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Carcinogenesis of 4-Aminobiphenyl in BALB/cStCrlfC3Hf/Nctr Mice

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Abstract—*Male and female (840 each) BALB/cStCrlfC3Hf/Nctr mice were given* 0, 7, 14, 28, 55, 110 *and* 220, *and* 0, 7, 19, 38, 75, 150 *and* 300 ppm, *respectively, of 4-aminobiphenyl in their drinking water. Necropsies on killed animals were performed at* 13, 26, 39, 52 *and* 96 weeks *on dose. Dose-related neoplasms were angiosarcomas, bladder urothelial carcinomas and hepatocellular neoplasms. The non-neoplastic dose-related lesions were left atrial thrombosis, bladder urothelial hyperplasia, splenic hemosiderosis and splenic erythropoiesis. The incidences of bladder carcinoma and atrial thrombosis were higher in the males and the incidences of hepatocellular neoplasms and angiosarcomas were higher in the females.*

INTRODUCTION

4-AMINOBIPHENYL is carcinogenic in man, rat, mouse, rabbit and dog. The compound causes bladder cancer in man [1], bladder and hepatocellular carcinoma in the mouse [2, 3], and bladder papillomas and carcinoma in the rabbit [4] and dog [5–8].

In this study the carcinogenesis caused by 4-aminobiphenyl was evaluated extensively in the mouse. Many doses (six doses per sex as well as a control group) were used in order to obtain as much information as possible at non-toxic dose levels. For each dose 120 animals of each sex were used to provide a sample size sufficient to detect low-incidence pathologies. A complete pathology work-up was done in order to obtain as much pathologic information as possible on the pathology induced by 4-aminobiphenyl administration.

MATERIALS AND METHODS

Four-week-old BALB/cStCrlfC3Hf/Nctr mice were placed four per cage in a room maintained at 22–24°C with 40–60% relative humidity. Sexes were segregated by cage. The 4-aminobiphenyl·HCl (synthesized by Illinois Institute of Technology Research, Inc., of Chicago, IL) was determined to be pure (99.5+%) at the limit of detection for impurities by gas chromatographic analysis [9]. The mass of 4-aminobiphenyl was confirmed by mass spectrometry. The test compound was administered in charcoal-filtered deionized drinking water adjusted to pH 2.0 (the pH of the 300 ppm-dosed water) with reagent grade HCl (0.1 N). The dosed water was used if within ±10% of the target dose as determined by a spectrophotofluorometric assay method [9]. Doses (in ppm) refer to the monohydrochloride salt. Mice received autoclaved pellet diet (Lab Chow® 5010 pellets, Ralston Purina Co., St. Louis, MO) and were transferred weekly to clean cages with fresh food and water. Polycarbonate cages containing hardwood chip bedding (Ab-Sorb Dri®, Lab Products, Garfield, NJ) were used. All cages were capped with polyester filter bonnets (Lab Products, Garfield, NJ). About 17 changes of outside air per hour were supplied to the room.

The mice used in this study were obtained from the NCTR breeding colony. Every 2 weeks the breeding colony room was checked for the presence of pathogenic micro-organisms (bacteria, mycoplasma, fungi, viruses, protozoa and rickettsia). Breeding colony rooms were decontaminated if unacceptable levels of pathogenic micro-organisms were found. Prior to room allocation of mice, the room was decontaminated.

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Thereafter, room swabs were tested for the presence of pathogenic micro-organisms. Microbiological evaluation of room air was performed every 2 weeks and waste samples from used animal cages were cultured on a weekly basis for the detection of pathogenic micro-organisms.

Male and female mice (840 each) were given 0, 7, 14, 28, 55, 110 and 220, and 0, 7, 19, 38, 75, 150 and 300 ppm, respectively, of 4-aminobiphenyl in their drinking water. The high dose for 1 week in the males (220 ppm) and females (300 ppm) was equal to the LD_{50} (in mg/kg given by gavage all at one time; unpublished results from this laboratory) under the assumption that a 30-g mouse drinks 28 g of water per week. Necropsies on scheduled terminated animals were performed at 13, 26, 39, 52 and 96 weeks on dose (see Table 1 for the number of mice designated for each termination time). Mice dying on study and mice *in extremis* were also necropsied. Detailed necropsies were performed, and macroscopic and microscopic findings were collected on approximately 45 tissues or organs of each animal [10, 11]. After fixation in Bouin's solution for 18–24 hr, the tissues were trimmed, placed in cassettes, processed in an automatic tissue processor, embedded in paraffin and submitted for histopathology. Sections were cut at 5 μm and stained with hematoxylin and eosin. Gomori's staining method for iron was used to demonstrate hemosiderin in the spleen. Other special stains were utilized when required.

Statistical tests for dose-response trends cannot be conducted on crude tumor rates (number of animals with lesions divided by the number of animals on study) because animals at the higher doses may die due to competing risks of other diseases before lesions develop. To adjust for this possibility, tumor rates for fatal lesions are based upon the number of animals at risk (alive) at any point in time. These rates are then tested for a positive trend (increase in tumor rate with increasing dose) over the duration of the experiment by the method given by Peto *et al.* [12]. For non-fatal lesions, tumor rates are based upon the number of animals killed or dying due to causes other than the lesion of interest during a time interval. These rates are then tested for a positive trend over the duration of the experiment by the method given by Peto *et al.* [12]. Finally, the dose-response trend test statistics are combined for fatal and non-fatal tumors. The pathologist decided whether or not a lesion was fatal. For statistical purposes, all lesions found at the scheduled terminations were considered non-fatal because the cause of death for killed animals was the termination. Furthermore, for statistical purposes only the primary microscopic lesion in each dead or moribund animal was listed as the cause of death or morbidity. When a cause of death or morbidity could not be determined, it was listed as unknown. If an animal was removed as dead or moribund before its projected termination time, it was included in the dead and moribund animals for purposes of statistical analyses. Statistical tests were conducted for both sexes for lesions that showed a significant ($P \leqslant 0.05$) positive-trend test: angiosarcoma, urinary bladder carcinoma, urothelial hyperplasia, hepatocellular carcinoma, hepatocellular adenomas and carcinomas combined, splenic hemosiderosis, splenic erythropoiesis and left atrial thrombosis of the heart. In a carcinogenesis study, statistical tests are conducted for dose-response trends for non-neoplastic and neoplastic lesions in all tissues and organs. Where spontaneous background incidence rates are sufficiently high, this multiplicity of testing leads to a chance of obtaining a false-positive result. Furthermore, as the number of statistical tests increases, the chance of obtaining a false-positive result also increases. This problem of an increased risk of a false-positive can be remedied by using the Bonferroni inequality [13]. For those results in

Table 1. *Experimental design*

Dosage (ppm)		Termination time in weeks (scheduled number of mice per sex)				
Male doses	Female doses	13	26	39	52	96
0	0	24	24	24	8	40
7	7	24	24	24	8	40
14	19	24	24	24	8	40
28	38	24	24	24	8	40
55	75	24	24	24	8	40
110	150	24	24	24	8	40
220	300	24	24	24	8	40
Total		168	168	168	56	280
Total per sex						840

*Table 2. Incidence of lesions in BALB/c mice during chronic dosing with 4-aminobiphenyl**

	Female dosage (ppm)								Male dosage (ppm)							
	0	7	19	38	75	150	300	P†	0	7	14	28	55	110	220	P†
Angiosarcoma																
Non-fatal	1	2	3	1	6	3	2	≤0.00005	0	1	1	1	2	3	6	≤0.00005
Fatal	0	2	1	1	8	23	9	≤0.00005	1	0	0	1	2	2	8	≤0.00005
Combined	1	4	4	2	14	26	11	≤0.00005	1	1	1	2	4	5	14	≤0.00005
No. exam.	119	120	120	120	120	118	117		118	117	118	119	115	119	118	
Bladder carcinoma																
Non-fatal	0	0	0	1	0	5	1	—‡	0	0	1	0	6	14	14	≤0.00005
Fatal	0	0	0	0	0	0	0	—	0	1	0	0	0	1	9	≤0.00005
Combined	0	0	0	1	0	5	1	—	0	1	1	0	6	15	23	≤0.00005
No. exam.	118	118	119	118	118	117	117		116	117	118	118	115	118	118	
Hepatocellular carcinoma																
Non-fatal	0	0	2	3	7	7	2	≤0.00005	2	0	0	0	0	3	2	—‡
Fatal	0	0	0	1	3	7	5	≤0.00005	0	1	0	0	0	0	0	—
Combined	0	0	2	4	10	14	7	≤0.00005	2	1	0	0	0	3	2	—
No. exam.	117	120	120	119	119	118	117		118	117	118	117	114	118	117	
Hepatocellular adenomas and carcinomas combined																
Non-fatal	0	0	2	3	8	10	5	≤0.00005	2	1	0	å	å	3	3	≤0.0031
Fatal	0	0	0	1	3	7	5	≤0.00005	0	1	0	0	0	0	0	—
Combined	0	0	2	4	11	17	10	≤0.00005	2	2	0	1	1	3	3	≤0.0082
No. exam.	117	120	120	119	119	118	117		118	117	118	117	114	118	117	
Left atrial thrombus																
Non-fatal	0	0	0	0	0	2	0	—	0	2	0	0	1	8	1	≤0.015
Fatal	0	1	0	1	3	8	9	≤0.00005	1	0	0	0	0	16	29	≤0.00005
Combined	0	1	0	1	3	10	9	≤0.00005	1	2	0	0	1	24	30	≤0.00005
No. exam.	117	120	120	119	119	117	117		117	115	117	119	115	119	118	
Bladder hyperplasia																
Non-fatal	0	0	3	53	106	97	83	≤0.00005	0	4	19	71	108	107	102	≤0.00005
No. exam.	118	118	119	118	118	117	117		116	117	118	118	115	118	118	
Spleen hemosiderosis																
Non-fatal	20	60	94	106	103	100	114	≤0.00005	7	10	15	43	81	96	100	≤0.00005
No. exam.	117	120	120	120	120	117	116		116	115	117	118	115	118	116	
Spleen erythropoiesis																
Non-fatal	8	25	56	84	96	105	90	≤0.00005	12	20	22	40	63	77	89	≤0.00005
No. exam.	117	120	120	120	120	117	116		116	115	117	118	115	118	116	

*All animals on study (killed, dead and moribund) are included in this table. Only lesions that showed a significant ($P \leq 0.05$) treatment-related effect are included.

†P = level of statistical significance for positive trend test.

‡No. of animals with tumors too small to perform a valid statistical test.

Table 2 that achieved a high level of statistical significance ($P \leqslant 0.00005$), application of the Bonferroni inequality for multiple testing would still result in statistical signifiance. Dose-response trends for the animals in the final termination were tested by the Cochran-Armitage procedure [14].

RESULTS

Survival effects

The effect of 4-aminobiphenyl upon survival is shown in Fig. 1. Both males and females exhibited statistically significant trends for increasing mortality with increasing dose ($P \leqslant 0.0005$).

Pathologic effects

The incidences of treatment-related fatal and non-fatal lesions with significant ($P \leqslant 0.05$) positive-trend tests are shown in Table 2. Animals were excluded from the total number of animals examined where the target organ for the given lesion was not microscopically examined. The total number of tissues examined varies from 115 to 120 for each dose. Causes of death are listed in Table 3.

Bladder urothelial hyperplasia, splenic erythropoiesis and hemosiderosis were significant ($P \leqslant 0.05$) non-fatal lesions. For each lesion, statistically significant ($P \leqslant 0.00005$) positive trends for a dose response were observed for both females and males. Each of these lesions first occurred before 100 days on dose at the three highest doses. The urothelial hyperplasia was usually simple and diffuse. However, in a few cases it was nodular. In most cases the hyperplasia was accompanied by cytoplasmic vacuolation of the urothelial cells. Splenic erythropoiesis was severe and this extramedullary hematopoietic response occupied much of the red pulp and in several cases obscured the white pulp. This lesion was not diagnosed unless it exceeded the amount of erythropoiesis normally expected to occur in the spleen. Hemosiderin pigment was also present in an excessive amount in the red pulp and it too was not diagnosed unless this was so.

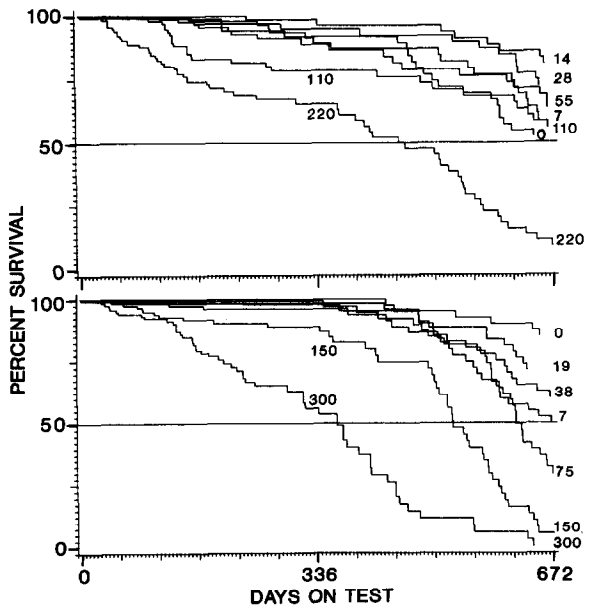

Fig. 1. Effect of chronic administration of 4-aminobiphenyl upon survival in male (top figure) and female (bottom figure) mice. Dose (in ppm*) is indicated.*

There was a statistically significant ($P \leqslant 0.00005$) increase in the rate of fatal bladder carcinoma with increasing dose for males. This was due to the large number of fatal tumors in the high-dose group (220 ppm). The combination of non-fatal and fatal bladder carcinomas in the males also showed a statistically significant ($P \leqslant 0.00005$) positive trend for dose. There were only non-fatal bladder carcinomas in females. Although there were too few females with bladder carcinoma for a valid statistical test, 5/117 animals had bladder carcinoma at 150 ppm. In the males all nine of the bladder transitional cell carcinomas invaded through the bladder wall and into the adjacent tissues. The bladder carcinomas in the females were confined to the mucosa or exhibited only limited invasion into the submucosa. The transitional cell carcinomas in the male contrasted with those of the females in that the male carcinomas were not as well differentiated. The deeply invasive carcinomas in the males had areas of anaplasia and squamous metaplasia. In the terminated animals (Table 4) only one female dosed at 38 ppm of 4-aminobiphenyl had a bladder carcinoma at the final termination (96 weeks on dose). Sixteen males had bladder carcinomas at the final termination and there was a significant ($P \leqslant 0.0005$) dose-response trend. One female (1/21) on 150 ppm and killed at 39 weeks had a bladder carcinoma.

There was a statistically significant ($P \leqslant 0.00005$) positive trend for fatal hepatocellular carcinoma vs dose in the females. There were too few hepatocellular carcinomas in the males to conduct a valid statistical test. If the hepatocellular carcinomas and adenomas were combined (Table 2), there was also a positive trend for fatal tumors ($P \leqslant 0.00005$) with respect to dose in the females but not in the males. In the final termination animals (Table 4) there was hepatocellular carcinoma only in the next to highest dose (110 ppm) in three males (3/22) and in one of the control males (1/21). There was a dose-response trend for hepatocellular carcinomas in the females ($P \leqslant 0.00005$) at the final termination,

Table 3. Causes of death following dosing with 4-aminobiphenyl

Sex	Dose (ppm)	Angiosarcoma	Hepatocellular carcinoma	Lung*	Lymphoma	RCS†	Bladder carcinoma	Multiple tumor	Other tumor	Thrombus	Unknown	Killed	Accidental	APD‡	No tissue	Total
	0	0	0	1	0	5	0	0	0	0	0	113	1	0	0	120
	7	2	0	1	3	9	0	1	2	1	2	99	0	0	0	120
	19	1	0	1	2	5	0	0	0	0	4	107	0	0	0	120
Female	38	0	0	2	0	4	0	2	2	1	6	103	0	0	0	120
	75	8	2	0	4	8	0	1	2	3	4	88	0	0	0	120
	150	20	4	0	2	0	0	3	1	7	6	75	0	1	1	120
	300	7	3	0	0	0	0	2	1	9	33	62	0	2	1	120
	Total	38	9	5	11	31	0	9	8	21	55	647	1	3	2	840
	0	1	0	4	1	1	0	0	0	1	14	96	0	2	0	120
	7	0	1	2	1	3	1	0	4	0	7	98	0	2	1	120
	14	0	0	1	0	1	0	0	2	0	4	110	0	1	1	120
Male	28	1	0	2	0	1	0	0	1	0	8	106	1	0	0	120
	55	2	0	1	1	2	0	0	2	0	9	98	0	3	2	120
	110	2	0	1	0	4	1	0	0	15	5	91	0	1	0	120
	220	6	0	0	3	1	7	3	0	29	9	60	0	1	1	120
	Total	12	1	11	6	13	9	3	9	45	56	659	1	10	5	840

*Lung neoplasms.
†Reticulum cell sarcoma.
‡Autolysis precludes diagnosis.

Table 4. Incidence of bladder carcinoma, hepatocellular carcinoma and angiosarcoma in the final termination (96 weeks on dose) animals

Sex	Dose (ppm)	Bladder carcinoma†	Hepatocellular carcinoma	Angiosarcoma†
Female	19	0/29	1/29(3)	0/29
	38	1/24(4)‡	2/24(8)	0/24
	75	0/12	4/12(33)	1/12(8)
	150	0/2	1/2(50)	0/2
Male	0	0/21	1/21(5)	0/21
	14	1/32(3)	0/32	0/32
	55	5/25(20)	0/25	0/25
	110	10/22(42)	3/22(14)	0/22

*Doses not shown did not have any animals with bladder carcinoma, hepatocellular carcinoma or angiosarcoma.
†The incidences of these lesions were low before 96 weeks on dose. The incidences for sacrifices before 96 weeks on dose are given in the test.
‡Incidence as a percentage.

with tumors appearing in four of the dose groups (19, 38, 75 and 150 ppm; Table 4). No female mice survived to the final termination in the high dose (300 ppm) and only two females survived at the second highest dose (150 ppm). One female (1/23) treated with 300 ppm of 4-aminobiphenyl and killed at 13 weeks on dose had hepatocellular carcinoma.

There was a statistically significant ($P \leqslant 0.00005$) positive trend for fatal angiosarcoma vs dose in both sexes. The females were observed to be more sensitive than the males to fatal angiosarcoma elicited by 4-aminobiphenyl when the dead, moribund and terminated animals were combined according to the statistical methods of Peto *et al.* [12]. In the terminated animals angiosarcoma appeared in only 1/12 females in the 75 ppm dose group at 96 weeks on dose. The angiosarcomas were not confined to a single location but were present in several different sites in the 90 mice with this neoplasm. These neoplasms were present in the following primary locations: 47 in the subcutaneous tissue, of which eight were multicentric, 27 in the abdominal cavity, eight in the liver, six in the spleen, one in the lung and one in the urinary bladder. Metastatic lesions in the lungs were present only in six cases but the larger sarcomas were frequently locally invasive. The histomorphology of these tumors varied from very well to poorly differentiated. This difference occurred between tumors and within the same tumor. The well-differentiated areas were composed of capillary spaces lined with enlarged endothelial cells; the poorly differentiated areas were more cellular and the endothelial cells were larger and aligned themselves close together. This close alignment of cells frequently formed pseudovascular walls that surrounded large blood-filled spaces. Hemorrhage and necrosis were frequent occurrences in the larger neoplasms and endothelial cells undergoing mitosis were frequently observed.

In addition to the fatal neoplastic lesions induced by administration of 4-aminobiphenyl, there were statistically significant ($P \leqslant 0.00005$) trends for a dose response for fatal left atrial thrombosis of the heart in both sexes. By 30 days on dose left atrial thrombosis was a cause of death or morbidity in the high-dose groups of both sexes. Sequellae to atrial thrombosis were infarcts in the brain and/or kidneys. These infarcts were present in 29/82 mice with atrial thrombosis. Infarcts were also present in the brain and/or kidneys of eight mice that did not have atrial thrombosis. Unfortunately, the left atria from two of these mice were not available for microscopic examination. Another associated secondary lesion was chronic passive congestion of the lungs. Both of these accompanying lesions were most frequently seen with those thrombi that were large, well-organized and widely adhered to the endothelium. In the terminated animals (Table 5) atrial thrombosis was observed in only two females and six males. There was not a significant positive dose-response trend for non-fatal atrial thrombosis (Table 2).

Table 5. Incidence of left atrial thrombosis in the terminated animals

Sex	Dose (ppm)	Termination (weeks) 13	26	39
Female	150	1/23(4)†	0/21	1/21(5)
Male	55	1/24(4)	0/24	0/21
	110	2/24(8)	2/22(9)	1/17(6)

*Doses and termination times not shown did not have any animals with left atrial thrombosis.
†Incidence as a percentage.

DISCUSSION

In earlier work [15] 0.2 ml of a 0.25% solution of 4-aminobiphenyl was given in arachis oil twice weekly for 9 months to mice (Ab × IF) via a stomach tube. The estimated total dose was 38 mg per animal. Of 17 mice so treated, 7/12 surviving to 90 weeks developed 'hepatomas' and three of these seven also developed bladder tumors, of which two were confirmed carcinomas. No control animals developed bladder tumors and the incidence of 'hepatomas' was not significantly different from the dosed animals. In a subsequent experiment [2] hybrid mice (C57 × IF) were given 0.2 ml of a 0.25% solution of 4-aminobiphenyl in arachis oil three times weekly for 50 weeks via a stomach tube. Of the 49 mice (28 female, 21 male) so treated, 17 developed malignant 'hepatomas', four developed tumors considered as 'probably malignant' and ten developed benign 'hepatomas'. One male animal developed a bladder carcinoma and one benign 'hepatoma' was observed in 50 control animals. In the present study in the BALB/c mouse, 4-aminobiphenyl caused dose-related bladder carcinoma in both males and females. Earlier studies in the mouse [2, 3] also found that 4-aminobiphenyl caused hepatocellular carcinoma. The results of one of these studies [2] have already been presented. In the other study [3], 50 3-day-old Swiss mice (25 male, 25 female) were injected (s.c.) with 200 mg of 4-aminobiphenyl in 0.02 ml of aqueous gelatin. Of these animals, 19/20 males and 4/23 females were found to have 'hepatomas' at the termination of the experiment, 48–52 weeks later. In the vehicle-treated control animals, 5/41 males and 2/47 females had 'hepatomas'. Hepatocellular neoplasms were also found in the present study. In the earlier studies hepatocellular neoplasms were present in greater magnitude and developed at an earlier age than in the current study. The differences between the carcinogenic responses in the earlier studies [2, 3, 15] and the present study might be related to pharmacokinetic differences between newborn and adult mice as well as to other factors (different strains, different routes of compound administration, etc.).

This is the first study to report left atrial thrombosis caused by administration of 4-aminobiphenyl. Atrial thrombosis of both sexes occurred predominantly in the two highest dose groups and was usually a fatal lesion because only eight animals with this lesion survived until their scheduled termination time. In the literature the highest reported spontaneous incidence of left atrial thrombosis is in inactive breeding BALB/c females [16]. The reported incidences of left atrial thrombosis in control NCTR BALB/c virgin female mice is 0.9% [17]. Another report of the incidence of left atrial thrombosis in the NCTR BALB/c virgin males and females is 0.7 and 0.1% respectively [18]. The cause of spontaneous atrial thrombosis is not understood. It is not known if the rebound of plasma prothrombin of 20–25% above normal following parturition in BALB/c mice may be related to the production of this lesion [19]. Left atrial thrombosis in aged Syrian hamsters has been reported to occur concomitantly with consumption coagulopathy [20]. The cause of left atrial thrombosis in the mice of this study is not known but it is suspected that there was a marked toxic effect on the hematopoietic system as indicated by the very evident extramedullary splenic erythropoiesis and splenic hemosiderosis. To what extent the coagulation factors may have been altered is not known because hematological profiles were not performed. The presence of infarcts in the brain and kidneys of animals without atrial thrombosis is an indication that the thrombosis could occur without a seeding thrombosis in the heart. The possibility that the thrombi in the atrium may have resolved in the animals without atrial thrombosis cannot be discounted.

In addition to being the first study to report left atrial thrombosis induced by 4-aminobiphenyl, this is also the first study to report angiosarcoma elicited by the compound. Several other compounds have been reported to induce angiosarcomas in the literature. Administration of 1,2-dimethylhydrazine produced angiosarcomas of the renal capsule in CBA mice [21]. 1,2-Dimethylhydrazine administration caused pararenal angiosarcoma in male but not female CBA mice [22]. Vinyl chloride administration caused angiosarcomas in the mouse's ear and subcutaneous tissues [23]. Administration of sterigmatocystin caused angiosarcomas in the liver and in the brown fat in BDF_1 mice [24]. The most common skeletal tumor in CBA and C3H mice after injection with strontium-90 was angiosarcoma [25]. Administration of 2-methyl-1-nitroanthraquinone produced subcutaneous, mesenteric and splenic angiosarcomas in $B6C3F_1$ mice [26]. Finally, administration of technical grade 2-aminobiphenyl in $B6C3F_1$ mice caused angiosarcomas at unspecified sites [27]. The presence in the technical grade 2-aminobiphenyl of 0.006–0.046% 4-aminobiphenyl, in view of the ability of 4-aminobiphenyl to elicit angiosarcomas in this study, may have been responsible for the angiosarcomas in that earlier study.

There were pathologic defects (bladder carcinoma and hepatocellular carcinoma) in this study in which one sex was much more sensitive to the pathologic lesion than the other sex. Even though there was a similar incidence of bladder

hyperplasia in both sexes, there was a much higher incidence of bladder carcinoma in the males than in the females. The higher incidence in the males could be due to the less complete emptying of the male bladder after urination compared to the female bladder [28]. Longer retention in the bladder of a cancer-causing agent in the males would be expected to elicit a higher incidence of carcinoma in the males. The higher incidence in the males could also be due to the longer survival on test of the males compared to the females. Bladder carcinoma is a late-appearing tumor and the females may have died before they had enough time on test to develop bladder carcinoma. Still another possibility is that the female bladder is resistant to the carcinogenic action of 4-aminobiphenyl. The high incidence of hepatocellular carcinoma in the females found in this study is difficult to explain. In the ED_{01} study conducted in this laboratory in female BALB/c mice (the same strain used in this study), 100 ppm of 2-acetylaminofluorene administered in the feed for 96 weeks (the duration of the current study) caused a 16% incidence of bladder carcinoma and a 5% incidence of hepatocellular carcinoma [29]. In other words, in the ED_{01} study hepatocellular carcinoma appeared after bladder carcinoma. In view of the earlier mortality observed in the females and in view of the customary finding that the male liver is usually more sensitive to carcinogens, the only apparent explanation for the higher incidence of hepatocellular carcinoma in the females in the current study is that the female liver is very sensitive to the carcinogenic action of 4-aminobiphenyl.

Future experimental work with 4-aminobiphenyl could use the compound as a known carcinogen to study the mechanism(s) of carcinogenesis. Des-gamma-prothrombin (an abnormal prothrombin) has been found to be elevated in human primary hepatocellular carcinoma and the elevated abnormal prothrombin level may be useful as a laboratory diagnosis for human hepatocellular carcinoma [30]. It would be of scientific interest to know if the abnormal prothrombin were elevated in chemically induced hepatocarcinogenesis. In future experimental work in mice, the possible elevation of the level of this abnormal prothrombin and the relationship of the time course of any elevation to the onset of hepatocellular carcinoma caused by 4-aminobiphenyl could be studied. The atrial thrombosis elicited by 4-aminobiphenyl in this study is consistent with an elevation of prothrombin level. Interferon induction in mice was inhibited by a single dose of 4-aminobiphenyl [31]. Inhibition of interferon induction is inhibition of a body defense mechanism. It is possible that such a reduction in a body defense mechanism precedes chemically induced carcinogenesis. In future experimental work in mice, the effect of multiple doses of 4-aminobiphenyl upon inhibition of interferon induction and the relationship, if any, between inhibition of interferon induction and appearance of bladder and liver carcinoma could be studied. Finally, the fact that 4-aminobiphenyl is found in cigarette smoke [31] suggests that an inhalation study on 4-aminobiphenyl might yield valuable scientific data. If 4-aminobiphenyl were administered by inhalation, it is possible that lung cancer could be elicited. In all future work a complete hematologic profile should be obtained because complete hematologic studies could help to explain the left atrial thrombosis, splenic hemosiderosis, splenic erythropoiesis and the morbidity and death in the high-dose group of females given 4-aminobiphenyl that showed no morphological evidence for morbidity.

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