Pharmacokinetic Factors Influencing Risk Assessment: Saturation of Biochemical Processes and Cofactor Depletion

Darrell D. Sumner and James T. Stevens

CIBA-GEIGY Corporation, Greensboro, North Carolina

Models generally consider risk to be a function of the hazard (toxicity) and exposure (dose). That function is best described by the dose response of the toxic effect. For any risk assessment system to be effective, it should consider that dose-response relationship. Saturation phenomena often produce nonlinear dose curves, and any risk assessment system should be able to address such effects. Physiologically based pharmacokinetics offer an approach to deal with these nonlinear responses. Some historic risk models and common saturable processes are discussed. The impact of maximum tolerated dose (MTD) on risk evaluation and the kinetics of some saturable processes are considered. Specific examples have been selected to demonstrate the importance of saturation of processes in assessing the hazard of chemicals. — Environ Health Perspect 102(Suppl 11):13-22 (1994)

Key words: hazard, maximum tolerated dose (MTD), dose-response, pharmacokinetics, nonlinear kinetics, modeling, risk assessment, logic, rational

Introduction

Risk assessment is an integral part of the regulation of synthetic chemicals in the United States. If the process is used properly, it helps define whether the benefits of a particular chemical outweigh the risks it might pose to society. Therefore, it is imperative that we develop and use the best methodologies available to assess risk. The impact of underestimating risk is often considered. However, if we overestimate risk, we can precipitate equally tragic results, particularly if the action impacts the prices of essential commodities such as food. Nearly 25 million people in the United States live below the poverty level. They spend more than one-third of their incomes on food.

The poor already suffer increased morbidity and mortality (1). If we overregulate and deprive society of useful chemicals, such as a pesticide that helps improve crop yield and quality, we could force an increase in food prices and exacerbate the nutritional problems of people below the poverty level.

Therefore, it is important to find risk assessment systems that consider potency, not just potential hazard. With increased awareness of nonlinear processes in biology, and the importance of these processes in the dose-response relationships, we must strive to develop models and approaches that embrace pharmacokinetic effects whenever appropriate.

The use of the linearized multistage (LMS) model (2), the model currently used as a default technique by the U.S. Environmental Protection Agency (U.S. EPA) (3), does not provide an adequate assessment of carcinogenic risk. It has become increasingly evident that the LMS model in many cases clearly overestimates risk. However, it is not well recognized that the LMS model in some instances may underestimate risk (4,5). Despite either scenario, it is clear that the model fails to distinguish adequately between compounds of different potencies (6). To the extent that pharmacokinetics modeling will improve risk assessment, it must be added to the tool chest of better understanding. Application of pharmacokinetic principles for all chemicals will most certainly be inappropriate for assessing the risk associated with all chemicals; however, when applicable, it can be invaluable.

Any useful system of risk assessment must be able to properly assess the risk posed by compounds with different shapes of dose-response curves. Compounds that have exponential and higher order dose-response curves are less hazardous at low doses than those with linear or one-hit type dose responses. If a risk assessment process does not distinguish dose response or potency, it can result in risk management that will be inappropriate. For example, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) forbids "misbranding" of pesticides (7); if a product that does not fit the criteria to be labeled with DANGER, it cannot carry the DANGER signal word. The proliferation of unjustified DANGER labels would erode the significance of this hazard classification. Similarly, carcinogens should be classified in a fashion that legitimately considers the dose-response relationships. In this manner we can ensure that the classification (risk assessment) is not devalued, and that the more hazardous materials are properly identified.

We will attempt to illustrate nonlinear oncogenic processes with several chemicals that, during their biokinetic processing, demonstrate saturation of biochemical processes or depletion of critical cofactors. Risk assessment of most of these agents may be improved by using pharmacokinetics; however, some will not benefit. Indeed, each chemical represents a challenge to develop and use the most appropriate risk assessment procedures to properly address the character of the dose response.

To address saturable processes and discuss examples of nonlinear risk relationships, it is appropriate to consider several areas relative to risk assessment. We briefly review some early risk models and their bases, the concept of maximum tolerated dose (MTD) as a saturation phenomenon itself, some biochemical processes and,
finally, some old and new examples of compounds which show these effects.

**Discussion**

Risk assessment is the actual integration of hazard, dose response, and exposure information into a useful tool for limiting human exposure to acceptable levels. This is not as simple as sometimes portrayed with the application of the LMS model (2) to the hazard data to derive a Q* value because the LMS model fails to capture the integrity of the dose response. An attempt to capture the dynamics of the process is given in Figure 1 (8). The simple product expression of risk is equal to hazard times exposure cannot hope to correctly address the essence of dose response.

From their infancy, mathematical models were applied as a means to express numerically the biological process. Most of these mathematical models have little foundation in biology. Some models that have been used for risk assessment illustrate some of the principles that still need to be considered. The equations for these theoretical models are presented in Table 1.

The one-hit model was developed as the one-hit theory of infectious titration, a biological impact of multiplying organisms. This was attractive because some types of radiation effects could be predicted with it (7). The model essentially predicts a linear dose-response curve at low doses asymptotically approaching 100%. This approach to 100% response is a common factor among risk models, and we all know that we seldom see tumor responses approaching 100% with xenobiotics.

The probit model (as proposed by Mantel and Bryan) is notable because it is characteristic of LD_{50} or ED_{50} determinations, or the log dose response (9). This feature is attractive as a model since it is experimentally demonstrable; but, of course, it cannot be demonstrated in the one-in-a-million range.

If we are to believe Paracelsus’ admonition that the dose makes the poison (15), then we must consider the processes involved and whether those processes are controlled by first-order or zero-order chemical kinetics. It is from those processes that we should establish a preference for linear dose or log-dose considerations.

The multistage model or one of its derivatives is probably the most commonly used model today (10). Its popularity may lie in its apparent capability to deal through the use of the quadratic equation with nonlinear processes (with sufficient numbers of dose groups). However, this feature is lost when the model is simplified to the LMS model that provides the notorious Q* value. This variant of the multistage model is so simplified that the overall limitations and uncertainties of our science may be lost on the public and the policy makers.

The Cornfield model was the only model that attempted to address saturable (enzymatic) processes in risk assessment (13). Cornfield’s model considers carcinogens to be simultaneously and reversibly activated and detoxified in the animal. The probability of an oncogenic response is proportional to the concentration of an activated toxin/substrate complex; this requirement is fulfilled when the dose (d) of the toxin is less than or equal to the substrate for detoxification (T). Dr. Cornfield was still working on this model at the time of his death in 1979. Further work perfecting this model has been very limited.

The extent to which nonlinear dose-response curves affect low-dose extrapolation is illustrated in a comparison of a “one hit” or linear dose–response curve with a fourth-order curve such as one would see with saccharin (Figure 2). The curves presented are Weibull distributions, a model that also has been proposed, but this is identical to a multistage model having only single dose terms (12). The difference between the two models is particularly evident at the extreme low end of the exposure curve—exactly where risk assessment is operating.

**Table 1.** Contemporary models for quantitative risk estimation: foundation and expression.

| Foundation                  | Biological                                                                 | Model                     | Expression                                                                 |
|-----------------------------|---------------------------------------------------------------------------|---------------------------|---------------------------------------------------------------------------|
| Tolerance distribution      | Log-dose response                                                        | Mantel-Bryan probit (9)   | \( P(d) = \phi(\alpha + \beta \log d^0(d)) \)                            |
| Relational-dose, latency and risk | Weibull (10)                                                            |                           | \( P(d) = 1 - e^{-d^a} \)                                                 |
| Quasi-stochastic (mechanistic) | Assumes no threshold; one hit, one tumor                                 | One-hit (11)              | \( P(d) = 1 - e^{-d^a} \)                                                 |
| Tumor development requires several stages | Multistage (12)                                                          | Multistage (12)           | \( P(d) = 1 - e^{-d^a} \)                                                 |
| Assumes linearity; one hit, one tumor | Linearized multistage (2)                                                | Cornfield (13)            | \( P(d) = k e^{d/(S+K)} \)                                               |
| Biokinetics                 |                                                                           |                           | \( P(d) = 1 - e^{T(a+b_d^c)} \)                                           |
| Mechanistic/distribution    | Time-to-tumor combined with a multiple step process of tumor formation   | Multistage Weibull (14)   | \( P(d) = 1 - e^{T(a+b_d^c)} \)                                           |
In addition to these models, there is a hybrid model that uses the Weibull model for correcting for time-to-tumor and the multistage model to plot the tumor response. This model, called the multistage-Weibull (14), is used to correct the projected incidence when an effect on survival is observed (5).

When considering saturable processes, one cannot ignore the concept of maximum tolerated dose (MTD) (16,17). The MTD, by regulatory definition (18), must be reached for feeding studies to be valid for risk assessment (3). However, the regulatory MTD, by nature of its alteration of the homeostasis of the animal, must be considered a saturating process (19). A nonlinear dose response of the toxic effect does not always occur at the MTD, but one must consider such potential effects to be possible. Timbrell reports in his recent book that xenobiotic metabolism cannot be separated from intermediary metabolism (20). This is perfectly logical and illustrates that we cannot ignore the physiologic state of the test animals. By focusing on processes that could be saturated at the MTD, one must consider several diverse options. These would include, but would not be limited to: a) saturating excretion mechanisms, b) saturating detoxification mechanisms, c) overwhelming DNA repair, d) overwhelming immunosurveillance, e) exceeding tissue repair capacity, and f) compromising health (8).

Excretion and detoxification have a direct effect on the toxins. DNA repair and immuno-surveillance, as well as tissue repair and replication, are corrective mechanisms that are indirectly compound related.

In the probit risk model, as well as classic pharmacology, xenobiotics are considered to act on the biological system in first order chemical processes; this leads to theoretical and experimental correlations with log-dose relationships. Current risk models usually use linear dose response considerations; this implies that zero-order processes are the rate limiting step in the toxic response. Several zero- and first-order processes are delineated in Table 2. Zero-order processes include active transport systems such as tubular reabsorption or gastrointestinal secretion, DNA repair, resynthesis of cellular components, and cellular replication. First-order processes include metabolic activation, detoxification, and passive routes of excretion (which are the most common). In addition, conjugation including cofactor depletion is a concentration-dependent process. All these processes are better described by log-dose considerations.

It is interesting to note that the zero-order processes are generally the repair and detoxification mechanisms while activation and toxic responses are first-order processes. It is then appropriate to question whether the use of a linear response as presented in the multistage model is appropriate. In general, pharmacokinetic models use standard kinetic expressions and deal more appropriately with both zero- and first-order processes. The integration of kinetic expressions into the simple risk models has not been routinely attempted. This means that the implications that the use of linear dose models on the value and direction of the risk assessment have to be examined.

Both zero-order and first-order processes are susceptible to saturation phenomena. It seems appropriate to comment briefly on some of the experimental observations that suggest nonlinear kinetics. Understanding these processes in the realm of the pharmacokinetics is truly an area that deserves consideration, particularly with the knowledge that chemicals are often tested at extremely high levels of administration in an attempt to achieve the regulatory MTD. At times it appears in today's science that basic elements of descriptive evaluation have been lost from the database. The application of physical-chemical properties (21), such as molecular weight, lipophilicity, and ionization potential (Figure 3), as well as the basic approaches to examination of absorption, distribution, metabolism, and elimination (ADME) is too often overlooked when the hazard is evaluated (4,22,23). Regarding the latter approach, some features, if observed experimentally, provide the clues to make a determination in regard to the nonlinearity of the processes. These include:

- elimination cannot be described by a single exponential process,
Chemical: Oxalic Acid
Description. Oxalic acid is present in many plants and vegetables where it occurs in the cell sap of the plant as the potassium or calcium salt (25). It is also the product of the metabolism of molds. Oxalic acid is used as an analytic reagent, a general reducing agent, cleaner, and decolorizer. In addition, it is the toxic metabolite of ethylene glycol used in auto antifreeze.

Chemical Properties. Oxalic acid is soluble in water and has a molecular weight of 90.04.

\[
\text{HOOC–COOH}
\]

Structure 1. Oxalic acid

Hazard Profile. Nephrotoxicity.

ADME. Oxalic acid is rapidly excreted by the kidney. At dose levels below the solubility of the calcium salt, appreciable toxicity has not been observed (26). The nonlinearity of the toxicity of oxalate is illustrated by the physiologic processes which occur with precipitation. Physiologically, calcium oxalate crystals in kidney tubules cause mechanical damage and increased liquid pressure. Increasing intratubular pressure leads to diminished glomerular filtration and renal blood flow. The resulting ischemia increases loss of renal cells.

A proper system of risk assessment must be able to distinguish the dose response of materials like oxalic acid. The incorporation of pharmacokinetic principles into a risk assessment model offer that distinction precisely.

Solubility: Formation of Bladder Calculi Resulting in Tumor Formation

Chemical: Melamine
Description. Melamine is a triaminos-triazine used as synthetic resin used to coat paper and paper products, as well as to make tabletopware (27).

Chemical Properties. Melamine is slightly soluble in water and insoluble in ether. The molecular weight of melamine is 126.13.

\[
\text{H}_2\text{NNHNH}_2
\]

Structure 2. Melamine

Hazard Profile. Melamine is a carcinogen in the rat and operates by a mechanism not dissimilar to calcium oxalate. Melamine produces bladder carcinomas in rodents (28).

ADME. Melamine is excreted unchanged by the kidney; but, when the concentration in the bladder reaches sufficient levels, the melamine precipitates to form calculi. These calculi roll around in the bladder and the resulting mechanical irritation results in the tumorigenic response. In the absence of calculi no tumors are formed.

One may argue that the mechanism is known and it is therefore unnecessary to use mathematical risk assessment with melamine. However, a proper risk assessment process should be able to recognize such a dose-response relationship, and that is better recognized with pharmacokinetic approaches than with the LMS model.

Saturation of Absorption Kinetics at High Levels of Administration

Chemical: Simazine
Description. Simazine is a symmetrical chlorotriazine herbicide used in agriculture for broadleaf-weed control. The mechanism for its herbicidal activity is inhibition of photosynthesis.

Chemical Properties. Simazine is relatively insoluble in water (3.5 ppm) and has a Log P value of 4.18. Its molecular weight is 201.7.

\[
\text{H}_3\text{C} \quad \text{Cl} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{NHCH}_2\text{CH}_3
\]

Structure 3. Simazine

Hazard Profile. Simazine is a triazine herbicide with low acute mammalian toxicity. The LD$_{50}$ is about 5 g/kg, while table salt is about 4 g/kg. The triazine herbicides inhibit photosynthesis, so it is not surprising that organisms that do not perform photosynthesis are not particularly susceptible. Simazine is not a mutagen nor a carcinogen in mice or male rats, but it does produce an increased incidence of mammary tumors in female Sprague-Dawley rats (29).

ADME. Simazine is rapidly metabolized by dealkylation and glutathione conjugation at the ring chloride. Its excretory distribution changes with dose as depicted in Figure 4. This response is relevant to this discussion. Simazine, when administered in low doses, is primarily excreted in the urine with little excreted in the feces. At higher doses the urinary excretion is diminished and an approximately 50/50 ratio is found in the urine and feces. Interestingly, simazine has a water solubility of 3 ppm. This is of course sufficient to be absorbed, but may reflect distribution characteristics preventing complete absorption from the intestine or increased biliary excretion.

Altered Metabolism at High Levels of Administration

Chemical: Primisulfuron
Description. Primisulfuron is a sulfonylurea herbicide used for selected weed control in corn. Its use rate is in the order of 10 to 20 g/acre.

Chemical Properties. Primisulfuron is moderately soluble in water (23 ppm). It has a Log P value of 1.15 and a pK$_a$ of 5.1. Primisulfuron has a molecular weight of 468.3, making it a candidate for biliary secretion.

\[
\text{H}_3\text{C} \quad \text{O} \quad \text{CH}_{2}\text{O} \quad \text{CHF}_2
\]

Structure 4. Primisulfuron

Hazard Profile. This product is not particularly acutely toxic; it is not a mutagen, a teratogen, or a reproductive toxin. Primisulfuron is not tumorigenic to rats. It has not been demonstrated to be a potential oncogen, but at doses above the MTD, liver tumors were seen in mice (30).

ADME. At doses below the MTD, the compound is metabolized by hydrolysis of the sulfonylurea and oxidation, as well as by conjugation. Feeding levels above 3000 ppm showed a new feces metabolite in mice. This compound seems to demonstrate activation of a secondary pathway of degradation at these high doses.

A histogram of the chromatography of the radioactive fecal metabolites of
primisulfuron is presented in Figure 5. This histogram shows that early eluting metabolites may change quantitatively with increasing dose but that the late eluting metabolite appears only at high doses. Interestingly, this late peak, eluting at fraction 36, seems to be less polar than the parent that elutes at fraction 28 in this reverse-phase column. It is further interesting to note that the metabolite seems to form only after repeat dosing, not with a single dose. The metabolite was identified after feeding mice radiolabeled primisulfuron for 28 days.

The structure of the unique high-dose metabolite is given in Figure 6. The high-dose metabolite was found to be a dimer of the original compound with a ring difluoromethoxy group replaced with a disulfide bridge between two molecules of the test material (T Capps, personal communication). The difluoromethoxy groups remain intact in other metabolites isolated from mice or rats. This suggests that the pathway is unique at these high doses. The presence of the disulfide bridge indicates that a sulfur-containing cofactor must have been involved. This presents possibilities that glutathione conjugation is one of the intermediate steps and its depletion could be involved in the toxic response. The disulfide could be formed in the liver or the gastrointestinal tract. If it is formed in the liver (we have not yet tried to cannulate the mouse bile ducts in subchronic feeding studies), the formation of a dimer dictates that second-order chemical kinetics must describe the reaction. If this were true, a log–dose response would not be appropriate to describe this material since it would require an exponent of higher power to describe the response.

The identification of this metabolite provides insight to understanding the toxicologic response of fluorosis observed at levels above 3000 ppm (Figure 6).

Saturation or Inhibition of Active Transport

Chemical: Sulfinpyrazone

Description. Sulfinpyrazone is a pharmaceutical agent that is indicated for the treatment of gout. It is a uricosuric agent.

Chemical Properties. Sulfinpyrazone is a strong organic acid with a \( pK_a \) of 2.8. It is rather soluble in water and has a molecular weight of 404.7.

Hazard Profile. Sulfinpyrazone belongs to the pyrazole class of agents. There are inconclusive results in animals suggesting sulfinpyrazone may be teratogenic. It has been shown to produce gastrointestinal irritation in some patients.

ADME. In a discussion of saturation in metabolism it is obligatory to present an inhibitor of renal tubular reabsorption. The half-life following intravenous administration is 4 hr. Sulfinpyrazone is an example of a compound that uses its ability to inhibit the pharmacokinetic process to achieve the therapeutic objective (32). Any risk assessment process should be able to properly consider the nonlinear processes produced by inhibition of reabsorption or secretion.

Reactive Metabolites

Chemical: Vinyl Chloride

Description. Vinyl chloride has been used as a chemical intermediate in plastics, as a refrigerant, and in organic synthesis.

Chemical Properties. Vinyl chloride is a colorless gas with a molecular weight of 62.5. It is sparingly soluble in water (2.5 ppm at 25°C).

Figure 5. Radiochromatograms of primisulfuron metabolites in the feces of male mice dosed orally.

Figure 6. Primisulfuron: high feeding level metabolite. It is clear that fluoride is lost in the fecal metabolite formation of the dimer.

Hazard Profile. Vinyl chloride is a human oncogen that has been rather thoroughly investigated in regard to its oncogenic dose response and metabolism (33). The compound produces angiosarcomas in humans exposed to high levels.

ADME. Vinyl chloride is metabolically activated by epoxidation of the vinyl group to produce an epoxide which opens to a reactive species, probably a carbonium ion (34). Gehring et al. (35) investigated the metabolism and kinetics of vinyl chloride in rats (Table 3). It was observed that when the incidence of tumors in rats was compared with the amount of vinyl chloride metabolized, a reasonable correlation was found (36). Metabolic production plateaued about 2500 ppm (37). Tumor incidence also seemed to plateau in about the same area (Table 4). The multistage model can be used to predict the incidence of tumors using the dose or using the amount of material metabolized (Table 5). This exercise is essentially the fitting of the dose–response curve to the maximum likelihood estimator of the model. As can be seen the predicted incidence, thus the fit of the curve, is much better with the kinetic data.

Table 3. Kinetics of vinyl chloride metabolism in rats.

| Exposure, ppm | Amount metabolized, \( \mu g/day \) | Observed incidence, % |
|---------------|---------------------------------|----------------------|
| 10,000        | 5.321                           | 14.8                 |
| 6,000         | 5.403                           | 21.7                 |
| 2,500         | 5.030                           | 22.0                 |
| 500           | 3.413                           | 11.9                 |
| 250           | 2.435                           | 6.8                  |
| 50            | 739                             | 1.7                  |

*From Gehring et al. (34).

Table 4. Calculated production of metabolites of vinyl chloride in rats.

| Exposure, ppm | Amount metabolized, \( \mu g/day \) | Observed incidence, % |
|---------------|---------------------------------|----------------------|
| 10,000        | 5.321                           | 14.8                 |
| 6,000         | 5.403                           | 21.7                 |
| 2,500         | 5.030                           | 22.0                 |
| 500           | 3.413                           | 11.9                 |
| 250           | 2.435                           | 6.8                  |
| 50            | 739                             | 1.7                  |

*From Gehring et al. (34), *From Maltoni and Lefemine (35).
Table 5. A comparison of the predicted versus observed incidences of angiosarcomas in rats exposed to vinyl chloride.

| Exposure | Incidence | Predicted (dose) | Predicted (kinetics) |
|----------|-----------|------------------|----------------------|
| 6,000    | 21.7      | 26.1             | 22.8                 |
| 2,500    | 22.0      | 11.9             | 20.6                 |
| 500      | 11.9      | 2.5              | 11.7                 |
| 250      | 6.8       | 1.3              | 7.3                  |
| 50       | 1.7       | 0.3              | 1.8                  |

Cofactor Depletion

Chemical: Acetaminophen

Description. Acetaminophen is an analgesic agent that is sold over the counter.

Chemical Properties. Acetaminophen is a weak acid which is only slightly soluble in water. Its molecular weight is 151.2.

Hazard Profile. This compound produces centrilobular hepatic necrosis with hepatocellular carcinomas in animals after prolonged administration. Bromobenzene is metabolized by epoxidation at the 2,3 or the 3,4 position (Figure 7). Although possibly more complex, in general, the 2,3 epoxide leads to 2-bromophenol which presents the lesser toxicity (40). The 3,4 epoxide may hydrolyze to a phenol or a diol, or it may react with glutathione. If glutathione has been depleted, the material covalently binds to macromolecules in the liver resulting in the hepatic toxicity (41).

Chemical: Methylene Chloride

Description. Methylene chloride is a solvent used in degreasing, cleaning agents, and in food processing.

Chemical Properties. Methylene chloride is a colorless liquid which has limited solubility in water (50 ppm at 25°C) and is miscible with alcohol and ether. It has a molecular weight of 84.94.

\[
\text{CH}_2\text{Cl}_2
\]

Structure 9. Methylene chloride.

Hazard Profile. Methylene chloride produces hepatocellular adenomas/carcinomas in inhalation studies, but not in studies where the test material is added to water (42).

ADME. The compound is metabolized by the hepatic mixed-function oxidase system in a saturable process (43). It is also conjugated with glutathione in an alternative pathway. Using pharmacokinetic models, it has been possible to demonstrate that the liver neoplasia are best correlated with the glutathione conjugation. The regulatory risk assessment has been modified to consider that pharmacokinetic process.

Review of the metabolism and toxicology data (Table 6) indicates that dose increases in drinking water produce essentially proportional increases in the amount of material metabolized by both of the prominent pathways. The tumor data indicate no increase in the background incidence of tumors at any of the doses tested. In the inhalation study, the amount of material metabolized by the mixed function oxidases did not change with increasing dose, but conjugated with glutathione increased proportionally. Tumor incidence increased with increasing dose. Andersen and Clewell (44) used physiologically based pharmacokinetic (PBPK) modeling studies to demonstrate the effects of this compound in relation to its metabolism (Figure 8). They used a PBPK model which considered separate compartments for gas exchange and metabolism in the lung. They, of course, included the usual distribution in tissues and metabolism in the liver.

Cellular Replication

Chemical: Metolachlor

Description. Metolachlor is a chloroacetanilide herbicide used to control many grass and broadleaf weeds.

Chemical Properties. Metolachlor is relatively soluble in water (530 ppm at

![Figure 7. Metabolism of bromobenzene.](image)

![Figure 8. Physiologically based pharmacokinetic model for methylene chloride.](image)
Hazard Profile. Metolachlor is not a teratogen, a reproductive toxin, or a mutagen. Metolachlor produces low increases in hepatocellular adenomas in female rats at high doses but not in male rats or mice (45). In test groups of 70 animals, two liver adenomas were found in controls and six primary hepatocellular tumors were found in animals consuming diets containing 3000 ppm of metolachlor.

ADME. The excretion of metolachlor is characterized as a complex mixture in both urine and feces. Multiple metabolites are observed with no individual metabolite exceeding a small percentage of the administered dose. Because of the hinderance and stability about the amide nitrogen, there is virtually no detectable hydrolysis of the amide. Most of the metabolism occurs about the chlorine, the methoxy and resulting alcohol, and to some extent on the side chains of the aromatic ring. Considering the complexities of the metabolic profile, the urinary excretion of radioactivity was evaluated and found to fit a first-order, one-compartment model at high doses (Figure 9). At lower doses, however, a subtle shift was observed and a two-compartment model best fit the data. The limitations on this process cannot be overemphasized. The use of total radioactivity data does not allow full consideration of just the types of dose–response changes discussed. Still, the shift suggested that something had changed in the metabolic process and the homeostasis of the animals may be compromised.

In the process of evaluating DNA repair in vivo, it was observed that there was an increase in S-phase cells in the livers of animals treated at carcinogenic doses. It appears from the dose–response curves, the mutagenicity and the presence of the S-phase cells in the animal livers, that the oncogenic response is due to hepatotoxicity. At sufficient doses that result in hepatotoxicity, hepatocellular death and regeneration occur in the rodent liver. Regeneration of the rodent liver is known to be a promoting phenomenon (46). This may be due to the fidelity of replication, since partial hepatectomy results in increased liver adenomas in rodents.

Binding

Chemical: Saccharin

Description. Saccharin is a noncaloric sweetener.

Chemical Properties. Saccharin is a crystalline solid that is highly soluble in water and in most solvents. It has a molecular weight of 183.18.

Hazard Profile. Saccharin has been shown to produce bladder carcinomas after lifetime administration in the diet at 5% or 50,000 ppm (47). This nongenotoxic carcinogen binds at high concentrations to urinary proteins which leads to the formation of silicate-containing precipitate and microcrystals in the urine of male rats (48,49). The crystals appear to produce superficial cytotoxicity to the bladder epithelium, cell death, and regenerative hyperplasia. With some diets, no tumor promotion is observed (50).

ADME. Saccharin is excreted unchanged in the urine.

The mechanism of tumor formation has been described as a multistage process involving binding, chemical reaction, and cellular mitogenesis. Fortunately the bioassay conducted with saccharin uses a sufficient number of feeding levels to characterize a multistage phenomenon. As a result, the multistage model predicts that a curve described by a fourth-order exponential expression that can be applied to the data (51), and this yields the best fit (Figure 10). The curve provides a reasonably good fit for this model, with a correlation coefficient of >0.95.

These data, along with maximum-likelihood-estimate (MLE), are presented in Table 7. The Q3* is equivalent to the upper 95% confidence interval for the linear slope in the linearized multistage model. At relatively high calculated risks the MLE and Q3* show a close correlation. But, at the lower risks (for example, at one-in-a-million), the difference between the MLE and Q3* is nearly five orders of magnitude. Such an observation raises questions about how the error is calculated. If, as with most curve-fitting routines, the error is dependent upon how far the data points differ from the fitted line, then a compound that fits the line more closely would show less error and the calculated Q3* be less severe. If such an event were to occur, it could result in the prediction of greater risk for a nonlinear compound such as saccharin than for a compound which better fits the LMS model.

Hormesis: A Paradoxical Response at Low-Dose Levels

Chemical: Propiconazole

Description. Propiconazole is a broad spectrum systemic, triazole fungicide that is used in agriculture for treatment of disease in wheat and rice.

Influence of Saturation

Figure 10. Multistage fit for the rat bladder tumor incidence observed after lifetime administration of saccharin.

Table 7. Comparison of Q* and best fit (MLE) of the dose response. For saccharin: P(d) = 1−e−Q4dQ4+Q4d2 where: Q3 = Q1 = Q2 = 0 and Q4 = 8.37 × 10−3.

| Q | Best fit, ppm | Q*(2 × 10^3), ppm |
|---|---|---|
| 0.5 | 59,600 | 50,000 |
| 1 | 83,000 | 5000 |
| 2 | 18,500 | 500 |
| 3 | 10,500 | 50 |
| 4 | 5,900 | 5.0 |
| 5 | 3,300 | 0.5 |
| 6 | 1,800 | 0.05 |
| 7 | 1,200 | 0.005 |
| 8 | 1,100 | 0.005 |

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Chemical Properties. Propiconazole is a viscous liquid. It has a solubility in water of 110 ppm (at 20°C). It has a Log P value of 3.3 and a molecular weight of 342.22.

Hazard Profile. The product is not mutagenic, teratogenic, or tumorogenic in rats or female mice. There was an increase in the incidence of liver tumors observed in male mice fed propiconazole at the highest feeding level of 2500 ppm; there is a significant reduction in tumor formation at 100 ppm, and no effect at 500 ppm (52). The feeding level of 2500 ppm greatly exceeded the MTD, as evidenced by a reduction in survival, a remarkable reduction in body weight gain, and severe liver damage.

ADME. Propiconazole is metabolized by the hepatic mixed-function oxidase system. In the rat and mouse the metabolic profile is complex. It has been shown that the male mouse and to a somewhat less degree the female mouse is capable of cleaving the dioxalane ring. The excercitary profile for the male mouse at high levels of administration is nonlinear. This pathway is less operative in the male rat. In addition, propiconazole has been shown to be a phenobarbital-type enzyme-inducing agent.

This final example speaks to the fact that quantitative risk assessment, as currently practiced, using LMS default model, is a giant step backwards. Figure 11 depicts four data scenarios. Scenario A provides the incidence of liver tumors observed in male mice fed propiconazole; this nonmonotonic response is impossible to fit using the LMS model (2). The beneficial effect at 100 ppm is thought to be real as this type of hormesis has been observed with other compounds exhibiting a similar biological profile to propiconazole.

Scenarios B, C, and D are hypothetical examples using the same feeding regimen but altering the incidence. Scenario B recreates an example of a more potent supralinear response where the incidence noted at 100 and 500 ppm is identical to that seen at 2500 ppm. This dose response should yield a large $Q_1^*$ value for the worst case. Scenario C sets the 100 and 500 ppm equivalent to the control value. Finally, Scenario D leaves the incidence at 2500 ppm fixed and projects a straight line back through zero, or a linearized response.

The data presented in each scenario are evaluated. Table 8 presents the estimated $Q_1^*$, the EPA version of the LMS model (2), and a second version of the LMS model, a quantal model developed by Sielken (53). The Sielken LMS is used in two modes. One mode is essentially configured to emulate the EPA version; the second mode is not so constrained so that it can fit the dose response. It can be seen that, regardless of the dose response, the EPA version of the LMS yielded essentially the same $Q_1^*$ value for all four dose–response scenarios. On the other hand, the Sielken unconstrained LMS version yielded conservative $Q_1^*$ values in the case of the more severe data sets and less conservative in the case of the propiconazole. Even the Sielken constrained LMS better reflected the dose response than did the EPA default model.

Conclusions
Risk assessment has the potential to provide a useful communication tool. Perhaps, like the ideal gas laws, we know they are not accurate, but they still are useful. Risk assessment needs to be as accurate as possible. Therefore, considering dose response, pharmacokinetic modeling may provide a mechanism to improve the accuracy in many cases. In the past decade there have been attempts to simplify the complex process of risk assessment to an extrapolation to a single estimator, the $Q_1^*$. This overly simplistic approach has provided a

| Scenario | Data | Sielken (54) Unconstrained | Constrained | Crump and Howe (2) |
|----------|------|---------------------------|-------------|-------------------|
| A        | Actual propiconazole | $2.7 \times 10^{-2}$ | $3.4 \times 10^{-2}$ | $5.0 \times 10^{-2}$ |
| B        | Supra linear, low- and mid-levels set to high-level incidence | 1.1 | $5.9 \times 10^{-1}$ | $4.6 \times 10^{-2}$ |
| C        | Supra linear, low- and mid-levels set to control incidence | $2.8 \times 10^{-1}$ | $2.6 \times 10^{-2}$ | $4.6 \times 10^{-2}$ |
| D        | Linear, control, low- and mid-levels set to straight line to high-level | $4.7 \times 10^{-2}$ | $4.1 \times 10^{-2}$ | $5.0 \times 10^{-2}$ |

$Q_1^*$ values expressed in 1/mg/kg/day human equivalent doses.
haven of reassurance for everyone who does not understand its limitations. This naive approach has not only created questionable science, but it has also created significant cost, both economically and in terms of the public trust. One may even raise questions concerning the impact of this approach, which is not used in the rest of the industrialized world, on the competitiveness of the United States. Can our nation afford such harmful extravagance? We think not: let us move forward and put our mistakes behind us. It is time to reexamine the biology and chemistry that is masked behind the endpoint of hazard, and to make the process as accurate as possible. After all, we all know it is indeed the dose that makes the poison.

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