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The Relationship Between Carcinogenic Potency and Maximum Tolerated Dose is Similar for Mutagens and Nonmutagens

Gay Goodman, Alexander Shlyakhter, and Richard Wilson

Correlations between carcinogenic potency (β or $1/TD_{50}$) and acute toxicity (LD_{50}) and between carcinogenic potency and maximum tolerated dose (MTD) have been described by several authors (1–5). The correlations have been attributed in part to a bias inherent in the carcinogenicity bioassay, namely, that the carcinogenic potencies of chemicals that are highly toxic and only weakly carcinogenic cannot be measured, since any such chemical would not produce excess tumors in the typically 50–100 experimental animals receiving it at the MTD (3). But a chemical at the opposite end of the spectrum, one highly carcinogenic relative to its MTD, could certainly be identified under the same bioassay conditions. If a chemical of the latter type were to produce tumors in 100% of the study animals at all doses tested (typically MTD, MTD/2, and MTD/4), its carcinogenic potency could not be determined using standard methods. However, potency could be estimated under these circumstances by incorporating time-until-tumor data, or another bioassay could be run at lower doses.

In fact, such chemicals are only rarely identified, most likely because few exist. Their absence from the data base amounts to evidence that carcinogenicity in the rodent bioassay is tied, presumably biologically, to toxicity (4). Given this observation, along with data on biochemical mechanisms of DNA damage and repair, Ames and co-workers (6,7) and others (8) suggested that for both genotoxic and nongenotoxic chemicals, toxic effects mediate the carcinogenicity observed in rodent bioassays.

Of the 928 chemicals (with Chemical Abstracts numbers) tested in long-term mouse or rat carcinogenicity bioassays and listed in the Carcinogenic Potency Data Base (CPDB) (9–11), we count 435 (280 for mice and 251 for rats) that have demonstrated carcinogenic potency at $P < 0.01$ (two-tailed test) in at least one target site; this is in general agreement with Gold et al.(12). We have arbitrarily chosen $P < 0.1$ as a cutoff for statistical significance; 521 of the 928 chemicals fall into this category (353 for mice and 318 for rats). Analysis in this report has been performed on subsets (explained below) of those chemicals defined by TD_{50} values significant at $P < 0.1$, $P < 0.05$, $P < 0.025$, or $P < 0.01$.

In lifetime rodent bioassays, chemicals are tested at the highest possible dose to maximize the probability that a significant site-specific excess of tumors will appear. The problem with testing at doses near the MTD is that some toxic effects may be inevitable. Indeed, as the bulk of papers presented in this symposium would indicate, it might be that many chemicals are carcinogenic at high doses primarily because of some mechanism related to their toxicity, hypothesized to be the result of cell death, oxygen-radical release, and cell proliferation (7,8,13). For several nongenotoxic chemicals, the evidence suggests that tumorigenesis occurs only when the dose is high enough to produce

quantifiable toxicity at the tumor target site; saccharin induction of bladder tumors in male rats is a notable example (14).

Do genotoxic chemicals cause cancer at high doses because they are genotoxic or because they are toxic? Since local toxicity at one or more sites is a probable consequence of dosing near the MTD, there may be synergistic effects due to toxicity (and consequent cell proliferation), even for chemicals that are carcinogenic *primarily* through genotoxicity. We approach the problem by asking whether the relationship between carcinogenic potency and MTD is weaker for mutagenic than for nonmutagenic agents. The maximum dose administered (MaxD) in a bioassay is usually fixed at the MTD; it consequently may be used as a surrogate for the MTD (2,5). In the work reported here, we addressed whether the TD_{50} has a different dependence on MaxD and on LD_{50} for mutagenic carcinogens than for nonmutagenic carcinogens. We also looked at the relationship between TD_{50} and MaxD in *Salmonella* mutagens as a function of the lowest effective dose (LED) for mutagenicity.

Methods

Two sets of chemicals were studied. The first comprised 222 chemicals tested by the National Cancer Institute/National Toxicology Program (NCI/NTP) and tabulated according to "structural alerts" (S/A) and mutagenicity (M) to *Salmonella* by Ashby and Tennant (15). Chemicals positive for both S/A and M were designated by Ashby and Tennant as +/+, chemicals negative for S/A and M were designated as −/−, and so forth. For concordant chemicals, i.e. those designated +/+ or −/−, we followed Ashby and Tennant's classification scheme. For the nonconcordant (+/− or −/+) chemicals, we made an assignment of mutagenicity or nonmutagenicity on the basis of (a) mutagenicity in *Salmonella* tests not considered by Ashby and Tennant, (b) mutagenicity in other bacterial systems, or (c) mutagenicity in some eukaryotic in vitro test, using *IARC Monographs Supplement 6* as a reference (16). If positive for S/A and untested for mutagenicity, a chemical was classified as mutagenic. In this manner, we categorized 117 chemicals as nonmutagens and 100 as mutagens; the remaining 5 could not be categorized.

The second set consisted of 245 chemicals that had tested positive for mutagenicity in various *Salmonella* strains, and for which quantitative information (i.e., revertant colonies at each dose level) was available. All data were from studies published by Zeiger and associates (17–19). From these data we estimated, for each chemical, the LED in each test, and we took the geometric mean of the LEDs over all tests. The chemicals were divided into three groups according to mean LED: low (LED <10 mg), intermediate (10 mg ≤ LED <100 mg), and high (LED ≥ 100 mg).

The minimum TD_{50}s at a given level of statistical significance were taken from the CPDB of Gold and colleagues (9–11). (For the NCI/NTP chemicals, the experiments yielding the appropriate minimum TD_{50} values were not necessarily those performed by the NCI/NTP. Note that "NCI/NTP dataset" here refers to the CPDB tabulation of all pertinent experimental results for these NCI/NTP chemicals and does not imply that the data came exclusively from NCI/NTP experiments.) Data from combined sites (tumor-bearing animals, abbreviated by Gold and co-workers as tba or TBA) were ignored. Data were obtaine
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control group for a given site exceeded 6
TD_{50} values were chosen to satisfy a give
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significance criteria as sets A, B, C, and D,
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from which the minimum TD_{50} was der

Tests for similarity

A dummy-variable method was u
regression lines are coincident. The da
performed for the model:

$$y = b_0 + b_1$$

where $\delta = 0$ for the first dataset and $\delta = 1$ fo
that the coefficients c_1 and c_2 are signifi
used to compute the statistical paramete

If the sample variances $s^1{}_2$ and $s^2{}_2$
distributions, then for comparison of the
determine the confidence with which we
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chemicals n_1 and n_2 in datasets 1 and 2.

The observed value r of the correlat
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For comparison of two values r_1 and r_2
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where σ_z is the standard error of the dif

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the null hypothesis, $H_0{:}(\rho_1 = \rho_2)$, is tru

TBA) were ignored. Data were obtained separately for mice and rats. Gender was ignored. Only oral and inhalation routes were considered. If the tumor incidence in the control group for a given site exceeded 60%, the TD_{50} at that site was disregarded. The TD_{50} values were chosen to satisfy a given statistical significance criterion: $P < 0.01$, $P < 0.025$, $P < 0.05$, or $P < 0.1$. We shall refer to the data selected according to these significance criteria as sets A, B, C, and D, respectively. Minimum LD_{50}s were obtained from the *Registry of Toxic Effects of Chemical Substances* (20); only oral and inhalation routes were allowed. The designated MaxD is the highest dose in the same experiment from which the minimum TD_{50} was derived.

Tests for similarity

A dummy-variable method was used to test the null hypothesis that a pair of regression lines are coincident. The datasets are combined and linear regression is performed for the model:

$$y = b_0 + b_1 x + c_1 \delta + c_2 \delta x,$$

where $\delta = 0$ for the first dataset and $\delta = 1$ for the second. A t-test is made of the probability that the coefficients c_1 and c_2 are significantly different from zero. (SAS software was used to compute the statistical parameters.)

If the sample variances $s^1{}_2$ and $s^2{}_2$ for datasets 1 and 2 are assumed to have χ^2 distributions, then for comparison of the two variances, an F test may be performed to determine the confidence with which we can reject the null hypothesis, $H_0{:}(\sigma_1{}^2 = \sigma_2{}^2)$, in favor of the alternative hypothesis, $H_1{:}(\sigma_1{}^2 \neq \sigma_2{}^2)$, where σ^2 is the underlying variance. The ratio $s_1{}^2/s_2{}^2$ is compared to the F statistic computed given the number of chemicals n_1 and n_2 in datasets 1 and 2.

The observed value r of the correlation coefficient ρ may be transformed to a new, approximately normal variable z_r, defined by

$$z_r = {}^1/_2[\ln(1 + r) - \ln(1 - r)].$$

For comparison of two values r_1 and r_2 obtained from independent samples of size n_1 and n_2, the variable Z is defined as

$$Z = \frac{z_1 - z_2}{\sigma_z},$$

where σ_z is the standard error of the difference between z_1 and z_2:

$$\sigma_z = \sqrt{\frac{1}{n_1 - 3} + \frac{1}{n_2 - 3}}.$$

Z is evaluated in terms of a standard normal distribution, yielding the probability that the null hypothesis, $H_0{:}(\rho_1 = \rho_2)$, is true (21).

Simulation

It has been argued by Rieth and Starr (22) that since the range of MaxDs "spans over six orders of magnitude," whereas the possible range of finite and significantly nonzero single-dose values of carcinogenic potency β at a given MaxD is, according to Bernstein et al., confined to a 30-fold range around 1/MaxD (2), then a high degree of correlation between β and MaxD is inevitable. This line of reasoning leads to a specific, answerable question: Is the relationship between β and MaxD stronger than what would be observed if the measured potency were randomly selected from the possible values that could arise under a given set of experimental constraints?

To examine the degree to which the quantitative relationship between β and MTD is an artifactual consequence of the bioassay conditions, we have simulated a simplified bioassay based on the complete experiments in the NCI/NTP datasets described above. Before performing the simulations, we calculated a carcinogenic potency based on partial data from the bioassay as follows. For each experiment that had provided a minimum TD_{50} value under the particular selection criterion (A, B, C, or D), we noted the control group tumor incidence a_0, the maximum tumor incidence a_m, and the total number of animals n_0 and n_m in the control and MaxD groups, respectively. A carcinogenic potency based on this pseudo single-dose experiment was calculated as

$$\beta = \ln\left[\frac{1-(a_0/n_0)}{1-(a_m/n_m)}\right]$$

(Note that this is the same formula for potency used by Bernstein et al. [2] in their simulation of the results of single-dose bioassays.) This value for β was plotted against 1/MaxD, and linear regression analysis was performed.

To simulate the pseudo single-dose experiment, a_m was allowed to take discrete integer values between $(a_0 + 1)/n_0$ and $(n_m - 1)/n_m$. The probability distribution of a_m was assumed to be uniform, and a value was chosen at random for calculation of carcinogenic potency according to the equation cited above. Note that no test for statistical significance was performed during this random selection process, and therefore the lowest values of simulated potencies would be expected to be lower than what would actually be allowed, at least at the higher significance levels (sets A and B). The method for calculating the statistical significance of TD_{50} values in the CPDB reflects the fact that the experiments are multidose rather than single dose (23). Using maximum-likelihood estimators, it allows for the significance of a dose trend even when the maximum number of total tumors is not by itself statistically significant at a given confidence level (24). Since it is not a small task to translate the TD_{50} significance criterion into a lower limit on potency in a single-dose experiment, we have elected to perform our analysis at this time without such an added restriction; a future report will deal with this problem (Shlyakhter, Goodman and Wilson, unpublished data).

Results

For the NCI/NTP data, $1/TD_{50}$ versus 1/MaxD is plotted in Figure 1 and

$1/TD_{50}$ versus $1/LD_{50}$ is plotted in Figu
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groups. Table 1 shows the results of obtai
linear-regression model

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where x is 1/MaxD or $1/LD_{50}$. The sl
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The slopes for mutagenic and nonr
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mutagenic chemicals.

Comparison of sample variances (s
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different. Pairwise comparison between
(≥90% confidence).

For completeness, in Table 2 we a
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We found that in every case in which
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This value for β was plotted against
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rather than single dose (23). Using
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to translate the TD_{50} significance
ose experiment, we have elected to
dded restriction; a future report will
Wilson, unpublished data).

/MaxD is plotted in Figure 1 and

$1/TD_{50}$ versus $1/LD_{50}$ is plotted in Figure 2 for mice and for rats and using different symbols for mutagenic and nonmutagenic chemicals. Data taken at the two extremes of statistical significance, sets A and D ($P < 0.01$ and $P < 0.1$), are plotted for the MaxD data. One level ($P < 0.025$) is plotted for the LD_{50} data. At higher statistical significance (i.e., $P < 0.01$), the comparison of LD_{50} datasets is less meaningful, since the number of points is so small (especially for the rat nonmutagens) that the sample is unlikely to be representative. Similarly, in the high-LED group of the Zeiger data, the small number of points sets a limit on the statistical significance level worth examining. Using $P < 0.025$ as the cutoff for the Zeiger mutagens, $1/TD_{50}$ versus 1/MaxD is plotted for mice and for rats if Figure 3, with different symbols for the low-, intermediate-, and high-LED groups. Table 1 shows the results of obtaining the least squares fit to the normal-error linear-regression model

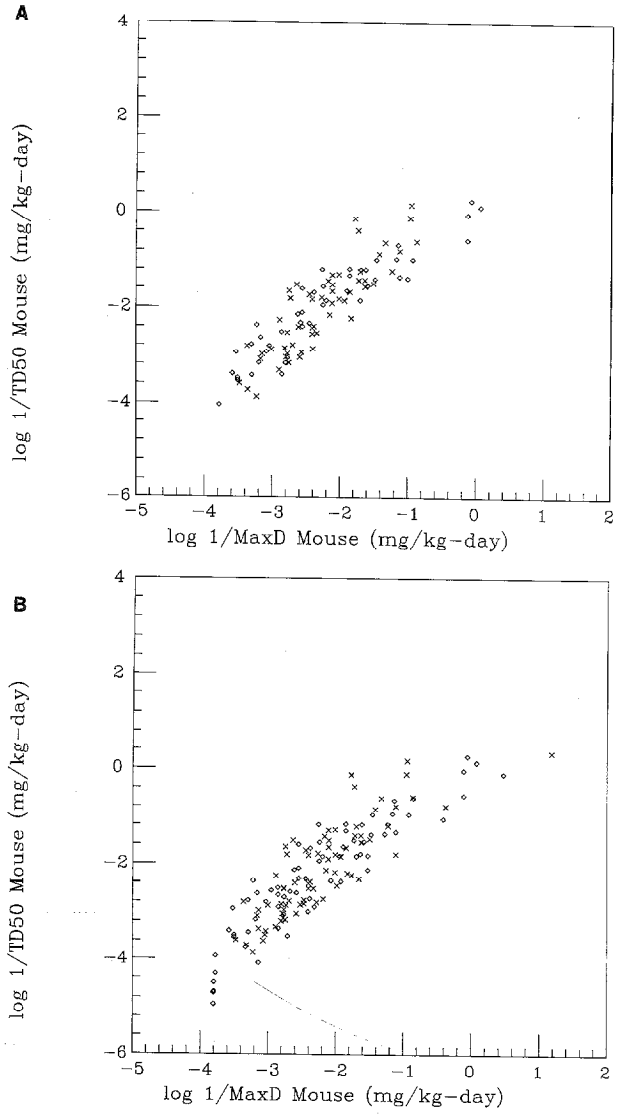
$$\log(1/TD_{50})_i = b_0 + b_1 \cdot \log x_i + \varepsilon_i,$$

where x is 1/MaxD or $1/LD_{50}$. The slope (±SD), zero intercept (±SD), observed correlation coefficient, number of points, and sample variance are given for each plot.

The slopes for mutagenic and nonmutagenic chemicals (NCI/NTP data, MaxD and LD_{50}) and for chemicals with low, intermediate, and high LEDs (Zeiger's *Salmonella* mutagens, MaxD) were compared (Table 2). All pairwise comparisons based on the MaxD resulted in failure to reject the null hypothesis of equal slopes (with ≥ 90% confidence), with the exception of the mouse dataset A, where it is rejected with 99.5% confidence. For the comparisons based on LD_{50}, the null hypothesis is rejected for the rat dataset (99.9% confidence). In both cases for which the slopes were significantly different, the intercepts also differed significantly (>99% confidence). Examination of the LD_{50} data (Fig. 2) suggests that a linear model may not be appropriate for the mutagenic chemicals.

Comparison of sample variances (s_2) between mutagens and nonmutagens and between pairs of LED groups is also shown in Table 2. In every case, the variance for the mutagens is greater than the variance for the nonmutagens. The sample variances based on the MaxD are significantly different for the most stringently selected mouse data, set A (90% confidence) and for all rat datasets: set A (90% confidence) and sets B, C, and D (95% confidence). Sample variances based on the LD_{50} were not significantly different. Pairwise comparison between LED groups reveals no significant difference (≥90% confidence).

For completeness, in Table 2 we also give a comparison of observed correlation coefficients for mutagens/nonmutagens and low/medium/high LED groups, although we think this is less informative than the comparison of sample variances. (The degree of correlation for a given sample may be high even when the variance is large, and for two samples with equal correlation coefficients, the variances might be quite different.) We found that in every case in which there was a significant difference in sample variances, there was also a significant difference in correlation coefficients. In two cases in which no significant difference in sample variances occurred, there was nevertheless a significant difference in correlation coefficients: the mutagen/nonmutagen comparison for mouse dataset D and the medium/high LED comparison for mice.



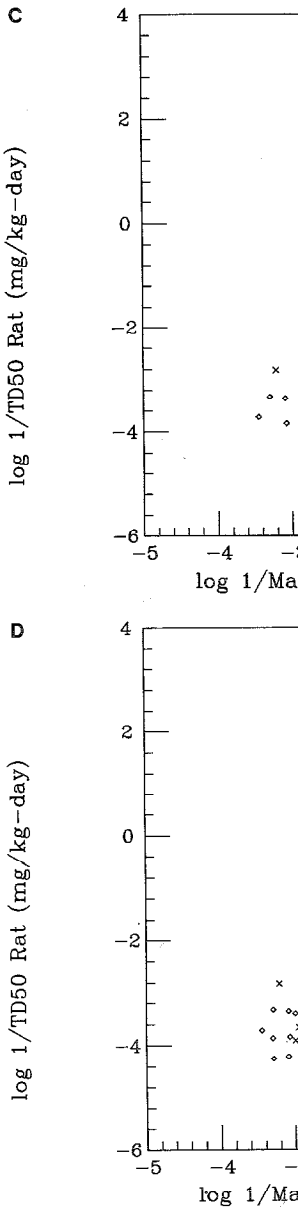
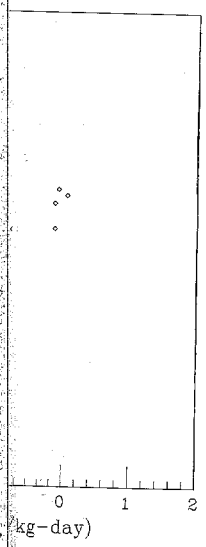
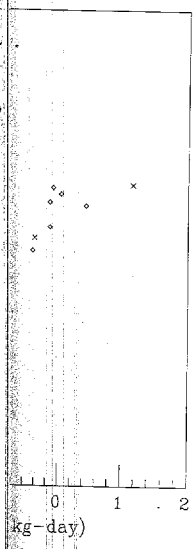


Figure 1. Log-log plot of $1/TD_{50}$ versus 1/MaxD for NCI/NTP chemicals tested in mice (A and B) and rats (C and D). ×, mutagens; ◇, nonmutagens. A and C, TD_{50} significant at $P < 0.01$; B and D, TD_{50} significant at $P < 0.1$. See text for details.

TD_{50} significance level: Effect on variance

As the significance level for selection of the minimum TD_{50} value is lowered, the sample variance increases. All comparisons were tested for significance at the 90% confidence level or higher. The variances
in mice (99% confidence) and the mutage
most stringently selected dataset (A) and





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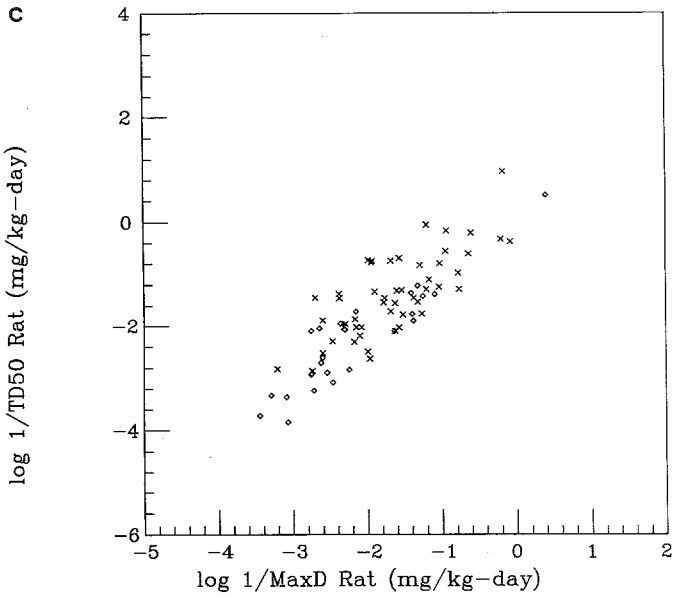
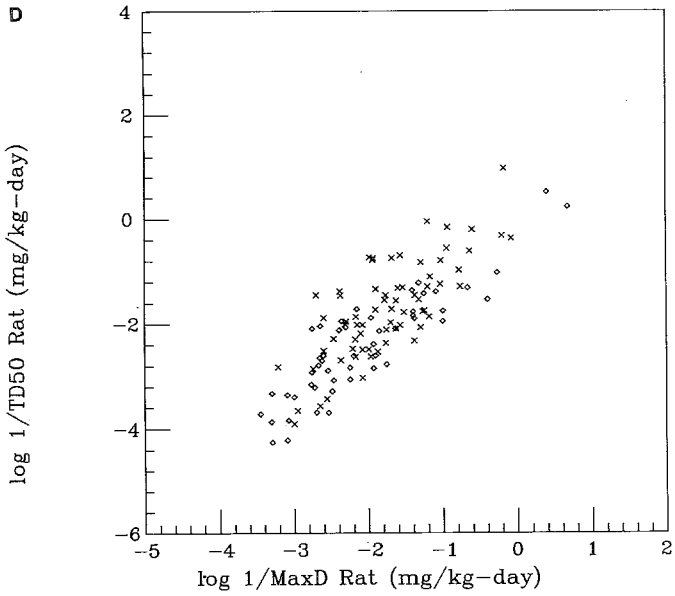




num TD_{50} value is lowered, the ed for significance at the 90%

confidence level or higher. The variances differ significantly for the nonmutagens tested in mice (99% confidence) and the mutagens tested in rats (95% confidence) between the most stringently selected dataset (A) and the other three sets, but the variances of these

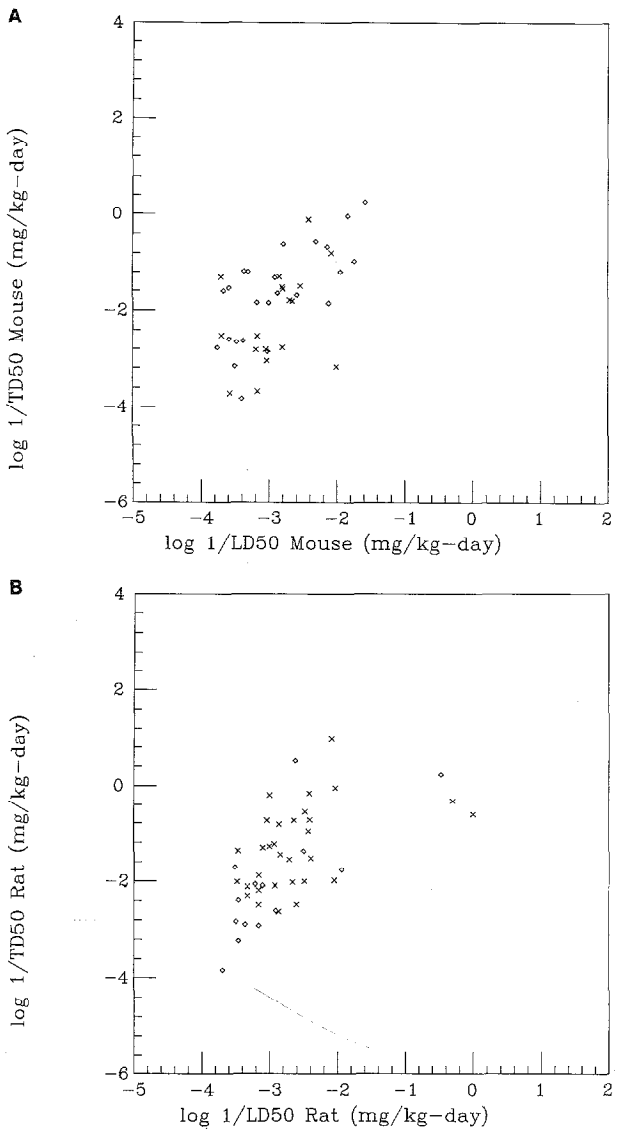


Figure 2. Log-log plot of $1/TD_{50}$ versus $1/LD_{50}$ for NCI/NTP chemicals tested in mice (A) and rats (B). ×, mutagens; ◇, nonmutagens. TD_{50} significant at $P < 0.025$. See text for details.

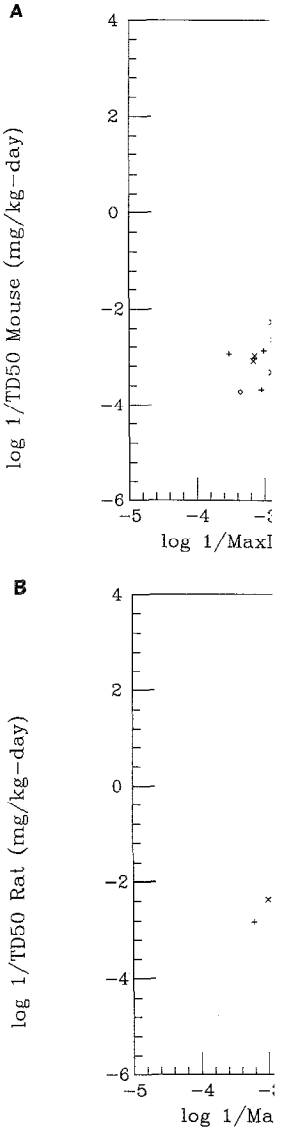
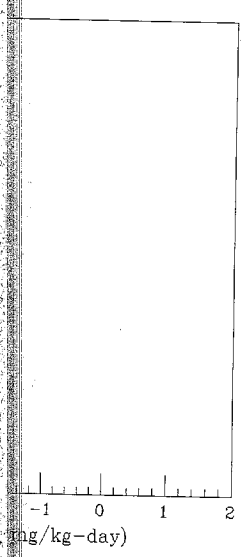
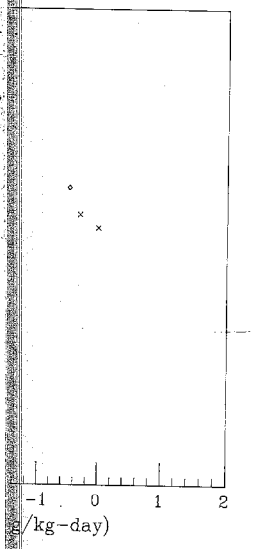


Figure 3. Log-log plot of $1/TD_{50}$ versus 1 in mice (A) and rats (B). +, LED < 10 r mg. TD_{50} significant at $P < 0.025$. Se

last sets do not vary significantly between themselves. For the mutagens tested in mice as well as the nonmutagens tested in rats, the increase in variance with decreasing significance-level selection becomes significant (95% confidence) only for comparison

of the least stringently selected set (D) v none of the comparisons was there a si observed correlation coefficients.





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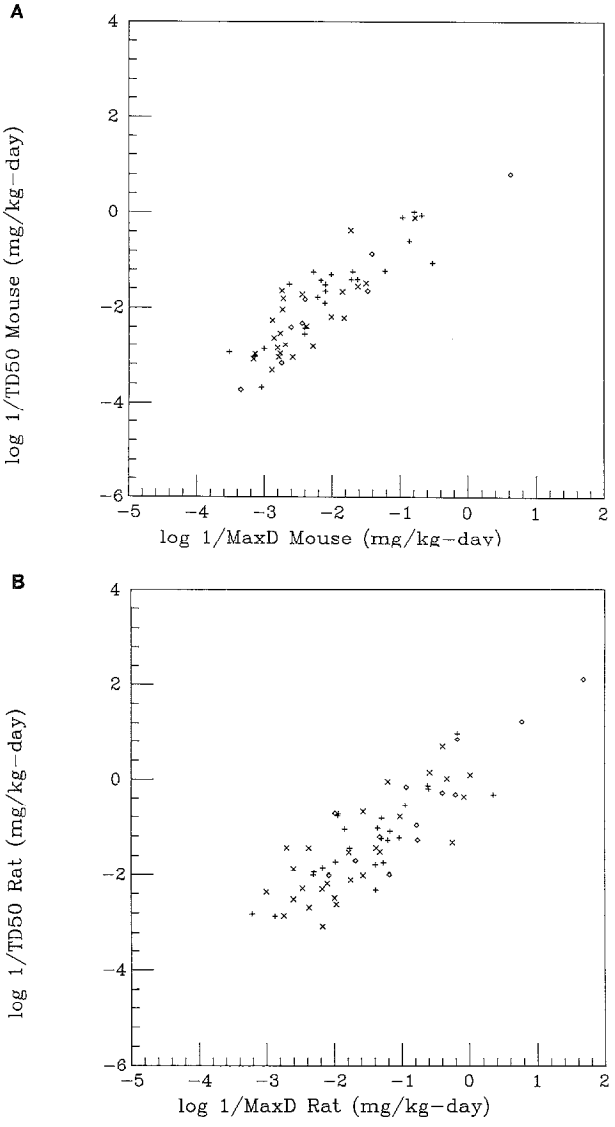

Figure 3. Log-log plot of $1/TD_{50}$ versus 1/MaxD for Zeiger *Salmonella* mutagens tested in mice (A) and rats (B). +, LED < 10 mg; ×, 10 mg ≤ LED < 100 mg; ◇, LED ≥ 100 mg. TD_{50} significant at $P < 0.025$. See text for details.

es. For the mutagens tested in mice
crease in variance with decreasing
% confidence) only for comparison

of the least stringently selected set (D) with the most stringently selected set (A). For none of the comparisons was there a significant difference (≥90% confidence) in the observed correlation coefficients.

Table 1. Linear Regression of Log(1/TD_{50}) Versus Log(1/MaxD) for NCI/NTP and Zeiger Datasets and Log(1/TD_{50}) Versus Log(1/LD_{50}) for NCI/NTP Datasets.

Type		TD_{50} significance[a]	Slope	Intercept	r	n	s^2
NCI/NTP carcinogens, MaxD							
Mouse mutagens		A	1.276 ± 0.100	0.882 ± 0.234	0.871	54	0.227
		B	1.189 ± 0.097	0.624 ± 0.226	0.850	60	0.259
		C	1.220 ± 0.093	0.641 ± 0.222	0.851	67	0.262
		D	1.056 ± 0.081	0.220 ± 0.191	0.841	72	0.290
Mouse nonmutagens		A	0.956 ± 0.056	0.131 ± 0.134	0.934	45	0.143
		B	1.009 ± 0.064	0.165 ± 0.155	0.912	53	0.210
		C	1.054 ± 0.062	0.212 ± 0.153	0.913	59	0.221
		D	1.041 ± 0.056	0.101 ± 0.138	0.916	69	0.234
Rat mutagens		A	0.855 ± 0.107	0.017 ± 0.189	0.757	50	0.274
		B	0.915 ± 0.116	0.035 ± 0.209	0.719	60	0.358
		C	0.972 ± 0.112	0.023 ± 0.204	0.740	65	0.358
		D	1.034 ± 0.109	0.094 ± 0.204	0.759	68	0.371
Rat nonmutagens		A	1.022 ± 0.092	0.069 ± 0.216	0.919	25	0.152
		B	0.982 ± 0.081	0.238 ± 0.186	0.919	29	0.190
		C	0.959 ± 0.070	0.381 ± 0.157	0.905	44	0.185
		D	0.956 ± 0.070	0.463 ± 0.158	0.892	50	0.214
NCI/NTP carcinogens, LD_{50}							
Mouse mutagens		B	0.830 ± 0.472	0.254 ± 1.386	0.402	18	0.895
Mouse nonmutagens		B	1.045 ± 0.223	1.340 ± 0.658	0.707	24	0.534
Rat mutagens		B	0.522 ± 0.186	0.054 ± 0.508	0.463	31	0.616
Rat nonmutagens		B	1.054 ± 0.282	1.017 ± 0.857	0.734	14	0.752
Zeiger *Salmonella* mutagens, MaxD							
Mouse	Low LED	B	1.032 ± 0.116	0.423 ± 0.247	0.889	23	0.206
	Medium LED	B	1.096 ± 0.182	0.416 ± 0.453	0.788	24	0.271
	High LED	B	1.105 ± 0.119	0.296 ± 0.273	0.967	8	0.151
	All	B	1.083 ± 0.078	0.423 ± 0.180	0.887	55	0.222
Rat	Low LED	B	0.874 ± 0.140	0.045 ± 0.235	0.800	24	0.294
	Medium LED	B	0.952 ± 0.140	0.046 ± 0.261	0.806	27	0.410
	High LED	B	1.052 ± 0.165	0.233 ± 0.205	0.887	13	0.373
	All	B	0.969 ± 0.077	0.137 ± 0.130	0.848	64	0.343

Abbreviations: LD, lethal dose; MaxD, maximum dose administered; n, the number of chemicals; NCI/NTP, National Cancer Institute/National Toxicology Program; r, the observed correlation coefficient; s^2, the sample variance (standard deviation squared); TD, tumor dose.
[a] TD_{50} statistical significance criteria: A, $P < 0.01$; B, $P < 0.025$; C, $P < 0.05$; D, $P < 0.1$.

Pseudo single-dose experiments and simulations

Linear regression was performed for each experimental dataset; the sample variances are given in Table 3, along with the observed correlation coefficients. There is no significant difference (≥90% confidence) between any pair of mutagen/nonmutagen variances obtained in the pseudo single-dose experiments, in contrast to the complete experiments (Table 2). The mutagen/nonmutagen comparison of observed correlation coefficients revealed significant differences for all mouse datasets (A, 99% confidence; B and C, 95% confidence; D, 90% confidence) and for rat datasets B, C, and D (95% confidence). Again, we suggest that the comparison of sample variances is a more meaningful indicator of the strength of the relationship between TD_{50} and MaxD; the failure

Table 2. Comparison of Slopes, Sample Varia
Linear Regression of Log(1/TD_{50}) Versus Log
and Nonmutagens (a and b) and Low/Mediu

Comparison	
(a) NCI/NTP carcinogens, MaxD	
Mutagen/Nonmutagen	Mou
	Rat
(b) NCI/NTP carcinogens, LD_{50}	
Mutagen/Nonmutagen	Mou
	Rat
(c) Zeiger *Salmonella* mutagens, MaxD	
Low/Medium LED	Mou
Low/High LED	
Medium/High LED	
Low/Medium LED	Rat
Low/High LED	
Medium/High LED	

Abbreviations: LD, lethal dose; LED, lowest e
NCI/NTP, National Cancer Institute/Natio
[a] Statistical significance criteria for A, B, C, a
[b] Probability is <0.5% that the two-dataset
consisting of mutagens alone.
[c] Probability of falsely rejecting H_0:($s_1^2 = s_2^2$
[d] Probability of falsely rejecting H_0:($s_1^2 = s_2^2$
[e] Probability of falsely rejecting H_0:($r_1 = r_2$) is
[f] Probability of falsely rejecting H_0:($r_1 = r_2$) is
[g] Probability of falsely rejecting H_0:($r_1 = r_2$) is

of the pseudo single-dose experiments t
found with the complete experiments inc
the latter.

Simulations were performed five ti
and observed correlation coefficients we
ulations. For two datasets (mouse mutag
simulation was performed 100 times, an
cients were averaged accordingly and cor
that the first five random number seeds
averaged, for these two datasets) sample
for the simulations are shown in Table
simulated and experimental pseudo sing

In every case except for rat muta
greater than the experimental sample var

axD) for NCI/NTP and Zeiger Datasets
ts.

tercept	r	n	s^2
92 ± 0.234	0.871	54	0.227
24 ± 0.226	0.850	60	0.259
41 ± 0.222	0.851	67	0.262
20 ± 0.191	0.841	72	0.290
51 ± 0.134	0.934	45	0.143
55 ± 0.155	0.912	53	0.210
12 ± 0.153	0.913	59	0.221
01 ± 0.138	0.916	69	0.234
17 ± 0.189	0.757	50	0.274
35 ± 0.209	0.719	60	0.358
28 ± 0.204	0.740	65	0.358
34 ± 0.204	0.759	68	0.371
59 ± 0.216	0.919	25	0.152
38 ± 0.186	0.919	29	0.190
31 ± 0.157	0.905	44	0.185
33 ± 0.158	0.892	50	0.214
34 ± 1.386	0.402	18	0.895
10 ± 0.658	0.707	24	0.534
14 ± 0.508	0.463	31	0.616
17 ± 0.857	0.734	14	0.752
33 ± 0.247	0.889	23	0.206
16 ± 0.453	0.788	24	0.271
16 ± 0.273	0.967	8	0.151
33 ± 0.180	0.887	55	0.222
15 ± 0.235	0.800	24	0.294
16 ± 0.261	0.806	27	0.410
13 ± 0.205	0.887	13	0.373
17 ± 0.130	0.848	64	0.343

nistered; n, the number of chemicals;
Program; r, the observed correlation
squared); TD, tumor dose.
025; C, $P < 0.05$; D, $P < 0.1$.

xperimental dataset; the sample
ed correlation coefficients. There
any pair of mutagen/nonmutagen
ents, in contrast to the complete
mparison of observed correlation
use datasets (A, 99% confidence;
or rat datasets B, C, and D (95%
sample variances is a more mean-
veen TD_{50} and MaxD; the failure

Table 2. Comparison of Slopes, Sample Variances and Observed Correlation Coefficients for the Linear Regression of Log(1/TD_{50}) Versus Log(1/MaxD) (a and c) or Log(1/LD_{50}) (b), for Mutagens and Nonmutagens (a and b) and Low/Medium/High LEDs (c).

Comparison		TD_{50} significance[a]	Slopes differ?	Variances differ?	Correlation coefficients differ?
(a) NCI/NTP carcinogens, MaxD					
Mutagen/Nonmutagen	Mouse	A	Yes[b]	Yes[c]	Yes[e]
		B	No	No	No
		C	No	No	No
		D	No	No	Yes[f]
	Rat	A	No	Yes[c]	Yes[f]
		B	No	Yes[d]	Yes[g]
		C	No	Yes[d]	Yes[g]
		D	No	Yes[d]	Yes[f]
(b) NCI/NTP carcinogens, LD_{50}					
Mutagen/Nonmutagen	Mouse	B	No	No	No
	Rat	B	Yes[b]	No	No
(c) Zeiger *Salmonella* mutagens, MaxD					
Low/Medium LED	Mouse	B	No	No	No
Low/High LED		B	No	No	No
Medium/High LED		B	No	No	Yes[f]
Low/Medium LED	Rat	B	No	No	No
Low/High LED		B	No	No	No
Medium/High LED		B	No	No	No

Abbreviations: LD, lethal dose; LED, lowest effective dose; MaxD, maximum dose administered; NCI/NTP, National Cancer Institute/National Toxicology Program; TD, tumor dose.

[a] Statistical significance criteria for A, B, C, and D as in Table 1.

[b] Probability is <0.5% that the two-dataset combination has the same slope as the dataset consisting of mutagens alone.

[c] Probability of falsely rejecting H_0:($s_1^2 = s_2^2$) is <10%.

[d] Probability of falsely rejecting H_0:($s_1^2 = s_2^2$) is <5%.

[e] Probability of falsely rejecting H_0:($r_1 = r_2$) is <10%.

[f] Probability of falsely rejecting H_0:($r_1 = r_2$) is <5%.

[g] Probability of falsely rejecting H_0:($r_1 = r_2$) is <1%.

of the pseudo single-dose experiments to replicate mutagen/nonmutagen differences found with the complete experiments indicates that the former are a poor surrogate for the latter.

Simulations were performed five times for each dataset, and the sample variances and observed correlation coefficients were averaged over these five independent simulations. For two datasets (mouse mutagens set D and mouse nonmutagens set A), the simulation was performed 100 times, and the sample variances and correlation coefficients were averaged accordingly and compared with the 5× averages, in order to check that the first five random number seeds were not atypical. The 5× averaged (or 100× averaged, for these two datasets) sample variances and observed correlation coefficients for the simulations are shown in Table 4, along with results of the comparison of simulated and experimental pseudo single-dose experiments.

In every case except for rat mutagens set D, the simulated sample variance is greater than the experimental sample variance. Only for mouse mutagens sets A and B

Table 3. Comparison of Sample Variances and Observed Correlation Coefficients for the Linear Regression of Log(1/TD_{50}) on Log(1/MaxD) for Mutagens (s_m^2 and r_m) and Nonmutagens (s_{nm}^2 and r_{nm}), Pseudo Single-dose NCI/NTP Data.

Pseudo single-dose dataset (Mutagen/Nonmutagen)[a]		s_m^2/s_{nm}^2	r_m/r_{nm}	Variances differ?[b]	Correlation coefficient differ?
Mouse dataset	A	0.127/0.130	0.839/0.949	No	Yes[c]
	B	0.177/0.182	0.828/0.923	No	Yes[d]
	C	0.191/0.183	0.803/0.916	No	Yes[d]
	D	0.200/0.187	0.865/0.924	No	Yes[e]
Rat dataset	A	0.160/0.108	0.893/0.925	No	No
	B	0.218/0.839	0.1420/0.935	No	Yes[d]
	C	0.225/0.156	0.822/0.923	No	Yes[d]
	D	0.268/0.195	0.781/0.906	No	Yes[d]

Note: The number of chemicals in each dataset is the same as for the corresponding complete dataset for mutagens or nonmutagens listed in Table 1.
Abbreviations: MaxD, maximum dose administered; NCI/NTP, National Cancer Institute/National Toxicology Program; TD, tumor dose.
[a] Data sets A, B, C, and D defined by statistical-significance criteria as in Table 1.
[b] Probability that H_0:($s_m^2 = s_{nm}^2$) is true is ≥10% in every case.
[c] Probability of falsely rejecting H_0:($r_m = r_{nm}$) is <1%.
[d] Probability of falsely rejecting H_0:($r_m = r_{nm}$) is <5%.
[e] Probability of falsely rejecting H_0:($r_m = r_{nm}$) is <10%.

and nonmutagens set A is the difference statistically significant at the 95% confidence level. For rat mutagens set A and for rat nonmutagens sets A and C, the difference is significant at the 90% level. No significant differences in observed correlation coefficients were found (≥90% confidence).

Discussion

Distribution of mutagens versus nonmutagens

Only for the most stringently selected mouse dataset ($P < 0.01$) were the data consistent with different $1/TD_{50}$ versus 1/MaxD distributions: both slope and intercept are significantly larger for the mutagens than for the nonmutagens. Examination of the data (Fig. 1A) shows that the difference appears when $1/MaxD > 10^{-2}$ (MaxD < 100 mg/kg-day), where the mutagens tend to have a higher carcinogenic potency relative to MaxD than do nonmutagens. The four chemicals with the lowest MaxDs, which presumably are the most toxic (reserpine, dieldrin, heptachlor, and aldrin), are all nonmutagens. For the chemicals with MaxD > 100 mg/kg-day, there is no apparent difference in the distributions.

Sample variances of mutagens versus nonmutagens

For the data based on MaxD, in the most stringently selected mouse dataset and in all the rat datasets the difference in sample variances between mutagens and nonmuta-

Table 4. Comparison of Sample Variances ar
Regression of Log(1/TD_{50}) Versus Log(1/Max
and r_s) Pseudo Single-dose NCI/NTP Data.

Pseudo single-dose dataset (Simulated/Experimental)[a]		s_s^2
Mouse mutagens dataset	A	0.237
	B	0.274
	C	0.248
	D	0.242
Mouse nonmutagens dataset	A	0.245
	B	0.244
	C	0.249
	D	0.249
Rat mutagens dataset	A	0.237
	B	0.251
	C	0.251
	D	0.241
Rat nonmutagens dataset	A	0.208
	B	0.215
	C	0.237
	D	0.253

Note: The number of chemicals is the same fo
as for the corresponding complete datase
Abbreviations: MaxD, maximum dose adminis
Toxicology Program; TD, tumor dose.
[a] Datasets A, B, C, and D defined by statistic
[b] Probability of falsely rejecting H_0:($s_s^2 = s_s^2$)
[c] Probability of falsely rejecting H_0:($s_s^2 = s_s^2$)

gens is significant at the 90% confidence
larger variance than nonmutagens, and t
dence) for three of the rat datasets (B, C,
TD_{50} is less tied to the MaxD than it is fo
mutagens are inducing neoplasms by mech
or if combined genotoxic and toxic mecha
ticipated, but the fact that it occurs to a la
data is puzzling. We do not understand th
rat mutagens with potencies that are low r
with higher relative potency to produce

Simulation of sample variance

Significant differences between san
of simulated and experimental pseudo si
correlation coefficients were found for
comparison of mutagens and nonmutag
correlation coefficient always accompanie
fact that this is not observed for the comp

Correlation Coefficients for the Linear
(s_m^2 and r_m) and Nonmutagens (s_{nm}^2

	Variances differ?[b]	Correlation coefficient differ?
49	No	Yes[c]
23	No	Yes[d]
16	No	Yes[d]
24	No	Yes[e]
25	No	No
35	No	Yes[d]
23	No	Yes[d]
06	No	Yes[d]

he as for the corresponding complete

TP, National Cancer Institute/National

e criteria as in Table 1.
case.

significant at the 95% confidence
ens sets A and C, the difference is
n observed correlation coefficients

dataset ($P < 0.01$) were the data
stributions: both slope and inter-
r the nonmutagens. Examination
rs when $1/MaxD > 10^{-2}$ (MaxD
ve a higher carcinogenic potency
hemicals with the lowest MaxDs,
drin, heptachlor, and aldrin), are
) mg/kg-day, there is no apparent

gently selected mouse dataset and
between mutagens and nonmuta-

Table 4. Comparison of Sample Variances and Observed Correlation Coefficients for the Linear Regression of Log(1/TD_{50}) Versus Log(1/MaxD) for Experimental (s_e^2 and r_e) and Simulated (s_s^2 and r_s) Pseudo Single-dose NCI/NTP Data.

Pseudo single-dose dataset (Simulated/Experimental)[a]		s_s^2/s_e^2	r_s/r_e	Variances differ?	Coefficient coefficients differ?
Mouse mutagens dataset	A	0.237/0.127	0.803/0.839	Yes[b]	No
	B	0.274/0.177	0.798/0.828	Yes[b]	No
	C	0.248/0.191	0.812/0.803	No	No
	D	0.242/0.200	0.848/0.865	No	No
Mouse nonmutagens dataset	A	0.245/0.130	0.901/0.949	Yes[b]	No
	B	0.244/0.182	0.908/0.923	No	No
	C	0.249/0.183	0.892/0.916	No	No
	D	0.249/0.187	0.902/0.924	No	No
Rat mutagens dataset	A	0.237/0.160	0.838/0.893	Yes[c]	No
	B	0.251/0.218	0.808/0.839	No	No
	C	0.251/0.225	0.818/0.822	No	No
	D	0.241/0.268	0.816/0.781	No	No
Rat nonmutagens dataset	A	0.208/0.108	0.899/0.925	Yes[c]	No
	B	0.215/0.142	0.915/0.935	No	No
	C	0.237/0.156	0.879/0.923	Yes[c]	No
	D	0.253/0.195	0.874/0.906	No	No

Note: The number of chemicals is the same for each pair of experimental and simulated datasets as for the corresponding complete dataset listed in Table 1.
Abbreviations: MaxD, maximum dose administered; NCI/NTP, National Cancer Institute/National Toxicology Program; TD, tumor dose.
[a] Datasets A, B, C, and D defined by statistical-significance criteria as in Table 1.
[b] Probability of falsely rejecting H_0:($s_s^2 = s_s^2$) is <5%.
[c] Probability of falsely rejecting H_0:($s_s^2 = s_s^2$) is <10%.

gens is significant at the 90% confidence level or better. The mutagens demonstrate a larger variance than nonmutagens, and this difference is more significant (95% confidence) for three of the rat datasets (B, C, and D). This suggests that for mutagens, the TD_{50} is less tied to the MaxD than it is for nonmutagens, which would follow if some mutagens are inducing neoplasms by mechanisms other than those mediated by toxicity, or if combined genotoxic and toxic mechanisms are prevalent. This would not be unanticipated, but the fact that it occurs to a larger extent for the less stringently selected rat data is puzzling. We do not understand this phenomenon, but perhaps it suggests that rat mutagens with potencies that are low relative to the MTD are more likely than those with higher relative potency to produce tumors by means of genotoxic mechanisms.

Simulation of sample variance

Significant differences between sample variances were found for the comparison of simulated and experimental pseudo single-dose data. No such differences between correlation coefficients were found for this comparison. Recall, however, that for comparison of mutagens and nonmutagens in the complete datasets, a difference in correlation coefficient always accompanies a difference in sample variance (Table 2); the fact that this is not observed for the comparison of simulated and experimental pseudo

single-dose data is therefore disturbing. It is possible that the differences in sample variance might be a spurious result of the absence of selection criteria in the simulation. Unfortunately, this finding sheds no light on the more interesting question of whether simulation of the complete experiments would reveal a similar lack of difference in sample variances. Based on the sample-variance differences between mutagens and nonmutagens in the complete sets, we suggest that simulation of the complete data would show that the simulated sample variance is larger than the experimental sample variance, at least for nonmutagens with TD_{50} values significant at $P < 0.01$.

The pseudo single-dose model described here, which is equivalent to that analyzed by Bernstein et al. (2), does not approximate the actual distribution of $1/TD_{50}$ versus 1/MaxD closely enough to be useful for examining artifacts in the apparent correlation of these two variables. Both simulated and actual pseudo single-dose experiments fail to account for the significantly different sample variances for mutagens and nonmutagens that arise when the complete experiments are considered. This may be because differences in tumor response between mutagens and nonmutagens appear in the sub-MaxD dose groups more often than in the MaxD dose group. For both mutagens and nonmutagens, at the MaxD the tumor response might be converging toward the same dependence on toxicity.

Conclusions

In the linear regression of $1/TD_{50}$ on 1/MaxD, the sample variance for mutagens is slightly or in some cases significantly elevated relative to nonmutagens. The fact that there exists a significant difference depending on mutagenicity, which is an unrelated variable, suggests that at least a portion of the correlation is nonspurious. Our work provides evidence that the Bernstein et al. pseudo single-dose simulation (2) is not detailed enough for describing the actual relationship between TD_{50} and MaxD; we are engaged, therefore, in a more complete simulation using Monte Carlo methodology (Shlyakhter, Goodman, and Wilson, unpublished data). However, we have not ruled out the possibility, especially for mutagens, that there is little more (or no more) quantitative information to be gained from the relationship between carcinogenic potency and MTD than is already contained in (a) the statistical significance level at which the potency is chosen, and (b) the fact that chemicals producing a 100% level of tumors at the MTD are rare. In this we concur with much of what Bernstein et al. (2) and Rieth and Starr (22) have previously concluded. The carcinogenic potency is more strongly associated with the MTD for nonmutagens than for mutagens. But differences between sample variances for mutagens and nonmutagens are small, and probably not very useful for predictive purposes, overall. Our findings are consistent with the premise that, even for most mutagens, at high doses carcinogenicity is associated mechanistically with toxicity.

The implications of our findings are far from obvious. Although often assumed, it is by no means certain that most mutagens and other genotoxic agents induce cancer in humans by means of genotoxic mechanisms. Most epidemiologic evidence for chemical carcinogenesis in humans comes from industrial or medical exposures in which the dose levels were high, approaching the MTD in many cases. Thus, toxicity could have been a real factor in these cases as well. The best-studied agent known to cause human cancer is tobacco smoke, which
respiratory system at all levels of usage. It
is high for the duration of inhalation, reg
smoked per day. For this reason, toxic effe
or even as the main cause of smoking-
tobacco smoke contains potent mutagen
that toxic effects are as important or mo
in the production of tumors in the rodent
associated human cancer as well. We the
carcinogens into the categories "primary"
would seem, for the present, an unsuital

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Data sets, including chemical na
request.

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human cancer is tobacco smoke, which produces acute toxic effects in the lungs and respiratory system at all levels of usage. It may be argued that the target-tissue dose level is high for the duration of inhalation, regardless of how few or how many cigarettes are smoked per day. For this reason, toxic effects cannot be ruled out as a contributing cause or even as the main cause of smoking-related carcinogenesis, despite the fact that tobacco smoke contains potent mutagens. Our results are in line with the suggestion that toxic effects are as important or more important than mutagenic events not only in the production of tumors in the rodent bioassay, but in the etiology of environmentally associated human cancer as well. We therefore agree with Benigni (25) that division of carcinogens into the categories "primary" (genotoxic) and "secondary" (nongenotoxic) would seem, for the present, an unsuitable basis for risk assessment.

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Notes

Data sets, including chemical names, are available from G. Goodman upon request.

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Chemicals, Cell Proli
and Multista

Henry C. Pitot, Yvonne P. Dra
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The rate, duration, and charact
critical factors in the development of nec
during the process of carcinogenesis m
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nisms that do not structurally alter the g
be reconciled with the multistage natu
possible relationship of cell proliferatio
hepatocarcinogenesis in the rat.

Cell proliferation in multistage hepatoca

Although this discussion is co
multistage hepatocarcinogenesis, the co
multistage carcinogenesis models such a
embryo cells (2,3), rat kidney (4–6), an

Initiation. The characteristics c
carcinogenesis have been previously de
restrictive definition of this first stage i
chemical, physical, or biologic agent irr
reaction gives a cell the potential to dev
cells during the stages of promotion or

This definition is based on the
analogous to a genomic DNA mutation.
correlated with mutagenesis (11–13),
members exhibit mutagenicity under
correlation (14). Furthermore, the nece
to occur in the presence of the initiatin
analogous to mutation fixation in micro
the presence of the carcinogenic agen
carcinogenesis and in other systems (19,
importance of the time point in the cell
it relates to the chemical's metabolism t
adduct. While cell division during initiat
ization that tissues with relatively high