1.65	.500	.090
0.25	4.82	2.24	1.93	1.65	.555	.090
0.50	3.06	2.20	1.93	1.63	.540	.088
0.75	7.74	2.28	1.89	1.57	.515	.085
0.80	....	2.27	....	....	....	....
0.90	....	2.22	....	....	....	....
1.00	8.28	2.11	1.74	1.45	.480	.082
1.50	3.40	1.38	1.09	1.02	.385	.072
2.00	1.20	0.74	0.67	0.61	.285	.063
3.00	0.28	0.24	0.23	0.22	.145	.050

For convenience, the radius of the circular source is here taken as unity. We see from this table that for $\rho = 1$ the film is quite uniform as far out from the center as $a = 1$. This case was realized in the 40-inch aluminizing tank by a circular array of twelve of the standard coils (see Fig. 12) spaced around a circle 36 inches in diameter, 18 inches above the face of the astronomical reflector to be coated (Fig. 22). Tests of transmission of a film produced with partially loaded coils confirmed the calculation, since the coat exhibited the expected uniformity.

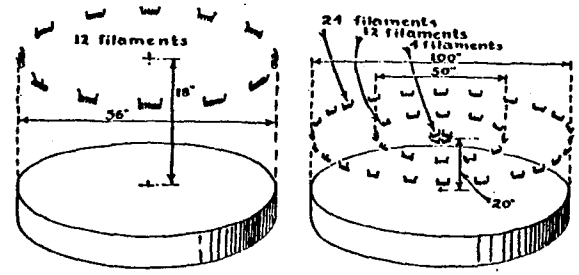

Fig. 22. Arrangements of evaporation coils for large mirrors.

TABLE A-1

REMOVAL OF ANIMALS — CD-1

(d_x = developed a tumor, w_x = died tumor-free; n_x = alive, tumor-free at start of period, ns = non-solar, s = solar)

	1-A-ns			3-A-s			5-B-s			7-B-ns			9-C-ns			11-C-s			13-D-s			15-D-ns		
Weeks	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x
0-4																								
5-8																				1	50			
9-13		1	50		1	50					1	50								1	49			
14-17			49		1	49		2	50			49				1		50			48			
18-21			49			48		3	48			49		1	50		1	49		1	48		1	50
22-26		1	49			48			45	1	1	49			49			48			47		1	49
27-30		2	48			48		1	45		2	47	1	1	49			48			47		1	48
31-34			46			48			44	3	1	45	1	1	47			48		2	47			47
35-39		1	46		2	48		2	44	1	3	41	1	2	45	1	2	48			45		1	47
40-43	1	2	45		1	46		2	42	2	2	37	1	1	42			45	3	2	45	3	1	46
44-47	1	1	42			45		2	40	1		33		2	40		3	45		1	40	3	1	42
48-52			40		24	45	3	17	38		1	32	2	3	38		1	42		4	39	2		38
53-56		4	40	1	3	21		1	18	1	1	31	3	4	33	2	5	41	1	4	35	1		36
57-60	3	8	36	1	5	17	1		17	6	4	29		1	26	1	7	34		4	30	7	3	35
61-65	1	3	25		5	11		5	16		4	19	2	5	25	2	4	26		8	26		2	25
66-69		8	21		2	6	1	3	11	3	4	15	3	5	18	1	5	20	1	6	18	2	5	23
70-73		4	13	1	1	4		2	7	2	2	8		4	10		7	14	1	1	11	1	5	16
74-78	2	7	9	1		2	2		5	1	2	4		2	6	3	4	7		3	9	1	3	10
79-82			0		1	1		3	3		1	1		4	4			0		6	6		6	6
Totals	8	42		4	46		7	43		21	29		14	36		11	39		6	44		20	30	

TABLE A-1 (continued)

REMOVAL OF ANIMALS — CD-1

(d_x = developed a tumor, w_x = died tumor-free; n_x = alive, tumor-free at start of period, ns = non-solar, s = solar)

	17-E-ns			19-E-s			21-F-s			23-F-ns			25-G-ns			27-G-s			29-H-s			31-H-ns		
Weeks	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x
0-4																								
5-8																								
9-13		1	50		2	50					1	50	1		50		1	50					1	50
14-17		2	49			48					2	49			49			49		1	50			49
18-21	1		47		2	48						47			49		1	49			49		1	49
22-26			46			46		1	50		1	47			49		1	48		1	49			48
27-30	1	1	46	1	1	46		1	49	2	1	46		1	49		1	47	1		48	5		48
31-34	2		44	1		44	1	4	48	4	1	43	1	1	48		1	46		1	47	1		43
35-39	10	1	42	4		43	1		43	2	1	38	5	3	46	2	2	45		2	46	8		42
40-43	11	2	31	1	3	39	2	1	42	11	1	35	8	1	38		2	41			44	2		34
44-47	6		18	4		35	1	3	39	9		23	4		29		2	39	4	1	44	3	1	32
48-52	2	1	12	2	2	31		4	35	4	2	14	5	2	25	8	1	37	10	4	39	11		28
53-56	1	1	9	4	3	27	1	4	31	3	1	8	7	1	18	5	6	28	5	7	25	1	1	17
57-60	3	1	7	6	3	20	3	2	26			4	5	2	10	6	4	17	3	1	13	3	2	15
61-65	1		3	2	2	11	6	1	21	2		4	1		3	2	2	7	1		9	4		10
66-69		1	2	1		7	5	3	14	1		2		1	2		1	3	2	1	8	2	2	6
70-73		1	1		1	6	1		6		1	1		1	1		1	2			5	1	1	2
74-78			0	3		5	2	1	5			0			0		1	1	2	2	5			0
79-82					2	2		2	2									0		1	1			
Totals	38	12		29	21		23	27		38	12		37	13		23	27		28	22		41	9	

TABLE A-1 (continued)

REMOVAL OF ANIMALS — CD-1

(d_x = developed a tumor, w_x = died tumor-free; n_x = alive, tumor-free at start of period, ns = non-solar, s = solar)

	33 solvent-ns			35 solvent-s			37 Cage			39 B(a)P-ns			41 B(a)P-s			43 Comb.-ns			45 Comb.-s			47 s-only		
Weeks	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x
0-4														1	50						50			
5-8		1	50		1	50					1	50			49		2	50		1	50		2	50
9-13			49			49						49			49			48			49			48
14-17			49		1	49						49			49		3	48		3	49			48
18-21		2	49			48						49			49			45	1	1	46			48
22-26			47		1	48				2		49	1		49	1		45		2	44			
27-30		1	47			47		1	50	3		47		1	48		1	43			42		1	48
31-34			46		2	47			49	6		44	1		47	4	2	42	1	1	42		1	47
35-39			46		1	45	1	1	49			38			46	6		36			40		1	46
40-43		1	46			44		5	47	7		38		2	46	4	1	30	2	1	40		1	45
44-47			45			44		2	42	1	1	31	2	4	44	2	1	25	1		37			44
48-52		5	45		1	44		2	40	12		29	3	2	38	6	1	22	2	2	36			44
53-56		4	40		3	43		7	38	5	4	17		2	33	3	1	15	2	2	32		3	44
57-60		6	36		9	40		1	31			8	1	3	31	2	2	11	1	5	28		6	41
61-65		6	30		6	31		3	30		1	8	2	3	27	3	3	7	1	1	22		1	35
66-69		5	24		5	25		6	27	1		7	2	6	22	1		1	2	6	20		8	34
70-73		9	19		10	20		5	21	3		6	1	5	14			0	2	4	12		12	26
74-78		10	10		10	10		16	16		3	3		8	8					4	6		14	14
79-82			0			0			0			0			0						2			0
Totals	0	50		0	50		1	49		40	10		13	37		33	17		15	35		0	50	

TABLE A-2

REMOVAL OF ANIMALS — C3H

(d_x = developed a tumor, w_x = died tumor-free; n_x = alive, tumor-free at start of period, ns = non-solar, s = solar)

	2-A-ns			4-A-s			6-B-s			8-B-ns			10-C-ns			12-C-2			14-D-s			16-D-ns		
Weeks	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x
0-4																								
5-8											1	50												
9-13					1	50		1	50		1	49					2	50		1	50			
14-17						49	1	2	49		1	48		2	50			48		1	49		3	50
18-21		1	50			49			46			47	2	1	48		2	48	1	2	48		1	47
22-26			49			49		2	46	2	1	47		2	45			46		2	45			46
27-30	1	1	49		1	49	3		44	3	1	44	1		43		3	46		3	43	1	1	46
31-34	3		47	2		48	3		41	10		40	2		42	1	1	43	1		40	2	2	44
35-39	5		44	4		46	5		38	4		30	8	1	40	1		41	4	1	39	7	3	40
40-43	4		39	3		42	6		33	12		26	2		31	3		40	8		34	11		30
44-47	3	1	35	6		39	12		27	9		14	6		29	5		37	4		26	3		19
48-52	10		31	9		33	8		15	2		5	11	1	23	11	1	32	8		22	6		16
53-56	5		21	5		24	3		7	1		3	5		11	8	1	20	5		14	8		10
57-60	8		16	2	1	19	2		4	1		2	1		6	1	1	11	4		9	1		2
61-65	4		8	5		16	1		2			1			5	2	2	9			5			1
66-69	2		4	1		11			1			1	1		5	2		5			5	1		1
70-73	1		2	1	1	10		1	1	1		1	1		4			3			5			0
74-78	1		1	3		8			0			0	2		3		3	3	3	2	5			
79-82			0	2	3	5								1	1			0			0			
Totals	47	3		43	7		44	6		45	5		42	8		34	16		38	12		40	10	

TABLE A-2 (continued)

REMOVAL OF ANIMALS — C3H

(d_x = developed a tumor, w_x = died tumor-free; n_x = alive, tumor-free at start of period, ns = non-solar, s = solar)

	18-E-ns			20-E-s			22-F-s			24-F-ns			26-G-ns			28-G-s			30-H-s			32-H-ns		
Weeks	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x
0-4																								
5-8		1	50		1	50		2	50											2	50			
9-13		2	49		1	49			48		1	50		1	50		2	50		1	48			
14-17			47			48			48		2	49		3	49	1	1	48			47		3	50
18-21			47		1	48		2	48			47		3	46	0	1	46			47			47
22-26		1	47		1	47		1	46	1	1	47	1	1	43	0		45		1	47			47
27-30			46			46		1	45			45		1	41	0	2	45		2	46	3	1	47
31-34	5	1	46			46			44	2		45	2	1	40	2		43		2	44		1	43
35-39	11	3	40	8	1	46	11		44	21		43	17	1	37	19		41	3		42	23		42
40-43	10		26	16		37	15		33	18	1	22	17		19	10		22	9		39	13		19
44-47	12		16	13		21	8		18	1		3	2		2	7		12	9		30	2		6
48-52	4		4	5		8	4	1	10	2		2			0	3		5	4	1	21	2		4
53-56			0	3		3	2		5			0				2		2	11		16	2		2
57-60						0	1		3									0	1	1	5			0
61-65							2		2										3		3			
66-69									0												0			
70-73																								
74-78																								
79-82																								
Totals	42	8		45	5		43	7		45	5		39	11		44	6		40	10		45	5	

TABLE A-2 (continued)

REMOVAL OF ANIMALS — C3H

(d_x = developed a tumor, w_x = died tumor-free; n_x = alive, tumor-free at start of period, ns = non-solar, s = solar)

Weeks	34 solvent-s			36 solvent-s			38 cage			40-B(a)P-ns			42-B(a)P-s			44 Comb.-ns			46 Comb.-s			48 s-only		
	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x	d_x	w_x	n_x
0-4																								
5-8											2	50		1	50		1	50		1	50		2	50
9-13		2	50		2	50			50		4	48		1	49		1	49		3	49			48
14-17			48		1	48			50			44			48			48			46		1	48
18-21		1	48		1	47		2	50			44			48			48	1	2	46		3	47
22-26		2	47			46			48		1	44		3	48	1		48		1	43			44
27-30			45			46			48			43			45	1	1	47	2	1	42		1	44
31-34		2	45		1	46		1	48		1	43			45	9	2	45	2		39			43
35-39		2	43		1	45			47	4	1	42	2		45	13	1	34	7	1	37			43
40-43			41	1		44		1	47	3	1	37	1	1	43	15		20	10		29		2	43
44-47		2	41		1	43			46		1	33	1	1	41	2		5	5		19		1	41
48-52		1	39			42			46	3		32		1	39	1		3	8		14			40
53-56		1	38		1	42		1	46	7		29	2	1	38	1		2	3		6			40
57-60			37		1	41		4	45	6	1	22	3		35			1	2		3		3	40
61-65		3	37			40		1	41	8		15	6	1	32	1		1	1		1			37
66-69		6	34			40		1	40	2		7	7	1	25			0			0		2	37
70-73		3	28		6	40		2	39	5		5	11	3	17								2	35
74-78		2	25	1	6	34			37			0	2	1	3								1	33
79-82		23	23		28	28		37	37						0							1	31	32
Totals	0	50		1	49		0	50		38	12		35	15		44	6		41	9		1	49	

TABLE A-3

Animal Weights — CD-1
(grams)

Even Weeks (E)	Odd Weeks (O)	GROUP* 1 (E) X̄	1 (E) SD	3 (E) X̄	3 (E) SD	5 (E) X̄	5 (E) SD	7 (E) X̄	7 (E) SD	9 (O) X̄	9 (O) SD	11 (O) X̄	11 (O) SD	13 (O) X̄	13 (O) SD	15 (O) X̄	15 (O) SD	17 (O) X̄	17 (O) SD	19 (O) X̄	19 (O) SD	21 (O) X̄	21 (O) SD	23 (O) X̄	23 (O) SD
t o		35.4	2.8	35.5	2.5	34.1	3.0	35.2	2.7	34.0	3.1	34.8	3.2	35.9	2.7	34.4	2.7	34.1	2.6	34.1	2.6	34.6	2.8	35.5	2.5
2	1	35.7	2.8	36.4	2.6	35.6	2.7	36.4	3.0	34.9	2.8	35.8	2.8	36.2	3.2	36.0	3.1	**		**		**		**	
4	3	36.0	3.1	36.2	3.2	37.5	3.1	37.5	2.9	36.4	2.8	35.8	2.8	36.5	3.0	33.6	3.3	36.6	2.6	37.3	3.0	37.4	2.9	38.1	2.8
6	5	37.9	2.6	38.4	2.8	38.0	3.2	38.6	3.4	38.0	2.9	37.6	3.0	37.6	3.1	37.3	3.3	36.7	2.8	37.2	2.8	37.0	3.0	38.1	3.3
10	7	40.6	3.2	40.9	3.2	39.8	3.4	40.3	3.4	37.9	3.0	37.5	2.9	38.0	3.3	37.5	3.6	37.3	3.0	36.8	2.6	37.3	2.8	37.8	3.5
14	11	39.6	3.6	39.8	3.5	39.0	3.6	38.6	3.7	39.7	3.4	38.8	3.6	39.6	4.1	39.5	4.5	40.5	3.4	38.7	3.1	40.0	3.5	40.8	3.9
18	15	40.3	5.0	39.3	5.0	38.4	4.3	39.4	4.4	38.9	4.0	38.1	4.0	39.8	4.2	39.0	4.6	39.4	4.1	37.4	4.5	39.6	3.9	41.0	4.4
22	19	41.1	5.4	41.1	4.7	39.7	4.2	38.4	5.2	39.5	4.5	39.3	4.7	39.8	4.9	39.5	5.2	40.2	4.4	38.4	4.1	39.6	4.3	41.7	5.6
26	23	38.4	5.3	40.7	4.9	37.7	4.4	37.4	4.0	39.6	4.0	38.6	4.5	37.2	4.3	39.0	5.5	39.0	4.4	36.3	3.7	37.8	4.0	39.7	5.7
30	27	39.8	5.4	40.0	4.8	38.1	4.5	37.9	4.1	39.8	4.6	38.4	4.0	38.3	4.6	39.1	6.1	39.2	4.4	36.9	4.2	37.3	4.4	39.2	6.2
34	31	40.2	5.2	40.0	3.6	37.4	4.4	38.3	3.7	38.3	4.3	37.6	5.1	37.0	4.5	38.3	6.1	38.2	4.5	35.8	2.9	36.7	4.1	39.2	5.0
38	35	38.7	5.6	38.5	3.6	37.5	4.4	37.3	3.8	37.2	4.6	37.3	4.6	36.0	3.3	37.8	6.1	37.3	3.9	35.6	2.9	37.5	3.6	37.5	5.1
42	39	37.9	5.8	37.0	3.5	35.7	4.2	36.5	3.4	37.1	4.3	36.8	4.6	36.3	3.7	37.6	5.7	36.6	4.0	34.7	2.7	36.1	3.6	36.9	4.9
46	43	38.2	5.4	38.1	3.4	37.0	4.0	37.3	3.6	37.6	4.5	36.6	3.7	35.5	3.1	36.8	5.2	35.5	3.8	34.0	2.7	36.7	3.2	37.4	4.7
50	47	39.3	5.3	37.4	3.8	38.1	5.0	38.1	3.8	36.3	4.4	36.5	4.5	35.7	2.6	37.2	5.2	36.1	3.1	35.5	2.8	36.6	3.0	37.1	3.7
54	51	39.4	5.0	39.4	3.1	38.6	5.2	37.6	3.4	37.1	3.5	36.7	4.2	35.1	3.4	36.1	4.9	36.1	3.1	34.1	3.4	35.2	3.7	36.5	3.8
58	55	38.0	4.5	36.9	2.2	38.3	4.2	37.4	3.2	37.6	4.1	36.8	4.2	35.1	3.4	36.3	4.6	35.7	2.9	35.9	3.0	35.9	3.5	37.3	3.2
62	59	39.1	5.2	34.6	2.5	37.1	4.8	37.3	4.0	36.1	4.5	36.8	4.4	34.4	3.3	37.9	3.9	36.2	4.1	35.6	4.2	35.8	3.6	37.3	2.6
66	63	37.9	5.3	36.7	3.0	36.9	6.0	38.6	3.9	39.6	4.0	36.6	4.3	34.2	3.6	37.3	4.4	36.2	5.0	35.2	3.7	35.4	4.2	37.0	3.3
70	67	38.2	6.7	35.5	2.9	37.7	4.2	36.1	4.7	37.2	5.0	35.4	4.2	35.3	2.4	37.6	4.8	35.8	2.4	35.9	3.6	36.3	3.5	36.5	2.3
74	71	39.8	7.4	37.0	2.6	38.6	3.2	36.4	4.4	37.9	4.4	35.3	4.8	34.8	2.3	37.1	4.5	34.0	1.7	35.7	3.8	35.9	2.9	34.4	2.6
78	75	37.9	5.7	36.7	1.5	37.6	2.2	36.5	3.3	37.7	4.4	36.9	5.0	34.3	3.1	33.5	3.1	Term.		36.0	3.0	35.8	2.9	Term.	

*(E) and (O) indicate, for each experimental group, the time-table on which its weights were taken.

**Data not available.

"Term." = experimental group terminated.

TABLE A-3 (continued)

Even Weeks (E)	Odd Weeks (O)	GROUP* 25 (O) X̄	SD	27 (O) X̄	SD	29 (O) X̄	SD	31 (O) X̄	SD	33 (O) X̄	SD	(O) 35 X̄	SD	37 (E) X̄	SD	39 (E) X̄	SD	41 (E) X̄	SD	43 (O) X̄	SD	45 (O) X̄	SD	47 (O) X̄	SD
t_0		35.7	2.6	36.2	2.8	36.3	3.1	36.0	3.6	35.8	2.7	35.4	2.9	34.2	2.4	35.2	2.6	35.6	3.2	33.2	3.6	35.1	3.2	35.6	2.8
2	1	**		**		**		**		35.7	2.9	35.2	3.6	36.1	2.7	37.0	2.6	37.6	3.4	**		**		37.7	3.3
4	3	36.9	5.7	36.0	2.8	36.2	3.8	36.2	3.5	36.8	3.3	35.8	3.6	37.4	3.3	37.9	2.4	38.4	3.5	37.0	2.9	36.5	3.3	38.1	3.6
6	5	37.4	2.9	36.7	2.6	36.4	2.8	37.3	3.4	38.3	3.8	37.6	3.6	38.3	4.4	38.3	2.6	38.9	3.4	37.6	2.7	37.1	3.4	39.3	3.7
10	7	38.2	2.8	37.3	2.4	38.1	2.8	39.1	2.7	39.4	4.2	38.2	3.8	39.2	5.5	38.8	2.8	39.2	4.0	37.5	2.7	37.4	3.5	40.2	5.0
14	11	39.1	3.3	37.3	3.2	39.4	2.9	39.7	3.5	40.5	5.5	38.1	4.2	40.7	6.4	39.7	3.5	40.1	5.3	37.8	2.8	38.8	4.0	42.5	6.4
18	15	39.7	3.8	38.3	2.8	39.0	2.5	40.6	4.4	42.1	5.9	39.7	4.4	41.6	6.5	41.0	4.1	42.3	6.5	39.8	3.4	39.4	4.3	44.5	7.4
22	19	39.6	4.2	37.9	4.2	39.4	3.4	41.3	3.8	42.7	6.5	40.6	5.5	42.4	6.7	40.7	4.1	41.8	5.9	39.4	4.1	38.6	4.6	45.6	8.1
26	23	38.3	4.6	36.2	3.0	37.2	3.1	39.7	3.9	41.5	6.4	40.1	6.0	43.3	7.1	41.0	4.6	42.4	6.2	39.5	4.2	40.1	5.0	46.9	8.8
30	27	38.3	5.2	36.6	3.4	38.2	2.9	39.6	4.0	43.4	7.4	41.4	6.9	42.0	7.3	41.2	5.0	43.1	7.0	38.3	4.7	38.8	5.6	46.3	9.0
34	31	38.0	5.0	35.6	3.3	37.7	2.9	39.4	4.5	42.5	6.6	41.6	7.1	41.0	7.2	40.8	5.0	41.6	5.7	38.8	4.5	38.6	5.2	45.6	8.9
38	35	35.6	3.7	34.2	3.1	36.0	2.9	38.3	4.0	41.1	5.5	41.0	7.7	41.5	7.4	40.4	5.3	41.7	6.6	38.2	4.0	38.4	5.2	47.0	10.0
42	39	36.2	3.4	35.0	3.0	36.6	3.2	39.1	4.0	41.6	5.9	42.5	7.8	40.9	6.7	39.2	5.2	39.9	6.1	37.1	4.2	37.7	5.7	46.9	9.9
46	43	33.4	2.2	33.8	3.1	35.6	2.4	36.4	3.9	41.3	6.4	41.8	8.2	41.2	8.0	39.6	6.0	38.8	6.0	36.4	4.0	37.3	4.6	47.8	11.3
50	47	36.6	2.3	35.3	3.1	36.2	2.8	38.0	3.4	41.0	6.4	43.1	8.0	40.4	7.9	39.4	5.7	38.2	4.9	35.7	3.9	37.0	4.4	47.0	10.1
54	51	33.4	2.5	33.1	2.9	31.7	2.6	34.0	3.9	39.8	6.2	41.5	6.8	39.2	6.3	37.2	5.1	37.0	4.7	36.2	3.8	36.6	3.8	49.3	9.8
58	55	36.1	3.1	35.1	3.3	35.2	3.5	37.1	3.2	40.6	5.9	41.8	6.6	40.1	6.6	38.4	4.2	38.8	3.7	37.8	3.4	34.8	3.7	45.0	8.8
62	59	37.6	3.6	33.4	3.4	35.8	3.9	37.5	3.3	38.6	5.3	41.0	6.9	40.4	6.2	38.4	5.0	38.2	4.5	38.2	5.3	36.0	3.9	44.8	9.5
66	63	35.6	2.4	34.8	3.5	36.5	3.0	36.8	4.3	38.0	4.9	37.6	5.6	40.8	6.1	37.0	4.6	38.0	4.7	36.9	4.2	37.1	4.5	45.4	8.7
70	67	35.2	2.3	34.3	3.4	35.6	3.1	37.3	4.3	39.3	5.6	39.0	3.8	38.6	6.1	37.1	4.3	37.1	5.0	37.4	4.0	37.5	4.5	43.6	9.8
74	71	34.8	3.1	34.1	4.1	34.4	3.4	36.4	3.4	37.5	4.8	37.8	4.2	38.5	5.7	37.4	3.9	38.5	4.5	37.5	4.9	37.4	4.5	42.5	8.6
78	75	36.6	1.6	Term.		34.7	2.3	Term.		38.4	5.3	36.9	4.2	36.3	5.6	34.5	3.8	34.7	5.1	Term.		38.2	3.6	40.4	7.7

*(E) and (O) indicate, for each experimental group, the time-table on which its weights were taken.

**Data not available.

"Term." = experimental group terminated.

TABLE A-4

Animal Weights — C3H/HeJ
(grams)

Even Weeks (E)	Odd Weeks (O)	GROUP* 2 (E) X	2 (E) SD	4 (E) X	4 (E) SD	6 (E) X	6 (E) SD	8 (E) X	8 (E) SD	10 (O) X	10 (O) SD	12 (O) X	12 (O) SD	14 (O) X	14 (O) SD	16 (O) X	16 (O) SD	18 (E) X	18 (E) SD	20 (E) X	20 (E) SD	22 (E) X	22 (E) SD	24 (E) X	24 (E) SD
	t 0	23.2	1.8	24.7	2.0	24.5	1.9	23.9	1.7	26.2	1.6	24.3	2.3	25.9	1.6	25.5	2.2	25.4	1.8	25.2	1.8	24.9	1.5	25.4	1.9
2	1	26.1	1.8	26.4	2.3	26.7	1.7	26.5	1.7	26.3	1.7	25.8	1.9	27.2	1.9	25.7	2.4	26.8	2.0	26.8	1.9	26.7	1.4	26.9	1.8
4	3	26.3	1.9	26.4	2.0	27.8	1.6	26.4	1.8	27.3	1.6	26.8	1.9	27.9	1.9	27.0	2.1	27.4	1.9	27.8	2.1	27.5	1.3	27.8	1.8
6	5	27.7	1.6	27.8	1.9	28.3	1.9	28.1	1.5	27.8	1.5	27.6	1.9	27.3	1.9	28.1	2.2	28.4	1.8	28.2	1.9	28.4	1.3	28.8	1.6
10	7	28.3	1.7	28.2	2.0	28.6	1.9	27.8	1.7	28.9	1.5	28.5	1.8	28.6	1.5	28.6	1.9	28.7	1.7	27.7	1.8	27.9	1.3	28.1	1.8
14	11	28.3	2.4	28.7	2.1	28.6	1.9	29.1	1.7	28.6	1.6	27.8	2.2	28.4	1.7	28.7	2.1	28.8	1.8	28.0	1.7	28.4	1.3	29.2	1.8
18	15	29.6	1.8	29.1	2.5	28.9	1.8	29.3	2.0	28.2	1.6	28.1	1.6	28.0	1.8	28.5	2.1	28.8	2.1	28.5	2.0	28.4	1.7	30.0	2.1
22	19	30.1	1.9	29.2	2.1	30.2	3.6	29.9	1.9	29.6	1.7	28.1	2.4	29.7	1.8	30.2	2.2	28.6	2.2	28.1	2.0	28.7	1.7	29.6	2.1
26	23	30.4	1.9	29.9	2.1	29.2	2.6	30.4	1.7	29.3	1.8	28.3	2.2	28.9	2.2	29.1	2.2	28.7	2.4	28.2	2.1	28.5	1.5	29.2	2.4
30	27	31.5	1.8	30.9	2.1	30.3	2.2	31.0	2.1	29.4	2.2	27.6	3.0	28.6	2.2	29.2	3.1	30.3	2.1	29.4	2.8	29.0	1.6	30.2	2.4
34	31	31.0	1.8	29.8	2.0	30.3	2.2	29.9	2.0	30.2	1.7	29.1	2.1	29.5	1.7	30.0	2.8	28.7	2.3	28.5	2.4	29.1	1.8	29.0	2.1
38	35	29.3	1.6	28.8	1.8	29.4	2.2	29.3	1.8	29.4	1.7	28.4	2.2	28.7	1.6	30.0	2.5	29.4	2.2	29.1	2.2	29.2	1.9	29.4	2.4
42	39	30.8	1.8	29.5	1.8	30.0	2.3	29.6	1.8	29.2	1.7	28.7	1.9	28.5	1.5	29.8	2.2	29.5	2.5	29.4	2.2	29.2	2.2	30.2	2.4
46	43	30.6	2.4	29.6	1.9	30.9	2.2	29.9	2.2	30.4	1.7	30.1	2.1	29.9	2.5	30.9	2.1	32.1	2.1	29.7	2.3	30.2	1.7	30.5	2.1
50	47	31.9	2.4	30.5	2.1	30.2	2.3	30.1	2.1	31.8	2.2	31.2	2.2	30.8	1.9	30.5	2.3	31.9	2.8	29.1	2.2	29.1	2.0	30.1	2.1
54	51	29.6	2.0	30.1	1.8	30.9	1.8	30.3	1.6	31.1	2.1	30.3	2.3	29.2	2.1	31.4	2.6	30.9	1.6	29.7	1.5	29.4	1.6	29.8	1.6
58	55	30.6	1.5	31.0	1.9	30.0	2.2	31.4	1.7	30.9	2.3	30.1	1.8	29.6	1.6	31.3	2.4	35.5	4.6	32.9	2.6	30.4	2.6	33.5	0.7
62	59	30.0	1.9	29.0	2.1	29.2	1.8	30.4	2.9	31.2	1.9	31.0	1.9	29.5	2.4	32.5	2.0	Term.		32.8	1.9	31.0	3.8	Term.	
66	63	30.8	1.7	30.1	2.1	29.9	2.9	30.6	2.5	30.1	1.2	30.1	1.8	30.0	1.9	31.8	2.2			Term.		Term.			
70	67	29.9	1.7	31.4	2.5	32.9	3.1	Term.		31.1	1.5	30.1	2.3	31.3	2.2	Term.									
74	71	31.6	1.9	30.9	1.7	Term.				31.9	1.4	30.2	1.5	Term.											
78	75	31.4	2.0	30.6	1.5					30.7	1.3	30.2	1.8												

*(E) and (O) indicate, for each experimental group, the time-table on which its weights were taken.

"Term." = experimental group terminated.

TABLE A-4 (continued)

Animal Weights — C3H/HeJ
(grams)

Even Weeks (E)	Odd Weeks (O)	GROUP* 26 (E) X̄	26 (E) SD	28 (E) X̄	28 (E) SD	30 (E) X̄	30 (E) SD	32 (E) X̄	32 (E) SD	34 (E) X̄	34 (E) SD	36 (E) X̄	36 (E) SD	38 (E) X̄	38 (E) SD	40 (E) X̄	40 (E) SD	42 (E) X̄	42 (E) SD	44 (E) X̄	44 (E) SD	46 (E) X̄	46 (E) SD	48 (E) X̄	48 (E) SD
	t_o	26.3	1.3	26.1	2.0	26.4	1.9	26.2	1.8	26.7	1.9	25.4	2.0	26.5	1.7	26.0	1.6	26.1	1.8	25.9	2.1	25.3	2.0	27.0	1.9
2	1	27.0	1.4	26.4	2.1	26.1	2.2	27.2	1.4	28.0	1.8	26.8	2.0	27.7	1.9	26.5	1.9	28.0	1.8	27.0	1.8	26.8	2.1	27.6	1.7
4	3	27.8	1.5	28.0	2.2	27.6	1.9	27.5	1.6	28.6	1.9	28.0	1.9	27.8	1.8	27.8	1.6	26.6	1.8	27.3	1.8	27.5	2.2	28.2	2.1
6	5	28.9	1.5	28.8	2.0	28.8	1.8	28.0	2.1	28.9	1.9	27.4	1.8	28.4	1.9	27.6	1.8	28.3	1.7	28.6	1.7	28.4	2.2	28.8	2.4
10	7	28.6	1.4	28.4	2.1	28.8	1.8	29.3	1.7	29.7	1.7	30.1	2.0	28.6	2.5	29.8	1.8	29.7	1.7	28.0	1.5	28.6	1.9	29.6	2.1
14	11	29.4	1.8	29.2	2.0	29.4	1.7	29.4	1.9	29.8	1.9	28.9	2.0	30.4	2.8	29.3	1.8	28.9	2.1	28.8	2.0	29.0	1.9	30.5	2.4
18	15	29.4	2.0	29.7	2.1	29.4	2.0	30.4	1.9	31.1	2.2	29.1	2.4	30.2	3.2	29.6	3.0	29.9	1.9	29.8	1.7	29.4	2.6	30.2	2.7
22	19	29.3	1.8	29.1	2.1	29.1	2.3	28.9	1.6	29.3	2.3	28.9	2.2	30.4	2.5	29.0	2.1	29.1	2.4	29.3	1.9	29.0	1.8	30.7	2.3
30	27	29.7	1.8	30.3	2.6	29.1	2.6	29.3	1.6	30.2	2.0	28.9	2.0	29.5	2.6	28.5	2.3	28.5	1.8	29.3	2.2	28.8	2.1	30.2	2.4
34	31	30.0	1.9	29.5	2.1	30.1	1.9	29.5	1.6	30.7	2.0	29.8	2.1	30.1	2.7	29.9	2.3	29.0	1.7	29.9	1.8	29.0	2.3	30.7	2.3
38	35	31.3	1.8	30.2	2.0	29.5	2.2	29.9	2.0	30.0	1.8	29.3	2.3	29.9	2.8	29.3	2.7	29.7	1.8	30.0	2.1	29.3	2.2	30.2	2.5
42	29	30.6	2.1	30.1	2.1	29.8	2.2	29.7	1.6	30.3	2.0	29.6	2.4	30.8	2.6	29.8	2.2	29.2	1.9	29.6	1.9	29.2	2.4	30.5	2.3
46	43	30.7	1.8	29.9	1.9	31.1	2.4	30.0	2.2	30.6	2.2	30.1	2.2	32.0	4.7	30.9	2.2	29.8	2.1	29.5	2.3	29.9	2.3	32.0	2.5
50	47	30.6	1.8	29.7	2.2	29.6	2.4	30.5	2.0	29.6	1.9	29.3	2.2	29.5	2.6	30.5	2.5	25.6	1.8	28.0	4.7	29.5	2.0	29.7	2.1
54	51	31.7	1.2	30.4	1.6	29.0	2.2	30.3	1.4	28.0	1.5	27.4	2.0	30.0	1.4	29.3	1.8	26.9	2.2	30.0	1.4	31.0	2.0	30.2	1.5
58	55	Term.		32.5	2.5	31.2	2.0	33.4	1.9	27.9	2.3	29.1	2.2	29.3	1.8	29.1	2.1	29.9	1.8	31.4	2.1	31.0	2.7	30.0	2.1
62	59			Term.		31.0	1.5	Term.		30.0	1.5	28.9	1.7	29.5	1.7	30.0	1.9	30.6	1.4	30.3	2.3	31.0	2.7	31.5	1.8
66	63					Term.				31.4	2.1	30.0	1.9	30.3	2.4	30.2	2.2	29.9	1.9	Term.		Term.		30.4	2.2
70	67									30.4	2.1	29.8	1.8	29.7	2.2	29.9	1.6	29.6	2.2					30.0	2.3
74	71									29.9	1.8	29.5	1.1	30.0	2.3	29.3	1.1	29.8	1.5					30.0	2.1
78	75									Term.		29.3	2.4	30.2	2.0	Term.		30.4	1.4					29.2	2.2

*(E) and (O) indicate, for each experimental group, the time-table on which its weights were taken.

"Term." = experimental group terminated.

TABLE A-5

DETAILS OF GROSS PATHOLOGY BY INDIVIDUAL TEST GROUP — CD-1 MICE
(Entries are number of mice out of original group of 50 showing the lesion)

Test Material	Solar	Group No.	Liver			Spleen		Lung
			En-larged	Pale	Out-growth	En-larged	Mottled	Out-growth
A	-	1	3	20	2	11	8	7
	+	3	30	44	2	14	9	7
B	-	7	9	30	2	14	5	7
	+	5	14	29	2	12	11	6
C	-	9	4	22	5	11	5	5
	+	11	4	22	4	8	5	3
D	-	15	8	17	9	11	6	4
	+	13	6	18	2	7	1	3
E	-	17	7	26	1	15	12	5
	+	19	8	20	1	14	5	4
F	-	23	11	30	3	24	13	7
	+	21	5	18	2	12	10	10
G	-	25	9	20	4	15	9	5
	+	27	10	21	3	10	6	4
H	-	31	6	23	2	17	13	6
	+	29	5	22	1	7	3	1
Combination	-	43	6	20	2	17	9	6
	+	45	7	20	3	10	7	7
Benzo(a)-pyrene	-	39	10	20	3	21	13	7
	+	41	5	16	1	14	7	5
Controls Solvent	-	33	5	24	2	5	5	9
	+	35	1	17	0	7	3	3
Solvent (Cage)	-	37	4	19	3	8	6	4
Untreated	+	47	2	25	7	10	5	5

TABLE A-6

DETAILS OF GROSS PATHOLOGY BY INDIVIDUAL TEST GROUP — C3H MICE
(Entries are number of mice out of original group of 50 showing the lesion)

Material	Solar	Group No.	Liver			Spleen		Lung
			Enlarged	Pale	Outgrowth	Enlarged	Mottle	Outgrowth
A	-	2	6	22	16	28	28	17
	+	4	5	18	14	24	31	13
B	-	8	6	24	20	27	35	15
	+	6	5	22	19	26	35	14
C	-	10	7	24	11	28	30	15
	+	12	6	23	20	22	26	11
D	-	16	6	31	10	26	31	12
	+	14	3	23	12	20	23	5
E	-	18	6	25	7	27	35	12
	+	20	4	35	8	30	36	22
F	-	24	14	31	12	26	37	16
	+	22	4	32	12	26	32	14
G	-	26	11	31	10	19	35	10
	+	28	3	32	5	28	37	17
H	-	32	6	33	11	23	35	14
	+	30	5	25	10	25	25	15
Combination	-	44	2	26	12	22	32	14
	+	46	3	26	6	23	32	20
Benzo(a)-pyrene	-	40	1	26	9	24	30	12
	+	42	6	19	21	22	22	5
Controls Solvent	-	34	3	7	20	1	2	4
	+	36	2	18	12	3	3	7
Solvent (Cage)	-	38	3	11	16	1	2	6
Untreated	+	48	3	22	13	1	1	6

TUMOR INCIDENCE

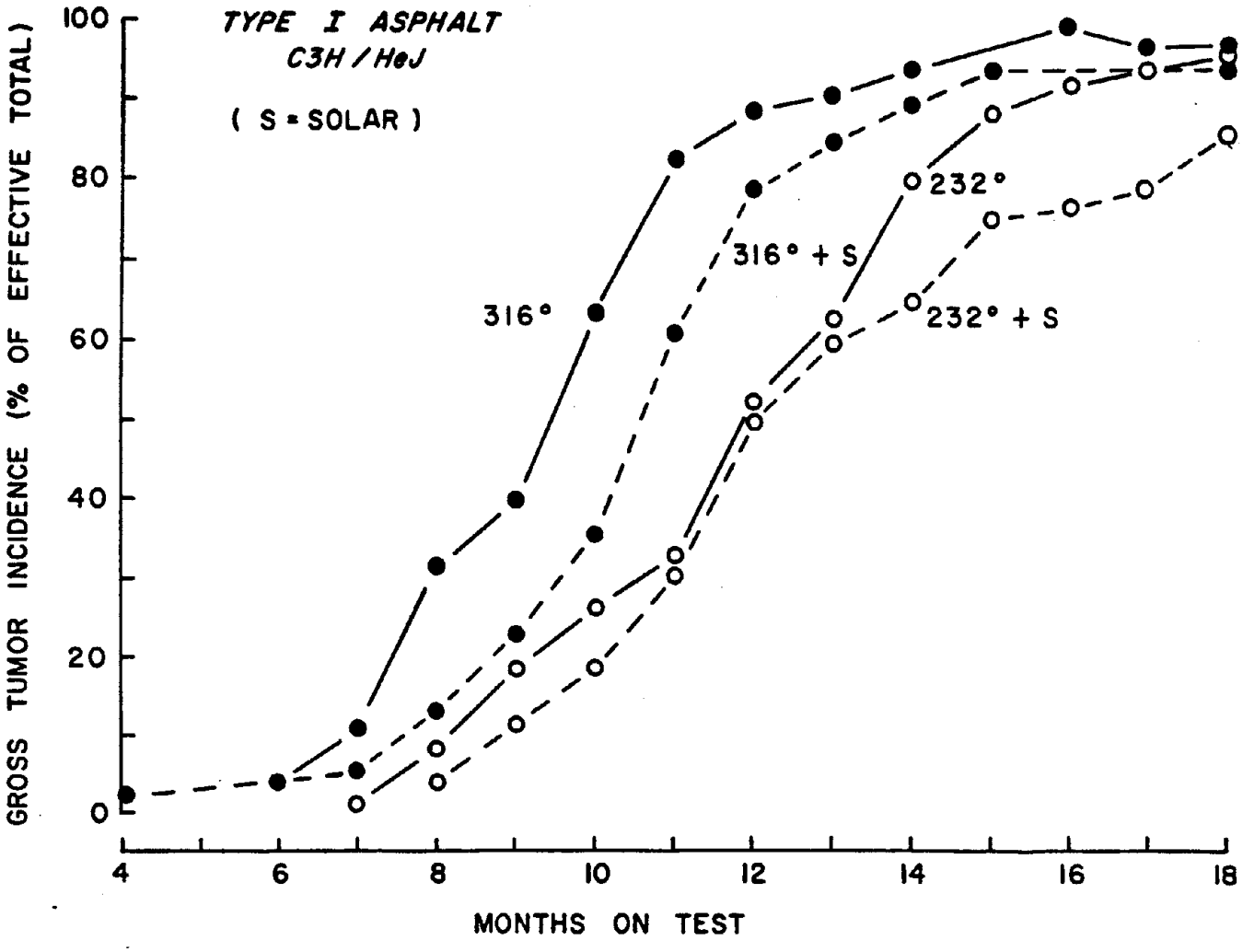

FIGURE A-1

TUMOR INCIDENCE

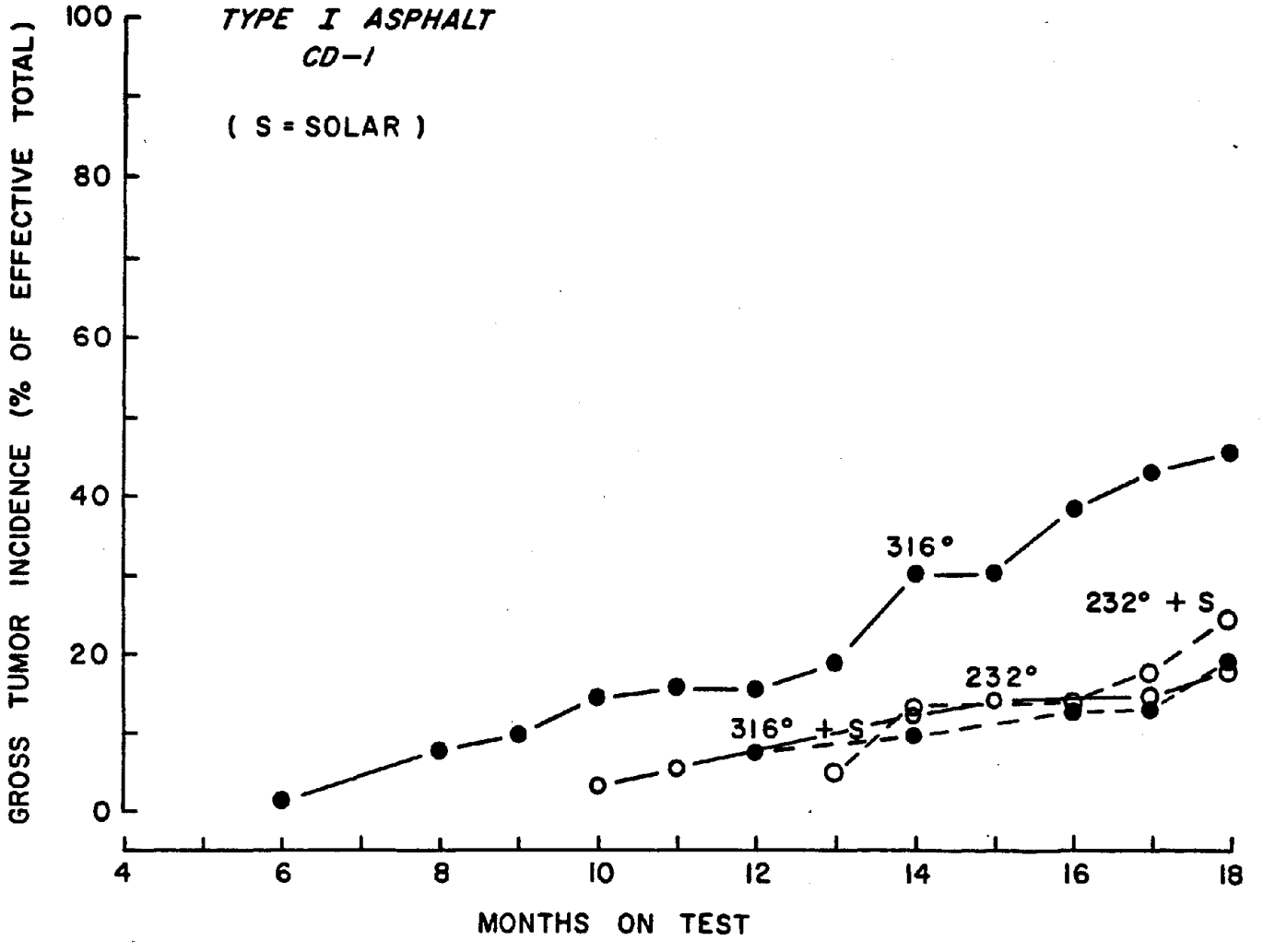

FIGURE A-2

TUMOR INCIDENCE

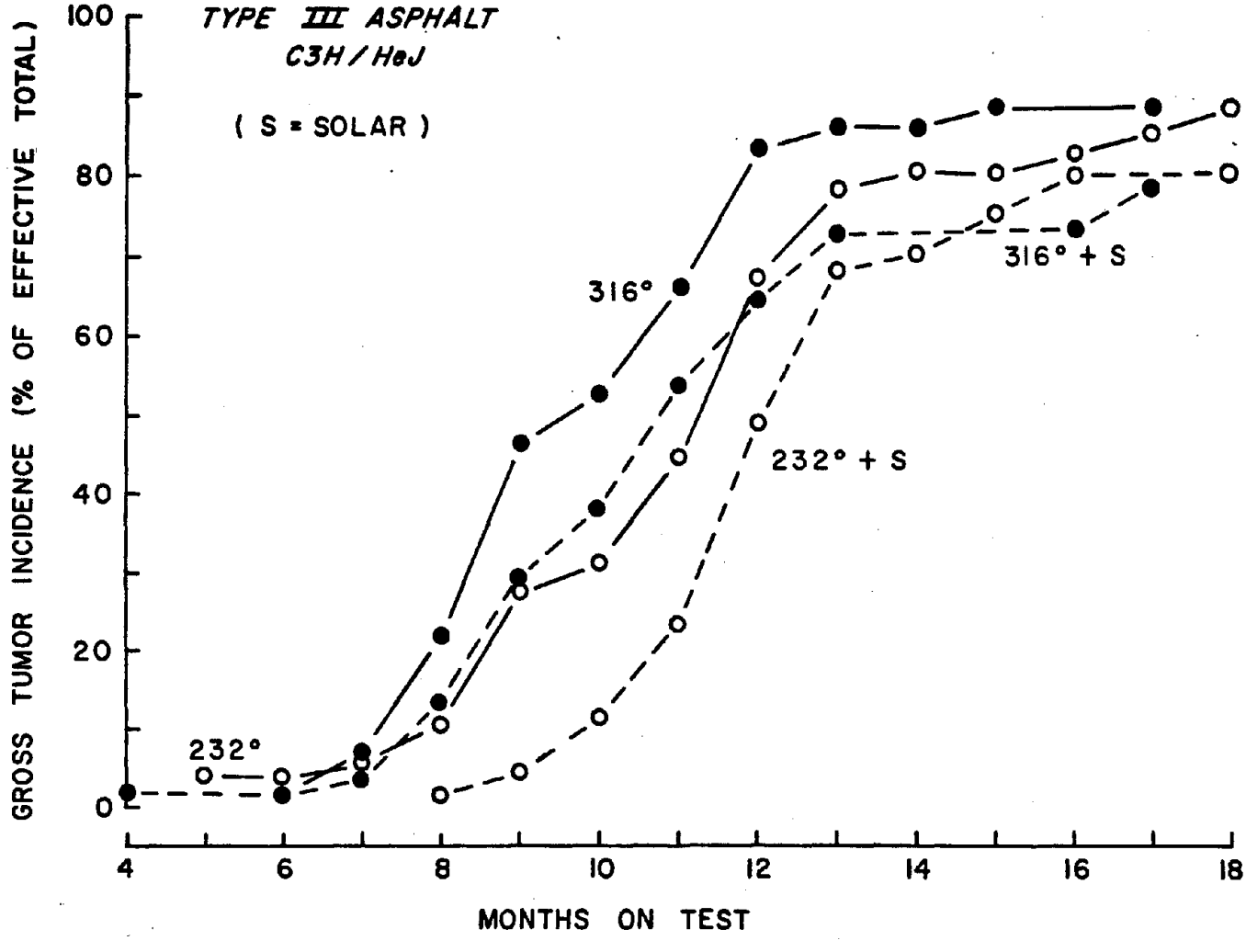

FIGURE A-3

TUMOR INCIDENCE

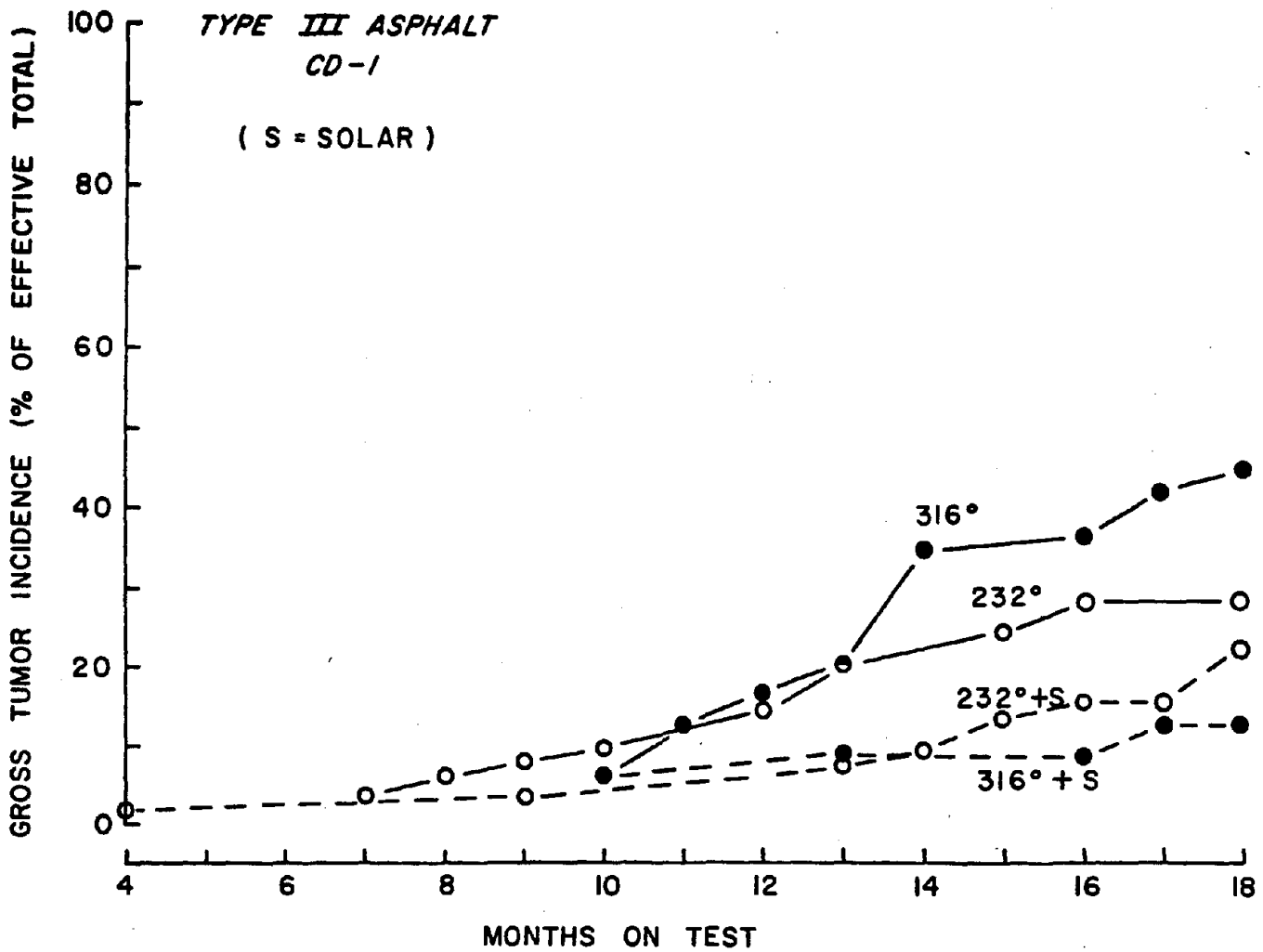

FIGURE A-4

TUMOR INCIDENCE

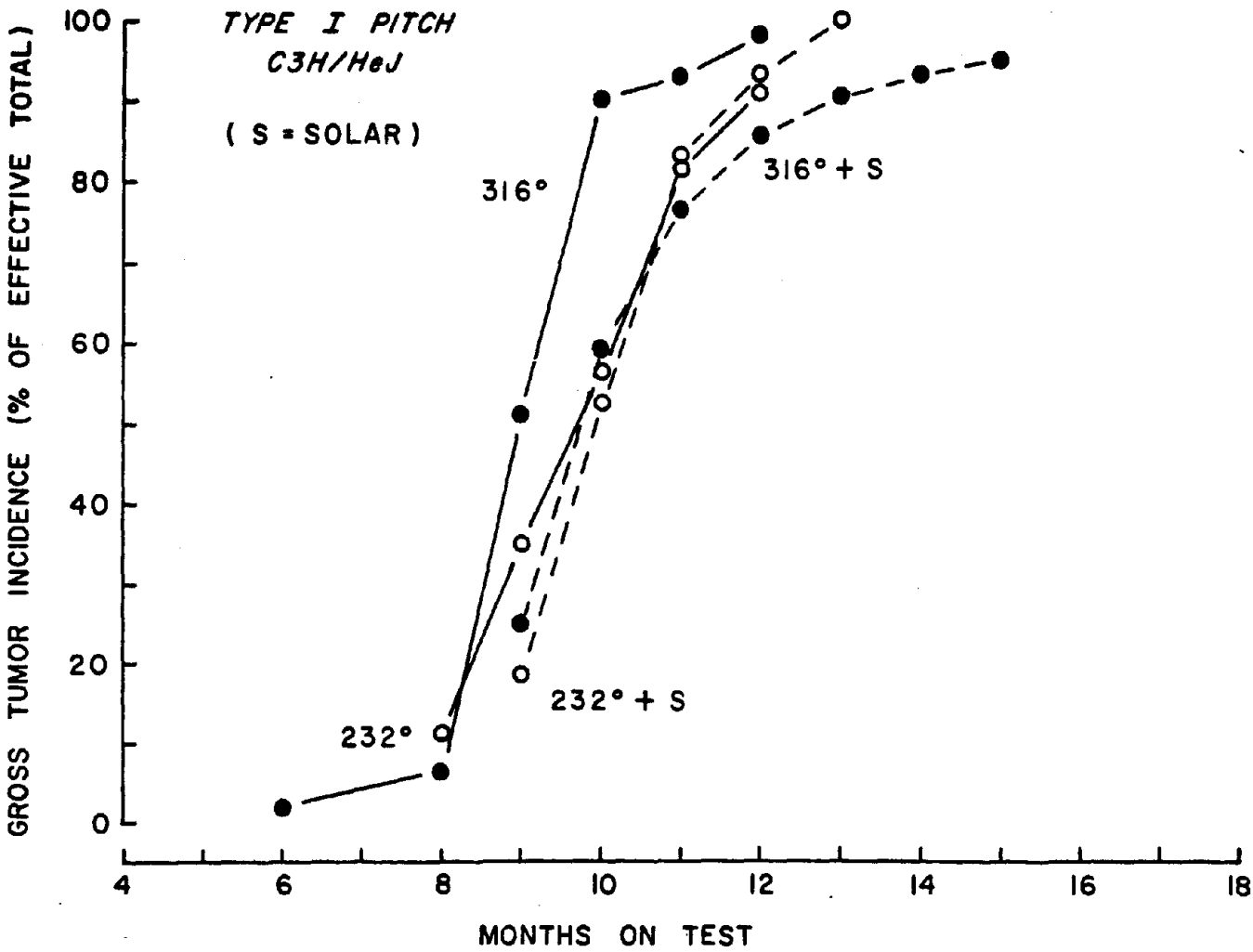

FIGURE A-5

TUMOR INCIDENCE

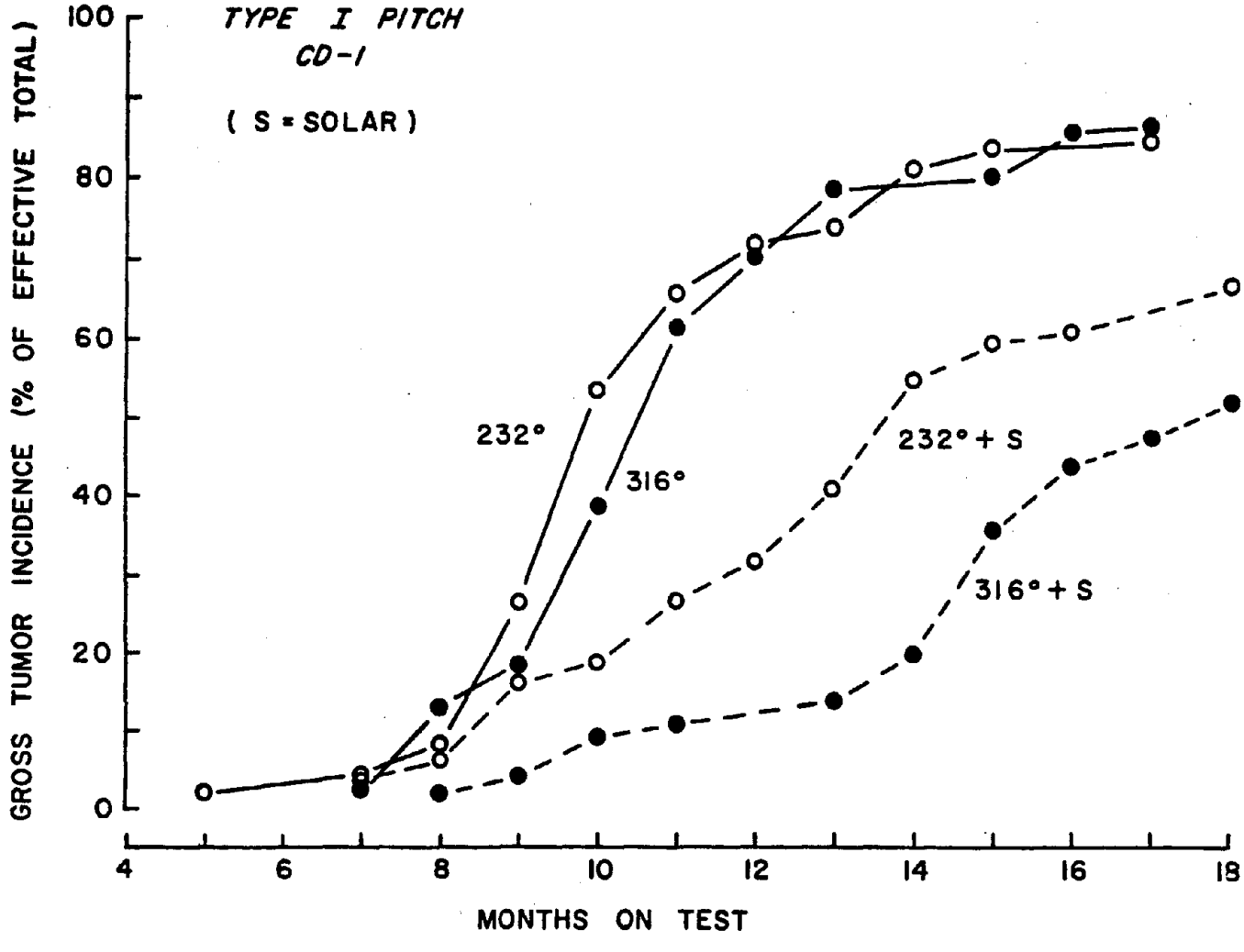

FIGURE A-6

TUMOR INCIDENCE

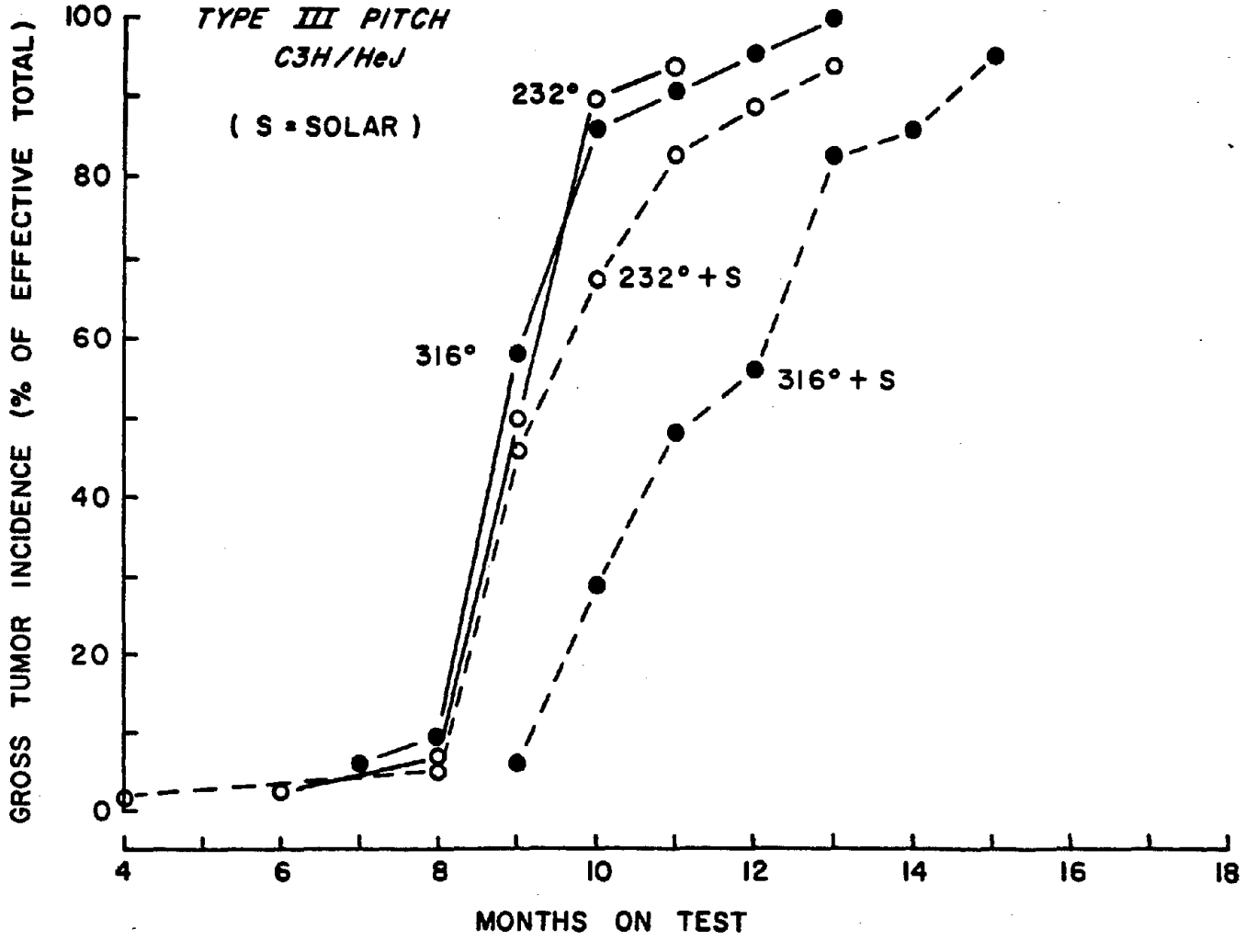

FIGURE A-7

TUMOR INCIDENCE

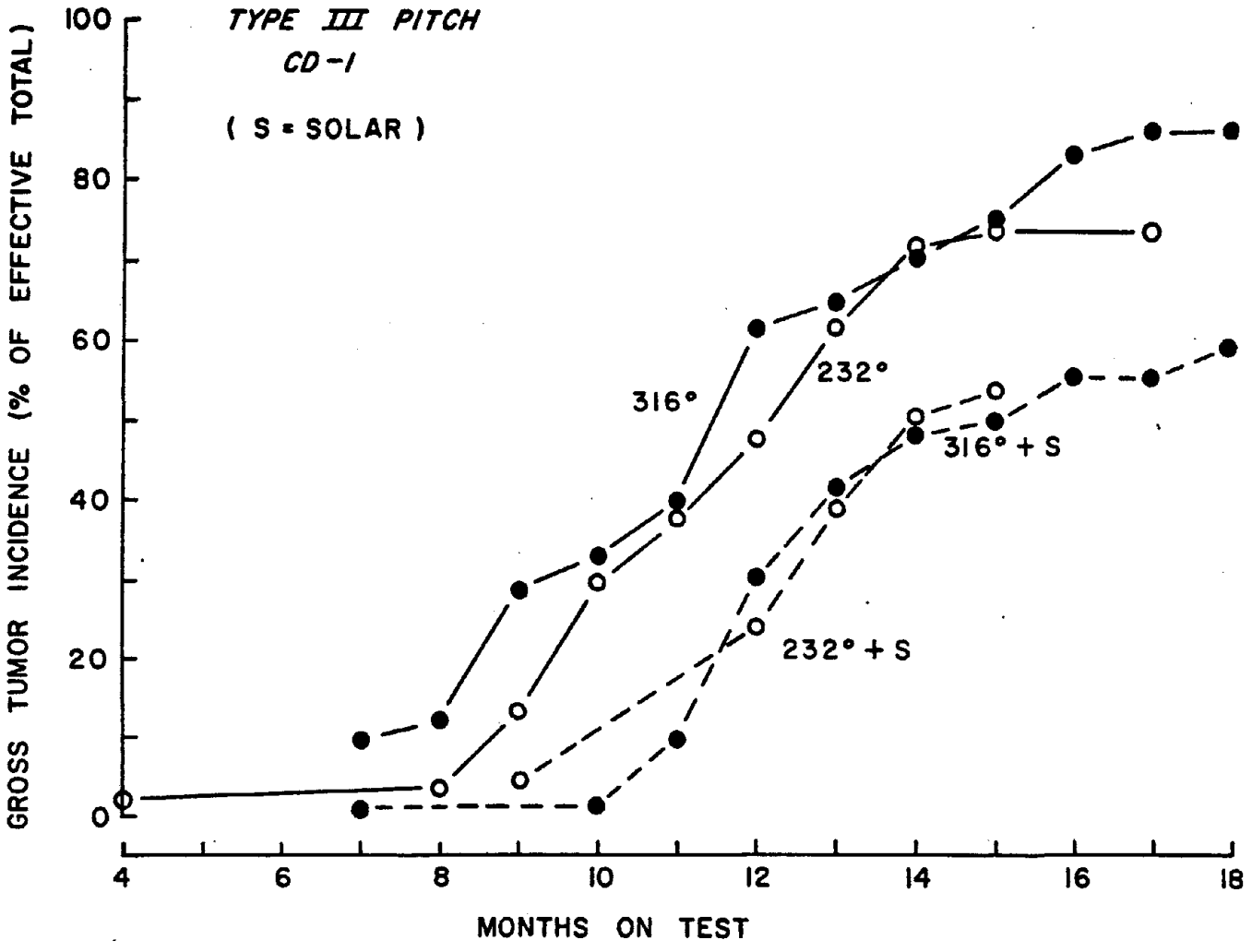

FIGURE A-8

TUMOR INCIDENCE

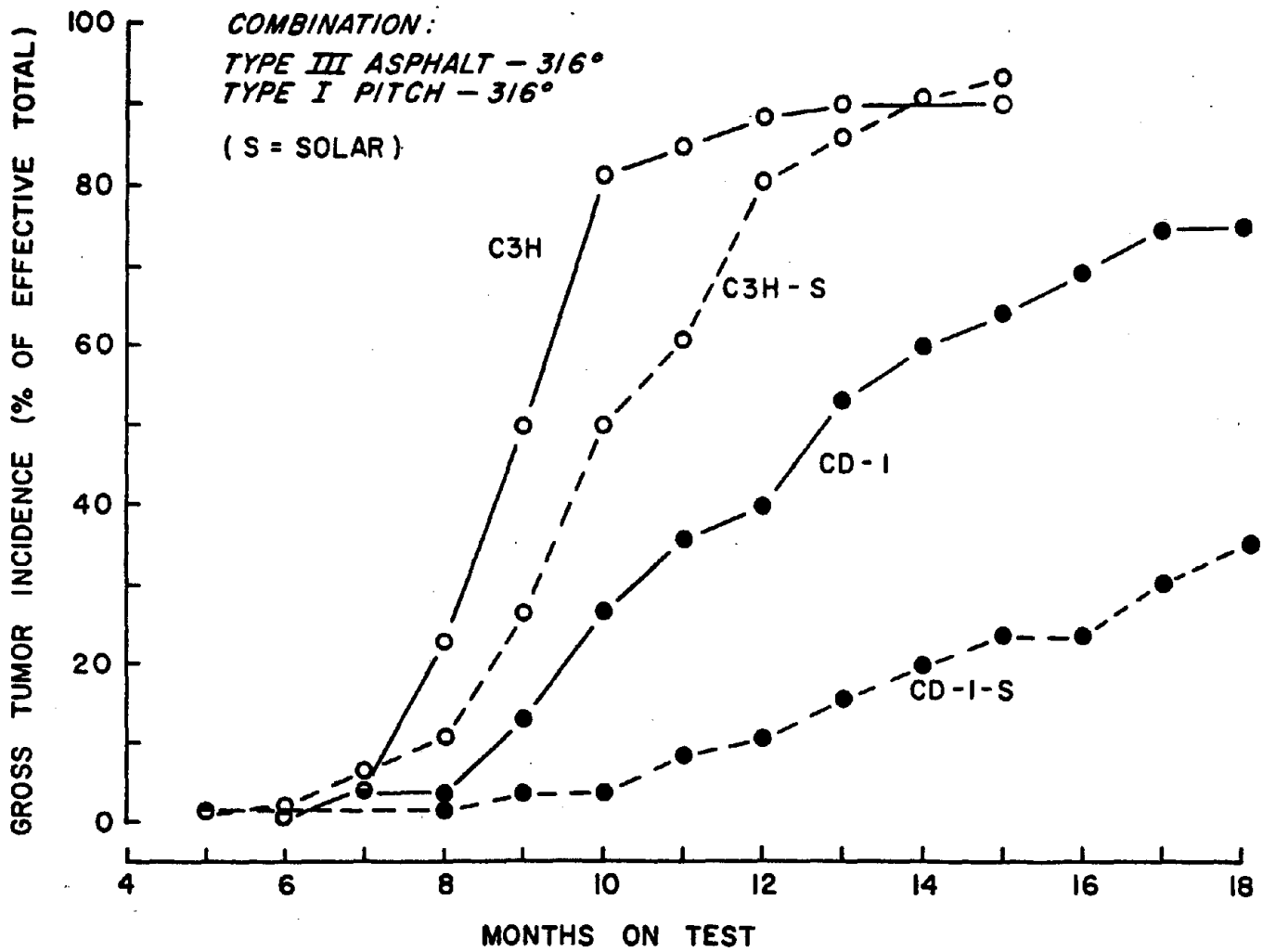

FIGURE A-9

TUMOR INCIDENCE

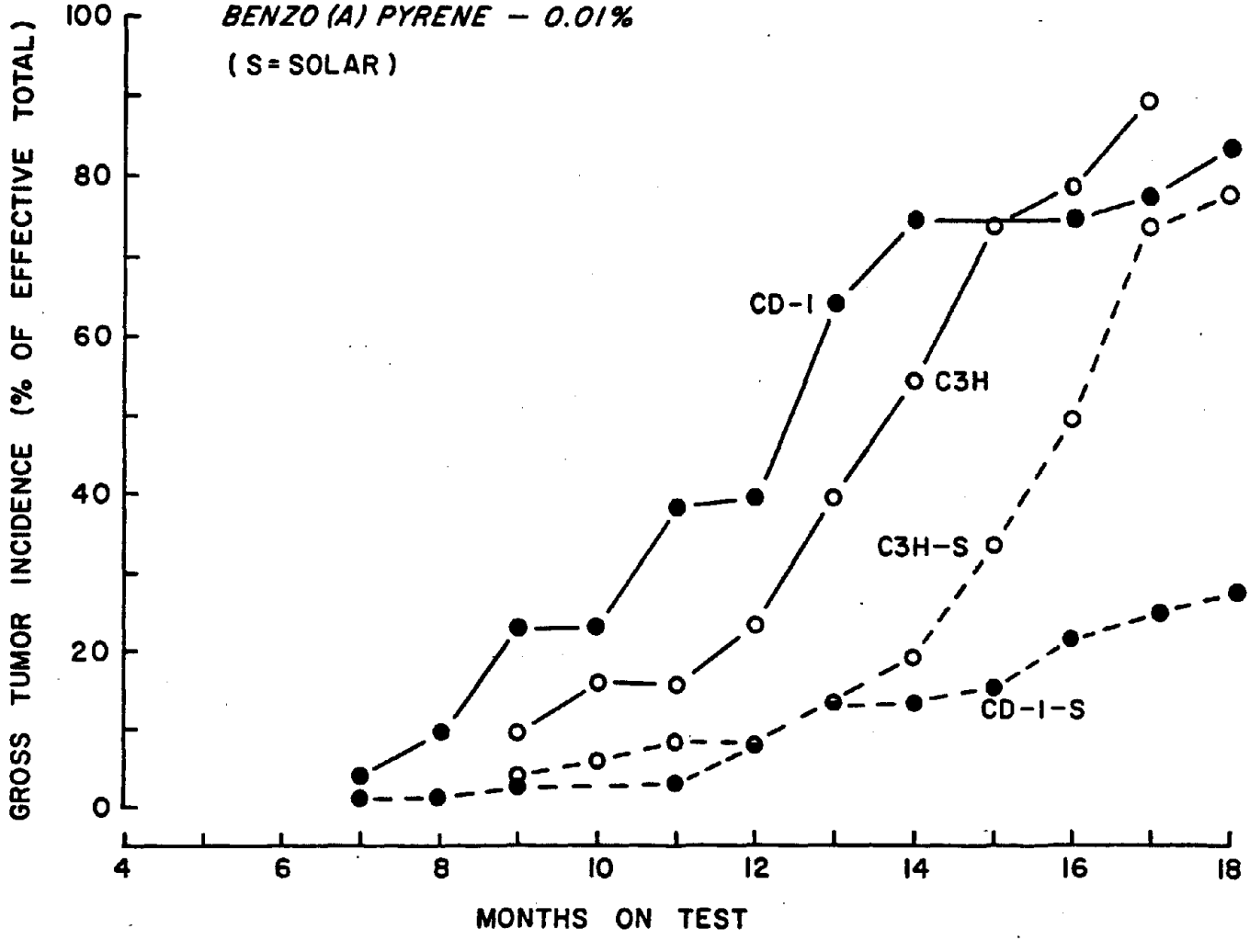

FIGURE A-10