ASSESSMENT OF THE COCARCINOGENIC/PROMOTING ACTIVITY OF ASPHALT FUMES

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ABSTRACT

Condensed roofing asphalt fumes, generated at 316°C, were collected by cold trap condensation and fractionated by preparative high performance liquid chromatography. The fractions obtained were: (A) alkanes, alkylated benzenes and alkylated naphthalenes, (B) olefins, alkylated aryl thiophenes and alkylated phenanthrenes, (C) alkylated phenylethanones and alkylated dihydrofuranones, (D) alkylated phenols and alkylated ketones, and (E) C6-C22 alkylated ketones and alkylated naphthols and phenols. The skin application carcinogenesis bioassay was conducted by twice weekly application of test materials in acetone: cyclohexane (1:1) for 104 weeks in male C3H/HeJ or Sencar mice with 30 mice per test group. The fractions were applied at a mass in proportion to their amount in the neat asphalt fumes.

Neat asphalt fumes produced similar and statistically significant increased tumor yields in both strains as compared to respective vehicle controls. Recombination of all fractions resulted in a tumor response similar to neat asphalt fumes. Among individual fractions, C was most potent with a tumor incidence of 100%, followed by B with an incidence of 60% based on the gross observed tumor incidence calculated as the fraction of mice at risk bearing carcinomas and papillomas. The other single fractions were without significant tumorigenic activity. Combinations containing fractions B and C were most active among the mixtures that were assayed and no evidence of enhancement of tumorigenesis in the mixtures was found. No significant cocarcinogenic or tumor promoting activity was observed with fractions A, D, or E and benzo(a)pyrene. Raw unheated asphalt produced a few tumors in C3H mice, but no tumors were seen when raw asphalt heated to 316°C, with the fumes permitted to escape, was applied.

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This research investigation on the performed Assessment of the Cocarcinogenic/Promoting Activity of Asphalt Fumes was performed by Arthur D. Little, Inc. (Cambridge, Massachusetts), under contract to the U.S. Department of Health and Human Services, Public Health Service, National Institute for Occupational Safety and Health's Division of Biomedical and Behavioral Science Center for Disease Control. Particularly helpful in the conduct of the work and preparation of this report were Mr. Dennis Lynch, Project Director; Dr. Richard Niemeier, Alternate Project Director, Mr. Barry Belinky, Chief Chemist for NIOSH, Dr. Steven Simon, Statistics, and Dr. Richard Solomon, Pathology. The Principal Investigator for Arthur D. Little, Inc. was Dr. Andrew Sivak and Mr. Kevin Beltis had primary responsibility for the chemistry related to the project.

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QUALITY ASSURANCE UNIT STATEMENT

ASSESSMENT OF THE COCARCINOGENIC/PROMOTING ACTIVITY OF ASPHALT FUMES

ADL REFERENCE: 50043

NIOSH CONTRACT NO. 210-78-0035

The conduct of this study has been subjected to periodic inspections by the Quality Assurance Unit on the dates listed below.

PHASE	DATE
Protocol development - fume generation	1/20/84
Fume generation	2/29/84
Fume fractionation	9/5/85
Pooling and concentration of fractions	2/25/86
Protocol development - bioassay	3/16/86
Preparation of dosing solutions	6/12/86
Randomization of mice	7/3/86
Administration of test and control substance to mice	10/20/86
Clinical observation	2/12/87
Body Weight determination	2/12/87
Administration of test and control substances to mice	5/26/87
Clinical observations	5/26/87
Administration of test substance to mice	9/10/87
Necropsy	12/28/87
Administration of test substance to mice	4/7/88
Necropsy	7/20/88
Data audit	9-11/88
Review of draft report	8/89

The Quality Assurance Unit reported its findings to the Study Director and Management on August 25, September 17, October 27, 1986, June 8, July 28, December 31, 1987, May 11, 1988, July 25, and September 14, 1989.

Histopathological evaluation and statistical analysis were conducted by the study sponsor and were not reviewed by the Arthur D. Little, Inc. Quality Assurance Unit.

Denise Hayes
Quality Assurance Officer

January 27, 1990
Date

INTRODUCTION

This project was initiated by the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control, to address several issues that emerged from a previous study supported by the Institute in our laboratories (Contract 210-78-0035). The purpose of that work was to examine a set of variables that might be of relevance to the assessment of the carcinogenic potential of volatile components of roofing asphalts and pitches (1). The variables examined were:

- Mouse strain (CD-1, C3H/HeJ)
- Type of roofing material (Asphalt, Types I and III; Coal tar pitches, Types I and III)
- Generation temperature for volatiles (232°C and 316°C)
- Exposure to simulated sunlight or absence thereof (Filtered Atlas 6.5 KW xenon arc)

The conclusions of that study were (2):

- 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 volatile, there is a pronounced effect of temperature of preparation, the 316°C preparations being the more active than the 230°C preparations.

- The pitches do not show this effect, but since the 316°C preparations were applied at lower concentrations and each shows tumorigenicity equivalent to that of the corresponding 232°C preparation, it is inferred that the former have a higher specific activity.

- The combination treatment shows effects that might be expected from the individual activities of the two 316° preparations used, without evidence of additivity or synergism.

The key conclusion that provided the impetus for the present study was that the carcinogenic activity of the asphalt fume materials could not even approximately be explained on the basis of their benzo(a)pyrene

content in contrast to the pitches. It was hypothesized at the time that cocarcinogenic effects of aliphatic hydrocarbons, which may be major components of the asphalt fumes generated in the study, may be responsible for this enhancement activity.

The present study was designed to examine this possibility as well as to investigate the individual biological activity of a number of chemically distinct fractions of the asphalt volatile condensate. The steps followed in the study were (1) to collect the Type III asphalt fumes generated at 316°C, (2) to fractionate the collected fume condensate by a method developed and provided by the National Institute for Occupational Safety and Health, and (3) to test the materials individually and in combination with each other and with benzo(a)pyrene for direct carcinogenic activity, cocarcinogenic activity, and tumor-promoting activity.

MATERIALS AND METHODS

TASK I - FUME GENERATION

Preparation of Asphalt Volatiles Samples

Generation

Type III roofing asphalt, supplied by NIOSH, was used in the generation of the asphalt volatiles or "fume" for preparation of the skin painting solutions for the mouse bioassay. The "Type III" or "steep" asphalt was of the same type used in a previous study conducted by Arthur D. Little, Inc. in 1978/1979 under NIOSH Contract No. 210-78-0035, purchased from an Exxon, Inc., Roofing Products distributor (Beacon Sales, Inc., Somerville, MA). The asphalt was produced by a process using distillation and enhanced aeration of Arabian crude oil.

The asphalt volatiles were generated by heating the raw asphalt to the point of fuming. An electric heating mantle capable of reaching 450°C was used to heat the roofing material to the desired generation temperature; mantle temperatures were controlled with a Variac® and a Honeywell controller. The specifications for all components are given in Table 1. This system is identical to that used under the NIOSH contract indicated above with the single exception that the third trap was chilled with a dry ice/isopropyl alcohol slurry in place of a liquid argon bath. The original system, picturing the liquid argon dewar, is shown in Figure 1.

TABLE 1

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-L
Oven:	Blue M, Inc., Model OV
Variable Voltage Regulator:	Variac®
Temperature Recorder:	Black Angus® Multichannel
Thermocouples:	Type J, fabricated by contractor

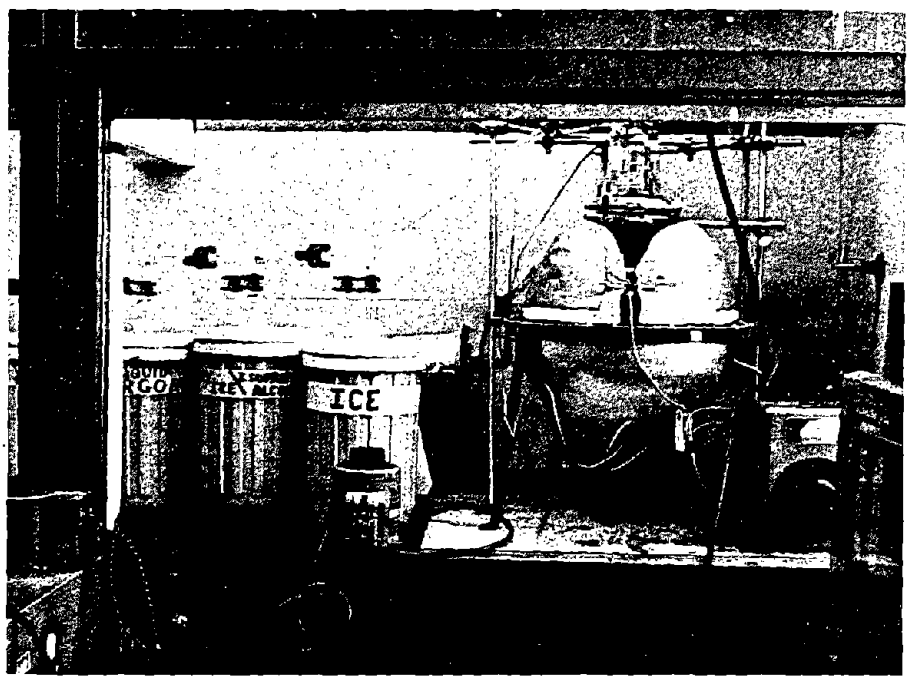

Figure 1 Generator/Collection System

The generation/collection system was contained in a laboratory hood whose lighting was filtered through yellow cellulose acetate-butylate filters to reduce exposure to ultraviolet light. The step wise protocol used for the generation procedure is given in Appendix I. Part A details the "Filling of the 12-L Flask with Asphalts", Part B details the "Generation of Asphalt Fumes", and Part C details the "Cleaning of Generator Parts."

The flasks used in generating the fumes were filled with raw asphalt in advance of the daily generations. The scheme used to fill the flask was as follows: The roofing asphalt was broken up into small pieces and placed in the 12-L flask. The flask was then placed in a forced air oven (Figure 2) at 150°C (302°F) to soften the asphalt for the filling until approximately 10-L of the material was present. The covered flask was then allowed to cool to room temperature and weighed. The flask and contents were stored at room temperature until used.

A routine was established for filling the flasks on the evening prior to each generation. A 12-L spherical reaction vessel (flask) was loaded with small pieces of the raw asphalt and the flask placed in a forced convection oven. The oven was preset to turn on at 0400 hours and heat to 150°C to soften the asphalt. At about 0730 hours, the heated flask was placed in the lower preheated mantle with a thermocouple under the flask. The upper preheated support mantle and a thermocouple were then put in place followed immediately by the reaction flask head, the stirrer, and the interior thermocouple (Figure 3).

Laboratory generation of the asphalt fumes and collection of the volatile materials by condensation was conducted using standard glass reaction vessels and impingers (Ace Glass Mfg., Vineland, NJ) for production of the required amounts of fumes. The 12-L flask was used to contain about 10-L roofing material during the individual fume generations. To permit uniform mixing, a stainless steel stirrer, driven by a pneumatic motor, was inserted through the center port of a three-hole glass reaction vessel head. The stirrer was sealed to the flask head with a Teflon® gland. One side port was fitted with another Teflon® gland to accomodate the conditioned inlet air used to entrain the asphalt volatiles. The other side port was fitted with a standard glass joint transfer tube to exhaust the fumes to the glass collection system which used a combination of cryogenic trapping and solvent impinging.

Ambient laboratory air supplied to the reaction vessel was cleaned and conditioned to preclude contamination from outside sources. Air was pulled through the system at 10 liters per mir (Lpm) with a vacuum pump (Gast, Inc.) and a flow limiting control orifice. To cleanse the air, it was passed through a high-efficiency filter (Filtrate 0.45-μm Micoflow cartridge), then through silica gel to remove water present due to ambient humidity, then through activated carbon to remove extraneous organics prior to entering a rotameter for flow monitoring . The air was then preheated to about 100°C by passing it through a muffler-

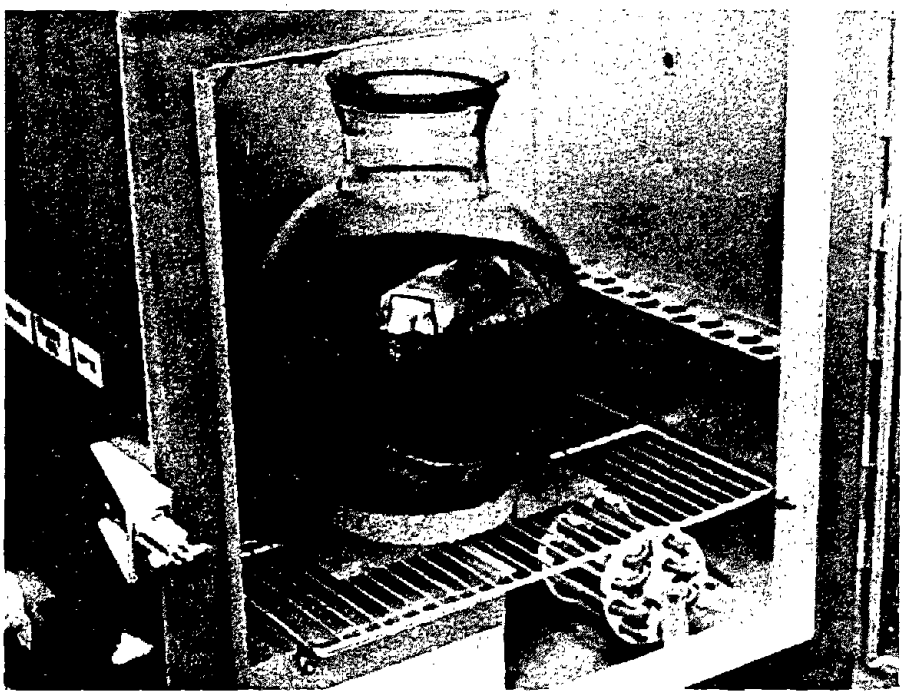

Figure 2 Preparing Material (Heating)

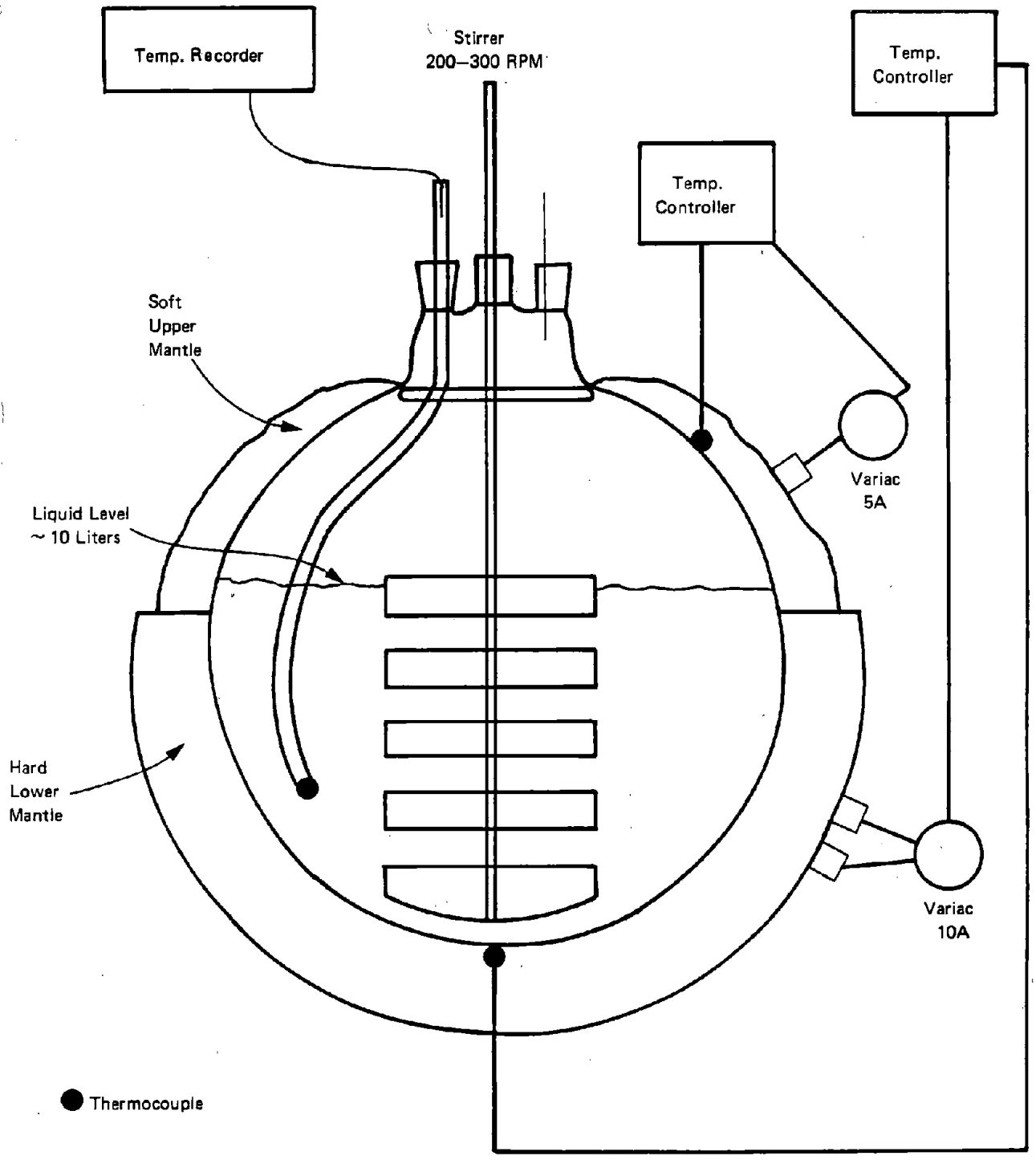

FIGURE 3 TEMPERATURE MONITORS

furnace heated coil immediately prior to entering the reaction vessel. Preheating the air prevented premature condensation of fumes on the reaction flask wall. The air was then passed through the reaction flask where it entrained the volatiles into the cryogenic traps.

Mixing was maintained at a stirring rate of about 200-300 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.

A pneumatic stirrer system was used to mix the asphalt during heating. The fume collection system was then attached. The system consisted of glass transfer tubes (20 mm OD) and glass impingers (Ace Glass, Inc.) placed in three individual cryotraps immersed successively in: ice (0°C), dry ice/isopropyl alcohol (-77°C), and dry ice/isopropyl alcohol (-77°C) (Figure 1*). Air exiting the reaction vessel initially passed through a large impinger (two liter capacity) immersed in ice to cool the air and principally condense any excess water. The air was further cooled by passing it through the two 750 mL impingers, immersed in the dry ice/isopropyl alcohol slurry to condense more of the volatiles. As the final collection sequence the effluent was passed through a 750 mL impinger containing ca. 500 mL of a 50%/50% solution of acetone/cyclohexane (HPLC grade solvents, obtained from Fisher Scientific).

Fumes were collected during heating to a temperature of 316°C (650°F) for a total period of about 7-10 hours. This temperature represents the high overheat temperature reached in asphalt kettles in the field (2). The asphalt temperature was maintained at 316°C and not allowed to exceed 326°C during the generation.

For the initial set of fume collections, a total of 22 generations were conducted to provide what was considered to be sufficient condensed material of about 8L of skin painting solution. Of the 22 runs performed, three were not used. The fumes collected from the Run #1 were not used as this was considered a test run and the maximum temperature was exceeded. Similarly, the fumes from Runs #3 and #16 were not used as the asphalt temperature exceeded the upper limit of 326°C (619°F). For the purposes of statistical records, there were 19 acceptable generations yielding 4906 grams of asphalt fume (or ca. 258 g/generation). The amount of fumes collected in each generation is reported in Table 2.

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*NOTE -- The photo used in Figure 1 shows the system from the original contract which used liquid argon in the third trap vs. the dry ice/isopropyl alcohol used in this program.

TABLE 2

ASPHALT FUME GENERATION
COLLECTION RATES
(SET I)

Run #	Asphalt Mass (g)	Air Flow Rate (L/min)	Run Time (hrs)	Volume Sampled (L)	Fume Mass (g) Organic Phase	Fume Mass (g) Aqueous Phase	Fume Mass (g) Total	Fume Conc. (g/L)
2	5700	-	6	-	162	10	172	-
4	4900	-	6	-	254	18	272	-
5	6500	-	6	-	184	10	194	-
6	6000	-	5	-	131	5	136	-
7	4400	-	3	-	117	3	120	-
8	7000	-	7	-	283	6	289	-
9	7000	-	9	-	248	5	253	-
10	5900	-	9	-	335	21	356	-
11	6100	5.6	8.25	2800	235	14	249	.089
12	6300	4.7	8.5	2400	269	17	286	.119
13	6500	4.4	8	2100	314	19	333	.159
14	5000	3.9	8.2	1900	391	21	412	.217
15	7000	1.3	9.2	720	171	4	175	.243
17	6700	3.5	8.8	1800	316	13	329	.183
18	6900	3.8	9	2100	287	10	297	.141
19	7000	3.3	8.7	1800	290	9	299	.166
20	7000	1.8	9	1000	152	4	156	.156
21	7000	3.1	9	1700	279	9	288	.165
22	7000	2.9	9	1600	281	9	290	.181
Total	119900				4699	207	4906	

Fume generation Set I averages -- .041 g fume/g asphalt, 258 g fume/run.

In June 1985, after receiving the formal documentation for the fractionation procedure and instruction in the use of the equipment specified in the protocol, fractionation of the fume began. As of October 16, 1985 all of the asphalt fume (2900 grams) from the first generation set had been fractionated using the Waters AutoPrep 500A LC supplied as GFP by NIOSH. Upon completion of these fractionations NIOSH determined that more fume material would be required. The NIOSH project team, comprised chiefly of Dr. Richard Niemeier and Mr. Barry Belinky, felt that the 2900 grams fractionated to that point might be insufficient to complete the intended animal studies, and therefore warranted the generation of additional fumes. Additional asphalt fume was generated and subsequently fractionated through November and December, 1985.

After consultation with the Arthur D. Little,Inc., team members, it was decided that three additional kilograms of fumes should be generated to yield at least two kilograms of viable fume. From the two kilograms, one kilogram was to be fractionated, 500 grams would be used for direct skin painting, and 500 grams would be sent to NIOSH for chemical analysis. Of the one kilogram to be fractionated, at least 500 grams were expected to be recovered from the rotary evaporation of which 350 grams would be used for the skin painting and 150 grams would be sent to NIOSH for chemical analyses.

The second set of generation of asphalt fumes began again in late November, 1985. The asphalt used in this current generation was of the same batch as that used in 1984. A total of ten runs were completed, (not including the test run) and 3440 grams of material collected. In recovering the fumes after each of these generations, the aqueous phases from the first trap (and the small amounts from each of the subsequent traps) for each run were composited and treated as a separate sample. The "Aqueous Phase Composite" was allowed to dry and the organic material then combined with the individual run organic phases as described further below. Table 3 summarizes the second set of generations.

Once the mass of fumes were calculated at the end of each run, a 50% solution was prepared on a weight to volume basis (w/v)* in terms of grams per milliliter (g/mL) using a 1:1 cyclohexane:acetone solvent mixture. With the consent of Mr. Belinky of NIOSH, all of the fume solutions (10 runs including the previously separated organics from the aqueous phases) were pooled together and mixed well for re-distribution. The asphalt fume solutions were equally aliquoted into eight bottles. A summary of the program efforts is given in Table 4.

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*Note -- All designations of concentrations in this report are referenced to standard SI units (i.e., g/mL) unless otherwise noted in the text.

TABLE 3

ASPHALT FUME GENERATION
COLLECTION RATES
(SET II)

Run #	Asphalt Mass (g)	Air Flow Rate (L/min)	Run Time (hrs)	Volume Sampled (L)	Fume Mass (g)	Fume Conc. (g/L)
2	5500	4.5	10.5	2800	286	.102
3	6100	4.8	10.25	3000	248	.083
4	6100	4.6	7.5	2100	185	.088
5	6000	9.0	8.6	4600	311	.067
6	6000	9.7	6.7	3900	178	.046
7	6200	9.6	11.25	6500	352	.054
8	6700	10	9	5400	439	.081
9	6400	9.4	8.3	4700	375	.080
10	6100	9.3	11	6100	425	.070
11	6900	9.3	8	4500	436	.097
Total	62000				3238	
Aqueous Phase Composite					199	
Total					3437	

Fume Generation Set II Averages -- 0.055g fume/g asphalt, 344 g fume/run

TABLE 4

NIOSH ASPHALT FUME GENERATION/FRACTIONATION PROGRAM SUMMARY

- Asphalt Fume Generation - Set I
  4900 grams collected - 19 runs
  1/13/84 - 3/6/84

- Asphalt Fume Fractionation - Set I
  2887 grams fractionated
  2653 grams collected - ~320 runs (65 days)
  6/14/85 - 10/15/85

- Asphalt Fume Generation - Set II
  3440 grams collected - 10 runs
  11/18/85 - 12/6/85

- Asphalt Fume Fractionation - Set II
  969 grams fractionated
  906 grams collected ~80 runs (12 days)
  12/10/85 - 12/26/85

Solution Preparation

After the fumes were condensed and collected in the sampling train and the individual impingers and transfer lines were weighed, all material was quantitatively transferred to 1 liter amber storage bottles 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. The standard protocol detailing the "Preparation of Collected Fume Samples is given in Appendix II, Part A.

The organic phase and water phase which existed for each sample were separated by transferring the entire solution to a 2-L separatory funnel. After settling, the organic phase was transferred to a rotary evaporator (Buchler, Inc.) and the solvent removed at reduced pressure with a water aspirator at a temperature of 50°C. The solvent was discarded. For the fumes generated in 1984, the aqueous phase was transferred to an evaporating dish and the water removed in a vacuum oven at 690 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 so as to provide a 50% w/v (g/mL) solution of asphalt fumes in the solvent mixture. The cyclohexane and acetone were both HPLC grade (greater than 99% purity) (Fisher Scientific, Inc., Fair Lawn, NJ).

The fume solutions were stored in brown glass bottles (Amber Glass, Rockaway Glass Company, Inc., via PSG Scientific, Rockville, MD) at 4°C. For analysis, 1 mL of each solution was submitted to NIOSH for analysis by GC/MS for quantitation of selected polynuclear aromatic hydrocarbons (PAH's).

In preparation for fractionation on the Waters Autoprep 500A Liquid Chromatographic System, the fumes were solvent exchanged into a mixture of hexane and methyl t-butyl ether (MTBE) using the rotary evaporation techniques used previously in this program and filtered to 0.45μm. The acetone/cyclohexane system was replaced with hexane/MTBE as the acetone can adversely react with the stationary phase of the NH_2 Prep HPLC column. The basis of this reaction is further discussed in the TASK II-A section of this report which addresses fractionation of the fumes. The protocol for the preparation procedure is given in Appendix II, Part B - "Fume Preparation for Fractionation."

Filtration of the fume solutions was carried out in stages to prevent overloading of the filter system. The solutions were filtered under pressure with nitrogen using a Millipore 142 mm Teflon® coated stainless steel Hazardous Waste Filtration System (Cat. No. YT30 142 HW). The solutions were sequentially passed through Teflon® and Teflon® (PTFE)

coated polypropylene filters ranging from 10μm to 0.45-μm*. The acetone/cyclohexane fume solution was initially filtered through the 10-μm filter then solvent exchanged into the hexane/ MTBE system. Once dissolved into the hexane/MTBE system, the fumes were then filtered to 0.45-μm. After filtration through the 0.45-μm filter, the percent solids in the solution was determined by thermal gravimetric analysis (TGA). The mass of fume to be separated on the chromatographic system was determined by the volume injected multipied by the percent solids.

As a significant amount of material was lost at the filtration steps, the filters were retained under refrigeration in a clean glass jar for recovery by solvent rinsing in the event additional material would be required. After all the other solutions were filtered and fractionated, it was determined that additional material was required. The filters were rinsed with the 9:1 v/v hexane/ MTBE to collect any excess asphalt fume material. The resulting fume solution was filtered, rotary evaporated, and diluted with the hexane/MTBE to a 50% w/v (0.5 g/mL) solution and labelled "Filter Recovery" for fractionation. Table 5 chronicles the weights of the fume through each of the major steps of the fume generation/fractionation process.

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*NOTE -- Teflon 10-μm filter (Millipore LCWP Mitex 10.0-μm) 5 μm (Millipore LSWP 142 50), PTFE membrane on polypropylene 3.0- (Gelman P5PI 142 Zefluor 3μm), 1.0- (Gelman P5PL 142 Zefluor 1μm), and 0.45-μm (Gelman 66151 TF-450) filters obtained from Gelman Sciences, Inc.

TABLE 5

ASPHALT FUME TRACKING

Generation #	Original Mass (g)	Mass after Filtration 10.0 μm (g)	Mass after Filtration 0.45 μm (g)	Chromatography Injected (g)	Chromatography Recovered (g)
21	279	242	-	178	172
18	287	264	-	179	172
17	316	276	-	181	165
19	290	248	-	161	146
14	391	358	-	188	177
13	314	296	-	158	146
12	269	249	-	131	114
9	248	244	-	152	135
11	235	234*	-	136	123
8	283	272	-	188	175
7	117	110	-	77	71
5	184†	89	-	74	62
10	335	90*	-	47	45
4	254	233	-	176	156
15	171	148	-	111	105
2	162	166	133*	105	99
22	281	273	260	220	202
20	152	128	-	104	97
6	131	124	-	99	89
Filter Recovery		280	235	222	202
	4699	4324	-	2887	2653
Set II	1500+	1278	1137	969	906
TOTAL	6199	5602		3856	3559

* -- Noted some irrevocable loss of material during filtration.
† -- Portion of material sent to NIOSH for chemical evaluation.
+ -- 3436.9g generated, only 1500g was used for fractionation, balance of material used as reserve and for other testing.

Note -- Indicated masses for individual runs and totals may vary from other tables due to rounding errors.

TASK II-A -- FRACTIONATION

The scheme for fractionation of the collected fumes was determined by NIOSH (17), based upon Waters Associates preparative liquid chromatographic procedures. The Waters system used an Apple IIE Computer to control a Waters AutoPrep 500A preparative scale high performance liquid chromatograph (HPLC).

The separations (i.e., fractionations) were accomplished using a Waters Associates amine bonded C18 reverse phase packed column, the "NH_2 PrepPak Custom Column" (Cat. No. 10008). The flow of eluants and eluates were controlled through a combination of programming and manual changing of the solvent reservoirs.

To help maintain quality assurance and determine column efficacy through the fractionation phase of the program, a "test mixture" was run daily. The separation of the "test mixture" was conducted purely as a qualitative check of the chromatographic column, pumps, detectors and other chromatographic systems.

Two Waters UV detectors were used concurrently to monitor the effluent. The detectors used were a Model 440, monitoring 313 nm at 2.0 absorbance units full scale (AUFS), and a Model 481, monitoring 345 nm at 1.0 AUFS. The output from both detectors were recorded opposite one another on the same chart recording. The resulting chromatograms had limited utility and they were used primarily in evaluating column efficacy vs. the standard "test mixtures".

Upon visual inspection of the "test mixture" chromatograms, the analyst would make a determination of the column efficacy by comparison with previous chromatograms. If the components in the mixture, as shown by the chromatogram, appeared significantly shifted from their normal elution time, or poorly resolved or recoveries were lower than expected, the column was replaced. In all, ten columns were required to complete the fractionations. The protocol used for the preparation and determination of the "test mixture" is given in Appendix III, Part C.

The procedure, as detailed below, was able to separate and collect, i.e., fractionate, the various classes of compounds. The protocol (as given in Appendix III, Part A), yielded eight (8) different fractions, as listed in Table 6. For each fractionation run, 24-30 mL of a 50% w/v (g/mL) solution of the asphalt fumes was injected into the preparative HPLC system. Each run required approximately one hour to complete before starting the next.

TABLE 6

COMPOSITION OF FRACTIONS BY CHEMICAL CLASS

(AS IDENTIFIED BY GC/MS)

Fraction	Composition
1 - A	C9 to C35 Alkanes, alkenes and monocyclic alkanes Alkylated benzenes Alkylated naphthalenes Benzothiophenes Biphenyls Fluorenes Indanes, Indenes
2 - B	Alkylated benzo- and dibenzo-thiophenes Alkylated benzo-naphthothiophenes Alkylated anthracenes and phenanthrenes Benzo and dibenzo-furans C6 to C26 Olefins Pyrenes and fluoranthrenes Fluoranones
3 & 4 - C	Alkylated phenylethanones C2 to C11 Alkylated dihydrofuranones, dihydroindenones Alkylated cyclo ketones isobenzo furanones hydroxy benzenethiols chrysenes tricarbocyclic fused ring thiophenes
5 & 6 - D	Alkylated phenols Alkylated ketones and acids Carbazoles Furanones
7 - E	C6 to C22 Alkylated ketones and acids Alkylated naphthols and phenols Benzoic acids

The procedure for fractionating the asphalt fume started with eluting hexane (Fisher HPLC Grade) through the column at 250 mL/min. The **first fraction** collection (later designated **Fraction "A"**) was started only after the first compounds had eluted to the end of the column which was timed at one and three quarter minutes. The first fraction contained the alkanes, benzenes and napthalenes, and was collected for one minute and fifteen seconds. At this point, three minutes into the run, the collection of the **second fraction** (**Fraction "B"** -- containing the thiophenes, phenanthrenes, and olefins) was begun. The second fraction was collected for one minute and thirty seconds.

At the three minute and forty-five second mark, the solvent was changed to a solution of 90% hexane and 10% methyl t-butyl ether (MTBE). After allowing this solvent to elute through the column for forty-five seconds, the **third fraction** collection was begun at the four minute and 30 second mark and continued for one and one-half minutes. The third fraction contained the phenylethanones, PAH's, and oxy-napthalenes. The **fourth fraction**, which contained the dihydrofuranones and alkylated cycloalkenes, was collected beginning at the six minute mark for six and one-half minutes. Fractions three and four were later combined and designated **Fraction "C"**.

The solvent was changed at the ten and one-half minute mark to 100% MTBE. At the twelve minute thirty second mark collection of **fraction five** began and continued for seven minutes. The solvent was next changed to methylene chloride (as an intermediary solvent) at the fourteen and one-half minute mark followed by methanol at the eighteen and one-half minute mark. Fraction five was determined to contain di-, tri-, and tetramethyl phenols and aliphatic ketones. Collection of **fraction six**, which contained the C_1 - C_4 phenols, and intermediate chained ketones, began at the nineteen and one-half minute mark and continued for six minutes. Fractions five and six were later combined and designated **Fraction "D"**.

At the twenty minutes and thirty seconds mark, the normal solvent flow was switched to a regeneration solution of ~90% methanol, ~10% water and 0.05% triethylamine (pH adjusted to 8.5 with triethylamine) at a flow rate of 100 mL per min. The NH_2 Prep HPLC column was reconditioned with the alcohol/triethylamine solution to cleave any Schiff base products which may have formed on the amine column stationary phase as the aldehydes and ketones eluted off.

Collection of the final fraction of interest (**fraction seven**) began at twenty-five minutes and thirty seconds and continued for fifteen minutes. To begin returning the system to initial conditions, one hundred precent (100%) methanol (100 mL per minute) was run into the system at the thirty minute and thirty second mark. The normal flow of 250 mL per minute was restored at thirty-eight minutes. Fraction seven was later designated **Fracton "E"**.

The solvent was changed back to methylene chloride at the thirty-eight minute thirty second mark and collection of **fraction eight** was started

two minutes later. The solvent was switched to MTBE at the forty-one minute mark followed by the 9:1 Hexane:MTBE mixture at the forty-three minute mark and finally 100% hexane at the forty-four minute mark. Fraction eight was collected until the completion of the program at forty-eight minutes. In late July, 1985, Mr. Belinky communicated with us to indicate the the collection of fraction 8 was no longer required.

On a daily basis, all runs of a given fraction would be combined and the solvent evaporated. An aliquot of each of the daily composited fractions was then sent to NIOSH for evaluation by infrared analysis to determine the final acceptability of the fraction. Any fractions which were deemed unacceptable were re-fractionated. The compound classes are listed more specifically for the fractions 1-7 in Table 6. The designation of the fractions was later changed to simplify the preparation of the skin painting formulations.

As indicated earlier, two separate sets of fume generation and fractionation were performed to obtain sufficient material for all the chemical and bioassay procedures to be performed. The first set of fractionations of the fumes, which were generated in the first quarter of 1984, was conducted from 6/22/85 through 10/15/85 and included the fume which was recovered from the filtration step described in the previous section. These fractionation runs are summarized in Table 7. In that time period, approximately three kilograms of fume were fractionated requiring just over 300 separation runs.

TABLE 7

ASPHALT FRACTIONATION RUNS 3-318
6/27/85 - 10/15/85

Total Injected 2884.3g

Fraction	Cumulative Wt. (g)	% of Amount Injected
1	1672.7	57.9
2	228.1	7.9
3 & 4	288.2	10.0
5 & 6	298.9	10.4
7	143.6	5.1
8*	18.9	0.7
TOTAL	2652.5	92.0

From Asphalt Generations 1-22 (excluding 1, 3, and 16) and Filter Recovery.

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* Collection of Fraction 8 was discontinued on July 30, 1985.

Through late October and early November, 1985 NIOSH determined that additional material would be required, and more fume was generated, a second set of separations was made in December. As indicated in Table 8, an additional kilogram of fume was fractionated in just over eighty (80) runs yielding just over 900 grams of individual fractions.

TABLE 8

ASPHALT FRACTION RUNS 320-403
12/10/85 - 12/26/85

Total Injected 967.9 g injected

Fraction			Cumulative Wt. (g)	% of Amount Injected
1	-	A	594.4	61.4
2	-	B	66.8	6.9
3 & 4	-	C	83.7	8.6
5 & 6	-	D	107.2	11.1
7	-	E	53.5	5.5
TOTAL			905.6	93.5

At the completion of the fractionation of all the fume solutions, the removal of the hexane/MTBE solvents, and their subsequent approval for inclusion in the study by NIOSH, all of the "A" fractions from the two sets of generations/fractionations were composited, weighed, and diluted to 50% w/v (g/mL) with the 1:1 v/v acetone/cyclohexane. The other individual fractions were similarly combined (i.e., all of the "B" fractions, all of the "C" fractions, all of the "D" fractions and all of the "E" fractions).

Those fractions whose analysis were indicated to be out-of-specification by NIOSH as listed:

15-Jul-85	Fraction #1
29-Jul-85	Fraction #2, #3, #4
08-Aug-85	Fraction #5 & 6
19-Aug-85	Fraction #5 & 6
30-Aug-85	Fraction #5 & 6
16-Sep-85	Fraction #2
10-Oct-85	Fraction #3 & 4
18-Dec-85	Fraction #2

were recombined (15-Jan-86), and refractionated (16/17-Jan-86) before inclusion in the fraction composites used in the treatment formulations.

TASK II-B - APPLICATION SOLUTION FORMULATIONS

In a communication to Arthur D. Little, Inc., Dr. Niemeier of NIOSH indicated the proportioning of the various fractions (Appendix IV) to yield the required concentrations of the various compound classes. The proportioning of the fume fractions, indicated by Dr. Niemeier, were calculated to simulate the concentrations found in the whole asphalt fume as 0.5 g fume/mL solutions. The basis of this study being to reproduce, as closely as possible, the whole fume solutions prepared in the previous study on asphalt fume carcinogenicity performed at Arthur D. Little, Inc., (Contract 210-78-0035) and to further determine which components of the fume are the more potent carcinogens and/or cocarcinogens.

NIOSH had determined that forty-two (42) different combinations of the raw asphalt, asphalt fume fractions, solvent or cocarcinogens should be tested as outlined in Table 9. For each group, it was decided that thirty animals (30) would be tested over a two-year period (104 weeks). Over that period, the animals would be treated twice per week with fifty (50) microliters of a specific test solution. The volume of test solution required per group, therefore, would be 312 mL, which was increased to 400 mL as a hedge against potential losses. The volume assumptions are further based on all the animals surviving the full term of the experiment. In actuality, this was not expected to happen and would provide a greater margin. Therefore, all calculations regarding quantities of materials were based on 400 mL of a 50% w/v (g/mL) solution of unfractionated asphalt fume or 200 g of unfractionated asphalt fume, for each of the groups. These calculations were then corrected for recovery of fume from the fractionation process.

Solution Preparation

Based upon the mass of fume recovered from the HPLC fractionation process, NIOSH determined the following asphalt fume composition for reformulation:

TABLE 10

Fraction Recoveries and Recombination

Fraction	% of Total Recovered Mass	Theoretical Composition (mL from 400mL)	Practical Volume of Fraction (mL)
A	64.1	256.4	255
B	8.3	33.2	40
C	10.5	42.0	40
D	11.5	46.0	40
E	5.6	22.4	25
Total Fume	100.0	400.0	400

TABLE 9 - Asphalt Application Scheme

	Group																																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Mouse Type A - C3H/HeJ B - Sencar	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B
Raw Asphalt	x	x[1]	x[2]																																							
Asphalt Fume			x[2]	x																																					x	
Dilution Solvent (Vary)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x
Frac. A						x					x	x	x	x	x		x	x			x	x	x	x			x	x	x							x						
Frac. B							x				x	x				x	x	x	x	x		x																				
Frac. C								x			x		x			x	x	x	x	x	x																					
Frac. D									x		x			x		x	x		x		x	x	x							x	x	x					x				
Frac. E										x	x				x	x		x			x	x	x										x	x	x			x				
B(a)P .01% .001% .0001% .4%																								x	x	x	x	x	x	x	x	x	x	x	x	(x)	(x)	(x)	(x)	[3]		

[1] Heated asphalt less fumes

[2] Heated asphalt plus fumes

[3] Parentheses indicates a single initial treatment with 50 µl of 4.0 µg B(a)P/µL solution followed by regular treatment with the indicated fraction.

While Table 10 indicates the percentage which each fraction represented of the material recovered during fractionation, it was apparent that there was not 100% recovery of the material injected onto the column (as previously indicated in Table 5). The concentration of each fraction had to be adjusted to correct for the fume which was not recovered in the chromatography process. To reformulate the fume fractions into the various combinations for carcinogenicity testing at concentrations representative of the original fume, the actual concentration of each fraction in the unfractionated fume had to be determined.

NIOSH chose to make the determination based on the presence of a tracer or indicator compound in the asphalt fume. The tracer, n-dodecane, was chosen as it elutes in Fraction A. To discern the mass ratio of Fraction A to total fume based upon the n-dodecane concentration, a comparative analysis was performed by NIOSH for n-dodecane in both Fraction A and the unfractionated fume. The concentration in the total fume was found to be 1.6 mg n-dodecane/g fume. Based on the requirement for 200 g of asphalt fume per solution, 320 mg of n-dodecane would be present. As Fraction A was found to contain 3.4 mg n-dodecane/g, 94.1 g was used in those solutions requiring "A". Therefore, 94.1 g of Fraction A should be made up to 400 mL with a combination of the mixed solvent or other fractions to generate a concentration of Fraction A consistent with a 50% w/v (g/mL) concentration of unfractionated fume.

Since 15 test groups were to be treated with solutions containing Fraction A, 1410 g were required for the 15 solutions of 255 mL for a volume of 3825 mL. To simplify the preparation, a total volume of 4000 mL was made, requiring 1480 g of neat Fraction A. The mass of the remaining fractions needed in solutions containing those fractions was based on the ratio of the other recovered fractions to Fraction A. This preparation information is shown in Table 11. The theoretical volumes for these preparations was similarly increased as insurance against loss. Benzo(a)pyrene standard solutions were required for cocarcinogenic evaluation and prepared as shown in Table 12.

As the fractions had already been prepared as 50% w/v (g/mL) solutions and gravimetric analyses performed, the existing solutions were simply diluted further, as indicated in the table below, to achieve the desired concentration (Table 13).

The detailed protocol for application solution preparation is shown in Appendix V.

TABLE 11

MASS RATIOS OF FRACTIONS

Fraction	Mass Ratio Compared to Fraction A	Mass of Fraction to Yield Correct Solution	Proposed Volume of Individual Fractions	# of Solutions	Theoretical Solution Prep. Total Mass/Total Vol.	Proposed Solution Prep. Total Mass/Total Vol.
A	1.00	94.1	255	16	1410/3820	1480/4000
B	.124	11.7	40	9	105/360	117/400
C	.162	15.2	40	9	137/360	152/400
D	.174	16.4	40	13	213/520	246/600
E	.068	6.35	25	12	76.2/300	102/400
TOTAL		143.7	400			

TABLE 12

BENZO(a)PYRENE SOLUTIONS

Final B(a)P Conc.	Stock B(a)P Conc. (mg/ml)	Stock Vol. (ml)	Stock Mass B(a)P (mg)	Final Solution Vol. (ml)	Final B(a)P ug/ul (ppth)
.01%	4.00	10 ml	40	400	0.1
.001%	0.40	10 ml	04	400	0.01
.0001%	0.04	10 ml	0.4	400	0.001
.4% (Single-dose)	4.0	50 µl	200 µg		

TABLE 13

FRACTION FORMULATIONS

Fraction	Gravimetric Analysis of Stock (g/mL)	Mass of Fraction Required (g)	Volume of Fraction Required (mL)	Final Dilution Volume (mL)
A	.500	1480	2960	4000
B	.464	117	252	400
C	.509	152	299	400
D	.456	246	539	600
E	.526	102	194	400

TASK III - BIOASSAY

Methods and Procedures

The specific details of the bioassay are described in the protocol which is shown in Appendix VI along with the protocol amendments executed during the study.

The specific chemical fractions isolated in Task II for use in the bioassay task are identified in Table 13. In addition to these fractions alone, in combination with each other and with benzo(a)pyrene, the following samples were tested:

- Raw asphalt diluted 50% w/v (g/mL),
- Residue of raw asphalt heated to 316°C for seven hours with escape of fumes diluted 50% w/v (g/mL),
- Raw asphalt heated to 316°C for seven hours as described above recombined with fumes diluted 50% w/v (g/mL)
- Neat asphalt fume diluted 50% w/v (g/mL).

The solvent used throughout was acetone:cyclohexane (1:1), HPLC grade. No further analysis for contaminants was performed.

The mice used for the study, except for 2 groups with male Sencar mice to test for strain variation in the response were male C3H/HeJ mice obtained from the Jackson Laboratories, Bar Harbor, Maine. The specific treatment groups are shown in Table 14. Groups 1 through 4 examined the responses following treatment with the starting asphalt material and combinations of fume and fume residue. Group 5 was the solvent control. Groups 6 through 23 tested the effects of single fractions obtained in the fractionation process and various combinations of the 5 fractions. Groups 24 through 35 tested for the cocarcinogenicity of three fractions (A, D and E) with three different doses of benzo(a)pyrene prepared on a weight to volume basis (w/v) in terms of grams per milliliter (g/mL) (i.e., 0.01%, 0.001% and 0.0001%). Groups 37 through 39 tested for the tumor promoting activity of fractions A, D and E following initiation with a single treatment of 200 μg of benzo(a)pyrene. Group 40 was the sentinel group for monitoring the health of the C3H/HeJ mice during the course of the study and 5 animals per group were tested for serum viral antibody titers before the study began and at the 6, 12, 18 and 24 month time points during the study. Groups 41 and 42 were Sencar mice used to test for strain variation in response to treatment with the neat asphalt fumes. Under some conditions, the Sencar mice have shown exceptional sensitivity to chemical induction of skin tumors.

TABLE 14

TREATMENT GROUPS

Group Number	Treatment	Group Number	Treatment
1	raw asphalt	24	.01% B(a)P
2	heated asphalt	25	.001% B(a)P
3	heated asphalt plus fume	26	.0001% B(a)P
4	neat asphalt fume	27	fraction A + .01% B(a)P
5	solvent control	28	fraction A + .001% B(a)P
6	fraction A	29	fraction A + .0001% B(a)P
7	fraction B	30	frcation D + .01% B(a)P
8	fraction C	31	fraction D + .001% B(a)P
9	fraction D	32	fraction D + .0001% B(a)P
10	fraction E	33	fraction E + .01% B(a)P
11	frctions A,B,C,D,E	34	fraction E + .001% B(a)P
12	fractions A,B	35	fraction E + .0001% B(a)P
13	fractions A,C	36	*Init then A
14	fractions A,D	37	*Init then D
15	fractions A,E	38	*Init then E
16	fractions B,C,D,E	39	*Init alone
17	fractions A,B,C,D	40	Sentinal mice
18	fractions A,B,C,E	41	Sencar fume
19	fractions B,C,D	42	Sencar control
20	fractions B,C		
21	fractions A,C,D,E		
22	fractions A,B,D,E		
23	fractions A,D,E		

*Mice were initiated with a single application of 200 μg of benzo(a)pyrene (50 μL of 4.0 μg B(a)P/μL) followed by twice weekly application of the fractions.

In order to provide for the large number of mice on the study, the C3H/HeJ mice were received in two shipments one week apart. The treatments for groups 1 through 20 started on July 18, 1986, and for groups 21 through 40, the treatment started on July 11, 1986. The Sencar mice were started on treatment on July 3, 1986. Initially, the mice were gang housed with 4 mice per cage, but it was noticed shortly into the study that the aggressive behavior of the mice could compromise the results, and it was decided to house the mice singly for the duration of the experiment. The mice were weighed prior to the start of treatment for randomization and assignment to groups. The mice were then weighed weekly for the first six weeks of treatment, and every four weeks thereafter until the last weighing at sacrifice. Clinical observations were made once a week and data recorded onto computer data files generated and prepared by NIOSH in an IBM-PC format. Floppy discs with data for each week were sent to NIOSH weekly during the course of the study. In addition, duplicate discs and print-outs of the data were kept at the contractor's facility.

Observations of the skin condition and the tumors as they occurred were carried out using a standard set of descriptors shown in Table 15. The data from these observations were also included on the floppy discs sent to NIOSH weekly. Confirmation of gross diagnosis for papillomas and carcinomas was carried by the study director (Case Leader) approximately every two weeks. Unusual lesions were examined and evaluated as soon as they were observed. Because the tumor burden in some test animals became quite large, it was decided to establish a sacrifice criterion for animals bearing grossly diagnosed carcinomas that were more than 3 cm in diameter and had persisted for more than 4 consecutive weeks.

All animals dying or sacrificed during the study and at the termination of the experiment at 24 months were subjected to gross necropsy. Only the skin tumors and adjacent normal skin samples were taken for histopathological analysis. The skin tumor sections were prepared and stained at the contractor's laboratory. A detailed summary of the observed gross skin lesions is presented in Appendix IX. The size and location of each lesion (mass) on the day of death or sacrifice is summarized on a mouse and treatment group basis.

Histopathological diagnosis was carried out by NIOSH. The skin sections were initially evaluated by a NIOSH pathologist and then independently reviewed by a second pathologist. Both reviews were conducted blind with the evaluators not knowing the identity of the treatment group of the tissue section. After both independent reviews were completed, the pathologists met and reached a consensus definitive diagnosis on all skin lesions. Diagnostic criteria used to assess the skin specimens (slides) and a detailed listing of the histopathology results per mouse and per treatment gropu are presented in Appendix X.

The statistical evaluation of data was carried out by NIOSH in accordance with the protocol. The data elements subjected to statistical analysis were body weights, survival, and tumor yields. For

TABLE 15

GLOSSARY OF TERMS USED FOR THE DESCRIPTION OF SKIN APPEARANCE

Depilation	Loss of hair caused by treatment with test substance.
Pared epidermis	Adventitious removal of section 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.
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 lateral and/or ventral extensions: regression unlikely.

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

the purpose of tumor analysis, carcinomas and sarcomas were combined and these malignant tumors are referred to as "carcinomas". Similarly, "papillomas" include papillomas, keratoacanthomas and fibromas.

Analysis of mortality involved computation of the product limit (Kaplan-Meier) survival curve. Terminal sacrifices were counted as censored observations. Comparison of the treatment groups to their relevant controls used the modified Wilcoxon (Breslow) test. No attempt was made to adjust for multiple comparisons. All p-values in this test were two-sided, implying that either an increase or a decrease in mortality was of interest. Because of the volume of data on weights, analyses focused on weights on or near days 100, 200, ... 700. A separate one factor analysis of variance (ANOVA) was run at each of the seven time points and no adjustment was made for multiple comparisons at this stage. If the ANOVA was significant at 0.05, then comparisons to the relevant control using a two-sided Dunnett's test (which does adjust for multiple comparisons) were calculated. Analysis of tumor response was similar to analysis of mortality. The time to first tumor (or first carcinoma or first papilloma) was recorded. A censored observation represented an animal who died or was sacrificed prior to developing a tumor. All comparisons of treatment groups used the modified Wilcoxon (Breslow) test. Again, no attempt was made to adjust for multiple comparisons and the p-values reported are two-sided.

Detailed statistical reports summarizing the analysis of the in-life and histopathologically confirmed skin tumors are presented in Appendix VII and Appendix VIII, respectively.

The development of the protocol and the conduct of the study were carried out in accordance with the Good Laboratory Practice rules of the U.S. Food and Drug Administration. The study was monitored by the Quality Assurance Unit of the test laboratory and audited by the sponsor.

RESULTS

Mortality

The survival data for the study are shown in Table 16. The primary perturbing factor that contributed to a reduction in mortality in certain test groups of the C3H mice was the occurrence of carcinomas that resulted in sacrifice of animals in accordance with the criteria established in protocol amendment number 4. The groups receiving repeated doses of benzo(a)pyrene at 0.01% (Groups 24, 27, 30 and 33) were most strongly affected in this way. There were no survivors to the end of the two year treatment interval in any of these groups. Similarly, all treated groups that were administered a combination of fractions B and C (Groups 11,16,17,18,19,20) also exhibited high tumor yields, and thus, reduced mean survival times. The reduced mean life spans of mice in groups 36, 37 and 38 and 39 were the result of a number of fatalities due to infection very early in the study. To reduce the possibility of infection, animals were given acidified water to drink from the third month of the study until its termination.

Sentinal Animal Screening

Animals from the sentinal group were testing in groups of 5 for serum viral antibodies before the study began and at 6, 12, 18, and 24 months. Titers for the following viruses were uniformly negative throughout the study: pneumonia virus of mice, reovirus type 3, Theiler's encephalitis virus (GD7), polyoma, ectromelia, sendai and lymphocyte choriomeningitis virus. At 12 months titers for mouse hepatitis virus became elevated and remained elevated at the 18 and 24 month assay. Titers for minute virus of mice became elevated at 18 months and remained elevated at 24 months. It is unlikely that these changes had any influence on the skin tumor response.

Body Weights

The data for body weights at 100 day intervals of the treatment period are shown in Table 17. At day 200, several groups showed statistically significant lower body weights as compared to controls, and at days 400 and 500 a few groups showed statistically significant increased body weights as compared to controls. The small magnitude of these changes and their random distribution in terms of time and treatment regimen strongly indicate that they have no biological relevance to the results of the study. The Sencar mice were substantially heavier than the C3H mice throughout the entire study.

TABLE 16

ANALYSIS OF MORTALITY

Group Number	Treatment	Mean Survival (Days)	Number Died	50th Percentile Survival (Days)*
1	raw asphalt	610	15	698
2	heated asphalt (less fume)	655	12	---
3	heated asphalt (plus fume)	692**	9	---
4	neat asphalt fume	526***	28	573
5	solvent control	607	19	629
6	fraction A	690**	11	---
7	fraction B	643	18	678
8	fraction C	610	24	659
9	fraction D	572	23	588
10	fraction E	629	17	675
11	fractions A,B,C,D,E	555***	29	533
12	fractions A,B	608	22	601
13	fractions A,C	583	27	573
14	fractions A,D	660	15	702
15	fractions A,E	622	20	656
16	fractions B,C,D,E	580	29	589
17	fractions A,B,C,D	551***	28	559
18	fractions A,B,C,E	582	29	583
19	fractions B,C,D	577	25	573
20	fractions B,C	566***	29	570
21	fractions A,C,D,E	597	23	615
22	fractions A,B,D,E	593	15	684
23	fractions A,D,E	562	22	530

TABLE 16 (continued)
ANALYSIS OF MORTALITY

Group Number	Treatment	Mean Survival (Days)	Number Died	50th Percentile Survival (Days)*
24	.01% B(a)P	449***	30	464
25	.001% B(a)P	666	15	732
26	.0001% B(a)P	630	15	727
27	fraction A + .01% B(a)P	538***	30	540
28	fraction A + .001% B(a)P	620	18	589
29	fraction A + .0001% B(a)P	692**	10	---
30	fraction D + .01% B(a)P	504***	30	491
31	fraction D + .001% B(a)P	623	15	705
32	fraction D + .0001% B(a)P	641	12	---
33	fraction E + .01% B(a)P	447***	30	459
34	fraction E + .001% B(a)P	553	17	526
35	fraction E + .0001% B(a)P	608	18	607
36	B(a)P 200 μg then A	466***	22	495
37	B(a)P 200 μg then D	480***	21	504
38	B(a)P 200 μg then E	500***	25	513
39	B(a)P 200 μg alone	530***	22	512
40	Sentinal mice			
41	Sencar fume	571	25	592
42	Sencar control	672	12	---

* Groups not having an entry had more than half the animals in a group surviving to the end of the study. The upper bound is 732 days due to terminal sacrifice.

** Survival significantly longer (P = 0.05) than corresponding control.

***Survival significantly shorter (P = 0.05) than corresponding control. The shortened survival of Groups 36, 37, 38 and 39 was the result of early fatalaties due to urinary tract infections.

TABLE 17

ANALYSIS OF BODY WEIGHTS
(mean body weight of survivors, grams)

Group Number	Treatment	Treatment Day 100	200	300	400	500	600	700
1	raw asphalt	26.97	26.69	25.93	28.58	24.76	22.83	24.34
2	heated asphalt	26.74	26.21	25.26	27.97	24.84	22.88	24.07
3	heated asphalt plus fume	26.70	27.35	25.70	28.27	24.80	22.96	24.49
4	neat asphalt fume	26.33	25.99	24.40	27.81	23.58	20.15	17.75
5	solvent control	27.28	26.96	26.14	27.25	23.27	21.01	22.24
6	fraction A	27.23	27.37	26.64	27.88	24.50	21.14	23.95
7	fraction B	27.20	26.05	26.38	27.59	23.06	21.89	23.56
8	fraction C	26.92	27.03	27.16	27.66	24.40	21.26	22.32
9	fraction D	26.07	26.52	26.32	26.50	22.60	20.96	22.33
10	fraction E	26.41	26.73	26.28	27.01	23.21	20.77	23.15
11	fractions A,E	27.15	27.55	27.18	27.99	24.19	20.49	20.62
12	fractions A,B	26.22	27.02	26.27	26.95	23.06	20.14	22.31
13	fractions A,C	25.89	26.28	27.04	27.44	21.69	19.18	19.82
14	fractions A,D	26.00	25.85	26.84	26.90	23.31	20.19	23.35
15	fractions A,E	25.62	25.81	26.93	26.22	22.97	20.86	22.78
16	fractions B,C,D,E	26.62	26.68	27.61	27.21	23.68	20.35	18.96
17	fractions A,B,C,D	27.17	27.00	27.49	27.55	25.62	21.46	21.30
18	fractions A,B,C,E	26.96	27.15	27.38	27.82	25.37	20.57	21.00
19	fractions B,C,D	26.73	26.26	26.61	27.12	24.83	20.45	22.99
20	fractions B,C	27.43	27.07	27.62	27.51	26.27(2)	20.80	21.60
21	fractions A,C,D,E	26.52	25.38	25.92	27.73	24.49	20.75	23.05
22	fractions A,B,D,E	26.00	25.28(1)	25.28	27.18	23.42	21.42	24.06
23	fractions A,D,E	26.90	25.71	25.33	26.94	23.91	21.65	24.20

(1) Average significantly less than corresponding control.
(2) Average significantly greater than corresponding control.

TABLE 17 (Continued)

ANALYSIS OF BODY WEIGHTS
(mean body weight of survivors, grams)

Group Number	Treatment	Treatment Day 100	200	300	400	500	600	700
24	.01% B(a)P	27.09	25.48	25.29	27.98	25.04	22.98	---
25	.001% B(a)P	26.96	23.48(1)	24.93	26.30	24.10	21.61	22.75
26	.0001% B(a)P	27.12	24.02(1)	25.13	26.88	24.44	22.02	22.93
27	fraction A + .01% B(a)P	27.31	24.62(1)	25.52	26.18	24.05	20.11	24.75
28	fraction A + .001% B(a)P	26.89	24.76(1)	26.26	26.28	23.63	21.22	24.18
29	fraction A + .0001% B(a)P	27.08	26.56	26.13	30.03(2)	24.69	21.78	23.90
30	fraction D + .01% B(a)P	27.18	25.71	25.44	29.13(2)	21.71	20.58	23.07
31	fraction D + .001% B(a)P	27.60	25.46	26.71	27.68	23.42	20.86	23.07
32	fraction D + .0001% B(a)P	26.89	25.70	26.99	29.08	24.92	22.74	24.14
33	fraction E + .01% B(a)P	26.29	25.23(1)	26.76	26.99	22.69	20.38	24.20
34	fraction E + .001% B(a)P	26.58	25.49	26.43	28.20	23.34	22.59	24.79
35	fraction E + .0001% B(a)P	27.00	25.44	27.48	26.03	23.29	20.54	22.74
36	B(a)P 200 µg then A	27.17	25.59	25.97	26.69	23.12	20.84	22.75
37	B(a)P 200 µg then D	26.75	25.88	26.34	29.36(2)	24.22	22.32	23.48
38	B(a)P 200 µg then E	26.70	25.62	26.35	29.50	23.71	21.10	21.07
39	B(a)P 200 µg alone	26.47	25.86	26.82	28.28	23.62	20.40	24.53
40	Sentinal mice	26.71	25.63	26.09	28.82	24.56	22.68	22.80
41	Sencar fume	40.00	39.33	40.98	40.31	40.37	39.56	38.28
42	Sencar control	38.52	37.82	40.11	39.85	39.67	39.53	39.25

(1) Average significantly less than corresponding control.
(2) Average significantly greater than corresponding control.

Clinical Responses

Several clinical responses were noted which occurred randomly among the test groups and with very low incidence (3-15%). Among these responses were lanquid behavior, soft feces, lacrimating eyes, and dyspnea. A hunched stature was seen at 50% incidence in one test group (#6), with incidences of 20 to 30% in 14 other test groups. There appeared to be no discernable pattern of test material exposure related to this behavior, and the solvent control group (#5) had an incidence of 30%. With respect to skin lesions, Group 24, the highest dose of benzo(a)pyrene (0.01%) applied repeatedly alone, had an incidence of 80% of scabs and sores. Group 33, treated with benzo(a)pyrene at 0.01% and fraction E, also had a high incidence (50%) of similar lesions. In contrast, Groups 27 and 30, which had the same dose of benzo(a)pyrene with fractions A and D, respectively, showed no unusual skin lesions. None of the fractions, either singly or as mixtures induced a notable skin response.

Tumor Response

The discussion of the tumor responses in this study is best presented in terms of the various questions that were posed in the original design of the study. The most important set of variables was the tumorigenic action of the various fractions derived from the HPLC separation. The incidences of papillomas and carcinomas per group, the average time to tumor, and the time to 50% tumors, are presented in Table 18. It is important to note that many skin tumors exhibited progressive changes from benign (papillomas or keratoacanthomas) to malignant variants (carcinomas). Figures 4 through 8 present the gross tumor incidence observed in selected groups during the in-life phase of the study. The gross tumor incidence was calculated as the fraction of mice at risk (survivors plus dead tumor bearers) bearing papillomas and carcinomas based on clinical observations summarized on a monthly basis.

It is clear that fractions B and C accounted for the majority of the tumorigenic activity in the collected asphalt fumes, with fraction C being somewhat more potent based on the total yield of carcinomas. Fractions A, D, and E showed no tumorigenic activity. Combination of fraction A with either fraction B or C in binary mixtures did not markedly influence the tumor response, although the combination of fraction A with fraction C did reduce the carcinoma yield as compared to the response with fraction C alone. The test groups with 4 fractions and also containing Fractions B and C (Groups 16,17, and 18) did not alter the response seen with the mixture of Fraction B and C (Group 20). Thus, mixtures of pairs of fractions using one from the inactivate (Fractions A,D and E) did not influence the response with the mixture of Fractions B and C.

TABLE 18

TUMOR RESPONSE

Group Number	Treatment	Time to First Tumor (Days) Papilloma	Time to First Tumor (Days) Carcinoma	Mice With Carcinoma	Average Time (Days) to Carcinoma	Time (Days) To 50% Carcinoma
1	raw asphalt	378	456	3	709	
2	heated asphalt					
3	heated asphalt plus fume					
4	neat asphalt fume	378	378	20	517	525
5	solvent control					
6	fraction A					
7	fraction B	343	406	10	688	728
8	fraction C	441	479	17	599	590
9	fraction D					
10	fraction E	606*				
11	frctions A,B,C,D,E	343	378	19	528	525
12	fractions A,B	378	479	8	682	714
13	fractions A,C	406	479	11	627	590
14	fractions A,D					
15	fractions A,E	571*				
16	fractions B,C,D,E	305	378	15	565	569
17	fractions A,B,C,D	277	378	18	560	562
18	fractions A,B,C,E	277	378	21	542	532
19	fractions B,C,D	406	479	15	605	591
20	fractions B,C	305	406	24	514	532
21	fractions A,C,D,E	312	406	14	620	649
22	fractions A,B,D,E	406	479	7	695	
23	fractions A,D,E	486				

Blanks in the table indicate that these parameters could not be obtained because of the absence of papillomas or carcinomas in particular groups.

*Papillomas were observed grossly in these groups, but were not confirmed histologically.

TABLE 18 (Continued)

TUMOR RESPONSE

Group Number	Treatment	Time to First Tumor (Days) Papilloma	Time to First Tumor (Days) Carcinoma	Mice With Carcinoma	Average Time (Days) to Carcinoma	Time (Days) To 50% Carcinoma
24	.01% B(a)P	312	385	27	391	397
25	.001% B(a)P	486	521	3	717	
26	.0001% B(a)P	486*				
27	fraction A + .01% B(a)P	385	385	23	486	482
28	fraction A + .001% B(a)P	641	641	1	741	
29	fraction A + .0001% B(a)P	669		1	742	
30	fraction D + .01% B(a)P	312	385	25	445	447
31	fraction D + .001% B(a)P	521				
32	fraction D +.0001% B(a)P	613		1	739	
33	fraction E +.01% B(a)P	312	385	23	429	419
34	fraction E +.001% B(a)P	578		2	739	
35	fraction E +.0001% B(a)P					
36	B(a)P 200 g then A					
37	B(a)P 200 g then D	613*				
38	B(a)P 200 g then E					
39	B(a)P 200 g alone	514*				
40	Sentinal mice					
41	Sencar fume	292	393	14	578	596
42	Sencar control					

*Papillomas were observed grossly in these groups, but were not confirmed histologically.

Figure 4

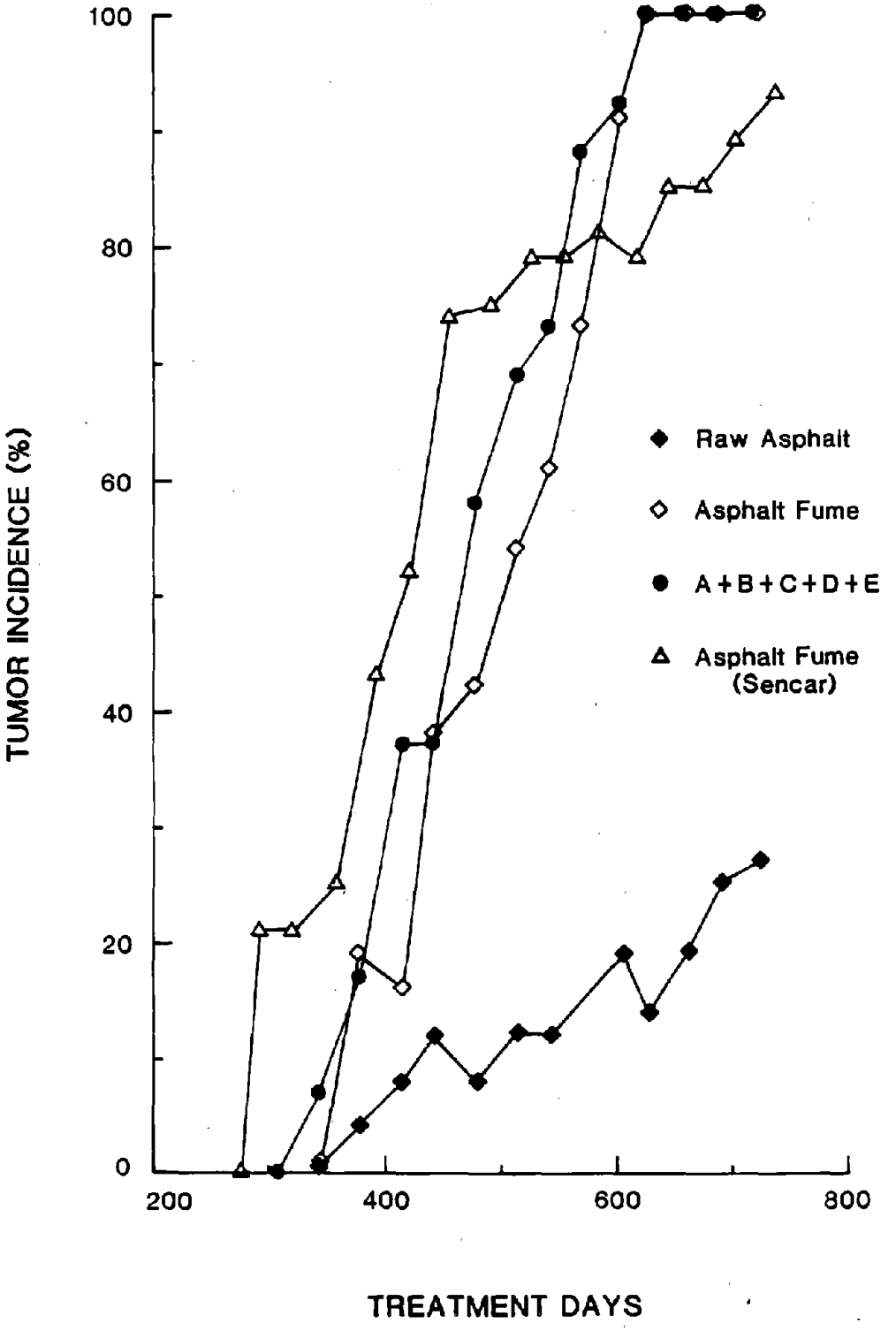

100
80
60
40
20
0
200
400
600
800
TUMOR INCIDENCE (%)
TREATMENT DAYS
Raw Asphalt
Asphalt Fume
A+B+C+D+E
Asphalt Fume (Sencar)

Figure 5

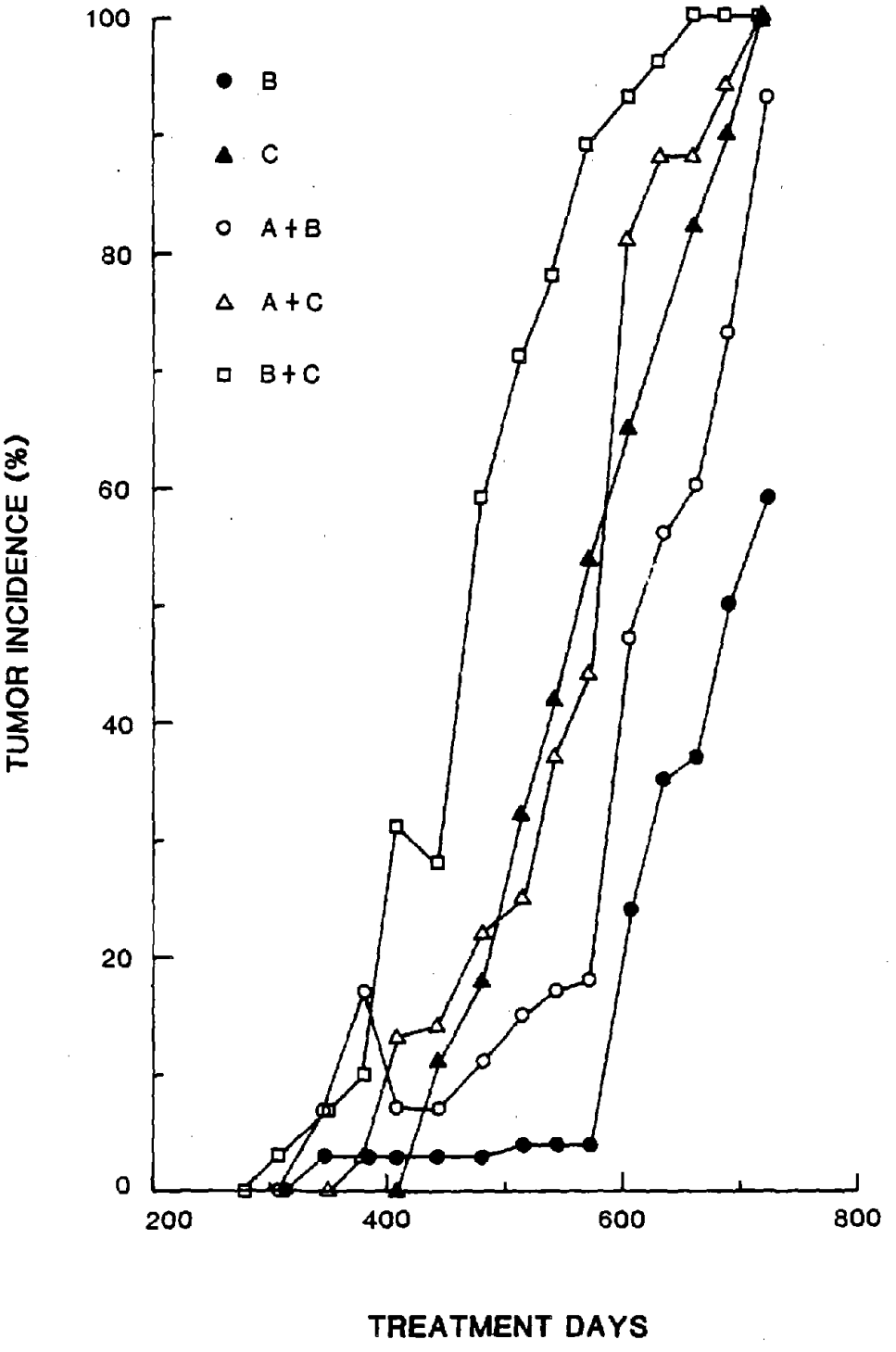

100
80
60
40
20
0
B
C
A+B
A+C
B+C
TUMOR INCIDENCE (%)
200
400
600
800
TREATMENT DAYS

Figure 6

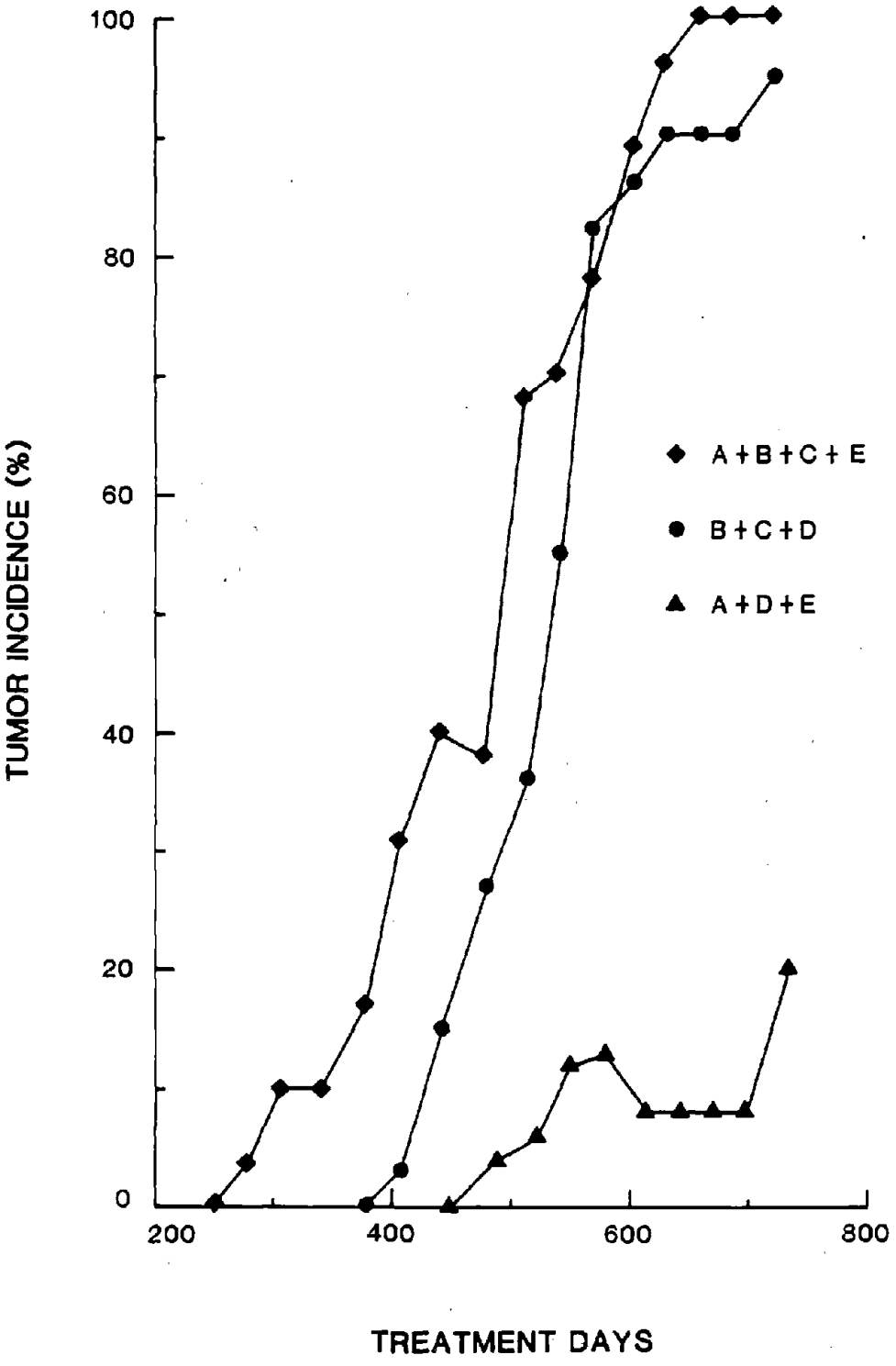

100
80
60
40
20
0
TUMOR INCIDENCE (%)
200
400
600
800
TREATMENT DAYS
A+B+C+E
B+C+D
A+D+E

Figure 7

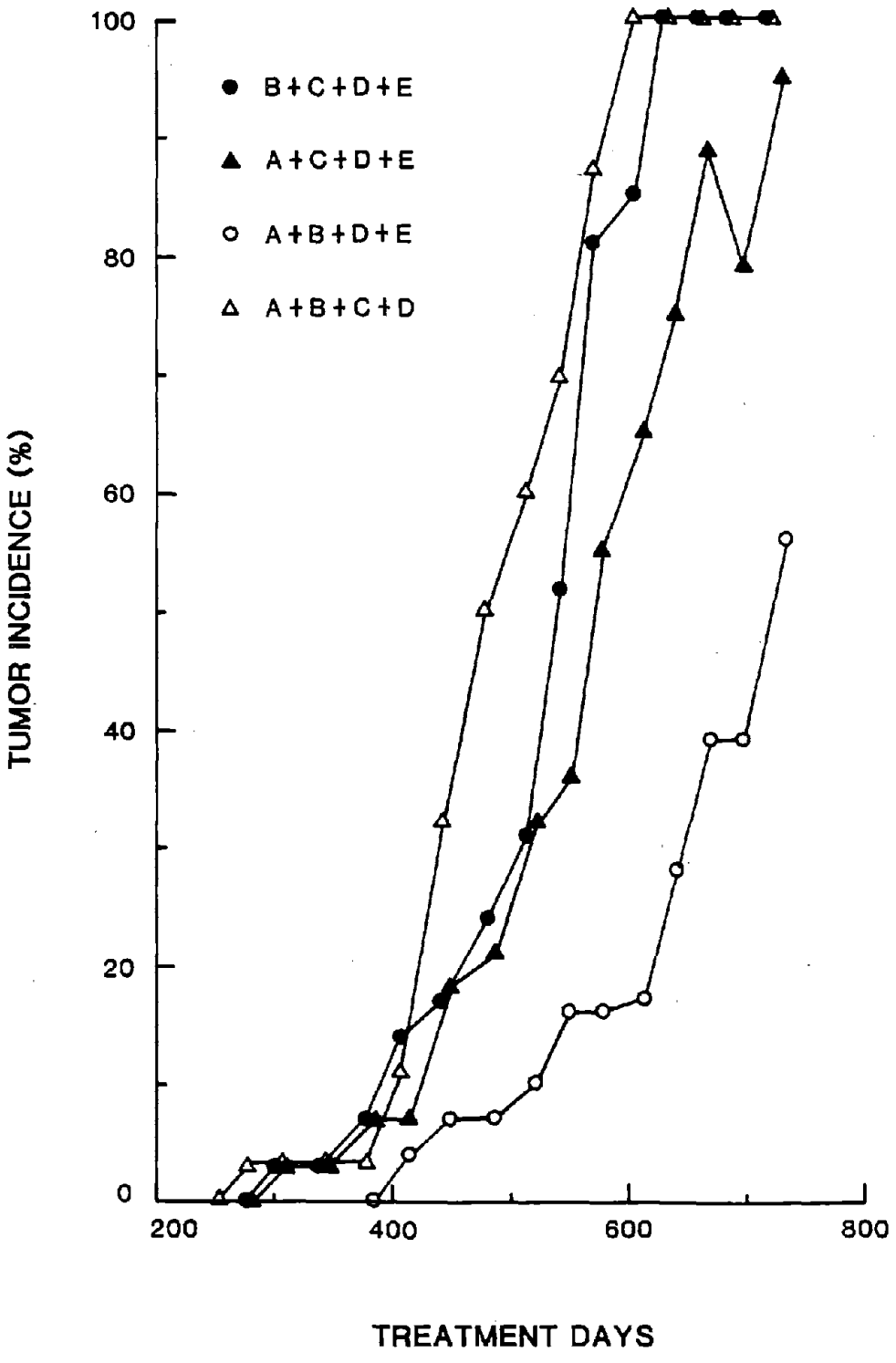
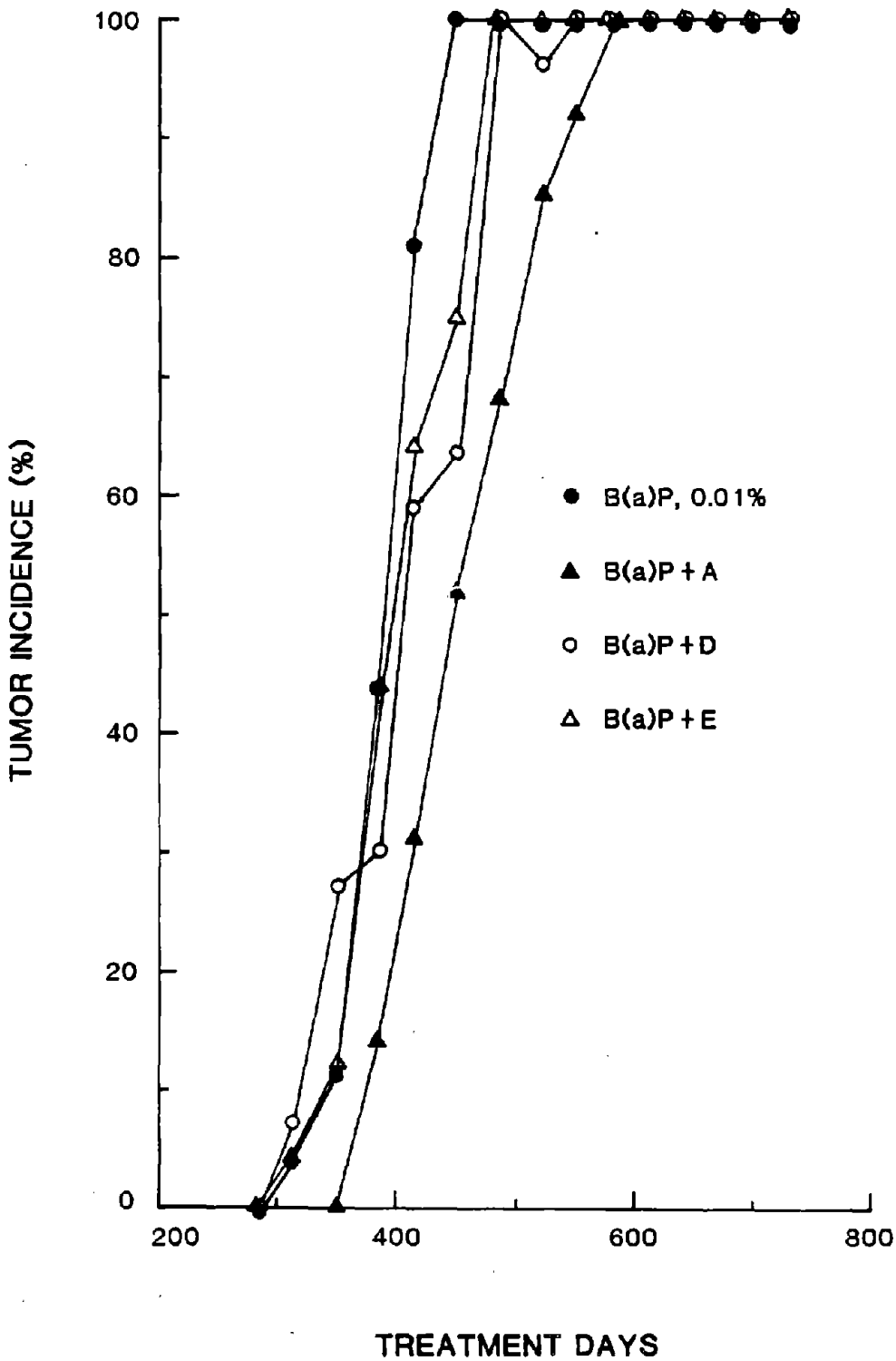

100
80
60
40
20
0
200
400
600
800
B+C+D+E
A+C+D+E
A+B+D+E
A+B+C+D
TUMOR INCIDENCE (%)
TREATMENT DAYS

Figure 8


100
80
60
40
20
0
200
400
600
800
TUMOR INCIDENCE (%)
TREATMENT DAYS
B(a)P, 0.01%
B(a)P + A
B(a)P + D
B(a)P + E

LEGENDS

Figure 4 - Tumor incidence in Groups 24, 27, 30 and 33. Gross Tumor incidence was calculated as the fraction of mice at risk (survivors + dead tumor bearers) bearing papillomas and carcinomas based on monthly clinical observations.

Figure 5 - Tumor incidence in Groups 7,8,12,13 and 20. Gross Tumor incidence was calculated as the fraction of mice at risk (survivors + dead tumor bearers) bearing papillomas and carcinomas based on monthly clinical observations.

Figure 6 - Tumor incidence in Groups 18,19 and 23. Gross Tumor incidence was calculated as the fraction of mice at risk (survivors + dead tumor bearers) bearing papillomas and carcinomas based on monthly clinical observations.

Figure 7 - Tumor incidence in groups 16,17,21 and 22. Gross Tumor incidence was calculated as the fraction of mice at risk (survivors + dead tumor bearers) bearing papillomas and carcinomas based on monthly clinical observations.

Figure 8 - Tumor incidence in groups 1, 4, 11 and 41. Gross Tumor incidence was calculated as the fraction of mice at risk (survivors + dead tumor bearers) bearing papillomas and carcinomas based on monthly clinical observations.

A comparison of the responses of Group 4 (neat asphalt fumes) and Group 11 (mixture of all fractions in appropriate proportions) reveals that the recombined fractions had essentially the same activity as the neat asphalt fume from which they were derived as determined by time to first tumor, average time to carcinoma, mice with carcinoma (Table 18), as well as overall tumor incidence and kinetics of tumor appearance (Figure 4). These results suggest that the fractionation procedure did not markedly alter the total biological activity of the neat asphalt fume and that the individual fractions were representative of the chemical entities as they occurred in the neat asphalt fume.

The cocarcinogenic and tumor-promoting activities of selected fractions (A,D and E) were tested because of the chemical nature of these fractions and the available information on the chemical nature of mouse skin cocarcinogens and tumor promoters. In the cocarcinogenesis segment (Groups 24 through 35), a dose response for tumor incidence and cancer yield with benzo(a)pyrene alone was obtained (Groups 24,25 and 26). There was no evidence that the fractions tested (A,D and E) at the concentrations used contributed any cocarcinogenic activity. The groups treated with benzo(a)pyrene at 0.01% (Groups 24, 27, 30 and 33) provided such a strong response due to the benzo(a)pyrene alone that the sensitivity for assessing cocarcinogenic activity was most likely reduced or eliminated (Figure 8). However, the weak response at 0.001% benzo(a)pyrene in Group 25 provided an ideal means to test for cocarcinogenicity of Fractions A,D and E (Groups 28, 31 and 34, respectively). No cocarcinogenic activity was observed.

The test of the tumor-promoting activity of Fractions A,D and E (Groups 36 through 39), showed no promoting activity for any of these fractions at the concentrations employed.

An examination of the results with the raw asphalt, the residue after heating and the fumes (Groups 1 through 4 and 41) (Table 18; Figure 4) indicates that the tumorigenic activity for mouse skin resides in the condensed fumes. The raw asphalt had only weak tumorigenic activity while the heated asphalt with the fumes allowed to escape had none.

The remaining variable, the responses of two different mouse strains, was tested by administering neat asphalt fume to C3H/HeJ (Group 4) and Sencar (Group 41) mice. Although the Sencar strain of mice has been bred for sensitivity to skin carcinogens, especially polycyclic aromatic hydrocarbons, the C3H/HeJ mice exhibited a stronger tumorigenic response than the Sencar strain in terms of cancer yield and rate of tumor occurrence. However, the strain responses were not statistically different for either individual tumor type or for combined tumors.

DISCUSSION

The premise that cocarcinogens and/or tumor promoters were responsible for at least a portion of the carcinogenic activity of roofing asphalt fume condensate on C3H mice was not borne out by the results of this study. Although there were both phenolic and aliphatic hydrocarbon compounds in the fractions tested for cocarcinogenic and tumor-promoting activity, the concentrations may not have been sufficient to result in a positive response under the conditions of this study. Earlier investigations examining the cocarcinogenic and tumor-promoting effects of pure chemical species of aliphatic hydrocarbons or phenols have shown that it requires substantial concentrations of these chemicals to yield a positive response on mouse skin (3,4,5).

The carcinogenic activity of the asphalt fume condensate resided essentially in two fractions, B and C. Fraction B contained a variety of aromatic thiophenes, some of which have been shown to have mutagenic activity in the Salmonella typhimurium based Ames assay (6,7). A variety of aryl thiophenes of three and four ring configurations have also been found in shale and coal derived petroleum products which also exhibit tumorigenic activity for mouse skin (8,9,10,11). Benzothiophenes have, in fact, been found specifically in vapors to which creosote workers have been exposed (12), and these types of compounds are probably found wherever sulfur containing petroleum products are used in association with substantial heating. In addition, there are examples of oxidation of aromatic thiophenes by environmental bacteria (13) and rat liver microsomes (14) to oxidized species, that include sultones, which are known mouse skin carcinogens (15). Another interesting feature of this class of chemicals is the antiestrogenic activity of some derivatives which have shown inhibition of carcinogen-induced mammary tumors (16).

The carcinogenic activity of fraction C is more difficult to explain. The primary chemical entities in this fraction are not known for their carcinogenicity. One possibility is that a small quantity of methylated four or five-ring polycyclic aromatic hydrocarbons could be present in this fraction, and these types of substances are known to be potent carcinogens. Further characterization of this set of chemicals will be required to obtain the information needed to explain its biological activity.

While the results of this study have provided some interesting new information about the specific chemicals responsible for the carcinogenic activity of asphalt roofing tar volatiles on mouse skin, the specific agents that were primary contributors to the carcinogenic

activity are not evident. Some chemical classes appear worthy of additional study. Based on the data available from this bioassay, it appears that the activity of the asphalt fume condensate was the result of the additive action of the fractions, and no evidence for cocarcinogenicity or tumor promoting activity was found.

The tumorigenic effects of the asphalt fume in the present study are consistent with the previous NIOSH sponsored study which reported that fumes from type III asphalt heated to 316°C and applied to the skin of C3H/HeJ and CD-1 mice resulted in a significant tumorigenic response (1,2). In addition, the tumorigenicity of the asphalt fumes was demonstrated in the Sencar mouse strain.

Since neither nitro nor amino compounds were present in any fraction and since the tumorigenic activity of the five recombined fractions was like that of the neat asphalt fume, it is unlikely that nitrogen-containing polycyclic aromatic hydrocarbons are important for the observed roofing asphalt fume carcinogenicity.

CONCLUSIONS

This study was designed to address issues that arose from an earlier project that examined the carcinogenicity for mouse skin of condensed volatiles of heated asphalt and pitch roofing tars. The finding in that study that the carcinogenicity of the asphalt tar volatiles could not be accounted for by the concentration of known carcinogens, primarily polycyclic aromatic hydrocarbons, in the test sample prompted the present investigation. The questions posed in this study were (1) is there either cocarcinogenic or tumor-promoting activity in any of the chemical fractions to account for the carcinogenic activity originally observed, and (2) which sets of chemical entities in the several fractions tested gave rise to a carcinogenic response.

The volatiles from heated asphalt roofing tar were collected and separated into five fractions. These fractions were tested separately and in combination for their carcinogenicity, and with benzo(a)pyrene for cocarcinogenic and tumor-promoting activity by skin application to C3H mice. Whole condensate was also tested in Sencar mice to examine interstrain sensitivity. The tumor responses in the test animals revealed no cocarcinogenic or tumor-promoting activity for the three fractions that were deemed most likely to show these kinds of activity based on the structures of the chemicals in these fractions (aliphatic hydrocarbons, alcohols and phenols). The direct carcinogenic activity that was observed occurred with two fractions, (B) olefins, alkylated aryl thiophenes and alkylated phenanthrenes, and (C) alkylated phenylethanones and alkylated difuranones. Synergism was not observed by treatment with combined fractions.

Based on this study, additional cocarcinogenesis and tumor-promotion experiments using a wider range of experimental variables would be desirable, since it was not possible to explore these parameters with the depth necessary to fully test the hypothesis. Moreover, further chemical separation of fractions B and C, which contained the bulk of the carcinogenic activity, and the evaluation of the resulting individual chemical substances, initially in short term genotoxicity assays followed by confirmation in carcinogenicity assays, would add substantially to the identification of the biologically active materials in asphalt roofing fumes. This identification could prepare the way for developing strategies to reduce or eliminate these active chemicals from the products used as roofing materials, and thus reduce or eliminate exposures of roofers to these agents in the workplace.

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APPENDIX I

ASPHALT FUME GENERATION PROCEDURE

PART A

STANDARD PROTOCOL

FILLING OF 12 L FLASK

1. Cool block of asphalt Type III to hardness prior to breaking up asphalt.

2. Weigh 12 L flask; record mass in notebook.

3. Split off small pieces of asphalt (approximately 4 cm x 4 cm) with hammer and chisel.

4. Discard asphalt from surface of new block.

5. Place pieces in flask; fill to top.

6. Place filled flask in oven at 150-160°C for several hours to soften.

7. Add more asphalt to warm flask and bring liquid level up to just over half full (about 6.5 Kg).

8. Let flask cool.

9. Weigh and record mass in notebook with flask number.

10. Store flask (covered) until ready for generation.

PART B

STANDARD PROTOCOL

GENERATION OF ASPHALT FUMES

1. Conduct generation in hood.

2. Place filled flask in oven on evening preceding generation; cover with watch glass.

3. Set timer to turn oven on at about 0400 hours and heat to 150-160°C.

4. When arriving in morning, turn temperature controllers, recorder and heating mantles on.

5. Controllers should be set at 600°F.

6. Set up four impingers in Dewar flasks.

7. Fill fourth impinger with 250 mL acetone/cyclohexane (1:1).

8. Connect transfer tubes and tighten joints with clamps.

9. Fill Dewars with cryogenic material:

   (a) ice
   (b) dry ice/propanol
   (c) dry ice/propanol
   (d) none

10. Put thermocouple from controller in bottom mantle.

11. Transfer heated asphalt flask to bottom mantle.

12. Insert stirrer; install flask cover and gasket with fittings.

13. Clamp cover to flask.

14. Cover with top mantle and insert thermocouple from top controller.

15. Start air stirrer; set at about 200 RPM.

16. Attach vacuum pump and turn on.

17. Note flow through last impinger and through dry gas meter at air inlet to system.

18. Measure flow through dry gas meter (should be at least 5-10 liters per minute).

19. Monitor system as temperature of asphalt approaches 316°C (600°F). Maintain system at 316°C ± 10°C.

20. Let system run for up to 8 hours after asphalt reaches 316°C. Record status hourly.

21. If flow through dry gas meter drops dramatically (below 5 LPM), check for plug in impingers (e.g., impinger 2 or 3).

22. Replace plugged impinger with clean impinger.

23. Continue to run system for desired period of time.

24. At the end of the generation, turn off pump and disconnect heaters.

25. Don protective mask.

26. Remove transfer tubes and impingers.

27. Let impingers and transfer tubes reach ambient temperature.

28. Remove air line, flask cap, thermocouple and stirrer. Cover flask with watch glass.

29. Weigh impingers and transfer tubes on Ohaus balance and record masses in notebook.

30. Transfer collected fumes to 1 L French square sample bottle.

31. Rinse impingers with acetone/cyclohexane (1:1) and decant into sample bottle.

32. Rinse transfer tubes with acetone/cyclohexane (1:1) until fumes are transferred to sample bottle.

33. Reweigh transfer tubes and impingers and record masses in notebook.

34. Set up clean impingers and transfer tubes for next generation.

35. Let 12 L flask cool for one hour and weigh on balance. Record mass in notebook.

36. Pour asphalt into empty paint cans for disposal.

PART C

STANDARD PROTOCOL

CLEANING OF GENERATOR PARTS

12 L Flask

1. Fill the empted, 12 L flask with toluene and let sit for 3-5 days.
2. Empty flask.
3. Ensuring that all residual asphalt has been removed. Rinse with acetone/cyclohexane.
4. Let air dry and refill flask with fresh asphalt for subsequent generation as per filling protocol.

Stirrer

1. Place used stirrer in pipet cleaner and fill with toluene.
2. After 12 hours, remove stirrer and scrape off residue.
3. Rinse with acetone/cyclohexane and dry stirrer for future use.

Impingers and Transfer Tubes

1. Rinse with acetone/cyclohexane.
2. Soak in chromerge (chromic acid).
3. Rinse with tap water.
4. Rinse with acetone.
5. Rinse with Mill-Q water and air dry for future use.

12 L Flask Cap

1. Put cap in paint can with toluene and let sit for 24 hours.
2. Rinse cap with acetone/cyclohexane to remove remaining asphalt.
3. Air dry for future use.

APPENDIX II

PREPARATION OF COLLECTED FUME SAMPLE

PART A

STANDARD PROTOCOL

PREPARATION OF COLLECTED FUME SAMPLE

1. Pour asphalt fume materials from 1-L storage bottles into 2-L separatory funnel.

2. Allow to settle for about 30-45 minutes to permit water phase to separate to bottom.

3. Drain aqueous phase into crystallizing dish.

4. Drain organic phase into tared 1-L round-bottom flask (or 2 1-L flasks as necessary).

5. Place crystallizing dish in vacuum oven (National Company) and remove water at about 15-20 inches vacuum and 45-55°C.

6. Transfer dried residue in crystallizing dish to 1-L round-bottom flask (Item 4 above) by rinsing with 1:1 acetone/cyclohexane.

7. Place 1-L round-bottom flask on Buchler rotary evaporator and remove solvent at reduced pressure with a water aspirator at 45-55°C.

8. Weigh the asphalt fumes in the round-bottom flask after no solvent remains.

9. Record final mass of fume and flask as well as net mass of fume in the notebook.

10. Transfer the asphalt fumes to a 500-mL graduated cylinder by rinsing the round-bottom flask with a volume of 1:1 acetone/cyclohexane mixture sufficient to bring the final volume up to exactly two times the mass of asphalt fumes.

11. Transfer to a 1-L brown bottle marked as follows:

    Case No.: 50043
    Generation Number:
    Date of Generation
    Mass Asphalt Fume:
    Volume Solution:
    Preparer's Number:

12. Transfer 1 mL of the final solution to a 1.8-mL Varian screw cap vial with Teflon-lined septum. Mark as shown in Item 11 and cover with aluminum foil.

13. Store the 1-L brown bottle and 1.8-mL vial at 4°C in the refrigerator in 15W/215.

14. Ship the sample in the vial to NIOSH as directed by the Project Manager. Use blue ice in a styrofoam chest for shipment by overnight delivery service (e.g., Federal Express).

PART B

I. FUME PREPARATION FOR FRACTIONATION

(Filtration and Solvent Exchange)

1. Allow the 1:1 acetone/cyclohexane solution to warm to room temperature.

2. Carefully decant the solution into the Teflon-coated stainless steel Hazardous Waste Filtration System (Millipore) which has been fitted with a 10.0-μm PTFE filter (Millipore). Filter under positive nitrogen pressure (not to exceed 100 psi).

3. Add 250 mL 1:1 acetone/cyclohexane to the sample bottle containing the insoluble material, sonicate for 10 minutes, decant again into the filtration device and filter under positive nitrogen pressure. Save the remaining insoluble material for possible use later.

4. Combine filtrate into a tared 1-L round bottom (r.b.) flask and rotovap to dryness (an oil). Remove the last traces of acetone by adding 150-200 mL cyclohexane and again rotovaping to dryness. Weigh the flask to determine the amount of asphalt fume.

5. Measure out a volume (mL) of 1:1 hexane/methyl t-butyl ether (MTBE) equal to the weight (g) of the fume and transfer the fume from the r.b. flask to a clean amber bottle with several washings of the solvent.

6. Transfer 20-25 mL of the fume sample in hexane/MTBE into the filtration device fitted with the precleaned 0.45-μm PTFE/ polypropylene filter (Gelman). Filter under positive nitrogen pressure. If the sample filters easily, then continue the filtration, changing the filter after each 100-150 milliliters or as necessary. If the sample is difficult to filter, discontinue the filtration after completing the initial portion. Change the filter to a 3.0-μm or a 1.0-μm PTFE filter (Gelman) and filter the sample under positive nitrogen pressure, changing the filter as often as necessary. Transfer 100-150 mL of the prefiltered (3.0-μm or 1.0-μm) fume sample in hexane/MTBE into the filtration device fitted with a precleaned 0.45-μm PTFE/polypropylene filter and filter under positive nitrogen pressure. Change the filter after each 100-150 mL portion or as necessary to rapidly filter the sample.

7. Determine the percent (%) solids in the final filtrate by Thermal Gravimetric Analysis.

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[1] The PTFE/polypropylene filters are precleaned by sonicating for 10 minutes in 1:1 hexane/MTBE prior to use.

- Gelman Sciences, 600 So. Wagner Rd., Ann Arbor, MI 48109, (800) 521-1520

APPENDIX III

ASPHALT FRACTIONATION PROTOCOL

ASPHALT FRACTIONATION PROTOCOL (Revised)
NIOSH FUME FRACTIONATION (PROGRAM #9)

Step[1]	Time (min:sec)	Program Function	Flow	Solvent	Fraction Collection	Volume (L)
1	0:00	Solvent Line 1	250	Hexane		
2	0:00	Recycle/Waste	250			
	1:00	Record Press.[3]				
3	1:45	Collect Line 1	250		1	0.313
4	3:00	Collect Line 2	250		2	0.375
5	3:45	Solvent Line 2	250	9:1, Hexane/MTBE		
6	4:30	Collect Line 3	250		3	0.375
	5:00	Chart Mark				
7	6:00	Collect Line 1	250		4	1.625
	8:00	Record Press.[3]				
8	10:30	Solvent Line 1	250	MTBE		
9	12:30	Collect Line 2	250		5	1.75
	13:30	Record. Press.[3]				
10	14:30	Solvent Line 2	250	$MeCl_2$		
	17:30	Record Press.[3]				
11	18:30	Solvent Line 1	250	MeOH		
12	19:30	Collect Line 3	250		6	.75
	20:15	Record Press.[3]				
13	20:30	Sample/Flow	100	Regeneration Soln.[2]		
14	25:30	Collect Line 1	100		7[5]	1.875
	27:00	Record Press.[3]				
15	30:30	Solvent Line 1	100	MeOH		
	37:30	Record Press.[3]				
16	38:00	Change Flow	250			
	38:30	Record Press.[3]				
17	39:00	Solvent Line 2	250	$MeCl_2$		
18	40:30	Collect Line 2	250		8	1.875
19	41:00	Solvent Line 1[4]	250	MTBE		
20	43:00	Solvent Line 2	250	Hexane/MTBE, 9:1		
21	44:00	Solvent Line 1	250	Hexane		
22	48:00	Recycle/Waste	250			
	49:00	Record Press.[3]				
23	52:00	Stop	0			

INSTRUCTIONS

1. Set up Waters Associates AutoPrep 500A according to the manufacturer's instructions. Monitor the column effluent at 313 nm (Waters Assoc. Model 440 UV detector), 2.0 AUFS and at 345 nm (Waters Assoc. Model 481 UV detector), 2.0 AUFS.

   a. Solvent/Reagents:
   Acetone -- Fisher Scientific, HPLC Grade (Cat. # A-949)
   Cyclohexane -- Fisher Scientific, HPLC Grade (Cat. # C-620)
   n-Hexane -- Fisher Scientific, HPLC Grade (cat. # H-302)
   Methyl t-Butyl Ether - Fisher Scientific, HPLC Grade (Cat# E-127)
   Methylene Chloride -- Fisher Scientific, HPLC Grade (Cat.# D-143)
   Methanol -- Fisher Scientific, HPLC Grade (Cat. # A-452)
   Triethylamine -- Fisher Scientific, HPLC Grade (Cat. # O-4884)

b. Load NIOSH Program #9 (from menu select Modify, Autoclean -- as above)
c. Initial Flow Rate = 250 mL/min.

2. Inject, via syringe, 3 x 8.0 ml of the fume solution.

3. Start Program #9.

4. Collected fractions should be rotovaped (water bath @ 50°C) in tared 1-L r.b. flasks. Fractions 3 and 4, and Fractions 5 and 6 may be combined. For each day's runs, all Fraction 1's (A) may be combined, as well as all Fraction 2's (B), all Fraction 3/4's (C), all Fraction 5/6's (D) and all Fraction 7's (E). Fraction 8's may be discarded. After determining the weights collected, 2-3 drops of each of the neat fractions is transferred to labelled Varian vials and shipped to NIOSH for infrared analysis. The remainder of each fraction is transferred to amber screw cap bottles with a volume (mL) of 1:1 acetone / cyclohexane equal to or greater than the weight (g) of the fraction. The bottles are labelled with fraction number, generation number, date fractionated, fraction weight, and volume of solvent, then stored in the refrigerator.

NOTES:

1 -- <u>All operations to be conducted using UV filtered (yellow) light.</u> At each step, press the chart mark switches on both detectors. Also, press chart mark at 5.0 minutes.

2 -- Regeneration solution. Combine 3600 mL MeOH, 400 mL dist. H_2O, and ca. 2 ml triethylamine. Adjust pH to 8.5 (using narrow range pH test paper) with triethylamine.

3 -- Record the column back pressure at time (t) = 1:00, 8:00, 13:30, 17:30, 20:15, 27:00, 37:30, 38:30, 49:00 minutes.

4 -- At Step 19, when moving solvent line 2 from the $MeCl_2$ reservoir to the Hexane/MTBE (9:1) reservoir, shake out the line so as not to contaminate the Hexane/MTBE.

5 -- Fraction #7 will contain the water (ca. 100 mL) from the regeneration solution. Separate the water from the organic phase in a separatory funnel. Collect the water into a separate container, and store the organics. Extract the organics from water by shaking with 3 x 50 mL of $MeCl_2$ in a small separatory funnel. Collect and dry the $MeCl_2$ layer over anhydrous sodium sulfate, filter and recombine with the organic phase of Fraction #7.

ASPHALT FRACTIONATION PROTOCOL (Revised)

II. PREP LC TEST MIXTURE (Revised)

NIOSH ASPHALT FUME FRACTIONATION (PROGRAM #4)

Step[1]	Time (min:sec)	Function	Solvent
1	0:00	Solvent Line 1	Hexane
2	0:00	Recycle/Waste	
	1:00	Record Press.	
3	1:45	Collect Line 1	
4	3:45	Solvent Line 2	Hexane/MTBE, (9:1)
5	5:00	Chart Mark	
6	7:45	Collect Line 2	
7	8:30	Solvent Line 1	MTBE
8	12:30	Solvent Line 2	$MeCl_2$
9	13:45	Collect Line 3	
10	14:30	Solvent Line 1	MeOH
11	18:45	Solvent Line 2	$MeCl_2$
12	19:45	Collect Line 1	
13	20:45	Solvent Line 1	MTBE
14	21:45	Solvent Line 2	Hexane/MTBE, (9:1)
15	22:45	Solvent Line 1	Hexane
16	26:45	Recycle/Waste	
	27:00	Record Press.	
17	32:45	Stop	

Instructions

1. Weigh out the following quantities of standards, transfer to a 1.0-L volumetric and dissolve in hexane/MTBE (1:1):

a.	Phenyldodecane	5.0 g	Aldrich Cat. No. 11, 322-9
b.	Pyrene	4.0 g	Aldrich Cat. No. 18, 551-5
c.	Benzo(a)pyrene	1.0 g	Aldrich Cat. No. B1,008-0
d.	Carbazole	0.8 g	Aldrich Cat. No. C308-1
e.	2-Naphthol	4.0 g	Aldrich Cat. No. 18,550-7

2. Set up Waters Associates AutoPrep 500A according to the manufacturer's instructions. Monitor the column effluent at 313 nm (Waters Assoc. Model 440 UV detector), 2.0 AUFS and at 345 nm (Waters Assoc. Model 481 UV detector), 2.0 AUFS.

   a. Load NIOSH Program #4 (from the menu select -- Test Mixture #3)
   b. Initial Flow Rate = 250 mL/min.

3. Inject, via syringe, 10.0 mL of the test mixture

4. Start Program #4, Mixture #3

5. Collected effluent may be discarded.

NOTES:

1 -- At each step, press the chart mark switches on both detectors. Also, press chart mark at 5.0 minutes.

2 -- At Step 11, when moving solvent line 1 $MeCl_2$ reservoir to the Hexane/MTBE (9:1) reservoir, shake out the line so as not to contaminate the Hexane/MTBE.

APPENDIX IV

FRACTION PROPORTIONING

(Communication from R. Niemeier)
(March 24, 1986)

The following fraction weights were obtained from the pre-December and December fume generation and fractionation:

Fraction	Weight (g)	Percent (%) Total
A	2281.8	65.47
B	283.0	8.12
C	369.1	10.59
D	397.5	11.40
E	154.1	4.42
Total Fume	3485.5	100.00

Analysis of the raw asphalt fume and Fraction A for two indicator compounds yielded the following results.

n-dodecane (μg/mg)		hexadecane μg/mg)	
Raw	Fraction A	Raw	Fraction A
1.7	3.7	1.5	3.7
1.6	2.7	1.4	2.8
1.5	3.2	1.2	2.3
	3.7		2.4
	3.7		2.7
Mean ± SD 1.6	3.4 ± 0.4	1.37	2.8 ± 0.5
% RSD	11.8		17.8

Using n-dodecane as the primary indicator compound, principally because of its biological significance as a potent cocarcinogen, the following weights of each fraction were calculated to be the equivalent of 200 g of raw asphalt fume, which is subsequently to be diluted to 400 mL with cyclohexane:acetone (1:1):

raw asphalt fume
200 g x 1.6 mg/g = 320 mg n-dodecane

Fraction A
320 mg/3.4 mg/g = 94.12 g of fraction A
94.12 x 15 = 1411.8 g needed

Fraction B
94.12 g X 8.12%/65.4% = 11.67 g of fraction B
11.67 x 9 = 105.0 g needed

Fraction C
94.12 g x 10.59%/65.4% = 15.22 g of fraction C
15.22 x 9 = 137.0 g needed

Fraction D
94.12 g x 11.40%/65.4% = 16.39 g of fraction D
16.39 x 13 = 213.1 g needed

Fraction E
94.12 g x 4.42%/65.47% = 6.35 g of fraction E
6.35 x 12 = 76.2 g needed

Mass balance of all fractions = 143.75 g (group 11)
vs. 200g (uncorrected) or 112.7g (corrected) of raw fume
(see below)

---

correction of raw fume mass balance
to compare to total recombined fractions

6268 g (total fume) - 3848 g (amount injected) = 2420 g (lost to filtration)
or 38.6%

200g (total raw fume) - 77.2 g (38.6% lost to filtration) = 122.8 g (net)

122.8 g (net) x 90.6% (recovery efficiency from HPLC) = 112.7 g

---

The following solutions should be made based on the previous calculations. The weight of the specified fraction in column AA was calculated based on the following formula:

$$AA = \frac{BB \times CC}{DD}$$

where

AA = weight (g) of fraction to be used for preparing the stock solution
BB = final volume (ml) of stock solution (dilution to the mark with cyclohexane:acetone -- 1:1)
CC = weight (g) of fraction required for each 400 ml of treatment solution
DD = volume of stock solution to be used in preparing each 400 ml of final treatment solution

Fraction	AA	BB	CC	DD
A	1476.4	4000	94.12	255
B	116.7	400	11.67	40
C	152.2	400	15.22	40

Fraction	AA	BB	CC	DD
D	234.1	500	16.39	35
E	84.7	400	6.35	30
Benzo(a)pyrene stock solutions --				
0.1%	0.4	100	0.040	10
0.01%	10 ml of 0.1%	100	0.004	10
0.001%	10 ml of 0.01%	100	0.0004	10
0.4%	use 0.1% stock solution			50 µl per treatment

Instruction for using preceeding table in formulating treatment solutions:

Example 1 -- to formulate treatment solution for group 11 mix the following:

	255 ml stock A
	40 ml stock B
	40 ml stock C
	35 ml stock D
	30 ml stock E
total volume	400 ml

Example 2 -- to formulate treatment solution for group 23 mix the following:

	255 ml stock A
	35 ml stock B
	30 ml stock C
	80 ml cyclohexane/acetone (1:1)
total volume	400 ml

Example 3 -- to formulate treatment solution for group 23 mix the following:

	30 ml stock E
	10 ml stock 0.001% B(a)P
	360 ml cyclohexane/acetone (1:1)
total volume	400 ml

APPENDIX V

APPLICATION SOLUTION PREPARATION SUMMARY

Application Solution Preparation Summary

Group	Formulation (diluted to 400 mL final volume with 1:1 acetone:cyclohexane)
1	200 g of raw asphalt
2	200 g of heated asphalt
3	189 g of heated asphalt + 11.3 g of asphalt fume
4	200 g of asphalt fume
5	400 mL 1:1 acetone:cyclohexane
6	255 mL Fraction A
7	40 mL Fraction B
8	40 mL Fraction C
9	40 mL Fraction D
10	25 mL Fraction E
11	255 mL Fraction A + 40 mL ea Fraction B,C,D + 25 mL Fraction E
12	255 mL Fraction A + 40 mL Fraction B
13	255 mL Fraction A + 40 mL Fraction C
14	255 mL Fraction A + 40 mL Fraction D
15	255 mL Fraction A + 25 mL Fraction E
16	40 mL ea Fraction B,C,D + 25 mL Fraction E
17	255 mL Fraction A + 40 mL ea Fraction B,C,D
18	255 mL Fraction A + 40 mL ea Fraction B,C + 25 mL Fraction E
19	40 mL ea Fraction B,C,D
20	40 mL ea Fraction B,C
21	255 mL Fraction A + 40 mL ea Fraction C,D + 25 mL Fraction E
22	255 mL Fraction A + 40 mL ea Fraction B,D + 25 mL Fraction E
23	255 mL Fraction A + 40 mL Fraction D + 25 mL Fraction E

Application Solution Preparation Summary (continued)

Group	Formulation (diluted to 400 mL final volume with 1:1 acetone:cyclohexane)
24	10 mL 4.0 mg/mL B(a)P
25	10 mL 0.4 mg/mL B(a)P
26	10 mL 0.04 mg/mL B(a)P
27	255 mL Fraction A + 10 mL 4.0 mg/mL B(a)P
28	255 mL Fraction A + 10 mL 0.4 mg/mL B(a)P
29	255 mL Fraction A + 10 mL 0.04 mg/mL B(a)P
30	40 mL Fraction D + 10 mL 4.0 mg/mL B(a)P
31	40 mL Fraction D + 10 mL 0.4 mg/mL B(a)P
32	40 mL Fraction D + 10 mL 0.04 mg/mL B(a)P
33	25 mL Fraction E + 10 mL 4.0 mg/mL B(a)P
34	25 mL Fraction E + 10 mL 0.4 mg/mL B(a)P
35	25 mL Fraction E + 10 mL 0.04 mg/mL B(a)P
36	255 mL Fraction A
37	40 mL Fraction D
38	25 mL Fraction E
39	400 mL 1:1 acetone:cyclohexane
40	No treatment
41	200 g asphalt fume
42	400 mL 1:1 acetone:cyclohexane

All solutions are to be prepared in a stoppered red Kimax® 500-mL Erlenmeyer flask and subsequently transferred to labelled amber vials for long-term storage and use.

Fume Fraction Stock Preparation

The following guidelines represent the actual directions used in the preparation of the solutions for this program.

Fraction A Pre-dilution concentration -- 0.500 g/mL. 1480 mL of Fraction A was measured using a 2-L graduated cylinder. This solution was diluted to 2.0 L with 1:1 acetone : cyclohexane (A/C), mixed well with a glass stirring rod, and transferred to a clean labelled 4-L amber solvent bottle. This procedure was repeated to prepare a total of 4.0 L of solution. The solution was stored under nitrogen and refrigerated until used.

Fraction B Pre-dilution concentration -- 0.464 g/mL. 252 mL of Fraction B was measured using a 500-mL graduated cylinder. This solution was diluted to 400 mL with A/C, mixed well with a glass stirring rod and transferred to a labelled 1-L plastic coated amber bottle. The solution was stored under nitrogen and refrigerated until used.

Fraction C Pre-dilution concentration -- 0.509 g/mL. 299 mL of Fraction C was measured using a 500-mL graduated cylinder. This solution was diluted to 400 mL with A/C, mixed well with a glass stirring rod and transferred to a labelled 1-L plastic coated amber bottle. The solution was stored under nitrogen and refrigerated until used.

Fraction D Pre-dilution concentration -- 0.456 g/mL. Note: This fraction contained material which precipitated with time. The bottle was shaken well to homogenize the solution before removing any aliquots. 539 mL of Fraction D was measured using a 1-L graduated cylinder. The solution was diluted to 600 mL with A/C, mixed well with a glass stirring rod and transferred to a labelled 1-L plastic coated amber bottle. The solution was stored under nitrogen and refrigerated until used.

Fraction E Pre-dilution concentration - 0.526 g/mL. 194 mL of Fraction E was measured using a 500 mL graduated cylinder. The solution was diluted to 400 mL with A/C, mixed well with a glass stirring rod and transferred to a labelled 1-L plastic coated amber bottle. The solution was stored under nitrogen and refrigerate until used.

Benzo(a)Pyrene [B(a)P]

4.0 mg/mL -- weigh 200 mg B(a)P. Transfer to a clean labelled 50-mL volumetric flask. Dilute to the mark with A/C.

0.4 mg/ml -- Pipet 5.0 mL of 4.0 mg/mL B(a)P into a clean 50-mL volumetric flask. Dilute to the mark with A/C.

0.04 mg/ml -- Pipet 5.0 mL of 0.4 mg/mL B(a)P into a clean 50-mL volumetric flask. Dilute to the mark with A/C.

Skin Application Solution - Preparation

General Directions

--Equipment Preparation

Pre-mark several 500-mL red Kimax® stoppered erlenmeyer flasks at the 400 mL level by etching with a carbide pencil or other permanent marking. Wash the flasks thoroughly and rinse with 50% acetone/50% cyclohexane (A/C) (HPLC grade solvent) before using.

A 250-mL graduated cylinder should be specially marked at the 255 mL level; (add 5.0 mL via volumetric pipet) for use in solution preparations involving Fraction A.

Pre-label the 14-mL amber septum sealed screw cap vials with appropriate study group and aliquot number.

--Solution Transfer

After the preparation of each of the individual solutions, aliquot 14 mL the solutions to the individual vials using a 25-mL graduated pipet. The solutions should be well mixed with mechanical stirring and/or sonification before transferring. Deliver 14 mL to each vial as labelled sequentially. The 400 mL of solution that has been prepared will require 29 vials. Each vial headspace should be purged with N_2 before sealing.

Specific Directions

Group 1 Weigh 200 g of raw asphalt (Type III). The asphalt should be broken into small pieces (ambient or chilled conditions) to fit into the preparation glassware. Transfer the asphalt to a pre-marked 500-mL Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 2 Weigh 200 g of the previously heated asphalt (Type III, Run 7, 12/2/85) The asphalt should be broken into small pieces (ambient or chilled conditions) to fit into the preparation glassware. Transfer the asphalt to a pre-marked 500-mL Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 3 Weigh 189 g of the previously heated asphalt (Type III, Run 7, 12/2/85). The asphalt should be broken into small pieces (ambient or chilled conditions) to fit into the preparation glassware. Deliver 22.6 mL composited asphalt fume solution (0.5 g/mL) via 25-mL graduated pipet to the pre-marked 500-mL Kimax® flask with the heated asphalt. Follow general directions for dilution, mixing and transfer.

Group 4 Using 400 mL composited 0.5 g/mL asphalt fume solution prepared previously, follow general directions for transfer.

Group 5 Using 1:1 A/C prepared previously, follow general directions for transfer.

Group 6 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A and transfer to a pre-marked Kimax® flask. Rinse the graduate with A/C and transfer the rinses (quantitative transfer) to the Kimax® flask to dilute to the 400-mL mark. Follow general directions for dilution mixing and transfer.

Group 7 Using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction B and quantitatively transfer to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 8 Using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction C and quantitatively transfer to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 9 Using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction D and quantitatively transfer to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 10 Using a 25-mL graduated cylinder, measure 25 mL of asphalt fume Fraction E and quantitatively transfer to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 11 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume fraction A; using 50-mL graduated cylinders, measure 40 mL each of Fractions B,C, and D; and using a 25-mL graduated cylinder, measure 25 mL of Fraction E. Transfer each to the pre-marked Kimax® flask. Allow graduates to drain several minutes, if necessary. As little or no solvent should be used in this particular transfer, reduce solvent evaporation by shielding or covering solutions in the transfer process. Follow general directions for mixing and transfer.

Group 12 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction B. Quantitatively transfer each (with solvent rinsing) to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 13 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction C. Quantitatively transfer each to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 14 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction D. Quantitatively transfer each to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 15 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using a 25-mL graduate cylinder, measure 25 mL of asphalt fume Fraction E. Quantitatively transfer each to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 16 Using 50-mL graduated cylinders, measure 40 mL each of asphalt fume Fractions B, C, and D; using a 25-mL graduated cylinder, measure 25 mL of asphalt fume Fraction E. Quantitatively transfer each to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 17 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using 50-mL graduated cylinders, measure 40 mL each of asphalt fume Fractions B, C, and D. Quantitatively transfer each (use solvent sparingly) to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 18 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using 50-mL graduated cylinders, measure 40 mL each of asphalt fume Fractions B and C; and using a 25-mL graduated cylinder, measure 25 mL of asphalt fume Fraction E. Quantitatively transfer each (use solvent sparingly) to the pre-marked Kimax® flask). Follow general direction for dilution, mixing and transfer.

Group 19 Using 50-mL graduated cylinders, measure 40 mL each of asphalt fume Fractions B, C, and D. Quantitatively transfer each to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 20 Using 50-mL graduated cylinders, measure 40 mL each of asphalt fume Fractions B and C. Quantitatively transfer each to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 21 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using 50-mL graduated cylinders, measure 40 mL each of Fractions B and D; and using a 25-mL graduated cylinder, measure 25 mL asphalt fume Fraction E. Quantitatively transfer each (use solvent sparingly) to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 22 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using 50-mL graduated cylinders, measure 40 mL each of Fractions B and D; and using a 25-mL graduated cylinder, measure 25 mL asphalt fume Fraction E. Quantitatively transfer each (use solvent sparingly) to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 23 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using 50-mL graduated cylinder, measure 40 mL of Fractions D; and using a 25-mL graduated cylinder, measure 25 mL asphalt fume Fraction E. Quantitatively transfer each (use solvent sparingly) to the pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 24 Using a 10-mL volumetric pipet, transfer 10 mL of 4.0 mg/mL B(a)P to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 25 Using a 10-mL volumetric pipet, transfer 10 mL of 0.4 mg/mL B(a)P to a pre-marked Kimax® flask. Follow general direction for dilution, mixing and transfer.

Group 26 Using a 10-mL volumetric pipet, transfer 10 ml of 0.04 mg/mL B(a)P to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 27 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using a 10-mL volumetric pipet, transfer 10 mL of 4.0 mg/mL B(a)P. Quantitatively transfer Fraction A to the Kimax® flask with the B(a)P. Follow general directions for dilution, mixing and transfer.

Group 28 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using a 10-mL volumetric pipet, transfer 10 mL of 0.4 mg/mL B(a)P. Quantitatively transfer Fraction A to the Kimax® flask with the B(a)P. Follow general direction for dilution, mixing and transfer.

Group 29 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A; using a 10-mL volumetric pipet, transfer 10 mL of 0.04 mg/mL B(a)P. Quantitatively transfer Fraction A to the Kimax® flask with the B(a)P. Follow general directions for dilution, mixing and transfer.

Group 30 Using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction D; using a 10-mL volumetric pipet, transfer 10 mL of 4.0 mg/mL B(a)P. Quantitatively transfer Fraction D to the pre-marked Kimax® flask with the B(a)P. Follow general directions for dilution, mixing and transfer.

Group 31 Using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction D; using a 10-mL volumetric pipet, transfer 10 mL of 0.4 mg/mL B(a)P. Quantitatively transfer Fraction D to the pre-marked Kimax® flask with the B(α)P. Follow general directions for dilution, mixing and transfer.

Group 32 Using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction D; using a 10-mL volumetric pipet, transfer 10 mL of 0.04 mg/mL B(a)P. Quantitatively transfer fraction D to the pre-marked Kimax® flask with the B(a)P. Follow general directions for dilution, mixing and transfer.

Group 33 Using a 25-mL graduated cylinder, measure 215 mL of asphalt fume Fraction E; using a 10-mL volumetric pipet, transfer 10 mL of 4.0 mg/mL B(a)P. Quantitatively transfer Fraction E to the pre-marked Kimax® flask with the B(a)P. Follow general directions for dilution, mixing and transfer.

Group 34 Using a 25-mL graduated cylinder, measure 25 mL of asphalt fume Fraction E; using a 10-mL volumetric pipet transfer 10 mL of 0.4 mg/mL B(a)P. Quantitatively transfer Fraction E to the pre-marked Kimax® flask with the B(a)P. Follow general directions for dilution, mixing and transfer.

Group 35 Using a 25-mL graduated cylinder, measure 25 mL of asphalt fume Fraction E; using a 10-mL volumetric pipet, transfer 10 mL of 0.04 mg/mL B(a)P. Quantitatively transfer Fraction E to the pre-marked Kimax® flask with the B(a)P. Follow general directions for dilution, mixing and transfer.

Group 36 Using the specially marked 250-mL graduated cylinder, measure 255 mL of asphalt fume Fraction A. Quantitatively transfer Fraction A to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 37 Using a 50-mL graduated cylinder, measure 40 mL of asphalt fume Fraction D. Quantitatively transfer Fraction D to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 38 Using a 25-mL graduated cylinder, measure 25 mL of asphalt fume Fraction E. Quantitatively transfer Fraction E to a pre-marked Kimax® flask. Follow general directions for dilution, mixing and transfer.

Group 39 Using 1:1 A/C prepared previously, follow general directions for transfer.

Group 40 No treatment

Group 41 Using the composited 0.50 g/mL composited asphalt fume prepared previously, follow general directions for transfer.

Group 42 Using 1:1 A/C prepared previously follow general directions for transfer.

APPENDIX VI

BIOASSAY PROTOCOL AND AMENDMENTS

PROTOCOL FOR THE ASSESSMENT OF THE COCARCINOGENIC PROMOTING ACTIVITY OF ASPHALT FUMES. TASK 3. BIOASSAY.

1. Purpose

To assist NIOSH in establishing cocarcinogenic and promoting activity of asphalt components found in the roofing industry. This is to be done by a chronic 24 month skin painting study in mice. The initiation and completion dates will be listed in the final report.

2. GLP Status

This study will be conducted according to Good Laboratory Practices regulations of the U.S. Food and Drug Administration with the exception of areas agreed upon in writing by NIOSH. This study will be entered in the Arthur D. Little, Inc., EPA Master Schedule Sheet.

3. Sponsor

The sponsor for this study is Center for Disease Control National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Cinicinnati, Ohio 45226, Contract No. 200-83-2612.

4. Justification for Selection of Test Systems

C3H/HeJ and Sencar
Historical Precedent

5. Address of Animal Facility

Arthur D. Little, Inc.
134 Main Street
Cambridge, MA 02142

6. Individuals to be Participating in Conduct of Study

1.	A. Sivak	Case Leader
2.	M. Goldman	Toxicology Laboratory Manager
3.	J. Fox	Veterinary Medicine
4.	A. Liberson	Veterinary Pathologist
5.	A. Ross	Senior Research Technician

Additional individuals will be added or substituted as the study progresses.

7. Test Article

To be administered as produced in a final form from fraction procedures which are still awaiting final development.

A. Name of Test Articles

(1) C3H/HeJ mice groups: Note that all dilutions will be made with acetone/cyclohexane (1:1) all HPLC grade used without further analysis for contaminants, designated below as A/C; further note that * means to deliver the fraction in a

concentration corresponding to the concentration of that fraction in the complete neat asphalt fume (group 4). The groups are as follows: (Each group consists of 30 animals)

#1: Raw Asphalt diluted 50/50

#2: Raw Asphalt heated to 316°C for 7 hours with permitted escape of fumes, diluted 50/50

#3: Same as group 2 but the fumes emitted over 7 hours are collected and recombined and the resultant mixture diluted 50/50

#4: Neat Asphalt Fume diluted 50/50

#5: Solvent Control consisting of A/C

#6: "HPLC Fraction #1" of asphalt fume, subsequently coded as "Skin Painting Code" (SPC) "A", diluted* (composition is primarily aliphatics with alkyl benzenes, alkyl napthalenes and olefins)

#7: "HPLC Fraction #2" of asphalt fume, subsequently coded as SPC "B", diluted* (composition is primarily thiophenes including benzo-, naptho-thiophenes, dibenzothiophenes, alkylthiophenes, other sulfur heterocyclics, low molecular weight PNA's and alkyl PNA's)

#8: "HPLC Fractions #3 and 4" of asphalt fume, subsequently coded as SPC "C", diluted* (composition is primarily phenylethanones and dihydrofuranones and includes some high molecular weight napthalenes and PNA's)

#9: "HPLC Fractions #5 and 6" of asphalt fume, subsequently coded as SPC "D", diluted* (composition is primarily phenols)

#10: "HPLC Fraction #7" of asphalt fume, subsequently coded as SPC"E", diluted* (composition is primarily aliphatic ketones isolated as the organic fraction from column regeneration)

#11: Recombination of materials from SPC A through E, diluted* (for comparison to complete activity of group #4 - neat asphalt fume)

The following groups will be used to test the cocarcinogenic activity of the aliphatic fraction in combination with other single SPC fractions

#12: Recombination of materials from SPC A and B, diluted*

#13 Recombination of materials from SPC A and C, diluted*

#14: Recombination of materials from SPC A and D, diluted*

#15: Recombination of materials from SPC A and E, diluted*

The following combinations will test the importance of potential cocarcinogenic fractions to the overall carcinogenic activity

#16: Recombination of materials from SPC B through E, diluted* (i.e., elimination of aliphatics)

#17: Recombination of materials from SPC A through D, diluted* (i.e., elimination of aliphatic ketones)

#18: Recombination of materials from SPC A through C and E, diluted* (i.e., elimination of phenols)

#19: Recombination of materials from SPC B through D, diluted* (i.e., elimination of aliphatics and aliphatic ketones)

#20: Recombination of materials from SPC B and C, diluted* (i.e., elimination of aliphatics, phenols and aliphatic ketones)

The following combinations will test the importance of the fractions which may serve as initiators (group 20 above could also be considered part of this subset since it may be assessing complete initiator activity).

#21: Recombination of materials from SPC A, C, D, and E, diluted* (i.e., elimination of sulfur compounds)

#22: Recombination of materials from SPC A, B, D, and E, diluted* (i.e., elimination of phenylethanones, dihydrofuranones and PNA's)

#23: Recombination of materials from SPC A, D, and E, diluted* (i.e., elimination of thiophenes, phenylethanones and dihydrofuranones)

The following groups will test the cocarcinogenic potential of fractions containing either aliphatics, phenols or aliphatic ketones.

Controls: #24: 0.01 percent B(a)P in A/C (100 mg/L:w/v)

#25: 0.001 percent B(a)P in A/C (10 mg/L:w/v)

#26: 0.0001 percent B(a)P in A/C (1 mg/L:w/v)

Aliphatics:

#27: Aliphatic Fraction (SPC A), diluted*, and containing 0.01 percent B(a)P

#28: Aliphatic Fraction (SPC A), diluted*, and containing 0.001 percent B(a)P

#29: Aliphatic Fraction (SPC A), diluted*, and containing 0.0001 percent B(a)P

Phenolics:

#30: Phenol Fraction (SPC D), diluted*, and containing 0.01 percent B(a)P

#31: Phenol Fraction (SPC D), diluted*, and containing 0.001 percent B(a)P

#32: Phenol Fraction (SPC D), diluted*, and containing 0.0001 percent B(a)P

Aliphatic Ketones:

#33: Aliphatic Ketone Fraction (SPC E), diluted*, and containing 0.01 percent B(a)P

#34: Aliphatic Ketone Fraction (SPC E), diluted*, and containing 0.001 percent B(a)P

#35: Aliphatic Ketone Fraction (SPC E), diluted*, and containing 0.0001 percent B(a)P

The following groups will address the assessment of the classical promoting potential of fractions:

#36: Initiation by single application of 0.05 ml of 0.4 percent (200 microgram) B(a)P in A/C (4 g/L:w/v) followed by twice weekly application of the aliphatic fraction (SPC A), diluted*

#37: Initiation by single application of 0.05 ml of 0.4 percent B(a)P in A/C followed by twice weekly application of the phenolic fraction (SPC D), diluted*

#38: Initiation by single application of 0.05 ml of 0.4 percent B(a)P in A/C followed by twice weekly application of the aliphatic ketone fraction (SPC E), diluted*

#39: Initiation by single application of 0.05 ml of 0.4 percent B(a)P in A/C followed by twice weekly application of solvent A/C (as used in group 5)

The following group will serve as sentinal animals.

#40: Sentinal untreated control mice for the animal disease screening program (see section 19, page 12) Ten extra animals (30 total) are included to ensure sufficient numbers of animals survive until the end of the study.

2. Sencar mice groups--sentinal animals not required.

#41: Neat Asphalt Fume diluted 50/50

#42: Solvent Control consisting of A/C

8. Test Article Identification, Purity, Stability

Samples of all test materials will be sent to NIOSH periodically for quality control analysis and stability estimations (approximately quarterly), according to the instructions of NIOSH.

A. Vehicle

Test solutions are as described in 7.A. No additional vehicles will be utilized.

B. Storage Conditions of Test Article

All samples will be stored in brown actinic glassware and refrigerated when not in use.

C. Disposition of Sample

Remaining sample will be returned to NIOSH, unless otherwise instructed.

9. Animals

A. Sources

C3H/HeJ male mice will be obtained from Jackson Laboratory, Bar Harbor, ME. in either one or two shipments (1200 plus 10% excess margin). The Sencar male mice will be supplied by Harlan Sprague Dawley, Inc. Indianapolis, IN. (60 plus 10% excess margin).

B. Quarantine

Mice will be quarantined for a minimum of 14 days to a maximum of 18 days under conditions simulating those in the test condition. The health of the animals will be assured during the last few days of quarantine. Unsuitable animals as determined by a veterinarian, will be discarded. A small, randomly selected number of mice, five (5) from each shipment, shall be sacrificed and examined grossly for disease and parasites by a laboratory animal veterinarian and/or veterinary pathologist. Lesions seen grossly should be

confirmed by histopathological examination and/or microbiological culture.

Mice will have to be received at 4 weeks (28 days) of age, in order to allow 14-18 days of quarantine (42-46 days of age), and to initiate treatment at 40-50 days of age. They will be held in stainless steel stock cages with wire mesh bottoms and under conditions of temperature, humidity, light, feeding and watering, as specified for the test conditions.

C. Randomization

Formal randomization by weight will be performed such that each experimental group of 30 animals will have approximately equal average weights based on the overall population weighed at the end of quarantine. All C3H/HeJ mice will be sorted by weight, using the computer program provided by NIOSH, and those mice deemed unsuitable will be discarded. The program is structured to allocate the mice to the 40 experimental groups by eliminating the extremes of the weight range and then assigning a sorted listing of mice in sequential order, i.e., groups 1 through 40. An identical procedure will be used to assign the Sencar mice to groups 41 and 42. All mice will be weighed on an electronic balance interfaced to the computer. Print-outs will be obtained and held as original data. In addition, a computer data file will be maintained and a copy sent to NIOSH.

Specific body weight range is not an important factor. It is important that each experimental group will have approximately equal average weights based on the overall population weighed at the end of quarantine.

D. Identification and Housing

We will use a base 3 derived ear-punch system, for identifying experimental animals, (Appendix C). The cages for this program are stainless steel, with wire-mesh bottoms, and the racks have excreta pans. Since their floor area is 64 in^2, we plan to house not more than 4 mice per cage (AAALAC guidelines), i.e., an experimental group of 30 animals might be housed in 6 cages with 4 mice each, and 2 with 3. If animals appear to have lesions from dominant fighting they will be separated. Each cage tag will have bar codes for the animals to facilitate data entry.

The C3H/HeJ and Sencar mice will be housed in separate rooms. Since each of our racks holds 60 cages, the C3H/HeJ experiments will require 5 racks, the Sencar, one rack.

Labelling of racks and cages will be done according to the Standard Operating Procedure of the facility. In addition, a system using colored tape will be used for a rapid, visual matching of test animal cages and test material bottles.

E. Diet

Laboratory feed will be certified commercial rodent diet. Analysis data will be preserved as well as mill run information. The lots will be as few as possible to keep diet variability at a minimum. According to analysis data there are no known contaminants in the food that may interfere with the study.

F. Feeding and Sanitation

Clean feeders with fresh feed will be supplied at least once weekly and feed should be provided as often as necessary to assure support of normal growth and maintenance. Dirty feeders should be soaked, if necessary, and then washed in at least one cycle of 180°F (83°C) water. At least twice weekly, paper in the excretion trays shall be changed. Racks and caging shall be sanitized every two weeks, washing each properly in a machine which provides at least one cycle of 180°F (83°C) water. Racks must be kept clean while in use and in particular the entire wheel surfaces must be cleaned when the floor is cleaned.

G. Water

Water bottles will be used. Dirty (used) bottles will be exchanged for clean, never refilled and reused. Empty bottles will not be filled in the animal room, but will be brought into the room already filled. Fresh sanitized water bottle, bottle stopper and sipper tube will be supplied at least twice weekly. Bottles, stoppers and sipper tubes will be soaked and washed promptly. Water bottles will be washed in water of at least 180°F (83°C). Stoppers and sipper tubes will be sterilized by autoclaving after washing. Cambridge tap water will be used. The City of Cambridge water analysis record is maintained in our files.

H. Air Supply

The temperature and humidity will be set and monitored at those settings that have been reported by AAALAC to be optimal for mice, i.e., temperature of 72 ± 2°F (23.3 ± 1.1°C) and a relative humidity of 50 ± 10 percent. Accuracy of thermometers will be checked quarterly. An automatic recording and an alert system will be used to monitor the ambient conditions. The qualitative direction of air flow (10 - 15 changes per hour) in each animal room will be recorded monthly.

Quantitative measurements of air flow rate will be made once in the cooling and once in the heating season. Reports of the measurements will be made in the monthly progress reports as appropriate.

Rooms are grouped into zones (2, 3 or 4 rooms) for control purposes and the mice for this program will all be housed in one zone, on the second floor of 134 Main Street. The zone has an alarm system for humidity and temperature.

Recording of temperature and humidity is done with Weathertronics hygrothermographs.

I. Lighting

Light cycles will be shifted 1 hour in the fall and spring to match normal time changes. The animal rooms are windowless, and each is provided with automatic control of light cycle. The 12 hours ON/12 hours OFF cycle will be set from 7 a.m. to 7 p.m., for operating convenience. We will use UV-screening filters (Crown Plastics Corp., No. FR213W), which, combined with the diffusers covering the fluorescent lights, to provide sufficient protection from the wavelengths of ultra-violet light that might perturb the study.

10. Experimental Conduct

Groups to be dosed as discussed in Section 7.A.

A. Dosing regimen

Each material will be applied in a 50 microliter volume, twice weekly to each mouse of the respective test group for 104 weeks. Specific day of dosing is not relevent to study, as long at the requirement of twice weekly is met.

B. Application of Test Material

All skin applications to C3H/HeJ mice shall be started at the beginning of the second resting (telogen) phase of the hair cycle (age 40 to 50 days - see Berenblum et al., 1958 Brit. J. Cancer 12:402-413). Young adult Sencar male mice to be used in groups 41 and 42 shall be handled according to this same procedure.

The hair will be removed from the dorsal interscapular area of each animal with a small electric clippers (#40 head) initially at least 2 days prior to treatment, and thereafter (generally weekly) as necessary to keep the area free of hair. Each material will be applied in a 50 microliter volume, using micropipet or syringe with a disposable tip, twice weekly to each mouse of the respective test group for 104 weeks. As tumors develop material will be applied to the base of the tumor and not directly on the growth. Different clipper heads and pipette tips will be used for each group. It is important that the dose be spread uniformly over a fixed area of skin, and that this area be the same size for each animal. Because the materials to be used in this effort are expected to be free flowing, use of a glass rod to spread the materials is not required or desirable.

C. Once tumors have begun to appear hair clipping will be done very carefully, since in the early stages, at least, the tumors are susceptible to being removed with the clippers.

11. Observations

A. Weight

Each mouse will be weighed prior to the initiation of the study for the purpose of randomization and group assignment and then before each first weekly application for six weeks. After 6 weeks, the weighings shall be performed every 4 weeks and at the terminal sacrifice.

Weights will be taken on a top-loading electronic recording balance, and reported to 0.1 g.

12. Monitoring Plan

A record, both computer print-outs and computer data files, will be maintained on all individual animals specifying clinical status, gross appearance, location, size, and enumeration of tumors, the dates and causes of deaths.

The computer data files generated by the NIOSH supplied program for the IBM-PC consist of a weekly observation file and a death file. Both files contain identical information on dates, mouse identification, weight, status, clinical observations, skin lesion type and location, etc.; however, the death file is a master file updated daily when additional mice are lost from the study. Weekly observation files contain information only on those mice that were alive during the particular observation week. As observations are entered into the computer, a print-out is made for the permanent written record; in addition, a computer data file is maintained and a copy is sent to NIOSH on a floppy disk (see attached example of a computer print-out).

Computer generated files and printouts of data will consist of a working copy, a NIOSH copy for direct mailing and an Archive copy. The Archive copy will be filed in a separate clean, dry area away from heat and magnetic fields such as telephones, dictation equipment, and electronic calculators, preferably in a grounded file cabinet. All disks will be labeled and write protected. A laboratory notebook will be established describing date, disc identification, description of data on disc, and signature.

Discs will be treated in the same confidential manner as all original data. (e.g. notebooks and data books)

Data Storage and Disc Handling - See S.O.P. #T-450 (Pages 1 and 2)

Daily observations will commence at the occurrence of the first death of any mouse from any group. The daily observation will consist of a brief inspection of each mouse early in the day to determine life status, i.e., alive, moribund, or dead. Dead animals

will be removed and refrigerated for subsequent pathological procedures. The cages of the moribund mice will be uniquely tagged by the observer for ease of recognition and recorded on the checklist. By the end of that same day the veterinarian will inspect those mice indicated in the record and determine whether they should be sacrificed. Data entry for the weekly observation computer file will be made once weekly unless the status of the mouse changes from the previously recorded for that week. This weekly data entry procedure may be substituted for one of the daily observations. Since the date of death is an important factor in data analysis, any status change in any mouse found during weekend observations will be entered directly into the computer data file. All monitoring personnel will be appropriately trained in the computer data entry procedure.

13. Clinical Observations

Clinical observations will be done once a week to each mouse of the respective test group for 104 weeks. Clinical observations shall include but shall not be limited to those listed in Table 2. If general toxic signs are noted, the project officer or his alternate shall be notified immediately by telephone and will be followed by written notification.

Surveillance for all the indicated abnormalities cited in Table 1 will be done by computer direct data acquisition (Appendix B).

Only non-normal observations will be recorded. Each record will be initialled, however, to indicate the animals have been checked.

14. Tumor Observations

The day of onset or regression of the first and each successive lesion (tumor), its (their) location(s) and physical description(s) will be recorded according to SOP 413A (Appendix A). Weekly updates of their progression or regression will be recorded. The criteria for identifying a gross tumor is defined in Table 2. A gross diagnosis of a carcinoma shall be based on a lesion that upon palpation is attached to underlying tissues, which generally indicates invasion of connective tissue or muscle layers.

15. Necropsy

All animals shall be treated and observed up to 24 months at which time the surviving animals will be killed and necropsied. In order to prevent loss of any tissues for histopathological examination from decomposition, all moribund animals which judged by the veterinarian or his designee will not survive until the next observation period will be killed and necropsied.

Scheduled gross necropsies will be performed in the presence of and under the supervision of the pathologist. Random or unscheduled

necropsies will be performed in the presence of the pathologist to the maximum extent possible. Animals are to be necropsied as soon after death as possible. Ideally, dead animals should be refrigerated for no longer than 8 hours prior to necropsy. Animals shall not be frozen. At necropsy, gross observations shall be made on those tissues specified in Table I. Those tissues shall be preserved for possible future histological examination. The entire mouse will be preserved in buffered formalin.

16. Quality Assurance

Critical Phase for GLP monitoring are:

A. Protocol review

B. Preparation and fractionation of asphalt fumes

C. Preparation of test solutions (including calculations pertaining thereto)

D. Body weight determination

E. Randomization and identification of test mice

F. Technique of skin application and recording thereof

G. Clinical observation of animals

H. Record-keeping, particularly in respect to clinical observations and tumor observations

I. Necropsy procedures

J. Audit of draft and final report

17. Histology

On all mice, both those that die or are sacrificed during the study, treatment-site skin tumors will be excised, placed on file card paper, circled and numbered for pathological examination and fixed in 10 percent buffered formalin for microscopic examination. Tissues will be trimmed in a period of not less than 48 hours nor greater than 12 weeks following necropsy.

18. Pathology

Tumors and skin lesions will be examined histologically by a Board-Certified Pathologist with a minimum of five (5) years experience in experimental tumor pathology. The pathologist will describe all lesions according to their location on the skin, cellular and extracellular composition, size and position in the tissue. Care and attention will be given during tissue block and slide preparation of skin lesions to assure that the histopathological evaluation of each can be traced back

to the individual gross observations of lesion appearance and location. The pathologist will review a sufficient number of known treatment groups to become familiar with the pathological response and then randomize the entire study for an "in blind" evaluation. The pathologist or his designee will maintain records on the incidence, identification, size, location, and disposition of the tumors as the study progresses. Attention should be given to the distribution between preneoplastic and neoplastic lesions such as fibrosarcomas, papillomas, squamous cell carcinomas, fibromas, keratoacanthomas, and other tumor and lesion types.

19. Animal Screening Program

Sentinel animals (group 40) will be clearly marked as sentinel and used only as sentinel animals and not as part of the animals used for the bioassay study. Serum samples will go for screening for the presence of antibodies to the following murine viruses:

Pneumonia virus of mice (PVM), reovirus type 3 (Reo 3), Theiler's encephalomyelitis virus (GD 7), minute virus of mice (MVM), polyoma, ectromelia, sendai, mouse hepatitis virus (MHV), and lymphocytic choriomeningitis virus (LCM).

Serum samples will be collected from the five mice killed during quarantine and five at the end of 6, 12, 18 and 24 months after the start of the administration of the test materials. As in the sampling of the quarantined animals, the remaining sentinel animals sampled for viral titers shall be examined grossly at the time of serum collection for evidence of disease and then disposed of.

The Murine Virus testing will be subcontracted by Arthur D. Little, Inc. to: Microbiological Associates, Inc.
Murine Virus Diagnostic Lab.
5221 River Road
Bethesda, MD 20816

TASK 4: RECORDS, ANALYSIS AND REPORTS

20. Tabulations and Calculations

All histopathologically confirmed tumors and lesions will be tabulated based on types (benign and malignant), locations, sizes, number of lesions, time of appearance (latent period in days since first treatment), and time to death for all members of each test group as well as the controls. A separate tabulation of non-treatment site lesions will be made.

21. Statistical Analyses

The experimental phase, including treatments, gross and histopathological evaluations shall be completed in 30 months. Statistical analyses to be used in the preparation of the final report will be performed by the NIOSH, DBBS Statistical Unit. Turnaround time of one month after complete data set receipt from NIOSH can be expected for the statistical reports. (1) One month after the final sacrifice a report will be generated on survival analysis, body weights, gross tumor incidence, and toxic signs; (2) A second report will be compiled and submitted to the contractor one month after the completion of the histopathological examination which will include mean tumor latency, analyses of variance for tumor types and treatment groups, life table analyses, gross necropsy findings and other appropriate correlations and analyses.

Andrew Sivak, March 28, 1986

Andrew Sivak, PH.D.
Vice President
Arthur D. Little, Inc.
Life Sciences Section

Richard W. Niemeier 4/10/

Dr. Richard W. Niemeier
Experimental Toxicology Branch
National Institute for Occupational
Safety and Health

Table 1

Tissues and Organs Examined at Gross Necropsy

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

TABLE 2

CLINICAL OBSERVATIONS

Animal and Housing

1. normal
2. abnormal

Animal Status
(six digit date required)

1. normal (alive)
2. found dead
3. moribund sacrifice
4. interval sacrifice/ terminal sacrifice
5. accidental death
6. animal escaped
7. cannibalized
8. replaced (explanatory text must accompany)
9. other

Behavior

1. normal
2. languid
3. anorexic
4. hyperactive
5. circling
6. prostrate
7. ataxia
8. other

Excretion

1. normal
2. vomit
3. salivation
4. soft feces
5. compound colored stool (abnormally colored)
6. other

Animal Condition

1. if normal
2. if abnormal

Housing, Food and Water

1. normal
2. spillage - wasting feed (5 animals)
3. no feed
4. no water
5. no food or water
6. other

Appearance

1. normal
2. hunched
3. thin
4. obese
5. malocclusion
6. head tilt
7. paralysis
8. tremors
9. other

Eyes
(Body locator codes must be used)

1. normal
2. squinted
3. opaque
4. lacrimation
5. chromodacryorrhea
6. exophthalmus
7. pupil dilation
8. other

Tissue Masses
(Body locator codes must be used)

1. no palpable tissue masses
2. small movable mass
3. small stationary tissue mass
4. large movable mass
5. large attached mass
6. other

TABLE 2 (continued)
CLINICAL OBSERVATIONS

Respiration

1. normal
2. wheezing
3. dyspnea (shallow)
4. polypnea (rapid)
5. rhinorrhea
6. epistaxis
7. other

Additional Objectives
(Body locator codes must be used)

0. normal
1. enlarged
2. small
3. red
4. pale
5. exudate
6. abcessed
7. necrotic
8. ulcerated
9. other

Skin and Pelage
(Should be used with body locator codes)

1. normal
2. urine stains
3. rough haircoat/discolored/ discolorations of hair/ skin with scab peeled off
4. sores/scabs/scab formation involving hair
5. cyanotic
6. desquamation
7. pilo-erection
8. other

BODY LOCATOR CODES

HED	1.	HEAD	ABD	20.	ABDOMEN
MTH	2.	MOUTH	BCK	21.	BACK, ANT.
TEE	3.	TEETH	BKP	22.	BACK, POST.
NSE	4.	NOSE	SDL	23.	SIDE-LEFT
JAW	5.	JAW	SDS	24.	SIDE-RIGHT
EAL	6.	EAR-LEFT	HPL	25.	HIP-LEFT
EAR	7.	EAR-RIGHT	HPR	26.	HIP-RIGHT
ELL	8.	EYELIDS-LEFT	AINL	27.	INGUINAL-LEFT
ELR	9.	EYELIDS-RIGHT	INR	28.	INGUINAL-RIGHT
NCK	10.	NECK	LHL	29.	LEG-HIND-LEFT
SHL	11.	SHOULDER-LEFT	LHR	30.	LEG-HIND-RIGHT
SHR	12.	SHOULDER-RIGHT	PHL	31.	PAQ-HIND-LEFT
XL	13.	AXILLA-LEFT	PHR	32.	PAW-HIND-RIGHT
AXR	14.	AXILLA-RIGHT	PNS	33.	PENIS
LFL	15.	LEG-FORE-LEFT	TSL	34.	TESTIS-LEFT
LFR	16.	LEG-FORE-RIGHT	TSR	35.	TESTIS-RIGHT
PFL	17.	PAW-FORE-LEFT	VAG	36.	VAGINA
PRF	18.	PAW-FORE-RIGHT	ANS	37.	ANUS
CHS	19.	CHEST	TAL	38.	TAIL

Tissue masses - Locator code

Other abnormalities - Adjective, locator code

Table 2

LESION GLOSSARY OF TUMOR OBSERVATIONS

1. depilated
2. pared epidermis
3. lesion
4. abraded lesion
5. atypical healing
6. suspicious area
7. thickened epidermis
8. spickle
9. horny outgrowth
10. suspicious wart-like
11. typical wart-like*
12. atypical wart-like*
13. suspicious bulbous*
14. typical bulbous*
15. papiloma
16. possible carcinoma
17. probable carcinoma
18. other (document)

* rate as is if 2 x 2 mm

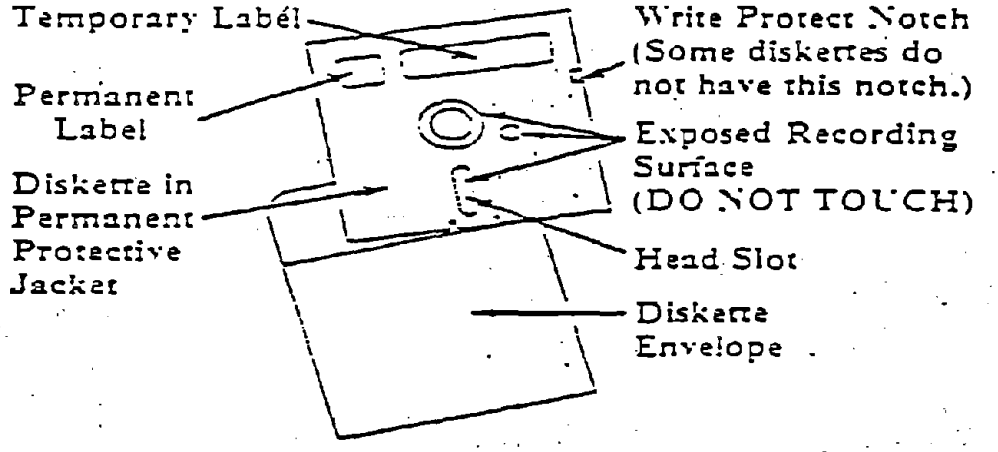
STANDARD OPERATING PROCEDURES T-450

Data Storage and Disc Handling

1. Do not touch the exposed recording surfaces.
2. Insert diskette into drive, label side up with a gentle push.
3. Do not force a diskette into a drive.
4. Avoid inserting or removing a diskette from the drive when the "in use" light is on.
5. Return diskettes to their protective sleeve as soon as you remove them from the diskette drive.
6. Don't lay heavy objects on top of diskettes.
7. If you stand diskettes on edge make sure they aren't bending or sagging.
8. Keep diskettes away from heat and magnetic field sources such as telephones, dictation equipment, and electronic calculators.
9. Be aware that small scratches, dust, food and tobacco particles may make diskette unusable.
10. Label diskettes to keep track of their contents.
11. After label is applied, write on label only with felt tip pen. Any pen is acceptable before label is attached to diskette.
12. Write protect diskettes of importance and make a back up copy of important data.
13. Establish a directory by name and number (i.e., telephone) so that a list of work sheets on file is readily available.
14. Establish a laboratory notebook describing Date, Disc Identification, Description of Data on disc and Signature.
15. Discs must be treated in same confidential manner as all original data (e.g., notebooks and data books).

STANDARD OPERATING PROCEDURES T-450 (continued)

Data Storage and Disc Handling (continued)



Rosalind C Anderson
Dec 3, 1985

Albert Thomas
Dec 2, 1985

APPENDIX A

Toxicology Unit

STANDARD OPERATING PROCEDURE T-413 --- A

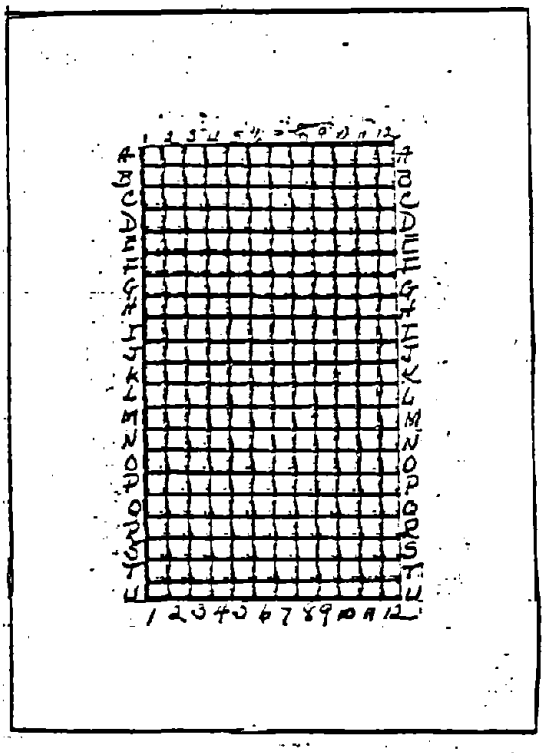
Computerized Skin Tumor Observation Record

A 1/8 inch grid will be used labeled:

A, B, C on Y axis
1, 2, 3 on X axis

Placing grid on back aligning bottom of grid (N-6) with midline of mouse using tail as center. Note location of tumor.

Documentation and descriptions are as attached.



—WEEKLY FILE REPORT for week 0003-- A.D. LITTLE, INC. CAMBRIDGE, MASS. 02140 REF:50003 NIOSH CONTRACT NO. 200-83-2612 (Asphalt Fumes) 'ASPHALT - SEMCAP

STUDY WEEK # 003 Starting Date of Study: 11-15-1985 Today's Date: 12-17-1985

JUL'N DAY	ANIMAL	GROUP	STATUS	WEIGHT	BEHAV	HOUSING	EXCRE	AFRNC	EYE	RESPIR	SKIN	MASS1	LOCATION #1	MASS2	LOCATION #2	ABNR1	LOCATION #1	ABNR2	LOCATION #2	S	SITE	SITE	LESION-TYPE	STUDY
20	1215	1		39	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1217	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1221	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1222	1		41	LANGD	NOFOOD	AB/FE	OBESE	PUPDL	WEEZ	DESQUM	SMAT	----------	NONE	----------	ABCES	testes lt	NONE	----------	-	----	----	----------	
20	1225	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1228	1		34	HYPER	NORM	NORM	THIN	SQINF	POLYP	NORM		----------		----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1234	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1237	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1238	1		38	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1239	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1241	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1243	1		40	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1248	1		32	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1263	1		38	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1265	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1268	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1270	1		40	NORM	FD/H2O	NORM	MALOC	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1271	1		29	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1274	1		39	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1277	1		32	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1278	1		40	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1281	1		38	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1282	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1283	1		50	PROST	NOFOOD	OTHER	OBESE	NORM	DYSP	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1284	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1286	1		37	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1291	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1294	1		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1297	1		39	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1216	2		35	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1218	2		22	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1220	2		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1224	2		33	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1226	2		37	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1227	2		38	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1231	2		38	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1236	2		34	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1245	2		40	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	
20	1246	2		36	NORM	NORM	NORM	NORM	NORM	NORM	NORM	NONE	----------	NONE	----------	NONE	----------	NONE	----------	-	----	----	----------	

12-17-1985 _____

Signature

APPENDIX 15

STANDARD OPERATING PROCEDURE

Disposal of Carcinogenic and Unknown Waste

(Amended 2/19/85)

All carcinogen and unkown chemical waste should be disposed of in the following manner. When at all possible, disposable labware should be used.

A. Biological Waste

(Biological Waste is defined as: animal carcasses, organs, tissues, tissue homogenates, excreta, tumors, and soiled animal litter; cells and cell media; microbiological media; and disposable glassware, pipettes, syringes, etc., which contain or have contained biological material.)

1. All biological waste which is contaminated with carcinogens should be triple-bagged (plastic), sealed and marked in large legible letters "Caution-Carcinogens, To Be Incinerated!" Be sure there is no leakage and that sharp objects will not tear the bag.

2. Do not accumulate carcinogen or chemical wastes in any lab. Ideally, waste should be disposed of at the end of every experiment.

3. All solids are to be collected in a triple-bagged (plastic) stainless steel container that has a lid. Container should be kept closed when not in use and stored in a hood.

4. All liquids are to be made solid by addition of agar or absorbent material. When agar is used, make sure that it has solidified before disposal. If absorbent material (Speedy Dry is obtained from the Stock Room) is used, fill a gallon (or other size) waste container with Speedy Dry and label "CAUTION! CARCINOGENS!" or "CAUTION! CHEMICAL WASTE!" This container should be stored on a metal tray under a hood. Do not remove! Collect liquids in this container and when full, dispose of as described below. CAUTION - this transfer of liquid should be done under a hood (and ONLY there).

5. ATTENTION! Disposed waste should not contain more than 2-5 lbs. of material (paper, plastics, etc.). Excess plastic from oversized bags should be trimmed off before disposing of material.

6. Sealed bags of biological waste should be placed in the room adjacent to the incinerator, enter through the side entrance (single door) to Main Street building, first door on your left.

7. A bin for collection of biological waste is located in the room with a Log Book attached. The contents of all bags must be entered in the Log Book with the following entries:

   - Sequential number of bag (also should be written on bag)

- Description of material (i.e., name of carcinogen if known or suspect carcinogen).
- Type of Material (petri dishes, plastics, glass, etc.)
- Your name
- Date of Disposal

8. NOTE: Do not put animal carcasses in this bin for incineration. They are to be deposited in sealed bags in the freezer provided in the Basement of Building 38, or in the freezer adjacent to the incinerator.

B. Non-Biological Waste

1. Non-biological waste shall be disposed of in metal barrels provided for this purpose.

2. A log with the name or I.D. No and the approximate amount of the compound discarded shall be kept next to the hood in which the disposal containers are stored.

3. All solids are to be collected in a triple-bagged (plastic) stainless steel container that has a lid. Container should be kept closed when not in use and stored in a hood.

4. All liquids are to be made solid by addition of absorbent material. When agar is used, make sure that it has solidified before disposal. If absorbent material (Speedy Dry is obtained from the Stock Room) is used, fill a gallon (or other size) waste container with Speedy Dry and label "CAUTION! CARCINOGENS!" or "CAUTION! CHEMICAL WASTE!" This container should be stored on a metal tray under a hood. Do not remove! Collect liquids in this container and when full, dispose of as described below. CAUTION - this transfer of liquid should be done under a hood (and ONLY there).

5. Full barrels shall be sealed and stored in the solvent shed. Inventory of barrel contents shall be given to the chairperson of the section safety committee.

Health and Safety Plan for NIOSH Study (Contract No. 50043-03): The Assessment of the Carcinogenic Promoting Activity of Asphalt Fumes

Any substance that is suspected of being carcinogenic or is under test for possible carcinogenicity should be considered as carcinogenic unless otherwise specified.

Clothing

All technicians will wear fully buttoned laboratory coats in all work areas. Protective gloves shall be used when handling chemicals and treated animals, and also when treating animals, or shaving animals in preparation for skin painting. Shoes are also mandatory. Sandals are not acceptable. Laboratory clothing shall not be worn outside the work areas either during or at the end of a working day. Hands and face shall be thoroughly washed at the end of each work period. Only designated authorized persons are allowed in chemical handling areas.

No smoking, eating, or drinking in any work area. No storage of food or of food and beverage containers or utensils, and no application of cosmetics in any work area.

No food or drink to be stored in refrigerators or freezers containing chemicals. All work surfaces shall be appropriately protected from contamination and thoroughly cleaned after use.

Hood

All work to be performed in a hood. The hoods are checked monthly and validated by an outside laboratory annually.

Disposal

For disposal of carcinogenic and unknown waste, see attached pages (from Safety Implementation Plan for Biomedical Research and Technology Section).

Protocol Amendment #1

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes
ADL Reference: 50043-03

To be amended:	Page 6, Section 9.D., first sentence.
	We will use a base 3 derived ear-punch system for identifying experimental animals (Appendix C).
To be changed to:	All mice will be tattooed with a specific animal number on the abdomen.
Reason for amendment:	Clarity and ease of animal identification.
Impact on study:	Improve data gathering and reduce technician error.
To be amended:	Page 9, Section 11.A. first sentence.
	Each mouse will be weighed prior to the initiation of the study for the purpose of randomization and group assignment and then before each first weekly application for six weeks.
To be changed to:	Each mouse will be weighed prior to the initiation of the study for the purpose of randomization and group assignment and then weekly for six weeks.
Reason for amendment:	It is unrealistic to weigh each animal prior to the first dosing. Weighing includes clinical observation on the computer which takes additional time. The slowness of the procedure would not allow dosing to occur as per the protocol.
Impact on study:	Allows the dosing and weighing to be performed in a manageable time frame. There should be no scientific impact.

Andrew Sivak, Ph.D.
Vice President
Life Sciences Section
Arthur D. Little, Inc.

9/15/86
(date)

Dr. Richard Niemeier
Deputy Director, Division of Document
Development & Technology Transfer
National Institute for Occupational
Safety & Health

9/18/86
(date)

Protocol Amendment #2

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes

ADL Reference: 50043

To be amended:	Page 6, Section 9.D. Identification and Housing We plan to house not more than 4 mice per cage. (NIH guidelines)
To be changed to:	All mice are being housed individually. (Effective date: August 27, 1986)
Reason for amendment:	Dominance in fighting has resulted in death of an animal.
Impact on study:	Reduce incidental mortality.

Andrew Sivak, Ph.D.
Vice President
Life Sciences Section
Arthur D. Little, Inc.

June 17, 1988
Date

Dennis Lynch
Project Director, Div. of Biomedical and Behavioral Science
National Institute for Occupational Safety and Health

6/29/88
Date

Protocol Amendment #3

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes

ADL Reference: 50043

To be Amended:	Page 7, Section 9.G. Water
To be changed to:	Cambridge tap water will be pH adjusted to pH 2-3 using approximately 12 ml of reagent Grade Hydrochloric Acid per 5 gallons of water. (Effective date: October 9, 1986)
Reason for amendment:	This is a therapeutic treatment to inhibit inner ear infections. There has been a number of deaths due to this problem.
Impact on study:	Reduce mortality due to inner ear infection.

Andrew Sivak, Ph.D.
Vice President
Life Sciences Section
Arthur D. Little, Inc.

June 17, 1988
Date

Dennis Lynch
Project Director, Div. of Biomedical and Behavioral Science
National Institute for Occupational Safety and Health

6/29/88
Date

Protocol Amendment #4

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes

ADL Reference: 50043

To be amended:	Page 10, Section D.15, Necropsy
Addition:	**Animals bearing tumors grossly diagnosed as carcinomas with a diameter of more than 3 cm and persisting for more than 4 consecutive weeks will be killed by carbon dioxide euthanasia and subjected to necropsy preserving the tumor and abnormal lesions will be perserved in buffered formalin.**
Reason for amendment:	When the tumor gets above 2 cm in diameter, the burden on the host becomes large and no additional useful information will be obtained by prolonging the life of the host under stressful conditions.
Impact on study:	None

Andrew Sivak, Ph.D.
Vice President
Life Sciences Section
Arthur D. Little, Inc.

June 17, 1988
Date

Dennis Lynch
Project Director, Div. of Biomedical
and Behavioral Science
National Institute for Occupational
Safety and Health

6/21/88
Date

Protocol Amendment #5

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes

ADL Reference: 50043

To be amended: Page 11, Section D.18 Pathology

To be changed to: Delete entries section D.18. replace by:

To be performed by NIOSH:

Tumors and skin lesions will be examined histologically by a veterinary pathologist at NIOSH. The pathologist will describe all lesions according to their location on the skin, cellular and extracellular composition, size and position in the tissue. The pathologist shall review a sufficient number of known treatment groups to become familiar with the pathological response and then randomize the entire study for an "in blind" evaluation. The pathologist or his designee will maintain records on the incidence, identification, size, location, and disposition of the tumors as the study progresses.

Attention should be given to the distribution between preneoplastic and neoplastic lesions such as fibrosarcomas, papillomas, squamous cell carcinomas, fibromas, keratoancanthomas, and other tumor and lesion types.

All histopathologically confirmed tumors and lesions will be tabulated based on types (benign and malignant), locations, sizes, number of lesions, time of appearance (latent period in days since first treatment), and time to death for all members of each test group as well as the controls. A separate tabulation of nontreatment site lesions will be made.

Protocol Amendment #5

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes

ADL Reference: 50043

Reason for amendment: To transfer work load from the contractor to the sponsor. Contract amendment #10 dated April 29, 1988.

Impact on study: None

Andrew Sivak, Ph.D.
Vice President
Life Sciences Section
Arthur D. Little, Inc.

June 17, 1988
Date

Dennis Lynch
Project Director, Div. of Biomedical
and Behavioral Science
National Institute for Occupational
Safety and Health

6/21/88
Date

Protocol Amendment #6

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes

ADL Reference: 50043

To be amended:	Page 6, Section D.9. Identification and Housing Each cage tag will have bar codes for the animals to facilitate data entry.
To be changed:	Delete above statement
Reason for amendment:	Bar code reader was incompatible with computer program supplied by NIOSH. Standard cage tags provide adequate identification of animals.
Impact on study:	None
To be changed to:	All mice will be weighed on a balance and the data will be recorded manually.
Reason for amendment:	Program supplied by NIOSH was not compatible with balance data entry systems.
Impact on study:	None

Andrew Sivak, Ph.D.
Vice President
Life Sciences Section
Arthur D. Little, Inc.

June 17, 1988
Date

Dennis W. Lynch
Dennis Lynch
Project Director, Div. of Biomedical and Behavioral Science
National Institute for Occupational Safety and Health

6/29/88
Date

Protocol Amendment #7

Protocol for the Assessment of the
Cocarcinogenic Promoting Activity of Asphalt Fumes

ADL Reference: 50043

To be amended:	Page 13, Identity of Study Sponsor
To be changed to:	Dennis Lynch
Reason for amendment:	Contract amendment No #10 dated April 29, 1988
Impact on study:	None

Andrew Sivak, Ph.D.
Vice President
Life Sciences Section
Arthur D. Little, Inc.

June 17, 1988
Date

Dennis Lynch
Project Director, Div. of Biomedical
and Behavioral Science
National Institute for Occupational
Safety and Health

6/29/88
Date

Deviations from the Protocol for the Assessment of the Cocarcinogenic Promoting Activity of Asphalt Fumes. Task 3 Bioassay.

These deviations are judged not to have affected the results of the study.

8. Test Article Identification, Purity, Stability

Samples were not sent to NIOSH periodically for quality control analysis and stability estimations.

9.H. Air Supply

Environmental conditions were outside the protocol specified ranges on some days during the study.

Qualitative direction of air flow was not determined monthly.

Quantitative measurements of air flow were not made twice each year.

9.I. Lighting

The ON/OFF cycle was set for 6 a.m. and 6 p.m., not 7 a.m. and 7 p.m.

12. Monitoring Plan

Moribund animals were not observed by a veterinarian prior to sacrifice. Animals were observed by an experienced animal technician.

15. Necropsy

Scheduled gross necropsies were not done in the presence of a veterinary pathologist. Detailed training on necropsy methodology was carried out by a veterinary pathologist and all necropsy technicians were so trained.

APPENDIX VII

NIOSH STATISTICAL REPORT FOR IN-LIFE DATA

Mortality
Body Weight
Clinical Signs

Tuesday, January 3, 1988

To: Chief, ASTS

Through: Chief, ETB

From: Chief, SU SS

Subject: Statistical report for asphalt fumes contract

Enclosed is a statistical report covering mortality, grossly diagnosed tumors, weights, and abnormal clinical signs. If you have any questions or comments, please stop by or call.

Statistical report for Asphalt Fumes contract

The following is a brief summary of the statistical analysis for asphalt fumes. This report covers four areas: analysis of mortality, analysis of grossly diagnosed tumors, analysis of weights, and analysis of other clinical signs.

This report focuses primarily on descriptive statistics. Therefore, these analyses try to bypass the complex analyses that adjust for multiple time points, multiple variables, and multiple comparisons. A second report, which will cover a complete inferential analysis of tumors, will be written after pathology results are in.

All inferential comparisons in this report are with the relevant control (group 5 for C3H and group 42 for Sencar). Clearly, there are other comparisons that are just as interesting and need to be examined for the second report. I have begun to discuss this issue with several people. A tentative list of relevant comparisons appears in Appendix E.

Analysis of mortality

Analysis of mortality involved computation of the product limit (Kaplan-Meier) survival curve. I have counted terminal sacrifices as censored observations (i.e., the animal's date of natural death is presumed to have occurred at some unspecified point later than the sacrifice date). From this survival curve, I produced estimates of mean survival time and percentiles (75th, 50th, and 25th). For certain groups with low mortality, some or all of these percentiles can not be estimated. For example, a group with only 40% mortality at the time of sacrifice does not allow you to estimate the median. I also include a comparison of each treatment group to the relevant control using the modified Wilcoxon (Breslow) test. I used the two-sided version of this test, implying that either an increase or decrease in mortality are of interest. These tests are not adjusted for multiple comparisons.

Three groups showed significantly less mortality than the control (i.e. a combination of fewer deaths and at later times). Thirteen groups showed significantly higher mortality (more deaths and at earlier times).

Detailed results for mortality appear in Appendix A.

Analysis of grossly diagnosed tumors

Two separate analyses of grossly diagnosed tumors are included: an analysis of carcinomas (tumor type 16=possible carcinoma or 17=probable carcinoma) and an analysis of papillomas plus carcinomas (tumor type 15=papilloma, 16=possible carcinoma, or 17=probable carcinoma).

Analysis of carcinomas involved computation of the product limit (Kaplan-Meier) survival curve. I have counted premature deaths as censored observations if the animal had no carcinomas at the time of death. This implies that the animal would have developed a carcinoma at some unspecified time later than its death if it had survived. From this survival curve, I produced estimates of mean survival time and percentiles (75th, 50th, and 25th). For many groups with low carcinoma rates, some or all of these percentiles can not be estimated. For example, a group with only 10% carcinomas does not allow you to estimate any of the three

percentiles. I also include a comparison of each treatment group to the relevant control using the modified Wilcoxon (Breslow) test. I used the two-sided version of this test, implying that either an increase or a decrease in tumor production is of interest. Although this test does make some adjustment for unequal mortality, caution is advisable for comparison of two groups with highly unequal mortality. In addition, these tests are not adjusted for multiple comparisons.

Analysis of papillomas plus carcinomas is identical.

For carcinomas, eighteen groups had significantly different responses (i.e. a combination of more grossly diagnosed tumors and at earlier times) than their respective controls. For carcinomas plus papillomas, the same groups plus treatment group #1 had significantly different responses.

Detailed results for grossly diagnosed tumors appear in Appendix B.

Analysis of weights

The large number of weighings makes a complete analysis of the weight data prohibitive. Instead, I focused the analysis on weights at days 100, 200, ..., 700. For those animals not weighed on a certain target date, I chose the nearest date prior to the target date. Weights for animals who died near the target date are not included.

I calculated a separate analysis of variance for each target date. No attempt was made to adjust for multiple time points. If a significant difference occurred at a certain date, then comparisons with the relevant control group were calculated. The test used here, Dunnett's test does adjust for multiple comparisons. I used the two-sided version of Dunnett's test, implying that either increases or decreases in weight are of interest.

No significant changes from the respective controls were found at days 100, 300, and 500. Six groups had significantly lower weights at day 200, four groups had significantly higher weights at day 400, one had significantly higher weights at day 500, and one had significantly lower weights at day 700. No treatment group appeared significant at more than one date. Note that there is less power at later dates because fewer animals are alive to be weighed.

Detailed results for weights appear in Appendix C.

Analysis of clinical signs

The large number of clinical signs, the variety of possible values, the predominance of "Other" categories, and the relative infrequency of many of the clinical events makes a formal analysis of clinical signs impossible. Descriptive tables listing the number of animals in each group found with certain clinical signs at some point in time are attached. The "other" category used under some clinical signs is not included in these descriptive statistics.

Detailed results for clinical signs appear in Appendix D.

Conclusion

Analyses of mortality, grossly diagnosed tumors, weights, and clinical signs are included. As the intent of this report was to provide primarily descriptive statistics, no attempt was made to correct for multiple time points, variables, or comparisons. Further, the analysis of grossly diagnosed tumors should be treated carefully as unequal mortality could affect these results.

Appendix A -- Analysis of mortality

Group(1)	Mean(2)	Number(3)	75%(4)	50%(5)	25%(6)
3(7)	692	9	684	---	---
29(7)	692	10	678	---	---
6(7)	690	11	607	---	---
25	666	15	607	732	---
14	660	15	580	702	---
2	655	12	610	---	---
7	643	18	546	678	---
32	641	12	679	---	---
26	630	15	503	727	---
10	629	17	552	675	---
31	623	15	509	705	---
15	622	20	514	656	---
28	620	18	504	589	---
1	610	15	533	698	---
8	610	24	545	659	722
35	608	18	509	607	---
12	608	22	555	601	---
5	607	19	537	629	---
21	597	23	509	615	722
22	593	15	501	684	---
13	583	27	516	573	635
18	582	29	524	583	632
16	580	29	516	589	639
19	577	25	524	573	659
9	572	23	499	588	729
20(8)	566	29	516	570	607
23	562	22	483	530	---
11(8)	555	29	499	533	624
34	553	17	482	526	---
17(8)	551	28	504	559	625
27(8)	538	30	504	540	588
39(8)	530	22	496	512	---
4(8)	526	28	477	573	624
30(8)	504	30	472	491	525
38(8)	500	25	233	513	670
37(8)	480	21	233	504	---
36(8)	466	22	234	495	---
24(8)	449	30	445	464	488
33(8)	447	30	414	459	492
42	661	12	672	---	---
418	571	25	519	592	653

Notes:

(1) Treatment group (see Appendix E for description).
(2) Average time in days that an animal lives since the start of the study (upper bound is 730 due to sacrifices at the end of two years.
(3) Number of animals who died prior to two year sacrifice.
(4) 75th percentile (time in days where 75% of animals are still alive).
(5) 50th percentile (time in days where 50% of animals are still alive). Undefined for groups with less than 50% mortality.
(6) 25th percentile (time in days where 25% of animals are still alive). Undefined for groups with less than 75% mortality.

(7) This group has survival times which are significantly longer (alpha=.05) than the corresponding control.
(8) This group has survival times which are significantly shorter (alpha=.05) than the corresponding control.

Appendix B -- Analysis of grossly diagnosed tumors

Carcinomas

Group(1)	Mean(2)	Number(3)	75%(4)	50%(5)	25%(6)
2	---	0	---	---	---
3	---	0	---	---	---
5	---	0	---	---	---
6	---	0	---	---	---
9	---	0	---	---	---
10	---	0	---	---	---
14	---	0	---	---	---
15	---	0	---	---	---
23	---	0	---	---	---
26	---	0	---	---	---
35	---	0	---	---	---
36	---	0	---	---	---
37	---	0	---	---	---
38	---	0	---	---	---
39	---	0	---	---	---
29	744	1	---	---	---
32	743	1	---	---	---
31	743	1	---	---	---
28	743	1	---	---	---
34	742	2	---	---	---
25	726	1	---	---	---
1	720	3	---	---	---
12(7)	703	6	693	---	---
7(7)	697	9	659	---	---
22(7)	696	7	692	---	---
13(7)	642	11	659	679	700
8(7)	635	16	582	679	699
21(7)	635	15	580	663	727
19(7)	599	15	563	576	651
16(7)	594	15	548	616	679
4(7)	550	19	475	547	643
18(7)	540	24	449	540	637
17(7)	535	22	448	548	610
20(7)	523	24	464	527	583
11(7)	512	22	435	504	568
27(7)	495	23	447	502	538
30(7)	445	27	405	440	462
33(7)	436	22	384	427	468
24(7)	413	26	369	411	440
42	737	0	---	---	---
41(7)	578	16	454	517	---

Notes:

(1) Treatment group (see Appendix E for description).
(2) Average time in days that an animal is diagnosed as having a carcinoma. This estimate is adjusted for mortality. Undefined for groups with no diagnosed carcinomas. Means based on small numbers of carcinomas should be interpreted with caution.
(3) Number of animals who are diagnosed as having a carcinoma.

(4) 75th percentile (time in days where 75% of animals are free from carcinomas). Undefined for animals with less than 25% carcinomas.
(5) 50th percentile (time in days where 50% of animals are free from carcinomas). Undefined for groups with less than 50% carcinomas.
(6) 25th percentile (time in days where 25% of animals are free from carcinomas). Undefined for groups with less than 75% carcinomas.
(7) This group has times to carcinoma which are significantly shorter (alpha=.05) than the corresponding control.

Papillomas plus carcinomas

Group(1)	Mean(2)	Number(3)	75%(4)	50%(5)	25%(6)
2	---	0	---	---	---
3	---	0	---	---	---
5	---	0	---	---	---
6	---	0	---	---	---
10	---	0	---	---	---
15	---	0	---	---	---
26	---	0	---	---	---
35	---	0	---	---	---
36	---	0	---	---	---
38	---	0	---	---	---
14	747	1	---	---	---
29	742	1	---	---	---
9	740	1	---	---	---
32	739	1	---	---	---
34	738	3	---	---	---
28	737	2	---	---	---
39	737	1	---	---	---
37	736	1	---	---	---
31	724	3	---	---	---
23	716	2	---	---	---
25	711	3	---	---	---
1(7)	702	4	---	---	---
7(7)	674	12	602	720	---
22(7)	665	10	621	698	---
12(7)	630	14	597	659	715
21(7)	571	19	510	552	657
13(7)	566	16	519	583	630
8(7)	558	21	491	568	630
19(7)	539	22	464	519	554
16(7)	524	21	519	540	583
4(7)	492	21	413	497	582
17(7)	490	25	435	484	562
20(7)	489	26	449	464	563
11(7)	473	25	392	476	562
18(7)	471	28	400	484	554
27(7)	450	25	404	447	502
33(7)	417	23	370	405	468
30(7)	401	29	349	405	427
24(7)	388	27	355	390	411
42	737	0	---	---	---
41(7)	484	21	359	442	643

Notes:

(1) Treatment group (see Appendix E for description).
(2) Average time in days that an animal is diagnosed as having a carcinoma or papilloma. This estimate is adjusted for mortality. Undefined for groups with no diagnosed papillomas or carcinomas. Means based on small numbers of papillomas and carcinomas should be interpreted with caution.
(3) Number of animals who are diagnosed as having a carcinoma or papilloma.

(4) 75th percentile (time in days where 75% of animals are free from papillomas and carcinomas). Undefined for animals with less than 25% papillomas or carcinomas.
(5) 50th percentile (time in days where 50% of animals are free from papillomas and carcinomas). Undefined for groups with less than 50% papillomas or carcinomas.
(6) 25th percentile (time in days where 25% of animals are free from papillomas and carcinomas). Undefined for groups with less than 75% papillomas or carcinomas.
(7) This group has times to carcinoma or papilloma which are significantly shorter (alpha=.05) than the corresponding control.

Appendix C -- Analysis of weights(1)

Target Day

Group(2)	100	200	300	400	500	600	700
11	27.15	27.55	27.18	27.99	24.19	20.49	20.62
6	27.23	27.37	26.64	27.88	24.50	21.14	23.95
3	26.70	27.35	25.70	28.27	24.80	22.96	24.49
18	26.96	27.15	27.38	27.82	25.37	20.57	21.00
20	27.43	27.07	27.62	27.51	26.27(4)	20.80	21.60
8	26.92	27.03	27.16	27.66	24.40	21.26	22.32
12	26.22	27.02	26.27	26.95	23.06	20.14	22.31
17	27.17	27.00	27.49	27.55	25.62	21.46	21.30
5	27.28	26.96	26.14	27.25	23.27	21.01	22.24
10	26.41	26.73	26.28	27.01	23.21	20.77	23.15
1	26.97	26.69	25.93	28.58	24.76	22.83	24.34
16	26.62	26.68	27.61	27.21	23.68	20.35	18.96
29	27.08	26.56	26.13	30.03(4)	24.69	21.78	23.90
9	26.07	26.52	26.32	26.50	22.60	20.96	22.33
13	25.89	26.28	27.04	27.44	21.69	19.18	19.82
19	26.73	26.26	26.61	27.12	24.83	20.45	22.99
2	26.74	26.21	25.26	27.97	24.84	22.88	24.07
7	27.20	26.05	26.38	27.59	23.06	21.89	23.56
4	26.33	25.99	24.40	27.81	23.58	20.15	17.75(3)
37	26.75	25.88	26.34	29.36(4)	24.22	22.32	23.48
39	26.47	25.86	26.82	28.28	23.62	20.40	24.53
14	26.00	25.84	26.84	26.90	23.31	20.19	23.35
15	25.62	25.81	26.93	26.22	22.97	20.86	22.78
23	26.90	25.71	25.33	26.94	23.91	21.65	24.20
30	27.18	25.71	25.44	29.13(4)	21.71	20.58	23.07
32	26.89	25.70	26.99	29.08	24.92	22.74	24.14
40	26.71	25.63	26.09	28.82	24.56	22.68	22.80
38	26.70	25.62	26.35	29.50(4)	23.71	21.10	21.07
36	27.17	25.59	25.97	26.69	23.12	20.84	22.75
34	26.58	25.49	26.43	28.20	23.34	22.59	24.79
24	27.09	25.48	25.29	27.98	25.04	22.98	.
31	27.60	25.46	26.71	27.68	23.42	20.86	23.07
35	27.00	25.44	27.48	26.03	23.29	20.54	22.74
21	26.52	25.38	25.92	27.73	24.49	20.75	23.05
33	26.29	25.23(3)	26.76	26.99	22.69	20.38	24.20
22	26.00	25.18(3)	25.28	27.18	23.42	21.42	24.06
28	26.89	24.76(3)	25.26	26.28	23.63	21.22	24.18
27	27.31	24.62(3)	25.52	26.18	24.05	20.11	24.75
26	27.12	24.02(3)	25.13	26.88	24.44	22.02	22.93
25	26.96	23.48(3)	24.93	26.30	24.10	21.61	22.75
42	38.52	37.82	40.11	39.85	39.67	39.53	39.25
41	40.00	39.33	40.98	40.31	40.37	39.96	38.28

Notes:

(1) Weighing was done on various days. These weights are the weights measured closest to and just prior to the target day.
(2) Treatment group (see Appendix E for description).
(3) This average is significantly less than the corresponding control.
(4) This average is significantly greater than the corresponding control.

Appendix D -- Analysis of clinical signs

Abnormal behavior

Group	Description	Frequency
3	prostrate	1
5	prostrate	1
7	anorexic	2
7	prostrate	1
8	circling	1
8	prostrate	1
9	languid	1
9	prostrate	2
11	languid	1
11	prostrate	1
12	anorexic	1
12	prostrate	1
14	languid	1
14	prostrate	1
15	languid	1
15	prostrate	3
16	prostrate	2
17	prostrate	1
19	languid	1
20	languid	1
21	languid	1
22	circling	1
24	languid	4
25	prostrate	1
26	languid	2
27	languid	2
29	languid	1
30	anorexic	1
32	languid	2
34	languid	3
34	hyperactive	1

35	circling	1
36	languid	5
37	languid	4
37	prostrate	1
38	languid	5
39	languid	1
40	languid	5
41	hyperactive	1
41	prostrate	1
42	prostrate	1

Abnormal excrement

Group	Description	Frequency
2	soft feces	1
4	soft feces	3
5	soft feces	1
10	soft feces	1
14	vomit	1
19	soft feces	1
22	soft feces	1
26	soft feces	2
31	soft feces	1
32	soft feces	1
33	soft feces	2
34	soft feces	5
36	soft feces	8
37	soft feces	1
39	soft feces	2
40	soft feces	5
41	soft feces	1

Abnormal feeding or drinking

Group	Description	Frequency
10	excess spilled food	2
14	excess spilled food	1
16	excess spilled food	1
33	excess spilled food	1
36	no food	1
36	no water	1
42	excess spilled food	1

Abnormal appearance

Group	Description	Frequency
1	hunched	6
1	thin	5
1	head tilt	1
2	hunched	8
2	thin	4
2	head tilt	1
3	hunched	9
3	thin	3
3	head tilt	1
3	tremors	1
4	hunched	1
4	thin	4
4	head tilt	2
5	hunched	8
5	thin	8
5	head tilt	3
6	hunched	17
6	thin	5
6	head tilt	1
7	hunched	9
7	thin	7
7	head tilt	1
8	hunched	7
8	thin	2
8	head tilt	3
9	hunched	6
9	thin	7
9	head tilt	1
10	hunched	3
10	thin	4
10	head tilt	1
11	thin	7
11	tremors	1
12	hunched	5
12	thin	6
12	head tilt	1
13	hunched	2
13	thin	10
13	head tilt	2
14	hunched	6
14	thin	9
14	head tilt	1

15	hunched	5
15	thin	12
16	hunched	1
16	thin	1
17	hunched	2
17	thin	7
17	tremors	1
18	hunched	8
18	thin	8
19	thin	7
20	hunched	4
20	thin	4
21	hunched	2
21	thin	7
22	hunched	5
22	thin	4
22	head tilt	1
23	hunched	4
23	thin	3
24	hunched	2
24	thin	3
25	hunched	6
25	thin	4
26	hunched	7
26	thin	5
27	hunched	1
27	thin	5
28	hunched	2
28	thin	5
29	hunched	6
29	thin	1
29	head tilt	1
30	hunched	1
30	thin	7
31	hunched	6
31	thin	5
32	hunched	5
32	thin	2
32	head tilt	2
33	thin	3
33	head tilt	1

34	hunched	6
34	thin	4
34	head tilt	1
35	hunched	8
35	thin	4
36	hunched	4
36	thin	2
37	hunched	2
37	thin	3
37	head tilt	1
37	paralysis	1
38	hunched	3
38	thin	3
38	head tilt	1
39	hunched	3
39	thin	2
40	thin	1
41	hunched	3
41	thin	1
41	head tilt	1
42	hunched	1
42	thin	2

Abnormality of the eyes

Group	Description	Frequency
2	squinted	3
2	opaque	1
6	lacrimating	1
7	squinted	2
7	lacrimating	1
9	squinted	2
10	squinted	2
15	squinted	1
15	lacrimating	1
17	lacrimating	1
19	squinted	
19	lacrimating	1
22	lacrimating	1
23	lacrimating	2
24	squinted	1
24	opaque	1
25	lacrimating	1
28	squinted	1
29	lacrimating	2
31	lacrimating	3
32	squinted	1
35	opaque	1
36	lacrimating	1
37	squinted	1
37	lacrimating	1
39	opaque	1
41	squinted	1
41	lacrimating	1
42	lacrimating	1

Abnormal respiration

Group	Description	Frequency
1	epistaxis	1
4	dyspnea	2
5	dyspnea	1
7	dyspnea	2
8	dyspnea	1
9	dyspnea	3
10	dyspnea	1
11	dyspnea	2
12	dyspnea	1
13	wheezing	1
14	dyspnea	2
15	dyspnea	2
16	dyspnea	2
17	dyspnea	1
19	polypnea	1
21	dyspnea	1
27	wheezing	1
29	epistaxis	1
30	dyspnea	1
39	dyspnea	1
41	wheezing	1
41	dyspnea	1
41	polypnea	1
42	dyspnea	1

Abnormality of the skin/fur

Group	Description	Frequency
3	scabs/sores	2
4	scabs/sores	1
5	scabs/sores	1
10	scabs/sores	1
17	scabs/sores	2
18	scabs/sores	1
24	scabs/sores	23
25	rough/discolored	1
25	scabs/sores	8
26	scabs/sores	6
33	scabs/sores	16
34	scabs/sores	10
35	scabs/sores	2
41	rough/discolored	1
41	scabs/sores	3
42	scabs/sores	1

Abnormal masses

Group	Description	Location	Frequency
2	small movable mass	abdomen	1
2	small movable mass	paw, hind rt	1
3	large movable mass	back, post	1
3	large movable mass	hip rt	1
4	large movable mass	abdomen	1
4	large movable mass	back, post	1
6	small movable mass	abdomen	2
8	small attached mass	leg, hind rt	1
9	small movable mass	chest	1
9	small movable mass	abdomen	1
10	small movable mass	abdomen	3
12	small attached mass	inguinal lt	1
14	small movable mass	abdomen	3
14	small movable mass	side rt	1
14	small movable mass	penis	1
15	small movable mass	mouth	1
15	small movable mass	chest	1
17	small movable mass	shoulder rt	1
19	small movable mass	jaw	1
20	small movable mass	abdomen	1
20	small movable mass	leg, hind rt	1
23	small movable mass	abdomen	1
23	small attached mass	teeth	1
25	small movable mass	abdomen	3
27	small movable mass	leg, hind rt	1
29	small movable mass	abdomen	2
29	small movable mass	hip rt	1
30	large movable mass	back, post	1
30	large movable mass	side lt	1
33	large movable mass	back, ant	1
33	large movable mass	back, post	1
33	large movable mass	side lt	1
34	small movable mass	hip lt	1
35	small movable mass	teeth	1

42	small movable mass	abdomen	2
42	small movable mass	side rt	1
42	small attached mass	back, post	1
42	large movable mass	abdomen	1
42	large movable mass	side rt	1
42	large attached mass	side rt	1

Additional abnormal masses

Group	Description	Location	Frequency
3	large movable mass	hip lt	1
12	small attached mass	inguinal rt	1

Other abnormalities

Group	Description	Location	Frequency
1	ulcerated	penis	1
1	ulcerated	tail	2
2	small	testes lt	1
2	necrotic	tail	1
2	ulcerated	tail	1
3	red	eye/lid rt	1
3	necrotic	tail	1
3	ulcerated	tail	2
5	small	tail	1
5	ulcerated	tail	2
6	small	penis	1
6	necrotic	tail	1
7	ulcerated	tail	1
8	ulcerated	tail	4
9	red	shoulder lt	1
9	abcessed	tail	1
9	necrotic	tail	1
10	small	tail	1
10	necrotic	tail	2
10	ulcerated	tail	2
11	ulcerated	tail	1
12	small	tail	1
12	necrotic	tail	2
12	ulcerated	penis	1
12	ulcerated	tail	3
13	ulcerated	tail	2
14	ulcerated	tail	2
15	ulcerated	tail	1
16	ulcerated	tail	1
17	ulcerated	tail	1
18	necrotic	tail	1
18	ulcerated	penis	1
18	ulcerated	tail	5
19	pale	tail	1
19	necrotic	tail	1
19	ulcerated	tail	3
20	ulcerated	tail	2

21	red	penis	1
21	ulcerated	tail	1
23	ulcerated	tail	1
25	necrotic	tail	2
25	ulcerated	penis	1
25	ulcerated	tail	1
26	necrotic	tail	2
28	red	abdomen	1
28	ulcerated	tail	1
29	ulcerated	tail	1
30	abcessed	tail	1
31	red	back, post	1
32	small	testes lt	1
33	abcessed	back, post	3
33	ulcerated	tail	1
36	ulcerated	tail	2
37	small	head	1
38	ulcerated	tail	1
39	small	testes lt	1
40	red	tail	1
40	ulcerated	tail	1
41	small	back, ant	1
41	ulcerated	back, post	1
42	ulcerated	leg, hind rt	1
42	ulcerated	tail	2

Additional cases of other abnormalities

Group	Description	Location	Frequency
1	small	tail	1
1	necrotic	tail	3
2	small	testes rt	1
2	necrotic	tail	4
2	ulcerated	tail	3
3	necrotic	tail	4
3	ulcerated	tail	1
4	ulcerated	tail	2
5	necrotic	tail	4
5	ulcerated	tail	1
6	necrotic	tail	1
6	ulcerated	tail	1
7	abcessed	penis	1
7	ulcerated	tail	2
8	necrotic	tail	1
8	ulcerated	tail	4
9	ulcerated	tail	2
10	small	tail	1
10	necrotic	tail	5
11	ulcerated	tail	2
12	necrotic	tail	2
12	ulcerated	tail	3
13	necrotic	tail	1
13	ulcerated	paw, fore lt	1
13	ulcerated	penis	1
14	necrotic	tail	1
14	ulcerated	tail	4
15	necrotic	tail	4
15	ulcerated	tail	8
16	ulcerated	tail	3
17	ulcerated	tail	2
18	necrotic	leg, hind rt	1
18	ulcerated	tail	1
19	necrotic	tail	1
19	ulcerated	tail	2
20	necrotic	tail	1

20	ulcerated	tail	2
23	ulcerated	tail	1
25	ulcerated	tail	1
32	small	testes rt	1
39	small	testes rt	1

Appendix E -- Tentative list of comparisons

Listing of group codes

```
 1='raw asphalt'
 2='heated asphalt'
 3='heated plus fume'
 4='neat asphalt fume'
 5='solvent control'
 6='A fraction'
 7='B fraction'
 8='C fraction'
 9='D fraction'
10='E fraction'
11='A-E fractions'
12='A,B fractions'
13='A,C fractions'
14='A,D fractions'
15='A,E fractions'
16='B,C,D,E fractions'
17='A,B,C,D fractions'
18='A,B,C,E fractions'
19='B,C,D fractions'
20='B,C fractions'
21='A,C,D,E fractions'
22='A,B,D,E fractions'
23='A,D,E fractions'
24='.01% B(a)P'
25='.001% B(a)P'
26='.0001% B(a)P'
27='A,.01% B(a)P'
28='A,.001% B(a)P'
29='A,.0001% B(a)P'
30='D,.01% B(a)P'
31='D,.001% B(a)P'
32='D,.0001% B(a)P'
33='E,.01% B(a)P'
34='E,.001% B(a)P'
35='E,.0001% B(a)P'
36='Init then A'
37='Init then D'
38='Init then E'
39='Init alone'
40='Sentinal mice'
41='Sencar fume'
42='Sencar control'
```

1 vs 4, 2 vs 4, 3 vs 4

These compare the effects of raw asphalt, heated asphalt, and heated asphalt plus fume to neat asphalt fume.

6 vs 4, 7 vs 4, 8 vs 4, 9 vs 4, 10 vs 4

These compare the effects of individual fractions of asphalt fumes to the neat asphalt fume.

11 vs 4

This compares the recombinations of the original fractions to the neat asphalt fume.

12 vs 7, 13 vs 8, 14 vs 9, 15 vs 10

This measures the effect of adding fraction A to each of the remaining four fractions.

16 vs 4, 17 vs 4, 18 vs 4, 19 vs 4, 20 vs 4, 21 vs 4, 22 vs 4, 23 vs 4

This measures the effect of deletion of certain fractions from the total.

27 vs 24, 28 vs 25, 29 vs 26

This measures the co-carcinogenic effect of fraction A.

30 vs 24, 31 vs 25, 32 vs 26

This measures the co-carcinogenic effect of fraction D.

33 vs 24, 34 vs 25, 35 vs 26

This measures the co-carcinogenic effect of fraction E.

41 vs 4, 42 vs 5

This compares the Sencar and C3H mice.

APPENDIX VIII

NIOSH STATISTICAL REPORT ON PATHOLOGY DATA

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
National Institute for
Occupational Safety & Health
Robert A. Taft Laboratories
4676 Columbia Parkway
Cincinnati OH 45226-1998

April 18, 1989

Anthony P. Graffeo, Ph.D.
Manager, Chemical and
Life Sciences Section
Arthur D. Little, Inc.
Acorn Park
Cambridge, Massachusetts 02140-2390

Dear Dr. Graffeo:

Enclosed is a copy of the statistical report on the pathology data from the asphalt fume study, Contract 200-83-2612, which was prepared by Dr. Stephen Simon of NIOSH. Please transmit the report to Dr. Sivak as soon as possible for his use in preparing the report on the in-life phases of the contract. Please have Dr. Sivak call me directly (513-533-8213) if he has any questions, or if he would be interested in receiving any graphical displays of these results. We are on schedule for completion of this contract effort, and we need to make a concerted effort do meet the remaining milestones in a timely and efficient manner. Please contact me immediately if any problems develop relating to this contract. I look forward to receiving the draft final report and the draft manuscript on July 15, 1989.

Sincerely yours,

Dennis W. Lynch

Dennis W. Lynch, M.S. Chief
Acute and Subchronic Toxicology Section
Experimental Toxicology Branch
Division of Biomedical
and Behavioral Science

Enclosure

To: Chief, Acute and Subchronic Toxicology Section

Through: Chief, Experimental Toxicology Branch RES

From: Chief, Statistics Unit SS

Enclosed is the second (and last) statistical report for the asphalt fumes study.

A large portion of the credit for this report must go to Mr. Al Stine. The quality of this effort occurred only through his tireless efforts, through his work with a variety of computer software, and through his sharp eye for detail. He had to process over 300 floppy disks, make thousands of comparisons, and run dozens of programs to get the data into a format where it could be properly analyzed. His work has gone far beyond what is normally expected of someone in his position; without him, timely completion of this report would have been impossible.

Although this report meets the contractual requirements on my part, I would be more than happy to perform any supplemental work (such as graphical display of these results) that you or Dr. Sivak may require. Please stop by if you have any questions or comments.

Statistical analysis of microscopically diagnosed tumors

This report presents the statistical analysis of tumors based on microscopic analysis provided by the Experimental Pathology Section. I am including tables of descriptive and inferential statistics for three categories of tumors, corresponding roughly to benign tumors alone, malignant tumors alone, and both types of tumors. In this report, comparisons include those among the treated groups as well as comparisons of all treated groups to the relevant control group.

In mid-February, I received a Lotus 1-2-3 spreadsheet file listing the animal codes, slide number and final diagnosis. The possible values for final diagnosis were

1. CAR	Carcinoma
2. PAP	Papilloma
3. FCYST	Follicular cyst
4. HYPER	Hyperatosis/Hyperplasia
5. KER	Keratoacanthoma
6. SAR	Sarcoma
7. FIB	Fibroma
8. NORM	Normal
9. OTHER	Other

and almost all the diagnoses fell into categories 1 or 2.

These data were matched with data in the clinical observations data set to obtain a time to tumor. I defined time to tumor using the first observation which reported any activity at or near the site where the tumor was located at the time of sacrifice. Any activity at another site or any activity that discontinued after a certain time frame was not used in determining time to tumor. For example, animal #5738 had a suspicious area at location A11 on day 707. Since this observation did not continue in any of the following weeks, this activity was not used in computing any time to tumor. As another example, animal #602 had a papilloma at location G8 on day 548 and a papilloma at location G11 on day 554. Since the only tumor at sacrifice time was located at H11, I set the time to tumor at 554, not 548.

Some slides were made of tumors found at sites other than the application site. Based on advice from Dr. Niemeier, I excluded these tumors from the analysis.

With nine categories, classification of tumors and comparison of groups is tricky. Using the suggestion of Dr. Salomon, I classified tumors as malignant if they were either 1 (carcinoma) or 6 (sarcoma). I classified tumors as benign if they were either 2 (papilloma), 5 (keratoacanthoma), or 7 (fibroma). The remaining categories were ignored in all data analyses. Using feedback from Dr. Lynch and Dr. Niemeier, I performed an anlysis counting mice with one or malignant tumors, one or more benign tumors, and one or more of either type of tumor. The second analysis could be subject to criticism, since mice with one or more benign tumors are compared to mice with no tumors combined with mice with only malignant tumors. Interpretation of the results for this case may be difficult.

Analysis of tumors involved computation of the product limit (Kaplan-Meier) survival curve. The number of mice with tumors, rather than the average number of tumors per mouse is the measure I used for all analyses. I have counted premature deaths as censored observations if the animal had no tumors of a specified type at the time of death. This implies that the animal would have developed a tumor at some unspecified time later than its death if it had survived. From this survival curve, I produced estimates of mean survival time and percentiles (75th, 50th, and 25th). For many groups with low tumor rates, some or all of these percentiles can not be estimated. For example, a group with only 10% tumors does not allow you to estimate any of the three percentiles. Further, the pattern of deaths in a group can influence whether it is possible to estimate certain percentiles.

I also include a comparison of certain groups to others using the modified Wilcoxon (Breslow) test. I used the two-sided version of this test, implying that either an increase or a decrease in tumor production is of interest. Although this test does make an adjustment for unequal mortality, caution is advisable for comparison of two groups with highly unequal mortality (refer to the previous report for mortality comparisons). In addition, these tests are not adjusted for multiple comparisons.

Table 1 -- Descriptive statistics on time to carcinoma (or sarcoma; i.e., malignant tumors)

	Group	Number of mice with carcinomas	Average time to carcinoma	Percentiles 75%	50%	25%
(24)	.01% B(a)P	27	391	362	397	411
(30)	D,.01% B(a)P	25	445	405	447	523
(20)	B,C fractions	24	514	457	532	563
(33)	E,.01% B(a)P	23	429	405	419	454
(27)	A,.01% B(a)P	23	486	440	482	530
(18)	A,B,C,E fractions	21	542	484	532	637
(4)	neat asphalt fume	20	517	421	525	615
(11)	A-E fractions	19	528	448	525	630
(17)	A,B,C,D fractions	18	560	484	562	651
(8)	C fraction	17	599	512	590	671
(16)	B,C,D,E fractions	15	565	526	569	623
(19)	B,C,D fractions	15	605	548	591	700
(41)	Sencar fume	14	578	426	596	---
(21)	A,C,D,E fractions	14	620	552	649	733
(13)	A,C fractions	11	627	555	590	---
(7)	B fraction	10	688	609	728	---
(12)	A,B fractions	8	682	643	714	---
(22)	A,B,D,E fractions	7	695	692	---	---
(25)	.001% B(a)P	3	717	---	---	---
(1)	raw asphalt	3	709	---	---	---
(34)	E,.001% B(a)P	2	739	---	---	---
(28)	A,.001% B(a)P	1	741	---	---	---
(29)	A,.0001% B(a)P	1	742	---	---	---
(32)	D,.0001% B(a)P	1	739	---	---	---
(42)	Sencar control	1	729	---	---	---
(2)	heated asphalt	0	---	---	---	---
(3)	heated plus fume	0	---	---	---	---
(5)	solvent control	0	---	---	---	---
(6)	A fraction	0	---	---	---	---
(9)	D fraction	0	---	---	---	---
(10)	E fraction	0	---	---	---	---
(14)	A,D fractions	0	---	---	---	---
(15)	A,E fractions	0	---	---	---	---
(23)	A,D,E fractions	0	---	---	---	---
(26)	.0001% B(a)P	0	---	---	---	---
(31)	D,.001% B(a)P	0	---	---	---	---
(35)	E,.0001% B(a)P	0	---	---	---	---
(36)	Init then A	0	---	---	---	---
(37)	Init then D	0	---	---	---	---
(38)	Init then E	0	---	---	---	---
(39)	Init alone	0	---	---	---	---

Note: --- implies that there were an insufficient number of malignant tumors to be able to estimate this statistic.

Table 2 -- Descriptive statistics on time to papillomas (or keratoacanthoma or fibroma; i.e., benign tumors)

	Group	Number of mice with papillomas	Average time to papilloma	Percentiles 75%	50%	25%
(18)	A,B,C,E fractions	14	601	509	569	---
(41)	Sencar fume	13	618	531	643	---
(11)	A-E fractions	12	602	497	706	---
(4)	neat asphalt fume	12	611	547	589	---
(17)	A,B,C,D fractions	12	618	526	590	---
(19)	B,C,D fractions	12	629	540	623	---
(30)	D,.01% B(a)P	11	556	468	---	---
(20)	B,C fractions	10	623	591	665	673
(12)	A,B fractions	7	710	679	721	---
(13)	A,C fractions	7	666	630	687	693
(27)	A,.01% B(a)P	5	637	---	---	---
(16)	B,C,D,E fractions	4	702	630	---	---
(21)	A,C,D,E fractions	4	707	719	---	---
(22)	A,B,D,E fractions	4	714	---	---	---
(8)	C fraction	3	720	---	---	---
(23)	A,D,E fractions	2	716	---	---	---
(25)	.001% B(a)P	2	723	---	---	---
(31)	D,.001% B(a)P	2	732	---	---	---
(7)	B fraction	2	741	---	---	---
(24)	.01% B(a)P	1	564	---	---	---
(33)	E,.01% B(a)P	1	675	---	---	---
(1)	raw asphalt	1	739	---	---	---
(28)	A,.001% B(a)P	1	744	---	---	---
(2)	heated asphalt	0	---	---	---	---
(3)	heated plus fume	0	---	---	---	---
(5)	solvent control	0	---	---	---	---
(6)	A fraction	0	---	---	---	---
(9)	D fraction	0	---	---	---	---
(10)	E fraction	0	---	---	---	---
(14)	A,D fractions	0	---	---	---	---
(15)	A,E fractions	0	---	---	---	---
(26)	.0001% B(a)P	0	---	---	---	---
(29)	A,.0001% B(a)P	0	---	---	---	---
(32)	D,.0001% B(a)P	0	---	---	---	---
(34)	E,.001% B(a)P	0	---	---	---	---
(35)	E,.0001% B(a)P	0	---	---	---	---
(36)	Init then A	0	---	---	---	---
(37)	Init then D	0	---	---	---	---
(38)	Init then E	0	---	---	---	---
(39)	Init alone	0	---	---	---	---
(42)	Sencar control	0	---	---	---	---

Note: --- implies that there were an insufficient number of benign tumors to be able to estimate this statistic.

Table 3 -- Descriptive statistics on time to benign or malignant tumor

	Group	Number of mice with tumors	Average time to tumor	Percentiles 75%	50%	25%
(30)	D,.01% B(a)P	29	433	405	440	468
(24)	.01% B(a)P	27	391	362	397	411
(18)	A,B,C,E fractions	27	495	414	492	569
(20)	B,C fractions	26	506	457	509	563
(11)	A-E fractions	25	476	392	491	562
(33)	E,.01% B(a)P	24	427	405	412	447
(27)	A,.01% B(a)P	24	475	419	468	530
(17)	A,B,C,D fractions	24	515	463	509	569
(4)	neat asphalt fume	21	495	421	497	582
(19)	B,C,D fractions	21	553	513	548	604
(41)	Sencar fume	20	533	405	510	727
(8)	C fraction	20	578	497	582	659
(16)	B,C,D,E fractions	19	539	526	555	623
(21)	A,C,D,E fractions	17	599	552	614	719
(13)	A,C fractions	15	586	548	590	630
(12)	A,B fractions	13	652	610	679	721
(7)	B fraction	11	683	609	728	---
(22)	A,B,D,E fractions	9	685	646	---	---
(25)	.001% B(a)P	5	707	---	---	---
(1)	raw asphalt	4	702	---	---	---
(23)	A,D,E fractions	2	716	---	---	---
(31)	D,.001% B(a)P	2	732	---	---	---
(28)	A,.001% B(a)P	2	738	---	---	---
(34)	E,.001% B(a)P	2	739	---	---	---
(42)	Sencar control	1	729	---	---	---
(32)	D,.0001% B(a)P	1	739	---	---	---
(29)	A,.0001% B(a)P	1	742	---	---	---
(2)	heated asphalt	0	---	---	---	---
(3)	heated plus fume	0	---	---	---	---
(5)	solvent control	0	---	---	---	---
(6)	A fraction	0	---	---	---	---
(9)	D fraction	0	---	---	---	---
(10)	E fraction	0	---	---	---	---
(14)	A,D fractions	0	---	---	---	---
(15)	A,E fractions	0	---	---	---	---
(26)	.0001% B(a)P	0	---	---	---	---
(35)	E,.0001% B(a)P	0	---	---	---	---
(36)	Init then A	0	---	---	---	---
(37)	Init then D	0	---	---	---	---
(38)	Init then E	0	---	---	---	---
(39)	Init alone	0	---	---	---	---

Note: ---implies that there were an insufficient number of tumors to be able to estimate this statistic.

Table 4 -- Inferential statistics for carcinoma (or sarcoma; i.e., malignant tumors)

First group	Second group	Generalized Wilcoxon	p-value	
Co-carcinogenic effects of fractions A, D, and E				
(29) A,.0001% B(a)P	(26) .0001% B(a)P	.826	.3634	
(28) A,.001% B(a)P	(25) .001% B(a)P	.745	.3880	
(27) A,.01% B(a)P	(24) .01% B(a)P	27.837	.0000	(b)
(32) D,.0001% B(a)P	(26) .0001% B(a)P	.913	.3393	
(31) D,.001% B(a)P	(25) .001% B(a)P	2.482	.1151	
(30) D,.01% B(a)P	(24) .01% B(a)P	11.442	.0007	(b)
(35) E,.0001% B(a)P	(26) .0001% B(a)P	----	----	
(34) E,.001% B(a)P	(25) .001% B(a)P	.117	.7319	
(33) E,.01% B(a)P	(24) .01% B(a)P	7.324	.0068	(b)
Additional activity due to fraction A				
(7) B fraction	(12) A,B fractions	.093	.7602	
(8) C fraction	(13) A,C fractions	.918	.3381	
(9) D fraction	(14) A,D fractions	----	----	
(10) E fraction	(15) A,E fractions	----	----	
Promotion capability of fractions A, D, and E				
(36) Init then A	(6) A fraction	----	----	
(36) Init then A	(39) Init alone	----	----	
(37) Init then D	(9) D fraction	----	----	
(37) Init then D	(39) Init alone	----	----	
(38) Init then E	(10) E fraction	----	----	
(38) Init then E	(39) Init alone	----	----	
Comparison of individual fractions to the total fume				
(4) neat asphalt fume	(6) A fraction	34.994	.0000	(a)
(4) neat asphalt fume	(7) B fraction	20.415	.0000	(a)
(4) neat asphalt fume	(8) C fraction	4.340	.0372	(a)
(4) neat asphalt fume	(9) D fraction	25.988	.0000	(a)
(4) neat asphalt fume	(10) E fraction	30.150	.0000	(a)
The effect of removing one fraction from the total				
(4) neat asphalt fume	(17) A,B,C,D fractions	2.252	.1334	
(4) neat asphalt fume	(18) A,B,C,E fractions	.537	.4637	
(4) neat asphalt fume	(22) A,B,D,E fractions	21.083	.0000	(a)
(4) neat asphalt fume	(21) A,C,D,E fractions	7.466	.0063	(a)
(4) neat asphalt fume	(16) B,C,D,E fractions	2.776	.0957	
Comparisons of other combinations to the total fume				
(4) neat asphalt fume	(11) A-E fractions	.307	.5797	
(4) neat asphalt fume	(23) A,D,E fractions	23.628	.0000	(a)
(4) neat asphalt fume	(19) B,C,D fractions	7.719	.0055	(a)
(4) neat asphalt fume	(20) B,C fractions	.031	.8599	
Comparison of asphalt fume to raw or heated asphalt				
(4) neat asphalt fume	(2) heated asphalt	32.578	.0000	(a)
(4) neat asphalt fume	(3) heated plus fume	36.190	.0000	(a)
(4) neat asphalt fume	(1) raw asphalt	16.810	.0000	(a)

Comparison of asphalt fume to control for Sencar				
(42) Sencar control	(41) Sencar fume	17.092	.0000	(b)
Interspecies comparisons				
(42) Sencar control	(5) solvent control	.846	.3576	
(41) Sencar fume	(4) neat asphalt fume	.845	.3580	
Comparison of all groups to the relevant control				
(5) solvent control	(24) .01% B(a)P	50.913	.0000	(b)
(5) solvent control	(33) E,.01% B(a)P	43.710	.0000	(b)
(5) solvent control	(30) D,.01% B(a)P	37.907	.0000	(b)
(5) solvent control	(27) A,.01% B(a)P	32.947	.0000	(b)
(5) solvent control	(20) B,C fractions	32.884	.0000	(b)
(5) solvent control	(4) neat asphalt fume	29.231	.0000	(b)
(5) solvent control	(18) A,B,C,E fractions	25.468	.0000	(b)
(5) solvent control	(11) A-E fractions	24.831	.0000	(b)
(5) solvent control	(17) A,B,C,D fractions	24.035	.0000	(b)
(5) solvent control	(19) B,C,D fractions	20.446	.0000	(b)
(5) solvent control	(8) C fraction	19.694	.0000	(b)
(5) solvent control	(16) B,C,D,E fractions	18.496	.0000	(b)
(5) solvent control	(21) A,C,D,E fractions	15.924	.0001	(b)
(5) solvent control	(13) A,C fractions	14.171	.0002	(b)
(5) solvent control	(7) B fraction	9.866	.0017	(b)
(5) solvent control	(12) A,B fractions	8.164	.0043	(b)
(5) solvent control	(22) A,B,D,E fractions	6.472	.0110	(b)
(5) solvent control	(1) raw asphalt	3.241	.0718	
(5) solvent control	(25) .001% B(a)P	2.328	.1271	
(5) solvent control	(34) E,.001% B(a)P	1.917	.1662	
(5) solvent control	(28) A,.001% B(a)P	.933	.3340	
(5) solvent control	(32) D,.0001% B(a)P	.913	.3393	
(5) solvent control	(29) A,.0001% B(a)P	.609	.4353	
(5) solvent control	(26) .0001% B(a)P	----	----	
(5) solvent control	(6) A fraction	----	----	
(5) solvent control	(14) A,D fractions	----	----	
(5) solvent control	(23) A,D,E fractions	----	----	
(5) solvent control	(15) A,E fractions	----	----	
(5) solvent control	(9) D fraction	----	----	
(5) solvent control	(31) D,.001% B(a)P	----	----	
(5) solvent control	(10) E fraction	----	----	
(5) solvent control	(35) E,.0001% B(a)P	----	----	
(5) solvent control	(2) heated asphalt	----	----	
(5) solvent control	(3) heated plus fume	----	----	
(5) solvent control	(39) Init alone	----	----	
(5) solvent control	(36) Init then A	----	----	
(5) solvent control	(37) Init then D	----	----	
(5) solvent control	(38) Init then E	----	----	

Supplemental analyses

(16) B,C,D,E fractions	(20) B,C fractions	3.058	.0803	
(17) A,B,C,D fractions	(20) B,C fractions	1.756	.1851	
(18) A,B,C,E fractions	(20) B,C fractions	0.523	.4695	
(19) B,C,D fractions	(20) B,C fractions	8.690	.0032	(b)
(21) A,C,D,E fractions	(8) C fraction	0.759	.3836	
(22) A,B,D,E fractions	(7) B fraction	0.397	.5285	

Notes:
(a) means that the first group showed significantly greater tumor activity (either more tumors or earlier tumors or both) than the second group.
(b) means that the second group showed significantly greater tumor activity (either more tumors or earlier tumors or both) than the first group.
---- means that no tumors were found in either group, making comparisons between the two groups unnecessary.

Table 5 -- Inferential statistics for papillomas (or keratoacanthomas or fibromas; i.e., benign tumors)

First group	Second group	Generalized Wilcoxon	p-value	
Co-carcinogenic effects of fractions A, D, and E				
(29) A,.0001% B(a)P	(26) .0001% B(a)P	----	----	
(28) A,.001% B(a)P	(25) .001% B(a)P	.261	.6093	
(27) A,.01% B(a)P	(24) .01% B(a)P	1.015	.3137	
(32) D,.0001% B(a)P	(26) .0001% B(a)P	----	----	
(31) D,.001% B(a)P	(25) .001% B(a)P	.098	.7545	
(30) D,.01% B(a)P	(24) .01% B(a)P	4.406	.0358	(b)
(35) E,.0001% B(a)P	(26) .0001% B(a)P	----	----	
(34) E,.001% B(a)P	(25) .001% B(a)P	1.257	.2622	
(33) E,.01% B(a)P	(24) .01% B(a)P	.059	.8074	
Additional carcinogenic effect of fraction A				
(7) B fraction	(12) A,B fractions	4.291	.0383	
(8) C fraction	(13) A,C fractions	1.026	.3111	
(9) D fraction	(14) A,D fractions	----	----	
(10) E fraction	(15) A,E fractions	----	----	
Promotion capability of fractions A, D, and E				
(36) Init then A	(6) A fraction	----	----	
(36) Init then A	(39) Init alone	----	----	
(37) Init then D	(9) D fraction	----	----	
(37) Init then D	(39) Init alone	----	----	
(38) Init then E	(10) E fraction	----	----	
(38) Init then E	(39) Init alone	----	----	
Comparison of individual fractions to the total fumes				
(4) neat asphalt fume	(6) A fraction	22.349	.0000	(a)
(4) neat asphalt fume	(7) B fraction	19.288	.0000	(a)
(4) neat asphalt fume	(8) C fraction	8.780	.0030	(a)
(4) neat asphalt fume	(9) D fraction	16.018	.0001	(a)
(4) neat asphalt fume	(10) E fraction	18.861	.0000	(a)
The effect of removing one fraction from the total fume				
(4) neat asphalt fume	(17) A,B,C,D fractions	.209	.6475	
(4) neat asphalt fume	(18) A,B,C,E fractions	.826	.3633	
(4) neat asphalt fume	(22) A,B,D,E fractions	15.002	.0001	(a)
(4) neat asphalt fume	(21) A,C,D,E fractions	9.663	.0019	(a)
(4) neat asphalt fume	(16) B,C,D,E fractions	8.630	.0033	(a)
Comparison of other combinations to the total fume				
(4) neat asphalt fume	(23) A,D,E fractions	8.056	.0045	(a)
(4) neat asphalt fume	(11) A-E fractions	1.564	.2110	
(4) neat asphalt fume	(20) B,C fractions	1.377	.2406	
(4) neat asphalt fume	(19) B,C,D fractions	.073	.7869	
Comparison of asphalt fume to raw or heated asphalt				
(4) neat asphalt fume	(2) heated asphalt	20.922	.0000	(a)
(4) neat asphalt fume	(3) heated plus fume	23.463	.0000	(a)
(4) neat asphalt fume	(1) raw asphalt	16.472	.0000	(a)

Comparison of asphalt fume to control for Sencar mice				
(42) Sencar control	(41) Sencar fume	18.533	.0000	(b)
Cross-species comparisons				
(42) Sencar control	(5) solvent control	----	----	
(41) Sencar fume	(4) neat asphalt fume	.014	.9042	
Comparison of all groups to the relevant control				
(5) solvent control	(4) neat asphalt fume	18.956	.0000	(b)
(5) solvent control	(18) A,B,C,E fractions	17.050	.0000	(b)
(5) solvent control	(17) A,B,C,D fractions	15.799	.0001	(b)
(5) solvent control	(19) B,C,D fractions	14.432	.0001	(b)
(5) solvent control	(11) A-E fractions	13.752	.0002	(b)
(5) solvent control	(30) D,.01% B(a)P	13.674	.0002	(b)
(5) solvent control	(20) B,C fractions	12.900	.0003	(b)
(5) solvent control	(13) A,C fractions	9.265	.0023	(b)
(5) solvent control	(12) A,B fractions	7.713	.0055	(b)
(5) solvent control	(27) A,.01% B(a)P	5.498	.0190	(b)
(5) solvent control	(16) B,C,D,E fractions	5.246	.0220	(b)
(5) solvent control	(21) A,C,D,E fractions	3.994	.0457	(b)
(5) solvent control	(22) A,B,D,E fractions	3.804	.0511	
(5) solvent control	(8) C fraction	2.997	.0834	
(5) solvent control	(31) D,.001% B(a)P	2.028	.1544	
(5) solvent control	(23) A,D,E fractions	1.925	.1653	
(5) solvent control	(7) B fraction	1.603	.2055	
(5) solvent control	(25) .001% B(a)P	1.449	.2287	
(5) solvent control	(33) E,.01% B(a)P	1.174	.2786	
(5) solvent control	(24) .01% B(a)P	1.125	.2888	
(5) solvent control	(1) raw asphalt	1.105	.2931	
(5) solvent control	(28) A,.001% B(a)P	.800	.3711	
(5) solvent control	(26) .0001% B(a)P	----	----	
(5) solvent control	(6) A fraction	----	----	
(5) solvent control	(29) A,.0001% B(a)P	----	----	
(5) solvent control	(14) A,D fractions	----	----	
(5) solvent control	(15) A,E fractions	----	----	
(5) solvent control	(9) D fraction	----	----	
(5) solvent control	(32) D,.0001% B(a)P	----	----	
(5) solvent control	(10) E fraction	----	----	
(5) solvent control	(35) E,.0001% B(a)P	----	----	
(5) solvent control	(34) E,.001% B(a)P	----	----	
(5) solvent control	(2) heated asphalt	----	----	
(5) solvent control	(3) heated plus fume	----	----	
(5) solvent control	(39) Init alone	----	----	
(5) solvent control	(36) Init then A	----	----	
(5) solvent control	(37) Init then D	----	----	
(5) solvent control	(38) Init then E	----	----	

Supplemental analyses

(16) B,C,D,E fractions	(20) B,C fractions	2.832	.0924
(17) A,B,C,D fractions	(20) B,C fractions	2.409	.1206
(18) A,B,C,E fractions	(20) B,C fractions	3.821	.0506
(19) B,C,D fractions	(20) B,C fractions	0.545	.4604
(21) A,C,D,E fractions	(8) C fraction	0.013	.9084
(22) A,B,D,E fractions	(7) B fraction	1.135	.2867

Notes:
(a) means that the first group showed significantly greater tumor activity (either more tumors or earlier tumors or both) than the second group.
(b) means that the second group showed significantly greater tumor activity (either more tumors or earlier tumors or both) than the first group.
---- means that no tumors were found in either group, making comparisons between the two groups unnecessary.

Table 6 -- Inferential statistics for benign or malignant tumors

First group	Second group	Generalized Wilcoxon	p-value	
Co-carcinogenic activity of fractions A, D, and E				
(29) A,.0001% B(a)P	(26) .0001% B(a)P	.826	.3634	
(28) A,.001% B(a)P	(25) .001% B(a)P	.987	.3205	
(27) A,.01% B(a)P	(24) .01% B(a)P	23.766	.0000	(b)
(32) D,.0001% B(a)P	(26) .0001% B(a)P	.913	.3393	
(31) D,.001% B(a)P	(25) .001% B(a)P	.803	.3703	
(30) D,.01% B(a)P	(24) .01% B(a)P	10.670	.0011	(b)
(35) E,.0001% B(a)P	(26) .0001% B(a)P	----	----	
(34) E,.001% B(a)P	(25) .001% B(a)P	.891	.3451	
(33) E,.01% B(a)P	(24) .01% B(a)P	7.039	.0080	(b)
Additional activity due to fraction A				
(7) B fraction	(12) A,B fractions	1.831	.1760	
(8) C fraction	(13) A,C fractions	1.265	.2607	
(9) D fraction	(14) A,D fractions	----	----	
(10) E fraction	(15) A,E fractions	----	----	
Promotion capability of fractions A, D, and E				
(36) Init then A	(6) A fraction	----	----	
(36) Init then A	(39) Init alone	----	----	
(37) Init then D	(9) D fraction	----	----	
(37) Init then D	(39) Init alone	----	----	
(38) Init then E	(10) E fraction	----	----	
(38) Init then E	(39) Init alone	----	----	
Comparison of individual fractions to the total				
(4) neat asphalt fume	(6) A fraction	38.159	.0000	(a)
(4) neat asphalt fume	(7) B fraction	25.450	.0000	(a)
(4) neat asphalt fume	(8) C fraction	5.044	.0247	(a)
(4) neat asphalt fume	(9) D fraction	29.526	.0000	(a)
(4) neat asphalt fume	(10) E fraction	33.312	.0000	(a)
The effect of removing one fraction from the total				
(4) neat asphalt fume	(17) A,B,C,D fractions	.549	.4587	
(4) neat asphalt fume	(18) A,B,C,E fractions	.021	.8856	
(4) neat asphalt fume	(22) A,B,D,E fractions	23.839	.0000	(a)
(4) neat asphalt fume	(21) A,C,D,E fractions	8.256	.0041	(a)
(4) neat asphalt fume	(16) B,C,D,E fractions	3.542	.0599	
Comparison of other combinations to the total				
(4) neat asphalt fume	(11) A-E fractions	.620	.4309	
(4) neat asphalt fume	(23) A,D,E fractions	22.284	.0000	(a)
(4) neat asphalt fume	(19) B,C,D fractions	3.837	.0501	
(4) neat asphalt fume	(20) B,C fractions	.097	.7553	
Comparison of asphalt fume to raw or heated asphalt				
(4) neat asphalt fume	(2) heated asphalt	34.758	.0000	(a)
(4) neat asphalt fume	(3) heated plus fume	38.774	.0000	(a)
(4) neat asphalt fume	(1) raw asphalt	17.834	.0000	(a)

Comparison of asphalt fumes to control for Sencar mice				
(42) Sencar control	(41) Sencar fume	28.076	.0000	(b)
Cross-species comparisons				
(42) Sencar control	(5) solvent control	.846	.3576	
(41) Sencar fume	(4) neat asphalt fume	.110	.7399	
Comparison of all groups to the relevant control				
(5) solvent control	(24) .01% B(a)P	50.913	.0000	(b)
(5) solvent control	(30) D,.01% B(a)P	47.997	.0000	(b)
(5) solvent control	(33) E,.01% B(a)P	45.703	.0000	(b)
(5) solvent control	(18) A,B,C,E fractions	37.111	.0000	(b)
(5) solvent control	(17) A,B,C,D fractions	36.397	.0000	(b)
(5) solvent control	(11) A-E fractions	36.287	.0000	(b)
(5) solvent control	(20) B,C fractions	36.250	.0000	(b)
(5) solvent control	(27) A,.01% B(a)P	35.338	.0000	(b)
(5) solvent control	(4) neat asphalt fume	32.918	.0000	(b)
(5) solvent control	(19) B,C,D fractions	30.680	.0000	(b)
(5) solvent control	(16) B,C,D,E fractions	26.016	.0000	(b)
(5) solvent control	(8) C fraction	24.448	.0000	(b)
(5) solvent control	(13) A,C fractions	20.741	.0000	(b)
(5) solvent control	(21) A,C,D,E fractions	20.366	.0000	(b)
(5) solvent control	(12) A,B fractions	14.395	.0001	(b)
(5) solvent control	(7) B fraction	10.950	.0009	(b)
(5) solvent control	(22) A,B,D,E fractions	8.727	.0031	(b)
(5) solvent control	(1) raw asphalt	4.335	.0373	(b)
(5) solvent control	(25) .001% B(a)P	3.865	.0493	(b)
(5) solvent control	(31) D,.001% B(a)P	2.028	.1544	
(5) solvent control	(23) A,D,E fractions	1.925	.1653	
(5) solvent control	(34) E,.001% B(a)P	1.917	.1662	
(5) solvent control	(28) A,.001% B(a)P	1.788	.1811	
(5) solvent control	(32) D,.0001% B(a)P	.913	.3393	
(5) solvent control	(29) A,.0001% B(a)P	.609	.4353	
(5) solvent control	(26) .0001% B(a)P	----	----	
(5) solvent control	(6) A fraction	----	----	
(5) solvent control	(14) A,D fractions	----	----	
(5) solvent control	(15) A,E fractions	----	----	
(5) solvent control	(9) D fraction	----	----	
(5) solvent control	(10) E fraction	----	----	
(5) solvent control	(35) E,.0001% B(a)P	----	----	
(5) solvent control	(2) heated asphalt	----	----	
(5) solvent control	(3) heated plus fume	----	----	
(5) solvent control	(39) Init alone	----	----	
(5) solvent control	(36) Init then A	----	----	
(5) solvent control	(37) Init then D	----	----	
(5) solvent control	(38) Init then E	----	----	

Supplemental analyses

(16) B,C,D,E fractions	(20) B,C fractions	3.049	.0808
(17) A,B,C,D fractions	(20) B,C fractions	0.139	.7097
(18) A,B,C,E fractions	(20) B,C fractions	0.166	.6839
(19) B,C,D fractions	(20) B,C fractions	3.628	.0568
(21) A,C,D,E fractions	(8) C fraction	0.852	.3561
(22) A,B,D,E fractions	(7) B fraction	0.162	.6874

Notes:
(a) means that the first group showed significantly greater tumor activity (either more tumors or earlier tumors or both) than the second group.
(b) means that the second group showed significantly greater tumor activity (either more tumors or earlier tumors or both) than the first group.
---- means that no tumors were found in either group, making comparisons between the two groups unnecessary.

APPENDIX IX

GROSS TUMOR INCIDENCE FROM TECHNICIAN'S OBSERVATIONS OF ANIMALS

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
1	142	1	21	H9	possible carcinoma	CAR
1	425	1	20	I7	probable carcinoma	CAR
1	459	1	2	G8	papilloma	PAP
1	487	1	23	H8	possible carcinoma	CAR
4	39	1	17	H8	probable carcinoma	CAR
4	85	1	5	D9	horny outgrowth	PAP
4	85	2	8	H6	possible carcinoma	OTHER
4	85	3	20	H9	possible carcinoma	CAR
4	154	1	14	F8	possible carcinoma	CAR
4	154	2	8	H8	possible carcinoma	PAP
4	157	1	18	F7	possible carcinoma	CAR
4	157	2	3	H10	papilloma	PAP
4	159	1	18	H7	possible carcinoma	CAR
4	159	2	5	E10	possible carcinoma	PAP
4	162	1	18	H9	possible carcinoma	CAR
4	165	1	13	G7	possible carcinoma	CAR
4	182	1	22	G8	possible carcinoma	CAR
4	183	1	11	F6	possible carcinoma	PAP
4	183	2	7	F8	possible carcinoma	PAP
4	183	3	4	G11	papilloma	PAP
4	183	4	3	H6	papilloma	PAP
4	183	5	3	H12	papilloma	PAP
4	183	6	2	I10	papilloma	PAP
4	267	1	27	E6	possible carcinoma	CAR
4	267	2	13	I9	possible carcinoma	CAR
4	343	1	22	F6	probable carcinoma	CAR
4	343	2	7	E11	possible carcinoma	CAR
4	343	3	2	G7	papilloma	PAP
4	343	4	3	H3	horny outgrowth	PAP
4	343	5	1	H11	papilloma	HYPER
4	345	1	6	H8	papilloma	CAR
4	345	2	8	I9	papilloma	CAR
4	345	3	2	J10	papilloma	PAP
4	349	1	10	F10	possible carcinoma	CAR
4	349	2	2	G11	papilloma	PAP
4	430	1	19	F11	possible carcinoma	CAR
4	430	2	7	F5	possible carcinoma	PAP
4	457	1	23	G8	possible carcinoma	CAR
4	471	1	25	I8	probable carcinoma	CAR
4	516	1	28	I7	probable carcinoma	CAR
4	561	1	4	E11	papilloma	HYPER
4	561	2	7	E9	possible carcinoma	PAP
4	561	3	5	E7	possible carcinoma	CAR
4	561	4	11	G8	possible carcinoma	CAR
4	561	5	10	I11	possible carcinoma	CAR
4	561	6	7	I8	possible carcinoma	PAP
4	580	1	6	G9	possible carcinoma	PAP
4	580	2	11	H9	possible carcinoma	CAR
4	580	3	4	H10	papilloma	PAP

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
4	580	4	7	H12	possible carcinoma	PAP
4	590	1	5	I8	papilloma	CAR
4	598	1	23	H8	probable carcinoma	CAR
4	598	2	3	G8	papilloma	PAP
7	10	1	25	G6	possible carcinoma	CAR
7	133	1	26	F9	possible carcinoma	CAR
7	163	1	2	F7	papilloma	CAR
7	171	1	4	H9	possible carcinoma	CAR
7	171	2	3	H11	possible carcinoma	PAP
7	180	1	9	H11	possible carcinoma	PAP
7	383	1	1	G5	papilloma	HYPER
7	383	2	8	F9	possible carcinoma	CAR
7	394	1	26	H7	probable carcinoma	CAR
7	431	1	18	G7	possible carcinoma	CAR
7	527	1	26	G9	possible carcinoma	CAR
7	540	1	15	G8	possible carcinoma	CAR
7	569	1	2	G9	papilloma	CAR
7	574	1	2	G7	papilloma	HYPER
8	35	1	24	G10	probable carcinoma	CAR
8	43	1	15	E9	possible carcinoma	CAR
8	86	1	30	H7	possible carcinoma	CAR
8	119	1	6	F7	papilloma	CAR
8	126	1	22	H6	possible carcinoma	CAR
8	126	2	7	H9	possible carcinoma	HYPER
8	127	1	24	H8	possible carcinoma	CAR
8	173	1	10	H10	possible carcinoma	KER
8	173	2	6	I5	possible carcinoma	PAP
8	177	1	32	G9	probable carcinoma	CAR
8	218	1	21	I8	probable carcinoma	CAR
8	247	1	2	I8	papilloma	FCYST
8	276	1	21	H11	possible carcinoma	CAR
8	334	1	26	H6	possible carcinoma	CAR
8	400	1	19	G9	possible carcinoma	CAR
8	432	1	5	F9	possible carcinoma	CAR
8	432	2	22	G7	possible carcinoma	CAR
8	453	1	22	F8	possible carcinoma	CAR
8	460	1	30	I9	probable carcinoma	CAR
8	485	1	5	G5	papilloma	CAR
8	513	1	31	H10	probable carcinoma	CAR
8	528	1	28	H7	possible carcinoma	CAR
8	543	1	8	H10	papilloma	PAP
8	582	1	2	F6	papilloma	PAP
9	322	1	3	H6	papilloma	HYPER
11	17	1	7	G9	papilloma	PAP
11	67	1	5	F7	possible carcinoma	PAP
11	67	2	6	G5	possible carcinoma	PAP
11	67	3	12	H8	possible carcinoma	PAP
11	80	1	33	H4	probable carcinoma	PAP
11	117	1	19	H8	possible carcinoma	CAR

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
11	117	2	8	F8	possible carcinoma	CAR
11	117	3	2	E11	papilloma	PAP
11	136	1	28	E8	possible carcinoma	CAR
11	214	1	29	I8	probable carcinoma	CAR
11	214	2	3	F9	papilloma	PAP
11	224	1	6	F9	possible carcinoma	PAP
11	224	2	17	I6	possible carcinoma	CAR
11	224	3	5	H8	papilloma	KER
11	257	1	22	G8	possible carcinoma	CAR
11	258	1	21	F9	possible carcinoma	PAP
11	258	2	5	H8	horny outgrowth	PAP
11	258	3	5	I2	horny outgrowth	PAP
11	258	4	3	I9	papilloma	KER
11	258	5	3	E11	papilloma	PAP
11	269	1	4	F4	papilloma	CAR
11	358	1	28	E5	possible carcinoma	CAR
11	373	1	22	D9	probable carcinoma	CAR
11	382	1	5	D10	papilloma	CAR
11	382	2	7	F8	papilloma	CAR
11	382	3	3	G7	papilloma	HYPER
11	386	1	23	H7	probable carcinoma	CAR
11	402	1	15	G8	possible carcinoma	PAP
11	408	1	10	F6	possible carcinoma	CAR
11	409	1	3	G7	horny outgrowth	PAP
11	409	2	24	H7	horny outgrowth	CAR
11	409	3	9	G11	possible carcinoma	CAR
11	424	1	21	H8	possible carcinoma	CAR
11	424		3	H11	horny outgrowth	NO SLIDE MADE[c]
11	462	1	2	F9	papilloma	PAP
11	462	2	10	G9	possible carcinoma	PAP
11	462	3	4	G10	papilloma	PAP
11	462	4	2	H7	horny outgrowth	PAP
11	462	5	8	H9	possible carcinoma	PAP
11	462	6	7	H11	possible carcinoma	CAR
11	462	7	9	I8	possible carcinoma	PAP
11	462	8	3	I10	papilloma	PAP
11	472	1	16	G10	possible carcinoma	CAR
11	472	2	3	G7	papilloma	KER
11	472		2	E4	papilloma	NO SLIDE MADE[d]
11	482	1	22	I12	probable carcinoma	CAR
11	490	1	26	H12	possible carcinoma	CAR
11	502	1	8	A10	horny outgrowth	CAR
11	502	2	25	G7	possible carcinoma	CAR
11	518	1	5	E8	papilloma	PAP
11	518	2	11	F9	possible carcinoma	PAP
11	518	3	11	G5	possible carcinoma	PAP
11	518	4	6	G9	horny outgrowth	PAP
11	518	5	4	G11	papilloma	PAP
11	518	6	6	H4	possible carcinoma	PAP

Group code	Animal code	Slide number	Size of tumor [a]	Location of tumor [b]	Gross diagnosis	Microscopic diagnosis
11	601	1	24	G8	possible carcinoma	CAR
12	118	1	5	G8	papilloma	PAP
12	124	1	15	E8	possible carcinoma	CAR
12	164	1	6	H8	papilloma	CAR
12	164	2	2	G11	papilloma	PAP
12	170	1	3	B6	papilloma	PAP
12	170	2	1	I9	papilloma	PAP
12	299	1	5	E9	papilloma	PAP
12	299	2	2	F8	papilloma	PAP
12	299	3	2	H9	papilloma	PAP
12	339	1	8	H9	possible carcinoma	CAR
12	397	1	5	G8	papilloma	PAP
12	434		4	G5	papilloma	NO SLIDE MADE [c]
12	496	1	16	F8	possible carcinoma	CAR
12	501	1	4	E9	papilloma	CAR
12	531	1	16	H6	possible carcinoma	CAR
12	531	2	3	I9	papilloma	PAP
12	534	1	28	H3	probable carcinoma	CAR
12	539	1	3	H7	papilloma	PAP
12	600	1	21	H7	possible carcinoma	CAR
13	20	1	3	I9	papilloma	PAP
13	20	2	4	H6	papilloma	CAR
13	36	1	4	H8	papilloma	CAR
13	36	2	4	I10	papilloma	CAR
13	36	3	3	G7	papilloma	CAR
13	36		2	A7	papilloma	NO SLIDE MADE [c]
13	56	1	5	H8	possible carcinoma	PAP
13	60	1	23	I7	probable carcinoma	CAR
13	66	1	16	G9	possible carcinoma	CAR
13	77	1	5	E7	papilloma	HYPER
13	123	1	5	G9	possible carcinoma	PAP
13	143	1	31	H9	possible carcinoma	CAR
13	145	1	25	E8	possible carcinoma	CAR
13	145	2	4	H11	papilloma	PAP
13	174	1	10	F5	possible carcinoma	CAR
13	199	1	32	G5	possible carcinoma	CAR
13	199	2	10	F8	possible carcinoma	CAR
13	253	1	3	G10	papilloma	CAR
13	298	1	8	H8	papilloma	KER
13	433	1	2	D9	papilloma	CAR
13	433	2	9	E4	possible carcinoma	CAR
13	433	3	5	E11	papilloma	PAP
13	433	4	5	F9	papilloma	PAP
13	433	5	7	G10	papilloma	PAP
13	433	6	13	H8	possible carcinoma	CAR
13	433	7	4	H11	papilloma	PAP
13	433	8	2	I6	papilloma	PAP
13	433	9	3	J9	papilloma	KER
13	523	1	9	F8	possible carcinoma	CAR

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
13	572	1	3	G7	possible carcinoma	PAP
14	565	1	5	H8	papilloma	FCYST
16	2	1	7	I8	papilloma	CAR
16	2	2	10	G7	possible carcinoma	CAR
16	16	1	23	F8	probable carcinoma	CAR
16	52	1	18	E8	possible carcinoma	CAR
16	81	1	6	H10	possible carcinoma	CAR
16	100	1	2	H10	papilloma	CAR
16	100	2	1	F11	papilloma	HYPER
16	156	1	18	F10	probable carcinoma	CAR
16	160	1	2	F8	papilloma	HYPER
16	176	1	27	F10	probable carcinoma	CAR
16	188	1	5	G9	papilloma	FCYST
16	191	1	8	H8	possible carcinoma	CAR
16	236	1	30	H9	possible carcinoma	CAR
16	239	1	6	G11	possible carcinoma	KER
16	239	2	5	G7	papilloma	KER
16	239	3	2	G12	papilloma	KER
16	239	4	2	H10	papilloma	PAP
16	239	5	3	I9	papilloma	PAP
16	308	1	26	I9	possible carcinoma	CAR
16	344	1	4	H9	papilloma	KER
16	370	1	2	G8	papilloma	PAP
16	413	1	27	G12	possible carcinoma	CAR
16	426	1	27	G5	probable carcinoma	CAR
16	440	1	20	G9	possible carcinoma	CAR
16	440	2	10	I6	possible carcinoma	CAR
16	440	3	1	B7	papilloma	HYPER
16	503	1	5	E8	papilloma	PAP
16	503	2	5	G8	papilloma	PAP
16	519	1	20	H12	possible carcinoma	CAR
16	560	1	4	E9	possible carcinoma	HYPER
16	560	2	12	G11	possible carcinoma	CAR
16	560	3	10	G4	possible carcinoma	CAR
17	14	1	2	E10	papilloma	KER
17	14	2	2	I6	papilloma	HYPER
17	38	1	30	H7	probable carcinoma	CAR
17	41	1	2	H6	papilloma	PAP
17	41	2	23	H8	possible carcinoma	CAR
17	44	1	31	H8	possible carcinoma	CAR
17	197	1	6	F8	possible carcinoma	PAP
17	197	2	10	G7	possible carcinoma	PAP
17	231	1	2	E8	papilloma	PAP
17	233	1	31	F8	probable carcinoma	CAR
17	275	1	32	I9	possible carcinoma	CAR
17	293	1	26	G7	probable carcinoma	CAR
17	293	2	3	I11	horny outgrowth	PAP
17	316	1	13	I11	possible carcinoma	CAR
17	316	2	7	J12	possible carcinoma	CAR

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
17	330	1	14	H4	possible carcinoma	CAR
17	332	1	19	H9	possible carcinoma	CAR
17	364	1	6	G8	papilloma	CAR
17	395	1	4	H6	papilloma	PAP
17	395	2	2	B2	papilloma	CAR
17	395	3	6	I10	papilloma	CAR
17	395	4	21	E8	probable carcinoma	CAR
17	422	1	2	F7	papilloma	PAP
17	422	2	20	I9	probable carcinoma	FCYST
17	444	1	16	E9	possible carcinoma	CAR
17	444	2	3	H9	papilloma	PAP
17	444	3	3	I11	papilloma	PAP
17	444	4	4	J8	papilloma	PAP
17	449	1	8	H9	possible carcinoma	PAP
17	480	1	15	E8	possible carcinoma	CAR
17	480	2	7	F12	possible carcinoma	HYPER
17	480	3	24	H7	possible carcinoma	CAR
17	491	1	33	H10	possible carcinoma	CAR
17	492	1	8	F7	possible carcinoma	PAP
17	492	2	5	G8	possible carcinoma	PAP
17	492	3	12	H6	possible carcinoma	CAR
17	492	4	9	I10	possible carcinoma	PAP
17	492		5	H12	possible carcinoma	NO SLIDE MADE[e]
17	515	1	26	H9	possible carcinoma	CAR
17	515	2	6	F7	possible carcinoma	PAP
17	526	1	30	F8	probable carcinoma	CAR
17	551	1	6	E9	possible carcinoma	OTHER
17	583	1	6	H8	possible carcinoma	CAR
17	585	1	3	I9	possible carcinoma	KER
18	1	1	23	H6	probable carcinoma	CAR
18	48	1	12	E9	possible carcinoma	CAR
18	48	2	4	F7	papilloma	PAP
18	48	3	4	G11	papilloma	KER
18	48	4	3	G7	papilloma	FCYST
18	70	1	21	H8	possible carcinoma	CAR
18	70	2			papilloma	CAR
18	73	1	6	H6	possible carcinoma	PAP
18	74	1	17	C7	possible carcinoma	CAR
18	74	2	15	H6	possible carcinoma	CAR
18	74		3	H8	papilloma	NO SLIDE MADE[c]
18	95	1	11	F7	possible carcinoma	PAP
18	95	2	4	H10	papilloma	PAP
18	95	3	2	I4	papilloma	PAP
18	112	1	6	E5	papilloma	PAP
18	112	2	3	E11	papilloma	PAP
18	200	1	6	F10	possible carcinoma	PAP
18	200	2	1	I8	papilloma	NORM
18	200	3	2	F9	papilloma	PAP
18	212	1	20	F4	possible carcinoma	CAR

Group code	Animal code	Slide number	Size of tumor [a]	Location of tumor [b]	Gross diagnosis	Microscopic diagnosis
18	212	2	3	D7	papilloma	PAP
18	212	3	13	E9	possible carcinoma	CAR
18	212	4	2	G9	papilloma	FCYST
18	232	1	2	I7	papilloma	HYPER
18	251	1	26	I10	possible carcinoma	CAR
18	251		3	J5	papilloma	NO SLIDE MADE [d]
18	270	1	3	F7	papilloma	PAP
18	287	1	27	H4	probable carcinoma	CAR
18	341	1	10	A7	horny outgrowth	CAR
18	341	2	22	H8	possible carcinoma	CAR
18	341	3	10	G12	possible carcinoma	CAR
18	381	1	22	E10	possible carcinoma	CAR
18	381	2	7	I7	possible carcinoma	CAR
18	412	1	24	G5	probable carcinoma	CAR
18	421	1	2	E10	papilloma	PAP
18	421	2	22	F7	possible carcinoma	CAR
18	452	1	15	D8	possible carcinoma	CAR
18	452	2	21	H9	possible carcinoma	CAR
18	468	1	5	I8	papilloma	PAP
18	481	1	6	E5	horny outgrowth	PAP
18	481	2	16	F8	possible carcinoma	CAR
18	486	1	29	G9	probable carcinoma	CAR
18	488	1	33	G5	probable carcinoma	CAR
18	545	1	18	G5	probable carcinoma	CAR
18	550	1	1	E8	papilloma	PAP
18	550	2	3	F11	papilloma	PAP
18	550	3	22	G10	probable carcinoma	CAR
18	550	4	3	H6	papilloma	CAR
18	550	5	5	I10	possible carcinoma	PAP
18	570	1	11	E6	possible carcinoma	PAP
18	570	2	14	H8	possible carcinoma	CAR
18	578	1	28	G8	probable carcinoma	CAR
18	607	1	6	E9	possible carcinoma	PAP
18	607	2	7	F7	papilloma	CAR
18	607	3	2	F10	papilloma	PAP
18	607	4	5	G11	papilloma	PAP
18	607	5	4	I8	papilloma	PAP
18	614	1	7	F9	possible carcinoma	CAR
18	614	2	5	H7	possible carcinoma	CAR
18	614	3	6	I5	possible carcinoma	PAP
18	614	4	9	I10	possible carcinoma	PAP
18	614	5	2	I8	papilloma	PAP
19	8	1	2	E9	papilloma	PAP
19	11	1	3	E8	papilloma	CAR
19	11	2	2	F10	papilloma	CAR
19	11	3	1	G7	papilloma	FCYST
19	11	4	4	H8	papilloma	CAR
19	22	1	1	H9	papilloma	CAR
19	65	1	6	C8	possible carcinoma	PAP

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
19	65	2	32	G10	possible carcinoma	CAR
19	87	1	30	H8	probable carcinoma	CAR
19	105	1	31	H7	probable carcinoma	PAP
19	140	1	3	H3	papilloma	PAP
19	140	2	23	H10	possible carcinoma	CAR
19	181	1	22	F8	possible carcinoma	CAR
19	181	2	12	G5	possible carcinoma	CAR
19	215	1	13	E8	possible carcinoma	CAR
19	215	2	7	I5	possible carcinoma	CAR
19	215	3	3	H10	papilloma	PAP
19	281	1	5	I8	papilloma	PAP
19	285	1	26	C9	probable carcinoma	CAR
19	285	2	3	G12	papilloma	PAP
19	285	3	7	I9	papilloma	CAR
19	328	1	4	H8	papilloma	FCYST
19	378	1	9	F8	possible carcinoma	CAR
19	378	2	7	I10	possible carcinoma	CAR
19	380	1	5	D10	papilloma	PAP
19	380	2	22	G8	possible carcinoma	CAR
19	385	1	3	H8	papilloma	PAP
19	414	1	33	H9	probable carcinoma	CAR
19	458	1	20	B8	possible carcinoma	CAR
19	458	2	21	F10	possible carcinoma	CAR
19	463	1	3	F8	papilloma	PAP
19	463	2	8	I6	possible carcinoma	CAR
19	536	1	5	F8	possible carcinoma	PAP
19	536	2	4	G9	papilloma	PAP
19	536	3	2	H9	papilloma	PAP
19	536	4	4	H7	papilloma	FCYST
19	549	1	3	F8	papilloma	KER
19	549	2	5	G10	papilloma	PAP
19	564	1	37	F8	probable carcinoma	CAR
19	602	1	26	H11	probable carcinoma	CAR
20	29	1	19	G11	probable carcinoma	CAR
20	29	2	5	I4	papilloma	PAP
20	63	1	20	H7	possible carcinoma	CAR
20	69	1	3	E8	papilloma	PAP
20	72	1	30	H8	possible carcinoma	CAR
20	82	1	34	H7	possible carcinoma	CAR
20	82	2	8	G9	papilloma	PAP
20	82	3	5	F8	papilloma	PAP
20	97	1	22	F8	possible carcinoma	CAR
20	97	2	8	G9	papilloma	KER
20	113	1	26	F6	probable carcinoma	CAR
20	113	2	5	F13	papilloma	HYPER
20	135	1	24	G8	possible carcinoma	CAR
20	144	1	20	I7	probable carcinoma	CAR
20	151	1	3	F8	papilloma	PAP
20	161	1	3	I8	horny outgrowth	HYPER

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
20	187	1	22	F8	probable carcinoma	CAR
20	227	1	31	H10	possible carcinoma	CAR
20	227	2	6	C8	horny outgrowth	PAP
20	234	1	5	F10	horny outgrowth	PAP
20	234	2	9	I8	probable carcinoma	CAR
20	246	1	25	I9	probable carcinoma	CAR
20	266	1	2	D9	papilloma	FCYST
20	266	2	12	H8	possible carcinoma	CAR
20	272	1	16	G8	possible carcinoma	CAR
20	272	2	5	H10	possible carcinoma	PAP
20	272	3	4	I8	possible carcinoma	PAP
20	292	1	17	F7	probable carcinoma	CAR
20	292	2	6	F8	possible carcinoma	CAR
20	292	3	5	F11	possible carcinoma	KER
20	292	4	14	H9	probable carcinoma	CAR
20	294	1	23	H7	probable carcinoma	CAR
20	312	1	5	G4	possible carcinoma	CAR
20	312	2	3	G9	papilloma	HYPER
20	312	3	3	I5	papilloma	HYPER
20	340	1	32	H4	probable carcinoma	CAR
20	363	1	22	G8	possible carcinoma	CAR
20	363	2	3	G6	papilloma	PAP
20	441	1	21	H6	possible carcinoma	CAR
20	447	1	8	I8	possible carcinoma	CAR
20	530	1	32	G11	possible carcinoma	CAR
20	576	1	2	G8	papilloma	HYPER
20	576	2	15	H7	possible carcinoma	CAR
20	587	1	35	H6	possible carcinoma	CAR
21	668	1	8	H9	possible carcinoma	CAR
21	716	1	28	E4	probable carcinoma	CAR
21	718	1	4	A7	papilloma	PAP
21	759	1	21	I7	possible carcinoma	CAR
21	778	1	6	H8	papilloma	PAP
21	788	1	9	F10	possible carcinoma	CAR
21	826	1	23	I3	possible carcinoma	CAR
21	848	1	2	E6	papilloma	CAR
21	848	2	3	F8	papilloma	KER
21	899	1	15	I9	possible carcinoma	CAR
21	929	1	19	E8	possible carcinoma	CAR
21	967	1	3	F3	papilloma	CAR
21	994	1	22	G10	possible carcinoma	CAR
21	1002	1	5	E8	possible carcinoma	HYPER
21	1002	2	1	I9	papilloma	HYPER
21	1087	1	32	G5	probable carcinoma	CAR
21	1186	1	32	E7	probable carcinoma	CAR
21	1200	1	15	F7	possible carcinoma	CAR
21	1254	1	10	E9	possible carcinoma	CAR
21	1274	1	21	F8	possible carcinoma	OTHER
21	1303	1	6	E9	papilloma	PAP

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
21	1303	2	3	F11	papilloma	FCYST
21	1303	3	2	F9	papilloma	HYPER
21	1303	4	9	G7	possible carcinoma	PAP
22	744	1	6	G7	possible carcinoma	CAR
22	796	1	13	G7	possible carcinoma	PAP
22	796	2	4	F9	papilloma	PAP
22	796	3	3	I8	papilloma	CAR
22	922	1	7	F9	papilloma	PAP
22	992	1	13	H9	possible carcinoma	CAR
22	992	2	2	F7	papilloma	FCYST
22	1023	1	8	H5	possible carcinoma	CAR
22	1057	1	3	G8	papilloma	PAP
22	1096	1	29	I10	possible carcinoma	SAR
22	1220	1	5	H11	papilloma	CAR
22	1294	1	9	I7	possible carcinoma	CAR
22	1294	2	2	I9	papilloma	PAP
22	1302	1	14	E10	possible carcinoma	HYPER
23	692		0	E3	suspicious area	NO SLIDE MADE[f]
23	692		0	F2	suspicious area	NO SLIDE MADE[f]
23	930	1	5	F9	papilloma	PAP
23	980	1	4	H6	papilloma	PAP
24	671	1	28	G8	possible carcinoma	CAR
24	677	1	35	H7	possible carcinoma	CAR
24	695	1	25	I8	probable carcinoma	CAR
24	736	1	30	F7	probable carcinoma	CAR
24	740	1	36	H8	probable carcinoma	CAR
24	797	1	37	H10	probable carcinoma	CAR
24	802	1	19	G5	possible carcinoma	CAR
24	806	1	22	G9	possible carcinoma	CAR
24	812	1	25	H8	probable carcinoma	CAR
24	824	1	32	H7	probable carcinoma	CAR
24	829	1	15	F8	possible carcinoma	CAR
24	849	1	26	H8	possible carcinoma	CAR
24	859	1	25	H10	possible carcinoma	CAR
24	889	1	24	I6	probable carcinoma	CAR
24	894	1	36	G8	probable carcinoma	CAR
24	954	1	30	H7	possible carcinoma	CAR
24	1019	1	23	G5	probable carcinoma	CAR
24	1025	1	18	F8	possible carcinoma	CAR
24	1050	1	34	H7	probable carcinoma	CAR
24	1051	1	6	I5	papilloma	CAR
24	1051	2	7	I9	suspicious bulbous	PAP
24	1094	1	26	H6	probable carcinoma	CAR
24	1128	1	20	I8	probable carcinoma	CAR
24	1128	2	14	D13	possible carcinoma	CAR
24	1134	1	21	G8	probable carcinoma	CAR
24	1153	1	27	H8	probable carcinoma	CAR
24	1178	1	23	G5	possible carcinoma	CAR
24	1183	1	37	H9	possible carcinoma	CAR

Group code	Animal code	Slide number	Size of tumor [a]	Location of tumor [b]	Gross diagnosis	Microscopic diagnosis
24	1184	1	31	H8	probable carcinoma	CAR
25	724	1	4	G8	papilloma	CAR
25	743	1	6	G7	papilloma	KER
25	985	1	6	G7	papilloma	PAP
25	1015	1	3	G8	papilloma	CAR
25	1036	1	15	F9	possible carcinoma	CAR
27	665	1	20	G7	possible carcinoma	CAR
27	665	2	4	H4	papilloma	PAP
27	705	1	28	G8	possible carcinoma	CAR
27	739	1	15	H10	possible carcinoma	CAR
27	739	2	10	F6	possible carcinoma	CAR
27	739	3	13	I8	possible carcinoma	CAR
27	805	1	12	H9	possible carcinoma	CAR
27	805	2	8	F8	possible carcinoma	CAR
27	811	1	7	D9	horny outgrowth	PAP
27	811	2	11	G11	possible carcinoma	CAR
27	855	1	4	I7	probable carcinoma	CAR
27	861	1	10	G7	possible carcinoma	CAR
27	876	1	4	I6	papilloma	PAP
27	876	2	4	H9	papilloma	CAR
27	876	3	3	G9	papilloma	PAP
27	876		3	F7	papilloma	NO SLIDE MADE [c]
27	895	1	29	G9	possible carcinoma	CAR
27	896	1	31	G8	probable carcinoma	CAR
27	943	1	30	I10	probable carcinoma	CAR
27	966	1	31	H7	possible carcinoma	CAR
27	1001	1	28	G8	probable carcinoma	CAR
27	1001	2	2	I8	papilloma	HYPER
27	1024	1	2	F8	papilloma	HYPER
27	1073	1	25	H8	probable carcinoma	CAR
27	1115	1	14	H8	probable carcinoma	CAR
27	1115	2	2	H6	papilloma	FCYST
27	1142	1	8	I8	possible carcinoma	PAP
27	1142	2	2	F8	papilloma	PAP
27	1147	1	38	H8	probable carcinoma	CAR
27	1149	1	30	H6	possible carcinoma	CAR
27	1149	2	6	G10	possible carcinoma	PAP
27	1165	1	27	I11	possible carcinoma	CAR
27	1167	1	15	F8	possible carcinoma	CAR
27	1167	2	7	H7	possible carcinoma	CAR
27	1195	1	11	F7	possible carcinoma	CAR
27	1195	2	23	I8	possible carcinoma	CAR
27	1210	1	32	I5	probable carcinoma	OTHER
27	1210	2	9	F9	possible carcinoma	CAR
27	1232	1	18	H8	probable carcinoma	CAR
27	1270	1	28	H6	probable carcinoma	CAR
28	1117	1	2	E11	papilloma	PAP
28	1125	1	11	H8	possible carcinoma	CAR
28	1125	2	5	E9	papilloma	NORM

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
29	670	1	9	H7	possible carcinoma	CAR
30	663	1	20	H12	probable carcinoma	CAR
30	663	2	4	G5	papilloma	KER
30	687	1	13	I8	possible carcinoma	CAR
30	687	2	5	H5	papilloma	HYPER
30	687	3	5	H8	papilloma	CAR
30	701	1	4	H8	papilloma	CAR
30	701	2	22	H5	probable carcinoma	CAR
30	701	3	4	F9	horny outgrowth	PAP
30	753	1	32	G5	possible carcinoma	CAR
30	763	1	16	H10	possible carcinoma	CAR
30	763	2	8	G11	possible carcinoma	CAR
30	763	3	5	F8	possible carcinoma	KER
30	779	1	3	H4	papilloma	PAP
30	794	1	23	G5	probable carcinoma	CAR
30	816	1	30	G9	possible carcinoma	CAR
30	823	1	4	F9	papilloma	PAP
30	823	2	4	G10	papilloma	KER
30	823	3	5	G7	papilloma	CAR
30	823	4	26	H4	possible carcinoma	CAR
30	879	1	3	F8	possible carcinoma	HYPER
30	879	2	22	H7	probable carcinoma	PAP
30	879	3	5	G8	papilloma	CAR
30	913	1	6	F2	possible carcinoma	KER
30	913	2	18	G8	possible carcinoma	CAR
30	938	1	21	F8	possible carcinoma	CAR
30	962	1	17	H7	probable carcinoma	CAR
30	978	1	20	H5	probable carcinoma	CAR
30	1035	1	4	G5	papilloma	PAP
30	1035	2	6	G8	papilloma	KER
30	1037	1	25	F7	possible carcinoma	CAR
30	1037	2	3	I8	papilloma	CAR
30	1046	1	18	G7	possible carcinoma	CAR
30	1065	1	12	H6	probable carcinoma	CAR
30	1065	2	15	F7	probable carcinoma	CAR
30	1084	1	22	H10	probable carcinoma	CAR
30	1084	2	3	G8	papilloma	KER
30	1084	3	8	G5	possible carcinoma	KER
30	1123	1	30	E6	possible carcinoma	CAR
30	1157	1	22	I9	possible carcinoma	CAR
30	1171	1	29	H8	possible carcinoma	CAR
30	1176	1	21	G12	probable carcinoma	CAR
30	1188	1	19	H12	probable carcinoma	CAR
30	1188	2	15	I4	probable carcinoma	CAR
30	1188		0	C12	suspicious area	NO SLIDE MADE[f]
30	1191	1	25	H9	probable carcinoma	CAR
30	1197	1	9	G10	possible carcinoma	CAR
30	1197	2	11	G6	possible carcinoma	CAR
30	1206	1	1	F9	papilloma	HYPER

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
30	1206	2	19	G9	probable carcinoma	KER
30	1239	1	20	G2	probable carcinoma	CAR
30	1239	2	4	G6	papilloma	CAR
30	1239		3	H7	papilloma	NO SLIDE MADE[g]
30	1247	1	7	G5	possible carcinoma	PAP
31	765		1	F8	papilloma	NO SLIDE MADE[c]
31	780	1	5	F6	papilloma	PAP
31	850	1	13	G9	possible carcinoma	PAP
32	925	1	20	E8	possible carcinoma	CAR
33	662	1	24	E8	probable carcinoma	CAR
33	675	1	20	H5	possible carcinoma	CAR
33	741	1	30	H8	probable carcinoma	CAR
33	751	1	30	F6	probable carcinoma	CAR
33	755	1	0	H7	suspicious area	PAP
33	756	1	30	H7	probable carcinoma	CAR
33	784	1	25	I6	possible carcinoma	CAR
33	787		0	G6	suspicious area	NO SLIDE MADE[f]
33	795		21	F8	possible carcinoma	NO SLIDE MADE[h]
33	833	1	25	H9	probable carcinoma	CAR
33	928	1	25	H7	probable carcinoma	CAR
33	955	1	35	H3	probable carcinoma	CAR
33	981	1	26	H5	possible carcinoma	CAR
33	995	1	20	H7	possible carcinoma	CAR
33	1034	1	25	H9	possible carcinoma	CAR
33	1053	1	18	H5	probable carcinoma	CAR
33	1063	1	25	G7	probable carcinoma	CAR
33	1083	1	31	H8	possible carcinoma	CAR
33	1090	1	27	I7	probable carcinoma	CAR
33	1098	1	25	H7	probable carcinoma	CAR
33	1137	1	25	H5	probable carcinoma	CAR
33	1148	1	3	G9	papilloma	CAR
33	1152	1	28	H4	possible carcinoma	CAR
33	1205	1	25	G7	probable carcinoma	CAR
33	1215	1	27	H9	possible carcinoma	CAR
33	1218	1	16	F7	possible carcinoma	CAR
34	688	1	8	E9	possible carcinoma	CAR
34	688	2	4	F6	papilloma	HYPER
34	799	1	3	G8	papilloma	FCYST
34	1048	1	7	F8	possible carcinoma	CAR
37	1160	1	3	F10	papilloma	FCYST
39	762	1	2	H3	papilloma	OTHER
41	1321	1	6	G7	papilloma	PAP
41	1321	2	4	G9	papilloma	PAP
41	1321	3	5	H10	papilloma	PAP
41	1322	1	10	F9	possible carcinoma	CAR
41	1327	1	4	H9	papilloma	PAP
41	1330	1	3	I7	papilloma	PAP
41	1330	2	1	H6	papilloma	HYPER
41	1332	1	38	H5	probable carcinoma	CAR

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
41	1332		0	E11	lesion	NO SLIDE MADE[i]
41	1336	1	0	G 4	lesion	HYPER
41	1339	1	45	H7	lesion	HYPER
41	1343	1	28	I12	possible carcinoma	CAR
41	1343	2	8	D12	possible carcinoma	CAR
41	1344	1	37	G7	probable carcinoma	CAR
41	1345	1	30	H7	suspicious area	OTHER
41	1348	1	6	I5	papilloma	PAP
41	1348	2	6	G4	possible carcinoma	PAP
41	1348		10	H2	papilloma	NO SLIDE MADE[j]
41	1348		9	I11	papilloma	NO SLIDE MADE[j]
41	1349	1	29	G11	probable carcinoma	SAR
41	1349	2	5	H8	papilloma	PAP
41	1353	1	0	G7	suspicious area	FCYST
41	1354	1	5	H11	papilloma	OTHER
41	1358	1	0	H8	suspicious area	HYPER
41	1358		0	H5	suspicious area	NO SLIDE MADE[f]
41	1358		0	H6	suspicious area	NO SLIDE MADE[f]
41	1358		0	H7	suspicious area	NO SLIDE MADE[f]
41	1358		0	H9	suspicious area	NO SLIDE MADE[f]
41	1358		0	G9	suspicious area	NO SLIDE MADE[f]
41	1363	1	20	F7	possible carcinoma	CAR
41	1363	2	4	E8	papilloma	PAP
41	1363	3	4	I7	papilloma	PAP
41	1363	4	15	H11	possible carcinoma	CAR
41	1363	5	2	H12	papilloma	PAP
41	1364	1	32	F4	probable carcinoma	CAR
41	1364	2	5	H7	papilloma	PAP
41	1364	3	4	F9	papilloma	PAP
41	1364	4	3	F10	papilloma	PAP
41	1364	5	3	F11	papilloma	PAP
41	1364	6	3	H8	papilloma	HYPER
41	1367	1	28	F12	probable carcinoma	CAR
41	1367	2	3	H6	papilloma	PAP
41	1369	1	29	I5	possible carcinoma	CAR
41	1369	2	6	I12	possible carcinoma	PAP
41	1369	3	2	F5	papilloma	PAP
41	1370	1	18	F6	possible carcinoma	CAR
41	1370	2	4	F10	papilloma	PAP
41	1371	1	30	F5	probable carcinoma	KER
41	1371	2	2	G7	papilloma	HYPER
41	1372	1	15	G7	possible carcinoma	CAR
41	1373	1	0	G6	suspicious area	HYPER
41	1373	2	0	G9	suspicious area	HYPER
41	1374	1	40	H8	probable carcinoma	CAR
41	1376	1	32	H3	probable carcinoma	CAR
41	1376	2	0	E12	lesion	HYPER
41	1380	1	3	E6	papilloma	PAP

Group code	Animal code	Slide number	Size of tumor[a]	Location of tumor[b]	Gross diagnosis	Microscopic diagnosis
41	1381	1	21	G8	possible carcinoma	CAR
41	1381	2	3	H7	papilloma	CAR
41	1381	3	3	I8	papilloma	PAP
42	1384	1			mass	SAR

[a] Size in mm.

[b] As per diagram on next page.

[c] Mass not found at necropsy.

[d] Mass lost following necropsy.

[e] H6-12 and H12-5 masses coalesced into a single mass in cassette.

[f] Suspicious area only, no mass.

[g] G6-4 and H7-3 masses coalesced into a single mass in cassette.

[h] Animal escaped, tissues lost.

[i] Lesion only, no mass.

[j] I5-6, H2-10 and I11-9 masses coalesced into a single mass in cassette.

APPENDIX A

Toxicology Unit

STANDARD OPERATING PROCEDURE T-413 --- A

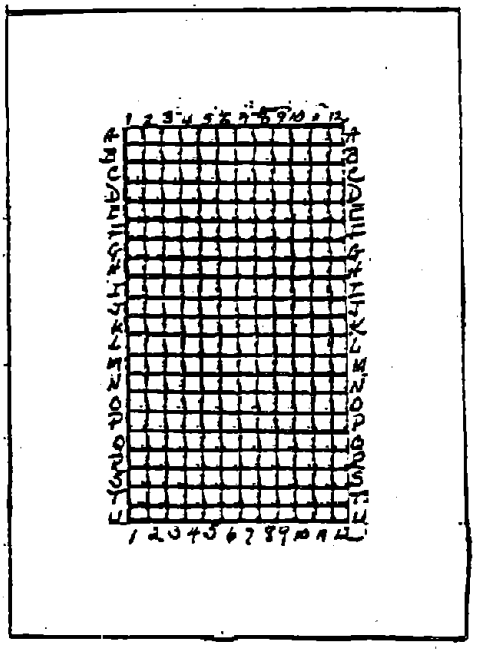
Computerized Skin Tumor Observation Record

A 1/8 inch grid will be used labeled:

A, B, C on Y axis
1, 2, 3 on X axis

Placing grid on back aligning bottom of grid (N-6) with midline of mouse using tail as center. Note location of tumor.

Documentation and descriptions are as attached.



APPENDIX X

DIAGNOSTIC CRITERIA AND INCIDENCE OF MICROSCOPICALLY CONFIRMED SKIN TUMORS

DEPARTMENT OF HEALTH & HUMAN SERVICES

Memorandum

Date September 18, 1989

From Chief, EPS (C-26)

Subject Histopathology Report For The Study "Assessment of the Cocarcinogenic/Promoting Activity of Asphalt Fumes"

To Mr. Dennis W. Lynch
Research Toxicologist (C23)
Through: Chief, ETB (C23)
Chief, ABB (C26)

This report includes tabulated histopathologic diagnoses for 669 skin specimens from C3H and SENCAR mice exposed via skin painting to volatile components of asphalt for two years. In vivo exposures, gross pathology and histotechnical procedures were conducted by Arthur D. Little, Inc. Histopathologic evaluation of H&E stained slides was conducted at NIOSH. A second, independent evaluation of each specimen was conducted by Dr. Richard H. Bruner of Pathology Associates, Inc., Wester Chester, Ohio. Both evaluations of the specimens were conducted in a blind fashion; i.e., without knowledge of the treatment groupings or other histopathology evaluation results. This report represents the consensus opinion from the two evaluations.

DIAGNOSTIC CRITERIA

General histomorphic features used to classify all significant skin lesions in this study are presented in the diagnostic catagories listed below. It should be emphasized that many skin tumors exhibited progressive changes from benign to malignant variants, and a critical objective in this review was to determine if some papillomas or keratoacanthomas contained regions where proliferating cells had undergone malignant transformation, making a diagnosis of carcinoma most appropriate. Not infrequently, final diagnoses were based upon collective evaluations of several characteristics which were equivocal in a single, H&E stained microslide.

1. Carcinoma - A malignant epithelial neoplasm which displayed one or more of the following characteristics:

   A) Marked anaplasia, B) Increased/atypical mitotic figures, C) Breach of basal lamina, D) Active invasion of the connective tissue stroma or muscle. Occasionally, anaplastic carcinoma cells were spindled-shaped and distinction from sarcoma was difficult without additional histotechnical procedures.

2. Papilloma - A benign epithelial neoplasm which often presented as one of two variants: A) pedunculated and B) sessile. Pedunculated variants were attached to the subjacent skin by a thin stalk (base) and the diagnosis

usually was considered as unequivocal. Sessile forms displayed a broadly based attachment and were sometimes difficult to distinguish from keratoacanthomas. Rarely, some papillomas were completely inverted and extended into the superficial dermis.

3. Follicular Cyst - Dilatation and plugging of hair follicles with keratin debris. No effort was made to distinguish follicular cysts from epidermal inclusion cysts in this review.

4. Hyperplasia/Hyperkeratosis - Specimens which exhibited thickened layers of viable epidermis (acanthosis) with or without thickened plaques of stratum corneum. With some focal areas of hyperplasia/hyperkeratosis, a benign papilloma or keratoacanthoma may have been present but out of the plane of section.

5. Keratoacanthoma - A benign neoplasm which was thought to originate from hair follicle structures and usually presented as a "crater-like" neoplasm filled with lamellated plugs of keratin. A sub-gross "cup-like" or "crater-like" appearance was used to differentiate keratoacanthomas from sessile papillomas with which they may be confused.

6. Sarcoma - A malignant spindle-cell neoplasm of connective tissue origin.

7. Fibroma - A benign spindled-cell neoplasm of connective tissue origin.

8. Other Diagnoses/Comments - Diagnoses in this group did not clearly fit into any of the other groups above and were primarily observations such as: A) "Mast Cells" In several specimens, small subcutaneous collections of "clear cells" were noted which were thought to be mast cells, B) "Sebaceous Cells" Occasionally, small collections of sebaceous cells were present and were thought to represent hyperplasia of sebaceous glands.

RESULTS

The table accompanying this report is a randomized listing of the animals/skin specimens as they were presented for histopathology evaluation with the diagnosis for each section. Only animals with skin lesions were examined and multiple lesions from the same animal are listed separately as an individual diagnosis. This table was provided to the Statistics Unit, DBBS, NIOSH for uncoding and separation into dosage groups.

In general, the majority of lesions fell into one of three groups: Carcinoma, Papilloma or Keratoacanthoma. As described above, carcinoma is a malignant tumor of epithelial origin, while papillomas and keratoacanthomas represent benign neoplasms of epithelial origin. Primary emphasis was placed on the

differentiation of benign and malignant lesions. In some cases, progression from benign to malignant lesions was noted. In animals with multiple lesions, combinations of diagnoses, including benign and malignant, were made on the same animal.

CONCLUSION

Due to the randomization and the large number of groups in this study, the Statistics Unit is to provide further analysis of the tumor data.

Richard A. Salomon

Richard A. Salomon, DVM

Attachment

11-Sep-89 FINAL DIAGNOSES--ASPHALT FUME STUDY

ANIMAL NUMBER	SLIDE NO.	Final DIAGNOSES 1
6034-3146		1
5939-6152		1
8364-4264		1
9440-1232	1	1
9440-1232	2	2
7206-8486		1
1720-4727		1
6189-7272	1	1
6189-7272	2	2
5730-5620	1	2
5730-5620	2	2
5730-5620	3	2
5730-5620	4	2
5730-5620	5	2
5730-5620	6	2
2056-7387	1	2
2056-7387	2	1
2056-7387	3	2
2056-7387	4	2
8565-7337	1	1
9593-9671	1	1
9593-9671	2	1
9593-9671	3	2
9593-9671	4	2
9593-9671	5	4
2455-6396	1	1
2455-6396	2	2
1905-1804	1	1
1905-1804	2	1
1905-1804	3	2
6460-3857	1	1
6460-3857	2	2
2851-8197	1	1
2851-8197	2	1
3969-1579	1	1
1051-4819	1	2
1051-4819	2	9
1051-4819	3	1
4204-6470	1	1
4204-6470	2	2
3977-8811	1	1
3977-8811	2	2

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

2355-5966	1		4
2355-5966	2		2
2355-5966	3		1
2355-5966	4		1
2355-5966	5		1
2355-5966	6		2
9003-2430	1		1
1281-1909	1		1
5182-7620	1		1
8838-4352	1		1
4655-9056	1		1
2341-3216	1		1
1247-9449	1		1
2118-9992	1		1
2118-9992	2		2
3652-8488	1		1
4370-5699	1		1
9162-1721	1		1
6657-9162	1		1
6657-9162	2		1
5772-8274	1		1
8804-6156	1		1
5313-2934	1		1
8853-5990	1		1
9783-5457	1		1
5744-2166	1		1
9165-2321	1		1
1124-5537	1		1
5449-8994	1		1
6114-2893	1		1
2509-6206	1		1
5519-5759	1		1
8594-4045	1		1
6145-4244	1		1
6143-8392	1		1
1822-9484	1		1
8117-2675	1		1
6371-2937	1		1
7247-2007	1		5
7247-2007	2		2
1847-5452	1		1
1511-3021	1		1
2777-4960	1		2
9481-1653	1		1
2106-2886	1		1
9650-7406	1		1
8402-4209	1		6
6097-6072	1		1

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

8110-4924	1	2
7354-9670	1	1
1444-2205	1	1
3591-5695	1	1
7775-7407	1	1
4722-4042	1	1
4722-4042	2	4
4269-1165	1	1
7664-5903	1	1
4813-4368	1	1
4813-4368	2	1
4933-6017	1	2
4933-6017	2	1
1276-7212	1	1
2347-7207	1	1
2347-7207	2	1
8553-5297	1	1
8553-5297	2	1
8553-5297	3	1
8105-9198	1	1
7956-3110	1	1
3944-4961	1	3
6216-8184	1	5
9299-1311	1	1
4995-2138	1	1
9144-9105	1	1
9527-8789	1	1
3240-7871	1	4
5221-9040	1	1
3488-5355	1	1
2263-2460	1	1
5715-4009	1	2
4729-4245	1	9
6836-3037	1	1
4115-6745	1	1
4115-6745	2	4
7763-8838	1	4
1513-1055	1	9
2052-1544	1	1
3651-6130	1	1
1782-1238	1	1
6507-4207	1	1
2345-5733	1	1
6640-4512	1	1
2725-7482	1	3
7196-9266	1	1
2253-5282	1	1
2253-5282	2	1

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

1947-1096	1	1
1947-1096	2	2
1947-1096	3	2
1947-1096	4	2
3672-4781	1	1
3672-4781	2	1
5100-2689	1	2
5100-2689	2	8
5100-2689	3	2
3992-8344	1	3
2822-5170	1	2
4117-1334	1	5
4117-1334	2	2
6984-6839	1	1
1430-9130	1	1
1430-9130	2	1
5410-6758	1	1
7589-5847	1	1
4908-6495	1	2
4908-6495	2	1
4405-7635	1	2
9738-3371	1	1
5479-2355	1	1
5394-5444	1	1
1635-8106	1	4
2488-5045	1	1
6655-8685	1	1
5947-8953	1	1
8102-7818	1	9
7784-7620	1	1
4450-2224	1	1
4450-2224	2	2
7523-4366	1	2
6832-8183	1	2
9093-5874	1	1
9963-6470	1	3
9963-6470	2	1
3091-9649	1	2
5763-5271	1	9
3187-8871	1	1
3206-7083	1	2

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

1850-7040	1		1
9718-7192	1		2
8910-9830	1		1
3737-6808	1		2
5244-1045	1		1
5244-1045	2		1
5244-1045	3		1
4008-9847	1		2
5954-6564	1		2
2442-6934	1		1
7713-3799	1		5
8192-9938	1		1
7952-8303	1		1
2467-5988	1		2
2467-5988	2		5
2467-5988	3		1
2467-5988	4		1
1070-1347	1		1
1070-1347	2		1
5568-8560	1		1
5568-8560	2		1
5568-8560	3		2
5568-8560	4		2
5568-8560	5		2
5568-8560	6		1
5568-8560	7		2
5568-8560	8		2
5568-8560	9		5
2708-5934	1		2
2830-8142	1		1
9240-6435	1		1
8911-8572	1		1
5875-8541	1		9
4656-3237	1		5
6306-5878	1		1
4990-9046	1		2
4990-9046	2		3
5422-6231	1		1
4117-4000	1		1
6357-1439	1		1
6357-1439	2		2
4053-4238	1		1
4053-4238	2		2
1965-8442	1		1
5486-3743	1		4

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

4251-2460	1	1
6713-4118	1	1
6713-4118	2	2
6713-4118	3	2
7934-7436	1	1
7934-7436	2	1
7934-7436	3	5
7934-7436	4	1
3115-9229	1	2
3115-9229	2	2
3115-9229	3	1
9829-9620	1	1
9829-9620	2	3
2292-1496	1	2
4776-5472	1	4
3503-3891	1	2
3503-3891	2	2
7335-8757	1	1
7335-8757	2	1
6444-4101	1	2
2878-1326	1	1
9646-6676	1	1
1170-3538	1	4
2086-8033	1	1
5987-3950	1	1
5987-3950	2	1
5987-3950	3	2
3922-7090	1	2
3922-7090	2	2
3922-7090	3	2
9124-5990	1	4
9124-5990	2	4
9088-6532	1	3
7801-8040	1	4
2508-5151	1	1
8045-4243	1	1
8045-4243	2	8
4905-4653	1	1
6489-2363	1	2
7245-3904	1	1
7245-3904	2	5
7245-3904	3	5
2977-6979	1	1
1467-4134	1	1
7173-2900	1	1

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

9739-2705	1	1
5473-8739	1	4
5473-8739	2	5
6245-5735	1	4
2250-4318	1	3
4873-4964	1	1
1548-1678	1	1
8797-5054	1	1
9243-3425	1	1
7351-6346	1	2
7351-6346	2	1
7351-6346	3	1
4213-9155	1	2
2439-5294	1	1
1062-8080	1	2
1062-8080	2	1
1062-8080	3	5
4753-6765	1	2
4753-6765	2	2
4753-6765	3	2
4753-6765	4	5
4753-6765	5	2
7004-9295	1	2
9304-9777	1	1
9304-9777	2	1
4374-2137	1	1
7340-8286	1	1
6399-2358	1	1
3763-9851	1	2
4864-5318	1	1
9650-1930	1	1
5197-8681	1	2
3379-4359	1	1
9587-7529	1	1
2759-5698	1	1
2720-1411	1	1
3376-1213	1	1
6628-5366	1	1
4992-7418	1	4
4992-7418	2	1
9727-6391	1	4
2683-9561	1	1
5927-4206	1	1
5927-4206	2	2
1869-7973	1	1
9309-6563	1	2
3483-6257	1	1

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

3809-5790	1	3
8452-9268	1	1
4824-9240	1	2
5485-7401	1	1
7300-5439	1	2
7941-5291	1	1
5080-6859	1	1
5080-6859	2	2
1361-6862	1	2
1361-6862	2	2
4635-3697	1	1
7368-7177	1	2
4903-5917	1	1
4903-5917	2	2
8288-5138	1	2
8288-5138	2	2
8288-5138	3	2
9816-5134	1	1
3499-9588	1	1
8209-1100	1	2
8209-1100	2	1
2806-4936	1	2
2806-4936	2	1
7033-7837	1	2
7033-7837	2	1
6047-5153	1	1
2390-7117	1	1
1974-6857	1	4
1974-6857	2	1
3683-7668	1	1
3683-7668	2	1
3683-7668	3	3
3683-7668	4	1
2976-2802	1	1
1751-8355	1	1
6836-9334	1	1
1074-8946	1	1
3115-5852	1	1
3115-5852	2	1
5023-3788	1	2
5023-3788	2	2
5023-3788	3	2
5023-3788	4	2
5023-3788	5	2

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPEREPATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

5023-3788	6	1
5023-3788	7	2
5023-3788	8	2
7974-8782	1	1
1411-8196	1	1
4831-9288	1	1
6550-5426	1	2
7824-9008	1	2
4032-6298	1	2
7704-9427	1	1
7704-9427	2	2
6394-9506	1	1
8033-4080	1	2
1339-5084	1	2
1339-5084	2	2
3960-2437	1	1
3960-2437	2	1
3960-2437	3	4
3550-3799	1	4
3550-3799	2	1
3550-3799	3	1
3776-1702	1	1
3776-1702	2	4
1294-2093	1	1
8980-7302	1	1
8980-7302	2	1
8119-4991	1	1
2720-2076	1	2
7163-7001	1	5
7163-7001	2	4
9789-6694	1	1
1920-9089	1	1
4826-7399	1	2
8565-3455	1	1
8565-3455	2	2
5525-1116	1	1
6102-6168	1	1
6102-6168	2	5
7871-4437	1	1
1008-3298	1	2
1008-3298	2	3
1008-3298	3	4
1008-3298	4	2
3055-4466	1	3
4256-6145	1	1
6370-8212	1	1
8628-6673	1	2

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERKERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

8628-6673	2	2
8628-6673	3	2
8628-6673	4	2
8628-6673	5	2
8628-6673	6	2
8877-4748	1	1
8877-4748	2	2
3434-5369	1	1
2868-4185	1	1
2868-4185	2	1
2868-4185	3	2
6091-1561	1	5
3824-7054	1	1
6150-6273	1	1
3758-6597	1	1
8229-8511	1	2
8229-8511	2	1
4937-2035	1	1
5672-3532	1	1
5672-3532	2	4
5672-3532	3	1
2172-7957	1	6
5273-3707	1	1
4952-9211	1	5
4952-9211	2	5
4952-9211	3	5
4952-9211	4	2
4952-9211	5	2
9705-2176	1	1
9705-2176	2	2
9705-2176	3	2
9705-2176	4	2
9705-2176	5	2
9705-2176	6	4
2062-1325	1	1
2062-1325	2	1
2513-4364	1	2
6616-4236	1	1
3161-5033	1	1
7648-3098	1	1
7648-3098	2	2

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

3990-5021	1	1
8771-6354	1	1
1787-8628	1	1
1787-8628	2	1
1787-8628	3	4
7289-3991	1	2
7289-3991	2	4
9083-6099	1	1
9083-6099	2	4
2156-3208	1	1
2156-3208	2	4
2156-3208	3	4
3239-6412	1	1
1903-5437	1	1
8124-5232	1	1
4111-9147	1	1
2394-7552	1	1
5723-1317	1	1
6704-5941	1	1
4516-8111	1	1
9534-7823	1	1
8903-1178	1	2
8903-1178	2	1
8503-6602	1	1
8503-6602	2	1
2891-4965	1	1
2891-4965	2	5
2771-4621	1	1
2771-4621	2	2
2480-8775	1	1
2480-8775	2	1
8291-8732	1	1
1213-3106	1	1
1213-3106	2	5
6216-4350	1	1
8973-4428	1	1
2032-1651	1	1
2220-2799	1	1
6491-4705	1	9
6491-4705	2	1
5697-2788	1	1
8408-9177	1	1
2843-1031	1	1
2843-1031	2	1
2843-1031	3	5
4427-2592	1	1
4427-2592	2	1
5476-7215	1	4

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

5476-7215	2		2
5476-7215	3		1
3275-8239	1		1
3275-8239	2		1
8305-6733	1		1
9951-9356	1		1
9596-4016	1		1
3446-4893	1		4
3446-4893	2		4
1319-3142	1		1
3684-7652	1		1
5765-2834	1		1
7923-8113	1		5
7923-8113	2		4
7393-3852	1		1
7393-3852	2		4
4621-6106	1		1
4621-6106	2		1
4621-6106		3	2
4268-7686	1		2
4268-7686	2		2
4268-7686	3		1
4268-7686	4		2
2055-5585	1		1
2055-5585	2		2
7654-5239	1		2
7654-5239	2		2
3093-8064	1		1
1560-1821	1		1
5581-3309	1		1
9364-2325	1		2
9364-2325	2		1
7253-6741	1		1
7253-6741	2		1
7253-6741	3		1
5738-4311	1		1
5738-4311	2		1
4316-5203	1		1
4408-7026	1		1
3489-5297	1		2
3489-5297	2		1
3489-5297	3		1
3489-5297	4		1
4975-5788	1		1
4975-5788	2		2
5890-9103	1		2
7760-5411	1		2
7760-5411	2		2

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

7760-5411	3	2
3938-9860	1	1
3938-9860	2	2
3938-9860	3	2
1075-2404	1	2
6623-3769	1	2
7897-7901	1	1
6123-5334	1	1
7485-3669	1	1
3831-3137	1	1
3831-3137	2	5
9105-9670	1	2
9105-9670	2	2
9105-9670	3	2
1572-9170	1	1
1572-9170	2	2
1572-9170	3	5
1572-9170	4	3
8636-6741	1	4
2469-4298	1	2
2469-4298	2	2
4164-4915	1	2
4164-4915	2	2
4164-4915	3	1
4164-4915	4	1
4164-4915	5	2
5932-3507	1	1
5932-3507	2	1
1255-7948	1	1
2716-7124	1	2
2787-6569	1	1
4718-8949	1	1
4718-8949	2	1
4718-8949	3	2
4718-8949	4	2
4718-8949	5	2
4505-1535	1	2
4505-1535	2	1
4505-1535	3	2
4505-1535	4	2
4505-1535	5	2
2857-5356	1	2
2857-5356	2	1
1471-7613	1	1

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPERERATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

1471-7613	2	2
1471-7613	3	1
1471-7613	4	3
2964-4524	1	1
2964-4524	2	1
1110-9438	1	1
6667-8298	1	1
1033-5995	1	1
5892-2223	1	1
5892-2223	2	2
9375-3315	1	1
9375-3315	2	4
7685-1463	1	4
7805-3341	1	1
9041-3455	1	1
9041-3455	2	2
2442-9506	1	1
5895-1779	1	2
5895-1779	2	2
6764-5774	1	2
6764-5774	2	1
5627-7971	1	1
5627-7971	2	3
6998-1313	1	1
3682-9867	1	2
3682-9867	2	1
3682-9867	3	2
6643-6713	1	1
6643-6713	2	2
6643-6713	3	2
7001-4469	1	1
6794-1848	1	1
2484-2080	1	1
6540-6628	1	1
4672-6795	1	1
2503-3653	1	1
6065-8753	1	2
4905-7905	1	1
6723-1425	1	6
6723-1425	2	2
3721-8865	1	2
4263-5294	1	2
5513-5882	1	1
5669-9789	1	2
5669-9789	2	2
5669-9789	3	2
5669-9789	4	3
4080-7650	1	1
4080-7650	2	1

1 CARCINOMA
2 PAPILLOMA
3 FOLLICULAR CYST
4 HYPEREPATOSIS/HYPERP
5 KERATOACANTHOMA
6 SARCOMA
7 FIBROMA
8 NORMAL
9 OTHER

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance[a]	Tumor diagnosis[b]
1	NO VIS. TUM.	31	226			
1	NO VIS. TUM.	51	748			
1	NO VIS. TUM.	57	22			
1	NO VIS. TUM.	132	748			
1		142	573	1	406	CAR
1	NO VIS. TUM.	150	748			
1	NO VIS. TUM.	152	545			
1	NO VIS. TUM.	153	748			
1	NO VIS. TUM.	229	698			
1	NO VIS. TUM.	230	748			
1	NO VIS. TUM.	297	748			
1	NO VIS. TUM.	321	513			
1	NO VIS. TUM.	326	681			
1	NO VIS. TUM.	352	748			
1	NO VIS. TUM.	356	132			
1	NO VIS. TUM.	377	748			
1	NO VIS. TUM.	384	412			
1	NO VIS. TUM.	390	575			
1	NO VIS. TUM.	393	748			
1		425	664	1	483	CAR
1	NO VIS. TUM.	439	748			
1	NO VIS. TUM.	442	330			
1		459	642	1	589	PAP
1	NO VIS. TUM.	474	748			
1		487	533	1	377	CAR
1	NO VIS. TUM.	494	748			
1	NO VIS. TUM.	525	748			
1	NO VIS. TUM.	535	748			
1	NO VIS. TUM.	605	524			
1	NO VIS. TUM.	606	748			
2	NO VIS. TUM.	15	720			
2	NO VIS. TUM.	18	748			
2	NO VIS. TUM.	116	530			
2	NO VIS. TUM.	147	748			
2	NO VIS. TUM.	186	748			
2	NO VIS. TUM.	190	748			
2	NO VIS. TUM.	198	748			
2	NO VIS. TUM.	204	748			
2	NO VIS. TUM.	213	748			
2	NO VIS. TUM.	238	610			
2	NO VIS. TUM.	256	692			
2	NO VIS. TUM.	279	447			
2	NO VIS. TUM.	302	748			
2	NO VIS. TUM.	315	748			
2	NO VIS. TUM.	333	748			
2	NO VIS. TUM.	335	748			
2	NO VIS. TUM.	347	629			
2	NO VIS. TUM.	351	239			
2	NO VIS. TUM.	359	537			
2	NO VIS. TUM.	361	503			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
2	NO VIS. TUM.	362	748			
2	NO VIS. TUM.	399	748			
2	NO VIS. TUM.	420	229			
2	NO VIS. TUM.	443	748			
2	NO VIS. TUM.	446	748			
2	NO VIS. TUM.	514	728			
2	NO VIS. TUM.	532	748			
2	NO VIS. TUM.	542	748			
2	NO VIS. TUM.	571	320			
2	NO VIS. TUM.	592	748			
3	NO VIS. TUM.	28	601			
3	NO VIS. TUM.	30	748			
3	NO VIS. TUM.	37	748			
3	NO VIS. TUM.	42	748			
3	NO VIS. TUM.	46	748			
3	NO VIS. TUM.	102	748			
3	NO VIS. TUM.	107	748			
3	NO VIS. TUM.	111	748			
3	NO VIS. TUM.	121	570			
3	NO VIS. TUM.	128	622			
3	NO VIS. TUM.	146	539			
3	NO VIS. TUM.	192	748			
3	NO VIS. TUM.	194	748			
3	NO VIS. TUM.	278	748			
3	NO VIS. TUM.	282	684			
3	NO VIS. TUM.	300	748			
3	NO VIS. TUM.	303	597			
3	NO VIS. TUM.	318	748			
3	NO VIS. TUM.	336	748			
3	NO VIS. TUM.	348	748			
3	NO VIS. TUM.	355	748			
3	NO VIS. TUM.	366	748			
3	NO VIS. TUM.	376	748			
3	NO VIS. TUM.	401	728			
3	NO VIS. TUM.	410	748			
3	NO VIS. TUM.	417	537			
3	NO VIS. TUM.	469	748			
3	NO VIS. TUM.	506	748			
3	NO VIS. TUM.	511	166			
3	NO VIS. TUM.	586	748			
4	NO VIS. TUM.	3	607			
4		39	513	1	356	CAR
4		85	652	1	553	PAP
4		85	652	2	596	OTHER
4		85	652	3	615	CAR
4	NO VIS. TUM.	96	232			
4	NO VIS. TUM.	106	109			
4		154	671	1	582	CAR
4		154	671	2	589	PAP
4		157	538	1	421	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
4		157	538	2	462	PAP
4		159	699	1	574	CAR
4		159	699	2	671	PAP
4		162	477	1	413	CAR
4		165	537	1	413	CAR
4		182	573	1	434	CAR
4		183	573	1	497	PAP
4		183	573	2	497	PAP
4		183	573	3	525	PAP
4		183	573	4	547	PAP
4		183	573	5	553	PAP
4		183	573	6	561	PAP
4	NO VIS. TUM.	184	607			
4	NO VIS. TUM.	185	454			
4	NO VIS. TUM.	210	235			
4		267	625	1	421	CAR
4		267	625	2	434	CAR
4	NO VIS. TUM.	342	153			
4		343	602	1	497	CAR
4		343	602	2	503	CAR
4		343	602	3	547	PAP
4		343	602	4	547	PAP
4		343	602	5	582	HYPER
4		345	608	1	539	CAR
4		345	608	2	547	CAR
4		345	608	3	582	PAP
4		349	624	1	553	CAR
4		349	624	2	615	PAP
4	NO VIS. TUM.	368	509			
4		430	608	1	525	CAR
4		430	608	2	547	PAP
4		457	748	1	589	CAR
4		471	445	1	349	CAR
4		516	635	1	421	CAR
4		561	748	1	602	HYPER
4		561	748	2	643	PAP
4		561	748	3	650	CAR
4		561	748	4	678	CAR
4		561	748	5	678	CAR
4		561	748	6	693	PAP
4	NO VIS. TUM.	577	545			
4		580	573	1	497	PAP
4		580	573	2	497	CAR
4		580	573	3	497	PAP
4		580	573	4	547	PAP
4		590	393	1	342	CAR
4		598	502	1	370	CAR
4		598	502	2	497	PAP
5	NO VIS. TUM.	49	748			
5	NO VIS. TUM.	54	613			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
5	NO VIS. TUM.	68	748			
5	NO VIS. TUM.	110	104			
5	NO VIS. TUM.	125	509			
5	NO VIS. TUM.	149	590			
5	NO VIS. TUM.	155	719			
5	NO VIS. TUM.	195	748			
5	NO VIS. TUM.	196	748			
5	NO VIS. TUM.	206	748			
5	NO VIS. TUM.	249	748			
5	NO VIS. TUM.	286	699			
5	NO VIS. TUM.	291	658			
5	NO VIS. TUM.	304	630			
5	NO VIS. TUM.	310	615			
5	NO VIS. TUM.	323	748			
5	NO VIS. TUM.	331	615			
5	NO VIS. TUM.	337	237			
5	NO VIS. TUM.	346	748			
5	NO VIS. TUM.	360	537			
5	NO VIS. TUM.	388	444			
5	NO VIS. TUM.	391	483			
5	NO VIS. TUM.	398	545			
5	NO VIS. TUM.	403	748			
5	NO VIS. TUM.	450	748			
5	NO VIS. TUM.	451	239			
5	NO VIS. TUM.	538	629			
5	NO VIS. TUM.	566	504			
5	NO VIS. TUM.	579	748			
5	NO VIS. TUM.	603	609			
6	NO VIS. TUM.	40	749			
6	NO VIS. TUM.	71	607			
6	NO VIS. TUM.	83	580			
6	NO VIS. TUM.	108	748			
6	NO VIS. TUM.	114	749			
6	NO VIS. TUM.	167	748			
6	NO VIS. TUM.	168	748			
6	NO VIS. TUM.	217	748			
6	NO VIS. TUM.	222	570			
6	NO VIS. TUM.	240	530			
6	NO VIS. TUM.	261	659			
6	NO VIS. TUM.	271	749			
6	NO VIS. TUM.	274	748			
6	NO VIS. TUM.	277	749			
6	NO VIS. TUM.	283	748			
6	NO VIS. TUM.	288	546			
6	NO VIS. TUM.	296	748			
6	NO VIS. TUM.	301	748			
6	NO VIS. TUM.	306	489			
6	NO VIS. TUM.	311	567			
6	NO VIS. TUM.	324	748			
6	NO VIS. TUM.	407	749			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
6	NO VIS. TUM.	427	748			
6	NO VIS. TUM.	428	749			
6	NO VIS. TUM.	436	748			
6	NO VIS. TUM.	466	748			
6	NO VIS. TUM.	537	569			
6	NO VIS. TUM.	546	748			
6	NO VIS. TUM.	553	699			
6	NO VIS. TUM.	567	657			
7	NO VIS. TUM.	6	166			
7		10	749	1	582	CAR
7	NO VIS. TUM.	88	678			
7	NO VIS. TUM.	120	573			
7		133	669	1	602	CAR
7	NO VIS. TUM.	134	532			
7		163	749	1	728	CAR
7		171	749	1	687	CAR
7		171	749	2	706	PAP
7	NO VIS. TUM.	178	498			
7		180	749	1	664	PAP
7	NO VIS. TUM.	189	546			
7	NO VIS. TUM.	219	678			
7	NO VIS. TUM.	221	749			
7	NO VIS. TUM.	237	749			
7	NO VIS. TUM.	295	524			
7		383	749	1	615	HYPER
7		383	749	2	728	CAR
7	NO VIS. TUM.	392	636			
7		394	727	1	609	CAR
7		431	706	1	596	CAR
7	NO VIS. TUM.	438	749			
7	NO VIS. TUM.	465	588			
7	NO VIS. TUM.	493	628			
7	NO VIS. TUM.	500	749			
7	NO VIS. TUM.	521	498			
7		527	667	1	582	CAR
7		540	449	1	322	CAR
7	NO VIS. TUM.	555	749			
7	NO VIS. TUM.	558	526			
7		569	749	1	720	CAR
7		574	749	1	728	HYPER
8	NO VIS. TUM.	24	21			
8		35	664	1	582	CAR
8		43	749	1	421	CAR
8	NO VIS. TUM.	53	532			
8		86	569	1	413	CAR
8	NO VIS. TUM.	98	695			
8	NO VIS. TUM.	109	671			
8		119	570	1	518	CAR
8		126	749	1	643	CAR
8		126	749	2	664	HYPER

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
8		127	671	1	518	CAR
8		173	545	1	448	KER
8		173	545	2	476	PAP
8		177	695	1	590	CAR
8		218	476	1	413	CAR
8	NO VIS. TUM.	226	509			
8		247	749	1	630	FCYST
8	NO VIS. TUM.	248	595			
8		276	632	1	426	CAR
8	NO VIS. TUM.	329	337			
8		334	727	1	659	CAR
8		400	749	1	671	CAR
8		432	749	1	568	CAR
8		432	749	2	637	CAR
8		453	749	1	643	CAR
8		460	659	1	497	CAR
8		485	496	1	476	CAR
8		513	668	1	561	CAR
8	NO VIS. TUM.	522	574			
8		528	722	1	512	CAR
8		543	580	1	531	PAP
8		582	678	1	590	PAP
8	NO VIS. TUM.	599	513			
9	NO VIS. TUM.	4	749			
9	NO VIS. TUM.	26	106			
9	NO VIS. TUM.	32	749			
9	NO VIS. TUM.	50	749			
9	NO VIS. TUM.	99	330			
9	NO VIS. TUM.	115	723			
9	NO VIS. TUM.	130	189			
9	NO VIS. TUM.	172	588			
9	NO VIS. TUM.	179	487			
9	NO VIS. TUM.	193	499			
9	NO VIS. TUM.	245	577			
9	NO VIS. TUM.	252	569			
9	NO VIS. TUM.	289	612			
9	NO VIS. TUM.	317	749			
9	NO VIS. TUM.	319	656			
9		322	650	1	637	HYPER
9	NO VIS. TUM.	379	601			
9	NO VIS. TUM.	405	63			
9	NO VIS. TUM.	406	749			
9	NO VIS. TUM.	416	573			
9	NO VIS. TUM.	423	749			
9	NO VIS. TUM.	473	749			
9	NO VIS. TUM.	483	497			
9	NO VIS. TUM.	507	492			
9	NO VIS. TUM.	510	509			
9	NO VIS. TUM.	556	729			
9	NO VIS. TUM.	559	676			

roup code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
9	NO VIS. TUM.	596	577			
9	NO VIS. TUM.	608	686			
9	NO VIS. TUM.	612	524			
10	NO VIS. TUM.	9	580			
10	NO VIS. TUM.	19	628			
10	NO VIS. TUM.	25	511			
10	NO VIS. TUM.	34	720			
10	NO VIS. TUM.	61	749			
10	NO VIS. TUM.	62	749			
10	NO VIS. TUM.	122	749			
10	NO VIS. TUM.	141	582			
10	NO VIS. TUM.	169	574			
10	NO VIS. TUM.	216	601			
10	NO VIS. TUM.	254	552			
10	NO VIS. TUM.	259	482			
10	NO VIS. TUM.	263	749			
10	NO VIS. TUM.	265	749			
10	NO VIS. TUM.	273	510			
10	NO VIS. TUM.	325	582			
10	NO VIS. TUM.	354	239			
10	NO VIS. TUM.	367	509			
10	NO VIS. TUM.	375	749			
10	NO VIS. TUM.	396	675			
10	NO VIS. TUM.	411	679			
10	NO VIS. TUM.	418	749			
10	NO VIS. TUM.	435	749			
10	NO VIS. TUM.	470	749			
10	NO VIS. TUM.	475	501			
10	NO VIS. TUM.	504	218			
10	NO VIS. TUM.	529	749			
10	NO VIS. TUM.	581	749			
10	NO VIS. TUM.	584	749			
10	NO VIS. TUM.	613	749			
11		17	545	1	497	PAP
11		67	545	1	399	PAP
11		67	545	2	463	PAP
11		67	545	3	512	PAP
11	NO VIS. TUM.	79	443			
11		80	504	1	322	PAP
11		117	720	1	637	CAR
11		117	720	2	637	CAR
11		117	720	3	706	PAP
11		136	624	1	519	CAR
11	NO VIS. TUM.	202	417			
11		214	584	1	525	CAR
11		214	584	2	532	PAP
11		224	513	1	435	PAP
11		224	513	2	435	CAR
11		224	513	3	435	KER
11		257	749	1	568	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
11		258	519	1	386	PAP
11		258	519	2	392	PAP
11		258	519	3	422	PAP
11		258	519	4	435	KER
11		258	519	5	497	PAP
11		269	481	1	456	CAR
11		358	663	1	448	CAR
11	NO VIS. TUM.	371	532			
11		373	454	1	343	CAR
11		382	671	1	630	CAR
11		382	671	2	643	CAR
11		382	671	3	650	HYPER
11		386	574	1	422	CAR
11		402	702	1	590	PAP
11		408	513	1	392	CAR
11		409	499	1	364	PAP
11		409	499	2	378	CAR
11		409	499	3	435	CAR
11		424	509	1	392	CAR
11		462	590	1	371	PAP
11		462	590	2	483	PAP
11		462	590	3	497	PAP
11		462	590	4	519	PAP
11		462	590	5	519	PAP
11		462	590	6	562	CAR
11		462	590	7	568	PAP
11		462	590	8	582	PAP
11		472	533	1	504	CAR
11		472	533	2	519	KER
11		482	449	1	364	CAR
11		490	669	1	562	CAR
11		502	624	1	519	CAR
11		502	624	2	547	CAR
11		518	580	1	386	PAP
11		518	580	2	483	PAP
11		518	580	3	497	PAP
11		518	580	4	519	PAP
11		518	580	5	547	PAP
11		518	580	6	554	PAP
11	NO VIS. TUM.	533	444			
11	NO VIS. TUM.	573	473			
11		601	537	1	491	CAR
12	NO VIS. TUM.	7	518			
12	NO VIS. TUM.	12	596			
12	NO VIS. TUM.	58	582			
12		118	646	1	575	PAP
12		124	749	1	643	CAR
12	NO VIS. TUM.	129	646			
12	NO VIS. TUM.	138	516			
12		164	714	1	714	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
12		164	714	2	714	PAP
12		170	749	1	721	PAP
12		170	749	2	721	PAP
12	NO VIS. TUM.	203	618			
12	NO VIS. TUM.	225	242			
12	NO VIS. TUM.	260	499			
12	NO VIS. TUM.	280	589			
12	NO VIS. TUM.	284	749			
12		299	749	1	672	PAP
12		299	749	2	679	PAP
12		299	749	3	687	PAP
12		339	749	1	610	CAR
12	NO VIS. TUM.	374	117			
12		397	601	1	568	PAP
12	NO VIS. TUM.	434	722			
12	NO VIS. TUM.	464	496			
12	NO VIS. TUM.	495	580			
12		496	545	1	456	CAR
12	NO VIS. TUM.	498	580			
12		501	749	1	707	CAR
12		531	749	1	616	CAR
12		531	749	2	721	PAP
12		534	636	1	392	CAR
12		539	749	1	679	PAP
12	NO VIS. TUM.	563	555			
12		600	671	1	504	CAR
12	NO VIS. TUM.	609	567			
13		20	475	1	457	PAP
13		20	475	2	463	CAR
13		36	595	1	583	CAR
13		36	595	2	583	CAR
13		36	595	3	590	CAR
13		56	749	1	693	PAP
13		60	609	1	407	CAR
13		66	749	1	603	CAR
13		77	613	1	457	HYPER
13		123	707	1	687	PAP
13		143	668	1	583	CAR
13		145	749	1	555	CAR
13		145	749	2	659	PAP
13		174	609	1	427	CAR
13	NO VIS. TUM.	175	526			
13		199	566	1	498	CAR
13		199	566	2	539	CAR
13	NO VIS. TUM.	207	477			
13	NO VIS. TUM.	211	540			
13	NO VIS. TUM.	228	582			
13		253	530	1	519	CAR
13	NO VIS. TUM.	255	530			
13		298	622	1	532	KER

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
13	NO VIS. TUM.	314	595			
13	NO VIS. TUM.	350	495			
13	NO VIS. TUM.	419	533			
13	NO VIS. TUM.	429	446			
13		433	635	1	548	CAR
13		433	635	2	590	CAR
13		433	635	3	597	PAP
13		433	635	4	616	PAP
13		433	635	5	616	PAP
13		433	635	6	616	CAR
13		433	635	7	616	PAP
13		433	635	8	623	PAP
13		433	635	9	630	KER
13	NO VIS. TUM.	512	573			
13		523	678	1	590	CAR
13	NO VIS. TUM.	548	432			
13	NO VIS. TUM.	562	495			
13		572	692	1	630	PAP
13	NO VIS. TUM.	589	512			
13	NO VIS. TUM.	594	516			
14	NO VIS. TUM.	5	573			
14	NO VIS. TUM.	27	749			
14	NO VIS. TUM.	45	749			
14	NO VIS. TUM.	59	604			
14	NO VIS. TUM.	64	581			
14	NO VIS. TUM.	75	749			
14	NO VIS. TUM.	91	503			
14	NO VIS. TUM.	92	749			
14	NO VIS. TUM.	94	509			
14	NO VIS. TUM.	101	749			
14	NO VIS. TUM.	103	749			
14	NO VIS. TUM.	104	749			
14	NO VIS. TUM.	131	749			
14	NO VIS. TUM.	220	580			
14	NO VIS. TUM.	250	702			
14	NO VIS. TUM.	268	589			
14	NO VIS. TUM.	290	749			
14	NO VIS. TUM.	313	502			
14	NO VIS. TUM.	369	749			
14	NO VIS. TUM.	404	650			
14	NO VIS. TUM.	448	611			
14	NO VIS. TUM.	461	604			
14	NO VIS. TUM.	497	749			
14	NO VIS. TUM.	509	749			
14	NO VIS. TUM.	520	509			
14	NO VIS. TUM.	541	749			
14	NO VIS. TUM.	547	545			
14		565	749	1	714	FCYST
14	NO VIS. TUM.	575	509			
14	NO VIS. TUM.	588	749			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
15	NO VIS. TUM.	13	749			
15	NO VIS. TUM.	21	514			
15	NO VIS. TUM.	23	749			
15	NO VIS. TUM.	33	659			
15	NO VIS. TUM.	55	478			
15	NO VIS. TUM.	76	695			
15	NO VIS. TUM.	78	749			
15	NO VIS. TUM.	137	349			
15	NO VIS. TUM.	148	656			
15	NO VIS. TUM.	223	222			
15	NO VIS. TUM.	235	728			
15	NO VIS. TUM.	264	591			
15	NO VIS. TUM.	305	646			
15	NO VIS. TUM.	309	749			
15	NO VIS. TUM.	353	584			
15	NO VIS. TUM.	415	630			
15	NO VIS. TUM.	437	749			
15	NO VIS. TUM.	445	604			
15	NO VIS. TUM.	454	509			
15	NO VIS. TUM.	455	716			
15	NO VIS. TUM.	456	384			
15	NO VIS. TUM.	477	749			
15	NO VIS. TUM.	478	702			
15	NO VIS. TUM.	499	749			
15	NO VIS. TUM.	517	749			
15	NO VIS. TUM.	524	518			
15	NO VIS. TUM.	593	477			
15	NO VIS. TUM.	597	749			
15	NO VIS. TUM.	604	749			
15	NO VIS. TUM.	611	511			
16		2	643	1	575	CAR
16		2	643	2	597	CAR
16		16	639	1	413	CAR
16		52	749	1	623	CAR
16		81	591	1	540	CAR
16	NO VIS. TUM.	84	516			
16		100	628	1	569	CAR
16		100	628	2	623	HYPER
16		156	701	1	623	CAR
16	NO VIS. TUM.	158	516			
16		160	554	1	519	HYPER
16		176	672	1	555	CAR
16		188	566	1	540	FCYST
16		191	513	1	413	CAR
16	NO VIS. TUM.	201	502			
16	NO VIS. TUM.	205	596			
16		236	417	1	308	CAR
16		239	698	1	590	KER
16		239	698	2	665	KER
16		239	698	3	693	KER

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
16		239	698	4	693	PAP
16		239	698	5	693	PAP
16		308	589	1	448	CAR
16		344	663	1	630	KER
16	NO VIS. TUM.	365	512			
16		370	601	1	555	PAP
16		413	547	1	392	CAR
16		426	679	1	526	CAR
16		440	607	1	548	CAR
16		440	607	2	575	CAR
16		440	607	3	603	HYPER
16	NO VIS. TUM.	476	511			
16		503	603	1	526	PAP
16		503	603	2	569	PAP
16	NO VIS. TUM.	508	362			
16		519	537	1	378	CAR
16		560	612	1	513	HYPER
16		560	612	2	532	CAR
16		560	612	3	597	CAR
16	NO VIS. TUM.	568	545			
16	NO VIS. TUM.	591	545			
17		14	559	1	526	KER
17		14	559	2	554	HYPER
17		38	687	1	569	CAR
17		41	749	1	590	PAP
17		41	749	2	714	CAR
17		44	628	1	562	CAR
17		197	610	1	477	PAP
17		197	610	2	540	PAP
17		231	603	1	583	PAP
17		233	573	1	484	CAR
17	NO VIS. TUM.	243	497			
17		275	625	1	442	CAR
17		293	608	1	509	CAR
17		293	608	2	554	PAP
17	NO VIS. TUM.	307	539			
17		316	519	1	414	CAR
17		316	519	2	435	CAR
17		330	398	1	364	CAR
17		332	570	1	435	CAR
17		364	524	1	484	CAR
17		395	669	1	597	PAP
17		395	669	2	651	CAR
17		395	669	3	659	CAR
17		395	669	4	665	CAR
17		422	504	1	442	PAP
17		422	504	2	498	FCYST
17		444	510	1	463	CAR
17		444	510	2	463	PAP
17		444	510	3	463	PAP

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
17		444	510	4	509	PAP
17		449	539	1	477	PAP
17		480	705	1	562	CAR
17		480	705	2	575	HYPER
17		480	705	3	651	CAR
17	NO VIS. TUM.	489	68			
17		491	749	1	680	CAR
17		492	598	1	435	PAP
17		492	598	2	448	PAP
17		492	598	3	540	CAR
17		492	598	4	554	PAP
17		515	609	1	526	CAR
17		515	609	2	569	PAP
17		526	666	1	569	CAR
17		551	448	1	435	OTHER
17	NO VIS. TUM.	554	288			
17		583	497	1	484	CAR
17		585	491	1	477	KER
17	NO VIS. TUM.	610	504			
18		1	472	1	379	CAR
18		48	632	1	513	CAR
18		48	632	2	554	PAP
18		48	632	3	610	KER
18		48	632	4	616	FCYST
18		70	406	1	267	CAR
18		70	406	2	301	CAR
18		73	524	1	414	PAP
18		74	729	1	659	CAR
18		74	729	2	680	CAR
18		95	635	1	509	PAP
18		95	635	2	598	PAP
18		95	635	3	603	PAP
18		112	603	1	492	PAP
18		112	603	2	583	PAP
18		200	561	1	435	PAP
18		200	561	2	548	NORM
18		200	561	3	548	PAP
18		212	573	1	435	CAR
18		212	573	2	526	PAP
18		212	573	3	526	CAR
18		212	573	4	569	FCYST
18		232	617	1	617	HYPER
18		251	561	1	407	CAR
18		270	582	1	569	PAP
18		287	454	1	386	CAR
18		341	729	1	637	CAR
18		341	729	2	651	CAR
18		341	729	3	680	CAR
18		381	607	1	513	CAR
18		381	607	2	519	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
18		412	705	1	575	CAR
18		421	749	1	554	PAP
18		421	749	2	721	CAR
18		452	567	1	484	CAR
18		452	567	2	492	CAR
18		468	404	1	404	PAP
18		481	588	1	484	PAP
18		481	588	2	519	CAR
18		486	583	1	484	CAR
18		488	568	1	400	CAR
18	NO VIS. TUM.	544	489			
18		545	457	1	379	CAR
18		550	609	1	457	PAP
18		550	609	2	464	PAP
18		550	609	3	532	CAR
18		550	609	4	598	CAR
18		550	609	5	598	PAP
18	NO VIS. TUM.	557	510			
18		570	671	1	603	PAP
18		570	671	2	623	CAR
18		578	699	1	583	CAR
18		607	584	1	540	PAP
18		607	584	2	540	CAR
18		607	584	3	554	PAP
18		607	584	4	554	PAP
18		607	584	5	563	PAP
18		614	591	1	519	CAR
18		614	591	2	548	CAR
18		614	591	3	563	PAP
18		614	591	4	563	PAP
18		614	591	5	563	PAP
19		8	573	1	563	PAP
19		11	749	1	714	CAR
19		11	749	2	721	CAR
19		11	749	3	729	FCYST
19		11	749	4	729	CAR
19		22	749	1	729	CAR
19	NO VIS. TUM.	47	530			
19		65	749	1	616	PAP
19		65	749	2	700	CAR
19		87	581	1	442	CAR
19	NO VIS. TUM.	93	428			
19		105	575	1	484	PAP
19	NO VIS. TUM.	139	501			
19		140	749	1	623	PAP
19		140	749	2	680	CAR
19		181	537	1	386	CAR
19		181	537	2	519	CAR
19		215	610	1	554	CAR
19		215	610	2	563	CAR

oup ode	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
19		215	610	3	576	PAP
19		281	516	1	427	PAP
19		285	692	1	604	CAR
19		285	692	2	637	PAP
19		285	692	3	659	CAR
19		328	492	1	449	FCYST
19	NO VIS. TUM.	338	423			
19	NO VIS. TUM.	357	450			
19	NO VIS. TUM.	372	145			
19		378	610	1	519	CAR
19		378	610	2	583	CAR
19		380	613	1	492	PAP
19		380	613	2	591	CAR
19		385	556	1	519	PAP
19	NO VIS. TUM.	387	538			
19		414	573	1	449	CAR
19		458	749	1	569	CAR
19		458	749	2	659	CAR
19		463	573	1	519	PAP
19		463	573	2	548	CAR
19	NO VIS. TUM.	479	573			
19		536	595	1	540	PAP
19		536	595	2	563	PAP
19		536	595	3	569	PAP
19		536	595	4	576	FCYST
19		549	524	1	513	KER
19		549	524	2	513	PAP
19		564	701	1	548	CAR
19		602	659	1	563	CAR
20		29	511	1	364	CAR
20		29	511	2	509	PAP
20		63	573	1	555	CAR
20		69	516	1	464	PAP
20		72	607	1	555	CAR
20		82	653	1	563	CAR
20		82	653	2	591	PAP
20		82	653	3	623	PAP
20		97	612	1	509	CAR
20		97	612	2	576	KER
20		113	581	1	570	CAR
20		113	581	2	581	HYPER
20		135	610	1	457	CAR
20		144	454	1	400	CAR
20		151	524	1	477	PAP
20		161	510	1	509	HYPER
20		187	439	1	379	CAR
20		227	669	1	598	CAR
20		227	669	2	610	PAP
20		234	580	1	513	PAP
20		234	580	2	520	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
20		246	570	1	464	CAR
20		266	537	1	414	FCYST
20		266	537	2	449	CAR
20		272	749	1	651	CAR
20		272	749	2	688	PAP
20		272	749	3	688	PAP
20		292	712	1	623	CAR
20		292	712	2	637	CAR
20		292	712	3	673	KER
20		292	712	4	693	CAR
20		294	492	1	386	CAR
20		312	601	1	532	CAR
20		312	601	2	548	HYPER
20		312	601	3	576	HYPER
20		340	598	1	492	CAR
20		363	678	1	563	CAR
20		363	678	2	665	PAP
20	NO VIS. TUM.	389	589			
20		441	580	1	492	CAR
20		447	537	1	457	CAR
20	NO VIS. TUM.	484	393			
20		530	482	1	407	CAR
20	NO VIS. TUM.	552	531			
20		576	560	1	532	HYPER
20		576	560	2	555	CAR
20		587	545	1	414	CAR
21		668	615	1	523	CAR
21	NO VIS. TUM.	703	519			
21		716	722	1	649	CAR
21		718	622	1	594	PAP
21		759	635	1	418	CAR
21	NO VIS. TUM.	774	504			
21	NO VIS. TUM.	776	504			
21		778	590	1	461	PAP
21		788	733	1	566	CAR
21	NO VIS. TUM.	819	733			
21		826	481	1	292	CAR
21		848	733	1	719	CAR
21		848	733	2	719	KER
21	NO VIS. TUM.	868	582			
21		899	509	1	446	CAR
21		929	709	1	552	CAR
21		967	733	1	733	CAR
21		994	649	1	552	CAR
21		1002	733	1	657	HYPER
21		1002	733	2	727	HYPER
21	NO VIS. TUM.	1007	107			
21	NO VIS. TUM.	1013	427			
21	NO VIS. TUM.	1045	628			
21		1087	721	1	614	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
21	NO VIS. TUM.	1105	511			
21	NO VIS. TUM.	1126	488			
21	NO VIS. TUM.	1161	512			
21		1186	714	1	552	CAR
21		1200	504	1	426	CAR
21		1254	733	1	677	CAR
21		1274	509	1	397	OTHER
21		1303	733	1	657	PAP
21		1303	733	2	677	FCYST
21		1303	733	3	719	HYPER
21		1303	733	4	727	PAP
22	NO VIS. TUM.	679	733			
22	NO VIS. TUM.	681	733			
22	NO VIS. TUM.	685	504			
22	NO VIS. TUM.	694	237			
22	NO VIS. TUM.	726	492			
22		744	733	1	698	CAR
22	NO VIS. TUM.	745	733			
22		796	733	1	646	PAP
22		796	733	2	719	PAP
22		796	733	3	719	CAR
22	NO VIS. TUM.	820	588			
22	NO VIS. TUM.	840	495			
22	NO VIS. TUM.	888	509			
22	NO VIS. TUM.	906	733			
22	NO VIS. TUM.	912	537			
22		922	733	1	628	PAP
22	NO VIS. TUM.	924	483			
22		992	733	1	621	CAR
22		992	733	2	719	FCYST
22		1023	733	1	692	CAR
22	NO VIS. TUM.	1030	501			
22		1057	733	1	733	PAP
22		1096	530	1	461	SAR
22	NO VIS. TUM.	1108	66			
22	NO VIS. TUM.	1130	497			
22	NO VIS. TUM.	1141	733			
22	NO VIS. TUM.	1212	733			
22		1220	684	1	649	CAR
22	NO VIS. TUM.	1228	733			
22	NO VIS. TUM.	1237	45			
22	NO VIS. TUM.	1273	733			
22		1294	635	1	502	CAR
22		1294	635	2	614	PAP
22		1302	733	1	523	HYPER
23	NO VIS. TUM.	692	299			
23	NO VIS. TUM.	714	298			
23	NO VIS. TUM.	730	295			
23	NO VIS. TUM.	764	733			
23	NO VIS. TUM.	777	483			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
23	NO VIS. TUM.	814	734			
23	NO VIS. TUM.	818	509			
23	NO VIS. TUM.	825	497			
23	NO VIS. TUM.	898	548			
23	NO VIS. TUM.	911	670			
23		930	513	1	439	PAP
23		980	719	1	663	PAP
23	NO VIS. TUM.	989	734			
23	NO VIS. TUM.	1016	719			
23	NO VIS. TUM.	1017	734			
23	NO VIS. TUM.	1082	469			
23	NO VIS. TUM.	1102	573			
23	NO VIS. TUM.	1106	474			
23	NO VIS. TUM.	1114	530			
23	NO VIS. TUM.	1118	734			
23	NO VIS. TUM.	1158	509			
23	NO VIS. TUM.	1180	497			
23	NO VIS. TUM.	1189	705			
23	NO VIS. TUM.	1202	344			
23	NO VIS. TUM.	1219	734			
23	NO VIS. TUM.	1227	734			
23	NO VIS. TUM.	1242	734			
23	NO VIS. TUM.	1245	589			
23	NO VIS. TUM.	1278	509			
23	NO VIS. TUM.	1282	251			
24		671	447	1	362	CAR
24		677	570	1	474	CAR
24		695	472	1	355	CAR
24	NO VIS. TUM.	720	233			
24		736	504	1	418	CAR
24		740	497	1	397	CAR
24	NO VIS. TUM.	760	234			
24		797	436	1	369	CAR
24		802	446	1	348	CAR
24		806	445	1	397	CAR
24		812	488	1	411	CAR
24		824	445	1	348	CAR
24		829	495	1	440	CAR
24		849	414	1	307	CAR
24		859	552	1	446	CAR
24		889	444	1	355	CAR
24		894	475	1	411	CAR
24		954	451	1	411	CAR
24		1019	468	1	390	CAR
24		1025	511	1	440	CAR
24		1050	504	1	404	CAR
24		1051	446	1	411	CAR
24		1051	446	2	440	PAP
24		1094	483	1	376	CAR
24		1128	464	1	369	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
24		1128	464	2	440	CAR
24		1134	487	1	397	CAR
24		1153	446	1	369	CAR
24		1178	464	1	383	CAR
24		1183	437	1	362	CAR
24		1184	469	1	411	CAR
24	NO VIS. TUM.	1280	235			
25	NO VIS. TUM.	691	701			
25	NO VIS. TUM.	698	685			
25	NO VIS. TUM.	712	734			
25	NO VIS. TUM.	719	732			
25		724	607	1	573	CAR
25		743	734	1	663	KER
25	NO VIS. TUM.	754	537			
25	NO VIS. TUM.	798	731			
25	NO VIS. TUM.	827	734			
25	NO VIS. TUM.	834	734			
25	NO VIS. TUM.	846	601			
25	NO VIS. TUM.	851	509			
25	NO VIS. TUM.	878	525			
25	NO VIS. TUM.	908	734			
25	NO VIS. TUM.	937	734			
25	NO VIS. TUM.	940	734			
25	NO VIS. TUM.	965	734			
25	NO VIS. TUM.	969	734			
25	NO VIS. TUM.	972	734			
25	NO VIS. TUM.	976	734			
25		985	607	1	560	PAP
25		1015	734	1	727	CAR
25	NO VIS. TUM.	1020	734			
25		1036	588	1	453	CAR
25	NO VIS. TUM.	1049	734			
25	NO VIS. TUM.	1072	734			
25	NO VIS. TUM.	1100	677			
25	NO VIS. TUM.	1248	460			
25	NO VIS. TUM.	1261	669			
25	NO VIS. TUM.	1283	354			
26	NO VIS. TUM.	715	747			
26	NO VIS. TUM.	732	747			
26	NO VIS. TUM.	785	747			
26	NO VIS. TUM.	857	446			
26	NO VIS. TUM.	864	590			
26	NO VIS. TUM.	869	511			
26	NO VIS. TUM.	891	663			
26	NO VIS. TUM.	944	747			
26	NO VIS. TUM.	1004	456			
26	NO VIS. TUM.	1018	684			
26	NO VIS. TUM.	1058	747			
26	NO VIS. TUM.	1068	747			
26	NO VIS. TUM.	1089	747			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
26	NO VIS. TUM.	1099	430			
26	NO VIS. TUM.	1120	727			
26	NO VIS. TUM.	1145	747			
26	NO VIS. TUM.	1154	463			
26	NO VIS. TUM.	1155	747			
26	NO VIS. TUM.	1166	747			
26	NO VIS. TUM.	1185	238			
26	NO VIS. TUM.	1193	747			
26	NO VIS. TUM.	1196	470			
26	NO VIS. TUM.	1211	235			
26	NO VIS. TUM.	1223	747			
26	NO VIS. TUM.	1253	747			
26	NO VIS. TUM.	1256	604			
26	NO VIS. TUM.	1257	680			
26	NO VIS. TUM.	1287	747			
26	NO VIS. TUM.	1288	503			
26	NO VIS. TUM.	1297	747			
27		665	588	1	488	CAR
27		665	588	2	580	PAP
27	NO VIS. TUM.	678	537			
27		705	580	1	447	CAR
27		739	500	1	461	CAR
27		739	500	2	474	CAR
27		739	500	3	481	CAR
27	NO VIS. TUM.	786	478			
27	NO VIS. TUM.	804	542			
27		805	601	1	502	CAR
27		805	601	2	510	CAR
27		811	532	1	397	PAP
27		811	532	2	524	CAR
27	NO VIS. TUM.	830	504			
27		855	491	1	411	CAR
27		861	576	1	474	CAR
27		876	513	1	440	PAP
27		876	513	2	482	CAR
27		876	513	3	496	PAP
27		895	601	1	510	CAR
27		896	615	1	530	CAR
27		943	681	1	545	CAR
27		966	590	1	516	CAR
27	NO VIS. TUM.	970	235			
27		1001	552	1	419	CAR
27		1001	552	2	524	HYPER
27		1024	540	1	530	HYPER
27		1073	574	1	482	CAR
27		1115	511	1	411	CAR
27		1115	511	2	510	FCYST
27		1142	537	1	427	PAP
27		1142	537	2	502	PAP
27		1147	602	1	468	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
27		1149	560	1	440	CAR
27		1149	560	2	530	PAP
27		1165	504	1	404	CAR
27		1167	501	1	369	CAR
27		1167	501	2	440	CAR
27		1195	588	1	447	CAR
27		1195	588	2	516	CAR
27		1210	615	1	510	OTHER
27		1210	615	2	615	CAR
27		1232	432	1	362	CAR
27		1270	455	1	383	CAR
28	NO VIS. TUM.	683	747			
28	NO VIS. TUM.	689	723			
28	NO VIS. TUM.	747	747			
28	NO VIS. TUM.	769	747			
28	NO VIS. TUM.	791	371			
28	NO VIS. TUM.	838	460			
28	NO VIS. TUM.	885	537			
28	NO VIS. TUM.	890	589			
28	NO VIS. TUM.	902	747			
28	NO VIS. TUM.	907	487			
28	NO VIS. TUM.	916	504			
28	NO VIS. TUM.	949	747			
28	NO VIS. TUM.	952	533			
28	NO VIS. TUM.	963	747			
28	NO VIS. TUM.	964	509			
28	NO VIS. TUM.	974	476			
28	NO VIS. TUM.	1006	747			
28	NO VIS. TUM.	1060	483			
28	NO VIS. TUM.	1081	513			
28	NO VIS. TUM.	1085	747			
28	NO VIS. TUM.	1093	747			
28	NO VIS. TUM.	1107	503			
28		1117	721	1	712	PAP
28		1125	714	1	657	CAR
28		1125	714	2	685	NORM
28	NO VIS. TUM.	1133	581			
28	NO VIS. TUM.	1135	413			
28	NO VIS. TUM.	1143	747			
28	NO VIS. TUM.	1168	510			
28	NO VIS. TUM.	1213	747			
28	NO VIS. TUM.	1264	747			
29		670	714	1	635	CAR
29	NO VIS. TUM.	672	747			
29	NO VIS. TUM.	674	747			
29	NO VIS. TUM.	686	747			
29	NO VIS. TUM.	710	747			
29	NO VIS. TUM.	721	747			
29	NO VIS. TUM.	737	498			
29	NO VIS. TUM.	807	510			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
29	NO VIS. TUM.	808	678			
29	NO VIS. TUM.	810	747			
29	NO VIS. TUM.	835	603			
29	NO VIS. TUM.	837	747			
29	NO VIS. TUM.	884	747			
29	NO VIS. TUM.	886	747			
29	NO VIS. TUM.	887	747			
29	NO VIS. TUM.	905	537			
29	NO VIS. TUM.	914	531			
29	NO VIS. TUM.	936	747			
29	NO VIS. TUM.	941	509			
29	NO VIS. TUM.	961	747			
29	NO VIS. TUM.	997	513			
29	NO VIS. TUM.	1039	747			
29	NO VIS. TUM.	1104	747			
29	NO VIS. TUM.	1146	727			
29	NO VIS. TUM.	1173	747			
29	NO VIS. TUM.	1187	747			
29	NO VIS. TUM.	1203	747			
29	NO VIS. TUM.	1241	747			
29	NO VIS. TUM.	1246	747			
29	NO VIS. TUM.	1281	747			
30		663	474	1	370	CAR
30		663	474	2	454	KER
30	NO VIS. TUM.	684	401			
30		687	504	1	412	CAR
30		687	504	2	440	HYPER
30		687	504	3	475	CAR
30		701	489	1	440	CAR
30		701	489	2	462	CAR
30		701	489	3	475	PAP
30		753	565	1	447	CAR
30		763	476	1	447	CAR
30		763	476	2	462	CAR
30		763	476	3	475	KER
30		779	504	1	475	PAP
30		794	459	1	405	CAR
30		816	635	1	538	CAR
30		823	601	1	523	PAP
30		823	601	2	566	KER
30		823	601	3	566	CAR
30		823	601	4	566	CAR
30		879	488	1	349	HYPER
30		879	488	2	440	PAP
30		879	488	3	447	CAR
30		913	530	1	468	KER
30		913	530	2	475	CAR
30		938	504	1	440	CAR
30		962	472	1	398	CAR
30		978	459	1	313	CAR

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
30		1035	504	1	475	PAP
30		1035	504	2	496	KER
30		1037	601	1	531	CAR
30		1037	601	2	595	CAR
30		1046	464	1	292	CAR
30		1065	496	1	398	CAR
30		1065	496	2	440	CAR
30		1084	447	1	349	CAR
30		1084	447	2	412	KER
30		1084	447	3	440	KER
30		1123	530	1	440	CAR
30		1157	525	1	523	CAR
30		1171	601	1	475	CAR
30		1176	478	1	405	CAR
30		1188	516	1	349	CAR
30		1188	516	2	511	CAR
30		1191	496	1	405	CAR
30		1197	446	1	405	CAR
30		1197	446	2	412	CAR
30		1206	472	1	447	HYPER
30		1206	472	2	468	KER
30		1239	491	1	462	CAR
30		1239	491	2	482	CAR
30		1247	481	1	454	PAP
31	NO VIS. TUM.	728	504			
31	NO VIS. TUM.	748	747			
31	NO VIS. TUM.	752	747			
31	NO VIS. TUM.	765	601			
31	NO VIS. TUM.	771	705			
31		780	747	1	595	PAP
31	NO VIS. TUM.	822	459			
31	NO VIS. TUM.	832	520			
31	NO VIS. TUM.	836	747			
31		850	747	1	646	PAP
31	NO VIS. TUM.	934	489			
31	NO VIS. TUM.	945	747			
31	NO VIS. TUM.	946	747			
31	NO VIS. TUM.	1005	747			
31	NO VIS. TUM.	1026	272			
31	NO VIS. TUM.	1052	513			
31	NO VIS. TUM.	1071	733			
31	NO VIS. TUM.	1092	747			
31	NO VIS. TUM.	1119	747			
31	NO VIS. TUM.	1136	510			
31	NO VIS. TUM.	1144	509			
31	NO VIS. TUM.	1163	747			
31	NO VIS. TUM.	1221	511			
31	NO VIS. TUM.	1222	747			
31	NO VIS. TUM.	1235	504			
31	NO VIS. TUM.	1252	747			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
31	NO VIS. TUM.	1277	295			
31	NO VIS. TUM.	1285	595			
31	NO VIS. TUM.	1286	747			
31	NO VIS. TUM.	1296	484			
32	NO VIS. TUM.	696	747			
32	NO VIS. TUM.	700	295			
32	NO VIS. TUM.	709	747			
32	NO VIS. TUM.	768	233			
32	NO VIS. TUM.	789	747			
32	NO VIS. TUM.	792	510			
32	NO VIS. TUM.	803	747			
32	NO VIS. TUM.	866	747			
32	NO VIS. TUM.	872	230			
32	NO VIS. TUM.	874	747			
32	NO VIS. TUM.	875	510			
32	NO VIS. TUM.	881	230			
32	NO VIS. TUM.	897	747			
32	NO VIS. TUM.	903	712			
32	NO VIS. TUM.	917	747			
32	NO VIS. TUM.	923	679			
32		925	722	1	574	CAR
32	NO VIS. TUM.	933	747			
32	NO VIS. TUM.	999	727			
32	NO VIS. TUM.	1010	747			
32	NO VIS. TUM.	1031	747			
32	NO VIS. TUM.	1056	705			
32	NO VIS. TUM.	1059	747			
32	NO VIS. TUM.	1131	235			
32	NO VIS. TUM.	1151	747			
32	NO VIS. TUM.	1159	747			
32	NO VIS. TUM.	1214	747			
32	NO VIS. TUM.	1226	747			
32	NO VIS. TUM.	1229	747			
32	NO VIS. TUM.	1255	747			
33		662	477	1	427	CAR
33		675	423	1	405	CAR
33		741	436	1	370	CAR
33		751	687	1	589	CAR
33		755	414	1	412	PAP
33		756	503	1	370	CAR
33		784	391	1	349	CAR
33	NO VIS. TUM.	787	295			
33	NO VIS. TUM.	795	503			
33		833	477	1	405	CAR
33	NO VIS. TUM.	892	240			
33		928	459	1	412	CAR
33		955	451	1	384	CAR
33		981	464	1	405	CAR
33		995	503	1	447	CAR
33		1034	553	1	454	CAR

roup code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
33		1053	417	1	349	CAR
33		1063	476	1	447	CAR
33		1083	557	1	489	CAR
33		1090	482	1	447	CAR
33		1098	487	1	419	CAR
33	NO VIS. TUM.	1101	238			
33		1137	467	1	412	CAR
33		1148	492	1	475	CAR
33		1152	457	1	405	CAR
33		1205	459	1	427	CAR
33		1215	390	1	307	CAR
33		1218	560	1	482	CAR
33	NO VIS. TUM.	1234	306			
33	NO VIS. TUM.	1275	341			
34	NO VIS. TUM.	667	705			
34		688	747	1	692	CAR
34		688	747	2	698	HYPER
34	NO VIS. TUM.	702	488			
34	NO VIS. TUM.	708	240			
34	NO VIS. TUM.	725	236			
34	NO VIS. TUM.	731	513			
34	NO VIS. TUM.	767	747			
34	NO VIS. TUM.	775	747			
34	NO VIS. TUM.	781	234			
34	NO VIS. TUM.	782	495			
34		799	747	1	719	FCYST
34	NO VIS. TUM.	839	747			
34	NO VIS. TUM.	893	229			
34	NO VIS. TUM.	904	747			
34	NO VIS. TUM.	927	236			
34	NO VIS. TUM.	931	747			
34	NO VIS. TUM.	939	747			
34		1048	747	1	706	CAR
34	NO VIS. TUM.	1070	747			
34	NO VIS. TUM.	1075	747			
34	NO VIS. TUM.	1076	511			
34	NO VIS. TUM.	1078	526			
34	NO VIS. TUM.	1116	114			
34	NO VIS. TUM.	1170	235			
34	NO VIS. TUM.	1192	526			
34	NO VIS. TUM.	1207	495			
34	NO VIS. TUM.	1217	482			
34	NO VIS. TUM.	1268	747			
34	NO VIS. TUM.	1276	607			
34	NO VIS. TUM.	1300	747			
35	NO VIS. TUM.	682	461			
35	NO VIS. TUM.	690	747			
35	NO VIS. TUM.	704	504			
35	NO VIS. TUM.	706	518			
35	NO VIS. TUM.	757	503			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
35	NO VIS. TUM.	773	747			
35	NO VIS. TUM.	801	504			
35	NO VIS. TUM.	815	638			
35	NO VIS. TUM.	821	747			
35	NO VIS. TUM.	854	635			
35	NO VIS. TUM.	865	747			
35	NO VIS. TUM.	867	509			
35	NO VIS. TUM.	883	747			
35	NO VIS. TUM.	900	747			
35	NO VIS. TUM.	918	652			
35	NO VIS. TUM.	935	747			
35	NO VIS. TUM.	1008	503			
35	NO VIS. TUM.	1042	511			
35	NO VIS. TUM.	1069	747			
35	NO VIS. TUM.	1097	547			
35	NO VIS. TUM.	1109	607			
35	NO VIS. TUM.	1111	453			
35	NO VIS. TUM.	1175	747			
35	NO VIS. TUM.	1199	747			
35	NO VIS. TUM.	1208	747			
35	NO VIS. TUM.	1216	747			
35	NO VIS. TUM.	1240	109			
35	NO VIS. TUM.	1263	510			
35	NO VIS. TUM.	1267	601			
35	NO VIS. TUM.	1269	509			
36	NO VIS. TUM.	661	233			
36	NO VIS. TUM.	673	232			
36	NO VIS. TUM.	697	487			
36	NO VIS. TUM.	746	748			
36	NO VIS. TUM.	758	748			
36	NO VIS. TUM.	770	747			
36	NO VIS. TUM.	783	747			
36	NO VIS. TUM.	793	748			
36	NO VIS. TUM.	813	495			
36	NO VIS. TUM.	858	501			
36	NO VIS. TUM.	870	748			
36	NO VIS. TUM.	871	509			
36	NO VIS. TUM.	901	664			
36	NO VIS. TUM.	926	232			
36	NO VIS. TUM.	991	233			
36	NO VIS. TUM.	1009	233			
36	NO VIS. TUM.	1012	232			
36	NO VIS. TUM.	1014	747			
36	NO VIS. TUM.	1027	747			
36	NO VIS. TUM.	1033	326			
36	NO VIS. TUM.	1113	503			
36	NO VIS. TUM.	1124	628			
36	NO VIS. TUM.	1169	233			
36	NO VIS. TUM.	1201	513			
36	NO VIS. TUM.	1249	574			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
36	NO VIS. TUM.	1260	236			
36	NO VIS. TUM.	1279	234			
36	NO VIS. TUM.	1292	240			
36	NO VIS. TUM.	1298	238			
36	NO VIS. TUM.	1301	235			
37	NO VIS. TUM.	723	748			
37	NO VIS. TUM.	727	232			
37	NO VIS. TUM.	733	748			
37	NO VIS. TUM.	734	748			
37	NO VIS. TUM.	735	601			
37	NO VIS. TUM.	742	233			
37	NO VIS. TUM.	800	509			
37	NO VIS. TUM.	817	233			
37	NO VIS. TUM.	852	89			
37	NO VIS. TUM.	877	748			
37	NO VIS. TUM.	919	233			
37	NO VIS. TUM.	932	488			
37	NO VIS. TUM.	953	509			
37	NO VIS. TUM.	971	496			
37	NO VIS. TUM.	977	91			
37	NO VIS. TUM.	988	467			
37	NO VIS. TUM.	1047	215			
37	NO VIS. TUM.	1055	748			
37	NO VIS. TUM.	1064	748			
37	NO VIS. TUM.	1067	748			
37	NO VIS. TUM.	1121	608			
37	NO VIS. TUM.	1129	244			
37		1160	601	1	595	FCYST
37	NO VIS. TUM.	1179	232			
37	NO VIS. TUM.	1238	748			
37	NO VIS. TUM.	1243	232			
37	NO VIS. TUM.	1251	504			
37	NO VIS. TUM.	1262	608			
37	NO VIS. TUM.	1290	748			
37	NO VIS. TUM.	1291	233			
38	NO VIS. TUM.	680	748			
38	NO VIS. TUM.	828	637			
38	NO VIS. TUM.	843	511			
38	NO VIS. TUM.	844	573			
38	NO VIS. TUM.	845	601			
38	NO VIS. TUM.	847	510			
38	NO VIS. TUM.	942	509			
38	NO VIS. TUM.	960	236			
38	NO VIS. TUM.	973	233			
38	NO VIS. TUM.	982	513			
38	NO VIS. TUM.	1011	233			
38	NO VIS. TUM.	1022	444			
38	NO VIS. TUM.	1038	748			
38	NO VIS. TUM.	1044	482			
38	NO VIS. TUM.	1054	670			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
38	NO VIS. TUM.	1086	233			
38	NO VIS. TUM.	1091	232			
38	NO VIS. TUM.	1112	670			
38	NO VIS. TUM.	1138	232			
38	NO VIS. TUM.	1139	232			
38	NO VIS. TUM.	1156	748			
38	NO VIS. TUM.	1162	748			
38	NO VIS. TUM.	1181	616			
38	NO VIS. TUM.	1198	520			
38	NO VIS. TUM.	1209	748			
38	NO VIS. TUM.	1231	232			
38	NO VIS. TUM.	1233	705			
38	NO VIS. TUM.	1284	619			
38	NO VIS. TUM.	1289	233			
38	NO VIS. TUM.	1299	592			
39	NO VIS. TUM.	664	748			
39	NO VIS. TUM.	693	524			
39	NO VIS. TUM.	713	512			
39	NO VIS. TUM.	722	513			
39	NO VIS. TUM.	729	748			
39	NO VIS. TUM.	749	512			
39	NO VIS. TUM.	761	309			
39		762	748	1	589	OTHER
39	NO VIS. TUM.	766	503			
39	NO VIS. TUM.	809	601			
39	NO VIS. TUM.	841	447			
39	NO VIS. TUM.	856	748			
39	NO VIS. TUM.	880	531			
39	NO VIS. TUM.	882	748			
39	NO VIS. TUM.	921	748			
39	NO VIS. TUM.	947	748			
39	NO VIS. TUM.	957	232			
39	NO VIS. TUM.	958	496			
39	NO VIS. TUM.	984	748			
39	NO VIS. TUM.	998	500			
39	NO VIS. TUM.	1028	604			
39	NO VIS. TUM.	1062	513			
39	NO VIS. TUM.	1079	90			
39	NO VIS. TUM.	1088	504			
39	NO VIS. TUM.	1103	235			
39	NO VIS. TUM.	1164	509			
39	NO VIS. TUM.	1258	237			
39	NO VIS. TUM.	1266	495			
39	NO VIS. TUM.	1293	525			
39	NO VIS. TUM.	1295	509			
40	NO VIS. TUM.	699	210			
40	NO VIS. TUM.	707	391			
40	NO VIS. TUM.	738	391			
40	NO VIS. TUM.	772	504			
40	NO VIS. TUM.	790	162			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
40	NO VIS. TUM.	860	230			
40	NO VIS. TUM.	862	210			
40	NO VIS. TUM.	909	677			
40	NO VIS. TUM.	950	236			
40	NO VIS. TUM.	956	391			
40	NO VIS. TUM.	959	234			
40	NO VIS. TUM.	968	563			
40	NO VIS. TUM.	983	181			
40	NO VIS. TUM.	990	728			
40	NO VIS. TUM.	1003	235			
40	NO VIS. TUM.	1029	563			
40	NO VIS. TUM.	1041	210			
40	NO VIS. TUM.	1095	728			
40	NO VIS. TUM.	1122	210			
40	NO VIS. TUM.	1127	235			
40	NO VIS. TUM.	1132	563			
40	NO VIS. TUM.	1140	563			
40	NO VIS. TUM.	1177	391			
40	NO VIS. TUM.	1190	563			
40	NO VIS. TUM.	1194	513			
40	NO VIS. TUM.	1225	210			
40	NO VIS. TUM.	1230	235			
40	NO VIS. TUM.	1259	181			
40	NO VIS. TUM.	1265	391			
40	NO VIS. TUM.	1271	233			
41		1321	706	1	596	PAP
41		1321	706	2	596	PAP
41		1321	706	3	609	PAP
41		1322	736	1	629	CAR
41		1327	553	1	503	PAP
41		1330	650	1	643	PAP
41		1330	650	2	650	HYPER
41	NO VIS. TUM.	1331	636			
41		1332	581	1	405	CAR
41		1336	411	1	293	HYPER
41		1339	161	1	63	HYPER
41		1343	450	1	293	CAR
41		1343	450	2	447	CAR
41		1344	519	1	405	CAR
41		1345	163	1	55	OTHER
41		1348	444	1	286	PAP
41		1348	444	2	377	PAP
41		1349	554	1	321	SAR
41		1349	554	2	531	PAP
41		1353	736	1	359	FCYST
41		1354	512	1	359	OTHER
41		1358	653	1	482	HYPER
41		1363	557	1	489	CAR
41		1363	557	2	503	PAP
41		1363	557	3	510	PAP

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
41		1363	557	4	517	CAR
41		1363	557	5	531	PAP
41		1364	617	1	314	CAR
41		1364	617	2	370	PAP
41		1364	617	3	482	PAP
41		1364	617	4	482	PAP
41		1364	617	5	482	PAP
41		1364	617	6	602	HYPER
41		1367	576	1	489	CAR
41		1367	576	2	576	PAP
41		1369	574	1	377	CAR
41		1369	574	2	482	PAP
41		1369	574	3	568	PAP
41		1370	630	1	574	CAR
41		1370	630	2	609	PAP
41		1371	596	1	359	KER
41		1371	596	2	538	HYPER
41		1372	646	1	596	CAR
41		1373	736	1	736	HYPER
41		1373	736	2	736	HYPER
41		1374	626	1	426	CAR
41	NO VIS. TUM.	1375	380			
41		1376	592	1	510	CAR
41		1376	592	2	574	HYPER
41		1380	736	1	727	PAP
41		1381	674	1	454	CAR
41		1381	674	2	517	CAR
41		1381	674	3	553	PAP
41	NO VIS. TUM.	1383	736			
42	NO VIS. TUM.	1323	737			
42	NO VIS. TUM.	1324	78			
42	NO VIS. TUM.	1325	737			
42	NO VIS. TUM.	1326	737			
42	NO VIS. TUM.	1328	627			
42	NO VIS. TUM.	1329	737			
42	NO VIS. TUM.	1334	737			
42	NO VIS. TUM.	1337	181			
42	NO VIS. TUM.	1338	737			
42	NO VIS. TUM.	1340	706			
42	NO VIS. TUM.	1341	737			
42	NO VIS. TUM.	1342	737			
42	NO VIS. TUM.	1346	737			
42	NO VIS. TUM.	1347	737			
42	NO VIS. TUM.	1350	737			
42	NO VIS. TUM.	1352	737			
42	NO VIS. TUM.	1355	737			
42	NO VIS. TUM.	1356	483			
42	NO VIS. TUM.	1357	737			
42	NO VIS. TUM.	1359	461			
42	NO VIS. TUM.	1360	700			

Group code	Skin appearance	Animal code	Death/sac. day	Slide number	First appearance	Tumor diagnosis
42	NO VIS. TUM.	1361	737			
42	NO VIS. TUM.	1362	737			
42	NO VIS. TUM.	1365	622			
42	NO VIS. TUM.	1366	703			
42	NO VIS. TUM.	1368	737			
42	NO VIS. TUM.	1378	672			
42	NO VIS. TUM.	1379	727			
42	NO VIS. TUM.	1382	737			
42		1384	616	1	538	SAR

[a] Study day when abnormal skin first observed.
[b] CAR - carcinoma
PAP - papilloma
FCYST - follicular cyst
HYPER - hyperplasia/ hyperkeratosis
KER - keratoacanthoma
SAR - sarcoma
FIB - fibroma
NORM - normal (no lesion)
OTHER - mast cells and/or sebaceous cells (no neoplasm)

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15. Supplementary Notes

16. Abstract (Limit: 200 words) The carcinogenicity for mouse skin of condensed volatiles of heated asphalt (8052424) was evaluated. The possibility that either cocarcinogenic or tumor promoting activity existed in any of the chemical fractions which would account for the carcinogenic activity was investigated and an attempt was also made to identify which sets of chemical entities in the several fractions gave rise to a carcinogenic response. Volatiles from heated asphalt were collected and divided into five fractions. Fractions were applied to the skin of male C3H/HeJ-mice or Sencar-mice twice weekly for 104 weeks in proportion to the amount of the fraction in asphalt fumes. Direct carcinogenic activity was limited to two fractions: olefins, which contained alkylated aryl thiophenes and alkylated phenanthrenes; and alkylated phenylethanones and alkylated difuranones. Synergism was not observed by treatment with combined fractions. The authors conclude that, if these fractions can be further subdivided and studied, it may be possible to identify specific compounds and take more active steps toward limiting exposure at the worksite.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms NIOSH-Publication, NIOSH-Contract, Contract-200-83-2612, Laboratory-animals, Asphalt-fumes, Skin-exposure, Carcinogens, Carcinogenesis, Hydrocarbons

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