

Carcinogenic Risk Assessment: Comparison of Estimated Safe Doses for Rats and Mice

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Data from the National Cancer Institute/National Toxicology Program (NCI/NTP) carcinogenesis bioassays were examined to compare cancer risks in rats and mice. Only those bioassays where chemicals were administered orally were used. The ratios for rats to mice of the virtually safe dose (VSD) levels associated with a risk of 10^{-6} were compared. Comparisons of the ratios were made for those chemicals that NCI/NTP determined to be carcinogenic in at least one species and that showed a dose response trend in the same sex at the same tissue/organ site in the other species. In all, 69 comparisons from 38 carcinogens were performed. The overall geometric mean of the VSD ratios is 1.27 in terms of concentration (ppm); the mean and the standard deviation in logarithm are 0.24 and 1.83, respectively. The VSD ratios vary from 1:51 to 49:1. Without the restriction of the same sex and site, the geometric mean of the minimum VSDs is 1.38, and the standard deviation in logarithm is 1.79. By directly comparing the VSDs for rats and mice (as they are performed for risk assessment), this study showed a probability of 0.10 that the ratio of VSDs is greater than 10, and the ratio is greater than 20 with a probability of 0.05 when a chemical is carcinogenic in both species.

Introduction

From a scientific standpoint, it would be desirable to measure the carcinogenic effects of chemicals in human populations in order to obtain information for regulatory decisions. Because of the lack of human data for many chemicals, experiments in laboratory animals must generally be used to quantify risk as a function of dose. The question often arises as to the relevancy of the results from animal experiments for predicting cancer risks in humans. Several studies of interspecies comparisons of carcinogenic potencies have been conducted (1-5). Those studies, in general, showed interspecies correlations between the carcinogenic potencies in three rodent species: rats, mice, and hamsters.

For regulatory decision purposes, we are most interested in the comparisons of doses between species at the low response rates. Regulatory agencies are frequently using risk assessment approaches to establish safe levels of exposure of chemicals at a given level of low risk, e.g., 10^{-6} . The purpose of this paper is to compare the virtually safe dose levels of carcinogens in rats and mice from data obtained by the NCI/NTP bioassays. These comparisons provide an indication of the size of the uncertainty factor required to allow for difference in cancer risks between rats and mice.

Method

The data collected by the NCI/NTP carcinogenesis screening program is available to compare cancer rates in rats and mice. Only bioassays in which chemicals were administered orally were considered in this paper. These bioassays generally employed 50 animals each at the maximum tolerated dose (MTD), 1/2 MTD, and 0 dose controls in both sexes of rats and mice. Osborne-Mendel, Sprague-Dawley, or Fischer 344 rats were used. B6C3F1 hybrid mice were used in all cases.

From a risk assessment viewpoint, we are interested in quantifying the extra risk of exposure to a chemical at a certain dose level. Let $P(d)$ represent the risk (probability) that an animal will develop a tumor at dose d . The quantity $P(d) - P(0)$ is the extra risk of getting a tumor (over background) due to the added dose d . The tumor rate at dose d is defined here as the extra probability of getting a tumor given dose d .

The tumor rates which are of concern in risk assessment are usually at the 1% level or below. These rates generally correspond to dose levels lower than the experimental dose levels. Thus, estimates of the 1% level and below generally require extrapolation from the experimental data and depend on the mathematical model chosen. The generalized multistage dose response model of Crump et al. (6) and the linear extrapolation method for low dose assessment as pro-

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posed by Gaylor and Kodell (7) were used here to estimate a lower limit of the safe dose level for a risk of 10^{-6} . The estimated virtually safe dose (VSD) is given by

$$\text{VSD} = \frac{d_e}{u(d_e)} \times 10^{-6}$$

where $u(d_e)$, obtained by fitting the Crump multistage model, is the upper confidence limit at the lowest experimental dose d_e or the dose estimated to produce a 1% tumor incidence; whichever dose is larger.

The ratios of the VSDs in rats to VSDs in mice provide a measure of the relative potency of carcinogens. Crouch and Wilson (8) proposed using a lognormal model for the interspecies sensitivity ratio. Assume that the ratios of VSDs are lognormally distributed and represent a sample of all chemicals in use. The mean and standard deviation of the logarithms of the VSD ratios were computed to compare with the results of Crouch (2) and Gaylor and Chen (5), in which different measures for the relative potency of carcinogens were used. The mean and the standard deviation provide measures of an average and variability for the overall agreement between rats and mice. A study of the variations of individual VSD ratios, which are related to the particular dose response model and the low dose assessment procedure used in the computations, are beyond the context of this paper.

The geometric mean, which is the antilogarithm of the mean of the logarithms of the VSD ratios, is used for comparing the overall agreement between the two species. If the logarithms of the VSD ratios have a normal distribution with mean $\log u$, then the geometric mean is the maximum likelihood estimator of the median of the lognormal distribution, u .

Two hundred fifteen NCI/NTP chemical bioassays were examined. Comparisons of the VSD ratios were only made for those chemicals determined to be carcinogenic by the NCI/NTP in at least one species and that showed a dose response trend in the same sex at the same tissue/organ site in the other species. Thus, our investigation does not cover those cases where a chemical is carcinogenic in only one species, or both species, but not at the same site in the same sex. We assumed imposing the restriction that the tumors must be in the same tissue/organ site in the same sex would provide a good predictability when a chemical was potentially carcinogenic in both species and when the same chemical was tested with similar protocols in the different species.

Results

The ratios of the VSDs are given in Table 1. In all, 69 comparisons were made for 38 chemicals. Thirty-three comparisons are for females and 36 are for males. Six comparisons are for Sprague-Dawley rats, 11 comparisons are for Osborne-Mendel rats, and 52 comparisons are for Fischer 344 rats. Thirty-one comparisons are for liver tumors, and the remaining 38 are at a

variety of tissue sites. A summary of the results by sex, by rat strain, and by liver versus nonliver tissue site is given in Table 2.

The overall geometric mean of the VSD ratios is 1.27 in terms of concentration (ppm); this means that on the average, the VSD for the rat is 1.27 times higher than the VSD for the mouse. The VSD ratios vary from 1:51 to 49:1. The standard deviation of the logarithm of the ratio is about 1.83. The correlation between the logarithms of VSDs for rats and mice is 0.69, which is statistically significant.

The geometric mean ratio is higher for females (1.39) than for males (1.17). The standard deviation is higher for females (1.91) than for males (1.78). In 46 out of 69 comparisons, data in the same tissue/organ site were available for both males and females. Comparing the VSD ratios between males and females can provide an indication of the interaction effect of sex and species. The agreement between males and females is good; that is, the species difference between rats and mice is consistent whether males or females are evaluated. For 2 of 23 comparisons, or 9%, the ratios of the VSD ratio in males to the VSD ratio in females differ by more than a factor of 10. The chemicals in which this pattern was observed are phenoxybenzamine HCl (1:14.1), and HCDD (1:13.3).

The geometric means for Sprague-Dawley, Osborne-Mendel, and Fischer 344 rat strains are 1.78, 1.68, and 1.15, respectively. The geometric mean for rats to mice is higher for liver tumor (3.03) than for tumors at the other sites (0.63).

The agreement of VSDs between rats and mice was good, on the average. However, for individual compounds the agreement was not always good, even though carcinogenicity was observed in the same sex at the same tissue/organ site for rats and mice. For 16 of the 69 comparisons (23%), the VSD ratios differ by more than a factor of 10. A stem-and-leaf plot (9) of the VSD ratios is shown in Figure 1. The largest discrepancies (factors of 1/50, 1/35, 1/27, 1/22) for which the rats appear more sensitive than the mice are produced by the three compounds: *o*-Anisidine HCl, Tris, and Cupferon. The largest discrepancies (factors of 49, 37, 30) for which the mice appear more sensitive than the rats all occur in females and are produced by amino ethylcarbazole HCl, 2,4,5-trimethylamine, and 1,1,1,2-tetrachloroethane.

The ratios of VSDs differed by a factor of 1.27 on the average; without a restriction to the same sex and site, agreement of VSDs between rats and mice was similar. The minimum VSDs from the mixtures of sites and sexes in each species were computed for the 38 chemicals. The geometric mean of the ratios of the minimum VSDs was 1.37 and the standard deviation in logarithm was 1.79. The ratios of the VSDs varied from 1:47 to 26:1.

Discussion

The VSD levels were computed for a risk level of 10^{-6} by the linear extrapolation method. At the low risk

Table 1. Estimates of the ratios of VSDs in rats and mice.

Chemical	Site	Rate strain ^a	Sex	VSD ratio, rats:mice
Thio-tepa	Skin	SD	M	1.4
Thio-tepa	Lymph	SD	M	1.9
Thio-tepa	Lymph	SD	F	15.1
Phenesterin	Hematopoietic	SD	M	3.8
Phenoxybenzamine HCl	Peritoneum	SD	M	1/8.3
Phenoxybenzamine HCl	Peritoneum	SD	F	1.7
1,2-Dibromoethane	Forestomach	OM	M	1/2.4
1,2-Dibromoethane	Forestomach	OM	F	1/2.0
1,2-Dichloroethane	Subcutaneous	OM	M	1/1.9
1,2-Dichloroethane	Mammary	OM	F	3.1
1,4-Dioxane	Liver	OM	F	2.1
Sulfallate	Mammary	OM	F	1/1.1
HCDD	Liver	OM	M	14.8
HCDD	Liver	OM	F	1.1
TCDD	Liver	OM	M	16.1
TCDD	Liver	OM	F	2.1
TCDD	Thyroid	OM	F	1/1.1
NTA	Kidney	F344	M	6.7
Thiodianiline	Thyroid	F344	M	1/4.5
Thiodianiline	Thyroid	F344	F	1/12.7
TRIS	Kidney	F344	M	1/35.1
Diaminoanisole SO ₄	Thyroid	F344	M	1/2.8
Diaminoanisole SO ₄	Thyroid	F344	F	1/2.5
<i>o</i> -Anisidine HCl	Bladder	F344	M	1/50.3
<i>o</i> -Anisidine HCl	Bladder	F344	F	1/26.8
Hydrazobenzene	Liver	F344	F	3.4
Amino ethylcarbazole HCl	Liver	F344	M	9.4
Amino ethylcarbazole HCl	Liver	F344	F	48.9
(Chloromethyl) pyridine HCl	Bladder	F344	M	6.6
Amino ethoxyacetanilide	Thyroid	F344	M	1.3
Amino ethoxyacetanilide	Thyroid	F344	F	1/1.1
Dimethoxybenzidine diisocyanate	Hematopoietic	F344	M	1/3.7
Dimethoxybenzidine diisocyanate	Hematopoietic	F344	F	1/1.6
1,5-Naphthalenediamine	Thyroid	F344	M	2.5
1,5-Naphthalenediamine	Thyroid	F344	F	1.6
1,5-Naphthalenediamine	Lung	F344	M	1.4
2-Aminoanthraquinone	Liver	F344	M	2.2
2,4,5-Trimethylaniline	Liver	F344	M	15.2
2,4,5-Trimethylaniline	Liver	F344	F	37.4
2,4,5-Trimethylaniline	Lung	F344	F	5.0
Michler's ketone	Liver	F344	F	1/8.1
<i>p</i> -Chloroaniline	Spleen	F344	M	1/2.3
Fluometuron	Liver	F344	M	1.1
Cinnamyl anthranilate	Liver	F344	M	9.5
4,4'-Oxydianiline	Liver	F344	M	1/2.1
4,4'-Oxydianiline	Liver	F344	F	2.6
4,4'-Oxydianiline	Thyroid	F344	M	1/8.0
4,4'-Oxydianiline	Thyroid	F344	F	1/12.7
Di(ethylhexyl) phthalate	Liver	F344	M	7.7
Di(ethylhexyl) phthalate	Liver	F344	F	4.8
Dichloro- <i>p</i> -phenylenediamine	Liver	F344	M	2.9
Dichloro- <i>p</i> -phenylenediamine	Liver	F344	F	4.3
C.I. Disperse Yellow 3	Liver	F344	F	12.4
D&C Red No. 9	Liver	F344	M	1.5
C.I. Solvent Yellow 14	Liver	F344	M	1/4.9
C.I. Solvent Yellow 14	Liver	F344	F	1/2.3
1,1,1,2-Tetrachloroethane	Liver	F344	M	7.4
1,1,1,2-Tetrachloroethane	Liver	F344	F	30.0
Dimethylenedianiline DiHCl	Thyroid	F344	M	1/1.0
Dimethylenedianiline DiHCl	Thyroid	F344	F	1/2.4
Dimethylenedianiline DiHCl	Liver	F344	M	1.4
Dimethylenedianiline DiHCl	Liver	F344	F	3.2
<i>p</i> -Nitrosodiphenylamine	Liver	F344	M	1/1.4
Cupferron	Heart	F344	M	1/14.5
Cupferron	Heart	F344	F	1/11.8
Cupferron	Liver	F344	F	1/22.1
<i>p</i> -Cresidine	Bladder	F344	M	7.64
<i>p</i> -Cresidine	Bladder	F344	F	2.22
2,4-Diaminotoluene	Liver	F344	F	9.65

^aSD = Sprague-Dawley; OM = Osborne-Mendel; F344 = Fischer 344.

Table 2. Summary of the ratios of VSD in rats to VSD in mice.

Comparison groups	Number of comparisons	Geometric mean	Log mean	Log SD	Ratios	
					Minimum	Maximum
Males	36	1.17	0.16	1.78	1:50.3	16.1:1
Females	33	1.39	0.33	1.91	1:26.8	48.9:1
Sprague-Dawley	6	1.78	0.58	1.58	1:8.3	15.1:1
Osborne-Mendel	11	1.68	0.52	1.27	1:2.4	16.1:1
Fischer 344	52	1.15	0.14	1.96	1:50.3	48.9:1
Liver	31	3.03	1.11	1.67	1:22.1	48.9:1
Nonliver	38	0.63	-0.46	1.66	1:50.3	15.1:1
Total	69	1.27	0.24	1.83	1:50.3	48.9:1

Stem	Leaf	#
4	9	1
4		
3	7	1
3	0	1
2		
2		
1	5556	4
1	002	3
0	55777889	8
0	1111122222223333344	22
-0 ^a	111112222222334	16
-0	55888	5
-1	332	3
-1	5	1
-2	2	1
-2	7	1
-3		
-3	5	1
-4		
-4		
-5	0	1
		69

^a(-) represents a reciprocal ratio, e.g., -27 = 1/27.

Figure 1. Stem-and leaf plot of the VSD ratios.

levels (1% or below), the VSD was approximately proportional to the corresponding risk level. For example, the VSD for the risk of 10^{-5} was approximately a factor of 10 higher than the VSD for a risk of 10^{-6} . Thus, the ratio of the VSD for rats to mice generally was not affected by the choice of the level of risk.

Ninety-three of 215 chemicals were found to have no carcinogenic response in either species. These results indicated that the two species were compatible in their response to the chemicals. Thirty-eight chemicals in which a carcinogenic response was found in both species at the same site in the same sex were compared in this investigation. Our study does not cover the remaining 84 chemicals, 39 of which were negative in rats and positive in mice, 26 of which were positive in rats and negative in mice; and 19 of which were positive in both species but not at the same site. When a chemical is positive in one species and negative in the other, it cannot usually be decided on the basis of bioassay results alone as to whether such results represent true discrepancies between the two species.

The disagreement of VSDs between rats and mice in

the extreme cases might be related to the choice of dose response functions. For example, in the case of *o*-anisidine HCl, the observed responses for urinary bladder tumors in the male rat were 0/51, 50/54, and 51/52 for the control, low dose, and high dose, respectively. The dose-response curve is not well defined in this instance and may lead to imprecise VSD estimates. Specifically, if the dose-response curve is sublinear below the experimental dose region, then the VSD will overestimate the risk. If the dose-response curve is supralinear below the experimental dose region, the VSD will underestimate the risk. The multistage model has been used by the EPA and FDA for cancer risk assessment with different approaches for low-dose extrapolation. When the predicted excess risk at the lowest experimental dose level is 1% or greater, the VSD estimates calculated either by the linear extrapolation procedure of Gaylor and Kodell (7) or by the linearized multistage model of Crump et al. (6) differ little. For *o*-anisidine HCl, the two extrapolation procedures have the same VSD estimate, 0.0016.

Zeise et al. (10) noted a correlation between carcinogenic potency and the LD₅₀ (dose that produces 50% mortality). Bernstein et al. (4) suggested that the correlation between the maximum tolerated dose (MTD) and doses producing tumors may be fortuitous because chronic bioassays are generally conducted in a relatively narrow dose range below the MTD. Since the same dose levels were often used for rats and mice for any given chemical, differences in the VSDs for rats and mice are somewhat restrained. When tumors are increased by a chemical, these increases will tend to occur at nearly the same dose levels, resulting in similar VSDs.

A purpose of carcinogenesis bioassays is to assist extrapolation from animal to human risks. The VSD is chosen here for the comparison between rats and mice, as it has been used in risk assessment for predicting human cancers. Crouch (2) compared the estimated slope of the one-hit model between rats and mice for 66 compounds from the NCI/NTP bioassays. Additionally, Peto et al. (11) proposed using the TD₅₀ as a measure for the carcinogenic potency; Gaylor and Chen (5) compared the minimum TD₅₀ for 190 compounds administered in the diet from the database compiled by Gold et al. (3). The mean and the standard deviation of the logarithm (to base e) reported by Crouch (2) were 0.39

and 1.52, respectively; the mean and the standard deviation reported by Gaylor and Chen (5) were 0.24 and 1.90, respectively. In this study, the mean and standard deviation were 0.24 and 1.83, respectively. The comparisons of VSD ratios from the three different measurements are in fair agreement. Moreover, the restriction to the same tissue/organ does not appear to be much different from other investigations. In our study, the means and the standard deviations are essentially the same with or without the restriction.

The VSD ratio is assumed to have a lognormal distribution because it has a positive skew; that is, the values of VSD ratios are spread over a wide range (0 to 49), but most values are between 0 and 10. An analysis indicated that the lognormal model seemed appropriate for the distribution of VSD ratios. Assume that the logarithms of VSD ratios have a normal distribution with standard deviation s . If f denotes the factor for the interspecies extrapolation, then the probability that the VSD ratio of a typical chemical will fall within the factor f is $P = \Phi(a) - \Phi(-a)$, where $a = (\log f)/s$ and Φ is the standard normal distribution function. In this study, the estimated value of s is 1.83; thus, if $f = 10$, a factor of 10, then $P = 0.79$. This value is consistent with the result shown in Figure 1, where about 77% of the VSDs differ by less than a factor of 10.

Finally, we must consider the extrapolation from rodents to humans. Assume that the variability of the sensitive ratios of rats and mice shown in this study could represent the true variations between the rodents and humans. If the result of a rodent bioassay of a chemical showed a positive carcinogenic effect with estimated VSD, v , then the probability of the VSD for humans was greater than v/f was $P = \Phi(a)$ (P was about 0.90 for $f = 10$ and P was 0.95 for $f = 20$), provided that the chemical was carcinogenic for humans; but the approach would be conservative when the chemical was

not carcinogenic for humans. In either case, there was a justification for quantitative estimates of human cancer risk from animal studies. However, if the chemical was not carcinogenic for rodents, but it was carcinogenic for humans, no estimate of the VSD would be made and a cancer risk in humans would result.

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