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WHAT DO ANIMAL CANCER TESTS TELL US ABOUT HUMAN CANCER RISK?: OVERVIEW OF ANALYSES OF THE CARCINOGENIC POTENCY DATABASE

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By developing the Carcinogenic Potency Database (CPDB) [1], a standardized and systematic resource on the published results of chronic animal cancer tests, we have been able to address many questions about the use of animal bioassays in evaluation of potential cancer risks to humans [2]. The CPDB is readily amenable to secondary analyses of experimental results, and in several papers, our group has investigated issues such as the following: How well can one predict carcinogenicity in rats from carcinogenicity in mice? Does the correlation in carcinogenic potency observed between rats

and mice provide justification for extrapolation of potency from rats to humans? How reproducible are the results of rodent bioassays? What are the limitations of high-dose bioassay data in efforts to extrapolate to low-dose risk? Do target organs of carcinogenicity differ between rats and mice or between mutagenic and nonmutagenic chemicals? Do bioassay results suggest that synthetic industrial chemicals are likely to be important causes of human cancer at typical human exposure levels? How applicable are results of experiments conducted at the maximum tolerated dose (MTD) to the low doses of most human exposures? How do results from chemicals that occur naturally compare to results for synthetic chemicals? What human exposures to rodent carcinogens rank high as possible cancer hazards?

This paper presents an overview of our earlier published analyses, using the larger number of experiments and chemicals now reported in the CPDB [3]. Tabular results are presented for several of the updated analyses.

I. THE CARCINOGENIC POTENCY DATABASE

The CPDB is a standardized resource of chronic carcinogenesis bioassay results, including analyses of 1002 papers in the general literature and 403 Technical Reports of the National Cancer Institute/National Toxicology Program (NCI/NTP) [1]. Results are reported for 5152 experiments on 1298 chemical agents. About 30% of the chemicals were tested by NCI/NTP. The published results of the experiments reported in the CPDB constitute a diverse literature that varies widely with respect to experimental and histological protocols as well as to how and which information is reported in published articles [1]. No attempt has been made in the CPDB to evaluate whether or not a compound induced tumors in any given experiment; rather, the opinion of the published author is presented. For any single chemical, the number of experiments in the database may vary. Some chemicals have only one test in one sex of one species, whereas others have multiple tests including both sexes of a few strains of rats and mice, possibly using quite different protocols [1].

A numerical description of carcinogenic potency, the TD_{50} [4,5], is estimated for each set of tumor-incidence data reported in the CPDB, thus providing a standardized quantitative measure for comparisons. In a simplified way, TD_{50} may be defined as that dose rate in mg/kg body wt/day which, if administered chronically for the standard life span of the species, will halve the probability of remaining tumorless throughout that period. Put differently, TD_{50} is the daily dose that will induce tumors in half of the test animals that would have remained tumor-free at zero dose. We estimate TD_{50} using a one-hit model [4,5]. TD_{50} is analogous to LD_{50}, and a low value of

TD_{50} indicates a potent carcinogen, whereas a high value indicates a weak one. TD_{50} is often within the range of doses tested and does not indicate anything about carcinogenic effects at low doses because bioassays are usually conducted at or near the maximum tolerated dose (MTD). (The MTD is generally accepted to be defined as the maximum dose level which is not expected to shorten the normal longevity from non-neoplastic causes, and which is expected to result in no more than a 10% weight decrement in animals receiving this dose when compared to controls [6].)

The range of TD_{50}s is at least 10^7-fold for carcinogens in each sex of rat or mouse. For female rats, the range of carcinogenic potency is shown in Fig. 1, which reports the most potent TD_{50}s for a selected group of rodent carcinogens. In each case, we have indicated the value for the most potent TD_{50} for a target site that was evaluated as positive by the published author, and for which the statistical significance of TD_{50} is less than 0.01. The range is more than 10^8-fold in female rats.

Among chemicals that are positive in both species, potency values in rats and mice are within a factor of 10 of each other for 73% of the chemicals that are carcinogenic in both species. The TD_{50} in rats is more potent than the value in mice for 131, and less potent for 57.

II. INTERPRETATION OF THE 50% POSITIVITY RATE IN RODENT BIOASSAYS

A. Half the Chemicals Tested in Rodents Are Carcinogens

Approximately half the chemicals tested in rats or mice are positive in at least one experiment. Positivity rates of about 50% are shown in Table 1 for chemicals tested in NCI/NTP bioassays, in the general literature, or in either of these sources. Table 2 reports a similar positivity rate for several subsets of the CPDB: naturally occurring chemicals, synthetic chemicals, natural pesticides (the chemicals that plants produce naturally to defend themselves), mold toxins, and chemicals in roasted coffee. Moreover, among the 465 chemicals that have been tested for both mutagenicity in *Salmonella* and for carcinogenicity in rats and mice, 72% are either mutagens or carcinogens, or both (Table 3). (A chemical is classified as mutagenic in our analyses if it was evaluated in the *Salmonella* assay as either mutagenic or weakly mutagenic [7–9].)

Other results also suggest that a high proportion of all chemicals might be carcinogenic, if tested under the conditions of standard rodent bioassays. In the *Physician's Desk Reference* (PDR) 49% (117/241) of the drugs with

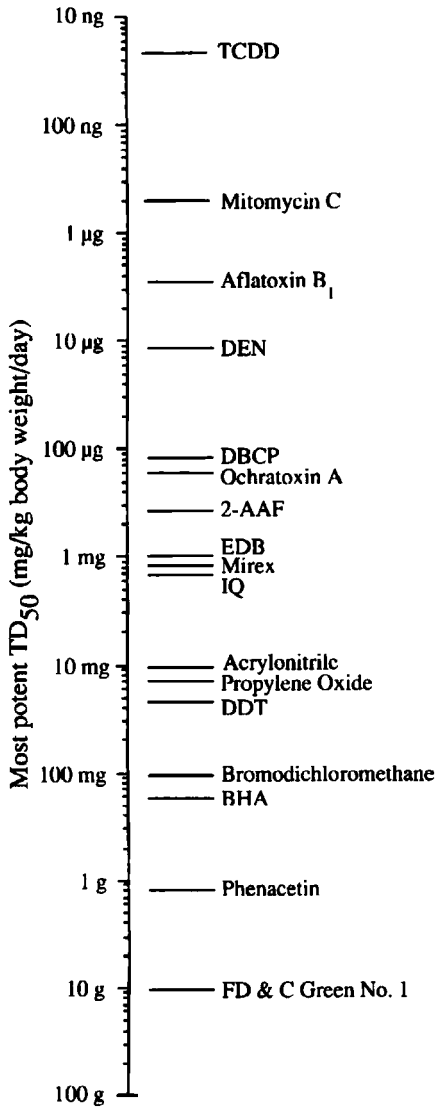

FIG. 1. Range of carcinogenic potency in female rats.

reports of animal cancer test results are carcinogenic in those tests [10]. Drugs that are selected for development and subsequently tested are not expected to be carcinogens. Because current drug development is primarily for chemicals that are not mutagenic in *Salmonella*, one would expect a

TABLE 1

Proportion of Chemicals in the Carcinogenic Potency Database Tested in Rats or Mice That Have Been Evaluated as Carcinogenic,[a] by Reference Source

Reference source	Proportion carcinogenic in rats or mice %	Proportion carcinogenic in rats %	Proportion carcinogenic in mice %
NCI/NTP or literature[b]	668/1275 (52%)	484/997 (49%)	372/847 (44%)
NCI/NTP	200/384 (52%)	144/366 (39%)	145/366 (40%)
Literature	505/1006 (50%)	361/699 (52%)	242/550 (44%)

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

[b]The number of chemicals is smaller than the sum of each source separately because some chemicals have been reported by both sources.

TABLE 2

Proportion of Chemicals Evaluated as Carcinogenic,[a] for Several Data Sets in the Carcinogenic Potency Database

Chemicals tested in both rats and mice	330/559 (59%)
Naturally-occurring chemicals	73/127 (57%)
Synthetic chemicals	257/432 (59%)
Chemicals tested in rats and/or mice	
Natural pesticides	35/64 (55%)
Mold toxins	14/23 (61%)
Chemicals in roasted coffee	19/28 (68%)

[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.

TABLE 3

Comparison of Mutagenicity and Carcinogenicity[a] for Chemicals Tested in Both Rats and Mice and for Mutagenicity in *Salmonella* in the Carcinogenic Potency Database

Carcinogenic	+			Carcinogenic	−
Mutagenic	+			Mutagenic	+
		165	43		
		127	130		
Carcinogenic	+			Carcinogenic	−
Mutagenic	−			Mutagenic	−

1. Of 465 chemicals, 45% are mutagens, 63% are carcinogens, and 72% are either mutagens or carcinogens or both (165 + 127 + 43)/465.
2. Mutagens are more likely to be carcinogenic 79% (165/208) than nonmutagens 49% (127/257).
3. Of 292 carcinogens, 43% are not mutagens, 125/(166 + 125).
4. Of 173 noncarcinogens, 25% are mutagens, 43/(43 + 130).

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

lower rate of carcinogenicity than for other chemicals; yet, 49% are positive. In a database of all pharmaceuticals tested for carcinogenicity for which a marketing authorization was applied for in Germany and the Netherlands since 1980, 48% (106/221) were positive [11].

Among chemicals to which humans are exposed, we estimate that 99.9% occur naturally [12]; however, among chemicals in the CPDB, only 22% (293/1298) are natural. Because half the natural chemicals tested are positive, human exposures to rodent carcinogens are likely to be ubiquitous (see Section VI).

Because the results of high-dose bioassays are routinely used to identify a chemical as a possible cancer hazard to humans, it is important to try to understand how representative the 50% positivity rate might be of all un-

tested chemicals. If half of all chemicals (both natural and synthetic) would be positive if tested, then the utility of a test to identify a chemical as a "potential human carcinogen" is questionable. To determine the true proportion of rodent carcinogens among chemicals would require a comparison of test results from a random group of synthetic chemicals to a random group of natural chemicals. Such an analysis has not been done.

It has been argued that the high positivity rate is due to selecting more suspicious chemicals to test, which is a likely bias because cancer testing is both expensive and time-consuming, and it is prudent to test suspicious compounds. In the general literature, however, chemicals are selected for testing for many reasons other than suspicion, including the extent of human exposure, level of production and occupational exposure, and scientific questions about carcinogenesis [13]; the positivity rate in the general literature is 50% (Table 1).

If chemicals were selected because they were likely to be carcinogens, then one would expect that they would primarily be mutagens, as mutagens are much more likely to be carcinogenic in bioassays than nonmutagens (Table 3). However, about half the chemicals tested in the NCI/NTP bioassay program or in the general literature are not mutagenic. Thus, prediction of positivity may often not be the basis for selecting a chemical to test.

The idea that chemicals are selected for testing because they are likely to be carcinogenic rests on an assumption that researchers have adequate knowledge about how to predict carcinogenicity and that there is consensus about the criteria (i.e., the idea that bias in the positivity rate is due to selection requires that there is shared, adequate knowledge of what is likely to be carcinogenic). However, although some chemical classes are more often carcinogenic in rodent bioassays than others—for example, nitroso compounds, aromatic amines, nitroaromatics, and chlorinated compounds—several results suggest that predictive knowledge is highly imperfect, even now, after decades of testing results have become available on which to base prediction of carcinogenicity. In 1990, a prospective prediction exercise was conducted by several experts in advance of the 2-year NTP bioassays. The accuracy of predicting a positive or a negative result in the subsequent 40 bioassays ranged widely among these experts, from 49% to 75% (the most accurate experts also had access to data on target organ toxicity in the 90-day study) [14]. There was wide disagreement among the experts on which chemicals they predicted would be carcinogenic when tested, thus indicating that predictive knowledge is highly uncertain.

Following the completion of bioassays on 379 chemicals, staff at the NCI/NTP retrospectively classified the chemicals according to the original rationale for their selection into the bioassay program before 1980. Each chemical was classified as to whether it was selected with a suspicion of carcinoge-

nicity (although other factors such as exposure may also have been involved) versus whether it was selected mainly, but not solely, on the basis of considerations of exposure and production volume [15]. Their classification indicated that 67% (253/379) of chemicals were selected due to suspicion, and of these, 68% were positive in bioassays. Of the remaining chemicals, 21% were positive in bioassays. The authors suggest that the high rate of carcinogenicity in NCI/NTP bioassays may be due to the "scientifically oriented selection of chemicals suspected of having carcinogenic activity." We have done an analysis to compare the predictive ability of this classification to the results obtained by the expert predictions described above that were done in advance of the bioassays. Of the 40 chemicals reported in the expert prediction exercise, 27 were included in the classification by NCI/NTP staff. Oddly, the predictive ability of the NCI/NTP classification, based on suspicion prior to 1980, was more accurate (81%) than any of the 1990 predictors for the same set of 27 chemicals, including the experts that used the 90-day toxicity data.

One predictive analysis for a randomly selected group of chemicals has been conducted. For 140 chemicals selected randomly from *The Merck Index* and *The Handbook of Chemistry and Physics*, the CASE/MULTICASE computer-automated structure evaluation system predicted that 46% (65/140) would be carcinogens if tested in a standard bioassay [16]. In other analyses of naturally occurring chemicals, the positivity rate was also predicted to be high: 40% (45/113) of phytoalexins and 37% (62/167) of other natural chemicals. The results of these CASE analyses suggest that a high proportion of chemicals, both natural and synthetic, might be carcinogenic under the conditions of the standard rodent bioassay.

One large series of mouse experiments by Innes et al. in 1969 [17,18] has been frequently cited [19] as evidence that the true proportion of rodent carcinogens is actually low. Innes tested 119 chemicals, selected primarily because they were the most widely used pesticides at that time; some industrial chemicals were also selected. Only 11 (9%) were judged to be carcinogens. We [13] have discussed that those early experiments lacked power to detect an effect because they were conducted only in mice (not in rats), they included only 18 animals in a group (compared with the usual 50), the animals were tested for only 18 months (compared with the usual 24 months), and the dose was usually lower than the highest dose in subsequent mouse tests of the same chemical.

We recently reexamined the Innes results using the CPDB to assess positivity in subsequent bioassays on the chemicals that Innes did not evaluate as positive (Table 4). Among 34 negative chemicals that were subsequently retested, 16 were carcinogenic (47%), which is similar to the proportion among all chemicals in our database [2]. Innes had recommended further

TABLE 4

Results of Subsequent Tests on Chemicals Not Found Carcinogenic by Innes et al. [17]

Retested Chemicals	% Carcinogenic When Retested		
	Mice	Rats	Either Mice or Rats
All retested	6/26 (23%)	13/34 (38%)	16/34 (47%)
Innes: Not Carcinogenic	3/10 (30%)	9/18 (50%)	10/18 (56%)
Innes: Needs Further Evaluation	3/16 (19%)	4/16 (25%)	6/16 (38%)

Note: Of 119 chemicals tested by Innes et al., 11 (9%) were evaluated as positive by these authors.

evaluation of some chemicals that had inconclusive results in their study. If those were the chemicals subsequently retested, then one might argue that they would be the most likely to be positive. Our analysis does not support that view: Among chemicals needing further evaluation 6/16 were positive when retested; among the other negatives, 10/18 were positive (Table 4).

B. Cell Division and the High Positivity Rate in Bioassays

What are the explanations for the high positivity rate in high-dose animal cancer tests? One plausible explanation, which is supported by many recent papers [20,21], is that the MTD of a chemical can cause chronic cell killing and cell replacement in the target tissue, a risk factor for cancer that can be limited to high doses. We (Ames and Gold) have discussed in detail the importance of cell division in mutagenesis and carcinogenesis [20,22–24]; several results in the CPDB are consistent with the idea that cell division increases carcinogenesis under the conditions of standard animal cancer tests.

Endogenous DNA damage from normal oxidation is enormous. The steady-state level of oxidative damage in DNA is about one million oxidative lesions per rat cell [25]. This high background suggests that increasing the cell division rate must be a factor in converting lesions to mutations and thus cancer [24]. Raising the level of either DNA lesions or cell division will increase the probability of cancer. Just as DNA repair protects against lesions, p53 guards the cell cycle and protects against cell division if the lesion level gets too high. If the lesion level becomes still higher, p53 can initiate programmed cell death (apoptosis). None of these defenses is perfect, however. The critical factor is chronic cell division in stem cells, not in cells that are discarded, and is related to the total number of extra cell divisions. Cell division is both a major factor in loss of heterozygosity

through nondisjunction and other mechanisms [22] and in expanding clones of mutated cells.

In animal cancer tests, the doses administered are near-toxic or minimally toxic (i.e., the MTD and half the MTD) and may result in cell division. *Ad libitum* feeding in the standard bioassay can also contribute to the high positivity rate [26], plausibly by increased cell division due to high caloric intake [24,26]. Although cell division is not measured in routine cancer tests, many studies on rodent carcinogenicity show a correlation between cell division at the MTD and cancer. For example, cell division rates at the bioassay dose were measured for 15 chemicals (8 mutagens, including pairs of mutagenic isomers, 1 of which was carcinogenic in the bioassay and 1 of which was not) and 7 nonmutagens [27,28]. In all 9 of the carcinogens, there was an increase in cell division in the target tissue, and in the 6 chemicals that did not cause tumors, there was no such increase. Extensive reviews of the experimental literature [22,25,29–31] indicate that chronic cell division can induce cancer, and reviews of the epidemiological literature indicate that increased cell division by hormones and other agents can increase human cancer [32].

Our analyses of the CPDB are consistent with the idea that in high-dose bioassays, cell division increases mutagenesis and, therefore, carcinogenesis. To the extent that increases in tumor incidence in rodent studies are due to the secondary effects of inducing cell division at the MTD, then *any* chemical is a likely rodent carcinogen; therefore, the high positivity rate in the CPDB overall, and for several subsets of chemicals (Tables 1 and 2), would be expected. Carcinogenicity results for mutagenic compared to nonmutagenic chemicals tested in rats and mice (Table 3) indicate that 43% of carcinogens are not mutagenic. For these chemicals, increased cell division is likely an important factor.

Mutagens can both damage DNA and increase cell division at high doses, thus having a mutiplicative effect on mutagenesis at high doses. Therefore, if cell division is important, one would expect stronger evidence of carcinogenicity for mutagens in rodent bioassays. Results of analyses of the CPDB are consistent with this idea. Mutagens are more likely to be carcinogenic than nonmutagens (Table 3). Mutagenic carcinogens compared to nonmutagenic carcinogens are more likely to be carcinogenic in both rats and mice rather than in only one species: Among chemicals tested in both rats and mice and carcinogenic in at least one test, 67% of mutagens (110/165) are positive in both rodent species compared to 41% (52/127) of nonmutagens. Moreover, mutagenic carcinogens induce tumors at more target sites in rodent bioassays than nonmutagens (Table 5). Analyses of the limited data on dose response in bioassays are consistent with the idea that cell division from cell killing and cell replacement is important. Among rodent bioassays with two doses and a control group, about half the sites evaluated

TABLE 5

Comparison of the Number of Positive Target Organs for Mutagens and Nonmutagens by Species,[a] in the Carcinogenic Potency Database

Number of target organs	Chemicals evaluated as carcinogenic in: Rats				Mice			
	Mutagens		Nonmutagens		Mutagens		Nonmutagens	
1	77	(40%)	58	(60%)	76	(48%)	69	(66%)
2	37	(19%)	25	(26%)	48	(31%)	22	(21%)
≥3	78	(41%)	13	(14%)	33	(21%)	13	(13%)
Total number of chemicals	192	(100%)	96	(100%)	157	(100%)	104	(100%)

[a]A target organ is classified by a author's positive opinion in any experiment. Experimental results are excluded if histopathological examination was restricted to a few selected tissues.

as target sites are statistically significant at the MTD but not at half the MTD ($p < .05$) [33]. This proportion is similar for the NCI/NTP bioassays and the general literature.

Thus, it seems likely that a high proportion of all chemicals might be "carcinogens" if tested in a standard bioassay at the MTD, but this would be largely due to the effects of high doses for the nonmutagens and a synergistic effect of cell division at high doses with DNA damage for the mutagens. Our results suggest that adding routine measurements of cell division to the 90-day prechronic study and the 2-year bioassay for each test agent would provide information that could improve dose setting, the interpretation of experimental results, and risk assessment. Without additional data on mechanism of carcinogenesis for each chemical, the relevance of a positive result in a rodent bioassay to low exposures is highly uncertain [34]. The carcinogenic effects may be limited to the high doses tested.

III. METHODOLOGICAL INVESTIGATIONS

A. Constraints on Estimation of Carcinogenic Potency

Rodent cancer tests are designed to maximize the chance of obtaining a positive result in a lifetime experiment with small numbers of animals; near-toxic doses (MTD and half MTD) are the dose levels that have been used for that purpose in the standard protocol of the NCI/NTP. This standard experimental design, with a narrow range of doses, was never intended to

provide information to quantitatively assess the risk to humans from chemical exposures at low doses. In regulatory policy, however, standard practice has been to assess risk by linear extrapolation to the human exposure level (i.e., risk = potency × human exposure).

In 1985, we [35] showed that statistically significant potency estimates based on the usual experimental design are constrained to a narrow range about the maximum dose tested, in the absence of tumors in all dosed animals (which rarely occurs). For an ideal-type experiment with 50 animals in a single-dose group and a 10% tumor rate in a large control group, the range of possible estimates for statistically significant potency values is about 32-fold about the MTD, which is a marked contrast to the more than 10^7-fold range of potency values across chemicals. The range of possible potency is widened somewhat in real bioassays with multiple-dose groups, variable control rates and group size, and lifetable analysis for potency estimation.

Other researchers later showed a similar result for q_1^* estimated from the linearized multistage model used in regulatory risk assessment [36]. Potency estimates based on standard bioassay design are highly correlated with the administered dose, regardless of whether the estimate is based on the one-stage, multistage, or Weibull model. This constraint on potency estimation contrasts with the enormous extrapolation that is required from the MTD in rodent bioassays to usual human exposure levels, often hundreds of thousands of times lower.

B. Artifacts in the Correlation of Potencies Between Rats and Mice

A strong correlation of carcinogenic potencies observed between rats and mice has been interpreted as a justification for quantitative extrapolation from rodents to humans. Bernstein et al. showed, however, that the correlation is largely an artifact [35]. Over large numbers of chemicals, the MTDs for rats and mice are highly correlated and span many orders of magnitude (Fig. 2); this is a biological correlation in toxicity between the two species. Because potency estimates are constrained to a narrow range about the MTD, the potency correlation between rats and mice necessarily follows statistically.

We [37] have investigated how much of the observed correlation in potencies between species is indeed artifactual. Our analysis involved two statistical models in which the impacts of various assumptions could be calculated. One model assumes that interspecies correlation of potencies is purely artifactual; it ignores the correlation between rats and mice of (potency × MTD), which is a rough measure of tumor yield. The second model incorporates the correlation in (potency × MTD) between rats and mice, which indicates that part of the interspecies correlation in potencies is real; that is,

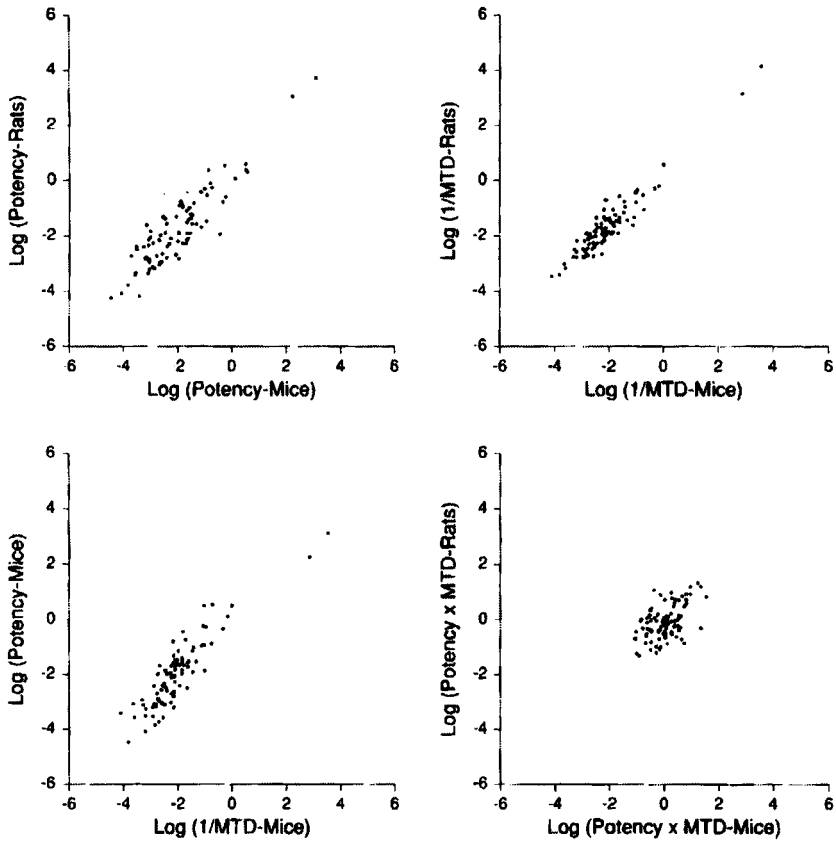

FIG. 2. *Top left*: The strong interspecies correlation of carcinogenic potencies; the horizontal axis shows log(potency–mice), and the vertical shows log(potency–rats). *Top right*: The horizontal axis shows log(1/MTD–mice), and the vertical shows log(1/MTD–rats); this correlation in toxicity is believed to be real. *Lower left*: A statistical artifact which drives the interspecies correlation of carcinogenic potencies; the horizontal axis shows log(1/MTD–mice), and the vertical axis shows log(potency–mice). *Lower right*: A weak interspecies correlation which seems to be real; the horizontal axis shows log[(potency × MTD)-mice], and the vertical shows log[(potency × MTD)–rats]. Each dot represents 1 of the 87 NCI/NTP bioassays where the chemical on test was significant at the 0.025 level (one-sided) in female mice and in female rats. Data are for females only. Logs are to base 10.

tumor yields in rats and mice are correlated among chemicals that are carcinogenic in both species. A comparison of the models and data suggests that over 80% of the interspecies correlation in carcinogenic potencies for chemicals positive in both rats and mice can be explained by the interspecies correlation in toxicity (MTD) and the correlation between log(potency) and log(MTD) (Fig. 2). This confirms the findings of Bernstein et al. [35] and indicates that although there may be some basis for extrapolation from rodents to humans, the interspecies correlation of potencies between rats and mice does not say much about the validity of that extrapolation.

C. Regulatory Risk Assessment and the Constraint on Potency Estimation

Standard practice in regulatory risk assessment for a given rodent carcinogen is to extrapolate from the high doses of rodent bioassays to the low doses of most human exposures by multiplying carcinogenic potency in rodents by human exposure. Strikingly, however, because potency estimates are constrained to lie within a narrow range about the MTD, the "virtually safe dose" (VSD) usually estimated by regulatory agencies to give one cancer in a million can be approximated simply by using the MTD as a surrogate for carcinogenic potency. The VSD can be approximated from the MTD. Gaylor and Gold [38] used the ratio MTD/TD_{50} and the relationship between q_1^* and TD_{50} found by Krewski et al. [36] to estimate the VSD. The VSD was approximated by the MTD/740,000 for NCI/NTP rodent carcinogens. This result questions the utility of bioassay results to estimate risk and demonstrates the limited information about risk that is provided by bioassay results. The MTD/740,000 was within a factor of 10 of the VSD for 96% of carcinogens. Without data on mechanism of carcinogenesis for a given chemical, the true risk of cancer at a low dose is highly uncertain and could be zero, even for rats or mice.

D. Lifetable Versus Summary Estimates of Potency

We have compared two methods of statistical analysis for estimating carcinogenic potency from carcinogenesis bioassays: one based on lifetable data and one based on summary incidence data (the crude proportion of animals with tumors) [39]. The lifetable analysis adjusts for the differential effects of toxicity among dose groups and for differences in the time pattern of tumor incidence, whereas summary incidence analysis does not. However, summary data are all that are usually available in the published results of animal cancer tests. Using results for NCI/NTP bioassays, which

provide full lifetable data, we compared lifetable and summary estimates of potency; our updated analysis for the CPDB includes 551 experiments with statistically significant lifetable TD_{50} values ($p < .01$). The most potent site represents an experiment; the starting number of animals was used in the summary analysis. For 91% of the experiments, the level of statistical significance is the same for lifetable and summary analysis. There is substantial agreement between the two methods in terms of potency estimation, although lifetable estimates are usually more potent. The median ratio of lifetable TD_{50} to summary TD_{50} is 0.69, and 85% of the ratios lie between 0.30 and 1.30.

The dose-response curve shape was compared by testing for linearity using results from the respective model goodness-of-fit test. Among experiments with two doses and a control, lifetable and summary methods agree on the shape of the dose response for 72% of the experiments. As expected, more curves are linear or curving upward for lifetable analysis, which takes survival and latency into account [39]. We note that with either method of analysis, the shape of the dose-response curve may differ for different target sites in experiments with the same test agent [39].

E. Reproducibility

Reproducibility of results in animal bioassays has been investigated in "near-replicate" comparisons consisting of two or more tests of the same chemical administered by the same route and using the same sex and strain of rodent [40]. The updated results continue to show good reproducibility. Among 166 comparisons, 84% (139/166) are concordant with respect to the published authors' opinions about whether tumors were induced in the experiments (Table 6). For rats and mice, in all but 3 of the 74 positive comparisons, at least 1 target site is identical. TD_{50} values are within a factor of 2 of each other in 47% of the positive comparisons, within a factor of 4 in 78%, and within a factor of 10 in 95% (Table 7).

F. Summary Measures of Carcinogenic Potency

For over half the carcinogens in rats or mice, there is more than one positive experiment, and it is desirable to have a summary measure of potency. We evaluated three summary measures of TD_{50} for these cases (arithmetic, geometric, or harmonic mean) to determine how different results would be from using the most potent site to summarize potency [41]. These measures differ according to the weight, given outlying results. Our analysis indicates that the most potent TD_{50} value is similar to the average values (Table 8). We have also compared the most potent to the least potent TD_{50} from different positive experiments, and found that the distribution of

TABLE 6

Summary of Reproducibility of Positivity in "Near-Replicate" Comparisons[a] of Chronic Exposure Carcinogenesis Bioassays in Hamsters, Mice, and Rats, in the Carcinogenic Potency Database

	Number of comparisons (%)			
	All species	Hamsters	Mice	Rats
Discordant	27 (16%)	0 (0%)	11 (20%)	16 (15%)
Concordant positive	80 (48%)	6 (75%)	29 (54%)	45 (43%)
Concordant negative	59 (36%)	2 (25%)	14 (26%)	43 (42%)
Total	166 (100%)	8 (100%)	54 (100%)	104 (100%)

[a]A comparison consists of the results for two or more experiments of the same chemical administered by the same route to the same strain and sex of rodent.

the ratio of least to most potent values for all chemicals was similar to that for near-replicate comparisons (Table 7). This similarity to the results for near-replicate tests suggests that discrepant results for a chemical within a species are not an artifact of combining across strains, routes of administration, and sexes. Thus, for various purposes, one may wish to use different summary measures; however, it generally makes little difference whether the choice is the most potent site or a mean.

TABLE 7

Ratio of Least to Most Potent TD_{50} from Different Positive Experiments for Near-Replicate Comparisons[a] and All Chemicals with More Than One Positive Experiment in the Carcinogenic Potency Database

Ratio of least potent TD_{50} to most potent	Rats				Mice			
	Near-replicate tests		All chemicals		Near-replicate tests		All chemicals	
1-1.99	21	(47%)	102	(41%)	14	(48%)	117	(50%)
2-2.99	10	(22%)	36	(14%)	9	(31%)	50	(21%)
3-3.99	4	(9%)	29	(12%)	0	(0%)	15	(6%)
4-9.99	7	(15%)	49	(19%)	5	(17%)	34	(14%)
≥10	3	(7%)	36	(14%)	1	(4%)	20	(9%)
Total	45	(100%)	252	(100%)	29	(100%)	236	(100%)

[a]A comparison consists of the results for two or more experiments of the same chemical administered by the same route to the same strain and sex of rodent.

TABLE 8

Ratio of Harmonic, Geometric, and Arithmetic Means to Most Potent TD_{50} for Chemicals Positive in More Than One Experiment in the Carcinogenic Potency Database

Ratio of mean TD_{50} to most potent	Rats N=252			Mice N=236		
	H %	G %	A %	H %	G %	A %
1-1.99	85	69	56	92	77.5	72
2-2.99	12	15	19	7	14	12
3-3.99	2	7	7	0.5	6	5
4-9.99	1	7	13	0.5	2.5	9
≥10	0	2	5	0	0	2
Total	100%	100%	100%	100%	100%	100%

Note: H = ratio of harmonic mean to most potent TD_{50}; G = ratio of geometric mean to most potent TD_{50}; A = ratio of arithmetic mean to most potent TD_{50}.

IV. EXTRAPOLATION OF CARCINOGENICITY BETWEEN SPECIES

A. Concordance Between Rats and Mice

The use of bioassay results in risk assessment requires a qualitative species extrapolation from rats or mice to humans. The accuracy of this extrapolation is generally unverifiable, as data on humans are limited. However, it is feasible to examine the accuracy of extrapolations from mice to rats. If mice and rats are similar with respect to carcinogenesis, this provides some evidence in favor of interspecies extrapolations; conversely, if mice and rats are different, this casts doubt on the validity of extrapolations from mice to humans.

One measure of interspecies agreement is concordance, the percentage of chemicals that are classified the same way as to carcinogenicity in mice and rats (i.e., either tumors are induced in both species or in neither). Observed concordance in the CPDB is about 75% (Table 9), which may seem low because the experimental conditions are identical and the species are similar. The observed concordance is just an estimate based on limited data. We [42,43] show, by simulations for NCI/NTP bioassays (which also have an observed concordance of 75%), that a variety of models with quite different true concordances are consistent with the observed results. The bias in observed concordance can be either positive or negative: An observed con-

TABLE 9

Comparison of Carcinogenic Response in Rats and Mice for Chemicals Tested in Both Species in the Carcinogenic Potency Database

Rats	+			Rats	−
Mice	+			Mice	+
		190	73		
		67	229		
Rats	+			Rats	−
Mice	−			Mice	−

1. Of 559 chemicals, 59% are positive in at least one test, (190 + 67 + 73)/559.
2. Of 559 chemicals, 75% are concordant in carcinogenicity between rats and mice, (190 + 229)/559.

cordance of 75% can arise if the true concordance is anything between 20% and 100% [42,43]. In particular, observed concordance can seriously overestimate true concordance: Due to lack of power in the bioassay, many chemicals that are truly discordant (i.e., positive in one species but negative in the other) are classified as negative in both species and, hence, concordant. Thus, it seems unlikely that true concordance between rats and mice can be estimated with any reasonable degree of confidence from bioassay data.

B. Comparison of Carcinogenicity in Rodents and Nonhuman Primates

Lifetime studies in cynomolgus and/or rhesus monkeys (lasting up to 29 years) are included in the CPDB for 16 rodent carcinogens for which monkey studies have been completed [44]. Experimental protocols for the studies in nonhuman primates varied, but generally included 5–20 dosed animals of 1 or both monkey species and a large colony control. Compared to other chemicals in the CPDB, there is strong evidence of carcinogenic activity in rodents for the chemicals that were selected for studies in monkeys; that is, the test agents induce tumors in a high proportion of rats or mice, often in

a short period of time, at multiple target sites, and all but one are mutagenic. In the monkey studies, tumors were induced by 10 of the 16 rodent carcinogens (aflatoxin B_1, *N*-nitrosopiperidine, procarbazine·HCl, urethane, IQ, sterigmatocystin, cycasin and methylazoxymethanol acetate, *N*-methyl-*N*-nitrosourea, *N*-nitrosodipropylamine, and *N*-nitrosodiethylamine). Lack of power may account for the negative results for the other chemicals. Whereas dosing in monkeys was usually long term, in four of the six negative studies chemical administration was ended after 5 years (the experiments were continued to 20–26 years): 2-acetylaminofluorene, *N,N*-dimethyl-4-aminoazobenzene, 3′-methyl-4-dimethylaminoazobenzene, and 3-methylcholanthrene. For an additional chemical (*N*-nitrosodimethylamine), the MTD was exceeded, and animals were all dead by 10 years due to toxicity. Only one chemical, urethane, was positive with the short-dosing (5-year) protocol. One chemical, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), was negative in monkeys with a protocol of lifetime dosing; MNNG is a potent mutagen, methylates DNA, and is a potent carcinogen in rats. Long-term bioassays in monkeys with a chronic-dosing protocol are currently being completed on an additional seven rodent carcinogens: four potent mutagenic chemotherapy agents (adriamycin, melphalan, azathioprine, and cyclophosphamide), as well as sodium saccharin, sodium cyclamate, and DDT.

The evaluation of carcinogenicity was the same in cynomolgus and rhesus monkeys for the 11 chemicals tested in both species, and the liver was the most frequent target site in both [1].

We note that the spontaneous tumor rate in the large-colony control of both cynomolgus and rhesus monkeys is low compared to the rate in rat and mouse strains generally used in carcinogenesis bioassays. In monkeys, the tumor incidence rate in controls increases markedly with age, as in other species.

C. Carcinogen Identification by Testing in Two Sex-Species Groups of Rodents Instead of Four

The standard protocol used to identify chemicals as carcinogens calls for testing in both sexes of rats and mice. We examined the accuracy of predicting positive chemicals on the basis of using two instead of four sex-species groups [13,45] for the subset of chemicals in the CPDB that have been tested in four groups and are positive in at least 1 ($N=254$). Under the conditions of these bioassays, a very high proportion of rodent carcinogens that are identified as positive by tests in four groups is also identified by results from 1 sex of each species (85–91%), as shown in Table 10. The fact that a higher proportion of carcinogens is identified by pairs consisting of one sex from each species, rather than by two sexes of the same species, is due to the fact that among chemicals positive in at least one group, there

TABLE 10

Predictive Value of Two Sex-Species Groups for Rodent Carcinogens Tested in Both Sexes of Rats and Mice, in the Carcinogenic Potency Database[a]

	NCI/NTP or literature	NCI/NTP	Literature
Sex-species groups used to identify carcinogens[b]	Number identified as carcinogenic at least once (N=254)[c]	Number identified as carcinogenic at least once (N=178)[c]	Number identified as carcinogenic at least once (N=71)[c]
FM, MR	231 (91%)	160 (90%)	67 (94%)
MM, MR	231 (91%)	159 (89%)	69 (97%)
FM, FR	218 (86%)	146 (82%)	65 (92%)
MM, FR	215 (85%)	142 (80%)	68 (96%)
FM, MM	201 (79%)	136 (76%)	60 (85%)
FR, MR	193 (76%)	131 (74%)	60 (85%)

[a]For chemicals tested in both sexes of rats and mice that were evaluated as carcinogenic in at least one experiment in the Carcinogenic Potency Database.

[b]FM = female mice, MM = male mice, FR = female rats, MR = male rats.

[c]A chemical is classified as a carcinogen in this analysis if it was tested in male and female rats and mice, and evaluated as positive in at least one experiment. Percentage in each column indicates the percentage of those carcinogens that would have also been identified as carcinogenic if the experiments had been conducted only in the two sex-species groups listed in the first column.

is greater agreement (and therefore redundancy of information) between sexes within a species than between species. Overall, the combination of male rats with either female or male mice gives results most similar to those obtained in tests of four sex-species groups (Table 10).

We repeated the analysis separately for chemicals tested in the literature or by NCI/NTP because, compared to the overall CPDB, NCI/NTP bioassays are overrepresented in the subset of chemicals tested in four sex-species groups (Table 10). The same mouse strain and only a few rat strains are used in NCI/NTP bioassays. In studies published in the general literature, the proportions identified by one sex of each species are similarly high; as many strains are used in literature studies, this result provides some confidence in generalizing the finding beyond this data set.

The U.S. Food and Drug Administration (FDA) recently proposed [46] a reduced protocol for 2-year testing: using both sexes of only one species. Table 10 indicates that this would identify fewer of the rodent carcinogens

than a protocol of one sex of each species. We have examined the impact of such a reduced protocol separately for mutagens and nonmutagens because pharmaceutical development is primarily for nonmutagens. Among nonmutagenic carcinogens identified by testing in four groups, only 66% (71/108) are identified by tests in only male and female rats; in contrast, 94% are identified by tests in male mice and male rats, and 89% are identified by testing female mice and male rats. Therefore, the FDA proposal would likely miss many of the chemical carcinogens that could be identified using a different two-group design.

Other bioassay results can also be accurately obtained with tests in one sex of each species. Chemicals that are classified as "two-species carcinogens" or "multiple-site carcinogens" on the basis of results from four sex-species groups are also identified as two-species or multiple-site carcinogens on the basis of tests in one sex of each species [45]. Carcinogenic potency (TD_{50}) values for the most potent target site are similar when based on results from two compared to four sex-species groups. Eighty-eight percent of the potency values are within a factor of 2 of those obtained from tests in four sex-species groups, 95% are within a factor of 4, and 99% are within a factor of 10 [45]. This result is expected because carcinogenic potency values are constrained to a narrow range about the maximum dose tested in a bioassay, and the maximum doses administered to rats and mice are highly correlated and similar [35].

A reduced protocol of two instead of four sex-species groups would lower cost, use fewer animals, and, therefore, make histopathological examination less time-consuming and result in cost savings that would be available to conduct more mechanistic studies of a chemical, which would provide information on the relevance of a high-dose test result to low-dose human exposures.

V. TARGET ORGANS OF CARCINOGENICITY

Chemical carcinogens in chronic bioassays induce tumors in a variety of target sites in each species. Researchers interested in results on a particular target site can use the "Summary of the Carcinogenic Potency Database by Target Organ," to identify particular chemicals that induce tumors at each of 35 target sites (e.g., all chemicals that induce lung tumors in mice are listed under lung) [47]. Target organ results are reported for rats, mice, hamsters, monkeys, bush babies, and dogs. A quick overview by chemical of all target sites in each sex-species group is given by chemical in "Summary of the Carcinogenic Potency Database by Chemical" [3], which summarizes carcinogenic potency, positivity, and all target organs in each sex-species tested.

We showed in Table 5 that it is common for a chemical to induce tumors at more than one target site, that this result is more frequent in rats than mice, and that it is more frequent among mutagens than nonmutagens. The greater frequency of target sites among mutagens is consistent with the hypothesis that in high-dose rodent tests, increased cell division at the MTD is important in the carcinogenic response: Mutagens have a multiplicative interaction for carcinogenicity because they can both damage DNA directly and cause cell division at high doses.

The frequency of target organs among carcinogens in rats or mice for all chemicals in the CPDB, and separately for mutagens and nonmutagens, is reported in Table 11. Because tissue distribution and pharmacokinetics would not be expected to differ systematically between mutagens and nonmutagens, one would not expect systematic differences in the particular organs in which tumors are induced [48]. Results do not support the idea that mutagens and nonmutagens induce tumors in different target organs [48]. Both mutagens and nonmutagens induce tumors in a wide variety of sites, and most organs are target sites for both (Table 11). Moreover, the same sites tend to be the most common sites for both: 81% or more of both mutagenic and nonmutagenic carcinogens are positive in rats and in mice in at least one of the eight most frequent target sites (liver, lung, mammary gland, stomach, vascular system, kidney, hematopoietic system, and urinary bladder).

The liver is the most common target site in both species, and among mutagens as well as nonmutagens. It is the predominant site in the mouse. Our analysis indicates a species difference in the predominance of the liver as a target site in mice compared to rats [49,50]. Among chemicals with positive results in the mouse, 55% (88/161) of mutagens compared to 70% (75/107) of nonmutagens induce liver tumors; in the rat, the respective proportions are 38% (76/199) and 33% (34/104). Thus, whereas the proportion of rat carcinogens that are positive in the liver is similar for mutagens and nonmutagens, in mice a higher proportion of nonmutagenic than mutagenic carcinogens are positive in the liver. This finding in mice reflects the fact that chlorinated compounds (composed solely of chlorine, carbon, hydrogen, and, optionally, oxygen) are frequently positive in the mouse liver and are usually not mutagenic in *Salmonella*. Excluding the chlorinated compounds, results in mice are similar for mutagenic and nonmutagenic carcinogens: 55% (81/147) of mutagens and 59% (44/74) of nonmutagens are mouse liver carcinogens.

Knowing a target site in a bioassay is not expected to provide information about specific chemicals that will increase human cancer rates *at that same site*. Our analyses of bioassays in rats, mice, hamsters, as well as comparisons between rodents and humans for known human carcinogens indicate that if a chemical induces tumors at a given site in one species, it is positive and induces tumors *at the same site* in the other species no more than 50% of the time [35,51].

TABLE 11

Frequency of Target Organs Among Carcinogens in Rats or Mice in the Carcinogenic Potency Database by Mutagenicity in *Salmonella*

	Chemicals evaluated as carcinogenic in:					
	Rats			Mice		
Target Organ	All Chemicals[a] (N=461)[b]	Mutagens (N=199)	Nonmutagens (N=104)	All Chemicals[a] (N=370)	Mutagens (N=161)	Nonmutagens (N=107)
Liver	183 (40%)	76 (38%)	34 (33%)	207 (56%)	88 (55%)	75 (70%)
Lung	50 (11%)	27 (14%)	2 (2%)	100 (27%)	47 (29%)	16 (15%)
Mammary gland	93 (20%)	57 (29%)	7 (7%)	19 (5%)	11 (7%)	5 (5%)
Stomach	74 (16%)	43 (22%)	8 (8%)	52 (14%)	30 (19%)	10 (9%)
Kidney	71 (15%)	25 (13%)	28 (27%)	21 (6%)	9 (6%)	8 (7%)
Vascular system	28 (6%)	16 (8%)	2 (2%)	54 (15%)	32 (20%)	9 (8%)
Hematopoietic system	52 (11%)	27 (14%)	15 (14%)	47 (13%)	22 (14%)	13 (12%)
Urinary bladder	43 (9%)	23 (12%)	13 (13%)	12 (3%)	8 (5%)	1
Nasal cavity/turbinates	40 (9%)	18 (9%)	3 (3%)	5 (1%)	5 (3%)	
Esophagus	35 (8%)	13 (7%)	1	7 (2%)	5 (3%)	
Ear/Zymbal's gland	39 (8%)	30 (15%)	1	2	1	1
Skin	31 (7%)	20 (10%)	3 (3%)	3	3 (2%)	
Large intestine	30 (7%)	20 (10%)	1	1		
Thyroid gland	26 (6%)	12 (6%)	8 (8%)	15 (4%)	9 (6%)	5 (5%)
Small intestine	27 (6%)	18 (9%)	1	4 (1%)	2 (1%)	1
Oral cavity	27 (6%)	16 (8%)	4 (4%)	2	2 (1%)	
Uterus	21 (5%)	10 (5%)	4 (4%)	11 (3%)	8 (5%)	2 (2%)
Central nervous system	21 (5%)	14 (7%)	2 (2%)	3	2 (1%)	1
Peritoneal cavity	20 (4%)	12 (6%)	4 (4%)	7 (2%)	2 (1%)	
Clitoral gland	17 (4%)	16 (8%)	1	2		1
Harderian gland				13 (4%)	8 (5%)	3 (3%)
Adrenal gland	14 (3%)	6 (3%)	6 (6%)	8 (2%)	3 (2%)	5 (5%)
Preputial gland	8 (2%)	5 (3%)	3 (3%)	11 (3%)	3 (2%)	2 (2%)
Pancreas	16 (3%)	7 (4%)	5 (5%)			
Pituitary gland	8 (2%)	2 (1%)	5 (5%)	7 (2%)	2 (1%)	2 (2%)
Subcutaneous tissue	10 (2%)	7 (4%)		3	2 (1%)	
Ovary				8 (2%)	4 (2%)	4 (4%)
Testes	10 (2%)	6 (3%)	3 (3%)	2		2 (2%)
Spleen	7 (2%)	4 (2%)	2 (2%)			
Gall bladder				4 (1%)		
Bone	4	2 (1%)	1			
Prostate	3		1			
Mesovarium	2					
Myocardium				2		1
Vagina	1	1				

[a]The CPDB does not have mutagenicity evaluations for 158 rat carcinogens and 105 mouse carcinogens.

[b]Percentage of rat carcinogens or mouse carcinogens that induce tumors at the given site. Many chemicals induce tumors at more than one site, and these are counted at each relevant target site. Therefore, many chemicals are counted more than once, and percentages cannot be added. For example, of 199 rat carcinogens that are mutagenic in *Salmonella*, 76 induce liver tumors (i.e., 38%).

Potency values of chemicals that induce tumors at each common target site vary widely, as expected [49].

VI. RANKING POSSIBLE CARCINOGENIC HAZARDS TO HUMANS

Epidemiological studies have identified several factors that are likely to have a major effect on lowering rates of human cancer: reduction of smoking, increased consumption of fruits and vegetables, and control of infections. Other factors include avoidance of intense sun exposure, increased physical activity, reduction of high occupational exposures, and reduced consumption of alcohol and possibly red meat. Risks of many forms of cancer can already be lowered, and the potential for further risk reduction is great. In the United States, cancer death rates for all cancers combined are decreasing if lung cancer—90% of which is due to smoking—is excluded from the analysis [20]. We have discussed these epidemiological results with an emphasis on cancer mechanisms [20]. (See the article by Ames and Gold in this issue.)

A. Human Exposures to Natural and Synthetic Chemicals

Current regulatory policy to reduce cancer risk is based on the idea that chemicals which induce tumors in rodent cancer tests are potential human carcinogens; however, the chemicals tested for carcinogenicity in rodents have been primarily synthetic [1]. The enormous background of human exposures to natural chemicals has not been systematically examined. This has led to an imbalance in both data and perception about possible carcinogenic hazards to humans from chemical exposures. The regulatory process does not take into account the following: (1) that natural chemicals make up the vast bulk of chemicals to which humans are exposed; (2) that the toxicology of synthetic and natural toxins is not fundamentally different; (3) that about half of the chemicals tested, whether natural or synthetic, are carcinogens when tested using current experimental protocols; (4) that testing for carcinogenicity at near-toxic doses in rodents does not provide enough information to predict the excess number of human cancers that might occur at low-dose exposures; (5) that testing at the maximum tolerated dose (MTD) frequently can cause chronic cell killing and consequent cell replacement (a risk factor for cancer that can be limited to high doses), and that ignoring this effect in risk assessment greatly exaggerates risks.

Of chemicals to which humans are exposed, we estimate that 99.9% are naturally occurring [12]. Yet, public perceptions tend to identify *chemicals* as being only synthetic, and only synthetic chemicals as being toxic; however, every natural chemical is also toxic at some dose. We estimate that the daily average U.S. exposure to burnt material in the diet is about 2000 mg, and to natural pesticides (the chemicals that plants produce to defend themselves against fungi, insects, and animal predators) about 1500 mg [12]. In comparison, the total daily exposure to all synthetic pesticide residues combined is about 0.09 mg, based on the sum of residues reported by the FDA in their study of the 200 synthetic pesticide residues thought to be of greatest concern [52]. We estimate that humans ingest roughly 5000–10,000 different natural pesticides and their breakdown products [12]. Despite this enormously greater exposure to natural chemicals, among the chemicals tested for carcinogenicity 78% (1007/1298) are synthetic (i.e., do not occur naturally).

It has often been assumed that humans have evolved defenses against natural chemicals that will not protect against synthetic chemicals. However, humans, like other animals, are extremely well protected by defenses that are mostly general rather than specific for particular chemicals (e.g., continuous shedding of surface cells that are exposed) [12]. Additionally, most defense enzymes are inducible and are effective against both natural and synthetic chemicals, including potentially mutagenic reactive chemicals [53].

Because the toxicology of natural and synthetic chemicals is similar, one expects and finds a similar 50% positivity rate for carcinogenicity among synthetic and natural chemicals (Table 2). Therefore, because humans are exposed to so many more natural than synthetic chemicals (by weight and by number), human exposures to natural rodent carcinogens as defined by high-dose tests are probably ubiquitous and unavoidable [12,54]. Concentrations of natural pesticides in plants are usually measured in parts per thousand or million rather than parts per billion, which is the usual concentration of synthetic pesticide residues or water pollutants. A diet free of chemicals that induce tumors in high-dose animal cancer tests is impossible.

Even though only a tiny proportion of natural pesticides have been tested for carcinogenicity, 35 of 64 that have been tested are rodent carcinogens (Table 2) and commonly occur in plant foods and spices [12,53,55]. (See Table 2 in the article by Ames and Gold in this issue.)

Humans also ingest large numbers of natural chemicals from cooking food. For example, more than 1000 chemicals have been identified in roasted coffee. Only 28 have been tested for carcinogenicity according to the most recent results in our CPDB, and 19 of these are positive in at least one test (Table 12) totaling at least 10 mg of rodent carcinogens per cup [56–59]. Among the rodent carcinogens in coffee are the plant pesticides caffeic acid (present at 1800 ppm) [56], and catechol (present at 100 ppm) [60,61]. Two

TABLE 12

Carcinogenicity Status of Natural Chemicals in Roasted Coffee

Positive: N = 19	acetaldehyde, benzaldehyde, benzene, benzofuran, benzo(*a*)pyrene, caffeic acid, catechol, 1,2,5,6-dibenzanthracene, ethanol, ethylbenzene, formaldehyde, furan, furfural, hydrogen peroxide, hydroquinone, limonene, sttyrene, toluene, xylene
Not positive: N = 8	acrolein, biphenyl, choline, eugenol, nicotinamide, nicotinic acid, phenol, piperidine
Uncertain:	caffeine
Yet to test:	~ 1000 chemicals

other plant pesticides, chlorogenic acid and neochlorogenic acid (present at 21,600 ppm and 11,600 ppm, respectively) [56] are metabolized to caffeic acid and catechol but have not been tested for carcinogenicity. Chlorogenic acid and caffeic acid are mutagenic [62–64] and clastogenic [65,66]. Some other rodent carcinogens in coffee are products of cooking [e.g., furfural and benzo(*a*)pyrene]. The point here is not to indicate that rodent data necessarily implicate coffee as a risk factor for human cancer, but rather to illustrate that there is an enormous background of chemicals in the diet that are natural and that have not been a focus of attention for carcinogenicity testing.

B. The HERP Ranking of Possible Carcinogenic Hazards

In the above, we discussed that rodent bioassays provide little information about mechanisms of carcinogenesis and low-dose risk. Additionally, there is an imbalance in bioassay data because the vast proportion of test agents are synthetic chemicals, whereas the vast proportion of human exposures are to naturally occurring chemicals. Moreover, potency estimates based on bioassay results are bounded by the doses administered; therefore, regulatory risk estimates based on linear extrapolation are also bounded. Given these results, what is the best use that can be made of bioassay data in efforts to prevent human cancer? In several papers, we have emphasized that gaining a broad perspective about the vast number of chemicals to which humans are exposed can be helpful when setting research and regulatory priorities [53,67–69].

One reasonable strategy is to use a rough index to *compare* and *rank* possible carcinogenic hazards from a wide variety of chemical exposures at levels that humans typically receive, and then to focus on those that rank highest [68–70]. Ranking is a critical first step that can help to set priorities for selecting chemicals for chronic bioassay or mechanistic studies, for

epidemiological research, and for regulatory policy. Although one cannot say whether the ranked chemical exposures are likely to be of major or minor importance in human cancer, it is not prudent to focus attention on the possible hazards at the bottom of a ranking if, using the same methodology to identify hazard, there are numerous common human exposures with much greater possible hazards. Our analyses are based on the HERP index (Human Exposure/Rodent Potency), which indicates what percentage of the rodent carcinogenic potency (TD_{50} in mg/kg/day) a human receives from a given daily lifetime exposure (mg/kg/day). TD_{50} values in our CPDB span a 10^8-fold range across chemicals (Fig. 1) [1].

In general, the ranking by the simple HERP index will be similar to a ranking of regulatory "risk estimates." As we discussed earlier, the VSD is approximately equivalent to the ratio of the high dose in a bioassay divided by 740,000 [38].

Overall, our analyses have shown that HERP values for some historically high exposures in the workplace and some pharmaceuticals rank high, and that enormous background of naturally occurring rodent carcinogens in typical portions of common foods that casts doubt on the relative importance of low-dose exposures to residues of synthetic chemicals such as pesticides [68,69,71]. A committee of the National Research Council (NRC) recently reached similar conclusions about natural versus synthetic chemicals in the diet and called for further research on natural chemicals [72].

Our earlier HERP rankings were for typical exposures. In this paper, we rank HERP values for *average* U.S. exposures to rodent carcinogens for which both concentration data and average exposure data were available.

The average daily U.S. exposures in the ranking (Table 13) are ordered by possible carcinogenic hazard (HERP). Results are reported for average exposures to 25 natural chemicals in the diet (in boldface) and to 28 chemicals for which the exposure is not natural. Of these 28 chemicals, 5 occur naturally, but human exposure is primarily or exclusively from anthropogenic sources (e.g., benzene, chloroform, formaldehyde, TCDD, and tetrachloroethylene).

Three convenient reference points in the HERP ranking are as follows: the median HERP value in Table 13 of 0.001%; the upper bound risk estimate used by regulatory agencies is one in a million (using the q_1^* potency value derived from the linearized multistage model), that is, the VSD, which converts to a HERP of 0.00003% if based on a rat TD_{50} and 0.00001% if based on a mouse TD_{50}; and the background HERP of 0.0003% for the average chloroform level in a liter of U.S. tap water, which is formed as a by-product of chlorination.

The HERP ranking maximizes possible hazards to synthetic chemicals because it includes historically high exposure values that are now much lower

TABLE 13

Ranking Possible Carcinogenic Hazards from Average U.S. Exposures

Possible hazard: HERP (%)	Average daily US exposure	Human dose of rodent carcinogen	Potency TD_{50} (mg/kg/day)[a] Rats	Mice
140	EDB: workers (high exposure) (before 1977)	Ethylene dibromide, 150 mg	1.52	(7.45)
17	Clofibrate	Clofibrate, 2 g	169	.
14	Phenobarbital, 1 sleeping pill	Phenobarbital, 60 mg	(+)	6.09
6.8	1,3-Butadiene: rubber workers (1978-86)	1,3-Butadiene, 66.0 mg	(261)	13.9
6.1	Tetrachloroethylene: dry cleaners with dry-to-dry units (1980-90)[b]	Tetrachloroethylene, 433 mg	101	(126)
4.0	Formaldehyde: workers	Formaldehyde, 6.1 mg	2.19	(43.9)
2.1	Beer, 257 g	Ethyl alcohol, 13.1 ml	9110	(–)
1.4	Mobile home air (14 hours/day)	Formaldehyde, 2.2 mg	2.19	(43.9)
0.9	Methylene chloride: workers (1940s-80s)	Methylene chloride, 471 mg	724	(918)
0.5	Wine, 28.0 g	Ethyl alcohol, 3.36 ml	9110	(–)
0.4	Conventional home air (14 hours/day)	Formaldehyde, 598 μg	2.19	(43.9)
0.1	Coffee, 13.3 g	Caffeic acid, 23.9 mg	297	(4900)
0.04	Lettuce, 14.9 g	Caffeic acid, 7.90 mg	297	(4900)
0.03	Safrole in spices	Safrole, 1.2 mg	(441)	51.3
0.03	Orange juice, 138 g	d-Limonene, 4.28 mg	204	(–)
0.03	Pepper, black, 446 mg	d-Limonene, 3.57 mg	204	(–)
0.02	Mushroom (Agaricus bisporus 2.55 g)	Mixture of hydrazines, etc. (whole mushroom)	–	20,300
0.02	Apple, 32.0 g	Caffeic acid, 3.40 mg	297	(4900)
0.02	Coffee, 13.3 g	Catechol, 1.33 mg	118	(244)
0.02	Coffee, 13.3 g	Furfural, 2.09 mg	(683)	197
0.009	BHA: daily US avg (1975)	BHA, 4.6 mg	745	(5530)
0.008	Beer (before 1979), 257 g	Dimethylnitrosamine, 726 ng	0.124	(0.189)
0.008	Aflatoxin: daily US avg (1984-89)	Aflatoxin, 18 ng	0.0032	(+)
0.007	Cinnamon, 21.9 mg	Coumarin, 65.0 μg	13.9	(103)
0.006	Coffee, 13.3 g	Hydroquinone, 333 μg	82.8	(225)
0.005	Saccharin: daily US avg (1977)	Saccharin, 7 mg	2140	(–)
0.005	Carrot, 12.1 g	Aniline, 624 μg	194[c]	(–)
0.004	Potato, 54.9 g	Caffeic acid, 867 μg	297	(4900)
0.004	Celery, 7.95 g	Caffeic acid, 858 μg	297	(4900)
0.004	White bread, 67.6 g	Furfural, 500 μg	(683)	197
0.003	Nutmeg, 27.4 mg	d-Limonene, 466 μg	204	(–)
0.003	Conventional home air (14 hour/day)	Benzene, 155 μg	(169)	77.5
0.002	Carrot, 12.1 g	Caffeic acid, 374 μg	297	(4900)
0.002	Ethylene thiourea: daily US avg (1990)	Ethylene thiourea, 9.51 μg	7.9	(23.5)
0.002	[DDT: daily US avg (before 1972 ban)]	[DDT, 13.8 μg]	(84.7)	12.3
0.001	Plum, 2.00 g	Caffeic acid, 276 μg	297	(4900)
0.001	BHA: daily US avg (1987)	BHA, 700 μg	745	(5530)
0.001	Pear, 3.29 g	Caffeic acid, 240 μg	297	(4900)
0.001	[UDMH: daily US avg (1988)]	[UDMH, 2.82 μg (from Alar)]	(–)	3.96
0.0009	Brown mustard, 68.4 mg	Allyl isothiocyanate, 62.9 μg	96	(–)
0.0008	[DDE: daily US avg (before 1972 ban)]	[DDE, 6.91 μg]	(–)	12.5
0.0007	TCDD: daily US avg (1994)	TCDD, 12.0 pg	0.0000235	(0.000156)
0.0007	Bacon, 11.5 g	Diethylnitrosamine, 11.5 ng	0.0237	(+)
0.0006	Mushroom (Agaricus bisporus 2.55 g)	Glutamyl-p-hydrazino-benzoate, 107 μg	.	277
0.0005	Jasmine tea, 2.19 g	Benzyl acetate, 504 μg	(–)	1440
0.0004	Bacon, 11.5 g	N-Nitrosopyrrolidine, 196 ng	(0.799)	0.679
0.0004	Bacon, 11.5 g	Dimethylnitrosamine, 34.5 ng	0.124	(0.189)

(continued)

TABLE 13 (Continued)

Possible hazard: HERP (%)	Average daily US exposure	Human dose of rodent carcinogen	Potency TD_{50} (mg/kg/day)[a] Rats	Mice
0.0004	[EDB: Daily US avg (before 1984 ban)]	[EDB, 420 ng]	1.52	(7.45)
0.0004	Tap water, 1 liter (1987-92)	Bromodichloromethane, 13 μg	(72.5)	47.7
0.0003	Mango, 1.22 g	d-Limonene, 48.8 μg	204	(–)
0.0003	Beer, 257 g	Furfural, 39.9 μg	(683)	197
0.0003	Tap water, 1 liter (1987-92)	Chloroform, 17 μg	(262)	90.3
0.0003	Carbaryl: daily US avg (1990)	Carbaryl, 2.6 μg	14.1	(–)
0.0002	Celery, 7.95 g	8-Methoxypsoralen, 4.86 μg	32.4	(–)
0.0002	Toxaphene: daily US avg (1990)	Toxaphene, 595 ng	(–)	5.57
0.00009	Mushroom (Agaricus bisporus, 2.55 g)	p-Hydrazinobenzoate, 28 μg	.	454[c]
0.00008	PCBs: daily US avg (1984-86)	PCBs, 98 ng	1.74	(9.58)
0.00008	DDE/DDT: daily US avg (1990)	DDE, 659 ng	(–)	12.5
0.00007	Parsnip, 54.0 mg	8-Methoxypsoralen, 1.57 μg	32.4	(–)
0.00007	Toast, 67.6 g	Urethane, 811 ng	(41.3)	16.9
0.00006	Hamburger, pan fried, 85 g	PhIP, 176 ng	4.29[c]	(28.6[c])
0.00005	Estragole in spices	Estragole, 1.99 μg	.	51.8
0.00005	Parsley, fresh, 324 mg	8-Methoxypsoralen, 1.17 μg	32.4	(–)
0.00003	Hamburger, pan fried, 85 g	MeIQx, 38.1 ng	1.99	(24.3)
0.00002	Dicofol: daily US avg (1990)	Dicofol, 544 ng	(–)	32.9
0.00001	Cocoa, 3.34 g	α-Methylbenzyl alcohol, 4.3 μg	458	(–)
0.00001	Beer, 257 g	Urethane, 115 ng	(41.3)	16.9
0.000005	Hamburger, pan fried, 85 g	IQ, 6.38 ng	1.89[c]	(19.6)
0.000001	Lindane: daily US avg (1990)	Lindane, 32 ng	(–)	30.7
0.0000004	PCNB: daily US avg (1990)	PCNB (Quintozene), 19.2 ng	(–)	71.1
0.0000001	Chlorobenzilate: daily US avg (1989)	Chlorobenzilate, 6.4 ng	(–)	93.9
<0.00000001	Chlorothalonil: daily US avg (1990)	Chlorothalonil, <6.4 ng	828[d]	(–)
0.000000008	Folpet: daily US avg (1990)	Folpet, 12.8 ng	.	2280[d]
0.000000006	Captan: daily US avg (1990)	Captan, 11.5 ng	2690[d]	(2730[d])

Note: Chemicals that occur naturally in foods are in bold. *Daily human exposure*: Reasonable daily intakes are used to facilitate comparisons. The calculations assume a daily dose for a lifetime. *Possible hazard*: The human dose of rodent carcinogen is divided by 70 kg to give a mg/kg/day of human exposure, and this dose is given as the percentage of the TD_{50} in the rodent (mg/kg/day) to calculate the Human Exposure/Rodent Potency index (HERP). TD_{50} values used in the HERP calculation are averages calculated by taking the harmonic mean of the TD_{50}s of the positive tests in that species from the Carcinogenic Potency Database. Average TD_{50} values have been calculated separately for rats and mice, and the more potent value is used for calculating possible hazard.

[a]A dot indicates no data in CPDB; a number in parentheses indicates a TD_{50} value not used in the HERP calculation because TD_{50} is less potent than in the other species. (–) = negative in cancer test; (+) = positive in cancer test(s) not suitable for calculating a TD_{50}.

[b]This is not an average, but a reasonably large sample (1027 workers).

[c]TD_{50} harmonic mean was estimated for the base chemical from the hydrochloride salt.

[d]Additional data from the EPA that is not in the CPDB were used to calculate these TD_{50} harmonic means.

(e.g., DDT, PCBs, occupational exposures). Additionally, the values for dietary exposures to synthetic chemicals are averages in the *total diet*, whereas for many natural chemicals, the exposures are for individual foods (i.e., the exposures for which concentration data were available).

Table 13 indicates that many ordinary foods would not pass the regulatory criteria used for synthetic chemicals. For many natural chemicals, the HERP values are in the top half of the table, even though natural chemicals are markedly underrepresented because so few have been tested in rodent bioassays. We discuss several categories of exposure below and indicate that mechanistic data are available for some chemicals, which suggest that the chemical would not be expected to be a cancer hazard at the doses to which humans are exposed; thus, their ranking by HERP would not be relevant.

C. Occupational and Pharmaceutical Exposures

Occupational and pharmaceutical exposures to some chemicals have been high, and most of the single-chemical agents or industrial processes evaluated as human carcinogens have been identified by high-dose exposures in the workplace [73]. HERP values rank at the top of Table 13 for chemical exposures in some occupations for which average exposure data were available: ethylene dibromide, 1,3-butadiene, tetrachloroethylene, and formaldehyde. When exposures are high, comparatively little quantitative extrapolation is required from high-dose rodent tests to those occupational exposures. The issue of how much human cancer can be attributed to occupational exposure has been controversial, but a few percent seems a reasonable estimate [20].

In another analysis, we used Permitted Exposure Limits (PELs) of the U.S. Occupational Safety and Health Administration as surrogates for actual exposures and compared the permitted daily dose rate for workers with the TD_{50} (PERP index, Permitted Exposure/Rodent Potency) [71,74]. We found that PELs for 9 chemicals were greater than 10% of the rodent carcinogenic dose, and for 27, they were between 1% and 10% of the rodent dose. For trichloroethylene, we recently conducted an analysis based on an assumed cytotoxic mechanism of action and using PBPK-effective dose estimates defined as peak concentrations. Our estimates indicate that for occupational respiratory exposures, the PEL for trichloroethylene would produce metabolite concentrations that exceed an acute no-observed-effect level for hepatotoxicity in mice. On this basis, the PEL is not expected to be protective. This contrasts with our finding that the EPA maximum concentration limit (MCL) in drinking water of 5 μg/L based on a linearized multistage model, is more stringent than our MCL based on a 1000-fold safety factor, which is 210 μg/L [75].

Some pharmaceuticals are also clustered near the top of the HERP ranking; we note that half the drugs reported in the *Physician's Desk Reference* with cancer test data are positive in rodent bioassays [10]. Most drugs, however, are used for only short periods and would not be comparable to HERP values, which are for lifetime exposures.

D. Natural Pesticides

Because few have been tested, natural pesticides are markedly underrepresented in our analysis. Importantly, for each plant food listed, there are about 50 additional untested natural pesticides. Although ~10,000 natural pesticides and their breakdown products occur in the human diet [12], only 64 have been tested adequately in rodent bioassays (Table 2). Average exposures to many natural-pesticide rodent carcinogens in common foods rank above or close to the median, ranging up to a HERP of 0.1%. These include caffeic acid (lettuce, apple, pear, coffee, plum, celery, carrot, potato), safrole (in spices), allyl isothiocyanate (mustard), *d*-limonene (mango, orange juice, black pepper), estragole (in spices), hydroquinone and catechol in coffee, and coumarin in cinnamon. Some natural pesticides in the commonly eaten mushroom (*Agaricus bisporus*) are rodent carcinogens (glutamyl-*p*-hydrazinobenzoate, *p*-hydrazinobenzoate), and the HERP based on feeding whole mushrooms to mice is 0.02%. For *d*-limonene, no human risk is anticipated because tumors are induced only in male rat kidney tubules with involvement of α_{2u}-globulin nephrotoxicity, which does not appear to be possible in humans [76,77].

E. Synthetic Pesticides

Synthetic pesticides currently in use that are rodent carcinogens and quantitatively detected by the U.S. FDA as residues in food are all included in Table 13. Most are at the bottom of the ranking, but HERP values are about at the median for ethylene thiourea (ETU), UDMH (from Alar) before its discontinuance, and DDT before its ban in the United States in 1972. These rank below the HERP values for many naturally occurring chemicals. For ETU, the value would be about 10 times lower if the potency value of the Environmental Protection Agency (EPA) were used instead of our TD_{50}; EPA combined rodent results from more than one experiment, including one at a lower dose in which ETU was administered in utero, and obtained a lower potency [78]. Additionally, EPA has recently discontinued some uses of fungicides for which ETU is a breakdown product, and consumption is

lower. DDT and similar early pesticides have been a concern because of their unusual lipophilicity and persistence, although there is no convincing epidemiological evidence of a carcinogenic hazard to humans [79]. Current exposure to DDT is in foods of animal origin, and the HERP value is low, 0.00008%.

In 1984, the U.S. EPA banned the agricultural use of ethylene dibromide (EDB), the main fumigant in the United States, because of the residue levels found in grain; HERP = 0.0004%. This HERP value ranks low, whereas the HERP of 140% for the high exposures to EDB that some workers received in the 1970s is at the top of the ranking [68].

Three synthetic pesticides, captan, chlorothalonil, and folpet, were evaluated in 1987 by the National Research Council (NRC) as being of relatively high risk to humans [80] and were also reported by FDA in the Total Diet Study (TDS). The contrast between the low-ranking HERP values for these pesticides (i.e., the lowest HERP values in Table 9) and the high-risk estimates of the 1987 NRC report is due to exposure estimates, which differ by more than 10^5-fold. Whereas the FDA used dietary intake estimates based on monitoring food as eaten, the NRC used the EPA Theoretical Maximum Residue Contribution (TMRC), which is a hypothetical maximum exposure estimate based on worst-case assumptions for the maximally exposed individual. For example, the EPA TMRC assumes that every pesticide registered for use on a food commodity is used on every crop, despite the fact that, for example, 54 insecticides are registered for tomatoes but the maximum used in California by any grower was 5, and among all growers, 52% used 2 or fewer insecticides and 31% used none [81]. Hence, using hypothetical maxima results in enormously higher risk estimates than using measured residues. We note that among synthetic pesticides, UDMH and ETU rank highest in HERP, and that exposures to these two chemicals are closer to the TMRC than other pesticides. Neither was measured in the TDS prior to 1990 in spite of positive rodent bioassays dating to 1968 for UDMH and 1973 for ETU [81].

F. Cooking and Preparation of Food

Cooking and preparation of food can also produce chemicals that are rodent carcinogens. Alcoholic beverages are a human carcinogen, and the HERP values in Table 13 for alcohol in beer (2.1%) and wine (0.5%) are high in the ranking. Ethyl alcohol is one of the least potent rodent carcinogens in the CPDB, but the HERP is high because of high concentrations and high U.S. consumption. Another fermentation product, urethane (ethyl carbamate), has a HERP value of 0.00001% in average beer consumption; for average bread consumption (as toast), the HERP would be 0.00007%.

Cooking food is plausible as a contributor to cancer. A wide variety of chemicals are formed during cooking. Rodent carcinogens formed include furfural and similar furans, nitrosamines, polycyclic hydrocarbons, and heterocyclic amines. Furfural, a chemical formed naturally when sugars are heated, is a widespread constituent of food flavor. The HERP value for furfural in average consumption of coffee is 0.02% and in white bread is 0.004%. Nitrosamines formed from nitrite or nitrogen oxides (NO_x) and amines in food can give moderate HERP values; for example, in bacon, the HERP for diethylnitrosamine is 0.0007% and for dimethylnitrosamine it is 0.0004%. A variety of mutagenic and carcinogenic heterocyclic amines (HA) are formed when meat, chicken, or fish are cooked, particularly when charred. Compared to other rodent carcinogens, there is strong evidence of carcinogenicity for HAs in terms of positivity rates and multiplicity of target sites; however, concordance in target sites between rats and mice is generally restricted to the liver [70]. Under usual cooking conditions, exposures to HA are in the low parts-per-billion range. HERP values for HA in pan-fried hamburger range from 0.00006% for PhIP to 0.000005% for IQ (Table 13). PhIP induces colon tumors in male but not female rats. A recent study indicates that whereas the level of DNA adducts in the colonic mucosa was the same in both sexes, cell proliferation was increased only in the male, contributing to the formation of premalignant lesions of the colon [82]. Therefore, there was no correlation between adduct formation and premalignant lesions, but there was between cell division and lesions.

G. Food Additives

Food additives can be either naturally occurring rodent carcinogens (e.g., allyl isothiocyanate and alcohol) or synthetic rodent carcinogens [butylated hydroxyanisole (BHA) and saccharin; Table 13]. The highest HERP values for average exposures to synthetic rodent carcinogens in Table 13 are for exposures in the 1970s to BHA (0.009%) and saccharin (0.005%), both nongenotoxic rodent carcinogens. For both of these additives, data on mechanism of carcinogenesis strongly suggest that there would be no risk to humans at the levels found in food.

BHA is a phenolic antioxidant that is Generally Regarded as Safe (GRAS) by the U.S. FDA. By 1987, after BHA was shown to be a rodent carcinogen, its use declined sixfold (HERP = 0.001%) [83]; this was due to voluntary replacement by other antioxidants and to the fact that the use of animal fats and oils, in which BHA is primarily used as an antioxidant, has consistently declined in the United States. The mechanistic and carcinoge-

nicity results on BHA indicate that malignant tumors were induced only at a dose above the MTD at which cell division was increased in the forestomach, which is the only site of tumorigenesis; the proliferation is only at high doses and is dependent on continuous dosing until late in the experiment [84]. Humans do not have a forestomach. We note that the dose response for BHA curves sharply upward, but the potency value used in HERP is based on a linear model; if the California EPA potency value (which is based on a linearized multistage model) were used in HERP instead of TD_{50}, the HERP values for BHA would be 25 times lower [85].

For saccharin, which has largely been replaced by other sweeteners, there is convincing evidence that the induced bladder tumors in rats are not relevant to human dietary exposures. The carcinogenic effect requires high doses of sodium saccharin which form calculi in the bladder, and subsequent regenerative hyperplasia. Thus, tumor development is due to increased cell division, and if the dose is not high enough to produce calculi, then there is no increased cell division and no increased risk of tumor development [86].

A recently compiled FDA database on food additives will permit expanded investigation of HERP values for chemicals that are rodent carcinogens [87].

H. Mycotoxins

Of the 23 fungal toxins tested for carcinogenicity, 14 are positive (61%) (Table 2). The mutagenic mold toxin, aflatoxin, which is found in moldy peanut and corn products, interacts with chronic hepatitis infection in human liver cancer development. There is a synergistic effect in the human liver between aflatoxin (genotoxic effect) and the hepatitis B virus (cell division effect) in the induction of liver cancer [88]. The HERP value for aflatoxin of 0.008% is based on the rodent potency. If the lower human potency value calculated by U.S. FDA from epidemiological data were used instead, the HERP would be about 10-fold lower [89]. Biomarker measurements of aflatoxin on populations in Africa and China, which have high rates of both hepatitis B and C viruses and liver cancer, confirm that those populations are chronically exposed to high levels of aflatoxin [90,91]. Liver cancer is rare in the United States. Although hepatitis B and C viruses infect less than 1% of the U.S. population, hepatitis viruses can account for half of liver cancer cases among non-Asians and even more among Asians [92].

Ochratoxin A, a rodent carcinogen, has been measured in Europe and Canada in agricultural and meat products. An estimated exposure of 1 ng/kg/day would have a HERP value at the median of Table 13 [93,94].

I. Synthetic Contaminants

Polychlorinated biphenyls (PCBs) and tetrachlorodibenzo-*p*-dioxin (TCDD), which have been a concern because of their environmental persistence and carcinogenic potency in rodents, are primarily consumed in foods of animal origin. In the United States, PCBs are no longer used, but exposure persists. Consumption in food in the United States declined about 20-fold between 1978 and 1986 [95,96]. The HERP value for the most recent reporting of the U.S. FDA Total Diet Study (1984–86) is 0.00008%, toward the bottom of the ranking, and far below many values for naturally occurring chemicals in common foods. It has been reported that some countries may have higher intakes of PCBs than the United States [97].

TCDD, the most potent rodent carcinogen, is produced naturally by burning when chloride ion is present (e.g., in forest fires). The sources of human exposure appear to be predominantly anthropogenic (e.g., from incinerators) [98]. TCDD has received enormous scientific and regulatory attention, most recently in an ongoing assessment by the U.S. EPA [98–100]. Some epidemiologic studies suggest an association with human cancer, but the evidence is not sufficient to establish causality. Estimation of average U.S. consumption is based on limited sampling data, and the EPA is currently conducting further studies of concentrations in food. The HERP value of 0.0007% is near the median of the values in Table 13. TCDD exerts many or all of its harmful effects in mammalian cells through binding to the Ah receptor. A wide variety of natural substances also bind to the Ah receptor (e.g., tryptophan oxidation products), and insofar as they have been examined, they have properties similar to TCDD [53]. For example, a variety of flavones and other plant substances in the diet and their metabolites also bind to the Ah receptor [e.g., indole carbinol (IC)]. IC is the main breakdown compound of glucobrassicin, a glucosinolate that is present in large amounts in vegetables of the *Brassica* genus, including broccoli, and gives rise to the potent Ah binder, indole carbazole [101].

Caution is necessary in drawing conclusions from the occurrence in the diet of natural chemicals that are rodent carcinogens. It is not argued here that these dietary exposures are necessarily of much relevance to human cancer. In fact, epidemiological results indicate that adequate consumption of fruits and vegetables reduces cancer risk at many sites, and that protective factors like intake of vitamin C and folic acid are important, rather than intake of individual rodent carcinogens. Our analysis does indicate that widespread exposures to naturally occurring rodent carcinogens cast doubt on the relevance to human cancer of low-level exposures to synthetic rodent carcinogens. Our results call for a reevaluation of the utility of animal cancer tests done at the MTD for providing information that is useful in protect-

ing humans against low-level exposures in the diet when a high percentage of both natural and synthetic chemicals appear to be rodent carcinogens at the MTD, when the data from rodent bioassays is not adequate to assess low dose risk, and when the ranking on an index of possible hazards demonstrates that there is an enormous background of natural chemicals in the diet that rank high, even though so few have been tested in rodent bioassays.

Our discussion of the HERP ranking indicates the importance of data on the mechanism of carcinogenesis for each chemical. For several chemicals, mechanistic data have recently been generated which indicates that they would not be expected to be a risk to humans at the levels consumed in food (e.g., saccharin, BHA, chloroform, *d*-limonene, as discussed above). Standard practice in regulatory risk assessment for chemicals that induce tumors in high-dose rodent bioassays has been to extrapolate risk to low dose in humans by multiplying potency by human exposure. Without data on the mechanism of carcinogenesis, however, the true human risk of cancer at low dose is highly uncertain and could be zero [22,68,102,103]. Adequate risk assessment from animal cancer tests requires more information for a chemical, about pharmacokinetics, mechanism of action, cell division, induction of defense and repair systems, and species differences. The EPA has recently proposed new cancer risk assessment guidelines [104] that emphasize a more flexible approach to risk assessment and call for use of more biological information in the weight-of-evidence evaluation and dose-response assessment. These proposed changes recognize the dose dependence of many toxicokinetic and metabolic processes and the importance of understanding cancer mechanisms for a given chemical. The proposed guidelines permit the use of nonlinear approaches to low-dose extrapolation if warranted by mechanistic data and a possible threshold of dose below which effects will not occur [104,105].

VII. FUTURE DIRECTIONS

Our analysis in this paper suggests several areas for further research into diet and cancer, including epidemiological, toxicological, and biochemical investigations. Further understanding of the role and mechanism of endogenous damage could lead to new prevention strategies for cancer. Present epidemiological evidence regarding the role of greater antioxidant consumption in human cancer prevention is inconsistent [20]. Nevertheless, biochemical data indicate the need for further investigation of the wide variety of potentially effective antioxidants, both natural and synthetic. Evidence supporting this need includes the enormous endogenous oxidative damage to

DNA, proteins, and lipids, as well as indirect evidence such as increased oxidative damage to human sperm DNA when dietary ascorbate is insufficient. Moreover, studies on the importance of dietary fruits and vegetables in cancer suggest the importance of further work on micronutrient deficiency as a major contributor to cancer. Studies in rodents and humans suggest further work on caloric intake and body weight, and the effects on hormonal status.

Because naturally occurring chemicals in the diet have not been a focus of cancer research, it seems reasonable to investigate some of them further as possible hazards because they often occur at high concentrations in foods. Only a small proportion of the many chemicals to which humans are exposed will ever be investigated, and there is at least some toxicological plausibility that high-dose exposures may be important. In order to identify untested dietary chemicals that might be a hazard to humans *if* they were to be identified as rodent carcinogens, we have used an index, HERT (the ratio of Human Exposure/Rodent Toxicity), which is analogous to HERP. HERT uses readily available LD_{50} values rather than the TD_{50} values from animal cancer tests that are used in HERP. This approach to prioritizing chemicals makes assessment of human exposure levels critical at the outset. The validity of the HERT approach is supported by three analyses. First, we have found that for the exposures to rodent carcinogens for which we have calculated HERP values, the ranking by HERP and HERT are highly correlated [55]. Second, we have shown that without conducting a 2-year bioassay, the regulatory VSD can be approximated by dividing the MTD by 740,000 [38]. Because the MTD is not known for all chemicals, and MTD and LD_{50} are both measures of toxicity, acute toxicity (LD_{50}) can reasonably be used as a surrogate for chronic toxicity (MTD). Third, LD_{50} and carcinogenic potency are correlated [106,107]; therefore, HERT is a reasonable surrogate index for HERP because it simply replaces TD_{50} with LD_{50} [55].

We have calculated HERT values using LD_{50} values as a measure of toxicity in combination with available data on concentrations of untested natural chemicals in commonly consumed foods and data on average consumption of those foods in the U.S. diet. We considered any chemical with available data on rodent LD_{50} that had a published concentration $\geq$ 10 ppm in a common food, and for which estimates of average U.S. consumption of that food were available. Among the set of 171 HERT values we were able to calculate, the HERT ranged across 7 orders of magnitude [55].

It might be reasonable to investigate further the chemicals in the diet that rank highest on the HERT index and that have not been adequately tested in chronic carcinogenicity bioassays in rats and mice. These include solanine and chaconine, the main alkaloids in potatoes, which are cholinesterase inhibitors that can be detected in the blood of almost all people [108–110], chlorogenic acid (a precursor of caffeic acid), and caffeine, for which no

standard lifetime study has been conducted in mice. In rats, cancer tests of caffeine have been negative, but one study that was inadequate because of early mortality showed an increase in pituitary adenomas [111].

Compelling theoretical reasons as well as data from a large body of experiments indicate that the prediction of carcinogenic risk to humans at low dose must take cell division into account. Just evaluating a chemical as a rodent carcinogen without considering the mechanism of action can be fundamentally misleading for low-dose risk assessment. Defenses are inducible at low doses, and even for mutagens, it may be that the increment in DNA damage over the enormous rate of endogenous background damage may not be significant. Many nonmutagens will have a threshold and there will be no risk at low dose. It is clear that the mechanisms of action for all rodent carcinogens are not the same. For some chemicals, there is evidence to support cell division effects unique to high doses (e.g., saccharin) and thus there appears to be a threshold. For others (e.g., butadiene and 2-acetylaminofluorene), there may well be multiplicative effects due to an interaction of cell division and DNA damage, but carcinogenic effects have been found considerably below the MTD. Sometimes, the mechanism leading to cell division and carcinogenesis in a rodent species has no analogy in humans (e.g., kidney tumors in male Fischer rats due to α_{2u}-globulin). Studies of mechanism in rodent bioassays would help to clarify such differences.

As currently conducted, rodent bioassays do not provide the information necessary to extrapolate from high to low dose.

It would be of particular interest to reevaluate some of the rodent carcinogens that are receiving extensive regulatory attention on the basis of standard risk assessment methodology (e.g., trichloroethylene). Measurement of cell division at and below bioassay doses in subchronic studies for these chemicals would permit a reinterpretation of the rodent data and an improved assessment of the potential risk to humans at low dose.

VIII. SUMMARY

Many important issues in carcinogenesis can be addressed using our Carcinogenic Potency Database, which analyzes and standardizes the literature of chronic carcinogenicity tests in laboratory animals. This review is an update and overview of our analyses during the past 15 years, using the current database that includes results of 5152 experiments on 1298 chemicals. We address the following:

1. More than half the 1298 chemicals tested in long-term experiments have been evaluated as carcinogens. We describe this positivity rate

for several subsets of the data (including naturally occurring and synthetic chemicals), and we hypothesize an important role in the interpretation of results for increased cell division due to administration of high doses.

2. Methodological issues in the interpretation of animal cancer tests: constraints on the estimation of carcinogenic potency and validity probloms associated with using the limited data from bioassays to estimate human risk, reproducibility of results in carcinogenesis bioassays, comparison of lifetable and summary methods of analysis, and summarizing carcinogenic potency when multiple experiments on a chemical are positive.
3. Positivity is compared in bioassays for two closely related species, rats and mice, tested under similar experimental conditions. We assess what information such a comparison can provide about interspecies extrapolation.
4. Rodent carcinogens induce tumors in 35 different target organs. We describe the frequency of chemicals that induce tumors in rats or mice at each target site, and we compare target sites of mutagenic and nonmutagenic rodent carcinogens.
5. A broad perspective on evaluation of possible cancer hazards from rodent carcinogens is given, by ranking 74 human exposures (natural and synthetic) on the HERP indes.

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REFERENCES

1. L. S. Gold, T. H. Slone, B. N. Ames, N. B. Manley, G. B. Garfinkel, and L. Rohrbach, in *Handbook of Carcinogenic Potency*

and Genotoxicity Databases (L. S. Gold and E. Zeiger, eds.), CRC Press, Boca Raton, FL, 1997, pp. 1–605.
2. L. S. Gold, T. H. Slone, and B. N. Ames, in *Handbook of Carcinogenic Potency and Genotoxicity Databases* (L. S. Gold and E. Zeiger, eds.), CRC Press, Boca Raton, FL, 1997, pp. 661–685.
3. L. S. Gold, T. H. Slone, and B. N. Ames, in *Handbook of Carcinogenic Potency and Genotoxicity Databases* (L. S. Gold and E. Zeiger, eds.), CRC Press, Boca Raton, FL, 1997, pp. 621–660.
4. C. Sawyer, R. Peto, L. Bernstein, and M. C. Pike, *Biometrics, 40*, 27–40 (1984).
5. R. Peto, M. C. Pike, L. Bernstein, L. S. Gold, and B. N. Ames, *Environ. Health Perspect., 58*, 1–8 (1984).
6. J. A. Sontag, N. P. Page, and U. Saffiotti. Guidelines for carcinogen bioassay in small rodents. 1976 (Carcinogenesis Tech. Rep. Ser. No. 1, DHEW Pub. No. (NIH) 76-801).
7. E. Zeiger, in *Handbook of Carcinogenic Potency and Genotoxicity Databases* (L. S. Gold and E. Zeiger, eds.), CRC Press, Boca Raton, FL, 1997, pp. 687–729.
8. L. E. Kier, D. J. Brusick, A. E. Auletta, E. S. Von Halle, M. M. Brown, V. F. Simmon, V. Dunkel, J. McCann, K. Mortelmans, M. Prival, T. K. Rao, and V. Ray, *Mutat. Res., 168*, 69–240 (1986).
9. A. E. Auletta, personal communication.
10. T. S. Davies and A. Monro, *J. Am. Coll. Toxicol., 14*, 90–107 (1995).
11. J. P. J. Van Oosterhout, J. W. Van Der Laan, E. J. De Waal, K. Olejniczak, M. Hilgenfeld, V. Schmidt, and R. Bass, *Regul. Toxicol. Pharmacol., 25*, 6–17 (1997).
12. B. N. Ames, M. Profet, and L. S. Gold, *Proc. Natl. Acad. Sci. USA, 87*, 7777–7781 (1990).
13. L. S. Gold, L. Bernstein, R. Magaw, and T. H. Slone, *Environ. Health Perspect., 81*, 211–219 (1989).
14. G. S. Omenn, S. Stuebbe, and L. B. Lave, *Molec. Carcinog., 14*, 37–45 (1995).
15. V. A. Fung, J. Huff, E. K. Weisburger, and D. G. Hoel, *Fundam. Appl. Toxicol., 20*, 413–436 (1993).
16. H. S. Rosenkranz and G. Klopman, *Mutat. Res., 245*, 51–54 (1990).
17. J. R. M. Innes, B. M. Ulland, M. G. Valerio, L. Petrucelli, L. Fishbein, E. R. Hart, A. J. Pallota, R. R. Bates, H. L. Falk, J. J. Gart, M. Klein, I. Mitchell, and J. Peters, *J. Natl. Cancer Inst., 42*, 1101–1114 (1969).
18. J. R. M. Innes, *Evaluation of Carcinogenic, Teratogenic, and Mutagenic Activities of Selected Pesticides and Industrial Chemicals.*

Volume 1: Carcinogenic Study, Bionetics Research Laboratories, Inc., Springfield, VA, 1968.
19. U. S. National Cancer Institute, *Everything Doesn't Cause Cancer: But How Can We Tell Which Things Cause Cancer and Which Ones Don't?* USNCI, Bethesda, MD, 1984.
20. B. N. Ames, L. S. Gold, and W. C. Willett, *Proc. Natl. Acad. Sci. USA, 92*, 5258–5265 (1995).
21. B. N. Ames, L. S. Gold, and M. K. Shigenaga, *Risk Anal., 16*, 613–617 (1996).
22. B. N. Ames and L. S. Gold, *Proc. Natl. Acad. Sci. USA, 87*, 7772–7776 (1990).
23. B. N. Ames and L. S. Gold, *Science, 249*, 970–971 (1990). Letters: *250*, 1498 (1990); *250*, 1645–1646 (1990); *251*, 12–13 (1991); *251*, 607–608 (1991); *252*, 902 (1991).
24. B. N. Ames, M. K. Shigenaga, and L. S. Gold, *Environ. Health Perspect., 101*(Suppl. 5), 35–44 (1993).
25. B. N. Ames, M. K. Shigenaga, and T. M. Hagen, *Proc. Natl. Acad. Sci. USA, 90*, 7915–7922 (1993).
26. R. Hart, D. Neumann, and R. Robertson, *Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies*, ILSI Press, Washington, DC, 1995.
27. M. L. Cunningham, L. L. Pippin, N. L. Anderson, and M. L. Wenk, *Toxicol. Appl. Pharmacol., 131*, 216–223 (1995).
28. J. J. Hayward, B. S. Shane, K. R. Tindall, and M. L. Cunningham, *Carcinogenesis, 16*, 2429–2433 (1995).
29. S. M. Cohen and L. B. Ellwein, *Cancer Res., 51*, 6493–6505 (1991).
30. S. M. Cohen, *Regul. Toxicol. Pharmacol., 21*, 75–80 (1995).
31. J. L. Counts and J. I. Goodman, *Regul. Toxicol. Pharmacol., 21*, 418–421 (1995).
32. S. Preston-Martin, M. C. Pike, R. K. Ross, and P. A. Jones, *Cancer Res., 50*, 7415–7421 (1990).
33. L. S. Gold, N. B. Manley, and B. N. Ames, *Risk Anal., 12*, 579–588 (1992).
34. L. S. Gold, *Risk Anal., 13*, 399–401 (1993).
35. L. Bernstein, L. S. Gold, B. N. Ames, M. C. Pike, and D. G. Hoel, *Fundam. Appl. Toxicol., 5*, 79–86 (1985).
36. D. Krewski, D. W. Gaylor, A. P. Soms, and M. Syszkowicz, *Risk Anal., 13*, 383–398 (1993).
37. D. A. Freedman, L. S. Gold, and T. H. Slone, *Risk Anal., 13*, 265–272 (1993).
38. D. W. Gaylor and L. S. Gold, *Regul. Toxicol. Pharmacol., 22*, 57–63 (1995).

39. L. S. Gold, L. Bernstein, J. Kaldor, G. Backman, and D. Hoel, *Fundam. Appl. Toxicol., 6*, 263–269 (1986).
40. L. S. Gold, C. Wright, L. Bernstein, and M. de Veciana, *J. Natl. Cancer Inst., 78*, 1149–1158 (1987).
41. L. S. Gold, T. H. Slone, and L. Bernstein, *Environ. Health Perspect., 79*, 259–272 (1989).
42. D. A. Freedman, L. S. Gold, and T. H. Lin, *Regul. Toxicol. Pharmacol., 23*, 225–232 (1996).
43. T. H. Lin, L. S. Gold, and D. Freedman, *Stat. Sci. 10*(4), 337–353 (1995).
44. R. H. Adamson and S. M. Sieber, in *Organ and Species Specificity in Chemical Carcinogenesis* (R. Langenbach, S. Nesnow, and J. M. Rice, eds.), Plenum Press, New York, 1982, pp. 129–156.
45. L. S. Gold and T. H. Slone, *J. Toxicol. Environ. Health, 39*, 147–161 (1993).
46. U.S. Food and Drug Administration, *Fed. Reg., 61*, 43297–43300 (1996).
47. L. S. Gold, N. B. Manley, and T. H. Slone, in *Handbook of Carcinogenic Potency and Genotoxicity Databases* (L. S. Gold and E. Zeiger, eds.), CRC Press, Boca Raton, FL, 1997, pp. 607–620.
48. L. S. Gold, T. H. Slone, B. R. Stern, and L. Bernstein, *Mutat. Res., 286*, 75–100 (1993).
49. L. S. Gold, J. M. Ward, L. Bernstein, and B. Stern, *Fundam. Appl. Toxicol., 6*, 677–690 (1986).
50. L. S. Gold and T. H. Slone, in *Fifth Workshop on Mouse Liver Tumors*, International Life Sciences Institute, Washington, DC, 1995, pp. 2–3.
51. L. S. Gold, T. H. Slone, N. B. Manley, and L. Bernstein, *Environ. Health Perspect., 93*, 233–246 (1991).
52. U.S. Food and Drug Administration, *J. Assoc. Off. Anal. Chem., 76*, 127A–148A (1993).
53. B. N. Ames, M. Profet, and L. S. Gold, *Proc. Natl. Acad. Sci. USA, 87*, 7782–7786 (1990).
54. B. N. Ames and L. S. Gold, *Chem. Eng. News, 69*, 28–32, 48–49 (1991).
55. L. S. Gold, T. H. Slone, and B. N. Ames, in *Food Chemical Risk Analysis* (D. Tennant, ed.), Chapman & Hall, London, 1997, pp. 261–295.
56. R. J. Clarke and R. Macrae (eds.), *Coffee*, Elsevier, New York, 1988, Vols. 1–3.
57. H. Maarse, C. A. Visscher, L. C. Willemsens, L. M. Nijssen, and M. H. Boelens (eds.), *Volatile Compounds in Foods. Qualitative and*

Quantitative Data. Supplement 5 and Cumulative Index, TNO-CIVO Food Analysis Institute, Zeist, The Netherlands, 1994.
58. Y. Fujita, K. Wakabayashi, M. Nagao, and T. Sugimura, *Mutat. Res., 144*, 227–230 (1985).
59. K. Kikugawa, T. Kato, and S. Takahashi, *J. Agric. Food Chem., 37*, 881–886 (1989).
60. R. Tressl, D. Bahri, H. Köppler, and A. Jensen, *Z. Lebensm. Unters. Forsch., 167*, 111–114 (1978).
61. W. Rahn and W. A. König, *J. High Resolut. Chromatogr. Chromatogr. Commun., 1002*, 69–71 (1978).
62. R. R. Ariza, G. Dorado, M. Barbanch, and C. Pueyo, *Mutat. Res., 201*, 89–96 (1988).
63. V. A. Fung, T. P. Cameron, T. J. Hughes, P. E. Kirby, and V. C. Dunkel, *Mutat. Res., 204*, 219–228 (1988).
64. A. F. Hanham, B. P. Dunn, and H. F. Stich, *Mutat. Res., 116*, 333–339 (1983).
65. H. F. Stich, M. P. Rosin, C. H. Wu, and W. D. Powrie, *Mutat. Res., 90*, 201–212 (1981).
66. M. Ishidate, Jr., M. C. Harnois, and T. Sofuni, *Mutat. Res., 201*, 89–96 (1988).
67. L. S. Gold, T. H. Slone, B. R. Stern, N. B. Manley, and B. N. Ames, in *Comparative Environmental Risk Assessment* (C. R. Cothern, ed.), Lewis Publishers, Boca Raton, FL, 1993, pp. 209–235.
68. L. S. Gold, T. H. Slone, B. R. Stern, N. B. Manley, and B. N. Ames, *Science, 258*, 261–265 (1992).
69. B. N. Ames, R. Magaw, and L. S. Gold, *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).
70. L. S. Gold, T. H. Slone, N. B. Manley, and B. N. Ames, *Cancer Lett., 83*, 21–29 (1994).
71. L. S. Gold, G. B. Garfinkel, and T. H. Slone, in *Chemical Risk Assessment and Occupational Health: Current Applications, Limitations, and Future Prospects* (C. M. Smith, D. C. Christiani, and K. T. Kelsey, eds.), Auburn House, Westport, CT, 1994, pp. 91–103.
72. National Research Council, *Carcinogens and Anticarcinogens in the Human Diet: A Comparison of Naturally Occurring and Synthetic Substances*, National Academy Press, Washington, DC, 1996.
73. L. Tomatis and H. Bartsch, *Exp. Pathol., 40*, 251–266 (1990).
74. L. S. Gold, G. M. Backman, N. K. Hooper, and R. Peto, *Environ. Health Perspect., 76*, 211–219 (1987).
75. K. T. Bogen and L. S. Gold, *Regul. Toxicol. Pharmacol., 25*, 26–42 (1997).

76. G. C. Hard and J. Whysner, *Crit. Rev. Toxicol., 24*, 231–254 (1994).
77. U.S. Environmental Protection Agency, *Report of the EPA Peer Review Workshop on Alpha$_{2u}$-globulin: Association with Renal Toxicity and Neoplasia in the Male Rat*, USEPA, Washington, DC, 1991.
78. U.S. Environmental Protection Agency, *Fed. Reg., 57*, 7484–7530 (1992).
79. T. Key and G. Reeves, *Br. Med. J., 308*, 1520–1521 (1994).
80. National Research Council (NRC), *Regulating Pesticides in Food: The Delaney Paradox*, National Academy Press, Washington, DC, 1987.
81. L. S. Gold, B. R. Stern, T. H. Slone, J. P. Brown, N. B. Manley, and B. N. Ames, *Cancer Lett., 117*, 195–207 (1997).
82. M. Ochiai, W. Masatoshi, K. Hiromi, K. Wakabayashi, T. Sugimura, and M. Minako, *Carcinogenesis, 17*, 95–98 (1996).
83. U.S. Food and Drug Administration, *Butylated Hydroxyanisole (BHA) Intake. DFCA Request of 1-15-91*, USFDA, Washington, DC, 1991 (memo from Food and Color Additives Section to L. Lin).
84. D. B. Clayson, F. Iverson, E. A. Nera, and E. Lok, *Annu. Rev. Pharmacol. Toxicol., 30*, 441–463 (1990).
85. California Environmental Protection Agency, *California Cancer Potency Factors: Update*, CalEPA, Sacramento, 1994 (memo).
86. S. M. Cohen and T. A. Lawson, *Cancer Lett., 93*, 9–16 (1995).
87. F. M. Clydesdale (ed.), *Food Additives: Toxicology, Regulation, and Properties*, CRC Press, Boca Raton, FL, 1997.
88. A. H. Wu-Williams, L. Zeise, and D. Thomas, *Risk Anal., 12*, 559–567 (1992).
89. U.S. Food and Drug Administration, *Assessment of Carcinogenic Upper Bound Lifetime Risk Resulting from Aflatoxins in Consumer Peanut and Corn Products. Report of the Quantitative Risk Assessment Committee*, USFDA, Washington, DC, 1993.
90. W. A. Pons, Jr., *J. Assoc. Off. Anal. Chem., 62*, 586–594 (1979).
91. J. D. Groopman, J. Q. Zhu, P. R. Donahue, A. Pikul, L. S. Zhang, J. S. Chen, and G. N. Wogan, *Cancer Res., 52*, 45–52 (1992).
92. M. C. Yu, M. J. Tong, S. Govindarajan, and B. E. Henderson, *J. Natl. Cancer Inst., 83*, 1820–1826 (1991).
93. T. Kuiper-Goodman and P. M. Scott, *Biomed. Environ. Sci., 2*, 179–248 (1989).
94. International Life Sciences Institute, *ILSI Europe Newsletter*, February 1996, p. 3.
95. M. J. Gartrell, J. C. Craun, D. S. Podrebarac, and E. L. Gunderson, *J. Assoc. Off. Anal. Chem., 69*, 146–161 (1986).
96. E. L. Gunderson, *J. Assoc. Off. Anal. Chem., 78*, 910–921 (1995).
97. World Health Organization, *Polychlorinated Biphenyls and Terphenyls. Environmental Health Criteria 140*, WHO, Geneva, 1993.

98. U.S. Environmental Protection Agency, *Estimating Exposure to Dioxin-Like Compounds (Review Draft)*, USEPA, Washington, DC, 1994.
99. U.S. Environmental Protection Agency, *Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds*, USEPA, Washington, DC, 1994.
100. U.S. Environmental Protection Agency, *Re-evaluating Dioxin: Science Advisory Board's Review of EPA's Reassessment of Dioxin and Dioxin-like Compounds*, USEPA, Washington, DC, 1995.
101. C. A. Bradfield and L. F. Bjeldanes, *J. Toxicol. Environ. Health, 21*, 311–323 (1987).
102. D. B. Clayson and F. Iverson, *Regul. Toxicol. Pharmacol., 24*, 45–59 (1996).
103. J. I. Goodman, *Regul. Toxicol. Pharmacol., 19*, 51–59 (1994).
104. U.S. Environmental Protection Agency, *Fed. Reg., 61*, 17960–18011 (1996).
105. National Research Council, *Science and Judgment in Risk Assessment*, National Academy Press, Washington, DC, 1994. Committee on Risk Assessment of Hazardous Air Pollutants.
106. L. Zeise, R. Wilson, and E. Crouch, *Risk Anal., 4*, 187–199 (1984).
107. C. C. Travis, S. A. Richter Pack, A. W. Saulsbury, and M. W. Yambert, *Mutat. Res., 241*, 21–36 (1990).
108. B. N. Ames, *Science, 221*, 1256–1264 (1983).
109. B. N. Ames, *Science, 224*, 668–670, 757–760 (1984).
110. M. H. Harvey, B. A. Morris, M. McMillan, and V. Marks, *Human Toxicol., 4*, 503–512 (1985).
111. T. Yamagami, H. Handa, T. Juji, H. Munemitsu, M. Aoki, and Y. Kato, *Surg. Neurol., 20*, 323–331 (1983).