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Perspectives on the Risk Assessment for Nongenotoxic Carcinogens and Tumor Promoters

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The issue of risk assessment for carcinogens that appear to act via nongenotoxic mechanisms or at the tumor promotion stage, respectively, is discussed in light of current information on biological mechanisms involved in carcinogenesis as well as interindividual variability in human response. Proposals to treat "nongenotoxic" carcinogens and tumor promoters as posing lower risks to humans are described and evaluated. It is concluded that, for purposes of risk assessment and regulation, there is currently no convincing scientific rationale for constructing categories of carcinogens according to their presumed mechanism or stage of action.

Introduction

Before tackling the question, Why is risk assessment for "nongenotoxic" and tumor-promoting carcinogens such an important and controversial issue, let me stress that the two terms are not synonymous. Rather, they reflect presumptions of mechanism ("nongenotoxic") and stage of operation (tumor promoter). Nevertheless, although the two classes are not congruent, they overlap in that many of the same chemicals have both characteristics. Thus, this review treats them as posing separate but related questions. By way of background, a significant number of carcinogens are inactive or only weakly active in conventional tests for genotoxicity such as assays for covalent binding to DNA and induction of mutagenicity in Salmonella (*1*). For example, an estimated 33% of the 138 rodent carcinogens tested by the National Toxicology Program (NTP) were negative in the Ames assay and were also negative for structural alerts to DNA reactivity (*2,3*). These carcinogens include commercially important industrial chemicals and man-made substances that represent significant environmental and occupational hazards by virtue of their high volume of production and release to the environment. Examples are halogenated organic compounds used as pesticides and herbicides, polychlorinated biphenyls, and asbestos. At the same time, a number of commercially valuable compounds, such as saccharin, phenobarbital, and di(2-ethylhexyl)phthalate, are inactive or weak initiating carcinogens but are capable of acting as tumor promoters in experimental systems. Therefore, debates over the extent of risk posed by such agents readily become politicized.

The mechanisms by which nongenotoxic compounds and tumor promoters induce cancer are less well understood than those for carcinogens that directly damage DNA. Despite a recent proliferation of research, the major focus in experimental and human systems has been on mechanisms by which model genotoxic agents exert their effects during the initiation and progression stages of chemical carcinogenesis. In the area of biomonitoring, for example, a battery of biologic markers is currently being validated to investigate these mechanisms in *in vitro* studies, in laboratory animals and in humans (*4,5*) (Table 1). By contrast, there are markedly fewer biologic markers that enable the parallel evaluation in experimental systems and in humans of nongenotoxic or indirect genotoxic mechanisms involved in carcinogenesis and of mechanisms specific to the promotion stage. This is a significant gap in research that may eventually be filled by biologic markers such as those reflecting indirect genetic toxicity (e.g., oxidative damage) and molecular events in tumor promotion (e.g., increased expression of certain genes implicated in growth control). For example, experimentally, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and other tumor promoters cause increased expression of genes related to cell proliferation, including c-*myc* and c-*fos*, ornithine decarboxylase (ODC), and phorbin (phorbol ester inducible gene), possibly as a result of binding to and activating protein kinase C (PKC) (*6*). Research is now underway to determine whether the human homolog of phorbin, erythroid potentiating activity (EPA), can be used as a biomarker in humans exposed to tumor promoters.

This great measure of biologic uncertainty concerning nongenotoxic carcinogens and tumor promoters quite naturally leads to heightened controversy. In this and in other such debates involving scientific uncertainty, there is a thin line between science and policy, with values playing a central, often unacknowledged, role (*7*). Thus, during the past decade, we have seen the evolution of two diametrically opposed views on the subject of risk assessment for carcinogens. The first is that nongenotoxic agents and tumor promoters are likely to have thresholds and therefore present less risk to humans than

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Table 1. Molecular effects used as biomarkers.

Biomarker	System	
	Laboratory	Human
Genotoxic carcinogens		
Covalent binding to DNA	√	√
DNA repair	√	√
Gene mutation	√	√
Chromosomal aberration	√	√
Oncogene activation	√	√
Nongenotoxic carcinogens[a]		
PKC induction	√	
Phorbin/EPA induction	√	
ODC induction	√	
Cell-to-cell communication	√	
Oncogene overexpression	√	

[a]PKC, protein kinase C; ODC, ornithine decarboxylase; EPA, erythroid potentiating activity.

genotoxic or initiating carcinogens (*8–10*) and, conversely, that nongenotoxic agents and promoters are of particular concern in terms of human risk (*11–14*).

The first position is based upon the assumption that unless agents are able to damage DNA and mutate genes, they cannot be considered to exert low-dose linearity. The opposite position holds that there is no reliable evidence of the presence or absence of an experimental or human threshold for any carcinogen regardless of its mechanism or stage of action (*14,15*) and that rapid benefits in terms of human cancer prevention can be achieved by controlling exposure to nongenotoxic and promoting agents (*11,14*). These authors cite the drastic reduction in human cancer achieved through control of cigarette smoking (both an initiator and a promoter) and exposure to estrogen (believed to act as a promoter) (*11*).

Traditionally, the major U.S. Federal and State agencies have used a nonthreshold model in risk assessment for carcinogens—regardless of their presumed mechanism of action (*16*). This decision has rested largely on two key assumptions, which were necessitated by the uncertainty about the true dose-response in humans. The first is that risk from any individual carcinogen will be at least additive upon background; the second assumption is that it is impossible to identify a threshold for a heterogeneous human population given the possible wide interindividual variability in response to carcinogens.

In 1984, I reviewed the literature pertaining to risk assessment for nongenotoxic agents in light of several proposals to amend Federal cancer policy by treating the agents as threshold-type toxicants (*17*). While many scientists shared the desire to fine tune the risk assessment process to make it more mechanistically relevant, the consensus at that time was that such proposals were premature given the current state of knowledge (*17*). This paper is intended to update that earlier review by assessing new information developed in the intervening 5-year period.

Chronology of Events

To recapitulate briefly, in 1980–1981, Williams and Weisburger (*8,9*) proposed that "epigenetic/nongenotoxic" carcinogens (defined as those negative in the Ames assay, the hypoxanthine [quanine] phosphoribosyl transferase [HPRT] gene mutation assay, the rodent hepatocyte assay for DNA damage and repair, and the assay for sister chromatid exchange) were likely to have thresholds and could be regulated less stringently than genotoxic carcinogens. A similar view was also espoused by Stott et al. (*18*), who singled out DNA alkylation *in vivo* and DNA repair as tests for genotoxicity. The Environmental Protection Agency (EPA) (*19*) proposed to amend its existing cancer policy by stipulating that only "genotoxic" carcinogens (those having positive results in several assays for gene mutation) would be regulated using the no-threshold linearized model. The no-observed-effect level (NOEL) safety-factor approach previously reserved for noncarcinogens would be applied to all others. The practical effect of this policy change would have been a relaxation of standards for certain waterborne carcinogens by 100- to 1000-fold compared to standards developed using the traditional linear risk extrapolation approach (*17*). After receiving overwhelmingly critical comments from the scientific community, EPA did not finalize the proposal.

Concurrently, the California Department of Health Services (*15*) carried out a similar deliberation and concluded: "Low dose linearity applies equally well to agents which are thought to act by either genetic or epigenetic mechanisms if one makes the reasonable assumption that similar mechanisms are already operating and contributing to the background incidence of cancer."

Similarly, working groups of the International Agency for Research on Cancer concluded in 1983 (and again in 1987) that there was insufficient information to implement a classification of agents according to their mechanism of action (*20,21*). This does not mean, however, that the scientific community has been unanimous on this question. In 1983, Kroes from the Netherlands (*10*) wrote:

> Current knowledge does not permit a rigid classification of carcinogens, but does warrant a subclassification into genotoxic and nongenotoxic compounds. Whereas for genotoxic compounds a real threshold cannot be expected on a theoretical basis, the existence of a threshold may well be expected for nongenotoxic compounds. In conjunction with other characteristics it may then be decided whether a genotoxic or nongenotoxic compound may be or may not be permitted in the human environment.

Two years later, in 1985, the Office of Science and Technology Policy (OSTP) grappled with this question in compiling an interagency review of the science regarding risk assessment for carcinogens (*22*). The report stated: "At the present state of knowledge mechanistic considerations such as DNA repair and other biologic responses, in general, do not prove the existence of, or the absence of, or the location of a threshold for carcinogenesis."

The EPA cancer policy, revised in 1986 (*23*), was consistent with OSTP in stating that: "At present, mechanisms of the carcinogenesis process are largely unknown and data are generally limited... In the absence of adequate information to the contrary, the linearized multistage model will be employed."

More recently, in 1989, the EPA has solicited comments from outside reviewers on the question of how to use mechanistic information relating to factors such as hormonal carcinogenesis, the role of cellular peroxide formation and cytotoxicity, promotion and, very importantly, genotoxicity (*24*). Thus, this is a recurrent theme that deserves serious further consideration by the scientific community.

Definitional Problems in Formulating Separate Risk Assessment Strategies for Nongenotoxic Carcinogens and Tumor Promoters

The first question is the definition of the term "nongenotoxic." Major confusion has arisen because nongenotoxic carcinogens are frequently, and mistakenly, equated with tumor promoters, and genotoxic carcinogens are often treated synonomously with initiating agents. As discussed by Yamasaki (*14*), the terms "initiation" and "promotion" refer to stages of operation, usually in the two-stage experimental model of carcinogenesis, whereas genotoxicity and nongenotoxicity refer to possible mechanisms by which agents exert their effects. There is no consensus in the scientific community as to the criteria for genotoxicity. This has led individual researchers to define these criteria arbitrarily. Thus, at one end of the spectrum the term has been narrowly defined as "positive in the Ames assay" while at the other extreme is the broadest definition, which includes the ability, directly or indirectly, to damage or alter the genetic material (*17,25,26*). In between are permutations shown in Table 2, which well illustrate the arbitrary nature of the exercise.

In addition, it is misleading to use these terms to suggest that a chemical acts only via a genotoxic or nongenotoxic mechanism (*14,27*). For example, polycyclic aromatic hydrocarbons (PAHs) are both genotoxic (binding covalently to DNA) and nongenotoxic (modulating membrane receptors) (*28*). Moreover, each of the carcinogens shown in Table 3 labeled "nongenotoxic" by various investigators (*1,8,9*) has displayed some genetic toxicity. Although in most cases, the majority of short-term test results have been negative, each has been positive in assays for chromosomal aberrations, sister chromatid exchange (SCE) and/or gene mutation, possibly as a result of induction of reactive superoxide radicals (*25,29,30*). It is generally agreed that the induction of chromosomal aberrations, SCEs, or gene mutation in well-designed, carefully controlled studies can provide evidence of genetic toxicity. A most striking finding has been the induction of a novel activating point mutation in the *ras* oncogene by furan and furfural, both of which are negative in the Ames assay (*31,32*).

Similarly, the term "tumor promoter" has been subject to widely varying definitions. The term is an operational one, referring to activity in the two-stage experimental model (usually in mouse skin or rat liver) in which the agent in question does not, by itself, significantly increase tumor formation but, when administered after an initiating agent or complete carcinogen, significantly enhances the induction of tumors. These studies have suggested that initiating agents directly interact with the genetic apparatus of cells, whereas the promoting agents encourage these latent initiated cells to expand clonally and form a benign tumor (*4,14,33–35*). During the past several years, evidence has developed that some type of heritable imprinting is also involved in tumor promotion, possibly via an indirect genetic mechanism (*14,25,33,35–39*). Recent studies indicate that tumor promoters can induce genomic changes—albeit indirectly—through induction of free radicals and superoxides that react with DNA and cause molecular lesions (such as thymine glycol and other altered nucleic acid structures) (*4,17,26,33,41,42*). Thus, indirect genotoxicity has been implicated in the induction of chromosomal aberrations by TPA (*25*) and in the formation of DNA strand breaks in human leukocytes by benzoyl peroxide, another potent tumor promoter (*43*).

Table 2. Definitions of genotoxicity.

Criteria	Reference
Mutagenicity (Ames, HPRT), DNA damage/repair, and sister chromatid exchange	(*8,9*)
DNA alkylation *in vivo* and DNA repair	(*18*)
Gene mutation: two assays, one eukaryotic or one whole mammal test	(*19*)
Direct or indirect genomic changes	(*14,17,25,26*)

Table 3. Nongenotoxic carcinogens with some evidence of genetic toxicity.

Agent[a]	End point/system	Reference
TCE	Mutation/bacteria and animal cells; SCE/human cells *in vitro*[b]	(*51*)
DDT	Chromosomal aberrations/human cells *in vivo*	(*52*)
	Mutation/animal cells *in vivo*	(*51*)
Ethyl alcohol	SCE and chromosomal aberrations/human cells *in vivo*	(*51,53*)
Asbestos	SCE and chromosomal aberrations/human cells *in vivo*	(*54*)
TPA	SCE and chromosomal aberrations/human cells *in vitro*	(*25*)
Furan and furfural	Point mutation in *ras* oncogene/rodent *in vivo*	(*31,32*)

[a]TCE, trichloroethylene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.
[b]SCE, sister chromatid exchange.

In addition, the terms "initiator" and "promoter" are not mutually exclusive. Many carcinogens can act as both initiators and promoters in the same tissue or organ. For example, PAHs act as complete carcinogens on mouse skin and therefore have both types of activity. TPA, which is a potent promoter in mouse skin, also acts as a weak complete carcinogen in that tissue (*14*).

There are also numerous examples of agents that can act at different stages in different organs. Urethane (ethyl carbamate) is not a complete carcinogen in mouse skin, although it is an effective initiator. In mouse liver, however, urethane is a complete carcinogen, presumably because cell division in the liver acts as an effective promotional stimulus (*33*). In humans, asbestos appears to act as a promoting agent or, at least, a "late-stage" carcinogen in lung cancer and as an initiating agent in mesothelioma (*20*). For all these reasons, Barrett and Wiseman (*33*) have concluded that one cannot predict the tumorigenicity of a compound based on its ability to act as a promoter in a two-stage model.

Regarding the question of dose-response for tumor promoters, it has been noted that apparent dose-response thresholds in experimental systems may actually reflect dose-schedule thresholds (*14,39,44–46*). Interestingly, in the mouse skin model and in a two-stage *in vitro* cell transformation system, respectively, treatment by benzo[*a*]pyrene or X-rays alone did not give a linear dose response. Linearity was seen only in the presence of the tumor-promoting agent TPA (*14,47,48*). These results suggest that available experimental data show a dose-schedule effect, not to be confused with a threshold, and that low-dose linearity of initiating agents may in some cases depend upon exposure to promoting agents.

Table 4. Interindividual variation in human cells (*4,55,56*).

Process	Maximum observed variation
Absorption/transport	?
Activation/deactivation	
P-450 isoenzymes	50–350 ×
Glutathione-*S*-transferase	100–200 ×
Reaction with target molecules	
DNA	50–200 ×
Components of signal transduction pathways	?
Oncogenes/anti-oncongenes	?
DNA repair	> 5 ×
Cell growth and differentiation	Severalfold

Difficulty in Identifying Population Thresholds for Any Carcinogen

From the above discussion, it appears that experimental data are too limited to conclude the absence or presence of thresholds for carcinogens, regardless of the presumed stage or mechanism of action. Human data are even more limited regarding this question. There is, however, a body of data concerning interindividual variability in response to carcinogens that supports the assumption of low-dose linearity. As shown in Table 4, significant interindividual variation has been observed in human cells *in vitro* or *in vivo* in terms of various steps in the biochemical handling of carcinogens. Each of these steps or processes reflects an interplay between acquired and/or genetic susceptibility (e.g., preexisting disease, nutritional imbalance, viral infection, hormonal status, immune surveillance) and environmental factors (e.g., exposures related to lifestyle, occupation, and the ambient environment). While at first glance, several of these steps (e.g., DNA binding and repair) appear to be irrelevant to assessing risk of tumor promoters, variation in any step or stage of the carcinogenic process will, in fact, affect an individual's risk from promoting agents. For example, the size of the initiated cell population at risk, dependent upon binding and repair, will directly determine the effect of exposure to a tumor promoter. It is also immediately obvious that many of the steps (absorption, activation/deactivation, immmune surveillance) are as relevant to response to promoting agents as they are to initiators. For example, like PAHs, hormones are oxidized by the cytochrome P-450 system so that variation in cytochrome P-450 activity may influence risk of breast cancer (*49*).

While there are few data that bear specifically on interindividual variability in tumor promotion, recent studies in human bronchial cells in culture have shown a several-fold variation in cell growth and differentiation (as evidenced by colony-forming efficiency) resulting from treatment with TPA (C. C. Harris, manuscript in preparation). In addition, in human volunteers, a marked intersubject variation was seen in TPA-induced epidermal ODC activity levels (*50*).

The fact that the human carcinogenic process extends over a prolonged period involving multiple stages increases the likelihood that interactions may occur between multiple factors, including combined actions of chemicals and viral agents (*6*). Indeed, there is considerable experimental evidence of synergistic interaction between genotoxic agents and viruses (e.g., PAH and papilloma virus on mouse skin), between tumor promoters and viruses (e.g., TPA and Epstein-Barr virus in transformation of human lymphocytes in cell culture) and between cellular oncogenes activated by chemical carcinogens and viruses (*ras* and HPV 16 or 18 in human epithelial cells) (*6*). Thus, in certain instances, the assumption of additivity on background may not be adequately conservative.

Conclusion

In summary, the multistage process of cancer development is now known to involve both mutagenic and nonmutagenic mechanisms. These mechanisms result in the induction of multiple direct and indirect genetic changes at target oncogenes or tumor-suppressor genes as well as alterations in signal transduction pathways involved in growth control. Much of the discussion of mechanisms for "nongenotoxic" carcinogens and "tumor promoters" in the context of policy and risk assessment has been fatally flawed by the lack of consistent terminology and an oversimplification of the underlying biological processes. The present review illustrates the impossibility of constructing broad categories according to more or less risky mechanisms or stages of action. There is a need to consider both the multiplicity of action of single agents and the influence of all agents to which humans are exposed simultaneously. This so-called background may include tumor promoting, initiating, or co-carcinogenic agents. The interindividual variation in cancer risk and in molecular or biochemical response among individuals with comparable exposure testifies to the complex interplay between genetic and acquired factors. This phenomenon deserves special attention in research since understanding the nature of interindividual variability and susceptibility is at the heart of cancer prevention. Development of biologic markers for studying nongenotoxic agents and tumor promoters *in vivo* holds promise for parallel laboratory and human studies. At the present time, one must conclude that there is no justification for systematically relaxing standards of health protection for carcinogens based on presumed mechanism or stage of action.

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