Risk Analysis, Vol. 12, No. 4, 1992

Extrapolation of Carcinogenicity Between Species: Qualitative and Quantitative Factors

Lois Swirsky Gold,[1,2,3] Neela B. Manley[2], and Bruce N. Ames[2]

Received April 14, 1992

Prediction of human cancer risk from the results of rodent bioassays requires two types of extrapolation: a qualitative extrapolation from short-lived rodent species to long-lived humans, and a quantitative extrapolation from near-toxic doses in the bioassay to low-level human exposures. Experimental evidence on the accuracy of prediction between closely related species tested under similar experimental conditions (rats, mice, and hamsters) indicates that: (1) if a chemical is positive in one species, it will be positive in the second species about 75% of the time; however, since about 50% of test chemicals are positive in each species, by chance alone one would expect a predictive value between species of about 50%. (2) If a chemical induces tumors in a particular target organ in one species, it will induce tumors in the same organ in the second species about 50% of the time. Similar predictive values are obtained in an analysis of prediction from humans to rats or from humans to mice for known human carcinogens. Limitations of bioassay data for use in quantitative extrapolation are discussed, including constraints on both estimates of carcinogenic potency and of the dose–response in experiments with only two doses and a control. Quantitative extrapolation should be based on an understanding of mechanisms of carcinogenesis, particularly mitogenic effects that are present at high and not low doses.

KEY WORDS: Interspecies prediction; human carcinogen; target site; cancer risk assessment; mitogenesis; HERP.

1. INTRODUCTION

Current strategies to prevent human cancer use chronic rodent bioassays as the major source of information to predict the risk to humans from chemical exposures. Two types of extrapolation are required in such an undertaking: (1) A qualitative extrapolation is necessary between a short-lived species such as rats or mice to humans, a long-lived species. (2) A quantitative extrapolation is necessary from the maximum tolerated dose (MTD) administered in bioassays to human exposure levels that are often hundreds of thousands of times lower. This paper reviews results on these two types of extrapolation, based primarily on analyses of our comprehensive, standardized database of chronic, long-term bioassays, the Carcinogenic Potency Database (CPDB).[1–5]

Results from rodent bioassays are routinely used to predict qualitatively whether a chemical is a potential human carcinogen. Ideally, one would like to know the accuracy of prediction from rats or mice to humans, but

[1] Life Sciences Division, Lawrence Berkeley Laboratory, 1 Cyclotron Road, Berkeley, California 94720.
[2] Division of Biochemistry and Molecular Biology, Barker Hall, University of California, Berkeley, California 94720.
[3] To whom all correspondence should be addressed.

0272-4332/92/1200-0579$06.50/1

because epidemiologic data are usually lacking and experiments cannot be conducted in humans, this knowledge is not available. We examine below the accuracy of prediction between the two closely related species, rats and mice, and between each of these two species and hamsters. This comparison reflects results obtained under similar experimental conditions, including administration of the MTD and laboratory diets fed *ad libitum*. Thus, qualitative prediction from one rodent species to another (i.e., prediction of positivity and prediction of target organ) can be examined without simultaneously having to address the issue of high- to low-dose extrapolation.[6,7] One would expect that the qualitative prediction of positivity and target organ from rats to mice would likely be much better than prediction from rats or mice to humans. The quantitative prediction from high dose in rodents to low dose in humans is much more uncertain. We also examine the bioassay results in rats and mice from the CPDB for the known human carcinogens, thus assessing the accuracy of prediction from humans to rats or humans to mice.

On quantitative extrapolation, we discuss reasons why standard rodent bioassays at high dose, as currently conducted, do not provide sufficient information to assess carcinogenic risk to humans at low dose. Such extrapolation should be based on knowledge of mechanisms of carcinogenesis, and should reflect the importance of mitogenesis. Cell division converts DNA lesions to mutations and thus, from first principles, cell division rates of the tumor precursor cells must be important. We have postulated and discussed the evidence that chronic administration of chemicals at the MTD increases chronic mitogenesis in cells that are not discarded, which in turn increases rates of mutagenesis and carcinogenesis.[8–10] Therefore, at the low doses of most human exposures where cell killing does not occur, the hazards to humans of rodent carcinogens may often be much lower than has commonly been assumed.

Table I. Prediction of Carcinogenicity and Target Site Between Rats and Mice Among Chemicals Tested in Both Species and Positive in at Least One

	Rat Carcinogens	Mouse Carcinogens
Positive in other species at same target site	108 (49%)	108 (47%)
Positive in other species but not at same target site	57 (25%)	57 (25%)
Negative in other species	59 (26%)	64 (28%)
Total	224 (100%)	229 (100%)

2. QUALITATIVE EXTRAPOLATION BETWEEN SPECIES

2.1. Prediction of Positivity Between Rodent Species

How well can one predict carcinogenicity from rats to mice, or from mice to rats? Table I reports results in the CPDB on prediction between rats and mice for the 479 chemicals that have been tested in both species.[4] If a chemical is positive in either rats or mice, it is positive in the other species about 75% of the time. This proportion is similar to results reported earlier for smaller numbers of chemicals.[6,7,11–13] However, we have also found that in each species about half the test chemicals are positive (224/479 in rats, 229/479 in mice); therefore, by chance alone one would expect a positive predictive value between species of about 50%.[6,7] Thus, the overall predictive values of 75% between rats and mice are significantly better than would be expected by chance, but provide only moderate confidence in interspecies extrapolation. Results for the limited number of compounds in the CPDB that have been tested in hamsters and rats, or hamsters and mice, indicate that prediction from rats to hamsters or from mice to hamsters (about 65%) is similar to, but slightly less accurate than, prediction between rats and mice.

In earlier work, we identified three factors that influence the accuracy of prediction of carcinogenicity between rats and mice. Predictive values are more accurate

[4] Our analyses are based on this database, which reports only results of chronic, long-term bioassays that are adequate to detect a carcinogenic effect or lack of effect and to estimate potency. More than 4400 experiments met the inclusion criteria of the database, but thousands of others did not [e.g., tests that lack a control group, that are too short or include too few animals to detect an effect, that use routes of administration not likely to result in whole body exposure (like skin painting or subcutaneous administration) or a dosing schedule which is not chronic, cocarcinogenesis studies, and bioassays of particulate or fibrous matters].

One-third of the chemicals in the database have been tested by the National Cancer Institute/National Toxicology Program, using standard protocols with tests in two species at the MTD. About half of the chemicals in the database, however, have been tested in only one species.

In this analysis, we classify the results of an experiment as either positive or negative on the basis of the author's opinion in the published paper and classify a chemical as positive if it has been evaluated as positive by the author of at least one experiment. We use the author's opinion to determine positivity because it often takes into account more information than statistical significance alone, such as historical control rates for particular sites, survival and latency, and/or dose–response. Generally, this designation by author's opinion corresponds well with the results of statistical reanalysis of the significance of the dose–response effect.[6]

for mutagens than nonmutagens; for chemicals that are toxic at lower doses compared to higher doses (as measured by the MTD); and for some chemical classes compared to others.[6] To compare the accuracy of prediction based on various target organs, we have examined the predictive value of individual sites in one species for positivity (at *any* site) in the second species.[6,7] Most sites are good predictors of carcinogenicity at *some* site in the other species. The least accurate predictors are the urinary bladder in the rat and the liver in the mouse.

2.2. Prediction of Target Site Between Rodent Species

If a chemical is positive in one species, how often will it be positive in the other species and at the *same* target site? Since many chemicals induce tumors at multiple sites, there is often more than one target site that is potentially a site in common between the two species, thus increasing the chance that there will be *some* target site in common. Site-specific prediction between rats and mice (Table I) is less accurate than overall prediction of positivity. Knowing that a chemical is positive at a site in one species gives about a 50% chance that it will be positive at the *same* site in the other species. Among the 108 chemicals that have a site in common between rats and mice (Table I), the liver is the only site in common for 47. Site-specific prediction from rats or mice to hamsters is similar to that between rats and mice.

2.3. Human Carcinogens

Ultimately, one wants to know whether chemicals that have been shown to be carcinogenic in experimental animals are also carcinogenic in humans. This question cannot be answered by reversing the question (i.e., by asking whether chemicals that are human carcinogens are also carcinogenic in a rodent species) because even if most human carcinogens are rodent carcinogens, the converse does not necessarily follow, as can be demonstrated by a simple probabilistic argument.[14] Nevertheless, some additional evidence about interspecies extrapolation can be obtained by asking how good a model the human is for the rat or the mouse, even though this will not provide direct evidence about how good a model the rat or mouse is for the human. The evaluations of the International Agency for Research in Cancer (IARC) list 55 known human carcinogens including industrial processes, therapeutic combinations, single chemicals, and mixtures such as tobacco smoke. For 35 of these human carcinogens, data in experimental animals have been evaluated by IARC.[15–18]

The CPDB includes only results of experiments on single chemicals that meet specified experimental-design criteria, including chronic, long-term administration of the chemical by routes that are expected to result in whole body exposure. Thus, some experiments that IARC has used in its evaluations are excluded. A search of the CPDB indicates that experiments are included for 18 human carcinogens, of which 17 have been tested in rats, and 16 in mice. Table II reports the CPDB results on positivity and target sites in rats and mice for these 18 human carcinogens, as well as the human target sites identified by IARC.

The overall predictive value in Table II from humans to rats is 76% (13/17) and from humans to mice is 75% (12/16). In the CPDB, *rat* experiments are all negative for four of the human carcinogens (arsenic/arsenic compounds, azathioprine, cyclosporin, and Myleran). IARC evaluates these four compounds as having limited (not sufficient) evidence of carcinogenicity in animals (Table II), and IARC does not report any evidence in rats for three of the four. For the fourth, azathioprine in rats, IARC reports only suspected target organs.[18]

Mouse experiments in the CPDB are all negative for four human carcinogens (aflatoxin, arsenic/arsenic compounds, cyclosporin, and nickel compounds). In comparison, IARC evaluations of carcinogenicity in animals for two of these four, arsenic and cyclosporin, are limited (not sufficient) evidence; for the other two, aflatoxin and nickel compounds, IARC evaluates the evidence as sufficient (Table II).[18] With respect to target organs in mice, IARC reports target organs for aflatoxin and cyclosporin, but only local target organs for nickel compounds and only suspected target organs for arsenic/arsenic compounds.[18]

Some rodent experiments on human carcinogens are used by IARC in their evaluations but are not included in the CPDB because of route of exposure (e.g., skin painting, subcutaneous injection, intratracheal instillation, implant), nonchronic dosing, cocarcinogenesis tests, use of infant animals, lack of controls, and screening assays on the accelerated induction of tumors.[16]

Table II also shows the accuracy of prediction to the *same* target site between humans and rats, and between humans and mice. There is a target site in common between humans and rats for 47% (8/17) of the human carcinogens, and a site in common between humans and mice for 37% (6/16). A variety of organs are represented among the sites in common. Table II thus indicates that the overall predictive values are similar to

Table II. Comparison of Positivity and Target Sites in Humans and Rats or Mice Among 18 Chemicals in the CPDB for Which IARC Found Sufficient Evidence of Carcinogenicity in Humans[a]

	IARC evaluation		CPDB Target Sites[c]	
Chemical	Animals[b]	Human target site	Rats	Mice
Alflatoxins	S	Liver	**Liver**, large intestine, kidney	Negative
Alcoholic beverages (Ethyl Alcohol)	I	Liver, oral cavity, pharynx, larynx, esophagus	**Liver**, adrenal gland, pancreas, pituitary gland	No test
4-Aminobiphenyl	S	Bladder	Mammary gland	**Bladder**, liver, angiosarcoma
Arsenic and arsenic compounds	L	Skin, lung	Negative	Negative
Azathioprine	L	Lymphoma, skin, mesenchy-tumors, hepatobiliary system	Negative	**Lymphoma**, uterus
Benzene	S	Leukemia	Zymbal's gland, nasal cavity, oral cavity, skin, stomach, angiosarcoma	Zymbal's gland, Harderian gland, lymphoma, lung, mammary gland, ovary, preputial gland
Benzidine	S	Bladder	Leukemia, liver, mammary gland	Harderian gland, liver, angioma
bis(Chloromethyl)ether and technical choro-methyl methyl ether	S	Lung	**Lung**, nasal cavity	**Lung**, peritoneum
1,4-Butanediol dimetha-nesulphonate (My-leran)	L	Leukemia	Negative	No test
Chlorambucil	S	Leukemia	**Leukemia**, Zymbal's gland, mammary gland, nervous system	Lymphosarcoma, lung
Cyclosporin	L	Lymphoma	Negative	Negative
Cyclosphamide	S	Bladder, leukemia	**Bladder, leukemia**	Lymphosarcoma, lung
Diethylstilbestrol	S	Cervix/vagina, breast, testis	Adrenal gland, pituitary gland	**Mammary gland**, thyroid gland
Melphalan	S	Leukemia	Peritoneum	Lung, lymphosarcoma
2-Napthylamine	S	Bladder	**Bladder**	Liver
Nickel compounds	S	Nasal sinus, lung	No test	Negative
Thiotepa	S	Leukemia	**Leukemia**, Zymbal's gland, skin	**Leukemia**, preputial gland, skin
Vinyl Chloride	S	Angiosarcoma (liver), liver, lung, brain, lymphatic and hematopoietic system	**Angiosarcoma[d], liver, lung, brain**	**Angiosarcoma, liver, lung**

[a] The IARC evaluation in animals and the human target sites are given for each chemical; target sites that IARC considers only "suspected" in humans are not included. For each rodent species, "no test" in the CPDB and only "negative" results in the CPDB are indicated. For positive chemicals in the CPDB, all target sites in each rodent species are reported, and the sites that match the human target site are given in boldface. Sites without boldface are targets in rats or mice but not in humans.

[b] S, sufficient; L, limited; I, inadequate.

[c] Rodent cancer tests are referenced in Refs. 1–5 and in the following: azathioprine (19); cyclosporin (20); diethylstilbestrol (21).

[d] In hamsters, vinyl chloride also induced angiosarcomas and tumors in the stomach, skin, and mammary gland. No other human carcinogens have tests in hamsters in the CPDB.

those reported above between rats and mice for the CPDB, and that the predictive value for target organ is slightly lower for mice.

We conclude, based on the experimental evidence from the CPDB involving prediction from rats to mice, from mice to rats, from rats or mice to hamsters, and from humans to rats and humans to mice, that one cannot assume that if a chemical induces tumors at a given site in one species it will also be positive and induce tumors at the same site in a second species; the likelihood is at most 49%.

3. QUANTITATIVE EXTRAPOLATION TO LOW DOSE FROM BIOASSAYS CONDUCTED AT HIGH DOSE

3.1. Limitations of Carcinogenesis Bioassay Data for Risk Estimation

Several recent analyses indicate that measures of carcinogenic potency estimated from standard rodent bioassays are restricted to a narrow range about the maximum dose tested for each chemical.[22–25] This narrow range contrasts with the 10 million-fold range in the test doses (MTDs) of different chemicals. In the CPDB, we use TD_{50} based on the one-hit model as the measure of potency (i.e., the tumorigenic dose rate for 50% of the animals at the end of a standard lifespan).[5,26,27] One reason for choosing the TD_{50} was that the concept is easily understood, particularly by analogy to the widely reported LD_{50}. Importantly, the TD_{50} is often within the range of doses tested; thus, the experimental results do not have to be extrapolated far to estimate TD_{50}.[25]

The statistical methods used to estimate TD_{50} do not matter greatly. There is substantial agreement between TD_{50} estimated by lifetable and summary analyses.[28] Additionally, among chemicals that are positive in more than one test in a species, the single most potent TD_{50} value from among all positive tests in the species is, with few exceptions, similar to other measures that average TD_{50} values (harmonic mean, geometric mean, or arithmetic mean).[29]

We showed several years ago that the potency (TD_{50}) calculated from bioassays as currently conducted, is constrained to be within a narrow range (~32-fold) about the maximum dose tested (in the absence of 100% tumors in all dosed animals).[22] Several papers that appeared later all confirmed this restriction.[30–32] Recently, Krewski showed that across chemicals, regardless of whether one uses the one-stage, multistage, or Weibull model to estimate TD_{50}, the correlation between the MTD and carcinogenic potency is greater than 0.9.[33] Thus, potency estimates are constrained to a limited range once one knows the MTD.

TD_{50} does not provide information about low-dose exposures. Thus, we have not attempted to say anything about the doses estimated to give tumors to one rat in a million. In contrast to TD_{50}, vastly different results would be obtained for such an undertaking, depending on what particular statistical model was fitted to extend the extrapolation to such a low level of exposure.[34-35] Whereas TD_{50} is close to the doses tested, an estimate of the dose to give tumors to a maximum of one animal in a million based on the linearized multistage model widely used for regulatory purposes, is, on average, 380,000 times below the high dose in the bioassay.[24] This enormous toxicological leap in the dark emphasizes the point that carcinogenesis bioassays were not designed to determine one-in-a-million risks.

A further limitation of bioassay data for quantitative extrapolation to low dose is the minimal information available about dose–response from an experiment with only two doses and a control. At the two high doses tested (MTD and 1/2 MTD), it is difficult to interpret the shape of the dose–response curve with three data points. A recent study tested for consistency of the dose–response with three different curves: linear, square-root, and quadratic.[36] Results of bioassays from the National Cancer Institute/National Toxicology Program (NCI/NTP) indicate that two thirds of the curves are consistent with all three models, and 83% are consistent with at least two models. An additional complication is the finding that the best fit curves for more than half the chemicals are not the same for different sex-species groups or different target organs within a single experiment. This variation in curves for the same chemical was also discussed earlier.[22,28]

The good correlation in carcinogenic potency between rats and mice at the high doses tested has been interpreted as a justification for quantitative extrapolation from rodents to humans. However, the MTDs of rats and mice for different chemicals are also very highly correlated, and as previously stated, they span a 10-million-fold range across chemicals; in contrast, the potency for a given chemical is constrained to a narrow range about the MTD.[22] These facts imply statistically that the potencies of chemicals that are positive in rats and mice will be highly correlated. Our recent analysis suggests that at least 80% of the correlation is tautological.[37] Thus, the study of potency correlations between rats and mice does not shed much light on the validity of quantitative prediction between species. The

biological basis for these correlations lies in part in the high correlation in the MTDs of the two species, and in part in the experimental finding that at the MTD and 1/2 MTD in experiments conducted using the standard bioassay design, it is uncommon to observe either a tumor incidence of 100% or a plateau in the dose–response curve. These latter results are consistent with the hypothesis that increased cell division, due to chronic administration of near-toxic doses, is an important factor in the carcinogenic response. The limitations of bioassay data for use in risk estimation underscore the importance of understanding mechanisms of carcinogenesis.

Several recent analyses have shown that quantitative risk assessments as currently conducted by regulatory agencies are also constrained to a narrow range about the MTD. Using data from the CPDB, Krewski and colleagues have shown that the unit risk factor Q_1^* derived from the linearized multistage model is restricted to a limited range about the MTD, that empirically the Q_1^* values for different chemicals are highly correlated with the MTD, and that linear extrapolation from the TD_{50} usually results in low-dose slope estimates that are similar to those based on the linearized multistage model.[25,33] Gaylor estimated the risk-specific dose (RSD) corresponding to a maximum risk of one cancer in a million based on the multistage model, and found that RSD averages 380,000 times below the MTD, and that 90% of the estimates are within a factor of 10 of that number.[24]

These are striking findings with broad implications for risk assessment: the dose usually estimated by regulatory agencies to give a maximum of one cancer in a million can be approximated merely by knowing the MTD, and a reasonable estimate of the Q_1^* can be made from the TD_{50} values in our published CPDB. Proposals based on these findings have been made to facilitate the regulatory process. While these proposals address the question of expediting regulation as it is currently done, they do not resolve the fundamental question of the vast biological uncertainties in extrapolating 380,000 times below the bioassay dose. Rather, they assume that the current methodology should be approximated.

Gaylor has proposed dividing the MTD of a given chemical by 400,000 to estimate the virtually safe dose; then, if the intended human exposure to a chemical is greater than the lowest virtually safe dose, the chemical cannot be accepted as safe. If the intended human exposure is below the lowest virtually safe dose, then conducting a bioassay may not be necessary because the predicted maximum risk will be below one in a million at the intended exposure level.[24] Rulis proposed a threshold of regulation for safety assessment of packaging materials based on the distribution of TD_{50} values in the CPDB.[38] This requires assuming that a substance is no more toxic than the most potent chemical carcinogen, and inferring a theoretical upper bound on potency below which risks would be trivial. The California Department of Health Services proposed that regulations for Proposition 65 be expedited by using the CPDB results; adjusted TD_{50} values could be used for chemicals that do not yet have a Q_1^* from either EPA or their agency. Zeise has shown that potency estimates derived from TD_{50} are reasonable estimates of potency values that have been proposed for Proposition 65.[39]

3.2. Ranking Possible Carcinogenic Hazards

Our approach has been to acknowledge the enormous limitations and uncertainties in quantitative risk assessment and to begin by ranking possible carcinogenic hazards to humans from typical exposures for a wide variety of chemicals.[40,41] This ranking can help to set priorities when selecting chemicals for chronic bioassay or mechanistic studies, for epidemiological research, and for regulatory policy. The current regulatory process needs to take into account several points that we have previously discussed in detail:[8–10,40–43] (1) An extrapolation from high to low doses should be based on an understanding of the mechanisms of carcinogenesis. (2) Testing at the MTD can frequently cause increased cell division (e.g. through chronic cell killing and consequent cell replacement), a risk factor for cancer that can be limited to high doses. Ignoring this mitogenesis effect can greatly exaggerate many low-dose risks. (3) About half of the chemicals tested at the MTD are positive, and about 40% of the positives are not mutagenic. This would be expected if mitogenesis is important in the carcinogenic response at the MTD. (4) About half of the *natural* chemicals tested chronically in rats and mice at the MTD are positive, and the natural world of chemicals makes up the vast proportion of chemicals that humans are exposed to. Thus, human exposures to rodent carcinogens (as defined by testing at the MTD) are likely to be common. (5) The toxicology of synthetic and natural toxins is not fundamentally different.

Together, these five points indicate that cancer-prevention strategies aimed at chemical carcinogens as potential causes of human cancer need to take a broad overview of chemical exposures, both natural and synthetic, to put possible hazards into perspective, and to focus on those exposures that rank highest in possible hazard. If there is an enormous natural background of "potential human carcinogens" as defined by rodent tests, then focusing regulatory attention on low-dose human

exposures to synthetic chemicals is not likely to reduce the human cancer burden significantly.

It is unlikely that the high proportion of carcinogens in rodent studies is due simply to selection of suspicious chemical structures: most chemicals were selected because of their use as industrial compounds, pesticides, drugs, or food additives. Moreover, historically the knowledge to predict carcinogenicity has been inadequate.[6] We have examined the proportion of chemicals positive in the CPDB for 10 different datasets, and in each case roughly half the chemicals are positive according to the published author's opinion in at least one test (Table III): all chemicals in the CPDB, NCI/NTP chemicals, NCI chemicals reported before 1979, literature other than NCI/NTP, chemicals tested in both rats and mice (and among these, natural chemicals only and synthetic chemicals only), natural pesticides, mold toxins, and 26 chemicals in roasted coffee.[6,8,9,29,40,43] Even if there is some selection bias, these results indicate that humans are likely to be living in a sea of rodent carcinogens as defined by testing at the MTD.

We have recently shown that even though only 57 of the 5000 or more naturally occurring plant pesticides in the diet have been tested, the 29 that are rodent carcinogens are present in many common foods and at concentrations that are commonly thousands of times higher than the concentrations of synthetic pesticide residues.[42] It is probable that almost every fruit and vegetable in the supermarket contains plant pesticides that are rodent carcinogens. A chemical pollutant should not be a high priority for concern with respect to carcinogenicity if, when ranked by the same methods as natural chemicals, its possible carcinogenic hazard appears to be far below that of many common food items.[40-42] That is not to say that these dietary exposures are necessarily of much relevance to human cancer, but rather that the background of exposures to natural rodent carcinogens may cast doubt on the relevance of far lower exposure levels to synthetic rodent carcinogens.

Our ranking of possible carcinogenic hazards is based on a simple measure, HERP (Human Exposure/Rodent Potency), which indicates what percentage of the TD_{50} (in mg/kg/day) a human gets from a daily lifetime exposure (in mg/kg/day) to a given chemical. We have also ranked possible carcinogenic hazards in the workplace based on the Permitted Exposure/Rodent Potency (PERP) index, using the OSHA Permitted Exposure Level (PEL) as a surrogate for estimates of exposure.[41] The HERP or PERP index uses the same animal results and similar statistical methods as the usual low-dose linear estimation of risk; however, our purpose is to *compare* possible carcinogenic hazards from a variety of naturally occurring and synthetic chemicals, not to perform risk assessments. As more theory is developed and more evidence is produced about the mechanisms of carcinogenesis, the ranking of hazards by the simple HERP index can be improved (as can risk assessment) by taking into account information for a given chemical about mechanism, pharmacokinetics, shape of the dose–response curve, and mutagenicity.

Our analysis of possible carcinogenic hazards has recently been expanded to 80 typical daily human exposures to rodent carcinogens from a variety of sources. Our results indicate that the possible hazards of synthetic chemicals ingested from pesticide residues or water pollution appear to be trivial relative to the background of rodent carcinogens from natural chemicals (e.g., from natural pesticides in plant foods or from the cooking of

Table III. Proportion of Chemicals Evaluated as Carcinogenic[a] for Several Datasets in the CPDB

1. All chemicals in CPDB	584/1117 (52%)
2. Chemicals tested in both rats and mice	288/479 (60%)
2a. Naturally occurring chemicals tested in both rats and mice	56/101 (55%)
2b. Synthetic chemicals tested in both rats and mice	232/378 (61%)
3. NCI/NTP chemicals[b]	
3a. NCI/NTP chemicals tested before 1979	60/117 (51%)
3b. NCI/NTP chemicals tested after 1979	105/198 (53%)
4. Chemicals tested in at least 1 speices	
4a. Natural pesticides	29/57 (51%)
4b. Mold toxins	12/20 (60%)
4c. Chemicals in roasted coffee	19/26 (73%)

[a] A chemical is classified as positive if the author of at least one published experiment evaluated results as evidence that the compound is carcinogenic.

[b] 94% (296/315) of NCI/NTP bioassays are conducted in both rats and mice.

food). Current synthetic pesticide residues are at the bottom of the HERP ranking, whereas HERP values for natural chemicals in foods occur throughout the ranking with many common foods in the top half.[44] Results are similar in a ranking of *average* daily consumption of natural pesticides and synthetic pesticide residues.

For occupational exposures, there is more than a 100,000-fold range in PERP values for rodent carcinogens that have PELs.[41] The permitted exposures to workers for several compounds are close to the TD_{50} value in rodents, indicating that these should be a high priority for regulatory attention. For high occupational exposures, little quantitative extrapolation is required to the high doses used in rodent bioassays, and therefore assumptions about extrapolation are less important. This contrasts with the large extrapolations required for the low human exposures to pesticide residues or water pollution.

Since only a tiny fraction of the chemicals to which humans are exposed will ever be tested in rodent bioassays, it is important to try and identify as-yet untested chemicals that might be of greatest possible hazard to humans. One strategy for choosing chemicals to test is to prioritize chemicals according to how they might rank in possible hazard *if* they were to be identified as rodent carcinogens. A useful first approximation is the analogous ratio Human Exposure/Rodent Toxicity (HERT). HERT would use readily-available LD_{50} values rather than the TD_{50} values used in HERP. LD_{50} is related to the MTD and the TD_{50},[45-46] and we have found that the rankings by HERP and HERT are similar (Spearman rank order correlation = 0.9). The number of people exposed is also relevant in attempting to prioritize systematically among chemicals. Chemicals with high HERT and population exposure could then be investigated in more detail as to mutagenicity, mitogenicity, pharmacokinetics, etc. Natural and synthetic chemicals should both be ranked, and if natural chemicals in foods such as chlorogenic acid in coffee, psoralens in celery, or indole carbinol in broccoli turned out to be important, they might be bred out; for processed foods such as coffee, they might be extracted.

3.3 Mechanisms of Carcinogenesis: Mutagenesis, Mitogenesis, and Carcinogenesis

The study of mechanisms of carcinogenesis is a rapidly developing field that can improve efforts to extrapolate from high to low dose. Both DNA damage and increased cell division (mitogenesis) are important aspects of carcinogenesis, and increasing either substantially can cause cancer.[40,47-51] Because there is an enormous background of endogenous DNA lesions,[9,10] any agent causing chronic mitogenesis can be indirectly mutagenic, and consequently carcinogenic, by increasing the probability of these endogenous DNA lesions being converted to mutations.

If one accepts that mutagenesis is important for carcinogenesis, it follows that mitogenesis rates must be important. When the cell divides, an unrepaired DNA lesion has a certain probability of giving rise to a mutation. Thus, an important factor in the mutagenic effect of an exogenous agent, whether it is genotoxic or nongenotoxic, is the increment it causes over the background cell division rate.[52] Those cells that appear to matter most for cancer are the stem cells, which are not on their way to being discarded. Increasing their cell division rate increases mutation and therefore cancer.

Mitogenesis can be caused by toxicity of chemicals at high dose (cell killing and subsequent replacement), by interference with cell–cell communication at high doses[53-56] by substances such as hormones binding to receptors that control cell division,[57] by oxidants (the wound healing response), by viruses, etc.[9] Chronic mitogenesis is important for many of the known causes of human cancer.[57,58]

Animal cancer tests are conducted at the maximum tolerated dose (MTD) and 1/2 the MTD of the test chemical for long periods of time, both high doses that can cause chronic mitogenesis.[9,22,49] Chronic dosing at the MTD may often be the equivalent of chronic wounding, which is known to increase tumor yields in rodent tests and to be a risk factor for cancer in humans.[59] If animal cancer tests are primarily measuring the effects of mitogenesis, then the dose–response would be expected to curve upward.[40,49,50,60-63] At doses too low to produce much mitogenesis, the cell-division rate would revert to something well within the normal range, and no significant enhancement would remain to multiply any other effects of the chemical; this would lead to an upward-curving dose–response for carcinogenicity, even for mutagens. Thus, a 10-fold reduction in dose would produce much more than a 10-fold reduction in risk, and a linear extrapolation from high to low dose would overestimate risk at low dose.

Recent analyses of dose-response in animal tests are consistent with the idea that mitogenesis from cell-killing and cell replacement at the high doses tested is important. Even at the two high doses tested, we have found that 44% of the positive sites in NTP bioassays are statistically significant at the MTD but not at 1/2 the MTD (among 365 positive sites). Moreover, the proportion positive only at the high dose is similar for mutagens and nonmutagens. Another analysis of the shape

of dose–response curves indicates that a quadratic dose–response is compatible with more of the data than a linear one for both mutagens and nonmutagens, thus suggesting that mitogenesis is an important factor at the MTD, even for mutagens.[36]

It is clear that the mechanisms of action for all rodent carcinogens are not the same, and that one cannot use a simple linearized risk assessment model for all of them. For some chemicals, there is evidence to support mitogenesis effects unique to high doses (e.g., formaldehyde, melamine, and saccharin). For others (e.g., butadiene), carcinogenic effects have been found considerably below the MTD. Further studies of mechanisms in rodent bioassays should help to clarify such differences. Adding routine measurements of mitogenesis to the 13-week toxicology study and the 2-year bioassay would provide information that would improve dose setting, interpretation of experimental results, and risk assessment. The 40% of rodent carcinogens that are not detectable mutagens should be investigated to see if their carcinogenic effects at high dose result from induction of mitogenesis; if so, then such rodent carcinogens would be unlikely to be a risk at low doses.

ACKNOWLEDGMENTS

This work was supported through the Lawrence Berkeley Laboratory by U.S. Department of Energy, contract DE-AC-03-76SF00098, and U.S. Environmental Protection Agency, agreement R-815619-01-0 to L.S.G., and through the University of California, Berkeley by National Institute of Environmental Health Sciences Center grant ESO1896, and by National Cancer Institute Outstanding Investigator grant CA39910 to B.N.A. We thank Daniel Krewski, Bonnie R. Stern, Thomas H. Slone and for suggestions on the manuscript, and Leah Slyder for technical assistance.

REFERENCES

1. L. S. Gold, C. B. Sawyer, R. Magaw, G. M. Backman, M. de Veciana, R. Levinson, N. K. Hooper, W. R. Havender, L. Bernstein, R. Peto, M. C. Pike, and B.N. Ames, "A Carcinogenic Potency Database of the Standardized Results of Animal Bioassays," *Environ. Health Perspect.* **58,** 9–319 (1984).
2. L. S. Gold, M. de Veciana, G. M. Backman, R. Magaw, P. Lopipero, M. Smith, M. Blumenthal, R. Levinson, L. Bernstein, and B. N. Ames, "Chronological Supplement to the Carcinogenic Potency Database: Standardized Results of Animal Bioassays Published through December 1982," *Environ. Health Perspect.* **67,** 161–200 (1986).
3. L. S. Gold, T. H. Slone, G. M. Backman, R. Magaw, M. Da Costa, P. Lopipero, M. Blumenthal, and B. N. Ames, "Second Chronological Supplement to the Carcinogenic Potency Database: Standardized Results of Animal Bioassays Published Through December 1984 and by the National Toxicology Program Through May 1986," *Environ. Health Perspect.* **74,** 237–329 (1987).
4. L. S. Gold, T. H. Slone, G. M. Backman, S. Eisenberg, M. Da Costa, M. Wong, N. B. Manley, L. Rohrbach, and B. N. Ames, "Third Chronological Supplement to the Carcinogenic Potency Database: Standardized Results of Animal Bioassays Published Through December 1986 and by the National Toxicology Program Through June 1987," *Environ. Health Perspect.* **84,** 215–286 (1990).
5. L. S. Gold, N. B. Manley, T. H. Slone, G. B. Garfinkel, L. Rohrbach, and B. N. Ames, "The Fifth Plot of the Carcinogenic Potency Database: Results of Animal Bioassays Published in the General Literature Through 1988 and by the National Toxicology Program Through 1989," *Environ. Health Perspect.* **100** (1992).
6. L. S. Gold, L. Bernstein, R. Magaw, and T. H. Slone, "Interspecies Extrapolation in Carcinogenesis: Prediction Between Rats and Mice," *Environ. Health Perspect.* **81,** 211–219 (1989).
7. L. S. Gold, T. H. Slone, N. B. Manley, and L. Bernstein, "Target Organs in Chronic Bioassays of 533 Chemical Carcinogens," *Environ. Health Perspect.* **93,** 233–246 (1991).
8. B. N. Ames and L. S. Gold, "Perspective: Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis," *Science* **249,** 970–971 (1990); *Letters* **250,** 1498 (1990); **250,** 1645–1646 (1990); **251,** 12–13 (1991); **251,** 606–608 (1991); **252,** 902 (1991).
9. B. N. Ames and L. S. Gold, "Chemical Carcinogenesis: Too Many Rodent Carcinogens," *Proc. Natl. Acad. Sci. U.S.A.* **87,** 7772–7776 (1990).
10. B. N. Ames and L. S. Gold, "Endogenous Mutagens and the Causes of Aging and Cancer," *Mut. Res.* **250,** 3–16 (1991).
11. E. Zeiger, "Carcinogenicity of Mutagens: Predictive Capability of the *Salmonella* Mutagenesis Assay for Rodent Carcinogenicity," *Cancer Res.* **27,** 1287–1296 (1987).
12. J. K. Haseman, J. E. Huff, E. Zeiger, and E. E. McConnell, "Comparative Results of 327 Chemical Carcinogenicity Studies," *Environ. Health Perspect.* **74,** 229–235 (1987).
13. I. F. H. Purchase, "Interspecies Comparisons of Carcinogenicity," *Br. J. Cancer* **41,** 454–468 (1980).
14. D. A. Freedman and H. Zeisel, "From Mouse to Man: The Quantitative Assessment of Cancer Risks," *Statistical Science* **3,** 3–56 (1988).
15. International Agency for Research on Cancer (IARC), *Overall Evaluations of Carcinogenicity* (Lyon, France, IARC, 1987), Suppl. 7.
16. International Agency for Research on Cancer, *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans,* Vols. 1–52 (Lyon, France, IARC, 1971–1991).
17. L. Tomatis, A. Aitio, J. Wilbourn, and L. Shuker, "Human Carcinogens So Far Identified," *Jpn. J. Cancer Res.* **80,** 795–807 (1989).
18. L. Tomatis and H. Bartsch, "The Contribution of Experimental Studies to Risk Assessment of Carcinogenic Agents in Humans," *Exp. Pathol.* **40,** 251–266 (1990).
19. A. Ito, M. Mori, and M. Naito, "Induction of Uterine Hemangioendothelioma and Lymphoma in (C57BL/6NXC3H/2N)F1 Mice by Oral Administration of Azathioprine," *Jpn. J. Cancer Res.* **80,** 419–423 (1989).
20. B. Ryffel, P. Donatsch, M. Madörin, B. E. Matter, G. Rüttimann, H. Schön, R. Stoll, and J. Wilson, "Toxicological Evaluation of Cyclosporin A," *Arch. Toxicol.* **53,** 107–141 (1983).
21. D. L. Greenman, B. Highman, J. Chen, W. Sheldon, and G. Gass, "Estrogen-Induced Thyroid Follicular Cell Adenomas in C57BL/6 Mice," *J. Toxicol. Environ. Health.* **29,** 269–278 (1990).
22. L. Bernstein, L. S. Gold, B. N. Ames, M. C. Pike, and D. G. Hoel, "Some Tautologous Aspects of the Comparison of Carcinogenic Potency in Rats and Mice," *Fund. Appl. Toxicol.* **5,** 79–86 (1985).
23. L. S. Gold and B. N. Ames, "The Importance of Ranking Possible Carcinogenic Hazards Using HERP," *Risk Analysis* **10,** 625–628 (1990).
24. D. W. Gaylor, "Preliminary Estimates of the Virtually Safe Dose

for Tumors Obtained from the Maximum Tolerated Dose," *Regul. Toxicol. Pharmacol.* **9,** 101–108 (1989).
25. D. Krewski, M. Szyszkowicz, and H. Rosenkranz, "Quantitative Factors in Chemical Carcinogenesis: Variation in Carcinogenic Potency," *Regul. Toxicol. Pharmacol.* **12,** 13–29 (1990).
26. C. Sawyer, R. Peto, L. Bernstein, and M. C. Pike, "Calculation of Carcinogenic Potency from Long-Term Animal Carcinogenesis Experiments," *Biometrics* **40,** 27–40 (1984).
27. R. Peto, M. C. Pike, L. Bernstein, L. S. Gold, and B. N. Ames, "The TD_{50}: A Proposed General Convention for the Numerical Description of the Carcinogenic Potency of Chemicals in Chronic-Exposure Animal Experiments," *Environ. Health Perspect.* **58,** 1–8 (1984).
28. L. S. Gold, L. Bernstein, J. Kaldor, G. Backman, and D. Hoel, "An Empirical Comparison of Methods Used to Estimate Carcinogenic Potency in Long-Term Animal Bioassays: Lifetable vs. Summary Incidence Data," *Fund. Appl. Toxicol.* **6,** 263–269 (1986).
29. L. S. Gold, T. H. Slone, and L. Bernstein, "Summary of Carcinogenic Potency (TD_{50}) and Positivity for 492 Rodent Carcinogens in the Carcinogenic Potency Database," *Environ. Health Perspect.* **79,** 259–272 (1989).
30. E. A. C. Crouch, R. Wilson, and L. Zeise, "Tautology or Not Tautology?" *J. Toxicol. Environ. Health.* **20,** 1–10 (1987).
31. J. P. Rieth and T. B. Starr, "Chronic Bioassays: Relevance to Quantitative Risk Assessment of Carcinogens," *Regul. Toxicol. Pharmacol.* **10,** 160–173 (1989).
32. J. P. Rieth and T. B. Starr, "Experimental Design Constraints on Carcinogenic Potency Estimates," *J. Toxicol. Environ. Health.* **27,** 287–296 (1989).
33. D. Krewski, D. W. Gaylor, A. P. Soms, and M. Syszkowicz, "Correlation between Carcinogenic Potency and the Maximum Tolerated Dose: Implications for Risk Assessment," *Risk Anal.* (in press).
34. Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation, "Panel on Carcinogenesis Report on Cancer Testing in the Safety Evaluation of Food Additives and Pesticides," *Toxicol. Appl. Pharmacol.* **20,** 419–438 (1971).
35. D. Krewski, M. J. Goddard, and J. Withey, "Carcinogenic Potency and Interspecies Extrapolation," In M. L. Mendelsohn and R. J. Albertini (Eds.), *Mutation and the Environment,* Vol. 340 (New York, Wiley-Liss, 1990), pp. 323–334.
36. D. G. Hoel and C. J. Portier, "Nonlinearity of Dose–Response Functions for Carcinogenicity," *Environ. Health Perspect.*, in press.
37. D. A. Freedman, L. S. Gold, and T. H. Slone, "How Tautological Are Inter-species Correlations of Carcinogenic Potencies?" *Technical Report No. 334* (Department of Statistics, University of California, Berkeley, 1992).
38. A. M. Rulis, "*De minimis* and the Threshold of Regulation," In C. W. Felix (Ed.), *Food Protection Technology* (Chelsea, MI, Lewis Publishers, 1986), pp. 29–37.
39. L. Zeise, "Issues in State Risk Assessment: California Department of Health Services," *Proceedings: Pesticides and Other Toxics: Assessing their Risks* (June 17, 1989).
40. B. N. Ames, R. Magaw, and L. S. Gold, "Ranking Possible Carcinogenic Hazards," *Science* **236,** 271–280 (1987); *Letters* **237**, 235 (1987); **237,** 1283–1284 (1987); **237,** 1399–1400 (1987); **238,** 1633–1634 (1987); **240,** 1043–1047 (1988).
41. L. S. Gold, G. M. Backman, K. Hooper, and R. Peto, "Ranking the Potential Carcinogenic Hazards to Workers from Exposures to Chemicals That Are Tumorigenic in Rodents," *Environ. Health Perspect.* **76,** 211–219 (1987).
42. B. N. Ames, M. Profet, and L. S. Gold, "Dietary Pesticides (99.99% All Natural)," *Proc. Natl. Acad. Sci. U.S.A.* **87,** 7777–7781 (1990).
43. B. N. Ames, M. Profet, and L. S. Gold, "Nature's Chemicals and Synthetic Chemicals: Comparative Toxicology," *Proc. Natl. Acad. Sci. U.S.A.* **87,** 7782–7786 (1990).
44. L. S. Gold, T. H. Slone, B. R. Stern, N. B. Manley, and B. N. Ames, "Rodent carcinogens: Setting Priorities," *Science,* **258,** 261–265 (1992).
45. L. Zeise, E. A. C. Crouch, and R. Wilson, "A Possible Relationship Between Toxicity and Carcinogenicity," *J. Amer. College Toxicol.* **5,** 137–151 (1986).
46. C. C. Travis, S. A. Richter Pack, A. W. Saulsbury, and M. W. Yambert, "Prediction of Carcinogenic Potency from Toxicological Data," *Mut. Res.* **241,** 21–36 (1990).
47. H. C. Pitot, T. L. Goldsworthy, S. Moran, W. Kennan, H. P. Glauert, R. R. Maronpot, and H. A. Campbell, "A Method to Quantitate the Relative Initiating and Promoting Potencies of Hepatocarcinogenic Agents in Their Dose–Response Relationships to Altered Hepatic Foci," *Carcinogenesis* **8,** 1491–1499 (1987).
48. E. Farber, "Possible Etiologic Mechanisms in Chemical Carcinogenesis," *Environ. Health Perspect.* **75,** 65–70 (1987).
49. B. E. Butterworth, T. J. Slaga, W. Farland, and M. McClain (Eds.), *Chemically Induced Cell Proliferation: Implications for Risk Assessment* (New York, Wiley-Liss, 1991).
50. S. M. Cohen and L. B. Ellwein, "Cell Proliferation in Carcinogenesis," *Science* **249,** 1007–1011 (1990).
51. H. A. Dunsford, S. Sell, and F. V. Chisari, "Hepatocarcinogenesis Due to Chronic Liver Cell Injury in Hepatitis-B Virus Transgenic Mice," *Cancer Res.* **50,** 3400–3407 (1990).
52. C. Tong, M. Fazio, and G. M. Williams, "Cell Cycle-Specific Mutagenesis at the Hypoxanthine Phosphoribosyltransferase Locus in Adult Rat Liver Epithelial Cells," *Proceedings of the National Academy of Sciences U.S.A.* **77,** 7377–7389 (1980).
53. J. E. Trosko, C. C. Chang, and A. Medcalf, "Mechanisms of Tumor Promotion: Potential Role of Intercellular Communication," *Cancer Invest.* **1,** 511–526 (1983).
54. J. E. Trosko, "Towards Understanding Carcinogenic Hazards: A Crisis in Paridigms," *J. Am. Coll. Toxicol.* **8,** 1121–1132 (1989).
55. J. E. Trosko, C. C. Chang, and B. V. Madhukar, "In Vitro Analysis of Modulators on Intercellular Communications: Implications for Biologically Based Risk Assessment Models for Chemical Exposure," *Toxicol. In Vitro* **4,** 635–643 (1990).
56. J. E. Trosko, C. C. Chang, B. V. Madhukar, and J. E. Klaunig, "Chemical, Oncogene, and Growth Factor Inhibition of Gap Junctional Communication: An Integrative Hypothesis of Carcinogenesis," *Pathobiology* **58,** 265–278 (1990).
57. S. Preston-Martin, M. C. Pike, R. K. Ross, and P. A. Jones, "Increased Cell Division as a Cause of Human Cancer," *Cancer Res.* **50,** 7415–7421 (1990).
58. B. E. Henderson, R. Ross, and L. Bernstein, "Estrogens as a cause of Human Cancer: The Richard and Hinda Rosenthal Foundation Award Lecture," *Cancer Res.* **48,** 246–253 (1988).
59. S. A. Weitzman and L. J. Gordon, "Inflammation and Cancer: Role of Phagocyte-generated Oxidants in Carcinogenesis," *Blood* **76,** 655–663 (1990).
60. J. A. Swenberg, F. C. Richardson, J. A. Boucheron, F. H. Deal, S. A. Belinsky, M. Charbonneau, and B. G. Short, "High- to Low-Dose Extrapolation: Critical Determinants Involved in the Dose Response of Carcinogenic Substances," *Environ. Health Perspect.* **76,** 57–63 (1987).
61. D. G. Hoel, J. K. Haseman, M. D. Hogan, J. Huff, and E. E. McConnell, "The Impact of Toxicity on Carcinogenicity Studies: Implications for Risk Assessment," *Carcinogenesis* **9,** 2045–2052 (1988).
62. F. H. Deal, F. C. Richardson, and J. A. Swenberg, "Dose Response of Hepatocyte Replication in Rats Following Continuous Exposure to Diethylnitrosamine," *Cancer Res.* **49,** 6985–6988 (1989).
63. B. E. Butterworth, "Consideration of Both Genotoxic and Nongenotoxic Mechanisms in Predicting Carcinogenic Potential," *Mut. Res.* **239,** 117–132 (1990).