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C_9-C_{12} Fractions Obtained From Petroleum Distillates

An Evaluation of Their Potential Toxicity

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THE INTRODUCTION of catalytic reforming operations into petroleum refining technology provides a convenient process for the conversion of low octane petroleum naphthas into high octane motor fuel components. These reformed naphthas are rich in alkyl aromatics and suitable fractions of these naphthas were found to have excellent solvent properties for special paints and surface coatings. Due to the lack of information regarding the toxicologic and physiologic effects resulting from exposure to these aromatic-rich naphthas,[1,2] the Medical and Health Committee of the American Petroleum Institute sponsored a research study focused on the wide spectrum of alkyl aromatics present in these naphthas. To gather information in this area, studies using various species of animals and methods of contact were planned and executed.

To obtain a representative sample of catalytic reformate as well as a sufficient amount of aromatic-rich fraction for this study, a large number of naphtha samples obtained from the participating companies were pooled and the pooled sample fractionated in commercial equipment. The resulting aromatic-rich fractions used in this study were alkyl benzenes, naphthalenes, indans, cycloparaffins, and paraffins. The C_9-C_{10} aromatic-rich fraction distilled off at 311 F-392 F, whereas the C_{11}-C_{12} aromatic-rich fraction came off at 392 F-480 F.

Our study included benzene and normal decane for comparative purposes.[3-6] Rats, mice, rabbits, and monkeys were used and exposures were carried on by: (1) inhalation, (2) skin application, and (3) subcutaneous injection

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The typical composition of materials used is given in Table 1.

Inhalation Studies

The exposure chambers (Fig 1) used consisted of three essential parts: (1) the Venturi mixing tube, (2) surge chamber with diffuser plate, and (3) inner chamber for holding animal cages. These parts were constructed of galvanized sheet metal and galvanized hardware screen. The Venturi mixing tube received incoming vapor from the generator and mixed it with the inlet air. From the Venturi, the air-vapor mixture was sucked into the surge chamber and diffused through the diffuser plate into the animal chamber. The outlet mixture of vapor and moisture from the exposed animals was exhausted to the outside atmosphere. A sampling port was provided in the center of the chamber. This was for sampling the air-vapor mixture to which the animals are actually exposed. The animal cages placed into the chamber were made of ¼ inch mesh hardware cloth. A 2 foot square door was placed on top of the chamber. Through this door the animal cages were placed into and removed from the chamber.

TABLE 1.—*Composition of Alkyl Aromatic Fractions*

Components	C_9-C_{10} Fraction BP: 311-392 F	C_{11}-C_{12} Fraction BP: 392-480 F
Paraffins	20 mol %	6 mol %
Cyclo paraffins	6 mol %	0 mol %
Alkyl benzenes	74 mol %	44 mol %
Indans	0 mol %	27 mol %
Naphthalenes	0 mol %	23 mol % *
Tricyclin	Omol %	0 mol %
Alkyl benzenes		
C_9	42 mol %	7 mol %
C_{10}	29 inol %	10 mol %
C_{11}	3 mol %	24 mol %
C_{12}	Omol %	5 mol %
C_{13}	0 mol %	1 mol %

* 13.5 mol % naphthalene, 9.5 mol % 2-methyl naphthalene.

The results obtained when animals were exposed by inhalation to C_9-C_{10} or C_{11}-C_{12} were compared to results obtained when the same species of animals were exposed to identical concentrations of benzene or normal decane.

Criteria used to detect changes from the normal in exposed animals were: (1) appearance and behavior of the animal, (2) body weight gains as compared to expected gains, (3) organ weights, **(4)** hematological findings, (5) bone marrow changes,[7] and (6) gross and microscopic pathological changes.

The chambers were so operated that there **was** a constant and known quantity of the test material fed into the vaporizers. Determinations of the concentrations of the vapor in each chamber were made each hour. The air flow into the chamber was measured with a thermo-anemometer and a dry-test meter (Table 2).

TABLE 2.—*Vapor Concentrations Employed* **for** *Inhalation*

Material	Concentration	Variation in Hours of Exposure	Maximum Days of Exposure	No. of Animals
		Rat Studies		
Benzene	1,000 ppm	122-1,865	105	38
	200 ppm	137-749	92	18
	50 ppm	256-756	90	23
C_9-C_{10}	1,000 ppm	272-1,428	78	38
	616 ppm	54-2,722	150	60
	200 ppm	72-739	93	IS
	50 ppm	697-715	90	IS
C_{11}-C_{12}	500 ppm	88-1,235	51	37
	200 ppm	730-744	93	17
	50 ppm	722-746	90	18
Ndecane	560 ppm	362-1,501	91	41
Control	No exposure			
		Monkey Studies		
C_9-C_{10}	200 ppm	630	90	3
	50 ppm	630	90	3
C_{11}-C_{12}	200 ppm	630	90	3
	50 ppm	630	90	3

Inhalation ***Studies*** *Using* Rats.—Benzene: Concentration of Benzene, 1,000 PPM. Rats exposed 23½ hours per day, seven days per week, appeared to be in "poor" condition, with a loss of body weight after 183 hours of exposure. They hemorrhaged from the nose and mouth; the stomach was distended; the gut was empty; and the blood vessels of the lungs, liver, kidneys, intestines, and omental tissues were engorged. The white blood cell count fell significantly; there was a reversal of the polymorphonuclear-lymphocyte ratio, and the deoxyribonucleic acid fell significantly. Microscopic studies of the bone marrow showed an increase in the proportion of erythrocyte precursors (stimulation of erythrocytic activity).

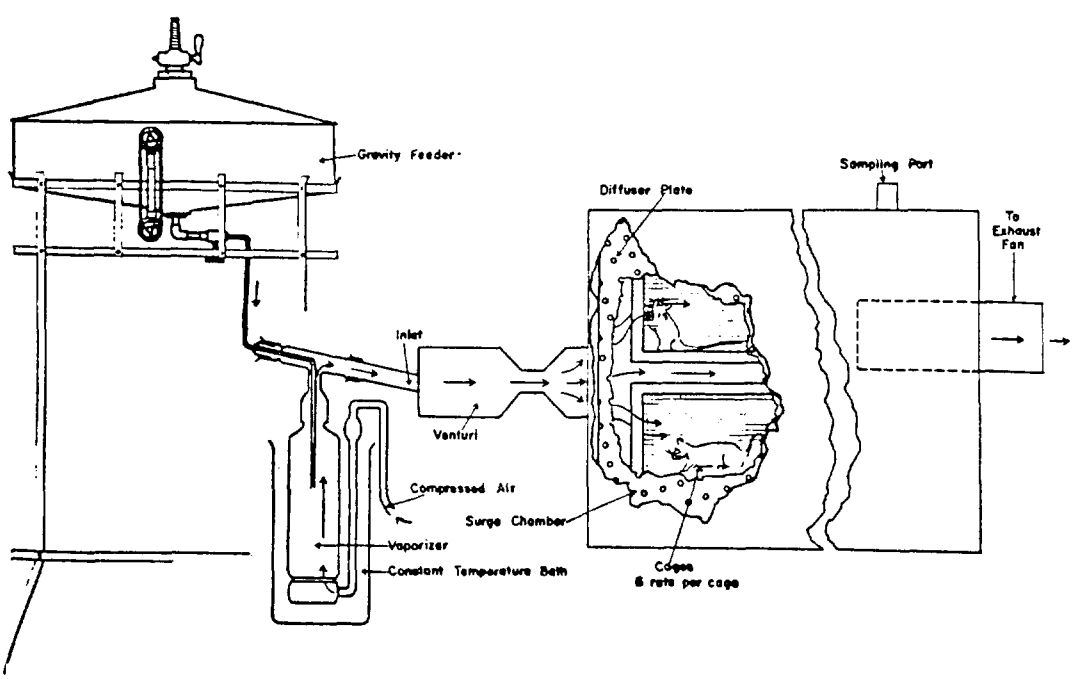


Fig 1.—Vaporizer and **exposure** chamber.

Rats exposed to this same concentration 18 hours per day, seven days per week for as long as 1,782 hours showed changes similar to the ones described above. Some of the rats were "set aside for observation" for 6½ months after 1,782 hours of exposure. All blood changes returned to normal values with the exception of the deoxyribonucleic acid which remained low.

Concentration of Benzene, 200 PPM. Rats exposed eight hours per day, five days per week, showed a fall in WBC after 750 hours of exposure with no change in the polymorphonuclear-lymphocyte ratio or expected weight gain. The microscopic examination of the bone marrow revealed some depression of myelocytic activity and a stimulation of erythrocytic activity.

Concentration of Benzene, 50 PPM. Rats exposed eight hours per day, five days per week for as long as 756 hours showed a fall in the white blood cell count with no other significant findings except that after 600 hours of exposure, 50% oi the rats developed bilateral cataracts. As with 200 ppm exposure, the rats developed lower DNA values and revealed depression in the bone marrow of myelocytic activity and a stimulation of erythrocytic activity.

C9-C10: Concentration of C_9-C_{10}, 1,000 PPM. Rats exposed 18 hours per day, seven days per week, developed congestive changes in the lungs and liver, large spleens, and hemorrhagic kidneys at the end of the first day of exposure. There was a significant lowering of the white blood cell count by the eighth day, as well as a shift in the polymorphonuclear lymphocyte ratio. DNA values were lowered and the expected body weight gains were not observed. There was an increased transparency and fragility of the femur of the exposed rats.

Concentration of C_9-C_{10}, 616 PPM. Rats exposed 18 hours per day, seven days per week, for as long as 2,424 hours showed a significant interference in expected weight gain as well as a significant fall in the total white blood cell count. There was evidence of congestion and hemorrhagic change in the lungs, liver. kidneys, spleen, and omental tissue after only a few days oi exposure. Focal, inflammatory changes in the lungs were noted as well as fatty changes in the liver after 54 hours of exposure. Afte: 400 hours of exposure there were hemorrhages around the nose and mouth; transitory blood changes occurred in the rats exposed for 2,424 hours. Seventy percent of the rats, which were set aside for two months with no additional exposure, developed bilateral cataracts. There were no cataracts observed in unexposed rats of the same age or in rats exposed to any concentration of n-decane.

The primary histological changes observed in the eye of the exposed rat occurred in the lens where one could observe, in focal areas, accumulations of enlarged epithelial cells with cytoplasm frequently vacuolated. Also, there were areas of proliferation of fibroblasts. There were no inflammatory cells. These changes in the rat were similar to those found in the lens of the chick embryo given dinitrophenol,[8,9] naphthalene,[10] maleic anhydride, or hydro-quinone.

There were no significant organ weight changes. Microscopic examination of the bone marrow indicated a stimulation of myelocytic activity.

Rats exposed to 616 ppm of C_9-C_{10} 23½ hours per day for seven days had blood in the urine and hemorrhagic changes in the kidneys, omental, and subcutaneous tissues.

Rats exposed continuously for 24, 36, 54, or 72 hours (one single exposure) to the above concentration of C_9-C_{10} showed only transitory blood changes.

Rats exposed to 616 ppm oi C_9-C_{10} on three alternating days for 18 hours each day and then set aside nine months for observation did not develop any cataracts. There were no significant changes from the normal observed.

Concentration of C_9-C_{10}, 200 PPM. Rats exposed eight hours per day, five days per week (90 exposures), showed no persistent or significant peripheral blood changes. weight gains, bone marrow or eye lens changes.

Concentration of C_9-C_{10}, 50 PPM. Rats exposed eight hours per day, five days per week. showed no detectable changes from the normal after 715 hours of exposure.

Inhalation Studies Using Monkeys.—C_9-C_{10}: Vapor concentration of C_9-C_{10}, 200 PPM. Three Rhesus (4 lb; 1.8 kg) monkeys exposed by inhalation to 200 ppm of C_9-C_{10} seven hours per day, five days per week, for a total of 90 exposures appeared "groggy" and sedated while being exposed. The white blood cell counts fell and there was a reversal in the polymorphonuclear-lymphocyte ratio. During the first week of the exposure the monkeys developed a noticeable tremor which became less pronounced as the exposure was continued. There was evidence of skin irritation with a loss of hair and the formation of a dry and "leathery" skin. The monkeys killed after 90 exposures showed no significant gross or microscopic changes from the normal other than those referred to but had a bone marrow suggestive of depression of both the myelocytic and erythrocytic activity.

Vapor Concentration of C_9-C_{10}, 50 PPM. Three monkeys exposed to 50 ppm of C_9-C_{10} seven hours per day, 5 days per week, for a total of 90 exposures showed no consistent significant changes by gross or microscopic examination except an increase in

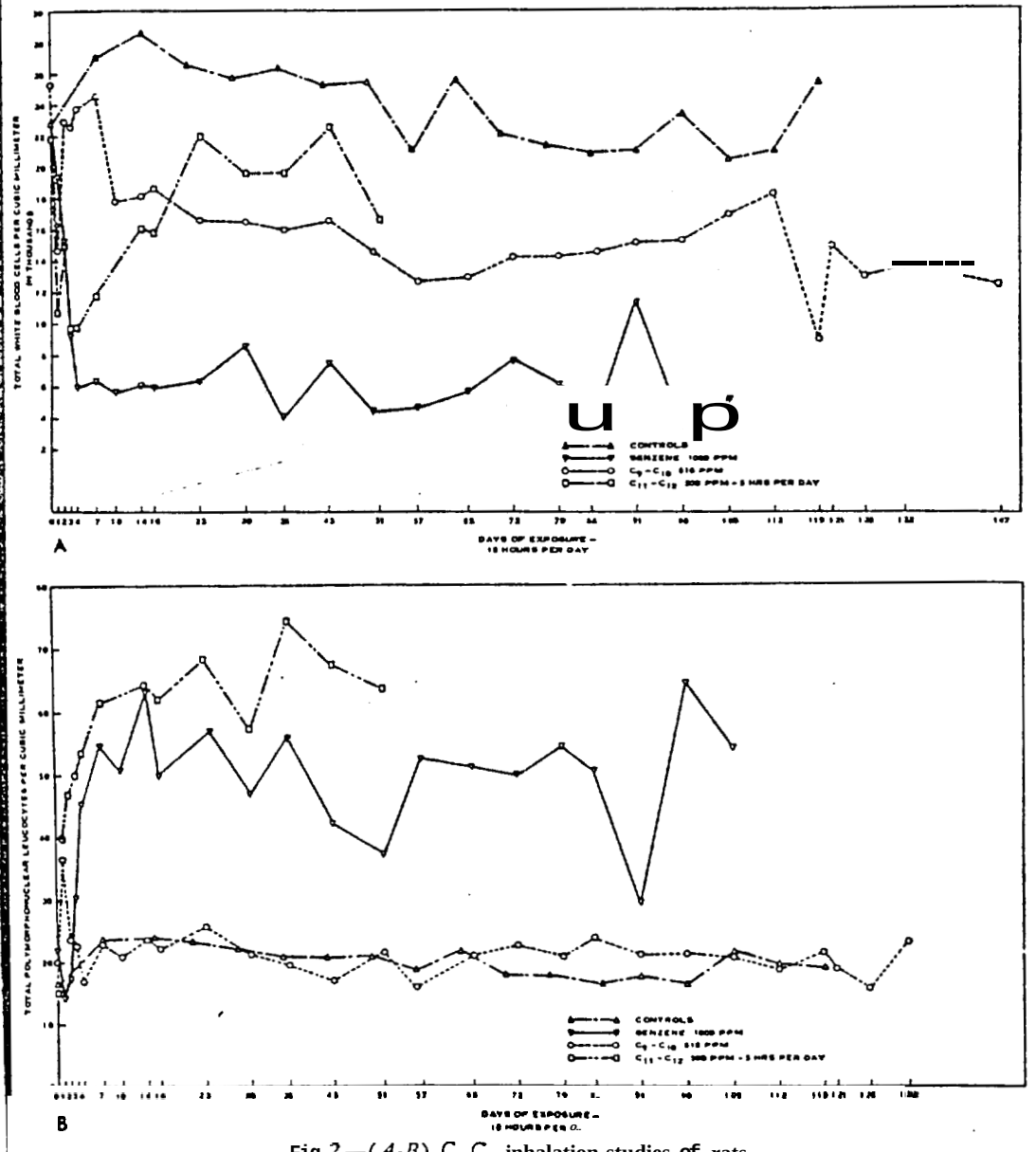


Fig 2.—(*A-B*) C_9-C_{12} inhalation studies of rats.

hematocrit readings and a shift in the polymorphonuclear-lymphoctyte ratio.

Inhalation Studies Using Rats.—C_{11}-C_{12}: Vapor Concentration of C_{11}-C_{12}, 500 PPM. Rats exposed 18 hours per day, seven days per week, did not tolerate such an exposure. Fifty percent of the rats exposed were dead at the end of the first 18 hours of exposure. Their weight loss was impressive. The organs were all engorged with blood, the spleen was small, and the intestines were filled with a liquid and a gaseous material.

When the exposure time was reduced to eight hours per day, seven days per week, the rats deteriorated rapidly (within 15 days) in appearance and weight loss. Postmortem changes noted included a small spleen, hemorrhagic lungs, and liver, and the intestines filled with a bloody liquid and gaseous material.

When the exposure time was reduced to five hours per day, seven days per week, the rats developed a dull and coarse hair (which tended to fall out), emaciation, and vesicles around the eyes, nose, and feet, They appeared narcotized. There was a

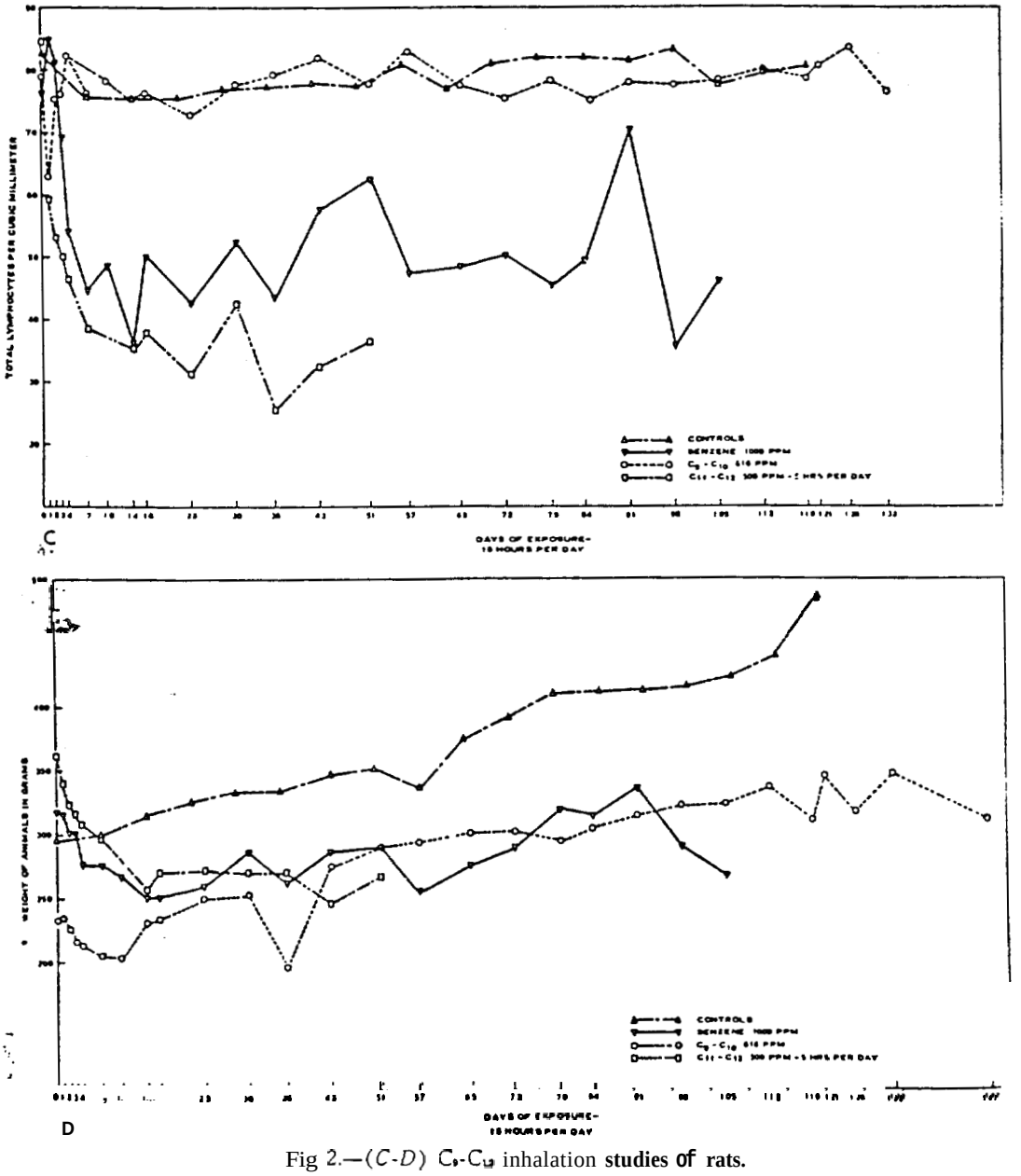
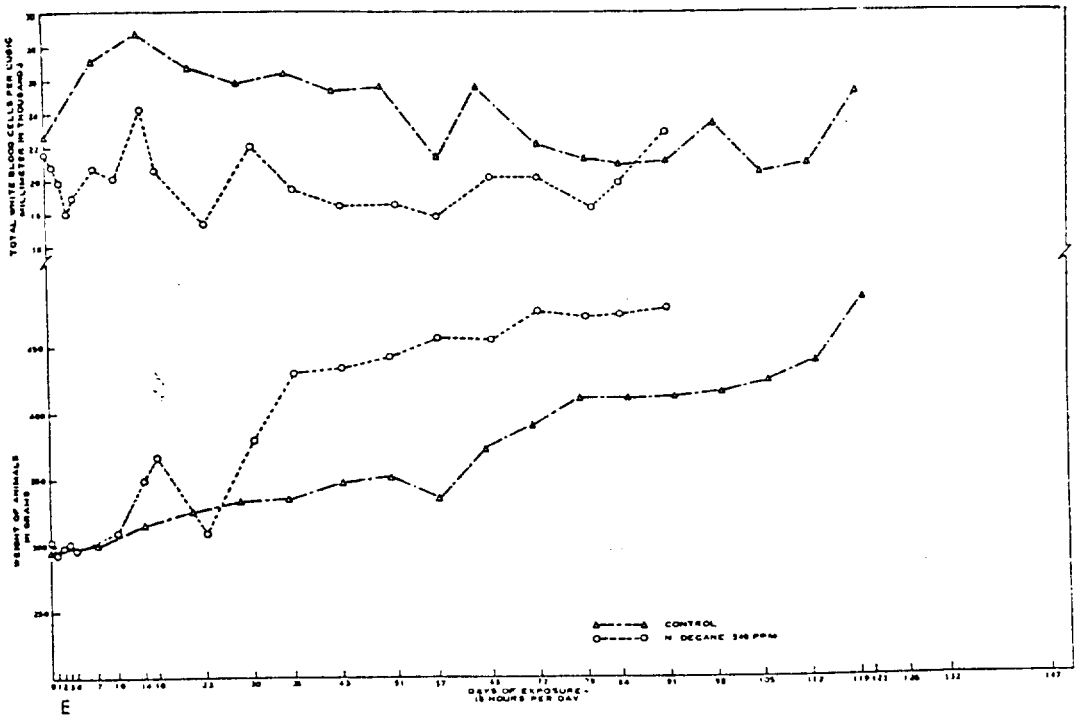


Fig 2.—(*C-D*) C_9-C_{12} inhalation studies of rats.

marked fall in the WBC, a reversal of the polymorphonuclear-lymphocyte ratio, and a very slight impairment of the expected weight gains.

When these rats were exposed as long as 1,683 hours the WBC and DNA decreased. Expected body weight gains were not observed; the spleens were small and the femurs appeared soft; the bone marrow was "watery." No cataracts were noted. Rats set aside for four months after the 1,683 hours of exposure appeared to make a good recovery.

Vapor Concentration of C_{11}-C_{12}, 200 PPM. Rats exposed for eight hours per day, five days per week, for 90 exposures developed a definite and gradual fall in the WBC and the expected body weight gains.



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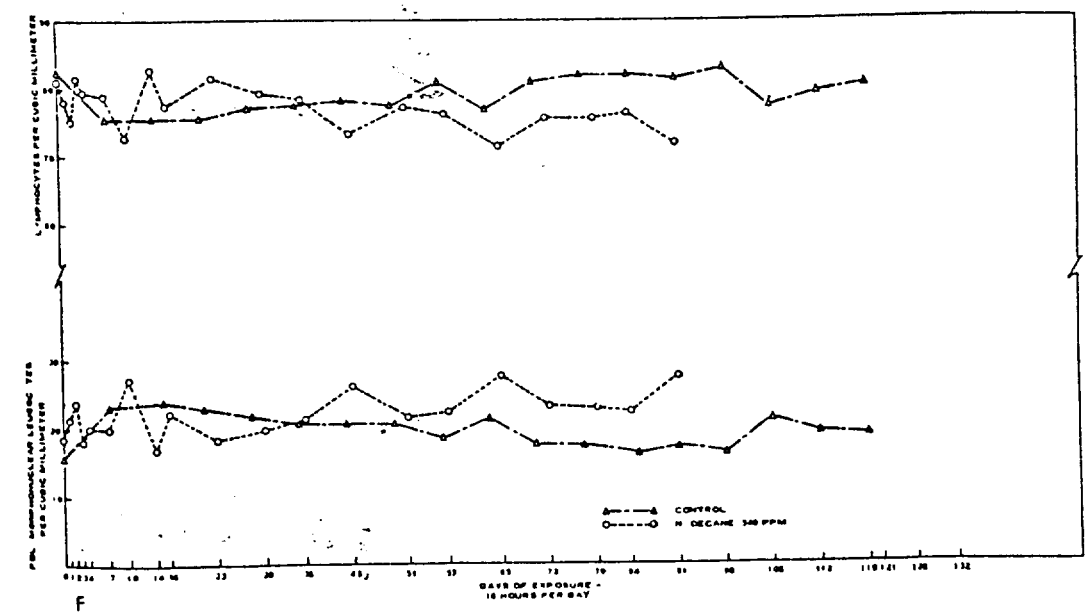

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Fig 2.—(*E-F*) C_9-C_{12} inhalation studies of rats.

TABLE 3.—*Growth and Hematological Changes in Rats Following Inhalation of Test Vapors**

Animal Croup	Preexposure Period No.	Mean Value	SD	End of 57 Days No.	Mean Value	SD	End of 105 Days No.	Mean Value	SD
Control animals									
Total leukocytes	20	22,760	3.060	20	21,055	1.610	17	20,510	2,120
Poly leukocytes	20	16	5	20	18	8	17	22	7
Total lymphocytes	20	82	6	20	81	9	17	77	6
Animal weight	20	295	33	20	335	94	17	423	23
Benzene animals									
Total leukocytes	14	22,650	750	12	4,625	2,045	6	5,425	4,440
Poly. leukocytes	14	22	9	12	52	16	6	54	13
Total lymphocytes	14	77	9	12	47	16	6	46	13
Animal weight	14	318	21	12	254	39	6	266	42
C_9-C_{10} animals									
Total leukocytes	30	25,380	1,685	28	12,680	1,190	26	16,920	1,103
Poly. leukocytes	30	20	8	28	16	5	26	21	7
Total lymphocytes	30	79	8	28	63	5	26	78	7
Animal weight	30	233	20	28	294	27	26	323	20
$C_{11}C_{12}$ animals									
Total leukocytes	25	21,790	1,940	5	16,570	5,000	Animals sacrificed		
Poly. leukocytes	25	15	10	5	64	7	because of poor health.		
Total lymphocytes	25	84	11	5	36	"			
Animal weight	25	36?	47	5	266	26			
							End of 91 Days		
Decane animals									
Total leukocytes	43	21,490	730	31	17,765	1,600	18	22,815	1,530
Poly. leukocytes	43	18	8	31	22	E	18	28	14
Total lymphocytes	43	81	8	31	76	6	IS	72	14
Animal weight	43	301	40	31	456	26	1s	476	35

* Benzene. 1,000 ppm; C_9-C_{10}, 616 ppm; C_{11}-C_{12}, 500 ppm; n-decane, 540 ppm.

One rat out of 17 developed a cataract. Examination of the bone marrow of the exposed rats revealed stimulation of erythrocytic activity and delayed depression of myelocytic activity.

Vapor Concentration of C_{11}-C_{12}, 50 PPM. Rats exposed eight hours per day, five days per week, for 90 exposures developed some very slight interference in the espected body weight gain; the DNA fell slightly. After having been set aside for observation for four months without further exposure, these same changes were observed. Bone marrow changes noted were the same as those observed in rats exposed to 200 ppm of C_{11}-C_{12}.

Inhalation Studies Using Monkeys.—C_{11}-C_{12}: Vapor Concentration of C_{11}-C_{12}—200 PPM. Four Rhesus monkeys exposed seven hours per day, five days per week (90 exposures) showed evidence of irritation on the face and in the eyes. Diarrhea was noted on the second or third day of exposure. There was no change in the expected body weight gain. There was, however, a reversal of the polymorphonuclear-lymphocyte ratio and the bone marrow showed evidence of a stimulation of erythrocytic activity arid depression of myelocytic activity.

Vapor Concentration of C_{11}-C_{12}, 50 PPM. Four monkeys exposed seven hours per day, five days per week, for 90 exposures developed diarrhea on the third day of exposure

TABLE 4.—*Significance of Growth and Hematological Changes in Rats From Inhalation of Test Vapors**

Blood Element	57 Day Exposure Period C vs B	C vs A.	C vs A_s	C vs D	Complete Exposure Period C vs B	C vs A_s	C vs A.	C vs D
Total leukocytes	—HS	—HS	—HS	—HS	—NS	—HS	NO	+HS
Poly. leukocytes	+HS	NS	+HS	NS	+HS	NS	Surviving	NS
Tot31 lymphocytes	—HS	NS	—HS	NS	—HS	NS	Animals	NS
Animal weight	—	NS	NS	+HS	—HS	—HS		+HS

*HS: P 0.001
S: P 0.01
NS: P 03
+ Increase in function
— Decrease in function

A. = C_9-C_{10} alkyl aromatic fraction (616 ppm)
A_s = C_{11}-C_{12} alkyl aromatic fraction (500 ppm)
C = Control animals
B = Benzene exposed animals (1,000 ppm)
D = N-decane exposed animals (540 ppm)

TABLE 5.—*Acute LC_{50} in Rats Weighing 150 Gm Each (96 Hours Waiting Period)*

Material	Total No of Rats Used	No. of Rats per Each Test Group	Exposure Time (Hr)	PPM V/V	Deaths in No. of Hours	
Benzene	70	10	1	6980	70 (7)	
C_9-C_{10}	70	10	7	2770	4 (7)	5 (24)
C_{11}-C_{12}	70	10	1	713	0 (7)	1 (96)

and irritation of the face and eyes. There was a reversal of the polymorphonuclear-lymphocyte ratio, a stimulation of erthrocytic activity of the bone marrow, and a depression of the myelocytic activity.

Inhalation Studies Using N-Decane.—N-Decade: Vapor Concentration of N-Decane, 540 PPM: Rats exposed to this concentration of n-decane by inhalation 18 hours per day, seven days per week showed a significant positive effect on the expected weight gains and a significant fall in the total WBC after 57 days of exposure. After 123 days of exposure there was still an increase in the expected weight gain and also an increase in the total WBC. There was no change in the polymorphonuclear-lymphocyte ratio at either of these times nor were there bone marrow changes or gross or microscopic organ changes of significance. Some rats set aside without additional exposure for one month showed no changes from the normal.

Some of the hematological findings in rats exposed to the various materials named are shown in Fig 2, *A-F*, and Tables 3 and 4.

It was noted that with benzene all rats which died were found dead at the end of seven hours of exposure (Table 5).

With C_9-C_{10} only four were dead at the end of the seven hours of exposure and one more died within 24 hours after the exposure ended.

With C_{11}-C_{12} no rats were dead at the end of the seven hours of exposure; one died within 24 hours after the exposure ended, and four more died within 96 hours after exposure ended.

TABLE 6.—*Acute LC_{50} in CFW Weighing 27 Gm Each*

Material	Total No. of Mice Used	No. of Mice in Each Test Group	Exposure Time (Hr)	PPM
Benzene	33	10 plus	3¾	13,100
C_9-C_{10}	50	10 plus	3¾	1,900
C_{11}-C_{12}	52	10 plus	3¾	526
N-decane	25	10 plus	3¾	540

As with rats, it was noted that with benzene all mice which died were found dead at the end of the 3¾ hours of exposure (Table 6).

With C_9-C_{10} four mice were dead at the end of the 3¾ hours of exposure. Fourteen more died within the next four hours and five additional mice died within 24 hours after the exposure ended.

With C_{11}-C_{12} no mice were dead at the end of the 3¾ hours of exposure. Nine died within the nest four hours after the exposure ended. Thirteen additional mice died within 24 hours after the exposure ended and four additional mice died within four days after the exposure ended.

With n-decane no mice were dead when the 3¾ hours of exposure ended. None died within the next 24 hours but four mice died before the end of the fourth day after the exposure.

The data for LC_{50} for rats and mice are similar showing the immediate effects of benzene and the delayed or cumulative effects of C_9-C_{10} and C_{11}-C_{12} (Table 7).

Recovery of Aromatics From the Blood of Rats Exposed by Inhalation *

For this study C_9-C_{10} and benzene were used in concentrations varying from 200-2,000 ppm for C_9-C_{10} and from 1,000-4,000 ppm for benzene. The blood of the exposed animal was extracted with spectrograde of iso-octane and the optical density (using the Beckman Du Spectrophotometer) was read. A review of the data reveals that the concentration of these hydrocarbons in rats' blood increased as the concentration of these materials in the inhaled air increased; also, that the blood levels of these materials fall

TABLE 7.—*The Relative Acute Inhalation Toxicity of These Hydrocarbon Vapors in Rats and Mice*

	Rats	Mice
Benzene	1	1
C_9-C_{10}	1.5	4.2
C_{11}-C_{12}	5.0	14.0

very rapidly after the exposure by inhalation ends.

It was possible to recover from 90% to 100% of these aromatics in the blood.

Skin Application

Benzene-C.P., C_9-C_{10}, C_{11}-C_{12}, and n-decane-CP were applied individually to the skin of 20 male C_3H mice, using a one-fourth inch brush and making one stroke up the middle of the mouse's back from the base of the tail to the base of the neck. About 0.10 to 0.15 gm of material was applied three times each week on alternate days. About 150 applications were made per mouse. The mice were observed closely for detection of any gross changes from the normal, and at the end of one year they were killed. A postmortem evaluation was done and all organs and tissues were examined microscopically.

Our findings were as follows:

A. Benzene - - mice (11.01 gm applied per mouse)

1. Pathology

(a) Gross—no detectable changes

(b) Microscopic

Skin:	Dermal fibrosis	40%
	Edema	20%
	Pigmentation	20%
	Hyperplasia	10%
Kidney:	Focal chronic inflammation	5%
Lungs:	Bronchopneumonia and bronchitis	5%
Spleen:	Lymphoid hyperplasia	10%
	Lymphocytopoiesis	5%
	Metaplasia	20%
	Pigmentation red pulp	5%

2. Hematology—no significant changes from the "normal" total WBC differential count were noted.

3. Expected weight gain—no effect

B. C_9-C_{10} fraction—79 mice (10.62 gm applied per mouse)

1. Pathology

(*a*) Gross—skin thick, dry, scaly. High incidence of lobulation oi kidney.

(*b*) Microscopic

Skin:	Hyperkeratosis	31%
	Atrophy of epidermis	24%
	Inflammatory reaction	41%
	Perikeratosis	23%
	Ulceration'	25%
Lungs:	Focal hemorrhage	12%
	Pigmentation	10%
	Inflammatory reaction	16%
	Atelectasis	7%
Spleen:	Amyloidosis	57%
Liver:	Focal. areas of necrosis	7%
	Amyloidosis	3.5%
Kidney:	Cortical scarring	35.5%
	Sclerosis	27%
	Necrosis of renal papilla	12%

2. Hematology—WBC increased gradually until at termination. it was double the initial count. The ratio of lymphocytes to polymorphonuclear leukocytes was variable but there was a relative increase in the number of polymorphonuclear leukocytes.

C. C_{11}-C_{12} fraction—85 mice (16.79 gm applied per mouse)

1. Pathology

(a) Gross—skin thick. dry and scaly. Higi: incidence of lobulation of the kidney (less than with C_9-C_{10}).

(b) Microscopic

Skin:	Hyperkeratosis	30%
	Perikeratosis	16%
	Atrophy of epidermis	29%
	Inflammatory reaction	23%
	Ulceration	6%
Kidney:	No significant findings	
Lungs:	Atelectasis	3%
	Focal hemorrhage	7%
	Pigmentation	17%
	Inflammation	17%
Liver:	Necrosis	16%
Spleen:	Amyloidosis	7%

2. Hematology—the WBC increased with increasing amounts of inaterial applied. The "poly" count increased slightly as painting was started, dropping back to its initial value and remained constant as the painting was continued to termination.

3. Expected weight gain—was impaired.

D. N-decane—65 mice (16.33 gm applied per mouse)

1. Pathology

(*a*) Gross—skin thick, dry, scaly. No other changes.

(b) Microscopic

Skin:	Fibrosis of dermis	43%
	Pigmentation	29%
	Ulceration	18%
	Hykerkeratosis	7%
	Perikeratosis	7%
Kidney:	Hemorrhage	33%
	Pigmeritation	33%
	Inflammation	18%

[1] Material supplementary to this article (Tables 8.13 and Fig 3 and 4) has been deposited as Document number 8721 with the ADI Auxiliary Publications Project. Photoduplication Service, Library of Congress. Washington 25, DC. A copy may be secured hy citing the Document number and by remitting f2.50 for photoprints. or $1.75 lor 35 mm microfilm. Advance payment is required. Make checks or money orders payable to: Chief. Photoduplication Service. Library of Congess.

Spleen:	Amyloidosis	10%
Lungs:	Hemorrhage	55%
	Pigmentation	24%
	Inflammation	28%
	Necrosis	11%

2. Hematology—No significant changes from the normal were observed.
3. Expected weight gains—no effect.

E, Controls—764 mice (no chemicals applied to the skin)

1. Pathology
   (a) Gross—no detectable changes
   (b) Microscopic (757 animals read)

Skin:	Dermal fibrosis	3.1%
	Edema	1.4%
	Pigmentation	0%
	Hyperplasia	0.7%
	Hyperkeratosis	1.6%
	Atrophy of epidennis	0.9%
	Inflammation	0%
	Perikeratosis	0%
	Ulceration	0%
Kidney:	Cortical scarring	3.8%
	Atelectasis	0%
	Amyloidosis	0%
	Focal chronic inflammation	18%
Lungs:	Bronchopneumonia	4.5%
	Bronchitis	0%
	Focal hemorrhage	0%
	Pigmentation	0%
	Inflammatory reaction	0%
	Atelectasis	0%
Spleen:	Lymphoid hyperplasia	3.4%
	Lymphocytopoiesis	0%
	Metaplasia	3.5%
	Pigmentation red pulp	3.7%
	Amyloidosis	1.7%
Liver:	Amyloidosis	0.5%
	Necrosis	070

2. Hematology—the WBC in nonexposed mice increased with increasing age (over a period of nine months). During this period there was no change in the differential count.
3. Expected weight gain—normal.

Subcutaneous Injection of Rats With C_9-C_{10}

The subcutaneous injection of 2.5-5.0 cc of C_9-C_{10} suspended in an equal amount of cottonseed oil into rats (Tables 11 and 12)† produced results which were not consistent. Often there was a "slough" formed at the site of the injection. This may have been preceded by extensive inflammatory changes and an effort to "wall off" the injected material by the formation of fibrous tissue. There may be an increase or a decrease in the total WBC count, or no change. The same effects were noted in rats injected subcutaneously with cottonseed oil alone.

Thirty male Wistar rats weighing 300 gm each were injected daily subcutaneously in the right and left groin for 14 successive days using an equal mixture of C_9-C_{10} and cottonseed oil with each injection (0.3 cc).

At the end of the eighth day (total injection being 2.4 cc of the mixture) two rats were killed. The WBC, hematocrit reading, and weight were determined before injections were started and when the animals were killed. There was a slight increase in the hematocrit evaluation of the rats killed and some increase in the WBC. This latter may have been the result of the irritation and inflammation.

At the end of the 14th day of the injection (total injection being 4.2 cc of the mixture) five more rats were killed. Again there was an increase in the WBC and the detection of 17.S ppm of C_9-C_{10} in the rats' blood.

Injections were terminated after the 14th day. Rats were killed on the 18th, 22nd, 31st, 37th, and 46th days. The WBC was increased in each case and the femur of the rats, killed on the 18th, 22nd, and 31st days, was found to be fragile as compared to the femur of control rats. For a period of time the fur of the experimental rats appeared coarse and discolored. A return to normal was noted as the last rats were killed. It was also noted that after the second or third day of injection the rats developed a urethral discharge which cleared up after two or three days. The urine in the urinary bladder appeared clear and a routine urinalysis of bladder urine revealed no significant abnormality. Also, the expected weight gain of the injected animals was slightly impaired.

The amount of C_9-C_{10} found in the rats' blood as injections were continued did not increase. This map have been due to an effective "walling off" as a result of the irritation, or the development of a method of detoxification, or of the metabolism of the injected material.

† Tables 11, 12, and 13 have been omitted and will be deposited with the ADI Auxiliary Publications Project.

Discrete pockets of the injected material were noted when post mortems were done.

A larger dose of an equal mixture of C_9-C_{10} and cottonseed oil was then injected subcutaneously in 12 male Wistar rats weighing 215-220 gm. A total of 2.5 cc of the mixture was injected daily. Four rats were killed 24 hours after 5 cc of the mixture was injected; four were killed 24 hours after 7.5 cc were injected: and four were killed 24 hours after 10 cc of the mixture were injected.

All rats injected showed an increase in the WBC prior to killing. There was a loss in weight of the last group of four killed. The second group of four rats killed showed some increase in the blood level for C_9-C_{10}. This value fell significantly in the blood of the third group of four.

DNA values for these rats were significantly increased as compared to control rats (Table 11).

This experiment was repeated using 16 female Wistar rats weighing 310 gm. It is noted that there was in the injected rats:

1. An increase in the WBC
2. A decrease in hematocrit values
3. Blood levels for C_9-C_{10} in the injected animals reached a peak of 11.87 ppm anti then fell off even though injections were continued. This, as previously suggested, may be due to an effective walling off or an effective method of metabolism (Table 12).

When rabbits or monkeys were injected subcutaneously with an equal mixture of cottonseed oil and C_9-C_{10} (20-80 cc) there was a distinct fall in the IVBC (Table 13).

Microscopic bone marrow studies revealed that:

1. With the smaller amounts of C_9-C_{10} injected repeatedly subcutaneously there was a stimulation of the myelocytic series reflected by an increase in the myelocytes arid "blast" cells. There was evidence of a depression of the erythrocytic series as reflected by a reduced number of normoblasts.
2. With the larger amounts of C_9-C_{10} injected repeatedly subcutaneously the changes noted above were more evident. Stimulation of the myelocytic series was revealed by an intrease in the number of (*a*) "stabs", (*b*) Juveniles, (c) myelocytes, and (*d*) "blast" forms. There was also a more significant reduction in the normoblast. This was comparable to the findings in rats inhaling C_9-C_{10}.

Odor Detection by Humans

The determination of odor detection was done in the Laboratory of Industrial Hygiene, Eastman Kodak Company, Rochester, NY, using the following method.

A total of 1 cc C_9-C_{10} was diluted with 100 cc of methyl alcohol, which dilution was then put into an air blender. The air flow through the blender was varied until a definite odor was perceived by the observer.

C_9-C_{10} has a minimum identifiable odor level of 11 mg/cu in. Assuming a molecular weight of 125, this level oi odor has a concentration of 2 ppm. Similarly, the minimum identifiable concentration oi C_{11}-C_{12} was found to be 2.5 mg/liter or 0.5 ppm on the basis of an assumed molecular weight of 150.

The minimum concentration which produced lacrimation and/or irritation of the eyes, skin, and mucous surfaces in humans was found to be 25 ppm for C_9-C_{10} and 19 ppm for C_{11}-C_{12}.

Conclusions

We believe that, under the conditions of our experiments, with the animals used the following conclusions are warranted:

1. The inhalation of C_9-C_{10} and C_{11}-C_{12} alkyl aromatics in a concentration oi 3.2 mg/liter (1,000 ppm for benzene, 616 ppm for C_9-C_{10}, and 500 ppm for C_{11}-C_{12}) leads to significant changes from the normal in rats (Table 4), but does not produce the myelotoxic effects characteristic of benzene.

2. The inhalation by monkeys of 50 or 200 ppm of C_9-C_{10} or C_{11}-C_{12} for 90 exposures of seven hours each led to skin irritation. a reversal of the polymorphonuclear-lymphocyte ratio, depression oi the myelocytic and stimulation of the erythro-

cytic activity of the bone. There was a pronounced early tremor and a fall of the WBC in those monkeys exposed to C_9-C_{10}.

3. The inhalation of n-decane in a concentration of 3.2 mg/ liter (560 ppm) leads to significant changes from normal insofar as total WBC and expected animal body weight gains are concerned. The blood changes are in the opposite direction to those produced by benzene, C_9-C_{10} and C_{11}-C_{12} (Table 4).

4. The ratio of the acute inhalation toxicity in rats (LC_{50}) for benzene, C_9-C_{10}, and C_{11}-C_{12} on the basis of equivalent Exposure times is 1:1.5:5. In mice this ratio is 1: 4.3:14.

5. The threshold of odor detection by humans is 2 ppm for C_9-C_{10} and 0.50 ppm for C_{11}-C_{12}, whereas the minimum concentration producing irritation to the eyes and mucous surfaces in humans was 25 ppm for C_9-C_{10} and 19 ppm for C_{11}-C_{12}.

6. These aromatics can be recovered quantitatively from the blood of rats inhaling them or into which subcutaneous injections have been made (using a 1:1 mixture in cottonseed oil).

7. These materials disappear rapidly from the blood of a rat which had ceased to inhale them.

8. Skin application of C_9-C_{10}, or C_{11} C_{12}, leads to pathological changes in the skin and internal organs of greater intensity and severity than that produced by benzene or normal decane.

9. The results of subcutaneous inoculation of these materials is not consistent with the inhalation toxicity or percutaneous toxicity of the same compounds.

10. It is suggested that a tentative concentration of 50 ppm for the C_9-C_{10} alkyl aromatic fraction and 25 ppm for the C_{11}-C_{12} alkyl aromatic fraction of the approximate composition used in this study be used as TLV values for eight hour inhalation exposures. Naphthas of greater or lesser aromatic content might be assigned correspondingly smaller or larger TLV values. These are not difficult to maintain due to the low volatility of these hydrocarbons at usual atmospheric temperatures.

11. These tests also suggest that eye contact and percutaneous exposures to these liquid hydrocarbon fractions be carefully avoided by the use of protective shields and clothing.

Summary of Findings

On the basis of our studies the alkyl aromatics present in these catalytic reformate fractions do not evert any myelotoxic effects characteristic of benzene. Exposure to these compounds by inhalation does lead to significant hemotologic changes which, however, are reversible if the exposure is terminated early enough. In terms of acute vapor exposure, the fraction richer in higher molecular weight aromatics is more toxic than the lower molecular weight aromatic fraction, but both fractions are more toxic than benzene or normal decane.

Percutaneous exposure repeatedly to these aromatic fractions may lead to significant systemic changes as well as to ulceration and atrophic changes of the skin.

Exposure of the eye to vapors of these fractions is irritating; liquid contact may lead to scar formation. Prolonged exposure may lead to the formation of cataracts.

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