

FYI-0794-000942



HASKELL LABORATORY

(A)

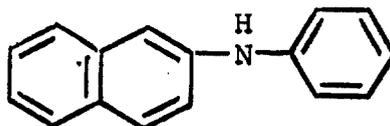
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This review reflects the available toxicity literature, both published and unpublished. Studies have not been evaluated for scientific merit.

Common Name: Phenyl β-Naphthylamine
Chemical Name: 2-Naphthalenamine, N-phenyl-
Synonyms: Neozone® D
PBNA

CAS Registry No.: 135-88-6

Chemical Structure:



84940000042



FYI-94-000942
INIT 07/26/94

Physical Properties:

Description:	Gray, non-dusting powder
Molecular Weight:	219.29
Boiling Point:	395-399.5°C @ 760 mm Hg
Melting Point:	106°C
Density/Specific Gravity:	1.24 (25/4°C)
Vapor Pressure:	13 mm Hg @ 237°C
Flash Point/Flammability:	300°F
Explosive Limits:	—
Solubility:	Insoluble in water Soluble in alcohol, ether, benzene and acetic acid

Conversion Factors: —

Exposure Standards:

None

DOT Classification:

None

EPA RCRA Status:

None

mm
9/25/95

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FDA Status:

- PBNA is cleared under 21 CFR for the following food-related uses:

Phenyl- β -naphthylamine free of β -Naphthylamine is cleared under §175.105 (adhesives).

Cleared as an antioxidant in dry rosin size, at a level not to exceed 0.4% by weight, under §176.170 (components of paper and paperboard in contact with aqueous and fatty foods) and §176.180 (components of paper and paperboard in contact with dry food).

Cleared as an antioxidant or antiozonant under §177.2600 (rubber articles intended for repeated use); total antioxidants and antiozonants are not to exceed 5% by weight of the rubber product.

Cleared under §178.3570 (lubricants with incidental food contact), alone or in combination with phenyl- α -naphthylamine, as an antioxidant in mineral oil lubricants, at a level not to exceed 1% by weight of the mineral oil.

TSCA Inventory:

Yes

TOXICITY

A. Acute

1. Oral

- LD50 (mice) = 1450 mg/kg (17).
- LD50 (rats) = 8730 mg/kg (17).

Effects: acute vascular changes in liver, lungs, and brain due primarily to venous congestion (17).

2. Skin

- Neozone® D was not corrosive in the standard DOT test (46).
- Neozone® D produced no irritation after being held on the skin of rabbits for 24 hours (46).
- Slight local irritation was observed in rabbits (17) and mice (19).
- PBNA has been reported to cause allergic skin reactions in workers (3,18,26,29,38,42).
- Of 221 cases of industrial dermatitis, 6 were positive to PBNA (31).

3. Eyes

- Neozone® D produced mild conjunctival irritation with no corneal or iritic effects when instilled into the rabbit eye (46).
- PBNA produced slight hyperemia in the rabbit eye (17).

4. Inhalation

No information available.

5. Intraperitoneal Injection

- LD50 (laboratory animals) = 8 mg/kg (25).

Effects: lesions of central nervous system (25).

B. Extended Studies

1. Oral

- Rats fed 1740 mg/kg/day (1/5 LD50) for 1 month became lethargic and exhibited pathological liver changes and an increase in methemoglobin (17).
- At levels of 20-200 mg/kg/day for 6-12 months Neozone® D exhibited significant cumulative properties in rats (35).
- Daily administration of 1 or 20 mg/kg from day 1 to 18 of pregnancy altered the frequency of malignant tumors in mouse offspring from 8 to 5-22%. The disturbance of water-salt metabolism in the embryos by 20 mg/kg caused a transient decrease in the weight of newborn mice (30).
- Rats receiving 1% Neozone® D in the diets for 71 weeks exhibited kidney damage. No bladder tumors were observed (22).
- Two strains of mice, 36 to a group, were administered 464 mg/kg/day for 21 days followed by administration of a diet containing 1206 ppm of PBNA for 18 months. The incidence of hepatomas, was statistically significant in male mice of one strain only. The incidence of other tumors was not compound-related (15,16).
- Daily doses of 100 mg/kg for 18 months in rats produced an increase in lung weights after 1 month and an increase in liver weights after 12 months. Changes in the gastrointestinal tract and in urinary function were also observed. In this test a level of 20 mg/kg caused no significant toxic effect (36).
- One dog fed 50 mg/kg/day for 13 months developed adrenal damage and voluntary muscle necrosis. Another dog receiving 100 mg/kg/day for 45 months exhibited cirrhosis of the liver. Bladder tumors were not observed in either animal (36).
- Three dogs were fed 540 mg/kg/day, 5 days a week for from 50-54 months. Two of the dogs were healthy at the time of sacrifice after 54 months on test. The third dog was sacrificed after 50 months following an intestinal hemorrhage which probably was secondary to an old cirrhosis of the liver (10,46).

- No compound-related tumors were seen in hamsters administered 37.5 mg/kg twice weekly for their lifetime (13).

2. Inhalation

- Rats exposed to an aerosol of 0.9 mg/L 2 hours/day for 14 days exhibited a decrease in weight, hypo- and hyperexcitability, tachypnia, slight erythrocytopenia, hyperemia of internal organs, pulmonary emphysema, and slight dystrophic changes in kidneys, liver, brain, and spleen (19).
- Exposure of rats to 0.73 or 4.23 mg/m³ or 5-6 hours/day for 4 months caused an increased ascorbic acid requirement (6).
- Inhalation of 0.012 mg/L PBNA plus 0.339 mg/L 1,3-butadiene 4 hours/day for 1 month caused leucopenia and respiratory tract irritation in mice (39).
- Experiments on rats established that the light-colored ingredients of the rubber mix most commonly used in tire production (zinc oxide, S, altax, captax, thiuram, Neozone[®] D, sulfenamide) are low-toxicity substances not having pronounced cumulative properties and not causing intoxication during long intake into the organism. Despite the absence of pronounced toxic properties the light-colored ingredients in combination with carbon black in the form of dust of a mixed composition have fibrogenic properties which lead to occupational diseases of the lungs (34).

3. Subcutaneous Injection

- Repeated injection of 5 mg crystals or 0.25 cc of a 5% suspension of technical grade phenyl-beta-naphthylamine produced no tumors in mice within 22 months (32).
- Two strains of mice, 36 to a group, were administered a single 464 mg/kg injection and observed for up to 80 weeks. In females of the first strain the frequency of tumor-bearing animals was significantly greater in comparison with control animals. There was also a statistically significant increase in the frequency of hepatomas in males of the second strain (15,16).

4. Intraperitoneal Injection

- Neozone® D administered to rats in doses of 100 and 1000 mg/kg/day for 4 months produced effects on red blood cells, liver and kidneys (41).

5. Urinary Bladder Implant

- A group of 35 mice was administered a paraffin pellet, containing 5-9 mg of technical grade PBNA, into their urinary bladder. The animals were observed until their spontaneous death, the last mouse dying 552 days after treatment. Seven mice developed bladder tumors. Another group of 18 mice was similarly administered a pellet containing pure PBNA. Three of these mice developed bladder tumors. Control groups administered the paraffin pellet only or a pellet containing 1% croton oil developed 0/14 and 3/45 bladder tumors, respectively. For comparison purposes the following amines were also studied.

<u>Compound</u>	<u>Bladder Tumors/ Total Number of Mice</u>
Methyl cholanthrene	9/25
4-Aminobiphenyl	8/35
α -Naphthylamine (technical)	13/34
β -Naphthylamine	9/30

(46)

C. Carcinogenic Potential

- Results of an experiment by oral administration in mice, indicate a statistically significant increase in the incidence of all tumors, and in particular of hepatomas, in males of one of the two tested strains. In addition, subcutaneous administration of this compound produced a significant increase in the total incidence of tumors in females of one strain and of hepatomas in males of the other strain (15,16). Oral administration to pregnant mice increased the frequency of malignant tumors in the offspring (30).
- No bladder tumors have been observed in rats (22, 36) or dogs (10,36,46) administered PBNA orally.

- An increased incidence of bladder tumors was observed in mice given bladder implants (46)
- No increase in the incidence of tumors was observed in hamsters administered PBNA orally (13).
- Repeated subcutaneous administration produced no tumors in mice within 22 months (32).
- A NCI-sponsored feeding test in rats and mice is underway at Litton-Bionetics (12).
- No evidence arises from several epidemiology studies to associate an increased incidence of bladder cancer with exposure to PBNA (9,14,20,28,44). In another study of workers who had no exposure to beta-naphthylamine an increased risk of bladder cancer was shown. Exposure was mixed but probably included PBNA (7,8).

D. Mutagenic Potential

- PBNA was not mutagenic in the Ames test in the presence of an activation system prepared from the liver of hamsters and mice (4).
- PBNA was not mutagenic in the Ames test using Salmonella typhimurium strains TA 1535, TA 1538, TA 98 and TA 100 and a S-9 activation system (9).
- PBNA was not mutagenic towards Salmonella typhimurium strains TA 98 and TA 100. When present in combination with norharman, it did show mutagenic activity (33).
- PBNA was not mutagenic in a mammalian cell transformation bioassay (40).
- PBNA was not active in an implant test with mice (24).
- PBNA was active in the degranulation bioassay using an isolated liver rough endoplasmic reticulum preparation incubated with aflatoxin B₁ (21).
- PBNA was active in the sebaceous-gland suppression bioassay in mice (23).
- PBNA was active in the tetrazolium-reduction bioassay with mice (45).

- See Related Reference 47 for additional information.

E. Embryotoxic Potential

No information available.

F. Other Reproduction Studies

- Reproductive function was impaired in rats fed 100 mg/kg/day for up to 18 months (36).

G. Aquatic

- Fathead minnows were exposed to 52.1 µg/L of PBNA for 32 days to determine its bioconcentration potential. The bioconcentration parameter a constant of proportionality between the concentration of the chemical in the fish and in the water was determined to be 0.3. This value indicates little potential for bioconcentration (43).

H. Human Exposure

- N-Phenyl-2-naphthylamine has been reported to produce leukoplakia, acne, and hypersensitivity to sunlight in 36 Polish workers (37). Other studies have not reported chronic effects (20). See also Section I (Epidemiology).

I. Epidemiology

- Mortality among 40,867 workers at a number of UK rubber and cable factories was studied. In a large group of workers not exposed to known carcinogenic materials; 33 deaths from bladder cancer occurred versus 22.7 expected at national rates, a statistically significant excess. This group of workers would have had mixed exposures to many rubber additives, but it may be assumed that a considerable number of men would have been exposed to PBNA. No evidence is found of a continued excess risk of bladder cancer in people who entered the industry after 1949 (7,8).
- In 1949, it was reported that there was an abnormal incidence of papilloma of the bladder among rubber workers and that the responsible agent appeared to be the antioxidant known as Nonox® S. Nonox® S was manufactured by a condensation reaction of paraldehyde with mixed alpha- and beta-naphthylamines; it contained about 2.5% unreacted naphthylamine, of

of which 2.25% was the alpha isomer and 0.05% was the beta isomer. Further investigation revealed that occupational cases do not make a major contribution to the frequency of bladder cancer and that there is no evidence that exposure to phenyl-beta-naphthylamine is associated with a bladder cancer hazard. Thus, exposure in industrial conditions to products containing impurities of carcinogenic amines up to 50 ppm appears to constitute a safe dose. Nonetheless, since 1953, National Insurance benefits have been awarded to British workmen who have worked with alpha or beta-naphthylamine or Nonox[®] S and who have subsequently developed papilloma of the bladder. It must be determined whether it is reasonable to regard exposure to a chemical which is not itself carcinogenic, but which contains trace impurities of a carcinogenic amine, as constituting employment involving exposure to that carcinogenic amine (28).

- The higher risk of bladder cancer and leukemia in rubber industry workers cannot be attributed to phenyl-beta-naphthylamine, as these workers are exposed to several other chemicals, notably benzene (9).

J. Metabolism

a) Animal Studies

- In dogs given single oral doses of 5 mg/kg [1,4,5,8-¹⁴C]-N-phenyl-2-naphthylamine following repeated doses of 400 mg/animal unlabelled N-phenyl-2-naphthylamine on 5 days a week for 4 weeks, more than 90% of the radioactivity was excreted over 3 days, mainly in the feces. Only 2.8% of the radioactivity was excreted in the urine. No increased excretion of labelled 2-naphthylamine was observed following 4 weeks' pretreatment with 400 mg/animal unlabelled N-phenyl-2-naphthylamine before administration of labelled compound. Metabolites of 2-naphthylamine, e.g., 2-naphthylhydroxylamine and 2-amino-1-naphthyl sulphate, were not detected in ether extracts or lyophilized fractions of urine (5).
- The metabolism of PBNA by liver microsomes of the rat, dog and man was studied in vitro. Two major metabolites were formed by all three microsome systems. These metabolites were identified by mass spectroscopy as ring-hydroxy N-phenyl-2-naphthylamines. All three species produced small amounts of

three additional metabolites and dog and rat microsomes each produced an unique metabolite. 2-Naphthylamine was not detected among the metabolic products in any of the microsomal incubations (1).

b) Human Studies

- In 19 volunteers given 10 mg N-phenyl-2-naphthylamine containing 8 ng 2-naphthylamine (0.8 mg/kg), from 0.4-3 μ g 2-naphthylamine were found in 24-hour urine samples from 7 subjects, 6 of which were nonsmokers. In 4 workers exposed to N-phenyl-2-naphthylamine dusts (estimated intake, 40 mg), which were estimated to contain 32 ng 2-naphthylamine, 3-8 μ g 2-naphthylamine were found in 24-hour urine samples (20).
- 2-Naphthylamine was found at a level of 3-4 μ g in 24-hour samples of urine from two volunteers who ingested 50 mg N-phenyl-2-naphthylamine containing 0.7 μ g 2-naphthylamine and from workers (unspecified number) estimated to have inhaled 30 mg N-phenyl-2-naphthylamine (27).

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RELATED REFERENCE

Mutagenicity

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"Mutagenicity testing of 36 chemicals including pesticides, food additives and drugs in the Salmonella/microsome system"

Susan W. Snyder:iml
April 30, 1976

Updated by:
Linda J. Ver Nooy:md
June 20, 1978

Updated by:
Richard C. Graham:md
September 25, 1981

RC Graham

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine

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HASKELL LABORATORY REPORT NO. _____

Materials Tested:

	Haskell Nos.:	Other Codes:
1) 2-Naphthylamine, phenyl-a)	1) 7698	1) "Neozone" D
2) Thermoflex Ab)	2) 7699	2) "MOCA"
3) Aniline, 4,4'-methylene bis(2-chloro-c)	3) 7700	3) Ethylene thiourea; NA-22
4) Imicazoline, 2-mercapto d)	4) 7702	

EYE IRRITATION TEST IN RABBITS

Procedure: The amount equivalent to 0.1 ml of each chemical^{e)} was placed into the right conjunctival sac of each of two albino rabbits. After 20 seconds, one exposed eye of each pair was washed with tap water for one minute. The other exposed eyes were not washed. Observations of the cornea, iris and conjunctiva were made with a hand-slit lamp at one and four hours and at one, two and three days; 5% aqueous fluorescein stain and a biomicroscope were used at examinations after the day of treatment.

Results:

Haskell Number and Dose	Treatment of Eyes	Ocular Effects	
		Cornea	Iris Conjunctiva
7698; 44.4 mg solid	Not washed	None	None Mild redness 1 hour-1 day; minimal swelling 1-4 hours; copious discharge at 1 hour, mild to minimal 4 hours- 1 day.
7698; 44.4 mg solid	Washed	None	Minimal to mild redness, swelling and discharge 1-4 hours.
7699; 58.8 mg solid	Not washed	None	Minimal to mild redness 1 hour-1 day; mild swelling at 4 hours; minimal to mild discharge 1-4 hours.
7699; 58.8 mg solid	Washed	None	Minimal redness 1-4 hours; minimal discharge at 4 hours.

Results: (Continued)

Haskell Number and Dose	Treatment of Eyes	Ocular Effects		
		Cornea	Iris	Conjunctiva
7700; 60.4 mg solid	Not washed	None	None	Mild redness 1 hour-1 day; mild swelling 1-4 hours; copious to moderate discharge 1-4 hours, minimal at 1 day.
7700; 60.4 mg solid	Washed	None	None	Minimal redness and swelling at 1 hour; minimal discharge 1-4 hours.
7702; 28.7 mg solid	Not washed	Localized area of micro- scopic surface sheen at 1 day.	Minimal congestion at 4 hours.	Mild redness 4 hours-1 day, minimal at 2 days; mild swelling 1-4 hours, minimal at 1 day; mild to moderate discharge 1-4 hours, minimal at 1 day.
7702; 28.7 mg solid	Washed	None	None	Mild redness 1 hour-1 day; mild swelling and mild to moderate discharge 1-4 hours, minimal at 1 day.

Summary: "Neozone" D, Thermoflex A and "MOCA" produced mild conjunctival irritation with no corneal or iritic effect in rabbit eyes. A rabbit eye treated with NA-22 showed localized microscopic corneal injury and mild conjunctival irritation with no significant iritic effect. An eye dosed with NA-22 and promptly washed had only mild conjunctival irritation with no corneal or iritic effect. All of the eyes exposed to these chemicals were normal within one to three days.

Although only temporary mild ocular effects occurred, good hygienic practice dictates copious flushing with water after any eye contact with these compounds.

- a) Grade: Regular - sample is routine commercial product (probably made in 1971).
Impurities: Contains trace of β-naphthylamine.
- b) Grade: Technical. Composition: 50% phenyl-beta-naphthylamine; 25% 4,4'-dimethoxy diphenylamine;
25% N,N'-diphenyl-paraphenylene diamine. Impurities: Contains a trace of beta-naphthylamine.
- c) Grade: Commercial. Impurities: Contains < 1% ortho-chloroaniline.
- d) Grade: Commercial.

Federal guidelines specify test amount equal to 0.1 ml loosely packed, but no more than 100 mg.
H-7698 and H-7699 were ground with a mortar and pestle before doses were calculated and eyes were dosed.

Report by: Karen M. Frank

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Material Tested

N-Phenyl-2-naphthylamine *

DEPARTMENT OF TRANSPORTATION SKIN CORROSION TEST ON RABBIT SKIN

Procedure: Six albino rabbits weighing between 2-3 kg were clipped free of hair on the back and placed in FDA-type stocks. 0.5 gm of the test material (as supplied) was applied under 1.5 by 1.5 inch, 12-ply cotton gauze pads and the trunk of each rabbit was then loosely wrapped with rubber sheeting. After four hours, the rabbits were removed from the stocks, the wrapping and gauze pads were removed and any skin reactions were evaluated. The test sites were then washed. Readings were again made at 24 and 48 hours after the initial application.

Results

<u>Route of Administration</u>	<u>Number of Rabbits</u>	<u>Dose Level</u>	<u>Number of Rabbits with Skin Corrosion</u>
Skin contact	6	0.5 gm	0/6

Conclusion: According to the regulations of the Department of Transportation, § N-phenyl-2-naphthylamine is not considered a corrosive material.

§ Department of Transportation, Hazardous Materials Regulations Board, Docket No. HM-57, Federal Register, Vol. 38, No. 28, Section 173.240, February 12, 1973.

* Active ingredient 96.9%.

Report by: *Doris F. Edwards*

Doris F. Edwards

Approved by: *Charles F. Reinhardt*

Charles F. Reinhardt
Assistant Director

HASKELL LABORATORY REPORT NO. _____

Materials Submitted	Haskell Nos.	Other Codes
1) 2-Naphthylamine, phenyl-†	1) 7698	1) "Neozone" D
2) Thermoflex A‡	2) 7699	2)
3) Imidazoline, 2-mercapto-‡	3) 7702	3) Ethylene thiourea

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SKIN IRRITATION TEST ON RABBITS

Procedure: H-7698 and H-7699 were dissolved in 50/50 acetone/dioxane solvent to give a 33 1/3% (wt/wt) solution.

H-7702, a white powder, was converted to a 50% (wt/wt) paste with propylene glycol.

Six male albino rabbits were clipped free of hair on the trunk and lateral areas and placed in FDA-type stocks. Doses of 0.5 ml of H-7698 and H-7699 solutions and approximately 0.5 g of the H-7702 paste were applied to intact skin under a 1" X 1" gauze square (double thickness). Rubber sheeting was then loosely wrapped around the trunk and secured with adhesive tape. After 24 hours, the rabbits were removed from the stocks, the patches taken off and the reactions observed. Observations were also made at 48 hours.

Results: No skin reactions were observed at any time with any material during this test.

Summary: The three materials tested (2-naphthylamine, phenyl-; Thermoflex A; and imidazoline, 2-mercapto-) produced no skin reactions on shaved intact rabbit skin at 24 hours or 48 hours after treatment. Although no dermatitis hazard is expected during the routine handling of these materials, good industrial hygiene practices should be followed when handling them and unnecessary contact should be avoided.

† Grade: regular. Sample is routine commercial product (probably made in 1971). Impurities: contains trace of β-naphthylamine.

‡ Grade: technical. Composition: 50% phenyl-beta-naphthylamine; 25% 4,4'-dimethoxy diphenylamine; 25% N,N'-diphenyl-paraphenylene diamine. Impurities: contains < 1% ortho-chloroaniline.

‡ Grade: commercial.

Report by:

John W. McBlack

Approved by:

Charles F. Reinhardt

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HL-4-42

IR

Aniline Tumors of the Bladder

Animal Work

Progress Report for 1941

Animal Studies

Over two and a half years have elapsed since a group of dogs received minimal damage to their bladders as a result of exposure to Beta Naphthylamine. Since that time they have not received additional exposure, save that due to a possible cross-contamination mentioned in the 1940 Progress Report. Up until the present, there is no sign of the development of bladder tumors in any of the animals.

We have not been able to produce bladder tumors in dogs as a result of exposure to any of the following compounds:

<u>Compound</u>	<u>Period of Exposure</u>
Alpha Naphthylamine (Technical and Pure)	21 months
Benzidine	30 months
Phenyl Beta Naphthylamine	22 months
Tolidine	6 months
Aniline	8 months

Whether these compounds will eventually produce tumors cannot be stated at the present time, but it can be stated that they are much less potent than Beta Naphthylamine in this respect.

A continuation of the exposure is necessary to establish the potency of these compounds as agents in causing bladder tumors.

Human Studies

During 1941 the urines on a group of workmen exposed to Beta Naphthylamine have been studied at frequent intervals following the procedure established at the Haskell Laboratory in 1940. Duplicate results of these examinations have been forwarded to the Laboratory and are being analyzed to obtain further data on the relation between the degree of exposure to Beta Naphthylamine and its action on the bladder.

HASKELL LABORATORY OF
INDUSTRIAL TOXICOLOGY

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Animal Work
Progress Report for 1941

Allan J. Fleming, M. D.

During the year the study of dogs exposed to the following compounds has been continued:

- (a) Beta Naphthylamine
- (b) Alpha Naphthylamine (Technical and Pure)
- (c) Benzidine
- (d) Phenyl Beta Naphthylamine

In addition, study on two new compounds, aniline and tolidine, was started.

The results obtained in experiments with these various compounds can be summarized as follows:

(a) Beta Naphthylamine

Three dogs given 300 mg. of Beta Naphthylamine daily by mouth between 3/9/39 and 4/28/39 (49 treatments) developed minimal irritation of the bladder as indicated by blood and excess growth promoting substances in the urine and confirmed by cystoscopy. These dogs have been under observation continuously for over 2 1/2 years without further treatment with Beta Naphthylamine and have shown no sign of developing bladder tumors. This experiment was instituted to show that, even should minimal damage to the bladder occur as a result of exposure to Beta Naphthylamine, if the individual is removed from exposure or the exposure is definitely reduced, no tumor will subsequently develop.

(b) Alpha Naphthylamine (Technical and Pure)

Five animals, two exposed to Technical Alpha Naphthylamine (containing 7-9% Beta) and the other three exposed to Pure Alpha Naphthylamine, have been given 300-320 mg. by mouth daily (5 times a week) for 21 months. On the last cystoscopy report by Dr. Wolfe (11/25/41) one dog in each group had abnormal areas of coloring in the bladder mucosa. None of the animals in either group have shown evidence of tumor formation by urinalysis or cystoscopy. Biopsies on suspicious areas in the bladders of 14 B₂ and G₂ (Alpha Technical) and 14 H₂ (Alpha Pure) on May 28, 1941 and November 25, 1941 were reported as lymphoid hyperplasia. These were unaccompanied by any changes in the bladder epithelium.

(c) Benzidine

A group of four dogs has been given 100-120 mg. of benzidine daily (5 times a week) since 6/25/39 without showing any evidence of bladder tumor formation.

(d) Phenyl Beta Naphthylamine

A group of three dogs has been given 540-550 mg. of Phenyl Beta Naphthylamine daily (5 times a week) since 2/28/40. No significant abnormality of the bladder has developed in any of this group.

(e) Tolidine

Three dogs have been given 250 mg. of Tolidine by mouth daily (5 times a week) since 6/19/41. Routine urinalysis, colpidium counts, and cystoscopies indicate that no irritation of the bladder has resulted.

(f) Aniline

Three dogs have been given 300 mg. of Aniline by mouth daily (5 times a week) since 5/14/41. Routine studies of the urine, colpidium counts, and cystoscopies, indicate the bladders so far have not been affected.

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Aniline Tumors of the Bladder

Studies of Urinary Bladder Tumors

Progress Report - February 1945

was originally approved in December, 1934, and experiments were started to determine whether beta naphthylamine could produce in the urinary bladder of dogs tumors of the type observed in industry. During the years 1934 to 1938, it was definitely established that beta naphthylamine fed to dogs would produce such bladder tumors. In addition, somewhat academic studies of the action of beta naphthylamine on isolated tissues were carried out.

It became obvious late in 1938 that a purely academic approach to the bladder tumor problem was giving little or no information of practical value in control of the industrial situation. As a basis for subsequent research, we planned to seek answers to a series of questions posed by Dr. Gehrmann following his visit to Europe in 1938. These questions in brief were:

- 1) What chemical or chemicals are responsible for the development of bladder tumors in the dye industry?
- 2) What is the minimal time of exposure necessary to produce such tumors?
- 3) What definite procedure should be followed with regard to changing the occupation of exposed men?
- 4) What are the safest, least annoying, and most accurate methods of diagnosis of bladder injury?

5) What are the most satisfactory and practical methods of prevention from:

- a) the standpoint of plant operation;
- b) the medical standpoint?

Answers more or less adequate have been obtained to most of these questions and are as follows:

1) Of eight chemicals studied, only beta naphthylamine has produced bladder tumors in dogs. The materials studied were:

Beta naphthylamine

Alpha naphthylamine (pure and technical)

Benzidine

Tolidine

Dianisidine

Aniline

Phenyl alpha naphthylamine

Phenyl beta naphthylamine

Dogs were fed benzidine (117 mg. daily five times a week), pure alpha naphthylamine (300 mg. daily five times a week), phenyl beta naphthylamine (540 mg. daily five times a week), for 4-1/2 to 5 years continuously and did not develop tumors. These dogs were killed in October, 1944, and at autopsy, no bladder tumors or bladder injury was noted.

Dogs exposed to the remaining compounds other than beta naphthylamine have been treated for 2-1/2 to 3-1/2 years without developing tumors.

While one cannot say positively that the compounds studied, other than beta naphthylamine, will never produce bladder tumors, they obviously afford much less hazard than beta naphthylamine.

2) Bladder tumors can be produced in dogs in the period of three to six months by feeding 300 mg. beta naphthylamine base daily on five days of each week.

3) Early observations of the formation of bladder tumors in animals treated with beta naphthylamine showed three stages:

a) A stage in which changes appear in the blood vessels and capillaries of the bladder wall, accompanied by hemorrhages in the submucosa, but usually without any change in the overlying mucosa. Between the affected areas the mucosa may appear quite normal, or there may be areas in which it is "ham colored". This stage may be accompanied by one or more symptoms of cystitis.

b) The stage of papilloma formation.

c) The stage of development of malignancy in the papilloma.

Any one of these stages may be of long or short duration, depending upon the degree of exposure and other variable factors.

If bladder tumors are to be prevented, bladder injury must not be allowed to pass beyond the first stage.

4) During 1939 and '40, it was shown that a proper routine examination of the urine of workers could indicate the condition of the bladder and the need for cystoscopy. A system of examinations has been recommended for use.

It includes observation for blood and blood corpuscles in the urine, the measurement of growth promoting substances, the estimation of free beta naphthylamine base excreted, and the total beta naphthylamine base and derivatives detectable by fluorescent photometry. Indices have been established to determine the degree of exposure and the ability of each individual to handle his exposure.

As yet we have no detailed report showing the extent to which these indices have been applied either to decide the need for cystoscopy or the need for reduction of the degree of exposure of any individual.

5) From both the operations and the medical standpoints, prevention of bladder tumors appears to us largely a matter of reducing exposure to beta naphthylamine to the point at which even the most susceptible individual does not excrete sufficient beta naphthylamine in the urine to irritate the bladder.

Should such work in this Laboratory show clearly that beta naphthylamine alone gives rise to tumors, steps might be taken to isolate the manufacture of this compound in buildings especially designed to reduce exposure to a minimum, and to facilitate the immediate removal of any accidental contamination by thorough washing. The present regulations governing personal hygiene, frequent washing and changing of clothes and drinking of water should still be enforced, and would probably be more effective when the possibilities of exposure were reduced.

Some of our studies during 1944 have suggested that the conversion of beta naphthylamine base to derivatives not irritating to the bladder might be assisted by feeding dl-methionine. We have evidence that injury to the liver, such as might be caused by disease or by poisonings, such as carbon tetrachloride, interferes with the ability of the dog to convert beta naphthylamine base into derivatives, and further, we have evidence that (dl-methionine) may improve the ability to change beta naphthylamine base even in the presence of an injured liver. An experiment demonstrating these points is represented by Figure 1.

B. Naphthylamine mg./100 cc. urino

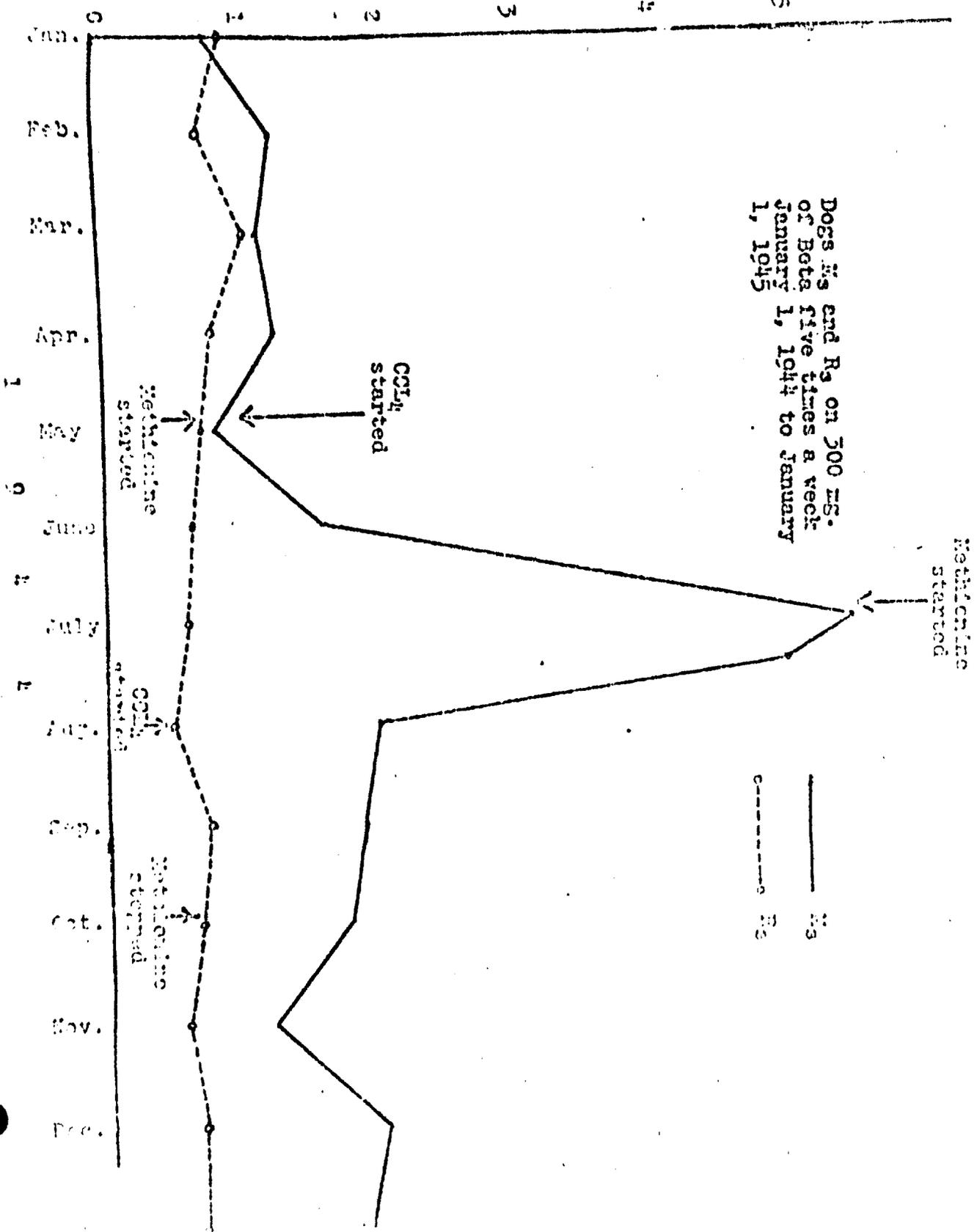


Figure 1

In the experiment summarized in Figure 1, there is shown (1) the ability of methionine to protect the liver of a dog against injury by carbon tetrachloride and so conserve its ability to convert beta naphthylamine into non-irritant derivatives, and (2) the ability of methionine to assist a liver already injured by carbon tetrachloride to recover a more or less normal activity so far as the handling of beta naphthylamine is concerned.

In Dog H₃ (solid line) the liver was deliberately injured by feeding small doses of carbon tetrachloride daily. Within two months, the average excretion of beta naphthylamine base had risen to about five times the normal level. Treatment with methionine was then started, the beta naphthylamine and carbon tetrachloride treatments being still given. The excretion of free beta naphthylamine dropped slightly in the following ten days and markedly during the next twenty days, and had returned almost to normal level by the end of three months. When sacrificed for autopsy about nine months after carbon tetrachloride was first given, this dog was found to have cirrhosis of the liver.

Dog R₃ (broken line) was given methionine for three months during which the average excretion of free beta naphthylamine fell slightly. At the end of August, treatment with carbon tetrachloride was started. Note the absence of a rise in the excretion of free beta naphthylamine base in the urine as compared with the rise shown by Dog H₃ treated with carbon tetrachloride, but not previously given a course of methionine. On October 27th, the treatment of Dog R₃ with methionine was discontinued, but carbon tetrachloride treatment still continued. However, the ability to convert beta naphthylamine to

non-irritant derivatives was maintained as indicated by the uniformly low excretion of free base in the urine, and on autopsy six months after commencing treatment with carbon tetrachloride, no gross injury to the liver of the dog was noted.

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