

INTERIM REPORT
API RESEARCH PROJECT MC-1

November 1, 1956



THE KETTERING LABORATORY
in the
Department of Preventive Medicine and Industrial Health
College of Medicine
UNIVERSITY OF CINCINNATI, CINCINNATI, OHIO

EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

LEGEND FOR DATA SHEETS ON INDIVIDUAL EXPERIMENTS

Symbols used in connection with average weight curves:

- ∇ Time of death (from disease) of i th tumor-free mouse.
 i Time of death (from disease) of i th tumor-bearing mouse.
 ∇ or i Time mouse killed.
 ~~$\times$~~ ~~Mouse missing from cage.~~

Gross and microscopic pathology:

- $\square$ Time of appearance of first papilloma in i th mouse.
When this symbol is first used for a given mouse after its death, the presence of non-invading carcinoma (intra-epithelial) or small areas of benign neoplasm was determinable only by histopathology.
- $\square$ Time of appearance of papilloma which regressed later or was not confirmed by histopathology.
- $\odot$ Time of appearance of gross changes indicative of malignancy in tumor of i th mouse. Diagnosis confirmed except in cases where no tissue section available. When this symbol is first used for a given mouse after its death, the invasive malignancy of the tumor was determinable only by histopathology.
- $\odot$ Gross diagnosis of malignancy of tumor not confirmed by histopathology.
- $\square$ or $\odot$ No tissue section available for histopathology because of extensive post-mortem decomposition or cannibalism.
- ~~$\times$~~ ~~Tumors not recorded during this week.~~
- * Painting discontinued for 1 week at each indicated time.

Cumulative dose-response curves (summation of data from all cages unless otherwise specified):

- $\cdot$ Experimental curve for benign papillomas.
- ~~$\odot$ Experimental curve for grossly malignant tumors.~~

FIGURE I

EXPERIMENT 220, SOLUTION OF METHYLCHOLANTHRENE, 1.034 PERCENT IN BENZENE

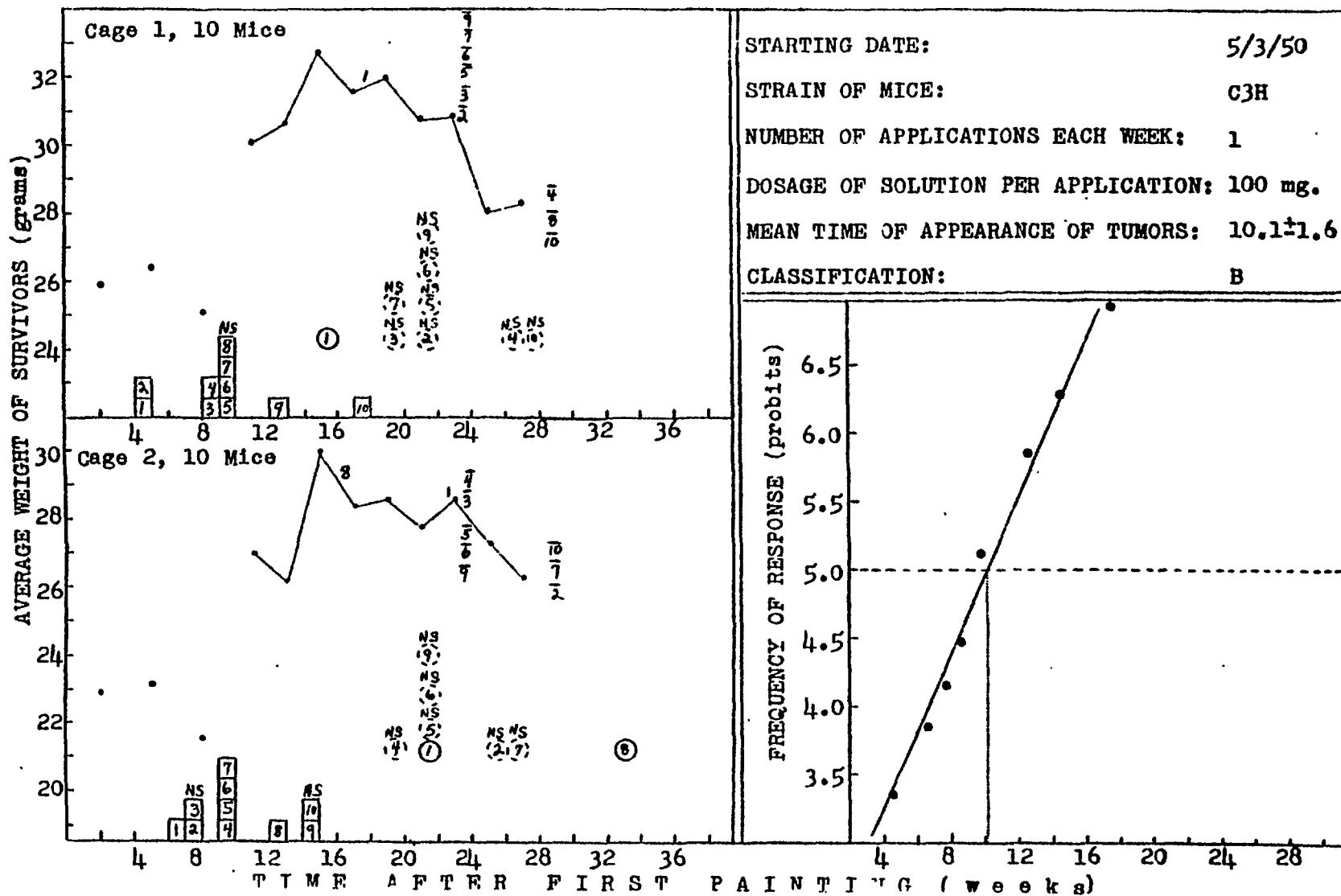


FIGURE II

EXPERIMENT 268, SOLUTION OF METHYLCHOLANTHRENE, 0.287 PERCENT IN BENZENE

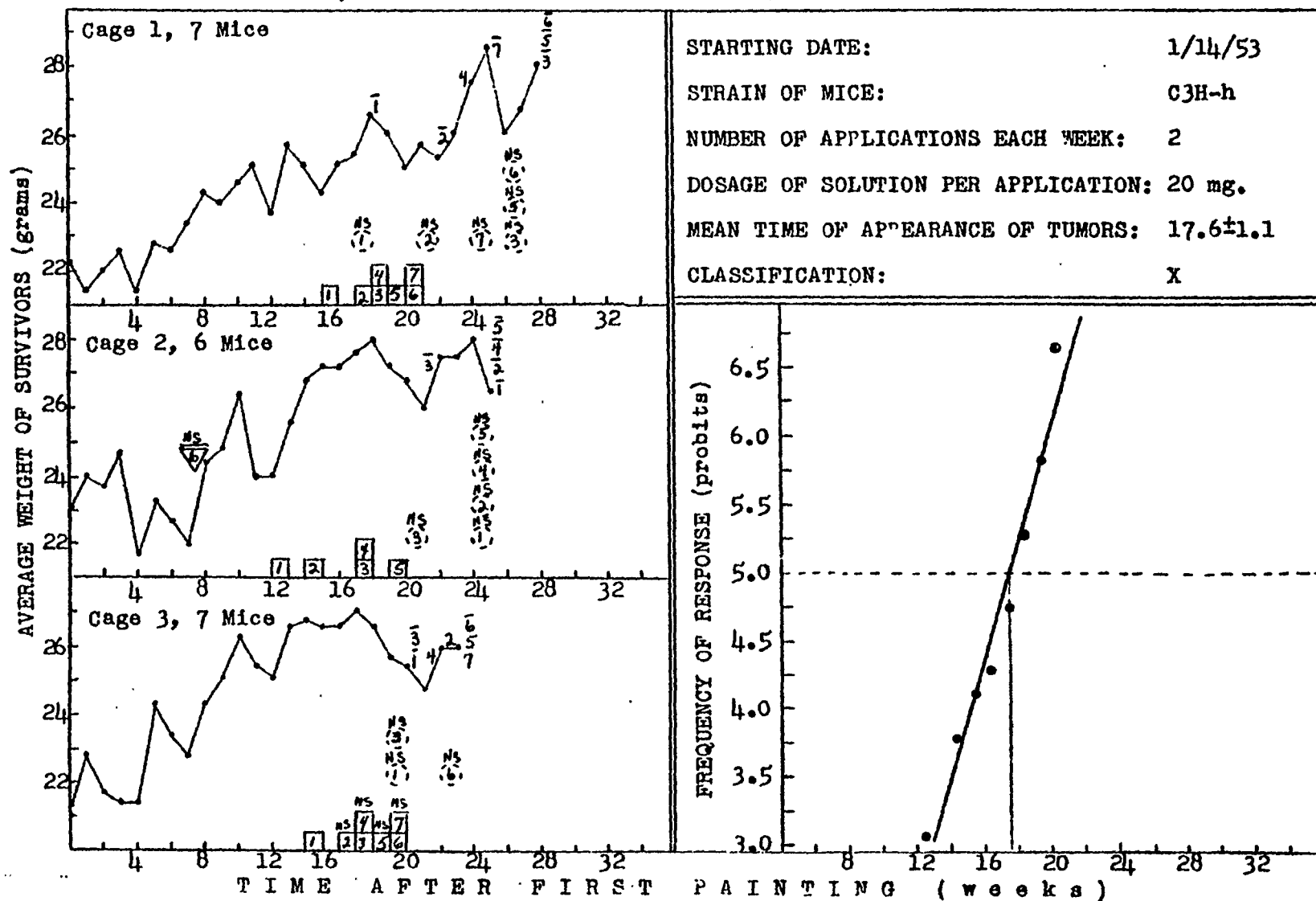


FIGURE III - TOTAL FEED TO DELAYED COKING UNIT

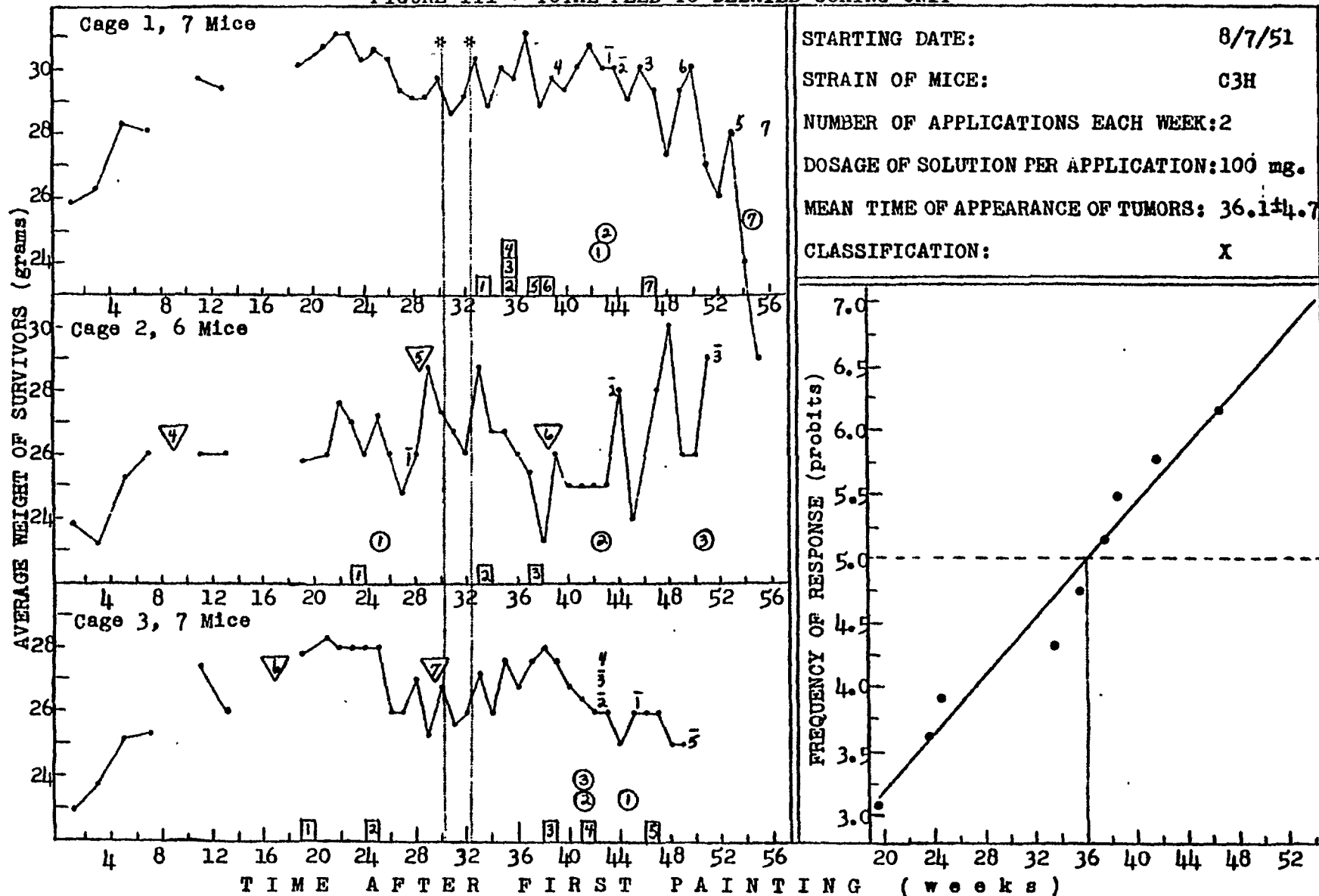


FIGURE IV

EXPERIMENT 243, SOLUTION OF METHYLCHOLANTHRENE, 0.15 PERCENT IN BENZENE

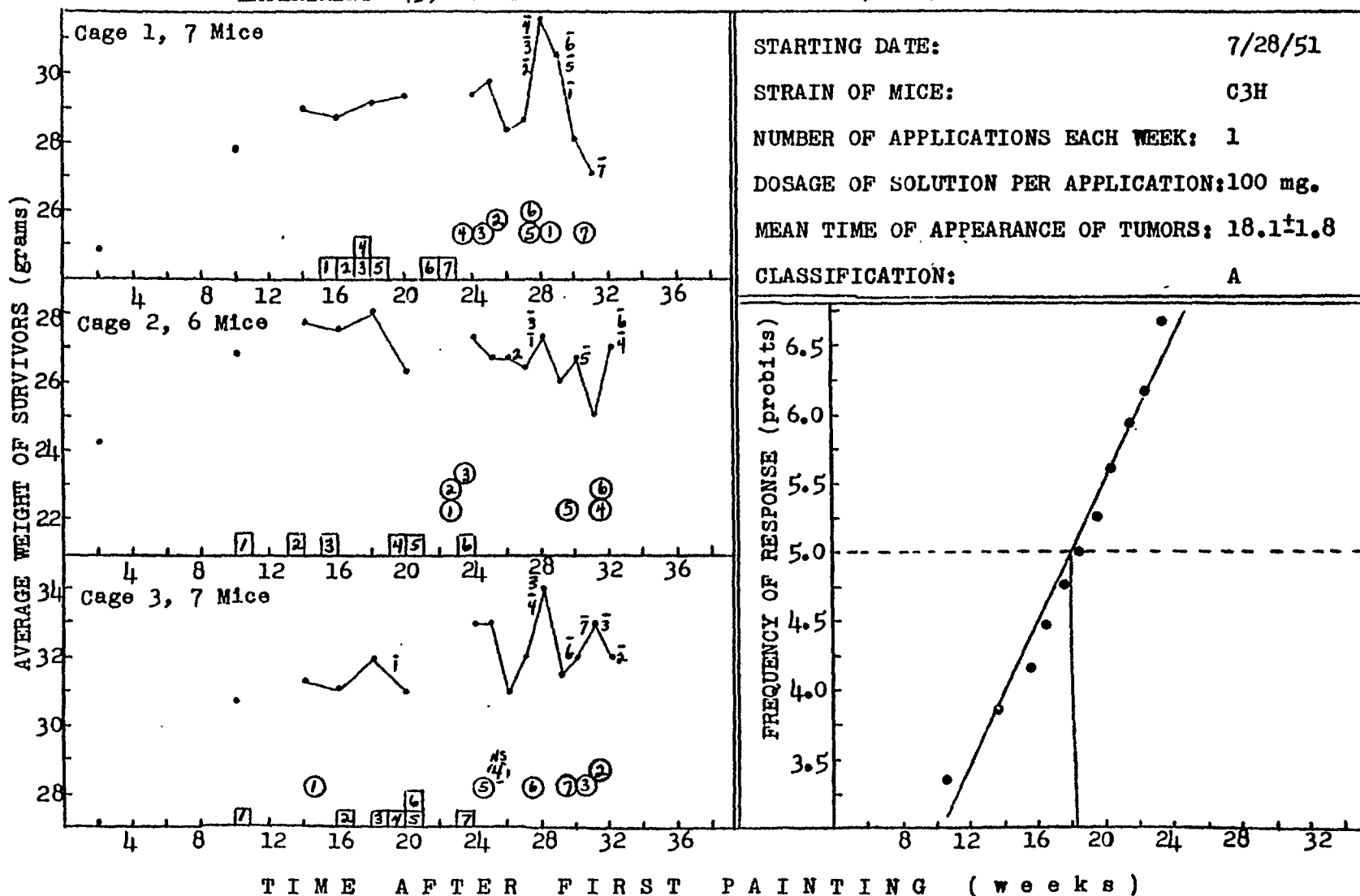
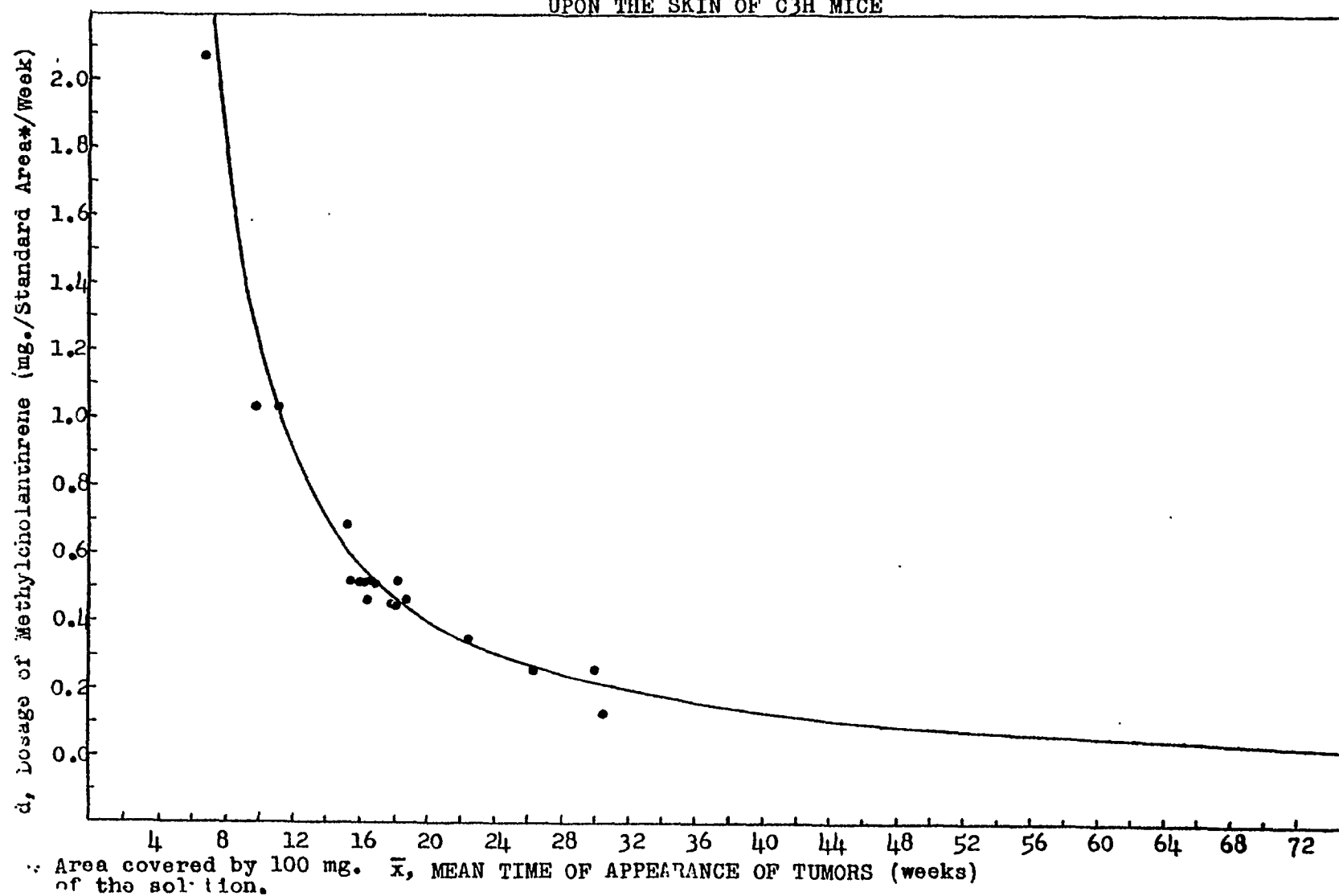


FIGURE V
EXPERIMENTS INVOLVING THE APPLICATION OF SOLUTIONS OF METHYLCHOLANTHRENE IN BENZENE
UPON THE SKIN OF C3H MICE



LEGEND FOR DATA SHEETS ON INDIVIDUAL EXPERIMENTS

Symbols used in connection with average weight curves:

- ▽ Time of death (from disease) of *i*th tumor-free mouse.
i Time of death (from disease) of *i*th tumor-bearing mouse.
▽ or $\overline{i}$ Time mouse killed.
▽ Mouse missing from cage.

Gross and microscopic pathology:

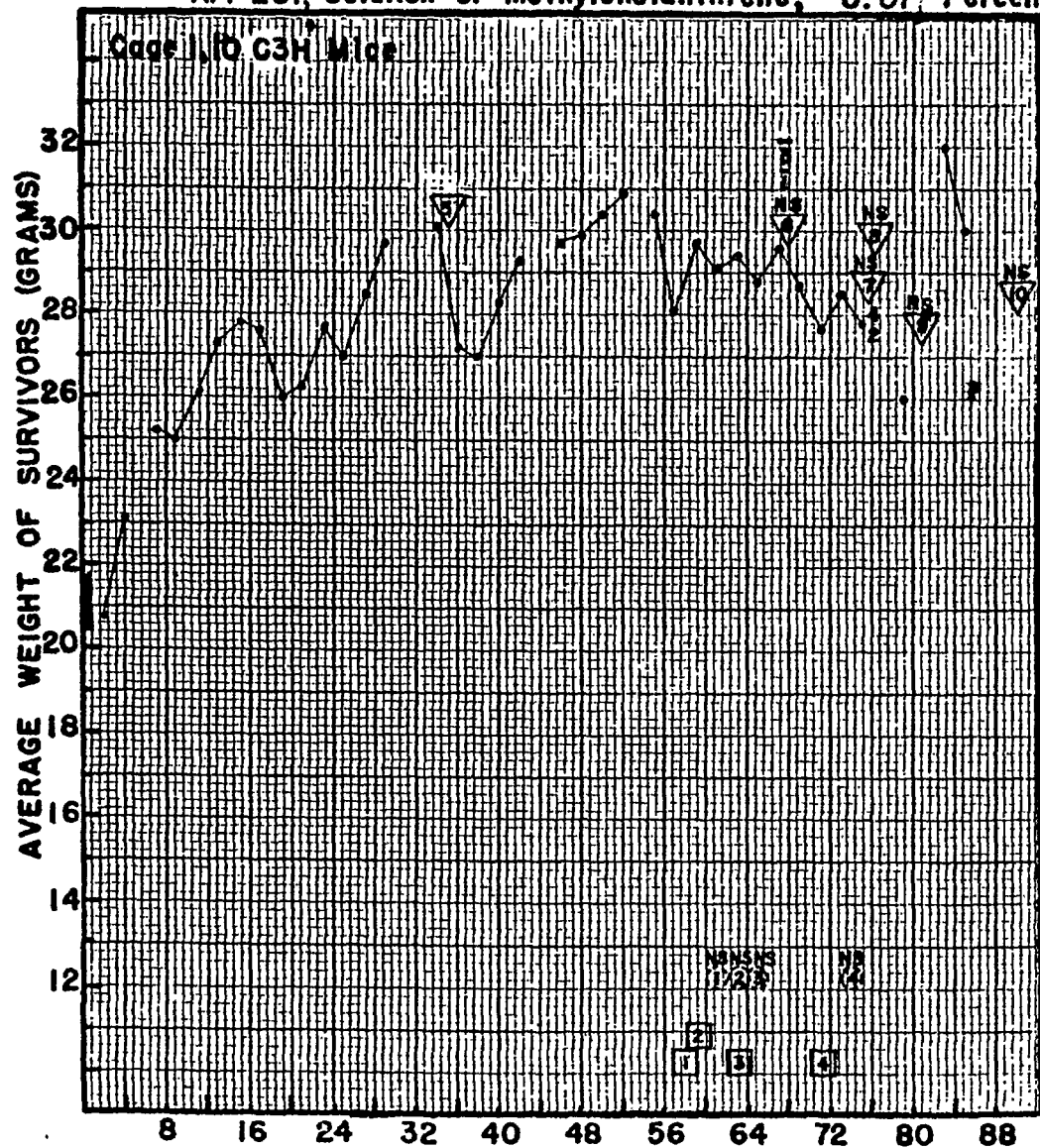
- [] Time of appearance of first papilloma in *i*th mouse. When symbol first used for a given mouse after its death, the presence of non-invading carcinoma (intra-epithelial) or small areas of benign neoplasm was determinable only by histopathology.
[] Time of appearance of papilloma which later regressed or was not confirmed by histopathology.
⊙ Time of appearance of gross changes indicative of malignancy in tumor of *i*th mouse. Unless otherwise indicated, diagnosis confirmed by microscopic examination of tissue sections. When symbol first used for a given mouse after its death, the invasive malignancy of the tumor was determinable only by histopathology.
⊙ Gross diagnosis of malignancy of tumor not confirmed by histopathology.
 $\overline{t}$ or $\overline{t}^{ns}$ No tissue section available for histopathology.

Cumulative dose-response curves (summation of data from all cages unless unless otherwise specified):

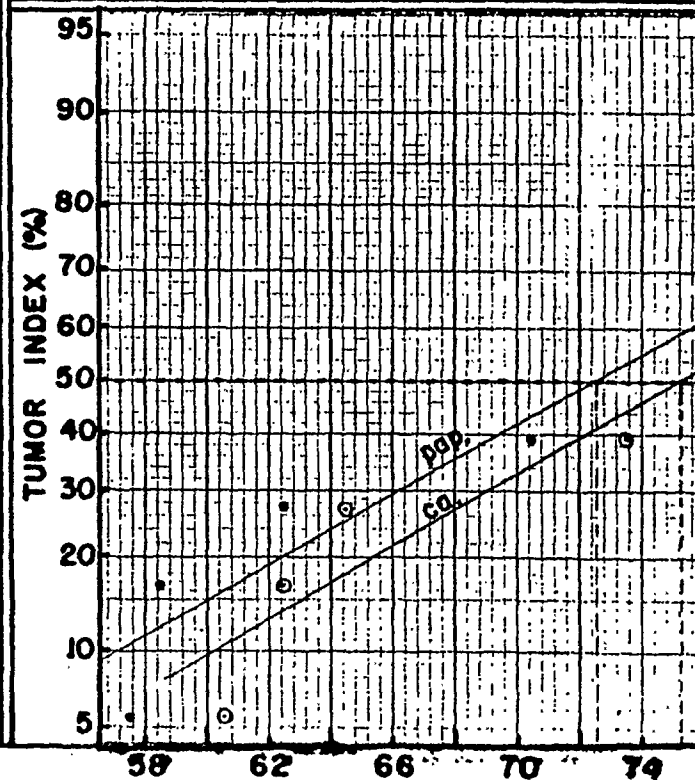
- — — — — • Benign papillomas.
⊙ — — — — ⊙ Grossly malignant tumors.

EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-201, Solution of Methylcholanthrene, 0.01 Percent by Weight in Benzene



STARTING DATE: 7-20-49
 STRAIN OF MICE: C3H
 NUMBER OF APPLICATIONS PER WEEK: 3
 DOSE PER APPLICATION: 100 mg.
 RELATIVE CARCINOGENIC POTENCY, $P_{MC} 0.01X$

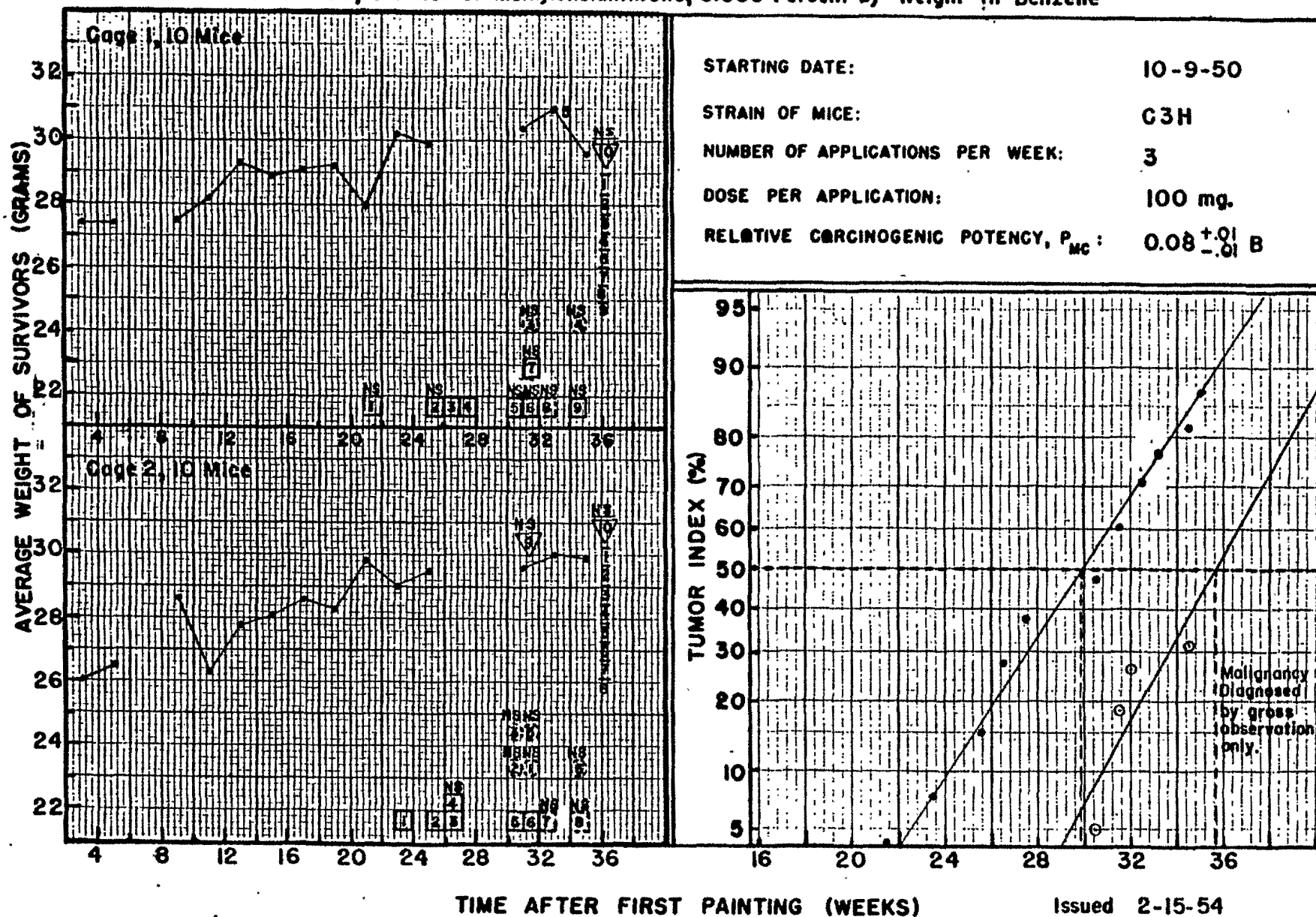


TIME AFTER FIRST PAINTING (WEEKS)

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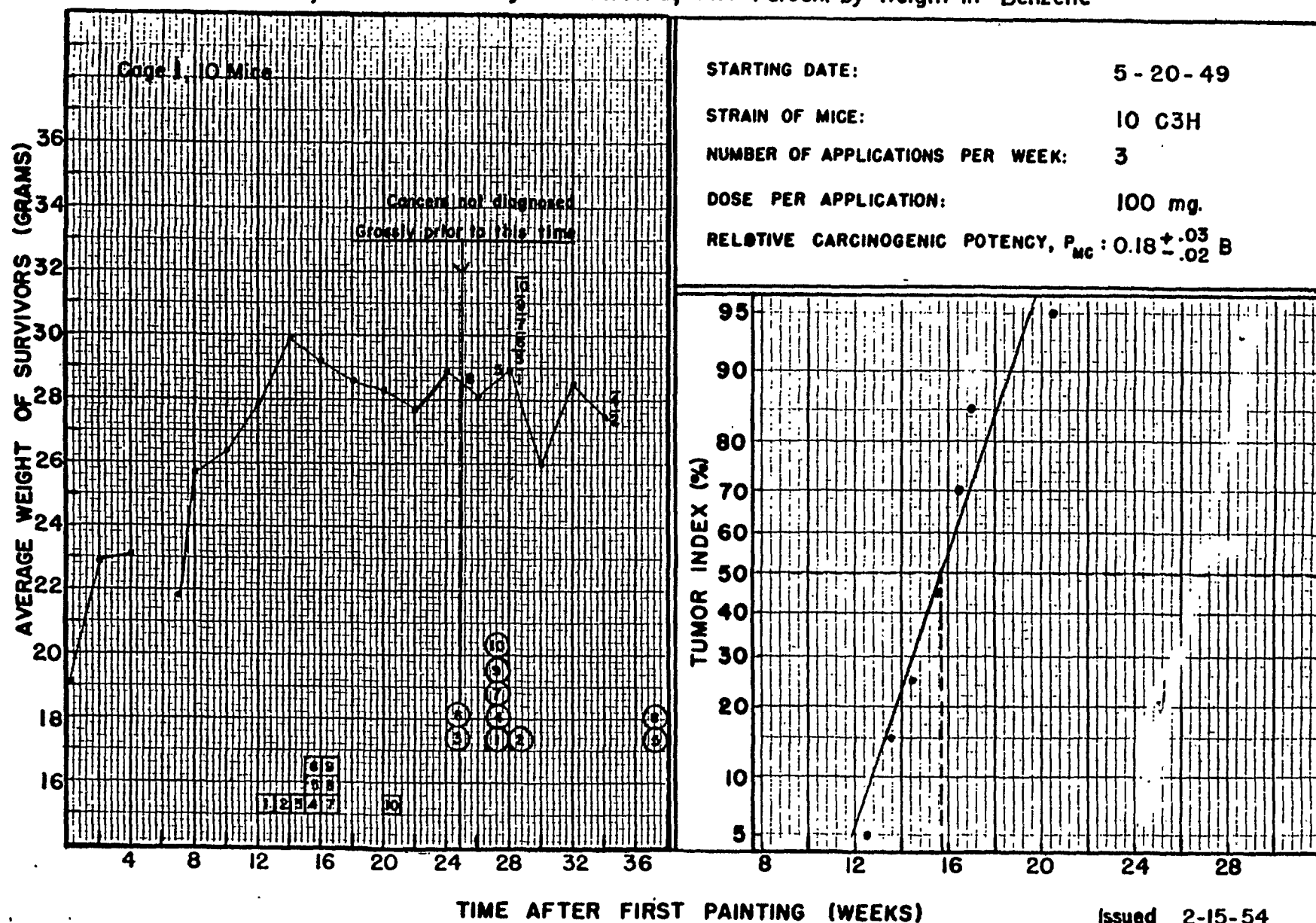
EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API - 203, Solution of Methylcholanthrene, 0.086 Percent by Weight in Benzene



EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

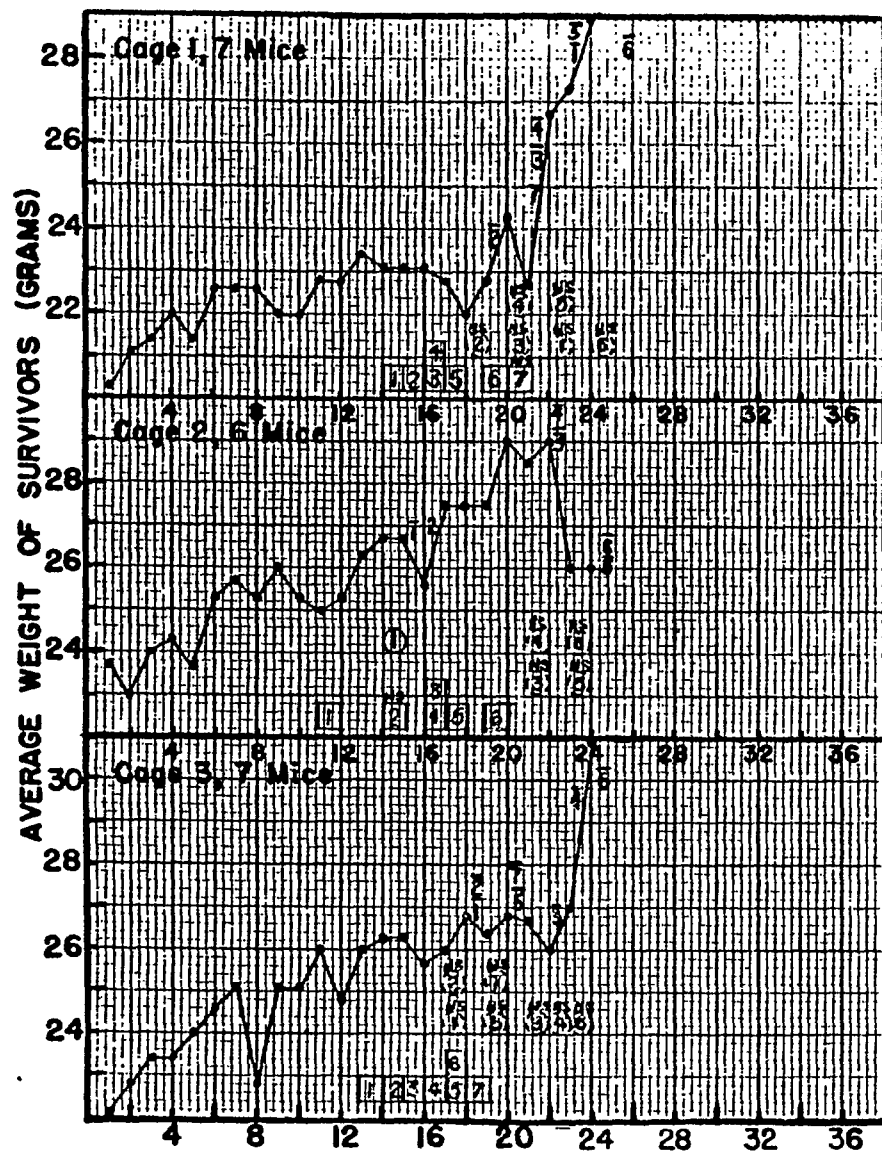
API-204, Solution of Methylcholanthrene, 0.17 Percent by Weight in Benzene



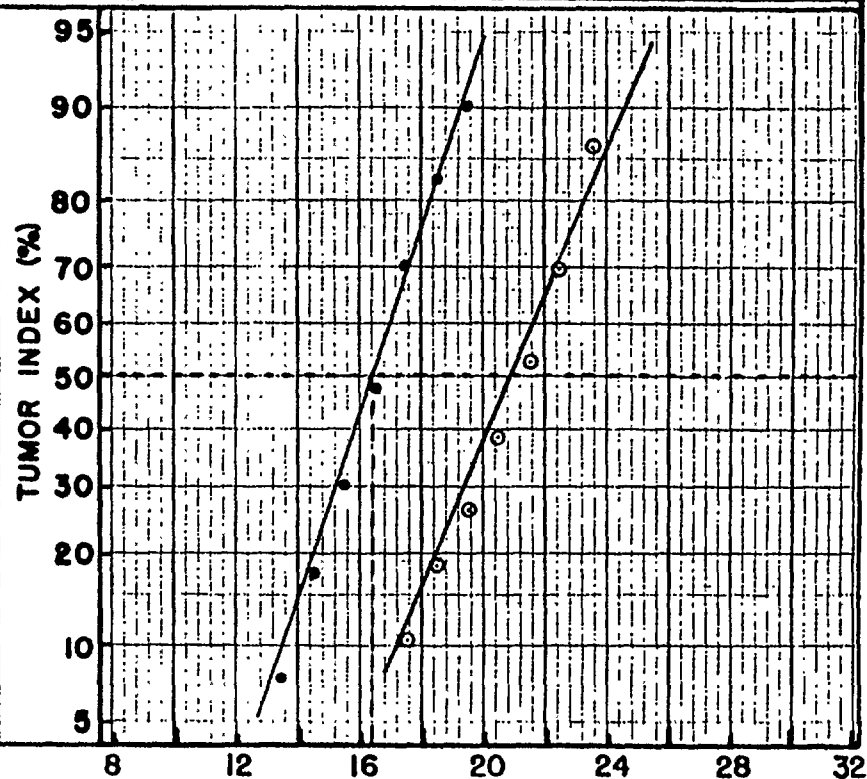
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EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-204, Solution of Methylcholanthrene, 0.17 Percent by Weight in Benzene



STARTING DATE: 10-4-52
 STRAIN OF MICE: 20 C3H-h
 NUMBER OF APPLICATIONS PER WEEK: 3
 DOSE PER APPLICATION: 20 mg.
 RELATIVE CARCINOGENIC POTENCY, $P_{MC} : 0.17 \pm 0.01 B$

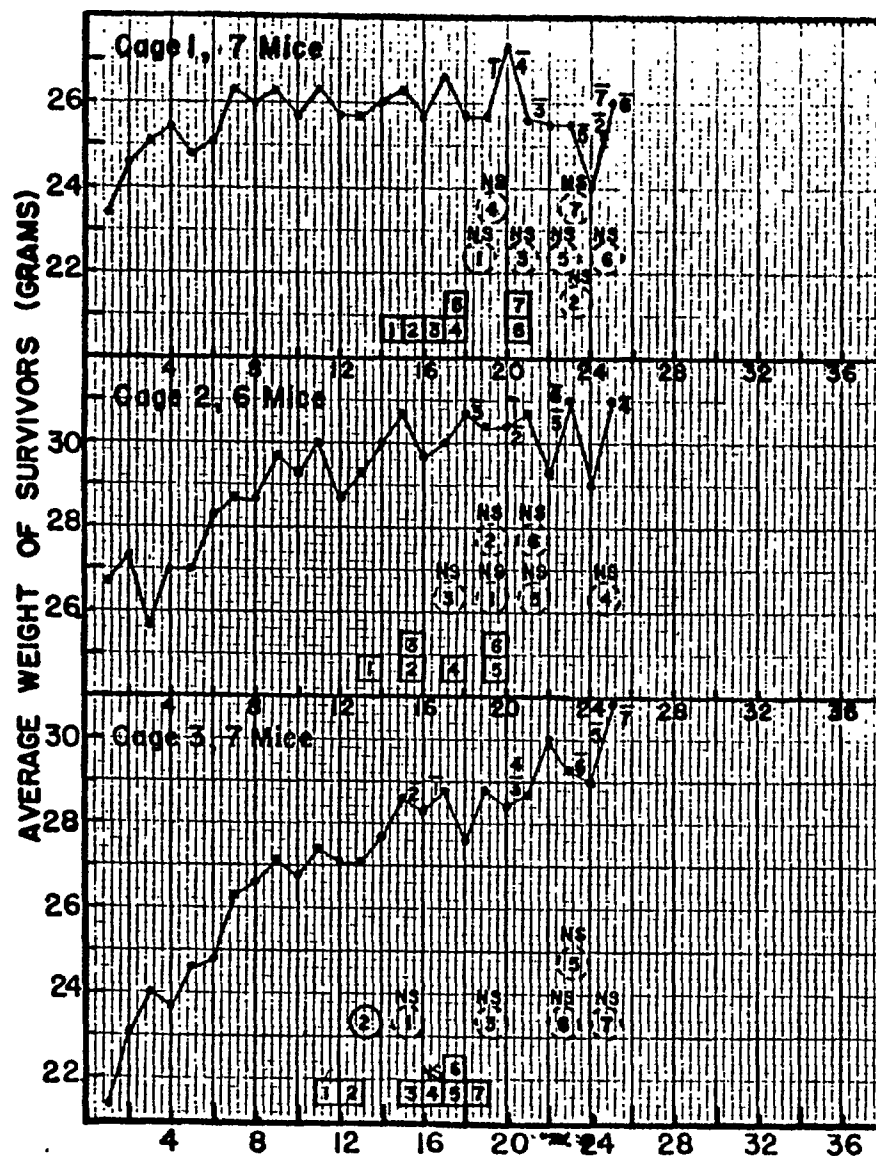


TIME AFTER FIRST PAINTING (WEEKS)

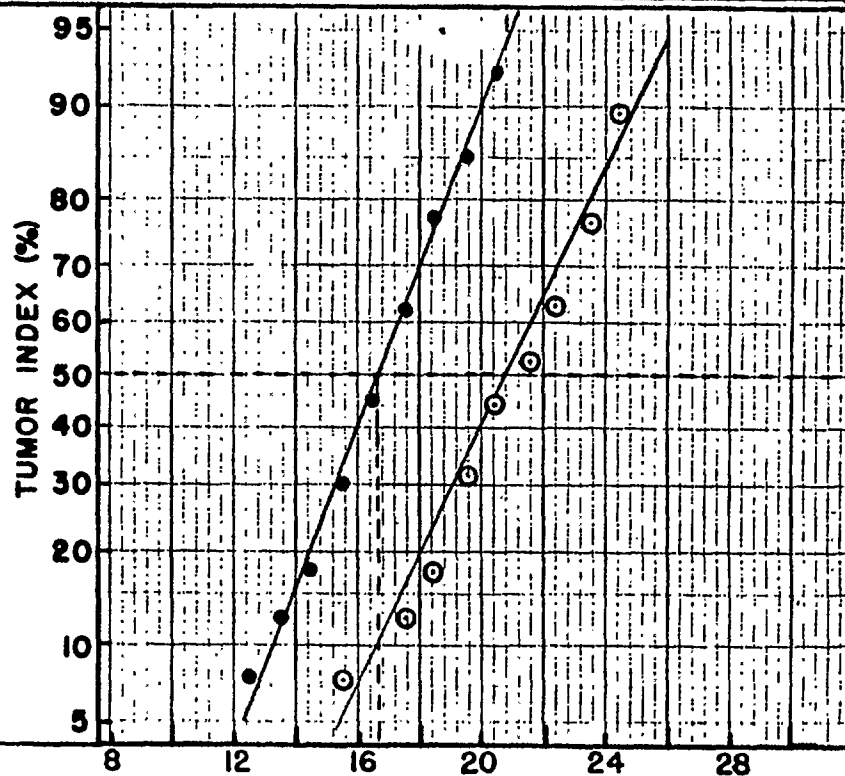
Issued 2-15-54

EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-204, Solution of Methylcholanthrene, 0.17 Percent by Weight in Benzene



STARTING DATE: 10-4-52
 STRAIN OF MICE: 20 C3H-h
 NUMBER OF APPLICATIONS PER WEEK: 3
 DOSE PER APPLICATION: 20 mg.
 RELATIVE CARCINOGENIC POTENCY, $P_{MC} : 0.17^{+0.01}_{-0.02} B$

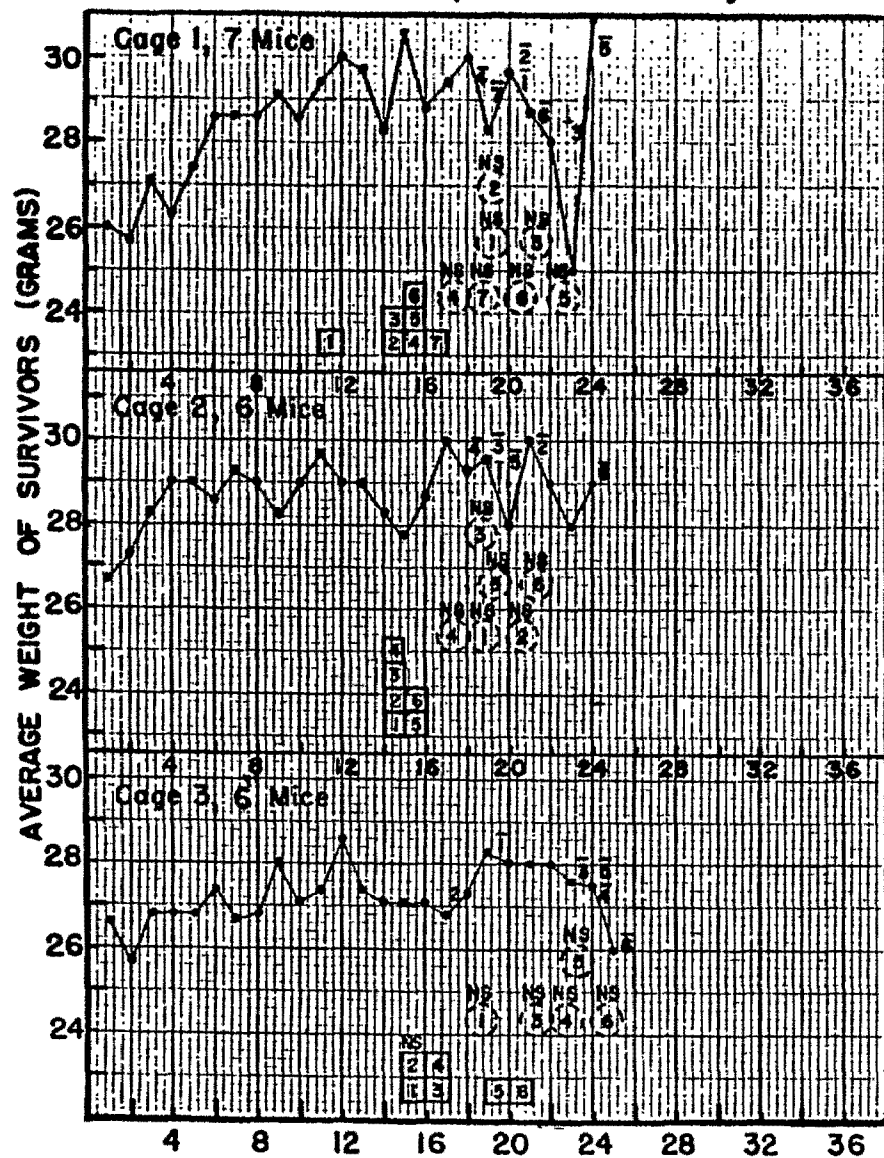


TIME AFTER FIRST PAINTING (WEEKS)

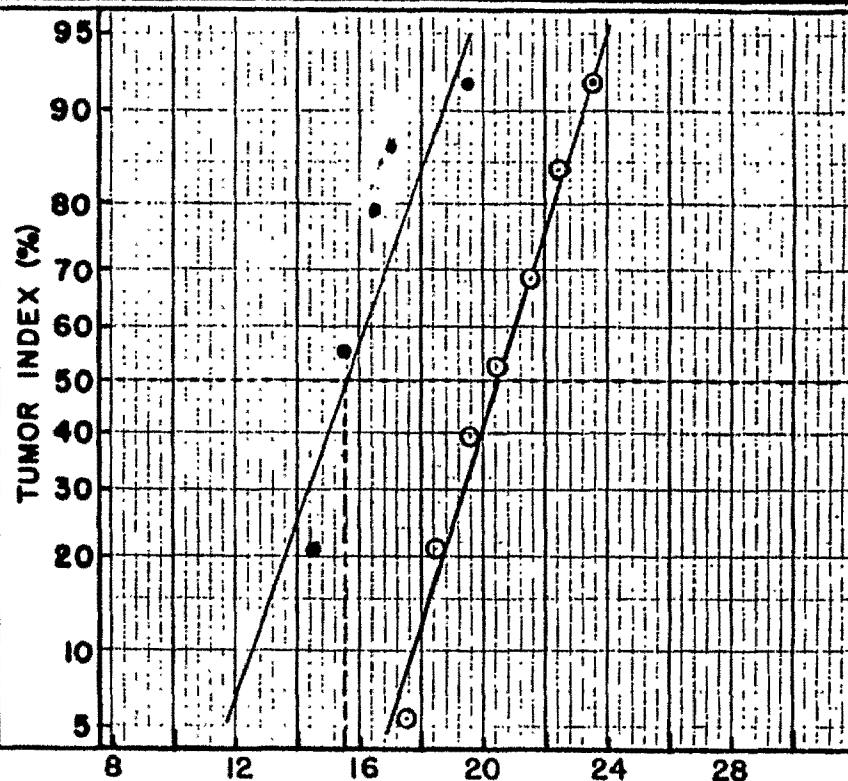
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EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-204, Solution of Methylcholanthrene, 0.17 Percent by Weight in Benzene



STARTING DATE: 10-28-52
 STRAIN OF MICE: 19 C3H-h
 NUMBER OF APPLICATIONS PER WEEK: 3
 DOSE PER APPLICATION: 20 mg.
 RELATIVE CARCINOGENIC POTENCY, $P_{Mc} : 0.18^{+0.02}_{-0.01} B$

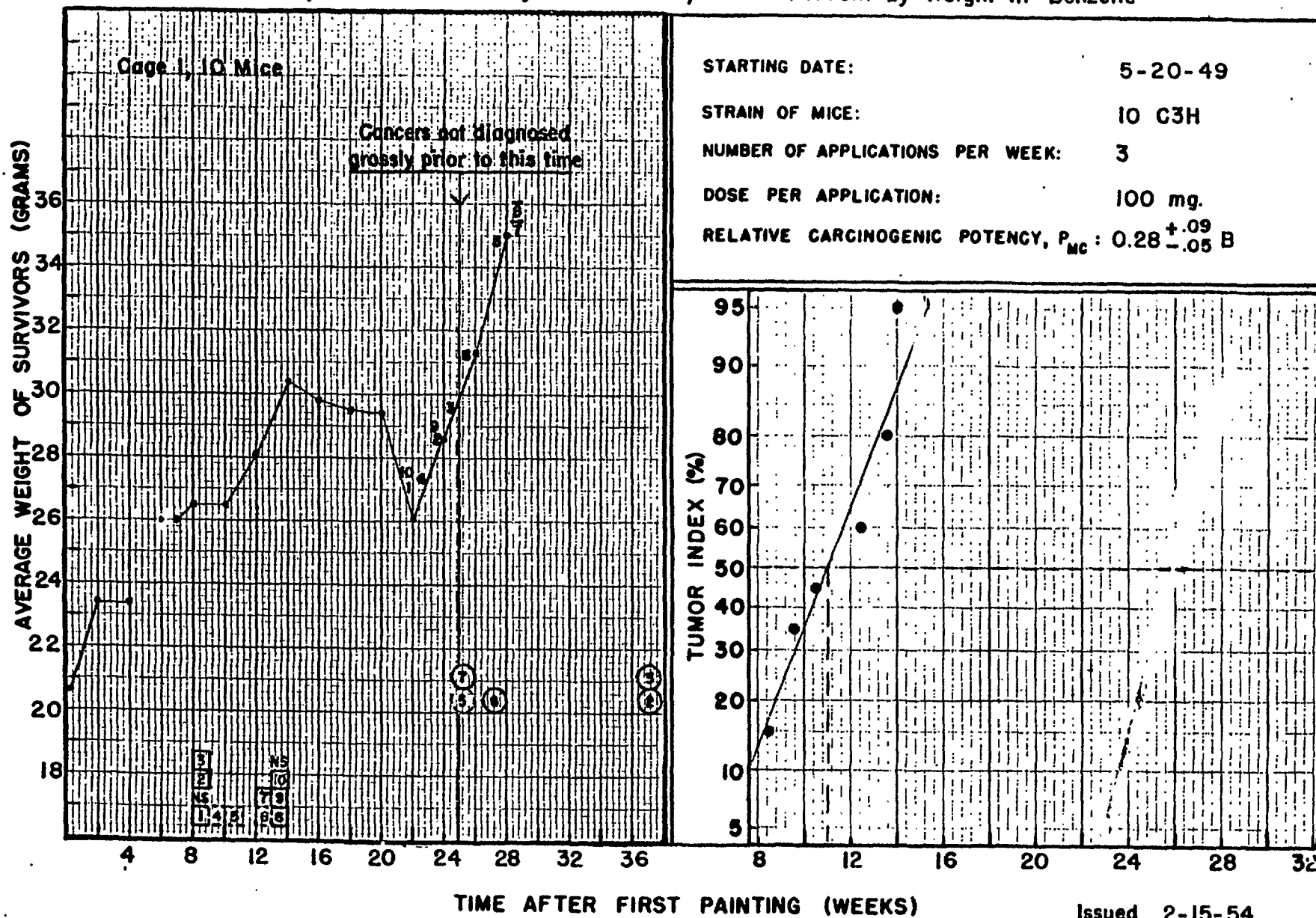


TIME AFTER FIRST PAINTING (WEEKS)

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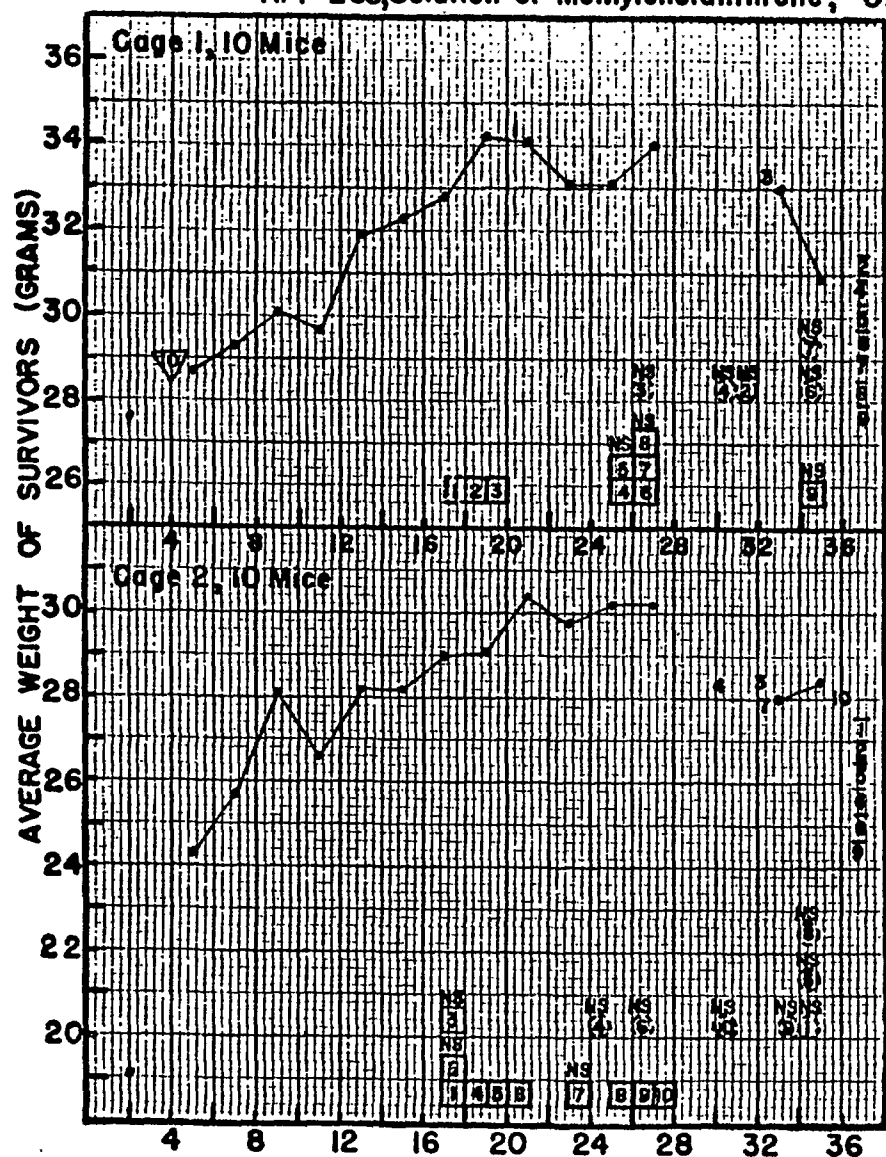
EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-205, Solution of Methylcholanthrene, 0.345 Percent by Weight in Benzene



EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-205, Solution of Methylcholanthrene, 0.345 Percent by Weight in Benzene



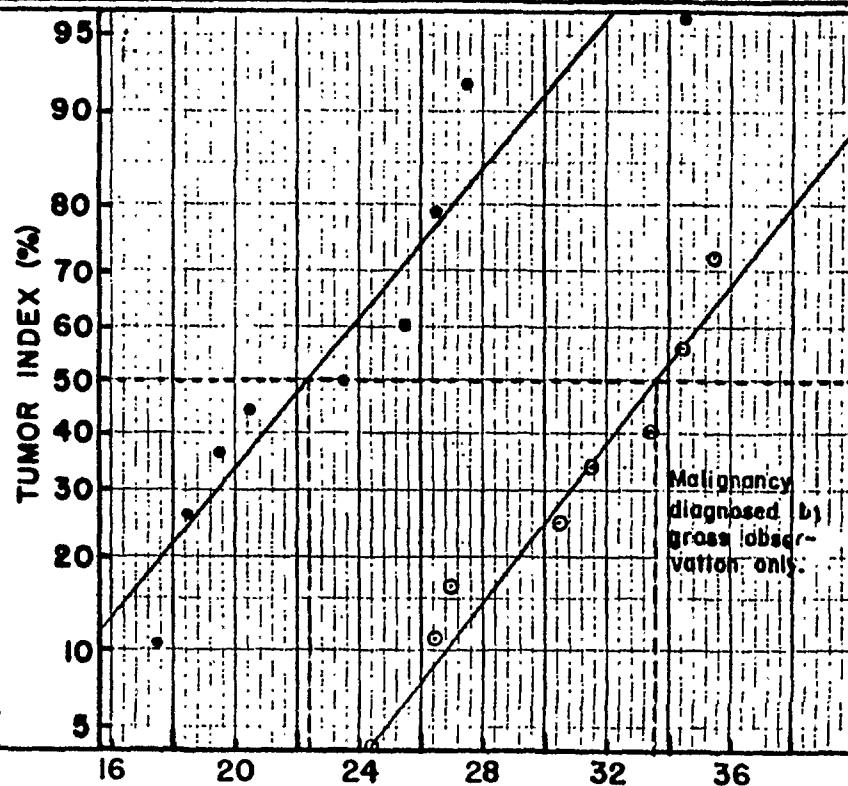
STARTING DATE: 6-13-50

STRAIN OF MICE: C3H

NUMBER OF APPLICATIONS PER WEEK: 1

DOSE PER APPLICATION: 100 mg.

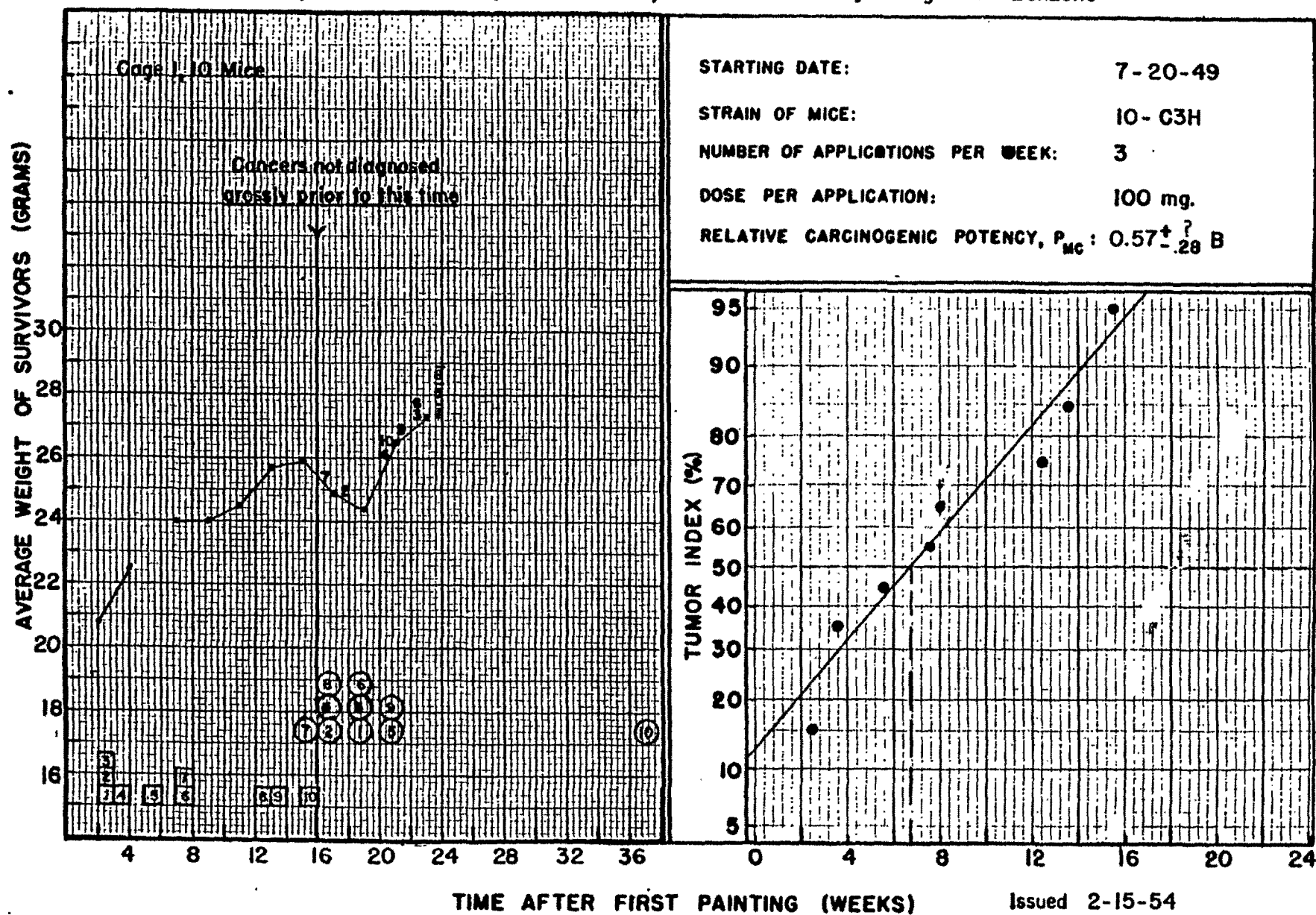
RELATIVE CARCINOGENIC POTENCY, P_{MC} : $0.34^{+0.06}_{-0.05}$ B



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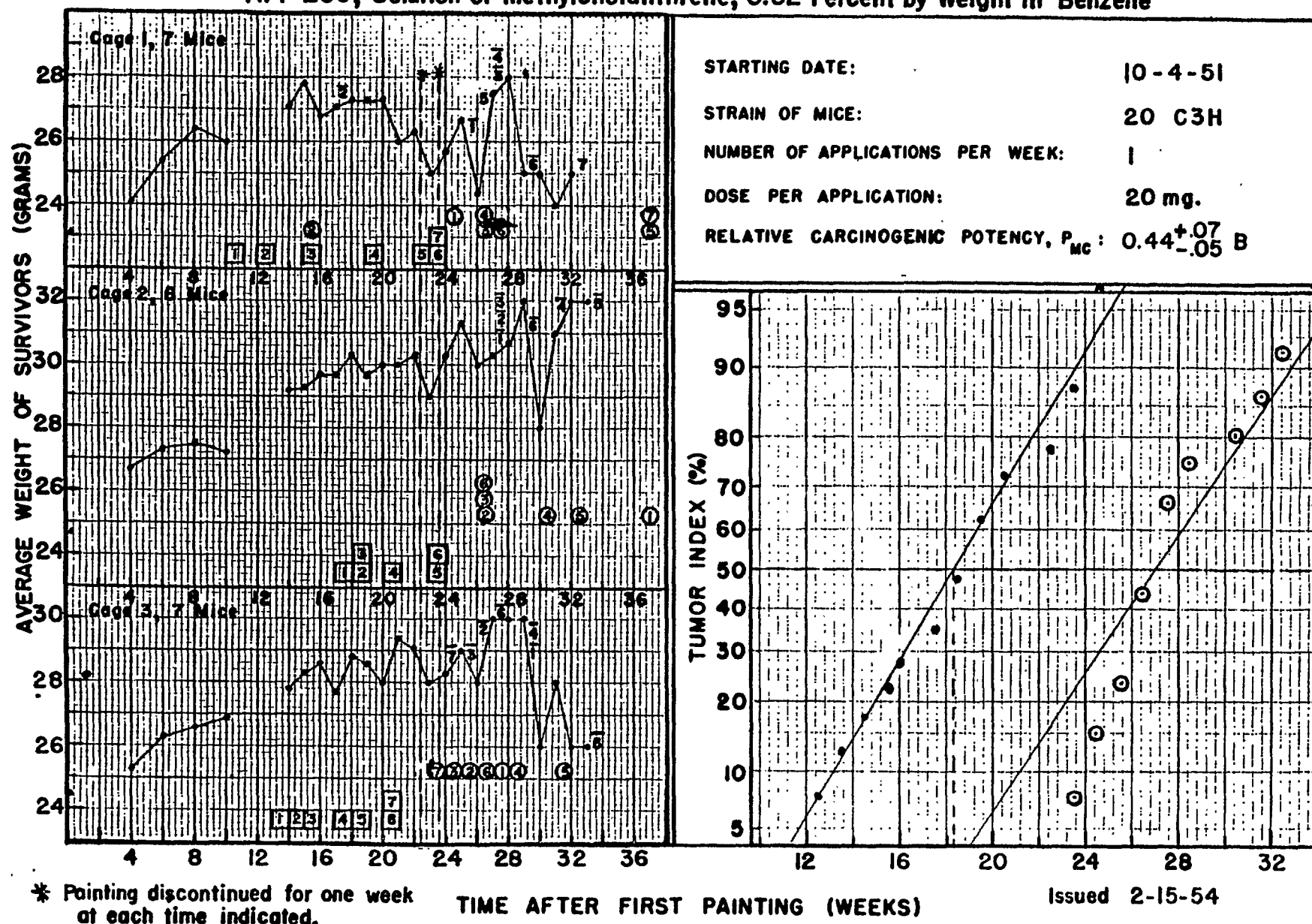
EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-206, Solution of Methylcholanthrene, 0.69 Percent by Weight in Benzene



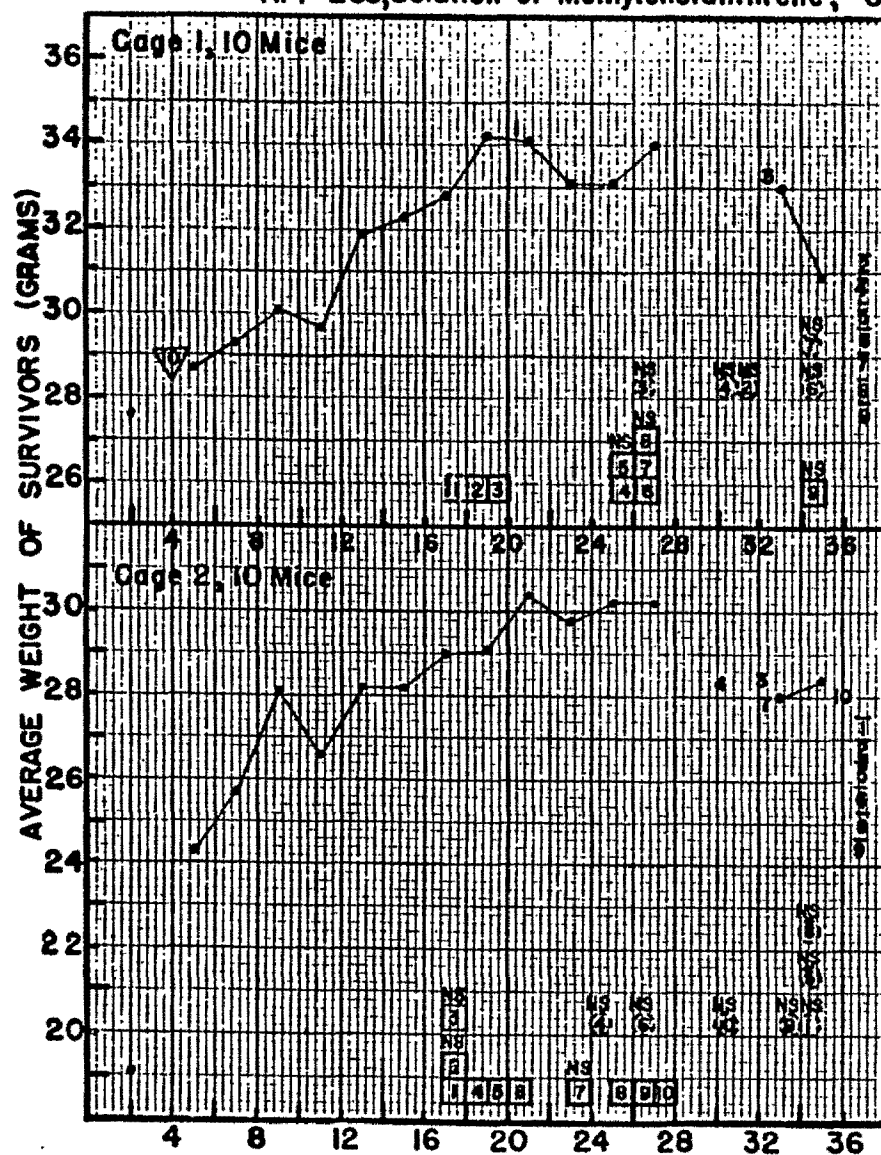
EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-209, Solution of Methylcholanthrene, 0.52 Percent by Weight in Benzene

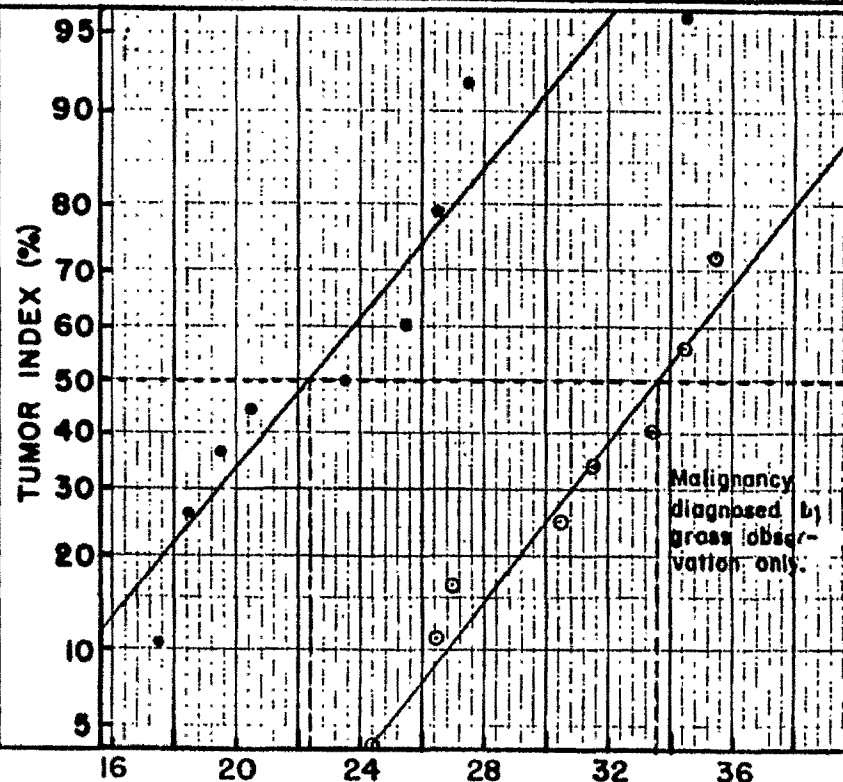


EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-205, Solution of Methylcholanthrene, 0.345 Percent by Weight in Benzene



STARTING DATE: 6-13-50
 STRAIN OF MICE: C3H
 NUMBER OF APPLICATIONS PER WEEK: 1
 DOSE PER APPLICATION: 100 mg.
 RELATIVE CARCINOGENIC POTENCY, P_{MC} : $0.34^{+.06}_{-.05}$ B

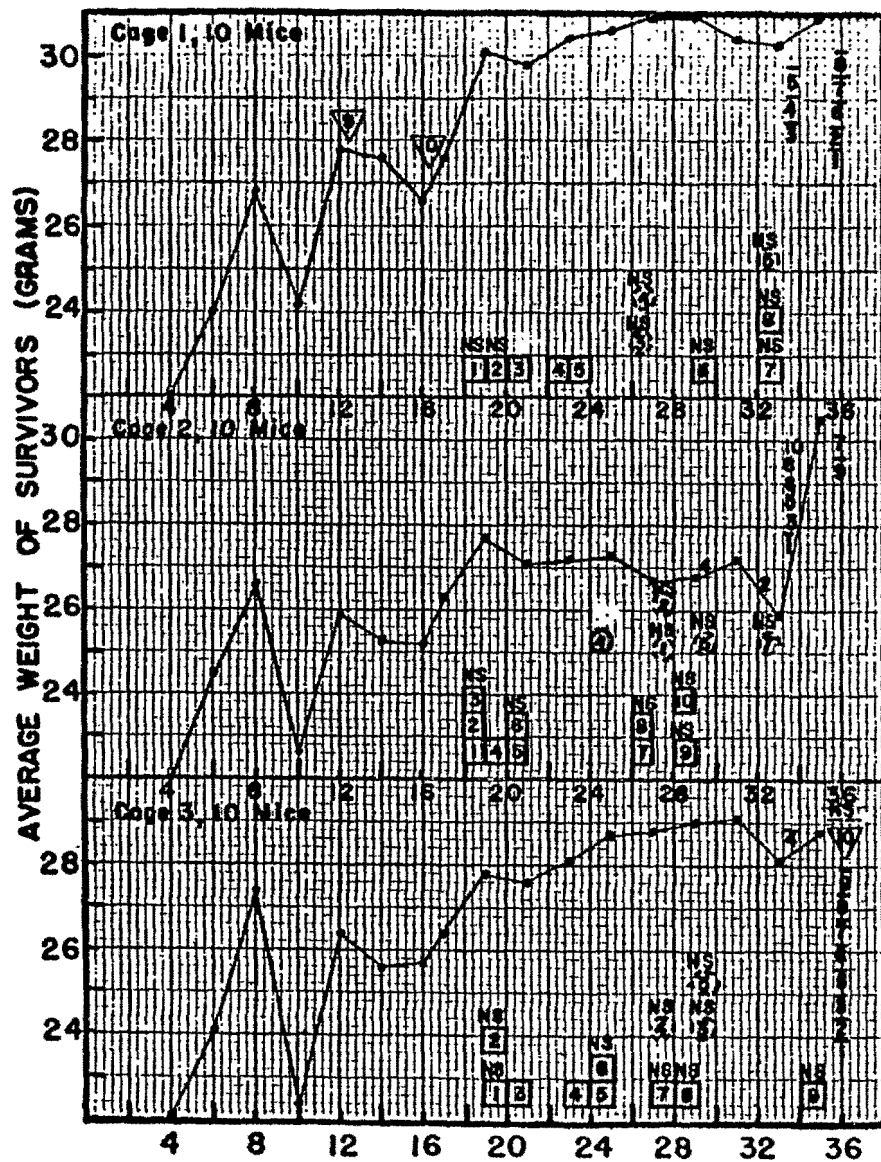


TIME AFTER FIRST PAINTING (WEEKS)

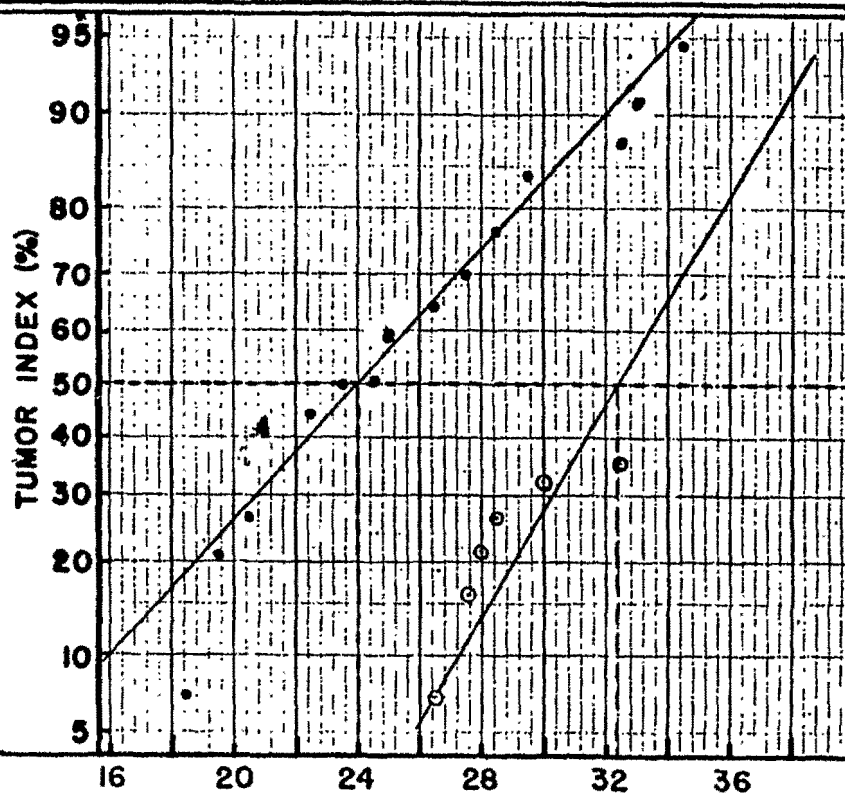
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EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-219, Solution of Methycholanthrene, 0.115 Percent by Weight in Benzene



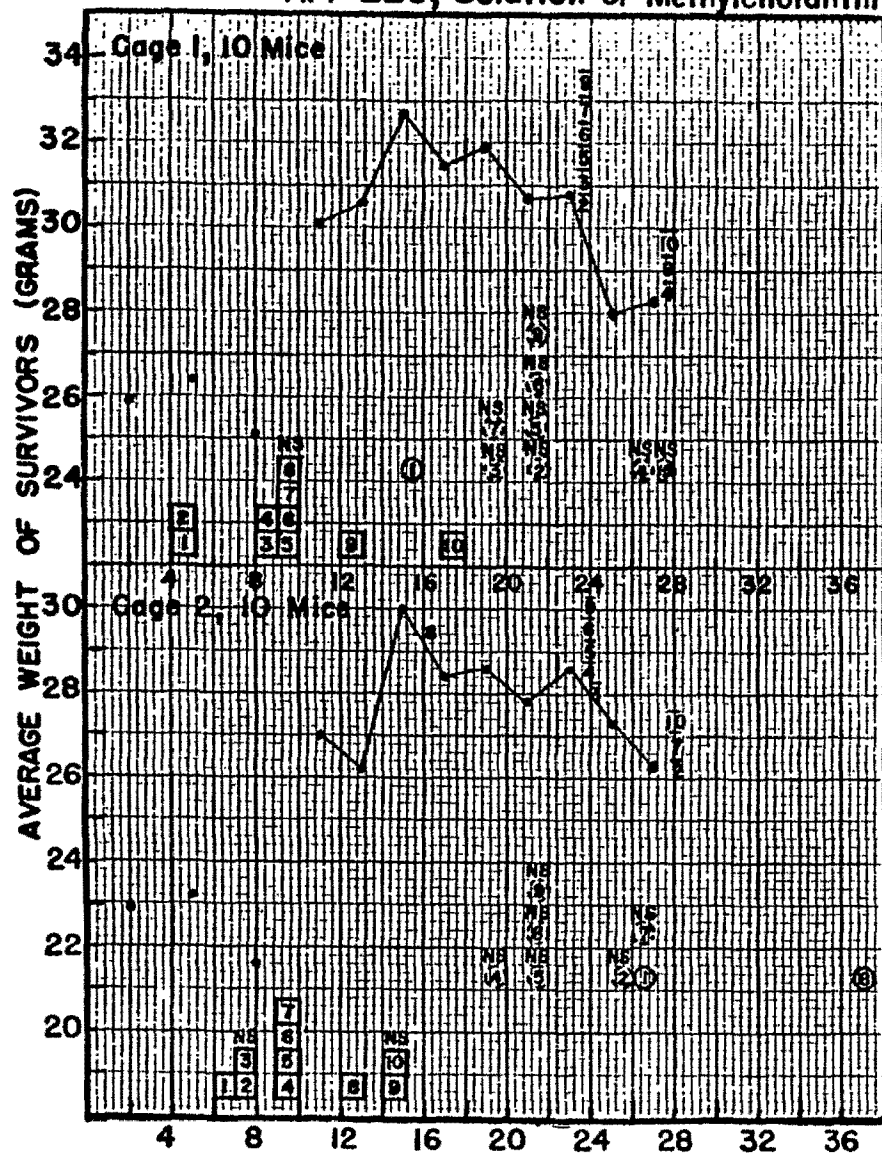
STARTING DATE: 4-14-50
 STRAIN OF MICE: C3H
 NUMBER OF APPLICATIONS PER WEEK: 3
 DOSE PER APPLICATION: 100 mg.
 RELATIVE CARCINOGENIC POTENCY, P_{Mc} : $0.10^{+0.02}_{-0.01} \times$



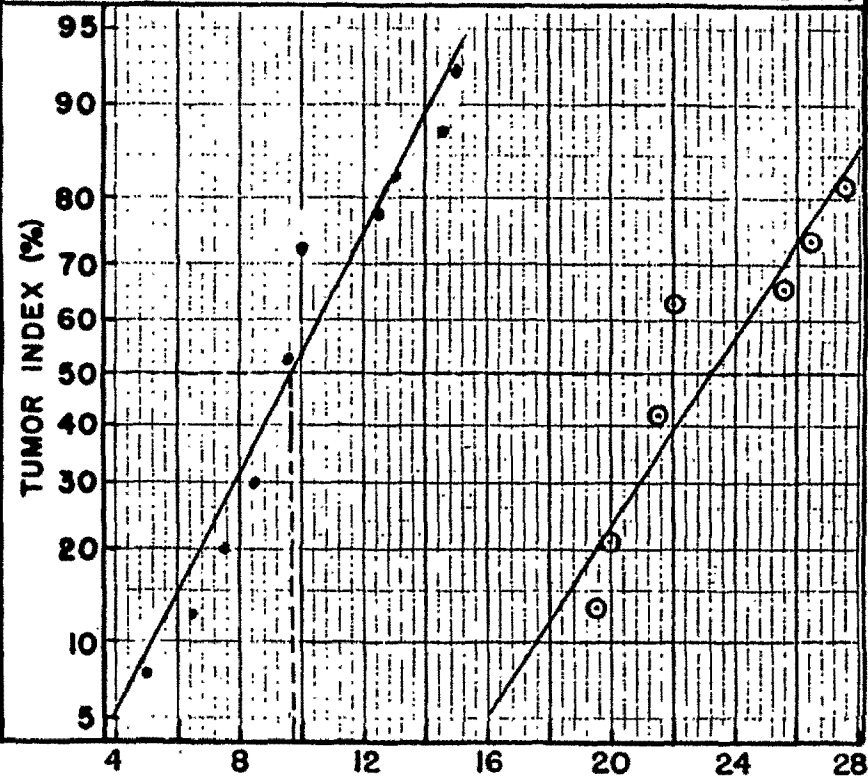
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EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-220, Solution of Methylcholanthrene, 1.034 Percent by Weight in Benzene



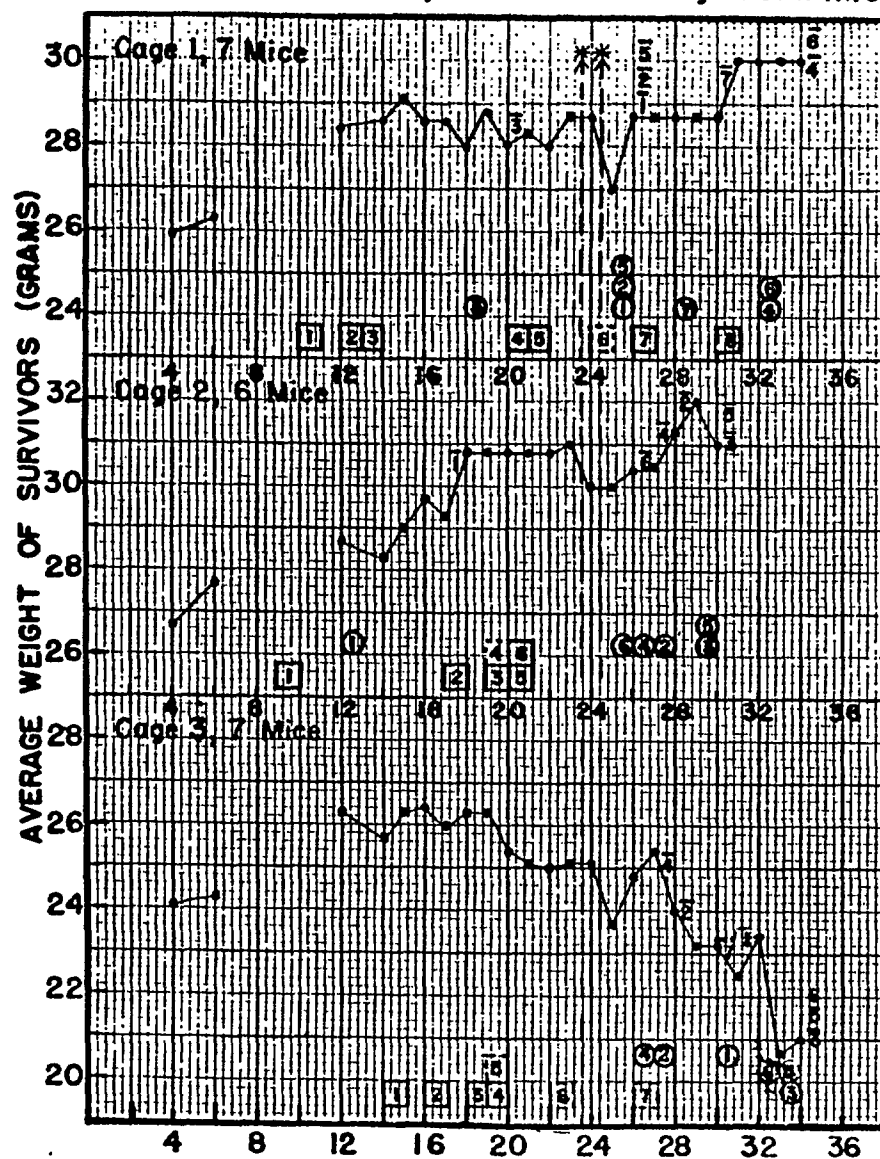
STARTING DATE: 5-3-50
 STRAIN OF MICE: 20 C3H
 NUMBER OF APPLICATIONS PER WEEK: 1
 DOSE PER APPLICATION: 100 mg.
 RELATIVE CARCINOGENIC POTENCY, P_{MC} : $1.01^{+0.28}_{-0.19}$ B



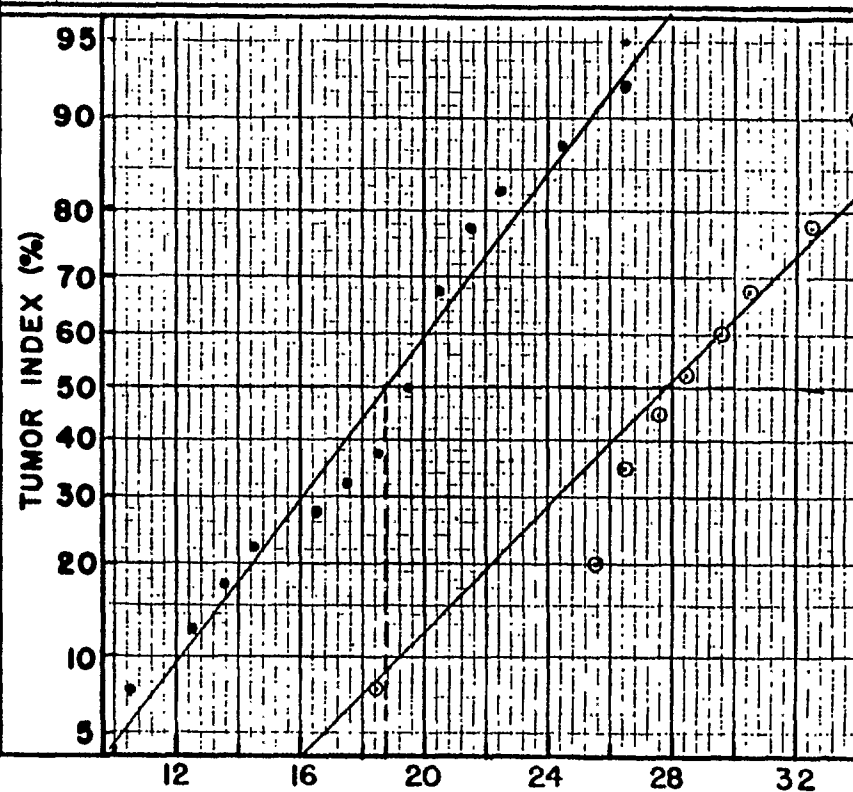
TIME AFTER FIRST PAINTING (WEEKS)

Issued 2-15-54

EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE
 API-238, Solution of Methylcholanthrene, 0.23 Percent by Weight in Benzene



STARTING DATE: 10-2-51
 STRAIN OF MICE: 20 C3H
 NUMBER OF APPLICATIONS PER WEEK: 2
 DOSE PER APPLICATION: 20 mg.
 RELATIVE CARCINOGENIC POTENCY, $P_{MC} : 0.21^{+0.04}_{-0.03} B$



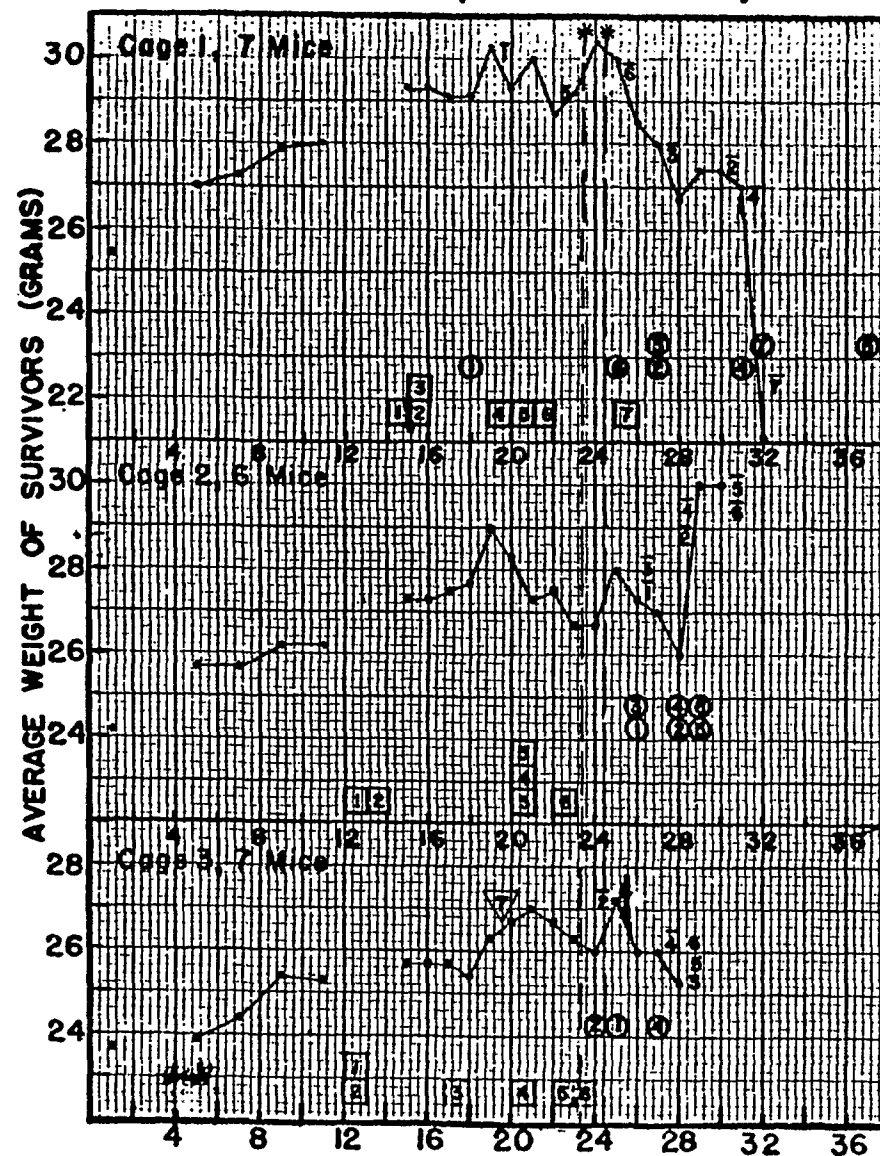
*Painting discontinued for one week at time indicated.

TIME AFTER FIRST PAINTING (WEEKS)

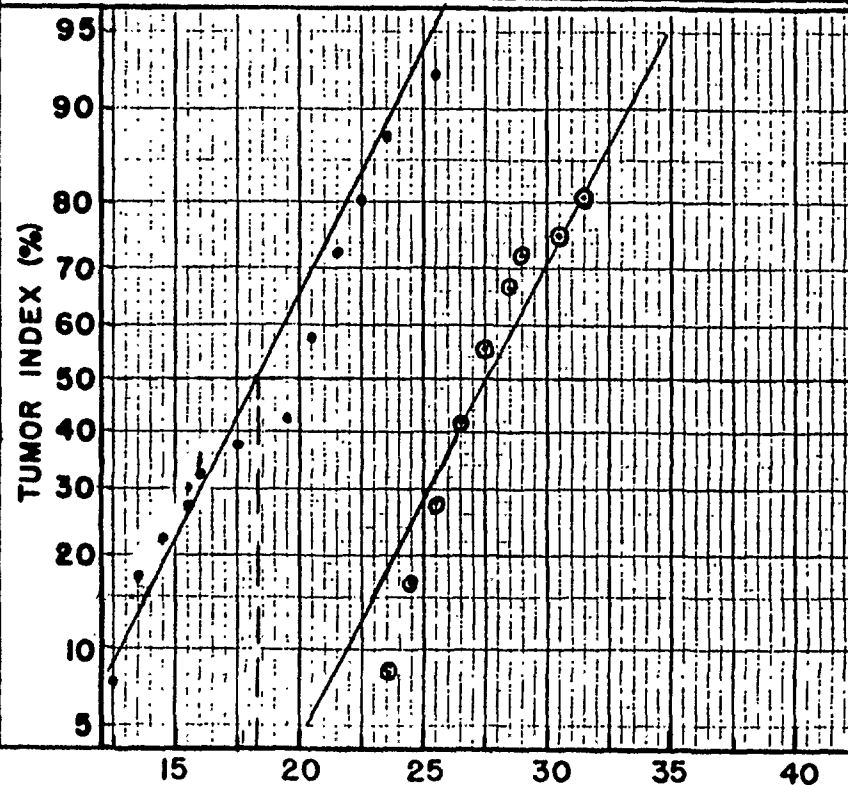
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EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-243, Solution of Methylcholanthrene, 0.15 Percent by Weight in Benzene



STARTING DATE: 10-1-51
 STRAIN OF MICE: C3H
 NUMBER OF APPLICATIONS PER WEEK: 3
 DOSE PER APPLICATION: 20 mg.
 RELATIVE CARCINOGENIC POTENCY, P_{Mc} : 0.15 ± 0.02 B



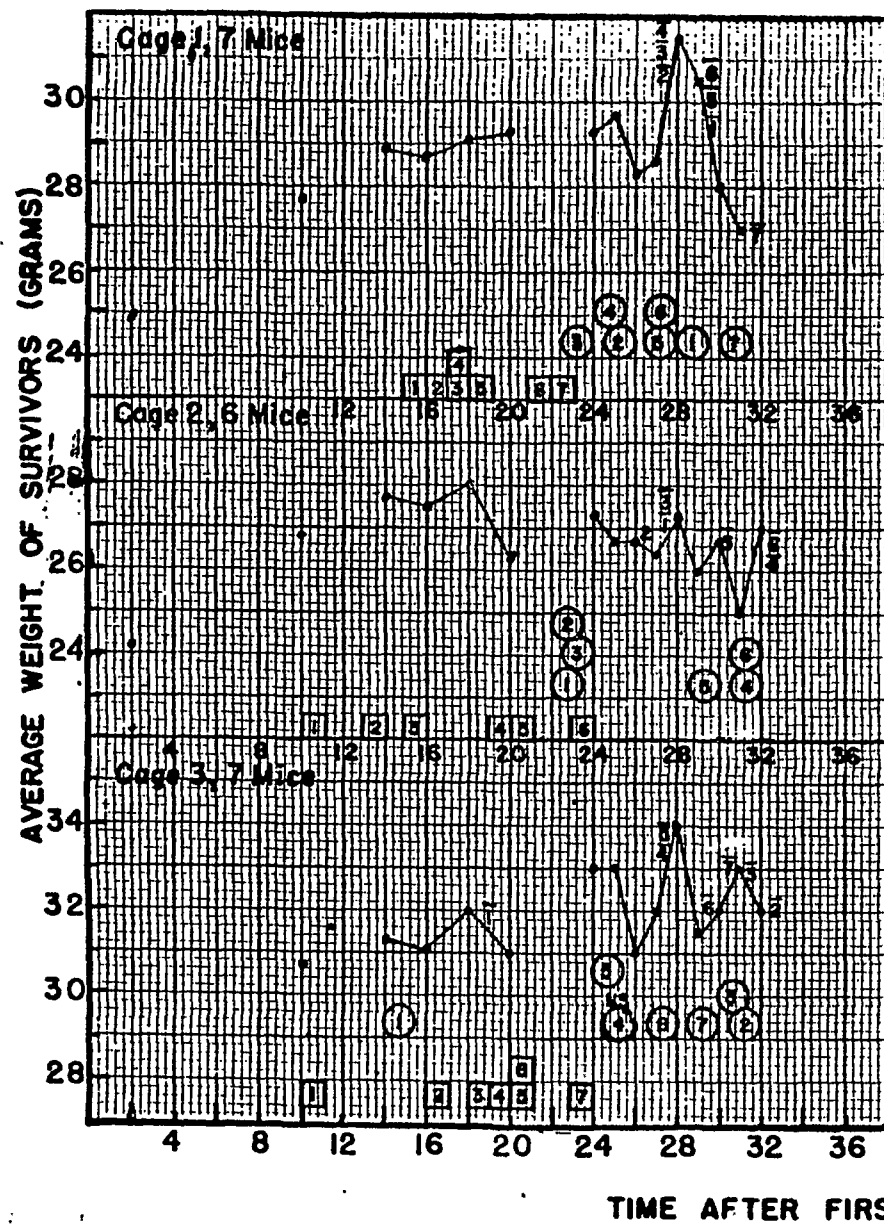
* Painting discontinued for one week at each indicated time.

TIME AFTER FIRST PAINTING (WEEKS)

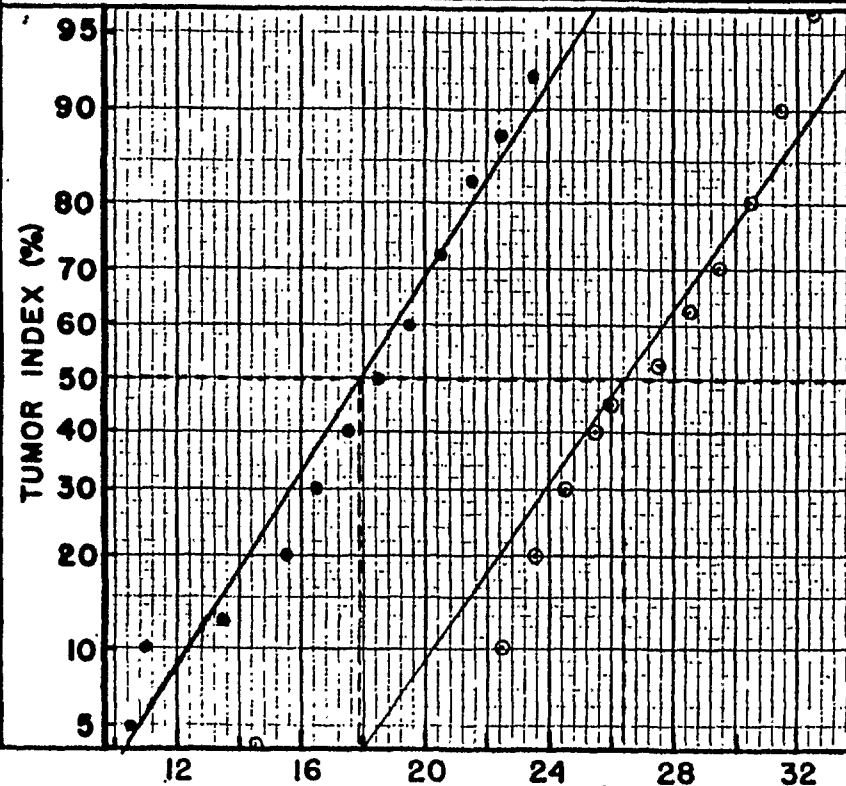
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EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-243, Solution of Methylcholanthrene, 0.15 Percent by Weight in Benzene



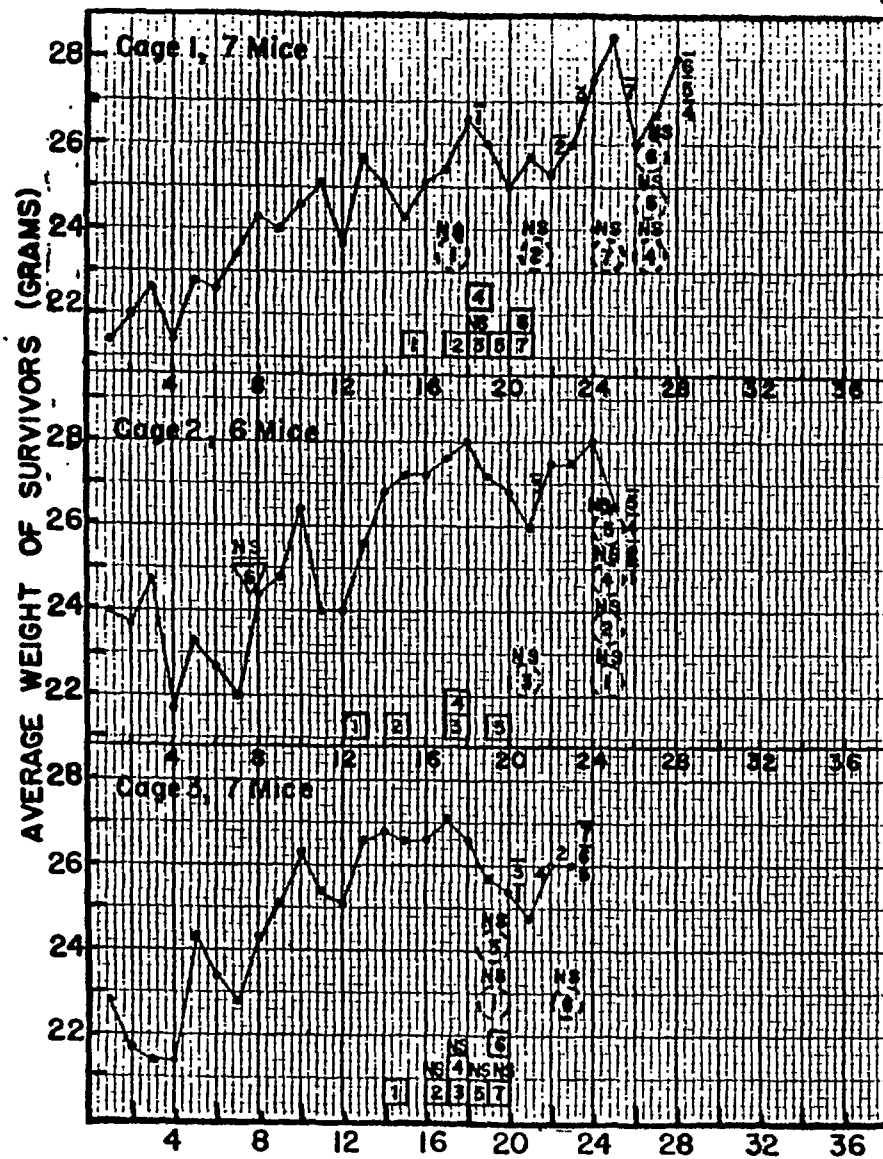
STARTING DATE: 7-28-51
 STRAIN OF MICE: C3H
 NUMBER OF APPLICATIONS PER WEEK: 3
 DOSE PER APPLICATION: 100 mg.
 RELATIVE CARCINOGENIC POTENCY, P_{MC} : 0.15 ± 0.03 A



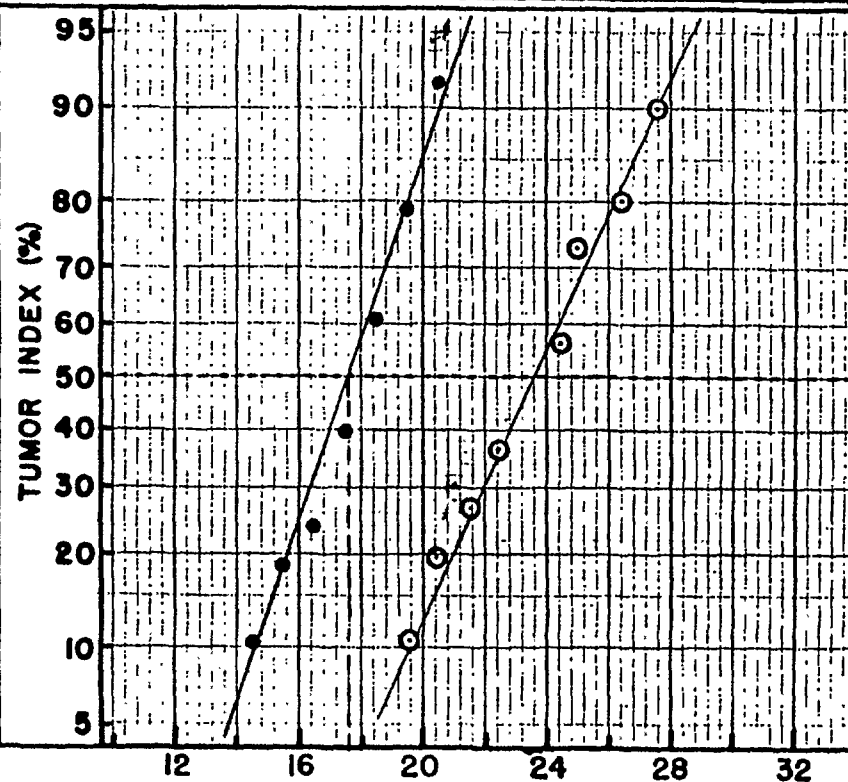
Issued 2-15-54

EXPERIMENTS INVOLVING THE APPLICATION OF MATERIALS TO THE SKIN OF MICE

API-268, Solution of Methcholanthrene, 0.29 Percent by Weight in Benzene



STARTING DATE: 2-13-53
 STRAIN OF MICE: 20 C3H-h
 NUMBER OF APPLICATIONS PER WEEK: 2
 DOSE PER APPLICATION: 20 mg.
 RELATIVE CARCINOGENIC POTENCY, P_{Mc} : $0.23 \pm 0.02 X$



TIME AFTER FIRST PAINTING (WEEKS)

Issued 2-15-54

INTERIM REPORT - API RESEARCH PROJECT MC-1

November 1, 1956

Certain factors, which were instrumental in bringing about a systematic investigation of potential hazards associated with carcinogenic materials encountered in various refining operations, were reviewed in some detail in an annual report dated October 1, 1955. This program has encompassed products from some 16 different cracking and lube refining processes. The carcinogenic potencies of the 120 samples, as gauged by the effects induced by their contact with the skin of mice, have varied over a wide range. As much as ten-fold differences have been observed between materials from similar processes, which were at least superficially comparable. These variations have demonstrated that the history of the sample, including the crude source and the operating conditions under which it was processed, were all-important in determining its final activity.

The need for rapid methods for estimating potency became apparent. Many empirical correlations between physical or chemical properties and potencies have been tested. Some of these have found limited applicability to certain classes of oils but no generally applicable analytical technique has thus far been developed. By 1951 it was evident that the complexity of the problem was such that a

detailed understanding of the relationships between the chemical composition of the different oils and their relative potencies would be required before it would be possible to set up the desired analytical procedures to replace the lengthy biological test. At the same time, the rapid changes which had taken place in this dynamic industry, since the initiation of the project in 1946, demonstrated that this type of fundamental understanding of the problem was essential for the recognition and control of the potential hazards associated with the handling of carcinogenic materials.

In the annual report for 1955 it was shown how the program of fractionation and biological testing of various components of selected carcinogenic oils had led to a useful hypothesis regarding the contributions of various classes of hydrocarbons to the effective potency of a given material. For the purpose of orienting the collection of experimental data these contributions may be expressed by the following equation:

$$P_{MC} = M_a (C_1 + C_2 + C_3).$$

The summation of C's represented the effective potency contributed by typical polycyclic carcinogens, such as benzo-pyrene. The contribution of the non-carcinogenic fraction of the oil, including the paraffins, cycloparaffins, and most of the aromatic constituents is represented by the multiplication factor, M_a . The considerable importance of these non-carcinogenic components is shown by the fact that

M_a may reach values as high as 10. To determine in a systematic manner the relative contributions of carcinogens and non-carcinogens to potency, a series of oils have been fractionated during 1956 by certain physical and chemical procedures in order to produce concentrates of the polycyclic carcinogens as free as possible of other components of the oils. By chromatographic procedures all of the carcinogens may be collected into a concentrate suitable for biologic test to provide values for the summation $(C_1 + C_2 + C_3)$. A further subdivision of the carcinogens has been possible through the process of extraction of the oils by maleic anhydride and subsequent chromatographic concentration of the components of the extract containing four or more rings. In Table 1 are shown the results of a series of biological tests on such fractions which are underway or have been completed during the current year.

It will be noted that only about 15 per cent of the effective potency of the heavy catalytic cycle gas oil, API-89, was contributed by polycyclic carcinogens (API-89-3). In contrast, the entire potency of the thermal tar, API-65, can be ascribed to such polycyclic components. In both instances 25 to 30 per cent of the basic potency due to polycyclic aromatics could be ascribed to compounds extracted by maleic anhydride, such as derivatives of benz(a)-anthracene. The contribution of the 5-ringed class, benzopyrenes, can be estimated from the content of this class as determined by direct chemical analysis. The third

major class of carcinogens, those of lowest molecular weight, are being investigated by a more direct approach, which will be discussed in a subsequent section.

Measurement of the multiplication factor, M_a , cannot be made by any direct method. This contribution due to accelerators may be estimated from the ratio of the effective potency of the oil to the base potency contributed by the polycyclic fraction. Thus, for oils API-89 and API-65, the estimated values of M_a would be 7 and 1, respectively.

The potential difficulties which preclude a direct measurement of M_a arise from the fact that a significant part of the non-carcinogens of the oils have physical properties which are quite similar to those of the carcinogens. Hence, separation of these aromatic non-carcinogens from the carcinogens requires highly efficient application of the techniques of distillation and chromatography. Such separations are being carried out with a few selected materials, but require much more time than the simpler separations which are being applied to quite a variety of oils.

It is possible, however, to obtain some useful information by direct tests of the effective accelerating properties of the non-carcinogenic components which are easily separated from the whole oil by chromatography. Thus, the fractions containing essentially all of the paraffins and cycloparaffins and monocycloaromatics, as well as most of the naphthalene derivatives, are under

test as the vehicles for 0.05 per cent of methylcholanthrene. In addition, tests are being started with solutions of methylcholanthrene in a medium devised to simulate as closely as possible the combination of the non-carcinogenic components of the original oils. These media are being blended from the non-carcinogenic chromatographic fractions together with certain liquid aromatic hydrocarbons to replace the polycyclics separated in the chromatography. These liquids, tetrahydronaphthalene and diphenylmethane, satisfy the theoretical requirement for introduction into the general medium of the aromatic system, with the potentially significant influence of its π -orbitals on the physical characteristics of the liquid phase, without the complications of insolubility, on the one hand, or high volatility, on the other, encountered in initial efforts to employ phenanthrene or benzene for this purpose.

It will be recalled from discussions in previous reports that, if the concentration of accelerators in an oil falls in the range 20 to 50 per cent (typical of catalytically cracked residua), some indication of the magnitude of their influence may be estimated by tests of the whole oils at dosage levels varying from 20 mg. to 100 mg. per application. One is, of course, limited by the toxic nature of an oil in determining the maximum effect of such accelerators. Hence, a direct test using appropriate fractions obtained in the chemical laboratory may serve this purpose better.

Since the results of tests on simple mixtures of relatively pure hydrocarbons normally lead to a more rapid understanding of the relative contribution of different types of components, a series of parallel tests are being carried out on solutions of benzopyrene in blended media, including a standard accelerator such as n-dodecane, together with cycloparaffins of high molecular weight (from white mineral oils), on the one hand, or with more volatile hydrocarbons of relatively low molecular weight, on the other. The results are shown in Table 2 and in Figure 1. It will be noted that, at the 50 mg. level of dosage, dilution of dodecane with 50 per cent of either of the non-accelerating solvents, decahydronaphthalene or beta-methylnaphthalene, did not reduce the accelerating activity of the n-paraffin. On the other hand, dilution with only 25 per cent of white mineral oil (viscosity, 340 S.S.U. at 100°F.) reduced the activity considerably. These results suggest that some major component of the white oils has the property of counteracting accelerating influences to a significant extent.

It has been shown previously that some of these white oils contain long-chain accelerators. The failure to obtain from them any significant adduct with urea indicates that these are not n-paraffins, but presumably isoparaffins and long-chain alkylcyclohexanes or cyclopentanes, the ring being near the end of the chain (1-cyclohexyldecane is an active accelerator, whereas 7-cyclohexyltridecane is almost inactive). It has been found that, to

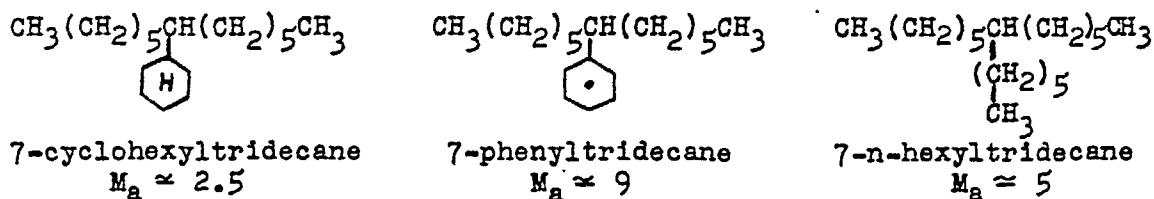
a useful extent, the monocycloparaffins can be extracted from the white oils by their formation of adducts with thiourea, the polycycloparaffins concentrating in the non-adduct. Such fractionations are being carried out on API-80 to obtain material with which to determine whether the polycycloparaffins are responsible for the inhibitory effect observed in the blends discussed above. Since it is suspected that the physiological as well as the physical properties of the liquid media are also influenced significantly by the presence of non-carcinogenic aromatics, suitable blends will also be tested containing such components together with the polycycloparaffins and the accelerators.

The preceding paragraphs indicate the necessity for consideration of the non-carcinogenic medium as a whole in the approach to the final goal of this phase of the project, a quantitative measure of the magnitude of the factor M_a under any given set of conditions of exposure. In addition, a continuing effort has been made to define the general characteristics of the components of this medium which are primarily responsible for its catalytic (accelerating) influence on carcinogenesis. The earlier results of tests of a variety of relatively pure hydrocarbons for their ability in this respect have been summarized in a paper entitled "Carcinogenesis of the Skin. II. The Catalytic Effects of Aliphatic and Related Hydrocarbons".

A fairly general relationship has been noted between the molecular length (in the extended configuration) of the solvent and its ability to accelerate the induction of tumors by polycyclic carcinogens. As shown in Fig. 2, activity seems to reach a maximum level at a length equivalent to that of the n-paraffins containing 12 to 14 carbon atoms. Further, the maximum effect for alkyl derivatives of benzene and naphthalene seems to be somewhat greater than that for saturated hydrocarbons.

The fairly active accelerating ability of triisopropylbenzene seems to be an exception to this relationship of molecular length and activity. As discussed previously, the initial sample used showed in its mass spectrum evidence of an impurity having a lower molecular weight. A pure sample of 1,3,5-triisopropylbenzene, kindly supplied by Dr. E. W. Adams, has now been tested. It has shown the same accelerating activity as the original sample ($M_a \approx 4$).

The shape of the molecule has also been considered as a potentially important characteristic in addition to its length. The saturate, 7-cyclohexyltridecane, demonstrated only a relatively low activity ($M_a \approx 2.5$), even though its molecular length should be optimum (see structures below). In contrast, however, the corresponding 7-phenyl and 7-n-hexyl derivatives have shown a significantly more active accelerating influence (these samples were kindly supplied by API Research Project 42 at Pennsylvania State College).



INVESTIGATION DIRECTED TOWARDS THE IDENTIFICATION OF THE
CARCINOGENS OF FRACTIONS, DISTILLING IN THE RANGE 720
TO 760°F., FROM CRACKED AND STRAIGHT RUN DISTILLATES OR
RESIDUA

These are the carcinogens responsible for the major part of the potency of heavy catalytic cycle gas oils, coker gas oils, and light lubricating stocks which contain little or no benzopyrene (occasionally the cracked gas oils have high enough end points to contain significant amounts of benzopyrene, but this seems to be the exception).

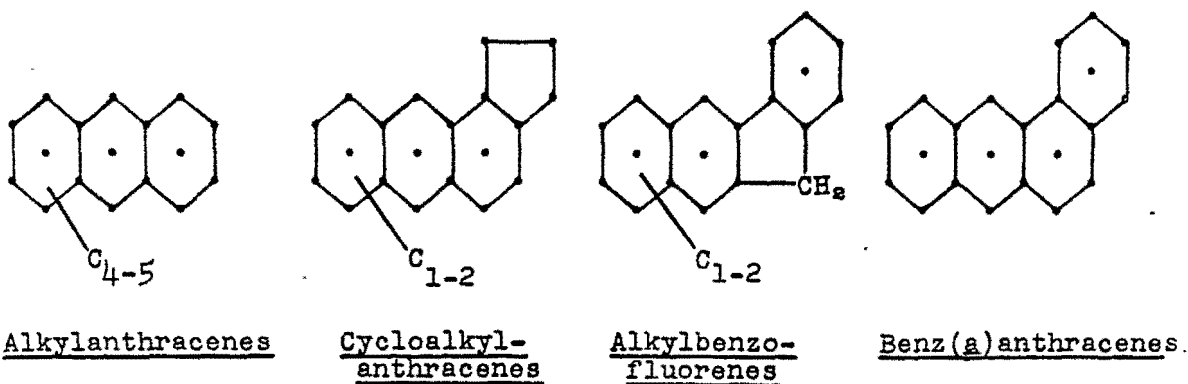
In a concentrated attack on this problem of the nature of the carcinogen of lowest molecular weight in petroleum oils, the polycyclic aromatics obtained by chromatography from a highly active residual product of Fluid Catalytic cracking, API-8, was fractionated through an 80-plate distillation column in 1955. The biological tests on narrow fractions distilling in the range, 650° to 760°, were completed in 1956. The results are shown in Table 3.

It has been demonstrated that all of these fractions contain accelerating components, although the activity in this respect apparently drops to a relatively low level by the time the concentration of carcinogen (in fraction 8-26-k or -l) has increased to a point capable of inducing tumors in 90 per cent of the C3H mice.

The carcinogenic fractions, 8-26-1, -m, and -n, (730 to 760°F.) were selected for further fractionation. In order to reduce the complexity of the system the three fractions were first extracted with aqueous acid and base and then with maleic anhydride. The concentration of the major components of the maleic anhydride adduct from 8-26-n was estimated from its mass spectrum (ionization potential, 7 volts) as follows:

<u>n</u>	<u>C_nH_{2n-18}</u>	<u>C_nH_{2n-20}</u>	<u>C_nH_{2n-22}</u>	<u>C_nH_{2n-24}</u>
17	0.7%	0.8%	0.3%	-
18	5.1	11.1	3.8	13.3%
19	6.4	11.8	6.4	2.2
20	3.1	5.6	2.3	0.8
21	1.1	1.8	0.8	0.3

Suggested structures:



On the basis of the mass spectrum at high voltage, the cyclopentano substitution is considered to be the most likely structure for the C_nH_{2n-20} hydrocarbons. A six-membered

cycloalkyl ring would be expected (on the basis of the spectrum of tetralin) to yield a strong fragment peak at m/e of 204 by loss of $-\text{CH}_2-\text{CH}_2-$. The intensity of the 204 ion in the spectrum of the mixture was negligible.

The components extracted by maleic anhydride apparently contributed about 1/6 of the potency of the original distillate, 8-26-n. About 1/5 of the accelerated potency of 0.10 shown by this fraction in the test 8-26-n-1 can be ascribed to benzanthrane. It seems likely that the major contribution came from the 4-ringed compounds having one partially saturated ring, but this cannot be stated with certainty until additional experimental evidence is available.

The combined material from 8-26-1; -m, and -n, which had been freed from the components soluble in aqueous acid and base and from the anthracenic compounds removed by maleic anhydride, was next subjected to chromatographic fractionation on activated alumina. Mass and absorption spectrometry were used to determine the distribution of the various classes of hydrocarbons through the chromatographic fractions (see Table 4). The biological test on the fraction which contained almost all of the 4-ringed chrysene and triphenylene (8-26-14) mixed with the more strongly adsorbed portion of the benzofluorenes, indicated the presence of only a trace of the carcinogens of the parent distillate. The test on the most strongly adsorbed fraction (8-26-15), that which includes nitrogen compounds such as derivatives

of carbazole, has shown mainly that this fraction is exceedingly toxic to the mice. No tumors have been produced in 34 weeks. One of the intermediate fractions (8-26-11), that containing cyclopentanophenanthrenes, fluoranthenes, and benzofluorenes, but essentially free of compounds having 4 fully aromatic rings, has shown some carcinogenic activity, but was not responsible for more than about 10 per cent of the potency of the original distillates.

The foregoing tests on fractions derived from 8-26-1, -m, and -n were accelerated by the use of dodecane as a solvent. This permitted a relatively rapid elimination of a possible 4-ringed aromatic carcinogen from consideration. Tests of the remaining fractions are being carried out at their original concentration (or at a doubled concentration) in benzene as a solvent in order to obtain a better measure of their relative potencies, P_{MC} , and for a more direct comparison with the potencies of the parent distillates, which were tested as 50 per cent solutions in benzene. These new tests, 8-26-7, -8, -9, -10, and -12, have been under way about four months. The results of these tests should indicate the particular classes of polycyclic hydrocarbons responsible for the major part of the potency in this distillation range, 720 to 760°F. According to the physical and chemical behavior of the classes indicated, a further concentration of the carcinogens will be carried out until their certain identification has been accomplished. Analytical techniques for the direct determination of this class in complex oils may then be developed.

API EXPERI- MENT NUMBER	DESCRIPTION	GRAVITY (°API)	FRACTION DISTIL- LING ≤750°F. (per cent)	BENZO- PYRENE (per cent)	RELATIVE CARCINO- GENIC POTENCY, P _{MC} (at 100 mg.)
89	Catalytically cracked sidestream (F.C.C. heavy cycle gas oil)	27.0	100	0.001	<u>0.3</u>
33	Coker gas oil	16.8	60	0.008	<u>0.4</u>
65	Thermal tar	6.6	43.8	0.04	<u>0.11X</u>
120	Straight run dis- tillate (naphthenic)	19.5	~52	≤.003	<u>0.07</u> (50 mg.)
105	Straight run dis- tillate (waxy)	28.9	72.9	0.0003	<u>0.11</u>
115	Straight run distil- late (naphthenic)	23.2	~50	.002	<u>0.03</u>

TABLE I

RELATIVE CONTRIBUTIONS OF CARCINOGENS AND NON-CARCINOGENS
TO THE EFFECTIVE POTENCY OF VARIOUS REFINERY STREAMS

API EXPERI- MENT NUMBER	CHROMATOGRAPHIC FRACTIONATION		Poly- cyclic Aromatics and Non-hydro- carbons (yield)	BASIC CONTRI- BUTION OF POLY- CYCLICS TO P _{MC} ¹	ESTI- MATED ACCELER- ATING FACTOR, M _a	FRACTION EXTRACTED BY MALEIC ANHYDRIDE		
	Saturates Thru Alkyl- naphthalenes					Yields		Contri- bution of 4+ Ring Fraction to P _{MC} ¹
	Yield	P _{MC} with 0.05% MC				2-3 rings	4+ rings	
89-1						1.64%		<u>0.00</u>
89-2							0.2%	<u>0.012</u>
89-3			19%	<u>0.04</u>	7			
89-4	81 ²							
89-6	81	<u>0.11</u>						
33-3						(2.1)	2.2	inc.
33-4			33	inc.				
33-5	67	inc.						
65-1			60	<u>0.12</u>	<u>1</u>			
65-2						(0.9)	1.1	<u>0.03</u>
65-3	40	inc.						
120-1						(1.2)	0.4	<u>~ 0.004</u>
120-2			33	inc.				
120-3	67	inc.						
105-1	84	inc.						
115-1	91	inc.						

1. Determined by biological test on solution of polycyclic fraction in benzene at appropriate concentration, three 20 mg. applications each week.
2. A direct test of this fraction has produced 1 benign papilloma in 36 weeks.

TABLE 2

INFLUENCE OF VARIOUS DILUENTS ON THE ACCELERATING ACTIVITY OF n-DODECANE

API EXPERI- MENT NUMBER	ACCELERATOR	DILUENT	CONCEN- TRATION OF BENZOPYRENE (% by weight)	RELATIVE SPREAD ¹	SCHEDULE OF APPLICATIONS (no./week - mg.)	MEAN TIME OF APPEARANCE OF TUMORS (weeks) ^{2,3}	RELATIVE CARCINO- GENIC POTENCY, P _{MC} ²
665	100% Dodecane	None	0.04	2.9	2 - 50	23.7	0.14
666	100% Dodecane	None	0.08	2.9	2 - 50	20.4	0.18
295	100% Dodecane	None	0.20	2.9	3 - 50	14.7	0.21
658	None	100% Decahydro- naphthalene	0.08	1.8 est.	2 - 50	~ 43	~ 0.05
659	50% Dodecane	50% Decahydro- naphthalene	0.08	2.4 est.	2 - 50	20.0	0.19
660	50% Dodecane	50% Beta-methyl- naphthalene	0.08		2 - 50	19.1	0.20
613	None	85% Beta-methyl- naphthalene, 15% Benzene	0.20		2 - 50	30.6X	≅ 0.10
614	75% Dodecane	25% white oil (340 SSU/100°)	0.20	2.65 est.	3 - 50	20.4	0.12
297	50% Dodecane	50% white oil	0.20	2.4 est.	3 - 50	20.2	0.12
296	None	100% white oil	0.20	1.8	3 - 50	27.9	0.07

1. Based upon relative spread of 1.0 for solutions of benzopyrene in benzene.

2. Experience indicates an experimental variation of $\pm 15\%$.

3. In these tests, essentially all of the C3H mice develop squamous cell papillomas and carcinomas if they survive.

TABLE 3

TUMOR-INDUCING ACTIVITY OF PRODUCTS OF FRACTIONAL DISTILLATION OF THE
POLYCYCLIC AROMATICS OF A CATALYTICALLY CRACKED OIL

API EXPERI- MENT NUMBER	FRACTION DISTILLED	ESTIMATED BOILING RANGE (In ° F. at 760 mm.)	INCIDENCE OF TUMORS (% - weeks)	RELATIVE CARCINO- GENIC POTENCY, P _{MC}
8-26-a ¹	28.5 - 30.8%	641 - 646°F.	0% - 80	-
8-26-b	30.8 - 32.0	646 - 651	0 - 80	-
8-26-c	32 - 34.1	651 - 656	0 - 80	-
8-26-d	34.1 - 36	656 - 662	0 - 80	-
8-26-e	36 - 38	662 - 668	0 - 80	-
8-26-f	38 - 40	668 - 674	7 - 80	-
8-26-g	40 - 43.9	674 - 686	28 - 80	-
8-26-h	43.9 - 46	686 - 693	12 - 80	-
8-26-i	46 - 50.3	693 - 709	36 - 80	~0.01
8-26-j	50.3 - 52.5	709 - 717	71 - 73	0.03±.01
8-26-k ¹	52.5 - 54.8	717 - 727	83 - 28	≅0.21 ¹
8-26-l	54.8 - 57.3	727 - 736	90 - 58	0.09 ⁺ .02 -.05
8-26-m ¹	57.3 - 60.4	736 - 747	87 - 45	0.29 ¹
8-26-n	60.4 - 62.4	747 - 759	100 - 46	0.15 ⁺ .02 -.07
<u>Contributions of Accelerators to Potencies of Fractions above</u>				
				Acceler- ating Factor
8-26-2	Solution of Benzopyrene in fraction ² of 8-26-g, -h, and -i; plus 20% benzene			5
8-26-4	Solution of Benzopyrene in fraction ² of 8-26-l, -m, and -n; plus 30% benzene			2

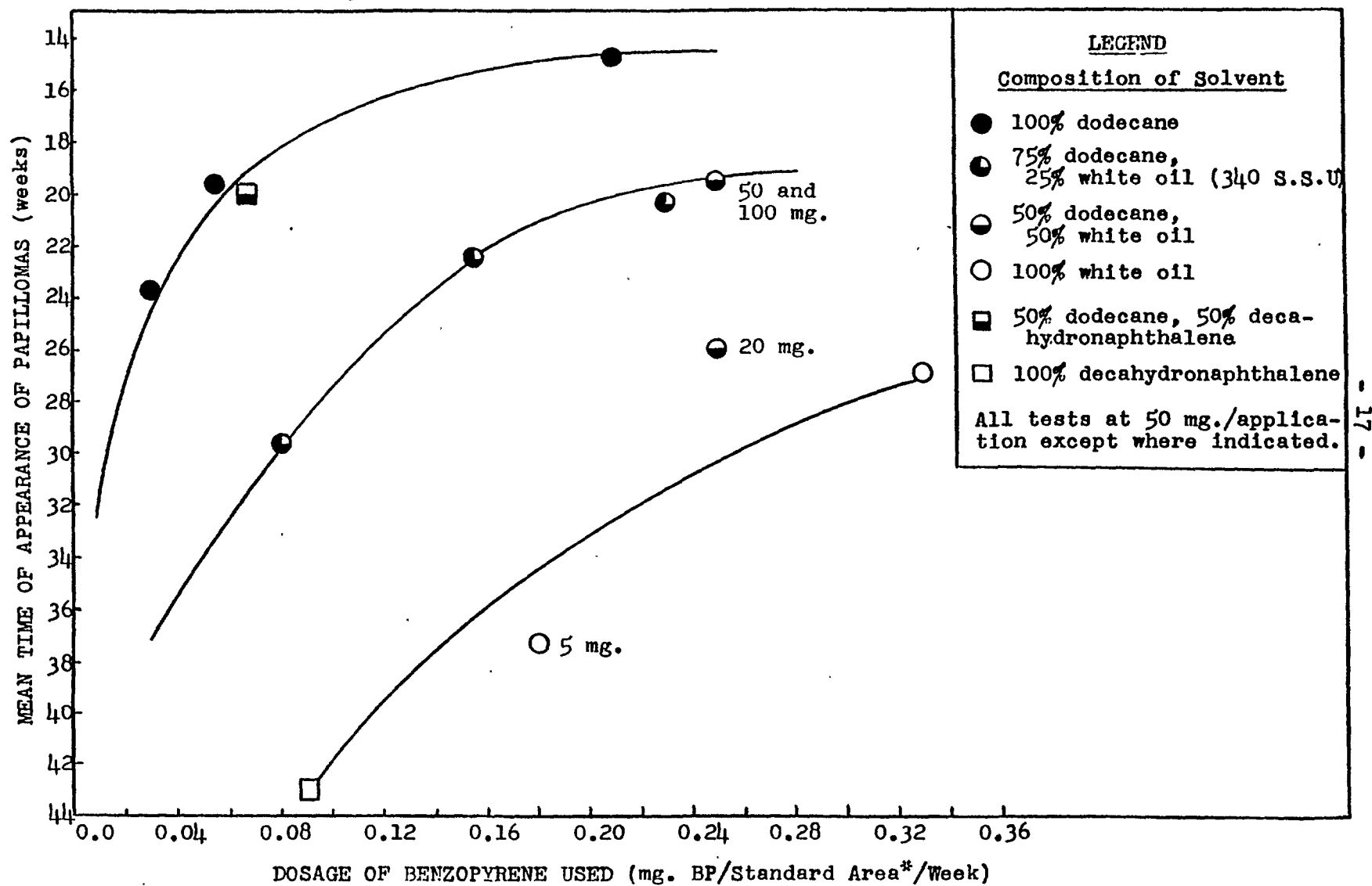
1. All fractions tested as 50% solutions in benzene except 8-26-k and -m, which were accelerated with 50% dodecylbenzene.
2. Chromatographic fractions including saturates through naphthalenes, 27% of 8-26-g, -h, and -i; 23% of 8-26-l, -m, and -n.

TABLE 4

RELATIVE POTENCIES OF CHROMATOGRAPHIC FRACTIONS OF CARCINOGENIC FRACTIONS OF LOWEST
MOLECULAR WEIGHT FROM CATALYTICALLY CRACKED RESIDUUM, API-8

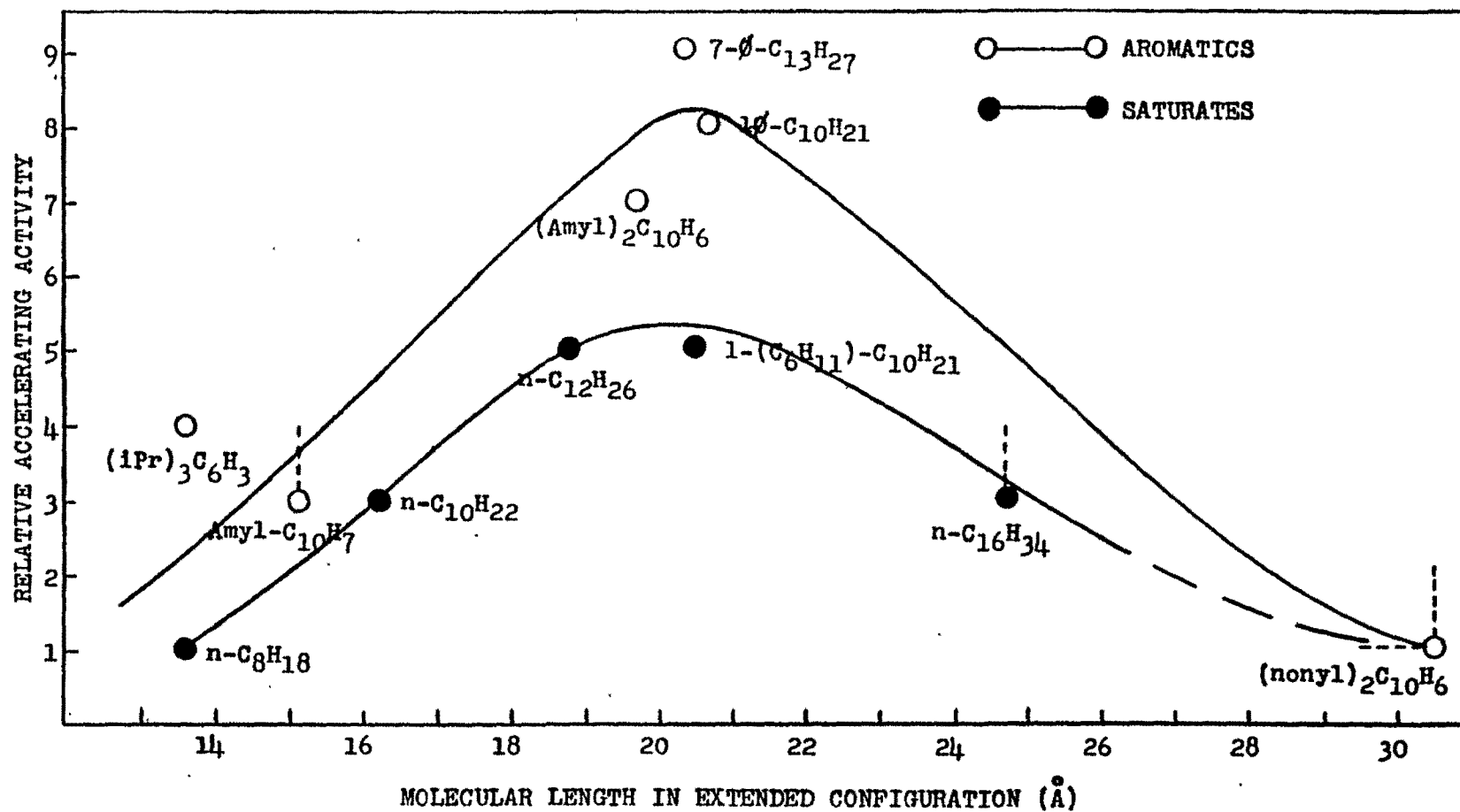
CHROMATOGRAPHY		BIOLOGICAL TESTS					
PERCENT ELUTED	APPARENT COMPOSITION	API EXPERIMENT NUMBER	PERCENT ELUTED	CONCENTRATION IN SOLUTION TESTED	SOLVENT	SCHEDULE OF APPLICATIONS (no./week - mg.)	INCIDENCE OF TUMORS (%-weeks)
0 - 22%	Saturates						
21 - 27	Naphthalenes						
25 - 90	Phenanthrenes	8-26-7	26 - 64%	33.4%	Benzene	3 - 15	0% - 19
50 - 87	Pyrenes	8-26-8	64 - 80	32.6	Benzene	3 - 15	18 - 19
80 - 91	Fluoranthenes	8-26-9	80 - 85	9.6	Benzene	3 - 15	0 - 19
84 - 94	Benzofluorenes	8-26-10	85 - 88	6.2	Benzene	3 - 15	0 - 19
		8-26-11	88 - 91	4.1	Dodecane	3 - 50	70 - 30
91 - 94	Chrysene, etc.	8-26-12	91 - 93	3.8	Benzene	3 - 15	0 - 19
		8-26-14	93 - 94	0.9	Dodecane	3 - 50	10 - 35
94 - 100	Non-hydrocarbons	8-26-15	94 - 100	10.4	Dodecane	3 - 50	0 - 35

FIGURE 1 - INFLUENCE OF CYCLOPARAFFINIC DILUENTS
ON THE ACCELERATING ACTIVITY OF n-DODECANE



* Area covered by 100 mg. BP in benzene.

FIGURE 2 - MOLECULAR LENGTH OF SOLVENT vs. ACCELERATING ACTIVITY



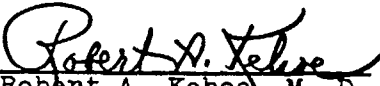
From The Kettering Laboratory in the Department of
Preventive Medicine and Industrial Health, College of
Medicine, University of Cincinnati, Cincinnati, Ohio.

Investigative Team:

Bernard H. Braun, Ph. D.
Frank P. Cleveland, M. D.
Ralph T. Denham, B. S.
Mary Jane Graf, B. S.
Francis F. Heyroth, Ph. D., M. D.
A. Wesley Horton, Ph. D.
John J. Phair, M. D.
Fred Shaffer, M. S.
Klaus L. Stemmer, M. D.
Russell Tye, M. S.
Waldo J. Younker, M. S.
Patricia Clapsaddle
Lenore Hull
Irvin Rapien
Effie West

Report:

A. Wesley Horton, Ph. D.

Approved: 
Robert A. Kehoe, M. D.
Director

Date: November 1, 1956