COMBINING PHARMACOKINETIC AND CANCER BIOASSAY DATA

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SIAM INSTITUTE FOR MATHEMATICS AND SOCIETY

HEALTH SCIENCES PROGRAM
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DEPARTMENT OF STATISTICS
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INTRODUCTION

Considerable uncertainties impede rational decisions concerning priorities for preventing or reducing human exposure to potentially toxic substances. The uncertainties arise from several sources. Relationships between emissions from industrial processes and ambient levels of air and water pollutants depend in a complex way upon temperature, wind speed and direction, precipitation and other emissions. Similarly, relationships between ambient pollutants and human exposures depend upon activity patterns, time spent indoors vs outdoors, air conditioning, exercise level and other factors. Relationships between human exposures and acute or chronic disease depend upon age, sex, metabolism, pre-existing illness, and exposures to other agents. Uncertainties arise because the independent variables in these complex relationships are unknown or measured with substantial error.

Despite these formidable sources of uncertainty, risk assessments are needed to avoid the extremes of either banning or allowing all exposures to potentially harmful chemicals. This paper addresses methods for reducing uncertainty in relationships between human exposures and disease, particularly cancer. Attention will focus on possible improvements in the use of animal experiments to predict human cancer risks.

Section 2 provides an overview of strengths and limitations of epidemiological studies and animal bioassays for risk assessment. Sections 3 and 4 discuss the principal limitations of the animal bioassay as currently conducted by the National Toxicology Program. Section 5 outlines how these limitations may be ameliorated through the use of data from carefully designed pharmacokinetic experiments.

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UNCERTAINTIES IN HUMAN AND ANIMAL EXPERIMENTS

Although epidemiological studies can examine the effects of realistic exposures to combinations of toxicants among various population subgroups, they have limited ability to specify which exposures at which levels produce risk. Exposures are seldom known with any degree of useful precision. In addition, they are insensitive to small increases in disease, particularly when the disease is prevalent in the population under study. All observational studies have at best limited control of interviewing factors such as cigarette smoking that can bias relationships of interest. However, those concerned with the carcinogenic effects of chemicals confront special difficulties due to the long period between exposure and disease.

Because the investigator working with animals has relatively close control over experimental conditions, causal relationships are more easily established in animal than in human studies. Further, animal experiments can examine biochemical and pathophysiological mechanisms for damage. They can also strengthen a causal basis for associations observed in epidemiological studies. Perhaps their greatest strength is their utility as early warning systems against unwitting human exposures to potent carcinogens.

However, experiments are limited by uncertainties in extrapolating downward from high experimental dose rates to low human exposures. Problems also exist in extrapolating animal results to man in the face of large interspecies variability in susceptibility to toxic effects. A third limitation of animal experiments is their inability to simulate the complex and changing chemical mix in man's environment.

We shall discuss how supplementing the cancer bioassay with pharmacokinetic data may improve its reliability by reducing uncertainties in extrapolating from high to low dose rates, and from rodents to man.

UNCERTAINTIES IN LOW DOSE RATE EXTRAPOLATION

A brief description of the rodent carcinogenesis bioassay conducted formerly by the National Cancer Institute and now by the National Toxicology Program (NTP) will illustrate the low dose extrapolation problem. In a typical bioassay 50 mice and rats of each sex receive a chronic dose rate of the test compound for 52 weeks (mice) or 104 weeks (rats). The route of administration is chosen for maximum compatibility with human exposures. Animals are exposed at two dose rates. Fifty additional rodents of each species and sex are assigned as vehicle controls. Acute and sub-chronic studies preceding the bioassay determine the high dose rate, chosen to be the
maximum tolerated by the animals without significant weight loss or premature mortality, and called the maximum tolerated dose rate (MTD). The low dose rate is typically taken to be one-third the MTD. A detailed description of the bioassay protocol and analysis can be found in any NTP carcinogenesis bioassay report, for example (1). The experiment is not cheap: even with as few as 50 animals per strain-sex-dose combination, it costs more than $600,000 (2).

The high dose rates used in the NTP bioassay indicate that it has been designed as a sensitive qualitative index of potential human carcinogenicity. Its use for quantitation risk assessment is limited by probable discrepancies between tumor occurrence at the MTD and at the very low dose rates comparable to human exposures. Determination of biologically plausible inferences about risk at these low rates from experimental results has baffled scientists for several decades. In light of this formidable problem, one might ask: why use such high dose rates?

Figure 1 provides an answer to this question. It shows hypothetical bioassay results expressed as percent animals with tumor at the MTD, at one-third the MTD, and at two very low dose rates (d₁ and d₂) comparable to human exposures. Note that none of the animals developed tumors at dose rates below the MTD/3. Thus if animals had been assigned only to the dose rates d₁ and d₂, the bioassay would produce no evidence of carcinogenicity. However, one can show that an absence of tumors in 100 animals could occur by chance in 5% of bioassays of a compound whose actual tumor probability at these dose rates is 3%. Thus an investment of more than one-half million dollars could with 5% probability produce a clean bill of health for a chemical whose exposure to the US population would cause 3% of 220 million or more than 6 million cancers. Bioassays at low dose rates are even less sensitive when the animals have a substantial spontaneous tumor rate.

The need to avoid even a 5% chance of such a false negative prompts the use of the MTD, and it necessitates the low dose rate extrapolation dilemma illustrated in Figure 2. The Figure shows two dose-response curves hypothetically fit to experimental data at four high dose rates. Although both curves may arise from biologically plausible carcinogenesis models and both adequately fit the data, they have substantially different predictions for risk at the low dose rates of interest to humans, as shown in the figure inset. Two commonly used curves are based on the probit and "one-hit" (i.e., linear) models (3). The one-hit model specifies that tumor probability is linear in dose rate at low dose rates. The dose-rate reductions required by these two models to reduce risk from 1:100 to 1:100 million can differ by four orders of magnitude. In other words, while the probit model requires dose rate reduction by a
Figure 1. Hypothetical plot of actuarially adjusted percent animals with tumor vs administered dose rate in a standard NTP rodent carcinogenesis bioassay. Vertical lines represent upper and lower confidence limits. Animals are typically assigned to two nonzero dose rates: a high dose rate, chosen as the maximum tolerated by the animals without premature mortality or weight loss (the MTD), and a low dose rate, taken to be one-third the MTD.

Figure 2. Dose-response curves from models 1 and 2 provide good fits to bioassay data but differ by several orders of magnitude in predicted "safe" dose rates $d_1$ and $d_2$ corresponding to tumor probability of $10^{-8}$, as shown in the inset.

factor of two thousand, the one-hit model requires reduction by a factor of one million. These large discrepancies are frustrating because their resolution is elusive and their consequences for the public health and the economy are great. They have prompted suggestions that quantitative risk estimates be replaced by chemical ranks with respect to their relative carcinogenic potencies (2-5). However, we show below how potency ranks also suffer from the uncertainties, which are due in part to
unknown differences between dose rates in chemical absorption, metabolism and elimination.

Ames et al. (4) have proposed the reciprocal of the TD$_{50}$ as a measure of potency. The TD$_{50}$ for a given compound, species, and sex is the chronic dose rate in mg/kg/day required to halve the percent of tumor-free animals at the end of the species' lifetime. When the species has negligible spontaneous tumor occurrence, the TD$_{50}$ is simply the dose rate needed to produce tumors in 50% of the animals. To simplify the discussion we shall assume in this paper that there is no spontaneous tumor occurrence. Then the dose rate TD$_R$ needed to cause tumors in R percent of the animals satisfies

$$100p(TD_R) = R$$

(1)

where p(d) is tumor probability among animals exposed to dose rate d.

The TD$_{50}$'s for two hypothetical chemicals are shown in Figure 3. Potency is based on the TD$_{50}$, rather than say, the dose rate TD$_1$ need to cause tumors in one percent of the animals, because the TD$_{50}$ usually falls between the high and low dose rates of the bioassay and obviates downward extrapolation. However, human protection requires potency ranks based on the TD$_R$, where the risk R is as low as $10^{-4}$ - $10^{-6}$ percent.

![Diagram showing TD$_{50}$ for two chemicals (A and B) whose dose response curves are linear.](image)

Figure 3. TD$_{50}$'s for two chemicals (A and B) whose dose response curves are linear.

Figure 3 shows that when the unknown dose-response curves for chemicals A and B are both linear, ranks based on the TD$_{50}$'s of the two compounds will agree with ranks based on the TD$_R$ for any arbitrarily low risk R. However, when the dose-response curve for even one compound is nonlinear, the ranks can be reversed at high and low dose rates (Figure 4).
Figure 4. TD$_{50}$ and TD$_{10}$ for two chemicals A and B, when the spontaneous tumor rate is zero and the curve for A is non-linear. The TD$_R$ is the dose rate in mg/kg/day required to reduce the percent of tumor-free animals by R percent.

UNCERTAINTIES IN INTERSPECIES EXTRAPOLATION

To illustrate the additional difficulties in extrapolating potency ranks from rodents to man we shall ignore the above dose-rate difficulties and assume that at low to moderate dose rates of a compound the probability $p$ of tumor occurrence is proportional to the dose-rate $d$:

$$p(d) = \beta d.$$  \hspace{1cm} (2)

In Equation 2 the slope constant $\beta = \beta$ (compound, species) depends on the chemical, the species, as well as sex, strain, and other factors. It follows from Equations 1 and 2 that $\beta$ is related to the TD$_R$ needed to cause R percent of tumors by:

$$\beta = R/(100 \ TD_R).$$

Thus $\beta$ is proportional to the potency of the compound. This implies that the ratio of betas for two species or two sexes equals the ratio of the corresponding compound potencies.

Figure 5 shows the results of the NCI chloroform bioassay for Osborne-Mendel rats and B6C3 F1 mice (5). The curves represent weighted least squares fits of the one-hit model (with intercept for spontaneous incidence). It is evident from the figure that chloroform is a more potent liver carcinogen for mice than for rats, and more potent for female rats than for male rats. Table 1 shows the estimated beta's by species and sex.
Figure 5. Liver tumor incidence vs chloroform dose rate in mg/kg/day for male and female Osborne-Mendel rats and male and female B6C3F1 mice. Fitted curves are based on the one-hit model given approximately by Equation 2 of the text. The parameter estimates were obtained by weighted least squares regression. Table 1 gives the estimated slope parameters beta.

<table>
<thead>
<tr>
<th></th>
<th>B6C3 F1 Mouse</th>
<th>Osborne-Mendel Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6.90</td>
<td>0.26</td>
</tr>
<tr>
<td>Female</td>
<td>40.26</td>
<td>0.97</td>
</tr>
<tr>
<td>Both sexes (geometric mean)</td>
<td>16.67</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Equation 2 also indicates that the one-hit slope beta determines the "safe" dose rate $d_{\text{species}}$ for a given animal species corresponding to, say, a risk of $10^{-8}$:

$$d_{\text{species}} = 10^{-8}/\beta_{\text{species}}.$$

Given an estimated safe dose rate for the mouse, how does one scale-up to a safe dose rate for man? In particular, how does one reconcile the conflicting results among rats and mice shown in Figure 5?

The traditional conservative approach has been to extrapolate to humans on the basis of surface area, body weight or parts per million (ppm) in food or air using the most
sensitive rodent species. This approach bases human chloroform risks on the murine potency estimates in Table 1.

Table 2 illustrates the problems with this procedure. Row A of the table shows the safe dose rates for man and rat estimated by extrapolating from the mouse on a body weight and surface area basis. Note that use of the mouse to predict the safe dose rate for the rat gives safe dose rates of 0.06 and 0.0045 mg/kg/day, roughly two orders of magnitude lower than the corresponding dose rates obtained directly from the rat data shown in Row B. Row B shows the substantially larger safe dose rates obtained by extrapolating to humans from the rat instead of the mouse.

Table 2
"SAFE" DOSE RATES (MG/KG/DAY) 10^-5
ESTIMATED FROM NCI CHLOROFORM BIOASSAY DATA

<table>
<thead>
<tr>
<th>Extrapolated to</th>
<th>Man</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body Weight</td>
<td>Surface Area</td>
</tr>
<tr>
<td>Predicted From</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Mouse</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>B. Rat</td>
<td>2.38</td>
<td>2.38</td>
</tr>
</tbody>
</table>

*Assuming mouse, rat and human weights of 0.03, 0.50, and 70.00 kg, respectively.

We outline in the next section how using pharmacokinetic data may provide some help in resolving both the low dose and interspecies extrapolation problems described above.

EXTRAPOLATION USING METABOLIC INFORMATION
Chloroform appears to belong to a class of compounds that requires metabolic activation for toxicity. Thus any species variation in the capacity to metabolize this compound could have a substantial effect on its relative carcinogenic potency. As noted by Reitz et al. (6) and shown in Table 3, there are considerable differences in recovery of unmetabolized chloroform when mice, rats, squirrel monkeys and humans receive a single dose of the chemical. Based on their review of the literature,
Table 3
ESTIMATED PERCENT OF CHLOROFORM METABOLIZED AFTER A SINGLE ORAL DOSE

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (Mg/Kg)</th>
<th>% Excreted</th>
<th>% Metabolized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>60</td>
<td>6</td>
<td>97</td>
</tr>
<tr>
<td>Rat</td>
<td>60</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Monkey</td>
<td>70</td>
<td>78</td>
<td>22</td>
</tr>
<tr>
<td>Man</td>
<td>7</td>
<td>17-66</td>
<td>34-83</td>
</tr>
</tbody>
</table>

Source: Reitz et al. (6).

Reitz et al. estimate that mice and rats metabolize approximately 94 and 80 percent, respectively, of a single 60 mg/kg oral dose of chloroform. These differences may account for the greater susceptibility of mice relative to rats shown in Figure 5 and Table 1.

There are many problems in attempting to combine pharmacokinetic and bioassay data to produce more reliable risk estimates, as discussed below. The following is a simplified outline for how such work could be done, what assumptions are needed, and what types of uncertainty may be reduced.

**Pharmacokinetic Data**

a. First, estimate from carefully designed radiolabeling experiments the steady-state body burden of parent compound or active metabolite, as percent of applied dose rate, in rodents and nonhuman primates exposed to various constant dose rates of the chemical. The rodent species and strains, the routes of administration, and the dose rates should be identical to those used in the cancer bioassay for the compound. One should also estimate body burden corresponding to additional dose-rates as low as feasible within the sensitivity limits of the radiolabeling procedure. Since the experiment need last only long enough to assure that steady-state kinetics have been achieved, its cost should be small relative to that of the bioassay.

b. Next, interpolate a curve between the data points obtained in (a) to estimate the relationship between applied dose rate and absolute concentration of body burden.
The relationship may be nonlinear as shown in Figure 6, and based on Michaelis-Menten or other kinetics. It may also vary among species, as shown in Figure 7. Note the many data points corresponding to the large number of dose rates available in the pharmacokinetic study, unlike the bioassay.

Figure 6. Applied dose rate vs body burden, expressed as percent applied dose rate, for hypothetical compound. Data points are obtained by measuring the amount of labeled compound excreted in urine, feces, and expired air under steady-state conditions. Solid line represents curve interpolated from Michaelis-Menten or other kinetic model.

Figure 7. Applied dose rate vs body burden, expressed as percent applied dose rate of hypothetical compound, for three different species. Curves are obtained by interpolating from pharmacokinetic data using Michaelis-Menten or other kinetics. Safe dose rate $d_*$ for primates corresponds to the safe body burden $c_*$ obtained by fitting one-hit model to rodent tumor incidence versus rodent body burden, as shown in Figure 8.
Figure 8. Rodent tumor incidence vs body burden, expressed as steady state concentration of compound for hypothetical compound. Data points are obtained from bioassay and pharmacokinetic study. Solid curve represents fit of one-hit model. Safe concentration $c_*$ corresponds to arbitrarily small tumor probability, such as $10^{-9}$.

**Low-Dose Extrapolation**

To predict animal risk at low dose rates:

- use the data obtained in (a) and (b) above to fit a one-hit curve to the graph of tumor incidence vs body burden, as shown in Figure 8. The pharmacokinetic data can be useful to the low dose extrapolation problem only to the extent that the one-hit model provides an adequate description of incidence as a function of body burden. Such adequacy of the one-hit model is an untestable assumption underlying the use of pharmacokinetics for low dose extrapolation. The assumption implies that all nonlinearities in the relationship between tumor incidence and applied dose rate are due to nonlinearities between body burden and dose rate, as pictured in Figure 6.

- Use the fitted one-hit curve to estimate the "safe" body burden $c_*$ corresponding to tumor incidence of $10^{-9}$ (see Figure 8).

- Determine the safe applied dose rate $d_*$ corresponding to $c_*$ from the curve interpolated for primates in step (b) above as shown in Figure 6.

If nonlinearities in the relationship between tumor incidence and applied dose rate are due to more efficient elimination and/or detoxification at low dose rates, the safe dose $d_*$ estimated by the above procedure can be several orders of magnitude larger than that obtained by ignoring pharmacokinetics. Figure 9 compares the estimated safe dose rate $d$ obtained by simply fitting a straight line to the bioassay data with the dose rate $d_*$ obtained using pharmacokinetics. The value $d$ will be smaller than $d_*$ when elimination and/or detoxification efficiency is increased at low relative to high dose rates. Decreased elimination efficiency at low dose rates would reverse the relationship between $d$ and $d_*$. 
Figure 9. Comparison of safe dose-rate $d$, obtained by fitting a straight line to bioassay data, with safe dose-rate $d_\star$, obtained by transforming applied dose rate to body burden, extrapolating downward, and transforming back to applied dose rate.

**Interspecies Extrapolation**

To scale safe dose rates from rodents to humans:

- Determine from the interpolated curve for primates, obtained in (b), the applied dose rate $d_\star$ corresponding to $c_\star$, as shown in Figure 7. The estimated safe dose $d_\star$ will be independent of rodent species only if the relationship between tumor incidence and body burden is constant across species, as shown in Figure 8. This testable assumption states that all interspecies differences in tumor susceptibility are due to differences in absorption, metabolism and excretion of the compound. The use of pharmacokinetics will resolve inconsistencies in interspecies extrapolation only to the extent that this assumption holds.

**DISCUSSION**

This paper has presented an outline for using pharmacokinetic data to improve estimates of human risks at low dose rates of carcinogens. The outline does not reflect the species-dependent and dose-dependent kinetics of carcinogenesis at the target organ, kinetics which limit its ability to eliminate extrapolation uncertainties. Nevertheless, it represents a step toward improving the reliability of the carcinogenesis bioassay, and as such, its potential utility should be investigated.

At present there are no published data relating applied dose rate to steady-state body burden for several species at several chronic dose rates. Such data could be obtained at relatively low cost for several animal carcinogens tested in the NTP system, and they could be used to examine the utility of incorporating pharmacokinetics into risk assessments.
REFERENCES


2. National Toxicology Program staff, personal communication.


