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The relevance of experimental carcinogenicity studies to human safety

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Abstract

The two year rodent bioassay has been the standard for carcinogenicity screening for 5 decades. However, numerous examples exist showing that a positive result is not predictive of human cancer risk because of lack of relevant dose and/or mode of action. Utilizing basic principles of carcinogenesis, evaluating chemicals for DNA reactivity, immunosuppression, estrogenic activity and increased cell proliferation provides a more scientific, rationally based process for evaluating risk, in conjunction with metabolism in human cell systems and dose response considerations. George Box once stated, "Models; all are wrong, some are useful." The two year rodent bioassay screen for carcinogenesis is no longer useful.

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1. Introduction

The environmental basis for many cancers was strongly suspected in the first half of the 20th century [1]. Initial discoveries of specific carcinogens were largely by observations in humans, often in occupational settings. By the 1950s, several specific chemicals were identified as carcinogens in animal models and humans. Considerable effort was made in the 1960s to develop a screen for detecting carcinogens so that they could be reduced or eliminated from the environment. This resulted in the two year rodent bioassay, initially developed at the National Cancer Institute (NCI) and then transferred to the National Toxicology Program (NTP) [2,3].

The two year bioassay became standard practice for screening numerous classes of chemicals including industrial chemicals, agrichemicals, consumer products, food additives, and pharmaceuticals. The procedures have largely remained the same for the past five decades, with some refinements regarding diet, pathology classification, statistical evaluations, and other aspects [2–6]. Reliance on the two year bioassay was based primarily on its strong record for detecting known human carcinogens, including aromatic amines, N-nitrosamines, polycyclic aromatic hydrocarbons, aflatoxins, and others.

However, several aspects of this screening process led to significant concerns, including the long time needed, use of large numbers of animals, and the expense. However, the major concern is its questionable predictivity for human carcinogenicity [7,8].

Most of the chemicals tested early in the two year bioassay were chemicals ultimately shown to be DNA reactive carcinogens. These agents are metabolically activated to reactive electrophiles, bind to DNA, lead to formation of adducts, and ultimately mutations [1]. This paradigm was seized upon by Ames in the 1970s to develop a quicker, less expensive, and non-animal assay involving mutagenicity screening in Salmonella, leading to the belief that "mutagenesis is carcinogenesis" [1,9]. An explosion of assays; *in vitro* and *in vivo*, were developed to screen for genotoxicity. The Ames assay was incorporated into the NTP program, and has subsequently been incorporated into screening processes for numerous commercial products.

However, concerns about the predictive value of such screens were raised, especially the number of chemicals identified as so-called promoters in the initiation-promotion model [1]. Many so-called promoters produced tumors by themselves in a full two year bioassay, and they were generally negative in the various genotoxicity assays. This ultimately led to the seminal publication by Weisburger and Williams [10] distinguishing two classes of chemical carcinogens, genotoxic and non-genotoxic.

2. Non-genotoxic carcinogens

A particular concern was the large percentage of chemicals being tested that were positive in the rodent two year bioassay. Also of concern were the increased incidence of tumors related to particular tissues in each species, such as the liver and lung in mice, and the liver, mammary gland, and various endocrine organs in rats [7,8]. Alarm continued to increase as several of the tumors identified in the rodent models were demonstrated to occur by modes of action that were not

relevant to humans, such as D-limonene-induced kidney tumors in male rats and saccharin-induced urinary bladder tumors in rats [1]. In the 1990s, Ames and Gold [11,12] highlighted this concern by focusing on chemicals that were present in food, natural or synthetic. Utilizing the cancer potency database [13], they demonstrated that if the two year bioassay was actually predictive of human carcinogenesis and potencies in the animal models were predictive, more than 99 percent of cancers were due to natural ingredients in foods rather than synthetic chemicals in foods or environmentally.

Numerous chemicals by the 1990s were identified as having a mode of action producing tumors in rodents that were not relevant to humans [1,7,8]. Furthermore, numerous chemicals were positive only at the highest dose used in the two year bioassay (maximum tolerated dose, MTD), raising concerns that the positive carcinogenicity results were related to the toxicity produced by the chemical rather than actual carcinogenic activity. Under such circumstances, extrapolation to lower non-toxic exposures in humans would be meaningless.

Any time an assay is performed in animals, there are two fundamental assumptions: 1) the animal model result is relevant to humans (interspecies extrapolation); and 2) the toxicological response at doses used in animal models are relevant to human exposure levels (dose extrapolation) [1,14]. The validity of these assumptions for the two year bioassay had been based on the tumor response for potent DNA reactive carcinogens. However, for non-genotoxic chemicals, one or both of these assumptions have been demonstrated to be incorrect.

Not only were numerous food ingredients, natural and synthetic, shown to be positive in the two year bioassay, but a large number of pharmaceutical agents, consumer products, and environmental chemicals were positive. Nevertheless, many have continued in commercial use because of significant differences between the mode of action in the animals versus humans and/or differences in exposure. For example, it has been estimated that approximately 60% of pharmaceutical agents listed in the Physicians' Desk Reference (PDR) tested in the rodent bioassay are positive [15]. Nevertheless, regulatory agencies have considered these not to be threats to human safety. Some of these positive results have been with agents that are widely used by humans such as statins (liver tumors) and proton pump inhibitors (stomach tumors).

Lack of carcinogenicity in humans for many of these drugs have been confirmed in large epidemiology studies.

3. Principles of carcinogenesis

Given the numerous examples of chemicals that produced positive results in the two year bioassay and yet are widely used by humans, there is significant doubt about its usefulness for predicting carcinogenic activity in humans [7,8]. It is a screening assay which is not founded on fundamental principles of carcinogenesis. The various genotoxicity assays, especially the Ames assay, are based on sound scientific theoretical considerations, but are only useful for chemicals that are DNA reactive. Development of structure activity relation (SAR) computer programs has further refined the predictive value of mutagenicity. These computerized programs provide rapid identification of structural alerts, and the Ames assay can be used as evaluation of such activity. This can be further evaluated with respect to specific metabolic pathways, with both qualitative and quantitative comparisons possible between the animal models and humans.

In the past century we have learned much about the carcinogenic process [14,16–18]. Cancer is a clonal disease; tumors arise from pluripotential (stem) cells in the target tissues. Cancer arises due to mistakes present in the DNA, with more than one genetic mistake required. Furthermore, every time DNA replicates mistakes can occur "spontaneously". These spontaneous errors appear to be due to the numerous endogenous processes that occur in cells every day, such as oxidative damage, exocyclic adducts, depurination, etc. These are predominantly repaired by an extensive and exquisite set of DNA repair enzymes, but some permanent mistakes occur with every DNA replication. Over time, all of the required mistakes can occur in a single cell and cancer arises [14,16–18].

Based on these concepts, there are fundamentally only two mechanisms by which an agent can increase the risk of cancer: 1) damage DNA directly so that more mistakes occur each time DNA replicates; and 2) increase the number of DNA replications increasing the opportunity for spontaneous mistakes [14,16–18]. Although some have referred to the latter process as "bad luck" [19], in reality, both of these processes can be influenced by environmental agents [14,16–18].

Chemicals that damage DNA directly, genotoxic carcinogens such as aflatoxin, have been identified as animal and human carcinogens, and are screened by genotoxicity assays [1,4]. However, it is the non-genotoxic chemicals that pose the greater challenge for identifying carcinogenic activity in humans. These are the chemicals that produce cancer by increasing the number of DNA replications. It has become apparent that the two year bioassay in rats and mice is of little predictive value for human carcinogenetic risk with respect to such agents, since there is an extremely high false positive rate based on either non-relevance of the mode of action or non-relevance of the doses required in the animal model. Extrapolation from the animal models