DNA Adduct Measurements and Tumor Incidence during Chronic Carcinogen Exposure in Rodents

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In an attempt to elucidate the relationship between DNA adduct formation and tumorigenesis, DNA adducts were measured in the livers and bladders of mice during chronic exposure to several different doses of 2-acetylaminofluorene (2-AAF) and 4-aminobiphenyl (4-ABP). Continuous oral administration of these compounds for 4 weeks produced an increase in DNA adduct formation during the first 2 weeks, followed by a plateau, which presumably occurred because the rate of adduct removal offset the rate of adduct formation. The quantity of DNA adducts present at equilibrium correlated directly with the carcinogen concentration; therefore, when exposure was continued for 4 weeks, DNA adducts that reflected the plateau level at each dose could be expressed as a function of dose. Liver and bladder DNA adduct profiles thus obtained during administration of multiple doses of 2-AAF (to female mice) and 4-ABP (to male and female mice) were compared to profiles for tumor incidences obtained during lifetime exposures to the same doses. These experiments demonstrated similar profiles for DNA adduct formation and tumorigenesis in liver. In the bladder, DNA adducts were linear, but tumors only appeared at the higher doses in conjunction with cell proliferation. In addition to these aromatic amines, similar data are available for aflatoxin B_1, diethylnitrosamine, and (methylnitrosamino)-1-(3-pyridyl)-1-butanone (also known as nicotine-derived nitrosoketone). Of the nine different biological situations (carcinogen/species/sex/organ) for which data are available, correlations between steady-state DNA adduct levels and tumorigenic response at the different doses were linear in five of the nine biological models. When the relationship between DNA adduct formation and tumor incidence was not linear, the profiles observed were considered to be the result of tissue-specific phenomena such as metabolic activation, cell proliferation, or cytotoxicity. — Environ Health Perspect 102(Suppl 6):161–165 (1994)

Key words: aromatic amines, 2-acetylaminofluorene, 4-aminobiphenyl, DNA adducts, tumorigenesis, rats, mice, chronic dosing

Introduction

Among the earliest cellular changes caused by chemical carcinogens are the formation of DNA adducts, which are covalent binding products of carcinogens with DNA (*1–3*). The weight of evidence from animal experimentation suggests that DNA adduct formation may constitute events that are necessary but not sufficient for tumorigenesis (*3,4*). DNA adduct studies that employ chronic carcinogen dosing over a range of carcinogen concentrations are relatively rare. However, most experiments of this type have demonstrated an early increase in adduct accumulation with time of exposure, followed by a plateau observed when the rate of adduct formation is offset by the rate of adduct removal (*5–9*). The plateau, indicating steady-state conditions, is frequently reached before 4 weeks of continuous carcinogen administration. The level of DNA adducts observed at the plateau phase usually correlates directly with the concentration of carcinogen in the diet (*6,7,10*) (Figure 1). Therefore, at a particular time (e.g., 4 weeks) of continuous carcinogen administration, it is possible to obtain a profile for steady state DNA adduct levels expressed as a function of different concentrations of carcinogen chronically given (Figure 2).

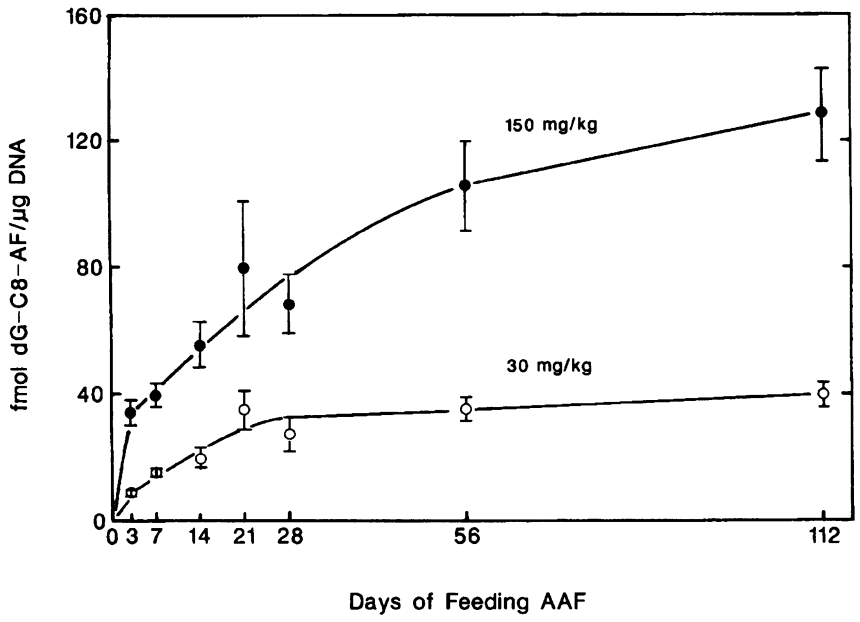

Figure 1. Female BALB/c mice were fed 2-AAF in the diet at concentrations of 30 (○) and 150 (●) mg/kg body weight for 3, 7, 14, 21, 28, 56, or 112 days; dG-C8-AF adducts were measured in liver DNA by radioimmunoassay.

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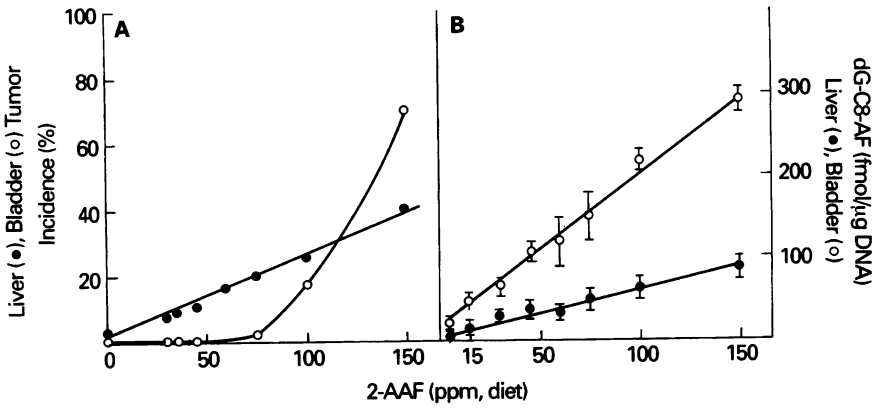

Figure 2. (*A*) Tumor incidence at 24 months and (*B*) dG-C8-AF at 28 days for livers (●) and bladders (○) of female BALB/c mice given continuous administration of 2-AAF in the diet. Concentrations of dietary 2-AAF for the tumor study were 0, 30, 35, 45, 60, 75, 100, and 150 ppm, and for the DNA adduct study were 0, 5, 10, 15, 30, 45, 60, 75, 100, and 150 ppm.

Most rodent tumorigenesis studies are conducted by lifetime carcinogen administration, but few of these are large enough to include many dose levels. In a few instances, rodent tumor incidences induced by lifetime carcinogen administration (24–36 months) have been compared to DNA adduct levels in target tissues at early times (1–2 months) of chronic exposure, over a wide range of doses. The profiles of adducts and tumorigenesis thus obtained are instructive in elucidating factors relevant to carcinogen-DNA interactions and tumor incidence in animals.

This review will discuss individual experiments in which chronic lifetime exposure over a broad dose range has been employed to induce tumors, and DNA adducts have been measured in target tissues of rodents given similar dosing for 1 to 2 months. The aromatic amines considered include 2-acetylaminofluorene (2-AAF) and 4-aminobiphenyl (4-ABP). These compounds were studied in livers and bladders of male and female mice. Other compounds for which similar experiments will be described include aflatoxin B_1 (AFB_1), diethylnitrosamine (DEN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (known as a nicotine-derived nitrosoketone or NNK). Overall patterns of tumor incidence and DNA adduct formation will be presented and significant aspects of tissue specificity will be discussed.

Aromatic Amines

In experimental animals, major target organs for tumor induction from aromatic amines include the liver and urinary bladder. In these two tissues in the mouse, 2-AAF (*10*) and 4-ABP (*11*) each form essentially only one DNA adduct, through covalent linkage of the amine nitrogen to the C8 of guanine. Thus, the tumorigenic effects of 2-AAF in mouse liver and bladder appear to be associated only with the *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF). Similarly, the tumorigenic effects of 4-ABP appear to be associated primarily with *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP).

In the mid-1970s an exceedingly large tumorigenicity experiment was conducted by feeding 2-AAF to approximately 24,000 female BALB/c mice (*12*). The compound was given at seven different dietary concentrations, and animals were killed at varying times between 9 and 33 months. Incidences of benign and malignant tumors obtained in both the liver and the urinary bladder at 24 months are shown in Figure 2*A*. In the livers of untreated animals the incidence of adenomas and carcinomas was 2.6%, and tumors increased linearly with concentration of 2-AAF in the diet, reaching 40% at the highest dose. In the bladder, by comparison, the tumor profile was nonlinear with a spontaneous rate of 0.3% that rose slowly to 2% at 75 ppm of dietary 2-AAF, and increased dramatically thereafter, resulting in incidences of 17 and 75% at doses of 100 and 150 ppm, respectively. A similar pattern of bladder hyperplasia preceded the bladder tumorigenesis in the ED01 study, which suggested that cell proliferation caused, or at least accompanied, the increase in tumorigenesis. This was further investigated by Cohen and Ellwein (*13*), who quantified cell proliferation associated with bladder hyperplasia at the same doses and hypothesized that a marked increase in proliferation at 100 and 150 mg/kg diet contributed to the elevated tumor incidence at these doses. In a parallel DNA adduct study (*10*), mice were fed 2-AAF in the diet at the doses used in the ED01 study plus 3 lower doses (Figure 2*B*). DNA adducts increased linearly with dose in both tissues; however, the bladder DNA adduct levels were consistently 2- to 4-fold higher than those observed in the liver. Thus, high levels of DNA adducts in the bladder appeared to be necessary for tumorigenesis but were not sufficient until the bladder cell proliferation rate reached a critical level, at doses above 75 mg/kg diet (*13*).

Another aromatic amine, 4-ABP, was chronically administered to male BALB/c mice at 6 doses between 7 and 220 ppm in the drinking water in both tumorigenesis and DNA adduct studies. Liver and bladder tumor incidences at 24 months (*14*) and DNA adducts at 28 days are shown in Figure 3. At the highest doses, 110 and 220 ppm, the liver tumor incidence did not reach 10% (Figure 3*A*), whereas in the bladder, the tumor increase was not significant at doses up to 28 ppm but was essentially linear from 55 to 220 ppm, reaching 82%. Bladder tumorigenesis was only significant at doses for which the incidence of bladder hyperplasia was >85% (*14*), suggesting that cell proliferation is a prerequisite for tumorigenesis in these male mice. In both tissues, DNA adducts (Figure 3*B*) increased linearly with dose and correlated well with tumorigenesis (i.e., adduct levels were 2- to 3-fold higher in bladder where the tumor incidence was also high, compared to liver where the tumor incidence was low). The relatively low DNA adduct levels in the male mouse liver (up to 125 fmoles/μg DNA) may reflect the presence of highly induced detoxification pathways. For example, there may be extensive *N*-glucuronidation of *N*-hydroxy-4-ABP, which could result in a larger effective dose to the bladder; concomitantly, less 4-ABP would be available for activation pathways leading to DNA adduct formation in the liver. In addition, kidney microsomes from male BALB/c mice apparently have a 10-fold greater capacity to metabolize the related aromatic amine, 3-methoxy-4-aminoazobenzene, to mutagenic metabolites than they do kidney microsomes from female mice (*15*). This sex-specific difference in metabolism may contribute to the high tumorigenesis and DNA adduct formation seen in bladders of male mice.

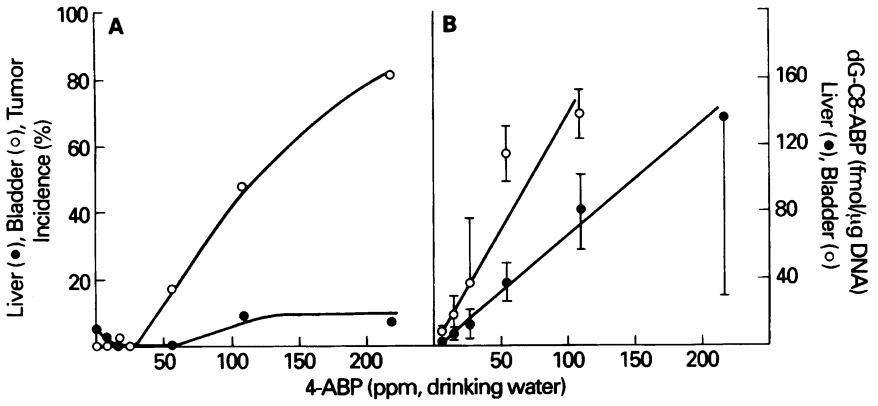

Figure 3. (*A*) Tumor incidence at 24 months and (*B*) dG-C8-ABP at 28 days for livers (●) and bladders (○) of male BALB/c mice given continuous administration of 4-ABP in the drinking water. Concentrations of 4-ABP for the tumor and DNA adduct studies were 0, 7, 14, 28, 55, 110, and 220 ppm.

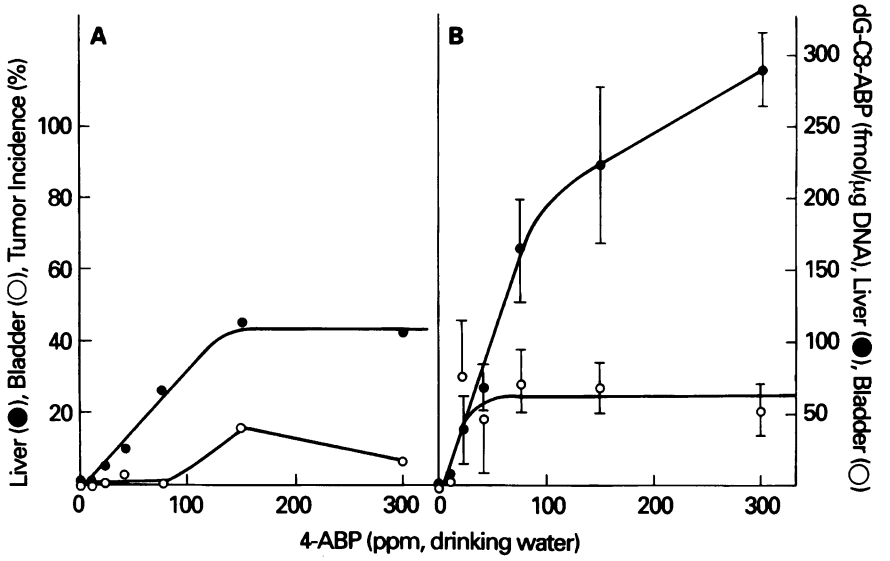

Figure 4. (*A*) Tumor incidence at 24 months and (*B*) dG-C8-ABP at 28 days for livers (●) and bladders (○) of female BALB/c mice given continuous administration of 4-ABP in the drinking water. Concentrations of 4-ABP for the tumor and DNA adduct studies were 0, 7, 19, 38, 75, 150, and 300 ppm.

Similar tumorigenesis and DNA adduct studies were conducted in female mice given 4-ABP in the drinking water at 6 doses between 7 and 300 ppm. The dose range for females was higher than that for males because of lower toxicity. The liver tumor incidence at 24 months (*14*) increased linearly with dose for the first 5 doses but was the same at 150 and 300 ppm (Figure 4*A*). The DNA adduct levels at 28 days (*11*) increased linearly for the first 4 doses and less than linearly at 150 and 300 ppm (Figure 4*B*). Thus, a saturation of the metabolic activation pathways leading to DNA adduct formation may have occurred in liver at 150 ppm and above. A factor confounding this interpretation is the considerable toxicity associated with the 300-ppm dose, with fewer than 50% of the mice surviving 2 years of treatment. The low adduct accumulation (<75 fmoles/μg DNA) (*11*) and low tumor incidence (<20%) (*14*) in female mouse bladder may reflect the reduced availability of compound for detoxification resulting from extensive activation and DNA adduct formation in the liver. Or they may result from the low metabolic capacity of BALB/c female kidney microsomes described above (*15*). Since the opposite metabolic relationship is seen in male mice (Figure 3), hormonal influences may contribute to the balance between activation and detoxification. There was significant bladder hyperplasia at 75 to 300 ppm (*14*), but in the absence of high levels of DNA adducts there was apparently insufficient stimulus for tumorigenesis.

Other Chemical Carcinogens

In three other situations, extensive data exist for tumorigenesis developing after chronic lifetime administration of multiple doses of chemical carcinogens and can be compared to the concomitant DNA adduct data for chronic dosing at similar concentrations for 1 to 2 months. Although these compounds are not aromatic amines, the data will be discussed because they contribute to conclusions that can be drawn from a complete overview of the area. The compounds in question are AFB_1, DEN, and NNK.

A linear increase in liver tumors at 24 months was observed in male Fischer rats given AFB_1 at five doses between 1 and 50 ppm in the drinking water (*16*), with the liver tumor incidence being 80% at the highest dose. A subsequent study by Buss et al. (*17*) investigated hepatic DNA adduct formation by administering radiolabeled AFB_1 to male Fischer rats in the drinking water at doses of 0.02, 0.6, and 20 ppm for 8 weeks. The increase in DNA adduct formation was linear, with the highest DNA adduct level being 2.7 fmoles/μg DNA. Thus, in the case of chronic administration of AFB_1 to rats, liver tumorigenesis is essentially linear, as is DNA adduct formation over a similar dose range. This situation is depicted in Figure 5, tumor and adduct curves B. The high carcinogenic potency of the aflatoxins may result in tumor formation at concentrations that do not saturate metabolic pathways, stimulate cell proliferation, or induce extensive toxicity.

Fifteen different doses of DEN, ranging between 0.033 and 16.9 ppm, were given in the drinking water in an extensive lifetime tumorigenesis study involving 1140 male Colworth (Wistar) rats (*18*). The lifetime liver tumor incidence, as a function of dose, was not linear, but approached a plateau at the higher doses. The experimental design of a DNA adduct study (*6,19*), measuring O^4-ethyldeoxythymidine (O^4-Et-dT) in liver DNA, was similar. This adduct accumulated in hepatocytes of rats continuously exposed to DEN and is considered to be the major promutagenic adduct responsible for the induction of

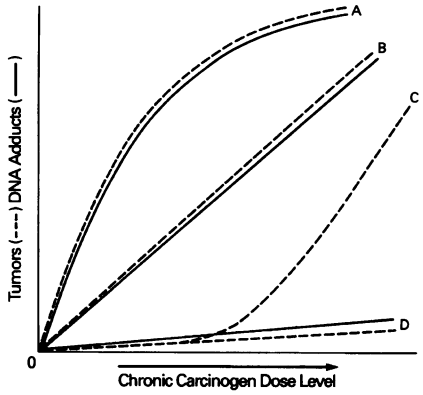

Figure 5. Schematic representation of supralinear (*A*), linear (*B*), sublinear (*C*) and low (*D*) profiles for DNA adduct formation (—) and tumorigenesis (– – –) in rodents given chronic administration of chemical carcinogens at multiple dose levels. Tumorigenesis is for lifetime exposure and DNA adduct data are obtained at 1 to 2 months.

hepatocellular carcinomas (*6*). The DNA adduct profile obtained with DEN was similar to the tumor profile, and both are depicted in tumor and adduct curves A of Figure 5 [designated by Swenberg (*7,19*) as supralinear curves]. This lack of linearity may result from a lower efficiency of adduct formation or an increased loss of the adduct. The latter hypothesis was supported experimentally. A study was done in which hepatocytes from rats given 40 ppm of DEN were shown to have a 43-fold increase in cell proliferation, as compared to controls (*20*). Thus, toxicity-related cell proliferation may be responsible for the lack of linearity of the DNA adduct profile at higher doses. The similar phenomenon in the tumorigenicity study may also be due to toxicity (*18*).

The nicotine-derived nitrosoketone, NNK, produces lung tumors as a result of chronic exposure in rats (*21*). Studies by Belinsky et al. (*21*) indicate that Clara cells appear to accumulate the highest levels of O^6-methyldeoxyguanosine (O^6-Me-dG), a procarcinogenic lesion. Therefore, lung tumorigenesis in male Fischer rats given subcutaneous injections of NNK 3 times a week for 20 weeks was compared with DNA adduct formation in the Clara cells of animals treated similarly for 4 weeks (*21*). Six doses were chosen, between 0.03 and 50 mg/kg bw, for the tumorigenicity studies, and lung tumors were evaluated at 31 months. The lung tumor incidence increased rapidly at low doses to give a 53% tumor incidence at 1 mg NNK/kg bw, and then more slowly to yield 73 and 87% tumors at 10 and 50 mg NNK/kg bw, respectively. For the DNA adduct studies, five doses were chosen between 0.1 and 50 mg/kg bw, and the profile for adduct formation was strikingly similar to that for tumorigenesis. The tumor and DNA adduct curves for NNK were supralinear (Figure 5, A curves), similar to DEN, but the increases at the lower doses were more pronounced. The biphasic nature of both tumor and DNA adduct curves was postulated to be caused by a high-affinity pathway that activates low doses of NNK to methylate DNA, forming the promutagenic adduct O^6-Me-dG. This pathway presumably becomes saturated at the higher doses (*8,22*). In addition, in animals given NNK doses higher than 1 mg/kg bw, there was significant evidence of lung hyperplasia and mild necrosis that may have contributed to the lack of linearity of DNA adducts with increasing dose (*21*).

Tumor-Adduct Profile Correlations

The different types of relationships between dose and tumorigenesis, and dose and DNA adduct formation, observed during chronic carcinogen administration at multiple doses in rodents are categorized in Figure 5 and summarized in Table 1. In addition to the linear category (B curves), the supralinear (A curves) and sublinear (C curves) categories originally defined by Swenberg (*7,19*), an additional category (D curves), termed "Low," is added here. Category D refers to situations in which, at all doses studied, there were few tumors and the levels of DNA adducts were low.

The data presented here, and summarized in Table 1, include nine different permutations of carcinogen, species, sex, and organ. Some issues that will be discussed include whether the tumorigenesis and DNA adduct profiles are both linear or nonlinear with dose, whether there are enough DNA adducts to produce tumors, and whether the tumorigenesis and DNA adduct profiles are similar to or different from each other.

In the simplest situation, both DNA adducts and tumors were linear with dose. This was observed with 2-AAF in female mouse liver and with AFB_1 in male rat liver. Representing two out of the nine possibilities, this straightforward relationship (Figure 5, B curves) is not the most frequent.

In two instances, with 4-ABP in male mouse liver and 4-ABP in the female mouse bladder, the DNA adduct levels were relatively low and the tumor incidence never reached 20%. This suggests that tumorigenic transformation, with or without toxicity or cell proliferation, might have occurred with higher DNA adduct levels. It implies the existence of effective hepatic detoxification pathways that may result in reduced activation to DNA adduct formation. In addition, in the case of female BALB/c mice given 4-ABP, the low level of kidney microsomal activation (compared to males) may contribute to the low DNA adduct levels (*15*).

In three instances, 4-ABP in female mouse liver, DEN in male rat liver, and NNK in male rat lung, the profiles for DNA adducts and tumor incidences were supralinear (Figure 5, A curves). In these situations, profiles for tumors and DNA adducts were similar, although the postulated underlying mechanisms for lack of linearity may have been different. For example, a metabolic saturation for 4-ABP activation may have occurred at the higher doses in female mice. In male rats given NNK, a high-affinity activation pathway with a low K_m for NNK is postulated to saturate at higher doses (*21*). For both NNK and DEN the higher doses were also considered to induce toxicity and cell proliferation that suppressed DNA adduct formation and tumorigenesis (*20,21*).

Table 1. Different patterns of rodent tumorigenesis and DNA adduct formation during chronic administration of chemical carcinogens at multiple dose levels.

				A - Supralinear		B - Linear		C - Sublinear		D - Low	
Compound	Species	Sex	Tissue	Tumors	Adducts	Tumors	Adducts	Tumors	Adducts	Tumors	Adducts
2-AAF	Mouse	F	Liver			*	*				
2-AAF	Mouse	F	Bladder				*	*			
4-ABP	Mouse	M	Liver				*			*	
4-ABP	Mouse	M	Bladder				*	*			
4-ABP	Mouse	F	Liver	*	*						
4-ABP	Mouse	F	Bladder							*	*
AFB_1	Rat	M	Liver			*	*				
DEN	Rat	M	Liver	*	*						
NNK	Rat	M	Lung	*	*						

Abbreviations: 2-AAF, 2-acetylaminofluorene; 4-ABP, 4-aminobiphenyl; AFB_1, aflatoxin B_1; DEN, diethylnitrosamine; NNK, nicotine-derived nitrosoketone; F, female; M, male.

In two instances, although DNA adduct formation was linear at all doses, tumorigenesis was sublinear (Figure 5, C curve) with no tumors formed at the lower doses but a significant jump to a high tumor incidence rate at higher dose levels. This occurred in the bladders of female mice given 2-AAF and male mice given 4-ABP. In the case of 2-AAF in the female mouse bladder, cell proliferation has been shown to be a necessary prerequisite for tumorigenesis (*12,13*). Although specific cell proliferation data are not available for male mice given 4-ABP, bladder hyperplasia (*14*) increased relative to dose and was highest at the tumorigenic doses (55–220 ppm).

Of the nine different biologic situations for which evidence has been considered above, profiles for DNA adduct formation reflected profiles for tumorigenesis in five cases, and in these instances linearity with dose for both tumors and DNA adducts appeared to be the norm at the lowest doses. In two situations the levels of DNA adducts formed were low, presumably below the threshold for extensive tumorigenesis. In two other situations, even though DNA adducts were linear, tumors were not formed at the lower doses; tumorigenesis appears to have required cell proliferation, in addition to DNA adduct formation, and this occurred only at the higher doses.

In conclusion, the chronic-dosing studies presented here indicate that DNA adduct formation is likely to be linear with dose, particularly at low, nontoxic doses. Tumorigenesis, however, may require factors in addition to DNA adduct formation, that are likely to be tissue-specific, and may include metabolic saturation, cell proliferation, and other manifestations of toxicity.

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