

# Use of Cell Proliferation Data in Modeling Urinary Bladder Carcinogenesis

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A multistage, probabilistic, biologically based model of carcinogenesis has been developed involving qualitative and quantitative aspects of the process. A chemical can affect the risk of cancer by directly damaging DNA and /or increasing the number of cell divisions during which errors in DNA replication can occur. Based on this model, carcinogens are classified as genotoxic versus nongenotoxic; nongenotoxic chemicals are further divided on the basis of whether or not they act through a specific cell receptor. Nongenotoxic compounds, particularly those acting through a nonreceptor mechanism, are likely to have dose and/or species-specific thresholds. This classification also implies the existence of chemicals that will be carcinogenic at high doses in animal models, but because of dose and/or mechanistic considerations, will not be carcinogenic to humans at levels of exposure. *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) and 2-acetylaminofluorene (AAF) are classical genotoxic bladder carcinogens that also cause proliferative effects at higher doses. Although there is an apparent no-effect level for the urinary bladder carcinogenicity of these two compounds at low doses, in reality, DNA adducts form at these low levels, and it is likely that there is a cancer effect (no threshold), but it is below the level of detection of the bioassay. These conclusions are based on studies involving multiple doses and time points in rodents, including results from the ED<sub>01</sub>. Pellets implanted directly into the rodent bladder lumen or calculi formed in the urine as a result of an administered chemical cause abrasion of the urothelium, and a marked increase in cell proliferation and cell number, and ultimately tumors. A threshold is readily definable based on physiologic, chemical, and pharmacokinetic considerations. Sodium saccharin also produces bladder cancer at high doses in rats, particularly males, if it is administered beginning at birth or earlier. The mechanism appears to be related to the formation of a silicate precipitate and/or crystals formed in the rat urine, which act as abrasives or cytotoxic materials, leading to increased cell proliferation and ultimately tumors. Numerous other sodium salts have similar effects. This effect is not observed in the mouse, hamster, or monkey, and epidemiological evidence suggests that it does not occur in humans. Thus, for sodium saccharin, even in the susceptible species, the rat, there appears to be a dose threshold, and extrapolation to humans appears inappropriate based on mechanistic considerations.

A multistage, probabilistic, biologically based model of carcinogenesis has been developed involving qualitative and quantitative aspects of the process (1-3). The risk of cancer can be affected by directly damaging DNA and/or increasing the number of cell divisions during which errors in DNA replication can occur. Based on this model, carcinogens are classified as

genotoxic versus nongenotoxic; nongenotoxic chemicals are further divided on the basis of whether they act through a specific cell receptor. Nongenotoxic compounds, particularly those acting through a nonreceptor mechanism, are likely to have dose and/or species-specific thresholds. This classification also implies the existence of chemicals that will be carcinogenic at high doses in animal models, but because of dose and/or mechanistic considerations, will not be carcinogenic to humans at anticipated levels of exposure.

*N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) is a bladder carcinogen in rats, mice, hamsters, and dogs and behaves as a classic genotoxic carcinogen (4). It is metabolically activated to a reactive intermediate that binds to DNA, producing DNA adducts leading to mutations and ultimately to cancer. If administered at doses above 0.01% of the diet, nearly 100% of the animals develop bladder tumors, whereas at lower doses,

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This paper was presented at the Symposium on Cell Proliferation and Chemical Carcinogenesis that was held January 14-16, 1992, in Research Triangle Park, NC.

there are no tumors produced within 2 years, despite the continuing presence of mutagenic activity in the urine. In addition to the genotoxic effects of FANFT metabolites interacting with DNA, FANFT administration produces increased proliferation of the urothelium, but only at the higher doses. Although the absence of tumors below the 0.01% dose suggests a no-effect level with respect to the bladder carcinogenicity of FANFT, in reality, this is merely a reflection of the limits of detection within the bioassay. The apparent no-effect level corresponds to the dose at which a cellular proliferative response is no longer present for synergistic interaction with the genotoxic effects of the compound. At doses where both effects are present, a detectable incidence of tumors is produced.

When a low dose of FANFT (0.005%) is administered simultaneously with a high dose of sodium saccharin (5%), there is a carcinogenic effect in the rat bladder, whereas when these chemicals given separately do not produce tumors (5). Sodium saccharin at 5% produces a proliferative response, which, in combination with the genotoxicity of FANFT even at a low dose, is probably adequate to produce an increase in bladder tumors within a 2-year experiment.

Similarly, 2-acetylaminofluorene (AAF) produced liver and bladder tumors when administered to mice. Its metabolism is well known, with production of reactive intermediates that bind to DNA producing DNA adducts, mutations, and ultimately tumors. In an experiment involving greater than 24,000 mice (the ED<sub>01</sub> experiment), administration of AAF at low doses (30–150 ppm) produced dose-response relationships that differed dramatically between the liver and bladder (6). In the liver, there was a detectable tumorigenic effect at the lowest doses, whereas in the bladder, there was no detectable effect below a dose of 60 ppm. Apparently, the genotoxic effect of AAF alone in the liver is sufficient to produce a detectable level of tumors because the doses used in the experiment were insufficient to result in an increased proliferative response in the liver.

In the bladder, however, a proliferative response is produced at doses of 60 ppm and above, the doses at which bladder tumors were detected. Although there is clearly a genotoxic effect produced by AAF as evidenced by the presence of DNA adducts to doses as low as 5 ppm of the diet (7), a detectable increase in tumors coincides with the doses having increased cell proliferation. In this regard, the dose-response relationship for the bladder with AAF is similar to that with FANFT: Doses producing proliferative response are adequate to generate a detectable incidence of tumors within the bioassay time period, whereas at lower doses, where only a genotoxic effect is present, the expected incidence is below the level of detection of the assay (8). With these genotoxic chemicals, the measured no-effect level is not a true threshold, but simply a reflection of the detection limits of the assay.

In contrast to these examples of genotoxic chemicals,

other examples deal with cases where proliferative events occur without accompanying genotoxicity. The simplest of these is implantation of pelleted material into the lumen of the bladder, which, depending on the roughness of the surface of the pellet, produces abrasion of the urothelium, regenerative hyperplasia, and ultimately tumors (3). The incidence of carcinoma increases with time, as demonstrated by Jull (9) for paraffin wax pellets: the incidence was 10.6% after 40–50 weeks, 26.8% after 70–80 weeks, and 53.8% after 100–110 weeks. A similar response is seen with other implanted materials, such as cholesterol pellets, glass, or stainless steel.

A similar phenomenon occurs with the production of calculi in the urine, whether due to administration of a chemical that leads to the production of calculi (3) or whether the necessary metabolic alterations are produced by other means, such as portacaval shunt (10), which can also lead to calculus production. Whenever calculi are produced, as with implanted pellets, there is abrasion of the urothelium, increased cell proliferation, and the increased risk of tumors. Calculi-producing chemicals include uracil, melamine, calcium oxalate, orotic acid, and glycine, as well as numerous others. Administration of these chemicals at doses high enough to produce calculi in the urine results in an increased risk of bladder tumors. If lower doses are administered, no calculi are generated and no bladder tumors are produced. In contrast to the examples of FANFT and AAF where the no-effect level for proliferative response is not a no-effect threshold for tumor production because of compound genotoxicity at low doses, the no-effect dose for these compounds is truly a threshold phenomenon. High-to-low dose extrapolation for risk assessment is inappropriate in these cases; what needs to be determined is the amount of chemical required to be ingested by the animal to produce calculi, subsequently producing proliferative and tumorigenic effects. At doses below which calculus formation occurs, no tumors are expected. The threshold cannot be determined by mathematical extrapolation but must be determined on the basis of physical chemical, pharmacokinetic, and physiological parameters.

A more subtle example occurs with sodium saccharin and numerous other sodium salts, such as sodium ascorbate, glutamate, erythorbate, citrate, aspartate, and others (2,3,11). Again, similar to chemicals that produce calculi where a high dose is required for the production of tumors, these chemicals are nongenotoxic. At high doses, there is increased cell proliferation, and at these doses, tumors occur. Although the details have not been completely established (12), it appears that the proliferative response occurs secondary to cytotoxicity produced by silicate-containing precipitate and/or crystals in the urine. The production of these silicates requires large amounts of protein, which is present in the rat, particularly the male. Consistent with this, there is a male predominance of tumors in the rat; the mouse, hamster, and monkey appear to be

resistant to the effects of these chemicals. Epidemiologic evidence suggests that there is no tumorigenic effect of sodium saccharin in humans (11), and preliminary studies also suggest that sodium saccharin ingestion does not produce increased cell proliferation of the bladder (13). In male rats, the most susceptible species and sex, a no-effect level appears at 1% of the diet. If this represents a true threshold, extrapolation to lower doses is inappropriate, and expected exposure levels by humans are unlikely to produce tumors. Similarly, if the mechanism for the production of these silicates occurs only in rats, and not in humans, interspecies extrapolation to human risk based on studies in the rat is inappropriate at any dose.

A similar difficulty occurs in estimating risk based on studies with sodium ascorbate. Sodium ascorbate affects the male rat in a fashion similar to sodium saccharin at comparable doses (14,15). If one calculates risk in humans based on data from male rats, a  $10^{-6}$  risk in humans is estimated as approximately 3 mg/day. This causes a serious problem because the minimum daily human requirement for vitamin C to prevent scurvy is 60 mg/day. In contrast, if one assumes a threshold phenomenon at approximately 1% of the diet in rats, the threshold for human consumption of ascorbate would be several orders of magnitude greater than the required 60 mg/day. Further, if the effect is truly confined only to the rat, then the cancer risk to humans for vitamin C consumption with respect to bladder cancer is zero, no matter what the dose.

The relationship of cell proliferation to carcinogenesis has produced confusion and seemingly conflicting results, many of which can be explained with more detailed examination of the biological phenomena occurring in a given model system. These are described in greater detail elsewhere (3). Of greatest importance with respect to risk assessment is that numerous nongenotoxic chemicals produce their effects by increasing cell proliferation, and it is likely, especially for those chemicals not acting through a specific cell receptor, that there is a threshold or a practical no-effect level for humans. In addition, because of mechanistic considerations, many of these chemicals will be capable of causing cancer in a specific animal model but would not be expected to have any tumorigenic effect in humans.

Evaluation of a chemical as a potential carcinogenic risk to humans using animal bioassays is based on two important assumptions: *a*) If a chemical causes cancer in mice and/or rats, it will cause cancer in humans (interspecies extrapolation) and *b*) if a chemical causes cancer at high doses, it will cause cancer at low doses (dose extrapolation). For genotoxic chemicals such as AAF and FANFT, these assumptions are likely to hold. On the other hand, for chemicals such as sodium saccharin and sodium ascorbate, neither of these assumptions appear to be valid. With the greater

understanding we now have with respect to the mechanisms involved in chemical carcinogenesis and cancer production in general, a more rational biological approach to risk extrapolation based on data from rodent bioassays is possible, supplementing blind, simplistic, mathematical extrapolation driven by the above two assumptions.

We greatly appreciate the assistance of Ginni Philbrick in the production of this manuscript. Our research is supported by Public Health Service grants CA32315 and CA36727 and by grants from the International Life Sciences Institute.

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