Ranking Animal Carcinogens: A Proposed Regulatory Approach

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Summary. The nature and extent of positive evidence associated with animal carcinogens vary widely, yet present regulatory policy does not permit adequate discrimination among the many carcinogenic substances. Most are treated as if they pose equal potential risk to humans, and this is not consistent with the available data. Without knowledge of carcinogenic mechanisms, the evaluation of responses in intact mammalian surrogates best reflects the potential levels of human risk. An example of a scoring system is proposed by which animal carcinogens are ranked according to the most relevant toxicological evidence derived from animal and genotoxicity studies. Different classes of animal carcinogens could thus be recognized and would permit several regulatory options and provide a means to establish priorities for public and scientific concerns.

The regulation of chemical carcinogens is based on several types of scientific evidence. Among these, well-conducted human epidemiological studies are the most persuasive and least controversial. Short-term studies in vivo and in vitro, including genotoxicity, neoplastic cell transformation, and chemical structure-activity relationships, may provide supportive or suggestive evidence. However, carcinogenicity testing in laboratory animals remains the primary basis for most regulatory decisions (*1*). In light of our ignorance of carcinogenic mechanisms and our inability to determine no-effect or threshhold levels, public health concern has required that all animal carcinogens be considered as potential human carcinogens. In the past decade, during which there were many animal carcinogenicity tests, it became evident that the nature and extent of positive evidence varied widely among different chemicals, as is true in other toxicological testing. Yet the existing all-or-none approach to carcinogen regulation requires that all animal carcinogens be treated as if they pose equal risk to humans. This is a difficult position to defend, and questions have been raised in the scientific and lay communities regarding the relevance of animal evidence to human risk. There has also developed a fatalistic disregard for experimental evidence, even among some of the best informed members of society. The assertion that all animal carcinogens pose equal threats to human health cannot continue without risking a greater skepticism for regulatory decisions.

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Illustrative of the problem is the fact that there is no acceptable regulatory procedure—particularly with regard to food additives—to permit distinctions to be made, based on the weight of evidence, about the potential cancer risks posed by such diverse substances as saccharin, 2-naphthylamine, nitrilotriacetic acid (NTA), chloroform, DDT, dimethylnitrosamine, aflatoxin, chlordane, vinyl chloride, and tris(2,3-dibromopropyl)phosphate (Tris). In this group are included examples of chemicals that vary widely in their carcinogenic potency and chemical characteristics. For example, NTA is not biotransformed, is biologically nonreactive, and is promptly excreted in the urine. It is carcinogenic only to the urinary tract of mice and rats at doses of 1.5 percent of the diet or above, administered for 2 years (*2*). This level is also very toxic to the kidneys. Similarly, saccharin, chloroform, chlordane, and DDT have shown positive results in only a few of several tests for genotoxicity, and they are carcinogenic in laboratory rodents only at very high levels of exposure through major portions of the animals' life-spans. By contrast, 2-naphthylamine, dimethylnitrosamine, aflatoxin, vinyl chloride, and Tris are genotoxic in vivo and in vitro in several different test systems. They are carcinogenic in multiple tissues, in more than one species, at very low doses, and—in some cases—relatively brief exposures. For example, liver tumors can be induced in rats with aflatoxin B_1 at a level of 0.000000001 percent in the diet (*3*).

A system by which selected animal carcinogens could be ranked semiquantitatively may be a useful regulatory alternative to current methods. It may not be possible to rank all animal carcinogens in a scientifically valid manner, even if this were desired. Many have had limited or inadequate testing. Others, such as hormones, may be considered to operate through a relatively unique (though unclear) mechanism, and a system based on traditional toxicological measurements may be considered inappropriate. In many instances, however, where testing has been adequate and equivalent, a ranking scheme may assist in interpreting toxicological data for health risk assessment and regulatory policy. In this article, I propose a possible approach to ranking animal carcinogens based on evidence derived primarily from the test animals themselves. Other ranking systems could be developed, and other factors and types of data will ultimately be included in an overall human risk assessment. However, test animals are the human surrogates in toxicology and will continue to be the basis for regulatory decisions for some time to come. The system is proposed on the assumption that the chemicals under consideration have not already been shown to be human carcinogens.

Current Efforts to Rank Carcinogens

The carcinogen standards of the Occupational Safety and Health Administration have separated potential carcinogens into three categories according to the level of evidence (*4*). However, most of the pertinent data are omitted from the criteria and all of the chemicals mentioned above would be classified as belonging to category 1. This is misleading in light of present knowledge and the nature of the available evidence.

A recent paper by Griesemer and

0036-8075/81/1120-0877S01.00/0

Table 1. Proposed system for ranking animal carcinogens.

Factor	Score
A. Number of different species affected	
Two or more	15
One	5
B. Number of histogenetically different types of neoplasms in one or more species	
Three or more	15
Two	10
One	5
C. Spontaneous incidence in appropriate control groups of neoplasms induced in treated groups	
Less than 1 percent	15
1 to 10 percent	10
10 to 20 percent	5
More than 20 percent	1
D. Dose-response relationships (cumulative oral dose equivalents per kilogram of body weight per day for 2 years)*	
Less than 1 microgram	15
1 microgram to 1 milligram	10
1 milligram to 1 gram	5
More than 1 gram	1
E. Malignancy of induced neoplasms	
More than 50 percent	15
25 to 50 percent	10
Less than 25 percent	5
No malignancy	1
F. Genotoxicity, measured in an appropriate battery of tests	
Positive	25
Incompletely positive	10
Negative	0

*Based on estimated consumption of 100 grams of diet per kilogram of body weight. Scoring could also be developed for inhalation or other appropriate routes.

Cueto (*5*) offers a more detailed classification of animal carcinogens from data derived from the National Cancer Institute's Testing Program (now the National Toxicology Program). The criteria used were based on those recently adopted by the International Agency for Research on Cancer (IARC) for use in the IARC monograph series that evaluates the carcinogenic risk of chemicals to humans (*6*). This method classifies the evidence of carcinogenicity in animal experiments as either "sufficient" or "limited." Sufficient evidence requires that animal experiments show an "increased incidence of malignant tumors: (i) in multiple species or strains, and/or (ii) in multiple experiments (routes and/or doses); and/or (iii) to an unusual degree (with regard to incidence, site, type, and/or precocity of onset). Additional evidence may be provided by data concerning dose-response, mutagenicity, or structure." Limited evidence is not precisely defined, but includes induction of "certain neoplasms, including lung tumors and hepatomas in mice, which are considered of lesser significance than neoplasms occurring at other sites for the purpose of evaluating the carcinogenicity of chemicals." This is a significant step toward ranking animal carcinogens according to the strength of experimental evidence. It does not, however, include the biological factors to be considered in assessing carcinogenic potency or potential human risk.

Other efforts to recognize the apparent differences among animal carcinogens have been expressed through proposals that two distinct categories be recognized: genetic and nongenetic (*7*). Such carcinogens would be considered either as initiators or as promoters (or modifiers). The danger in this dichotomous approach is that it may result in substitution of one rigid policy for another [namely, the Delaney clause (*8*)], both based on theoretical assumptions and yielding only two possible categories. Although the opinion prevails that a mutagenic-like event—that is, DNA damage—is the ultimate mechanism of neoplastic transformation (*9*), this remains hypothetical. Further development of genotoxicty tests and understanding of carcinogenic mechanisms may ultimately permit short-term studies to largely replace long-term animal bioassays. However, at present, the high correlation between genotoxicity and carcinogenicity is empirical and should not govern regulatory policy.

At certain exposure levels, most—if not all—animal carcinogens are toxic to the target cells. Neoplastic transformation could therefore be either all genetic or all nongenetic, and the differences observed in animal studies may be epiphenomena associated with detoxification, repair, or other adaptive mechanisms. Furthermore, if so-called modifiers or promoters act on initiated cells, a promotional effect on tissues such as breast, colon, or lung, where there exists a high background of cancer in humans, could produce a greater risk than would be produced by an initiator acting on the liver, for example, where human cancer rates in the United States are very low. Perhaps the most important consideration in the mechanism of neoplastic transformation is whether it is direct or indirect. If transformation is secondary to certain levels of toxicity, then no-effect or threshold levels would exist irrespective of whether carcinogenesis is genetic, nongenetic, or both.

Several animal carcinogens have not been shown to be mutagenic and give positive results in only a few of a large battery of other genotoxicity tests, according to current methods (*10, 11*); there is no firm evidence to explain the mechanism by which they induce cancer. Furthermore, there remains controversy over which short-term tests should constitute an appropriate battery for determination of mutagenic or carcinogenic potential (*11, 12*). While genotoxicity tests should be a significant part of the total assessment of carcinogenic potential, they should be considered as providing suggestive or supportive evidence—as are other short-term and in vitro methods—which may or may not add to the evidence derived from animal studies.

Extrapolation from animal results to potential human risk has recently centered on the use of mathematical models, partially in an effort to obviate the debate over threshold or no-effect levels. Several models have been developed, some of which are said to reflect certain biological events at low levels of exposure (*13*). However, no models can actually be based on the biological events, since these are not known for any carcinogens. For the same animal data, different models may predict levels of risk that vary widely (*14*), indicating the potential error involved in estimating carcinogenic potency or human cancer risks by such methods. Because of the uncertainty, regulatory agencies have tended to employ conservative models, for which low-dose linearity is assumed. These models are based on theoretical one-hit mechanisms, as in radiation-induced mutagenesis; such models may also be justified by assuming the additivity of background and induced tumors, which would yield low-dose linearity regardless of the mathematical model employed. Other less conservative models, such as

Table 2. Ranking animal carcinogens into five classes according to total factor score.

Total factor score	Carcinogen class	Regulatory options
86 to 100	I	Restrict or ban
71 to 85	II	
56 to 70	III	
41 to 55	IV	
Less than 41	V	Several options (no action, limited use, labeling, public education)

the multi-hit, do not presuppose low-dose linearity, but instead, depend on the shape of the dose-response curve in the observed range in animal tests.

Mathematical models also neglect most of the biological information relevant to human extrapolation. They reduce the risk assessment to counting animals with neoplasms on the unwarranted assumption that human response will be quantitatively comparable to that in test animals. As stated by Munro and Krewski (*15*), "We must not lose sight of the fact that animal studies serve primarily as qualitative surrogates for humans and that any attempts to quantify response beyond the realm of biological certainty are open to serious question." Extrapolation from animal data to potential human risk requires consideration of many factors, including biological data and the nature of the substance in question.

Proposed Method for Ranking Animal Carcinogens

The identification of an animal carcinogen requires long-term exposure of test animals, usually mice and rats, to the chemical in question. The design, conduct, and evaluation of the experiments are complex procedures, and these are discussed at length in several recent publications (*1, 11, 12*). It is assumed for the purpose of this discussion that animal carcinogens have been identified by testing at multiple doses in at least two species and that the adequacy or validity of the experiments and conclusions are not in serious question. If such testing requirements have not been met, this ranking system should not be applied. In fact, it is difficult at present to conceive of a method for comparing carcinogenic potentials of chemicals inadequately or unequally tested. Although testing in the past did not provide data for the use of such a ranking system, recent government and nongovernment guidelines recommend or require protocols that provide the necessary information.

Six factors (Table 1) are proposed in this example of a ranking system. They are based on evidence from long-term carcinogenicity studies in animals and from genotoxicity tests, and there is biological justification for including each of the factors. Some carcinogens that have been tested in several animal species have produced clearly positive results in two species or more (factor A), and some also induce more than one type of neoplasm (factor B). Examples include 2-napthylamine, nitrosamines, aflatoxin, and vinyl chloride. At other extremes, chemicals have been positive in only one tissue of one species and, sometimes, only in one sex, as with saccharin or DDT. Metabolism, pharmacokinetics, and detoxification may vary qualitatively and quantitatively among different species, and universality of toxicological responses is more likely to indicate an inherent property of a substance. It is biologically reasonable to assume that the greater the number of mammalian species and tissues that are affected in a similar manner by a toxic substance, the more likely it is that the human response will also be similar. Comparative metabolic and pharmacokinetic studies can be equally or more revealing, but they are often not available when decisions must be made.

The natural incidence in control animals of the type of neoplasm induced in treated animals must be considered (factor C). The high susceptibility of laboratory rodents to several types of carcinogenic effects provides a sensitive indicator for regulatory purposes and is probably based on genetic susceptibility, as suggested by the very high incidence of spontaneous tumors. All laboratory rodents have tumor rates far exceeding those of humans at most sites (*16*). Even so-called low tumor incidences of approximately 1 percent in animals would be major epidemics in the human population. Whatever the mechanism, there appear to be large populations of so-called initiated or latent neoplastic cells in certain tissues in laboratory rodents. Thus, the experimental induction of tumors that have high natural occurrences in the test animals is less relevant to human risk than is the induction of tumors that are normally rare in the test animals. It can be argued that tissue specificity is not always correlated between man and test animals. No biological rules are absolute. However, according to the IARC documents to date, there is an 80 percent correlation between tissue site susceptibilities in humans and in test animals among the 15 known carcinogens that have been adequately tested in animals by routes comparable to those of human exposure (*17*).

Table 3. Approximate rank of ten animal carcinogens based on the proposed system.

Carcinogen	Score	Rank
Aflatoxin	100	I
Dimethylnitrosamine	95	I
Vinyl chloride	90	I
Tris(2,3-dibromopropyl)-phosphate (Tris)	90	I
2-Naphthylamine	81	II
Chloroform	65	III
NTA	51	IV
Chlordane	40	V
Saccharin	36	V
DDT	31	V

Dose-response relationships (factor D) must also be considered. The amount of chemical required to induce a neoplastic response and the latency, or time before the tumor appears, are generally considered to reflect the potency of a chemical for the species being tested. A chemical that must be administered in massive or overtly toxic doses throughout a large proportion of a test animal's life-span in order to induce a neoplasm should be regarded differently than one for which low doses for relatively short periods of time are carcinogenic. Latency as such is not included in this scoring system because this determination requires that large numbers of animals be killed at various intervals to detect the onset of most neoplasms, and this is not a routine procedure in most testing programs. Latency is reflected, however, in the use of cumulative dose equivalents.

The implications of dose-response for the mechanism of action may be equally important. If genotoxic as opposed to nongenotoxic properties are directly related to a carcinogenic mechanism, those substances that induce neoplasms only after severe and prolonged tissue damage make subtle, irreversible one-hit type effects implausible.

The induction of malignant rather than benign neoplasms generally provides persuasive evidence for carcinogenic potential (factor E). As indicated earlier, this is a major criterion in the recent IARC approach. The inclusion of benign neoplasms in evaluation continues to be controversial. However, since there is evidence of progression from benign to malignant stages in the multi-stage devel-

opment of several epithelial cancers in humans and other animals (*18*), it is prudent from the regulatory aspect to include benign neoplasms. Consequently, they are included here but are weighted less than malignant neoplasms.

The criterion of genotoxicity (factor F) takes into consideration the prevailing theory of neoplastic transformation and the possibility of subtle, irreversible effects at low, nontoxic exposure levels that cannot be assessed in animal tests. Positive findings in all or in some tests in an appropriate genotoxicity battery could be required. Another approach is to assign a lower score to substances that give incompletely positive results, as illustrated in Table 1. In the final report of the Scientific Committee of the Food Safety Council (*12*), chemicals were classified as belonging to category A, B, or C, depending on the strength of the evidence for mutagenic potential; such a scheme could be developed to score chemicals in this proposed ranking system.

Regulatory Applications

As shown in Table 1, application of the scoring system to the six factors will result in total scores varying from 13 to 100. If results are positive in more than one species, sex, or experiment, data from the most sensitive responders would be used for scoring categories C, D, and E. There may be different—perhaps equally defensible—assignments of numerical values, and these scores may be grouped to rank animal carcinogens into any number of classes. The development of an ultimate scoring system would probably require the coordinated effort of a multidisciplinary panel or committee. As recommended here, however, five classes would permit an adequate spread and several regulatory options (Table 2). In Table 3, the ten chemicals listed above were scored by this method (*19*). These chemicals were chosen somewhat arbitrarily to present a range of scores, and because adequate experimental data were available. Of the several established human carcinogens, relatively few have been appropriately tested in animals to permit applying this system, and consequently the ultimate test of its validity is lacking.

Regulatory options would be influenced by the nature or intended use of a chemical, the estimated types and levels of human exposure, the number of persons exposed, and by considerations of health and economic benefit. In this system, a class I substance would represent the greatest potential hazard and may, in the case of an intentional food additive, trigger a total ban. Class I and II chemicals would also have the highest priority for regulation. Chemicals in classes III to V may permit many options including no action, approvals for limited uses, labeling, or public education programs.

Carcinogen class may also influence the selection of mathematical models if quantitative risk assessement is to be performed. Chemicals classified I or II, for example, might prompt a more conservative approach than chemicals classified III, IV, or V, regardless of other considerations.

Conclusion

In this article, I have considered only one aspect of carcinogenesis risk assessment and cancer prevention, that is, the evaluation of animal carcinogens. Continued epidemiological research and development may provide greater health benefits in the future. Also, educational efforts by government and the scientific community to create public awareness of the importance of life-style and the voluntary aspects of environmental control should be expanded. At present, however, and presumably for some time to come, testing in animal surrogates will continue to influence our cancer prevention efforts.

The proposed system is based on available data and the current state of knowledge for rational control of animal carcinogens. The emphasis is on test animal data, since without further knowledge of mechanisms, this information is the most relevant to human risk. Whatever experimental data are to be included, however, the weight of scientific evidence should be considered in an appropriate system of carcinogen classification. Concerns about animal carcinogens may thereby be put into better perspective.

References and Notes

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