Cancer Risk Assessment: Importance of Identifying Mechanisms of Action

Richard J. Bull

The process by which regulatory standards are established for drinking water contaminants is based on extrapolating animal toxicity data to humans using a standard mathematical model. The assumptions and judgments involved introduce a relatively high degree of uncertainty and conservativeness into both the qualitative and quantitative aspects of the process. Setting standards based on knowledge of specific mechanisms of carcinogenicity would decrease the uncertainty involved in risk assessment. Understanding these mechanisms is necessary for arriving at the most appropriate mathematical construct for calculating more rational standards.

The goal of risk assessment is to estimate the likelihood of adverse human health effects that could result from exposure to potentially hazardous substances or activities. Based on such an assessment, governmental regulatory agencies make decisions regarding risk significance and risk control. Potentially hazardous substances that are found in environmental media such as water are of particular concern, especially in regard to their potential to cause or induce cancer. Setting regulatory standards for such substances is a complex process involving both scientific and political judgment.

In developing standards for drinking water contaminants, the US Environmental Protection Agency (USEPA) follows a five-step process:

1. Identification of the contaminant in several drinking water supplies.
2. Identification of the contaminant's toxicological properties.
3. Development of appropriate dose-response data.
4. Estimation of acceptable concentrations (i.e., maximum contaminant level goals, or MCLGs) in treated water using the qualitative data in step 2 and the quantitative data in step 3, and
5. Establishment of enforceable standards (i.e., maximum contaminant levels, or MCLs) based on practical limitations to regulation at specific concentrations (e.g., analytical detection limits, cost of compliance, severity of associated adverse health effects).

The first four steps are components of risk assessment, whereas the last step is often referred to as risk management. A common perception is that most judgment in the development of standards is applied during the final step. The primary objective of this article is to identify areas in which judgments are made in risk assessment and to indicate how better data in the second and third steps of the process should substantially decrease the uncertainty involved in risk assessment. Appropriate data in these areas will help remove the conservative assumptions that are now made in risk assessments when insufficient data are available.

It is important to note that the formal rules for assessing health risks associated with relatively low concentrations of toxic chemicals in environmental media are still under development. The major difficulty associated with the process is that most data used in assessing human health risks come from animal studies.

### TABLE 1
Mathematical models used to estimate carcinogenic risks*

<table>
<thead>
<tr>
<th>Model</th>
<th>Major Assumptions</th>
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<tbody>
<tr>
<td>Probit</td>
<td>Assumes that each person has a tolerance to a carcinogen and a threshold value below which no tumor response occurs. The distribution of sensitivities is taken to be log normal. The stochastic (i.e., all-or-none) nature of converting a normal cell to a cancerous one suggests that thresholds may not exist.</td>
</tr>
<tr>
<td>Mantel-Bryan</td>
<td>Special case of the Probit model that forces the slope to 1 probit per 10-fold increase in dose and abolishes the notion of thresholds. Viewed as a conservative estimate of &quot;virtually safe doses.&quot; Selection of slope of 1 is conservative but arbitrary.</td>
</tr>
<tr>
<td>One-Hit</td>
<td>Carcinogenic risk is directly proportional to accumulated number of &quot;hits&quot; in the sensitive cells. At low levels of risk the proportionality between dose and risk approaches 1.0. Therefore, a single event can lead to cancer. Evidence that most cancers require multiple events is quite convincing.</td>
</tr>
<tr>
<td>Multihit</td>
<td>Models that require a series of &quot;hits&quot; or events to occur before cancer develops. Depending on the specific model, events may all have to occur in the same cell, in a particular sequence, or in any sequence. The probability of cancer tends to be proportional to dose to the power equal to the number of hits.</td>
</tr>
<tr>
<td>Multistage</td>
<td>A multihit model that requires hits to occur in a specific sequence. This leads to an upward-curving dose-response curve that reduces to a linear form at low response rates. Consistent with classical two-stage carcinogenesis, thought to have some biological relevance.</td>
</tr>
</tbody>
</table>

*See references 4-6 for further discussion of these models.

### TABLE 2
Conventions used by USEPA in estimating carcinogenic risks

<table>
<thead>
<tr>
<th>Convention</th>
<th>Quantitative Impact</th>
<th>Rationale</th>
</tr>
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<tbody>
<tr>
<td>95 percent upper bound confidence limit</td>
<td>Increase the estimate of risk per unit dose; usually differs from the maximum likelihood estimate by less than a factor of 2 or 3; can vary by several orders of magnitude.</td>
<td>Convention is less sensitive to small change in data (i.e., a more stable estimate).</td>
</tr>
<tr>
<td>Dose per unit of body weight corrected to the unit of surface area</td>
<td>Dose per unit of body weight in small animal represents a smaller dose per unit of surface area than the same dose per unit of body weight in humans.</td>
<td>Based primarily on toxic effect of cancer chemotherapeutic agents; no specific justification for response to carcinogens.</td>
</tr>
<tr>
<td>Percent metabolized</td>
<td>An adjustment made for the net metabolism of a chemical in different species.</td>
<td>Toxic effects of some chemicals depend on how rapidly they are metabolized.</td>
</tr>
<tr>
<td>Age of exposure</td>
<td>Exposure early in life is weighted more heavily.</td>
<td>Adjustment is made for prolonged latent period for development of cancer.</td>
</tr>
</tbody>
</table>

**Notes:**
- *See references 4-6 for further discussion of these models.*
animal-exposure tests, or bioassays. In general, extrapolating animal-exposure data to humans is justified, at least in qualitative terms, because known human carcinogens are also carcinogenic in test animals. However, there are cases in which extrapolation of toxicological data between mammalian species has been shown to be inappropriate. Consequently, the possibility must be considered that test-animal response to a specific chemical may be falsely positive or falsely negative relative to the chemical's effects on humans.

Another major difficulty in assessing risks associated with toxic chemicals in environmental media is that, in contrast to other risks, it is almost always necessary to estimate risks associated with relatively low exposures in humans based on test-animal exposure levels that are frequently several orders of magnitude higher. The life insurance industry, for example, estimates risk based on a sampling of past human experience, which gives the industry confidence in its predictions of future risks. Predicting cancer risks associated with chemicals, however, is seldom based on actual human experience; such predictions, in fact, are actually made with the hope that the experience needed to confirm the predictions will never arise.

The primary reason such an uncertain process is employed is that no acceptable alternatives currently exist. Human experience would provide the most direct information about chemical toxicity. Epidemiology studies (those that examine the actual occurrence of disease in a population) would provide the most practical source of data for setting chemical exposure standards, but such data are extremely limited and are rarely of sufficient sensitivity and specificity to provide information about the effects of low levels of chemicals generally found in drinking waters. Also, no responsible individual would advocate introducing contaminants into drinking water, either deliberately or by negligence, without some knowledge of their toxicity. There is, therefore, no choice but to continue to base most judgments concerning health risks associated with exposure to drinking water contaminants on animal-exposure data.

It is important, though, that the risk assessment process be continually improved. The most problematic outcome of not doing so is that regulatory decisions made using inadequate information will continue to be the basis for subsequent regulatory decisions. In other words, legal precedent could stand in the way of developing a more precise understanding of the hazards of chemical presents. If current conservative assumptions continue to be applied even in the face of new information, there is no incentive for chemical producers to characterize the hazards of their products better.

### Assessing cancer risk

There are two steps involved in cancer risk assessment. The first is a qualitative decision based on observations of human and animal responses to exposure; USEPA identifies this step as hazard identification. If the available data provide convincing evidence that the chemical in question represents a qualitative cancer risk to humans, then the second step is taken—estimation of the quantitative risk the chemical presents in different environmental circumstances. Quantitative estimates of a chemical's cancer risk are generally made using mathematical models. Models that have received the most attention are listed in Table 1. It is beyond the scope of this article to discuss the advantages and disadvantages of each of these models in detail; the major point is that objective data rarely exist to demonstrate that one model is more accurate than another for a given chemical.

Deciding which model to use, then, is a relatively arbitrary process based, at least theoretically, on which model best reflects current general understanding of how chemically induced cancers are produced. USEPA uses the linearized multistage model to quantify acceptable human exposure levels to toxic chemicals. Inasmuch as multistage carcinogenesis appears to be a general phenomenon in cancer development, USEPA's decision to use the linearized multistage mathematical model to determine cancer risk was a scientific one. Inasmuch as there is little scientific proof that the linearized multistage model is more accurate than others, USEPA's decision is a matter of policy.

Calculating cancer risk using mathematical models is a relatively simple matter; some programs can be run on a personal computer equipped with a math coprocessor and require only some judgment on what data to input. Varying degrees of "conservativeness" (i.e., assumptions that tend to increase the estimate of hazard) can be introduced to the calculations via various conventional calculations. The most important of the conventions utilized by USEPA are described in Table 2.

The two conventions that most affect risk assessment are the body-weight-to-surface-area correction and the 95 percent upper-bound confidence limit correction. The body-weight-to-surface-area correction is approximated by a factor proportional to the 2/3 power of the ratio of the two species' body weights. For purposes of these calculations, average human body weight is 70 kg, average mouse body weight is 0.03 kg, and average rat body weight is 0.35 kg. Thus, the correction factor for extrapolating cancer data from mice to humans approximates

$$\frac{70 \text{ kg}}{0.03 \text{ kg}} \approx 175$$

and the factor associated with rats and humans approximates

$$\frac{70 \text{ kg}}{0.35 \text{ kg}} \approx 200$$

Thus, if doses that are equivalent per unit of body weight produce similar tumor responses in mice and rats, the data that are obtained in mice will result in a five times greater risk than the data from rats.

In most cases, the use of the 95 percent confidence limit does not yield risk values much different from the maximum-likelihood-estimate (MLE) of risk because most animal cancer bioassays only employ two high-dose groups plus a control group. The MLE is the value actually predicted by the extrapolation model. The 95 percent confidence limit is based on a curve below which MLEs calculated from replicated experiments fall 95 percent of the time.

However, if a chemical displays a particularly steep dose-response curve in a species other than the one used in a bioassay, the differences in risk values can be significant. Unfortunately, a number of prominent drinking-water contaminants have such steep dose-response curves.

### Mechanisms of carcinogenicity

A mathematical model of the dose-versus-response characteristics of carcinogens always has mechanistic implications. A model can be viewed as describing how a chemical reaction proceeds as the concentrations of the reactants increase; however, cancer is not produced by a single reaction but by a series of reactions or stages. Each stage has some probability of occurring that is a function of the dose of a chemical that affects the stage. All chemical carcinogens do not, however, affect the same stage to the same extent. Ultimately, the probability that cancer will occur is a summation of the kinetics of absorption, distribution, metabolism, and the nature of the interaction of the chemical at the body site at which it produces its effect. In addition, it is most important to understand how the interaction leads to cancer and whether the effect is reversible. Therefore, before more rational risk estimates can be developed, the molecular, biochemical, and physiological mechanisms by which a chemical causes or induces cancer must be understood. In recent years it has become increasingly apparent that the mechanisms by which different chemicals produce cancer vary consid-

*[GLOBAL82, TOX-RISK: K.S. Crump Div., Clement Associates, Ruston, La.]*
erably and that employing a standard treatment (e.g., the linearized multistage model) for all chemicals that produce cancer is naive.

In making risk assessments, USEPA assumes that all chemical carcinogens act initially by altering DNA (deoxyribonucleic acid, or the molecular basis of heredity). This alteration might be induced directly by the chemical in its ingested form, or it may require metabolism of the chemical to a new chemical that is capable of altering DNA.

For DNA alteration to be irreversible (i.e., a mutation) and thus to induce cancer, it must cause misreading of DNA information during cell replication such that the mutation can be passed to daughter cells. Such misreading can be caused by obstruction of normal DNA-replication interactions or by errors in DNA-repair processes. Mutations can only occur when a DNA-altered cell divides at least once after the initial DNA alteration is produced.

It is important to understand that the process of DNA alteration and mutation underlies the linear extrapolation of risks to zero dose and explains why USEPA uses the linearized multistage model. Once DNA alteration is replicated and mutation occurs, the mutation will persist in an irreversible form. Unless the mutated cell dies, the potential for tumor growth exists, so this first step is usually referred to as tumor initiation, and the chemicals capable of producing this step are known as tumor initiators.

The presence of cells in which tumors have been initiated does not always lead to cancer, however. Although most mutated cells remain dormant, some can potentially progress to cancerous growth. A variety of endogenous factors determine whether an affected cell will begin to replicate more rapidly than normal and produce a benign tumor (one that grows but does not form secondary tumors or damage other tissue) or whether it will undergo changes that lead to cancerous, or malignant, tumors (those that can form secondary tumors and damage other tissue). Essentially, a mutant-initiated cell must possess a selective advantage over other cells in the tissue in which it resides; it may be more sensitive to internal growth factors and hormones, or conversely, it may be less sensitive to endogenous or exogenous factors that cause cell death. In either case, more rapid growth occurs in these cells than in normal cells in the affected tissue. Examples of both endogenous (e.g., hormones) and exogenous (e.g., diet) mechanisms have been identified.

The process by which a chemically caused mutation induces rapid cell replication is referred to as tumor promotion. Tumor initiation is irreversible. Tumor promotion is reversible, at least to a point (i.e., when a malignant tumor has developed). Consequently, in most instances the effects of tumor promoters do not lead to dose-response curves that pass through zero.

In addition to tumor initiation and promotion, a third process that is clearly involved in the development of cancer is progression. In most situations, chemicals that induce mutations stimulate the growth of both benign and malignant tumors. Some of the benign growths, for example, hyperplastic nodules (a growth of rapidly dividing cells) in the liver, depend on continued promotion. If the hormone, growth factor, or exogenous tumor promoter is withdrawn, such tumors will regress. Other benign tumors are capable of relatively independent growth; they will remain and in some cases grow even larger. Generally, this growth is confined to the tumor itself and is differentiated from that of malignant tumors, which frequently spread into and damage adjoining tissues.

Finally, some cancerous cells eventually become capable of avoiding immune system activities and migrate via blood to colonize other tissues, a process known as metastasis.

Because there is a tendency for benign tumors to develop malignant areas within their boundaries and for some cancerous tumors to eventually metastasize, it is clear that initiated cells progress through several steps before developing into malignant tumors. This process is referred to as tumor progression or differentiation.

Progression to the malignant state can be viewed as a virtually random process that depends primarily on the number of initiated cells present. Therefore, the major impact of tumor promoters is to expand the number of initiated cells by stimulating cell division, which increases the probability of progression to malignancy. Progression to malignancy can occur very early, however, with a single or small number of initiated cells being malignant from the first round of cell division following initiation. It is possible, therefore, for a tumor to be malignant at the point it begins to develop.
Progression is also closely linked to what was originally described as the first stage of tumor promotion, which is also known as conversion. Conversion events have recently been closely linked with clastogenic events (i.e., chromosome loss or gain, breaking and rejoining of part of one chromosome to another). However, conversion has been demonstrated to be more slowly reversible than tumor promotion. Unlike tumor promotion, conversion can occur for a limited period prior to initiation. However, in the course of a few weeks the interaction with tumor initiation disappears.

Carcinogenesis can occur as a result of either the direct action of a particular chemical or its metabolites or by the activation by the chemical or its metabolites of certain endogenous systems. Such indirect activity can involve any of the steps recognized to occur in chemical carcinogenesis. For example, the body generates considerable amounts of reactive oxygen species in the course of normal metabolism, most specifically superoxide anion and hydrogen peroxide. These two active oxygen species are of concern primarily because of their sufficiency to react with free radicals, which damages lipids, proteins, and nucleic acids. The hydroxyl radical's ability to damage nucleic acids appears to be a potential means of initiating, promoting, or enhancing the progression of tumors. A relatively large number of chemicals can directly or indirectly increase the body's production of superoxide anion or hydrogen peroxide.

A second example of this indirect activity is found in the development of breast tumors. Frequently, the growth and maintenance of such tumors depend on estrogen. Thus, an endogenous hormone acts as a tumor promoter, and drugs that indirectly modify this activity may affect the course of the tumor's growth and development. Another example of such activity is the promotion of intestinal tumors by bile salts.

Diet can also influence tumor development. For example, unsaturated fats appear to promote development of tumors in the rat pancreas as well as certain mammary tumors in rats. Many other examples of this type illustrate that tumor induction is not peculiar to chemicals, and even under the most controlled of circumstances, carcinogenesis must be viewed as a process involving endogenous as well as exogenous factors.

This discussion illustrates that chemicals can act by a variety of mechanisms to increase the incidence of tumors in humans as well as in test animals. Once a chemical initiates an activity that accelerates one of the carcinogenesis steps, other endogenous and exogenous factors can translate the effect into cancer.

If chemicals were tested for carcinogenicity at actual environmental exposure levels, the differences between these various mechanisms would be of little importance. Regulatory policy decisions, however, are made regarding very low levels of human cancer risk (i.e., 1 in 100,000 to 1 in 1,000,000). For tests of chemical carcinogenicity at ambient exposure levels to be of any predictive value, huge numbers of test animals would have to be exposed—a practical impossibility. Carcinogenicity tests, therefore, routinely expose a small number of test animals to very high chemical doses that are generally several orders of magnitude greater than ambient concentrations. These very high doses generate dose-response data using manageable numbers of animals, but they often provide meaningful analysis of the mechanisms that might be involved in the induction of tumors by a specific chemical.

Conclusions

The current risk assessment process by which standards are set for drinking water contaminants involves scientific and political judgments. Because of the complexity of the problems involved in the process and the lack of sufficient knowledge of carcinogenic mechanisms, these judgments are based on multiple conservative assumptions.

The practical effect is that standards developed under the current regulatory process are based on inflated estimates of the actual calculated risks. If the process were to take into account the carcinogenic mechanisms of specific contaminants rather than treat all contaminants the same, the resulting standards could differ by orders of magnitude. More accurate assessments of actual human risks associated with chronic exposure to low doses of drinking water contaminants could be obtained by exposing huge numbers of test animals to such doses, but gathering such empirical data is clearly impossible. Obtaining better data regarding the actual carcinogenic mechanisms of drinking water contaminants is, therefore, the only practical means for arriving at the most appropriate mathematical construct for setting standards.

References


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