DNA Adducts in Target and Nontarget Tissues of 3,2'-Dimethyl-4-aminobiphenyl in Rats

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3,2'-Dimethyl-4-aminobiphenyl (DMAB) is a potent carcinogenic aromatic amine which demonstrates multiorgan tropism in rats. Using polyclonal antibodies against DMAB-DNA adducts, an immunohistochemical experiment was applied to investigate the relationship between DMAB-DNA adduct formation and tumorigenicity. Dose-related nuclear staining was observed 24 hr after application of the carcinogen but specificity in terms of sites of tumor development was lacking. No observable decrease in staining intensity was evident in most organs by 168 hr after administration of DMAB. Specific DNA lesions which could be responsible for carcinogenesis were not detected by the ³²P-postlabeling method. The tumorigenic response of the ventral prostate in five strains of rats was roughly paralleled by DMAB-DNA adduct levels generated in the tissue. Strong enhancement of bladder tumor development by combined administration of the antioxidants, butylated hydroxyanisole, or butylated hydroxytoluene, with DMAB, was well correlated with an increase in DNA adducts. Our findings so far suggest that DNA adduct formation itself does not determine the carcinogenic organotropism of DMAB. Other factors (including cell proliferation and promotion by exogenous agents) may play important additional roles. For individual target organs or tissues, however, there seems to be a correlation between adduct levels and carcinogenic potential. — Environ Health Perspect 102(Suppl 1):167-172 (1994)

Key words: 3,2'-dimethyl-4-aminobiphenyl, rat, DNA adducts, immunohistochemistry, carcinogenesis

Introduction

The process of chemically induced carcinogenesis generally is divided conceptually into initiation and promotion stages. In the initiation stage, reactive carcinogen and/or metabolite(s) covalently bind to DNA in target tissues to form DNA adducts. These modifications lead to development of DNA damage, resulting in the production of initiated cells. These cells are then susceptible to promotional stimuli, which leads to tumor formation. The initiation event is believed to be an irreversible process and therefore a key point in chemical carcinogenesis.

3,2'-Dimethyl-4-aminobiphenyl (DMAB) is a potent multiorgan carcinogenic aromatic amine that targets the colon, Zymbal gland, preputial gland, urinary bladder, prostate, pancreas, and subcutis (1-5). Tissue specificity is also exhibited within the prostate complex (4-6). The seminal vesicles are also targeted and develop atypical hyperplasias. Clarification of the relationships between the carcinogenicity of DMAB and DNA damage in specific organs or tissues is an important goal in understanding mechanism(s) of chemical carcinogenesis. Specific antibodies have recently been developed against such carcinogen-DNA adducts and used for the sensitive detection of adduct formation in tissues (7-9). An immunohistochemical approach using such specific antibodies makes possible the precise localization of adduct formation at the cellular level (10,11).

We have developed polyclonal antibodies specific for DMAB-DNA adducts. In this article, we describe the immunohistochemically visualized distribution of DNA adducts, and present a quantitative comparison of adduct levels in relation to tumor response.

Immunohistochemical Demonstration of DMAB-DNA Adduct

Polyclonal antibody specific for DMAB-modified DNA (DMAB-DNA) was raised in rabbits as described previously (12). The antibody recognizes DNA modified with N-OH-DMAB and N-hydroxy-4-aminobiphenyl, but not unmodified, 2-acetylaminofluorene- or 4-nitroquinoline 1-oxide-modified DNA, or free aminobiphenyl and DMAB derivatives. Relative affinities of the antibody were given previously (12).

Male F344 rats (Charles River, Japan), 9 weeks old, received DMAB (>98% purity) dissolved in 0.5 ml of corn oil by sc injection at various doses and 24 hr later all animals were killed and autopsied. The influence of orchietomy and chemical castration with estrogen on adduct formation was also studied. Organs or tissues were fixed in cold acetone for 1 week and processed routinely for preparation of paraffin sections. Adducts in paraffin sections were visualized immunohistochemically as described previously (13).

Tables 1 and 2 summarize data for tissue or organ specificity of carcinogenic action and dose-related staining intensities in rats given sc injections of 200, 50, and 10 mg DMAB/kg bw. Dose-dependent staining was observed with only very weak intensity at a level of 10 mg. At a dose of 200 mg/kg, almost all organs examined demonstrated positive staining, at least to a certain degree, regardless of their DMAB-carcinogenic target status (Figure 1). In the male accessory sex organs, strong staining was observed in the ventral and lateral prostate lobes; the seminal vesicles exhibited prominent nuclear staining, lighter staining being evident in the dorsal and anterior (coagulating gland) lobes (Figure 2). A positive reaction was found for both the acinar and
ductal epithelial cells of the prostate. Target sites of DMAB-carcinogenicity are the ventral prostate and seminal vesicles and other target organs, including the urinary bladder, colon, preputial glands, Zymbal glands, sebaceous glands, and pancreas. All exhibited positive staining.

However, nontarget organs such as the kidney, lung, brain, and salivary glands also exhibited positive staining. The positive sites in the liver were the hepatocytes and sinusoid endothelial cells distributed uniformly throughout the lobules and those in the kidney were the distal and collecting tubule cells. Positive binding in the salivary glands was located in the acinar cells of the submandibular gland and the duct epithelial cells of the sublingual glands. A weak positive staining was also found in primary spermatocytes of the testis and accessory glands of the skin. In the brain, glia cells and cholid plexus were positive, while neurocytes were negative. Heart muscle proved negative. Investigation of DMAB-DNA adduct persistence continued until

### Table 1. Immunohistochemical localization of DMAB-DNA adducts in rats 24 hr after a single sc injection of DMAB at various doses.

| Organ                              | Carcinogenicity<sup>b</sup> | Dose of DMAB, mg/kg bw |
|------------------------------------|----------------------------|------------------------|
|                                    | 200 | 50  | 10  | 0   |
| Prostate: Ventral                   | _+ +_ | +/± | ±   | -   |
| Lateral                            | _+ +_ | +/± | ±   | -   |
| Dorsal                             | _+ +_ | +/± | ±   | -   |
| Anterior                           | _+ +_ | +/± | ±   | -   |
| Seminal vesicles                   | _+ +_ | +/± | ±   | -   |
| Bladder                            | _+ +_ | +/± | ±   | -   |
| Colon                              | _+ +_ | +/± | ±   | -   |
| Ileum                              | _+ +_ | +/± | ±   | -   |
| Sebaceous glands                   | _+ +_ | +/± | ±   | -   |
| Pancreas                           | _+ +_ | +/± | ±   | -   |
| Liver                              | _+ +_ | +/± | ±   | -   |
| Tests                              | _+ +_ | +/± | ±   | -   |
| Kidney                             | _+ +_ | +/± | ±   | -   |

NE, not examined. aCarcinogenicity evaluated in our previous papers (4,5,7,16,23). bStaining intensity: –, negative; ±, trace to weakly positive; +, positive; ++, strongly positive. cIn parentheses are staining intensities of interstitial cells of the crypts.

### Table 2. Persistence of nuclear staining of DMAB-DNA adducts in rats given a single injection of DMAB.

| Organ                              | Carcinogenicity<sup>a</sup> | Hours after DMAB injection<sup>b</sup> |
|------------------------------------|----------------------------|----------------------------------------|
|                                    | 24  | 72  | 168 |
| Prostate: Ventral                  | _+ _ | +/ _ | _+ _ |
| Lateral                            | _+ _ | +/ _ | _+ _ |
| Dorsal                             | _+ _ | +/ _ | _+ _ |
| Anterior                           | _+ _ | +/ _ | _+ _ |
| Seminal vesicles                   | _+ _ | +/ _ | _+ _ |
| Bladder                            | _+ _ | +/ _ | _+ _ |
| Pancreas                           | _+ _ | +/ _ | _+ _ |
| Colon                              | _+ _ | +/ _ | _+ _ |
| Ileum                              | _+ _ | +/ _ | _+ _ |
| Bladder                            | _+ _ | +/ _ | _+ _ |
| Zymbal glands                      | _+ _ | +/ _ | _+ _ |
| Sebaceous glands                   | _+ _ | +/ _ | _+ _ |
| Kidney                             | _+ _ | +/ _ | _+ _ |
| Salivary glands                    | _+ _ | +/ _ | _+ _ |
| Lung                               | _+ _ | +/ _ | _+ _ |
| Heart                              | _+ _ | +/ _ | _+ _ |
| Brain                              | _+ _ | +/ _ | _+ _ |
| Epidermis                          | _+ _ | +/ _ | _+ _ |

NE, not examined. aThe dose of DMAB was 100 or 200 mg/kg bw. bCarcinogenicity evaluated in our previous papers (4,5,7,16,23). cIn parentheses are staining intensities of interstitial cells of the crypts. dTumors could be induced by administration of DMAB and then testosterone propionate. eGlia cell/ependymal cell.

![Figure 1](image1.png) **Figure 1.** Variation in staining in different organs. DMAB was given at a dose of 200 mg/kg bw. (A) colon, (B) bladder, (C) liver, and (D) kidney.

![Figure 2](image2.png) **Figure 2.** Variation in staining intensity in the prostate and seminal vesicles. (A) ventral lobe, (B) lateral lobe, (C) anterior lobe, (D) dorsal lobe, and (E) seminal vesicles.
168 hr after the administration of DMAB revealed no clear changes in staining intensity in organs other than the urinary bladder, colon, ileum, and duct epithelium of the preputial glands (Table 2).

Ovariec-tomy caused severe atrophy of all parts of the accessory sex organs associated with a marked to moderate decrease in staining intensity. Mild to moderate atrophy induced by chemical castration with ethinyl estradiol was accompanied by moderate to slight decrease in staining intensity.

**Strain Differences in Prostatic Carcinogenesis and Adduct Formation**

Tumorigenic response to DMAB in the prostates of F344, ACi, Lewis, CD, and Wistar strain rats was examined in relation to adduct formation (14). Rats received DMAB sc at a dose of 50 mg/kg bw 10 times, once every other week. The experiment was terminated at week 60. The final survival rates were 44, 28, 46, 27, and 0 % in F344, ACi, Lewis, CD, and Wistar rats, respectively. All Wistar strain rats died by week 50. Carcinomas of the ventral prostate were found in 46, 50, 5, 0, and 0 % of F344, ACi, Lewis, CD, and Wistar strain animals, respectively (Table 3).

The amounts of DMAB adducts in DNA samples isolated from the prostate and liver of the five strains of rats 24 hr after administration of a single sc injection of DMAB 200 mg/kg bw were quantitatively analyzed using ELISA. The results were calculated as numbers of adducts per 10^6 nucleotides (Table 3). Ventral prostate values for the 5 strains ranged from 2.75 adducts per 10^6 for F344 and Wistar animals, to 1.0 adduct per 10^6 nucleotides in Lewis rats. The dorso-lateral lobe levels were about 45 to 70% of those in the ventral lobes, depending on the strain. With regard to the liver, F344, ACi, and Wistar strain animals demonstrated similar numbers of adducts (about 1.6 times the respective values for Lewis and CD). Thus, ventral tumor yield correlated well with DMAB-DNA adduct formation (except in the case of Wistar rats which died very early from nephropathy).

**Alteration of Tumor Sites in the Prostate Complex by Administration of Testosterone Propionate after DMAB Treatment**

Promotion effects of testosterone propionate (TP) on prostate carcinogenesis were investigated in F344 rats given DMAB. One group of F344 rats treated sc DMAB injections at a dose of 50 mg/kg bw at 2-week intervals for a total of 10 times and then sc implantations of TP-containing silastic tubes for 40 weeks. Prostate carcinomas were found in the lateral and anterior prostate and seminal vesicles, as well as in the ventral prostate in rats so treated, while DMAB without subsequent TP administration induced only ventral prostate tumors (Table 4).

**Modification of DMAB Carcinogenesis by BHA and BHT and Relationship to Alteration of the Adduct Formation**

Modification of DMAB-multiorgan carcinogenesis by simultaneous treatment with BHA or BHT was studied using male F344 rats (15). Four-week-old animals were given DMAB (sc injection of 50 mg/kg bw once a week for 10 weeks) during administration of BHA (2.0 % in diet for 11 weeks) or BHT (1.0 % in diet for 11 weeks). The experiments were terminated at week 55, at which time the incidences of urinary papillomas and carcinomas in rats given DMAB plus BHA or BHT were more than 70 %, whereas no bladder tumors were evident in animals given DMAB alone (Table 5). The development of neoplastic lesions in the liver and pancreas, however, was reduced approximately one half by BHA and BHT. No modification by BHA or BHT was evident for these tumor inductions in other organs. The urinary bladders of rats given DMAB with BHA or BHT had two or three times the levels of DMAB-DNA adducts in rats given DMAB alone (Table 5). In contrast, the amounts of the adducts in the livers of rats given DMAB plus BHA or BHT were significantly lower than those given DMAB alone. Suppression of adduct formation in the liver by BHT was stronger than by BHA. Those in the colon were also decreased by the concomitant treatment with BHA or BHT.

### Table 3. Incidences (%) of neoplastic and neoplastic lesions of the ventral prostate in five strains of rats (F344, ACi/N, Lewis, CD, Wistar) and their relation to DNA adduct formation.

| Strain   | Number of rats | Atypical hyperplasia | Carcinoma   | Ventral prostate | Liver    |
|----------|----------------|----------------------|-------------|------------------|----------|
| F344     | 24             | 17 (71)              | 11 (46)     | 2.75             | 3.66     |
| ACi/N    | 24             | 19 (79)              | 11 (50)     | 2.22             | 3.56     |
| Lewis    | 24             | 7 (32)               | 1 (5)       | 1.00             | 2.22     |
| CD       | 13             | 0                    | 0           | 1.47             | 2.22     |
| Wistar   | 7              | 1 (14)               | 0           | 2.75             | 3.56     |

*All lesions were found in the ventral lobe except for one carcinoma located in the dorso-lateral lobe of a CD rat. Data obtained from an independent experiment and expressed as adducts/10^6 nucleotides.

### Table 4. Prostate tumor development in the lateral and anterior lobes after administration of DMAB and then testosterone propionate.

| Treatment  | No. of rats | Incidences of prostate tumor (%) |
|------------|-------------|----------------------------------|
| DMAB       | 17          | 5 (33.3)                         |
| DMAB→TP    | 15          | 1 (6.7) 3 (19.2) 5 (29.4)        |

TP, testosterone propionate.

### Table 5. Incidences of neoplastic and neoplastic lesions in the urinary bladder and other organs of rats given DMAB and BHA or BHT.

| DMAB + BHA | Number of rats | Incidences of tumors (%) | DNA adducts/10^6 nucleotides |
|------------|----------------|--------------------------|------------------------------|
| BHA 20     | 20             | 18.3 ± 0.70^b 12.3 ± 1.2^c 6.5 ± 0.8 |
| BHT 20     | 20             | 10.0 ± 1^d 3.5 ± 0.6^d 21.0 ± 0.2 |
| Control 20 | 20             | 16.3 ± 1.3 8.3 ± 1.8 ± 0.5 4.7 ± 0.4 |

*Means ± SE of four determinations. ▲Significantly different from the corresponding control value at p < 0.001. ▲Significantly different from the corresponding control value at p < 0.05. ▲Significantly different from the corresponding control value at p < 0.02.

| DMAB + BHT | Number of rats | Incidences of tumors (%) | DNA adducts/10^6 nucleotides |
|------------|----------------|--------------------------|------------------------------|
| BHA 20     | 20             | 18.3 ± 0.70^b 12.3 ± 1.2^c 6.5 ± 0.8 |
| BHT 20     | 20             | 16.3 ± 1.3 8.3 ± 1.8 ± 0.5 4.7 ± 0.4 |
| Control 20 | 20             | 16.3 ± 1.3 8.3 ± 1.8 ± 0.5 4.7 ± 0.4 |

*Means ± SE of four determinations. ▲Significantly different from the corresponding control value at p < 0.001. ▲Significantly different from the corresponding control value at p < 0.05. ▲Significantly different from the corresponding control value at p < 0.02.
Immunohistochemically, the nuclear staining intensity of the urinary bladders of rats given DMAB plus BHA or BHT was clearly stronger than that of rats given DMAB alone. In the livers, however, the intensities were weaker in the antioxidant groups. BHT suppressed the formation of the adducts particularly in the centrilobular areas, whereas BHA exerted diffuse effects.

**Demonstration of Specific DNA Lesions by the 32P-Postlabeling Method**

DMAB-DNA adducts were analyzed by the 1-butanol extraction method (17). DNA (10 μg) was digested to 3'-mononucleotides at 37°C for 3.5 hr with micrococcal nuclease and spleen phosphodiesterase as described previously (18). DMAB-DNA adducts were then extracted with water-saturated 1-butanol from 8-μg samples of DNA digests according to the method of Gupta (17), and then 32P-labeled by T4 polynucleotide kinase with 2.0 μM[y-32P]-ATP (7000 Ci/min) at 37°C for 1 hr. The labeled solutions were applied to a PEI-cellulose layer. Adducts were located by screen-enhanced autoradiography at -80°C for 2 days. Amount of total nucleotides were determined as described previously (17) and adduct levels were calculated from radioactivities of the spots for each adduct divided by the radioactivity for the total nucleotides (17).

Figure 3 illustrates autoradiograms of DNA adducts in the prostate complex and liver of rats exposed to 150 mg/kg of DMAB 24 hr before sacrifice. The levels were 37.1, 14.6, 6.8, 15.3, 6.7, and 39.7 adducts per 10^6 nucleotides in the ventral, lateral, dorsal, anterior lobes, the seminal vesicles, and the liver, respectively. Four spots usually were observed in both the prostate complex and the liver. The ratios of adduct levels of no. 2 and no. 3 spots to total adducts were lower in the prostate complex (about 10%) than the liver (about 30%). There were no clear differences in the ratio of each spot among prostate lobes, but no. 5 spot could only be clearly detected in the ventral prostate and not in other organs.

**Discussion**

Immunohistochemical staining of DNA-carcinogen adducts revealed that DMAB metabolites bind to DNA in a variety of organs. This approach has the advantage of allowing localization at the cellular rather than the tissue or organ levels. However, observed distribution of adducts did not obviously correspond to the reported and observed sites of tumor development in DMAB-exposed rats. While all target organs of DMAB examined, including the colon, small intestine, ventral prostate, seminal vesicles, preputial gland, urinary bladder, sebaceous gland, pancreas, and liver were positive, nontarget organs such as the kidney, salivary gland, testis, and anterior, lateral, and dorsal prostate lobes also demonstrated considerable formation of DNA adducts. Similar anomalous findings in nontarget tissues have been reported earlier (19). Quantitation of adduct levels also did not reveal any direct correlation with DMAB-organotropism (12). In addition, persistence of DMAB-DNA adducts up to 168 hr after the single carcinogen treatment in the present experiment did not allow an explanation of organotropism on the basis of differential repair.

DNA modification through covalent binding of carcinogen(s) is thought to be an essential step for the activity of genotoxic carcinogens but not in itself enough to initiate carcinogenesis. In addition to possible formation of DNA adduct types that are nonrelevent to carcinogenesis, a high efficiency of DNA repair and therefore inadequate fixation of heritable DNA lesions also have been suggested as complicating factors. The chemical natures of the main DNA-DMAB products were identified by Flammang et al. (20) as N-(deoxyguanosin-8-yl)-DMAB (60–70%), 5-(deoxyguanosin-N2-yl)-DMAB (2–3%) and N-(deoxyadenosin-8-yl)-DMAB (1–3%). At present, antibodies specific for individual isolated DNA adducts are not available, and detection of the forms primarily responsible for carcinogenesis awaits the development of such analytical tools.

32P-Postlabeling demonstrated five observable spots, indicating five different DNA adducts, and although each spot has not yet been characterized, nos. 1 to 3 seem to correspond to N-(deoxyguanosin-8-yl)-DMAB, 5-(deoxyguanosin-N2-yl)-DMAB, and N-(deoxyadenosin-8-yl)-DMAB, respectively. Warranting further attention is spot no. 5 that was detected only in the ventral lobe and seminal vesicles, which are susceptible to DMAB carcinogenesis.

Many carcinogenic compounds that have no effect under normal conditions induce liver carcinomas when applied during the period of regenerative cell proliferation after partial hepatectomy (22). DMAB was found to induce rat pancreatic tumors when given at doses necrogenic for the pancreas (13) and cell proliferation at the time of carcinogen action is generally accepted to play an important role in the initiation of carcinogenesis, presumably via fixation of heritable DNA lesions. Accordingly, one might expect development of liver tumors in rats given DMAB only in association with partial hepatectomy, since this carcinogen is not particularly toxic in this organ. Since carcinogenesis is a multistage process, a second complicating factor that should be recognized is that such promotion may be required for the development of visible tumors in organs with
DMAB-DNA adduct formation that are normally considered to be non-target tissues. A good example for this is apparent from experiment 3. Under normal conditions, DMAB induces prostate tumors only in the ventral lobe. However, long-term treatment with TP after DMAB injection results in tumor development also in the lateral and anterior prostate and seminal vesicles.

There is a possibility that the adduct pattern in different tissues might vary with single and multiple dosing and indeed our studies with DMAB have shown that carcinogenic response is related to the dosing schedule (5). The multiorganotropicity of DMAB carcinogenicity is also dependent on the strain of rat investigated. With respect to prostate tumorigenesis, F344 rats showed a high susceptibility not shared by other strains. N-OH-DMAB, a proximate form of DMAB, can be further metabolized by O-acetylation to yield a carcinogenic form that is capable of reacting with nucleic acids (20,23). However, the prostate tumorigenic response could not be correlated with the activity of this enzyme in the ventral lobe (24), suggesting that prostate-susceptibility to DMAB carcinogenicity is related to both activation within the target organ and disposition and excretion patterns of the chemical and/or its metabolites in the whole body. The observed differences in the numbers of DMAB-DNA adducts in the ventral prostate among the five strains of rats, on the other hand, did demonstrate a good correlation between these lesions and the tumorigenic response, except in the Wistar rat case, which had a poor survival period. The very weak staining in atrophied prostate tissue resulting from orchietomy might account for the decreased tumor response in the prostate under such conditions (21). Loss of activating enzyme(s) in the epithelial cells in this case may be one of the reasons why DMAB-DNA adduct formation is decreased.

The striking finding that a simultaneous administration of DMAB with BHA or BHT strongly enhanced urinary bladder tumor induction while inhibiting the appearance of prostatic plastic lesions in the liver and pancreas offered direct support for an adduct role. Thus, quantitative assay of carcinogen-DNA adduct formation clearly demonstrated that the amounts of DMAB-DNA in the urinary bladder, liver, and pancreas correlated well with the tumorigenic response; both BHA and BHT increased levels of DMAB-DNA adducts in the urinary bladder, but decreased those in the liver and pancreas. A discrepancy was observed only for the colon where a decrease in the amounts of DMAB-DNA adducts was found but no change in tumor incidence occurred. The reason might be that although the drop in DNA adducts in the experimental groups was statistically significant, it was not low enough to result in decreased tumor induction. In fact, no apparent difference in nuclear staining intensity was present in the immunohistochemically stained colon samples.

The mechanisms by which BHA and BHT modulate DMAB carcinogenesis seem to be the same as those proposed in the case of modulation of 2-AAF carcinogenesis by BHT (25), i.e., changes in carcinogen metabolism resulting in increased or decreased ultimate carcinogenic species, because BHA and BHT are inducers of drug metabolizing enzymes such as cytochrome P450s, epoxide hydrolase, glucuronyltransferase, and glutathione S-transferase (26). Increased excretion of glucuronate conjugates (27) of N-OH-DMAB resulting from an alteration of liver metabolism by BHA and BHT could have played an important role in enhancing urinary bladder carcinogenesis and inhibiting hepatic carcinogenesis in the present experiment. Another factor that could have acted in the enhancement of urinary bladder carcinogenesis is increased DNA synthesis in the urinary bladder epithelium induced by BHA and BHT (28,29). Therefore, synergistic effects of increased reactive metabolite(s) of DMAB in the urine and increased DNA synthesis in the urothelium are mediated as the mechanism behind strong enhancement of urinary bladder carcinogenesis.

In conclusion, the data regarding initial formation of DNA adducts by DMAB and their persistence suggest that DNA lesions themselves are not sufficient to explain tumor induction. Involvement of other factors, including cell proliferation and promotion, require further investigation. The findings on staining differences and BHA- or BHT-modulation, however, do indicate that there is a correlation between adduct levels and carcinogenic potential for individual target organs or tissues.

REFERENCES

1. Walpole AL, Williams MHC, Roberts DC. Bladder tumours induced in rats of two strains with 3,2'-dimethyl-4-aminobiphenyl. Br J Cancer 9:170–176 (1955).

2. Spijut HJ, Nooli MW. Experimental induction of tumours of the large bowel of rats. A review of the experience with 3,2'-dimethyl-4-aminobiphenyl. Cancer 28:29–37 (1971).

3. Nussbaum J, Fiia ES, Kulkarini B, El-Bayoumy K, Weisburger JH. In vivo metabolism of 3,2'-dimethyl-4-aminobiphenyl (DMAB) bearing its organotropism in the Syrian golden hamster and the F344 rat. Environ Health Perspect 49:233–231 (1983).

4. Shirai T, Sakata T, Fukushima S, Ikawa E, Ito N. Rat prostate as one of the target organs for 3,2'-dimethyl-4-aminobiphenyl-induced carcinogenesis: effects of dietary ethyl estradiol and methyltestosterone. Jpn J Cancer Res (Gann) 76:803–808 (1985).

5. Ito N, Shirai T, Tagawa Y, Nakamura A, Fukushima S. Variation in tumor yield in the prostate and other target organs of the rat in response to varied dosage and duration of administration of 3,2'-dimethyl-4-aminobiphenyl. Cancer Res 48:4629–4632 (1988).

6. Shirai T, Fukushima S, Ikawa E, Tagawa Y, Ito N. Induction of prostate carcinoma in situ at high incidence in F344 rats by a combination of 3,2'-dimethyl-4-aminobiphenyl and ethyl estradiol. Cancer Res 46:6423–6426 (1986).

7. Leng M, Sage E, Fuchs RPP, Daune MP. Antibodies to DNA modified by the carcinogen N-acetoxy-N-acetylaminofluorene. FEMS Lett 92:207–210 (1978).

8. Poirier MC. Guest editorial. Antibodies to carcinogen-DNA adducts. J Natl Cancer Inst 67:515–519 (1981).

9. Morita T, Ikeda S, Minoura Y, Kojima M, Tada M. Polyclonal antibodies to DNA modified with 4-nitroquinoline 1-oxide: application for the detection of 4-nitroquinoline 1-oxide-DNA adducts in vivo. Jpn J Cancer Res (Gann) 79:195–203 (1988).

10. Huitfeldt HS, Spangler EF, Hunt JM, Poirier MC. Immunohistochemical localization of DNA adducts in rat liver tissue and phenotypically altered foci during oral administration of 2-acetylaminoflourene. Carcinogenesis 7:123–129 (1986).

11. Nakagawa K, Tada M, Morita T, Utsunomiya J, Ishikawa T. Immunohistochemical detection of 4-hydroxyaminoquinoline 1-oxide-DNA adducts in mouse tissues in vivo. J Natl Cancer Inst 80:419–425 (1988).
12. Tada M, Aoki H, Kojima M, Morita T, Shirai T, Yamada H, Ito N. Preparation and characterization of antibodies against 3,2'-dimethyl-4-aminobiphenyl-modified DNA. Carcinogenesis 10:1397–1402 (1989).
13. Shirai T, Nakamura A, Fukushima S, Tada M, Morita T, Ito N. Immunohistochemical demonstration of carcinogen-DNA adducts in target and non-target tissues of rats given a prostate carcinogen, 3,2'-dimethyl-4-aminobiphenyl. Carcinogenesis 11:653–657 (1990).
14. Shirai T, Nakamura A, Fukushima S, Yamamoto A, Tada M, Ito N. Different carcinogenic responses in a variety of organs, including the prostate, of five different rat strains given 3,2'-dimethyl-4-aminobiphenyl. Carcinogenesis 11:793–797 (1990).
15. Shirai T, Fukushima S, Kawabe M, Shibata MA, Iwasaki S, Tada M, Ito N. Selective induction of rat urinary bladder tumors by simultaneous administration of 3,2'-dimethyl-4-aminobiphenyl (DMAB) and butylated hydroxyanisole or butylated hydroxytoluene is associated with increased DMAB-DNA adduct formation. Carcinogenesis 12:1335–1339 (1991).
16. Shirai T, Nakamura A, Wada S, Ito N. Pancreatic acinar cell tumors in rats induced by 3,2'-dimethyl-4-aminobiphenyl. Carcinogenesis 10:1127–1130 (1989).
17. Gupta RC. Enhanced sensitivity of 32P-postlabeling analysis of aromatic carcinogen: DNA adducts. Cancer Res 45:5656–5662 (1985).
18. Gupta RC, Reddy MV, Randerath K. 32P-Postlabeling analysis of nonradioactive aromatic carcinogen-DNA adducts. Carcinogenesis 3:1081–1092 (1982).
19. van Bentham J, Vermeulen Eden Engelse L, Wilmer JWFM, Scherer E. Organ-specific carcinogenesis by N-methyl-N-benzylnitrosamine (NMBzA) does not correlate with formation, persistence or accumulation of O3-methylguanine or 7-methylguanine in target and nontarget tissues of the rat: a quantitative immunocytochemical study. Proc Am Assoc Cancer Res 29:110 (1988).
20. Flammang TJ, Westra JG, Kadlubar FF, Beland FA. DNA adducts formed from the probable proximate carcinogen, N-hydroxy-3,2'-dimethyl-4-aminobiphenyl, by acid catalysis or S-acetyl coenzyme A-dependent enzymatic esterification. Carcinogenesis 6:251–258 (1985).
21. Shirai T, Tagawa Y, Fukushima S, Asamoto M, Ito N. Lack of tumorigenic response in the prostate gland of castrated F344 rats by 3,2'-dimethyl-4-aminobiphenyl given with methyltestosterone. Cancer Lett 35:1–6 (1987).
22. Craddock VM. Induction of liver tumors in rats by a single treatment with nitroso compounds given after partial hepatectomy. Nature 245:386–388 (1973).
23. Flammang TJ, Kadlubar FF. Acetyl coenzyme A-dependent metabolic activation of N-hydroxy-3,2'-dimethyl-4-aminobiphenyl and several carcinogenic N-hydroxyarylamines in relation to tissue and species differences, other acyl donors, and arylhydroxamic acid-dependent acetyltransferase. Carcinogenesis 7:919–926 (1986).
24. Yamada H, Shirai T, Ito N, Wang CY. Species- and strain-specific a-acylation of N-hydroxy-3,2'-dimethyl-4-aminobiphenyl by liver and prostate cytosol. In: Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes (King CM, Romano LJ, Schuetze D, eds). New York:Elsevier Science Publishing, 1988:223–2216.
25. Maeura Y, Weisburger JH, Williams GM. Dose-dependent reduction of N-fluorenylacetamide-induced liver cancer and enhancement of bladder cancer in rats by butylated hydroxytoluene. Cancer Res 44:1604–1610 (1984).
26. Kahl R. Synthetic antioxidants: biochemical actions and interference with radiation, toxic compounds, chemical mutagens and chemical carcinogens. Toxicology 33:185–228 (1984).
27. Kadlubar FF, Miller JA, Miller EC. Hepatic microsomal N-glucuronidation and nucleic acid binding of N-hydroxyarylamines in relation to urinary bladder carcinogenesis. Cancer Res 37:805–814 (1977).
28. Shibata MA, Yamada M, Tanaka H, Kagawa M, Fukushima S. Changes in urine composition bladder epithelial morphology, and DNA synthesis in male F344 rats in response to ingestion of bladder tumor promoters. Toxicol Appl Pharmacol 90:37–49 (1989).
29. Fukushima S, Shibata MA, Tamano S, Ito N, Suzuki E, Okada M. Aging and urinary bladder carcinogenesis induced in rats by N-butyl-N-(4-hydroxybutyl)nitrosamine. J Natl Cancer Inst 79:263–267 (1987).