A RASH Analysis of National Toxicology Program Data: Predictions for 30 Compounds to Be Tested in Rodent Carcinogenesis Experiments

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Relative potencies for 30 compounds scheduled for carcinogenic testing in the 2-year rodent bioassays were estimated based on comparisons with a wide variety of bioassay data for benzo[*a*]pyrene, nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide. Potential for oncogenic transformation of each of the compounds was estimated from short-term bioassays. Promoting strength was assigned on the basis of comparisons of the product of relative potency and test dose with the distribution of similar products obtained for 67 common compounds in the database of Gold et al. A potency class for promotion was assigned on the basis of whether the potency-adjusted test dosage was $>2\sigma$ below the mean, $>1\sigma$ below the mean, within $\pm\sigma$ of the mean, $>\sigma$ above the mean, or $>2\sigma$ above the mean, as determined from the 67 compounds. The underlying hypothesis is that a weak test dose may have a low probability of revealing a potential carcinogen, whereas a strong dose may have a high probability of producing false-positive results. Predictions are therefore directed at the central 68% of the log-normal frequency distribution according to the assumption that $\pm\sigma$ represents the ideal test dose. — Environ Health Perspect 104(Suppl 5):1017–1030 (1996)

Key words: rodent carcinogenesis, relative potency, promotion

Introduction

Goal

The goal of this analysis is to use a wide variety of existing data from several sources and relative-potency-based models in an effort to predict the outcome of carcinogenic testing of rats and mice in the standard 2-year bioassay as used by the National Toxicology Program (NTP).

Some of the existing bioassay data are used to estimate the compound-specific potential for initiation. For the compound-specific capacity to promote initiated carcinogenic lesions, a large volume of unedited data are compared to matched tests for reference carcinogens comprised of benzo[*a*]pyrene B[*a*]P, nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide. In addition to predicting carcinogenic outcome of the rodent bioassays, rank order is assigned for the 30 compounds as requested by the organizers from the National Institute of Environmental Health Sciences (NIEHS) and the NTP.

Tennant et al. (*1*) published an analysis on "Prediction of the outcome of rodent carcinogenicity bioassays currently being conducted on 44 compounds by the National Toxicology Program." In an editorial in that same issue of *Mutagenesis*, Parry (*2*) noted, "Readers will be aware of others who propose alternative methods for the prediction of the carcinogenicity of chemicals. I would like to take this opportunity to open the debate to other contributors."

Because we have successfully used a Rapid Screening of Hazard (RASH) chemical-scoring method for a variety of difficult applications (*3–7*), it was an excellent opportunity to test if the RASH-derived relative-potency estimates could be used for range-finding for the doses actually tested in the 2-year bioassays or if those estimates of relative potency could be used to predict the carcinogenic outcome of the tests.

Since RASH was based on the hypothesis that toxicity-induced compensatory cell proliferation may be a practical index of carcinogenic promotion for toxicological and radiological insults (*8,9*), there were no considerations of potency for carcinogenic initiation in any of the various RASH applications other than that by Jones and Easterly (*10*). The analysis of the 44 compounds (*10*) avoided the modeling of carcinogenic initiation by using the tabulations by Tennant et al. (*1*).

This effort will again attempt to demonstrate the range-finding utility of the RASH method by using data from the *Registry of Toxic Effects of Chemical Substances* (RTECS) (*11*) and short-term bioassay data from the NTP to estimate whether methods described in this exercise can expedite the NTP range-finding study by reducing the number of test animals required to determine the ideal test doses.

Compound-specific relative potency values and the database of Gold et al. (*12,13*) are used to model test doses considered to be of the proper magnitude to minimize the probability of obtaining false-positive and false-negative test results. This exercise attempts to demonstrate the utility of a simple, rapid, data-rich screening tool and will not resort to careful literature reviews to refine our initial predictions.

For this analysis, a prototype personal computer version of RASH (called CRASH) (*5*) has permitted more exhaustive relative-potency estimates than were possible from hand calculations (*10,14*). The relative potency from CRASH will be used in combination with an estimate of initiation potential as based on short-term test results to predict which test doses are too weak to express possible carcinogens, which test doses are so strong that equivocal

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Abbreviations used: B[*a*]P, benzo[*a*]pyrene; CRASH, computerized rapid screening of hazard; $D_{B[a]P}$, dose of benzo[*a*]pyrene; D_c, dose of test compound to be compared with a reference compound; FM, frmale mice; FR, female rats; HARM, hazard assessment rating methodology; kVp, kiloVolt potential applied to an X-ray machine; LD_{50}, dose lethal to 50% of a test group; MaxD, maximum dose tested in the NTP 2-year bioassay; MM, male mice; MR, male rats; NTP, National Toxicology Program; NIEHS, National Institute of Environmental Health Sciences; RASH, rapid screening of hazard; RP, relative potency; RTECS, Registry of Toxic Effects of Chemical Substances; R, risk; σ, estimated standard deviation of the log-probability distribution; $(Slope)_{B[a]P}$, risk coefficient for B[*a*]P.

results or false positives are possible, and which test doses are just right to produce a carcinogenic outcome that is consistent with other NTP test results in the database of Gold et al. (*12,13*).

Background

The Environmental Monitoring Plan for the U.S. Synthetic Fuels Corporation (*15*) stated that the corporation should have the burden of justifying the need to monitor specific unrelated substances and of providing threshold values above which those substances must be monitored. Somewhat overlapping in time, the U.S. Air Force sponsored the development of a hazard assessment rating methodology (HARM) that finally became known as the defense priority model (*16,17*) for site-specific screening of the Installation Restoration Program. Based on observed linear relationships between carcinogenic risk and either cytotoxicity or compensatory cell proliferation for both ionizing radiations (*9,18*) and carcinogenic chemicals (*8*), the RASH method was proposed for both of these applications (*19*).

Method

Because RASH has been documented exhaustively, it will be summarized very briefly with somewhat greater detail given to the differences between the tedious hand calculations associated with the original RASH and the prototype personal computer version used for this application, CRASH. Jones et al. (*14,19*) present numerical demonstrations of the RASH evaluations.

RASH. The definition of relative potency (RP) as used in RASH is the dose of a reference compound such as B[*a*]P divided by the dose of an "interviewing" compound (i.e., a compound being evaluated) or insult "*i*" that causes the same level of response in a common test system, i.e., $RP = D_{B[a]P}/D_i$. In the data used in this analysis, the doses are the lowest published values for a positive result in the test of comparison. The RP index is exactly analogous to the relative biological effectiveness factors used to compare various ionizing radiations to a standard such as 250-kVp X-rays or, to use another analogy, to an electric motor rated in terms of horsepower.

In this manner, it is possible to compute an equivalent toxic dosage of an interviewing compound about which little is known regarding carcinogenic or human risk in terms of a standard or reference compound for which the carcinogenic or human dose response is reasonably well known. For the linear model used to estimate carcinogenic risk, $(\text{Risk})_{B[a]P} = (\text{Slope})_{B[a]P} \times D_{B[a]P}$ and $(\text{Risk})_i = (\text{Slope})_{B[a]P} \times RP \times D_i$. More extensive discussion of fundamental concepts and other models of risk have been presented by Finney (*20*) and Owen and Jones (*7*). It is desirable for the reference compounds to have been tested extensively in various bioassays so that several relative-potency values can be computed for each new compound of interest. The median of the array of RP values should be a practical estimate of the composite toxicological potency (*14*). The distribution of RP values and the stability of the median provide useful information about the uncertainty in doses required to cause different biological effects. For most applications, we have used the interquartile range, i.e., the spread between the 25th and the 75th percentiles, as a practical measure of uncertainty due to random errors and variations in experimental design.

Most calculations that have been based on the RASH method have considered data unselectively from RTECS on mutagenesis, carcinogenesis, reproductive toxicity, tumorigenesis, acute toxicity, and even irritation, although the user could select bioassays considered to be most relevant to carcinogenic risk. From examples shown in previous publications, the different categories of test data for most compounds usually lead to similar distributions of RP values (*10,14*).

In previous analyses based on RASH, the compound-specific products of RP × Regulatory Benchmark are reasonably constant for a variety of compounds evaluated by similar considerations. Alternatively, the empirical behavior can be viewed as an inverse proportionality between relative potency and permissible exposure (*5,20*). After extensive testing of the RASH method by six investigators, each using assumptions in accordance with individual professional and academic backgrounds, the RASH process has been found to be quite robust to different users but somewhat less robust to additional test data, especially when RPs were computed from small numbers of previously matched comparisons.

This particular study is the second application of a CRASH program (*5*). The standardization and simplification required for the Windows version personal computer program are consistent with the previous findings in that significant changes from earlier publications are primarily due to new test data. Generally this imprecision results from user-specific choices or variations associated with standardized algorithms used in the CRASH program. In contrast, however, additional test data for a compound (that has previously been tested only by Ames tests) and one or two relevant tests for acute toxicity may cause estimates to vary by factors of 3 to 10, while compounds evaluated only from a couple of mutagenesis assays (perhaps Ames test results with and without S9 substrate) may change by factors of 100 or 1000.

CRASH. The CRASH code was designed to be as similar to the original RASH method (*14*) as possible. The goal was to match each bioassay available for the interviewing or test compound with a similar result for one and only one reference compound. Reference compounds included B[*a*]P as a primary standard and several secondary standards that were sometimes varied from study to study. Whenever a test result for the interviewing compound was matched successfully, the calculation proceeded to the next bioassay without considering whether matches with other secondary standards were possible. Because computers are almost infinitely faster than humans at matching bioassay results and computing relative potency ratios, more comparisons between the interviewing compound and the several reference compounds provide greater accuracy—provided that the results of many bioassays matched to many different primary reference compounds are used correctly. Used incorrectly, an impressive degree of precision is achieved, but the goal for accuracy is not achieved.

It was recognized from the beginning that the RASH method did not necessarily need to match compounds according to their mechanisms of action because the definition is analogous to that of work, namely, force applied and work achieved. However, the chemical's structure often controls the selection of the bioassays used to test its potency and it is readily seen that inorganic compounds are frequently tested by bioassays and protocols that are uncommon to organics.

For this application, the CRASH analyses will typically be based on matches of all the bioassay results for a particular test compound, with corresponding test results for each of five reference compounds used one by one. The median relative potency is taken for the interviewing compound relative to a particular reference compound. This produces five compound-specific scales, each of which is normalized to unity for the reference standard. At the next step

of the analysis, the five scales are standardized to a common scale by normalizing each to have unit potency for B[*a*]P.

It is imperative to match individual bioassays in this or a logically equivalent manner because if relative potencies are computed without any balance, Ames tests and LD_{50} results will propagate exponentially in numbers of matches and dominate the results. This uncharacteristic proliferation of "excessive matches" usually leads to great precision but can result in great inaccuracies.

For the same reason and because the CRASH program may be used by individuals who are relatively new to the process, the CRASH code does not run to completion when fewer than three matches are found between a particular test compound and a specified reference compound. Therefore, 3 of the 30 compounds were computed by hand according to the RASH methods—phenolphthalein, sodium xylenesulfonate, and isobutene.

Initiation. Results from mutation bioassays are available from RTECS and from the NTP battery of short-term screening tests, which was generously supplied to participants. Both sources will be used. Ames test results with and without metabolic activation have a long, complex trail as possible predictors of initiation and carcinogenesis. Ames test results from RTECS and from the NTP bioassays will be taken as one component of five considerations used to judge the probability of binary initiation, i.e., whether initiation processes can be successfully completed by any conceivable test protocol implementing that particular compound. The second component (indicated by "I" in column headings of Table 1) is based on NTP results for chromosome aberrations and sister chromatid exchanges and RTECS results for specific locus test; DNA damage, repair, synthesis, and inhibition of synthesis; gene conversion and mitotic recombination; cytogenetic analysis; sister chromatid exchanges; mutation in somatic mammalian cells; and oncogenic transformation. The third component, considered more closely related to intracellular dosimetry (indicated by "II" in Table 1), is based on NTP results from the mouse bone-marrow micronucleus assay, and RTECS data on body-fluid assay; dominant lethal test; micronucleus test; phage inhibition capacity; sex chromosome loss and disjunction; sperm morphology; and heritable translocation test. Positive results in this class without support from the other four classes are treated as questionable.

In addition, supplemental considerations were added as seen in Table 1 if existing RTECS data indicated that the compound is oncogenic in either animals or humans. Results given in Table 1 include the overall estimate (in the right-hand column) based on the strength of the total evidence for inducing positive results with respect to oncogenic transformation.

Although we have postulated that initiation has a binary, on/off behavior and is only qualitatively related to carcinogenic potency (*8,9*), data gaps for the 30 test compounds cause us to model initiation in a stepwise fashion for this application. We do not believe the process actually behaves in this manner, but based on the available data, there is a significant probability that we will not be able to classify a

Table 1. Summary of mutation bioassay results as available from NTP and RTECS for 30 compounds currently being tested in the 2-year mouse and rat studies.[a]

			Ames test			Class I			Class II					
No.	CAS no.	Test compound	NTP Ames	RTECS Ames	Sum	NTP I	RTECS I	Sum	NTP MN	RTECS II	Sum	Animal tumors	Human cancers	Composite of 5 classes
1.	6533-68-2	Scopalamine hydrobromide trihydrate	–		[–]									–
2.	76-57-3	Codeine	–		[–]									–
3.	147-47-7	1,2-Dihydro-2,2,4-trimethylquinoline	–		[–]				–		[–]			–
4.	75-52-5	Nitromethane	–		[–]				–	[–]				–
5.	109-99-9	Tetrahydrofuran	–		[–]	–		[–]	?	+	[+]			?
6.	1948-33-0	*t*-Butylhydroquinone	–		[–]		+	[+]						+
7.	100-41-4	Ethylbenzene	–		[–]		+	[+]	–		[–]			+
8.	126-99-8	Chloroprene	–	+	[+]	–	+	[+]	–	+	[+]			+++
9.	10026-24-1	Cobalt sulfate heptahydrate	Weak		[?]									?
10.	8003-22-3	D&C Yellow No. 11	Weak	+	[+]		+	[+]	–		[–]			++
11.	78-84-2	Isobutyraldehyde	?		[?]									?
12.	1313-27-5	Molybdenum trioxide	–		[–]							[+]		+
13.	127-00-4	1-Chloro-2-propanol	+	+	[+]				–		[–]			+
14.	111-42-2	Diethanolamine	–		[–]				–		[–]			–
15.	77-09-8	Phenolphthalein	–		[–]				+		[+]			?
16.	110-86-1	Pyridine	–	+	[+]					+	[+]			++
17.	1300-72-7	Sodium xylenesulfonate	–		[–]									–
18.	98-00-0	Furfuryl alcohol	–		[–]	–	+	[+]		+	[+]			++
19.	125-33-7	Primaclone	+	+	[+]		+	[+]	–	+	[+]			+++
20.	111-76-2	Ethylene glycol monobutyl ether	–		[–]									–
21.	1303-00-0	Gallium arsenide	–		[–]				–		[–]			–
22.	115-11-7	Isobutene	–		[–]									–
23.	93-15-2	Methyleugenol	–		[–]		+	[+]	–	+	[+]			++
24.	434-07-1	Oxymetholone	–		[–]								[+]	?
25.	84-65-1	Anthraquinone	+	+	[+]		+	[+]	–		[–]			++
26.	518-82-1	Emodin	+	+	[+]		+	[+]	+		[+]			+++
27.	5392-40-5	Citral	–		[–]				–	+	[+]			?
28.	7632-00-0	Sodium nitrite	+	+	[+]		+	[+]		+	[+]			+++
29.	104-55-2	Cinnamaldehyde		+	[+]		+	[+]	–	+	[+]			+++
30.	1314-62-1	Vanadium pentoxide	–		[–]					+	[+]			?

[a] Compounds are classified according to negative [–]; uncertain [?]; possible [+]; moderate [++]; and strong [+++] evidence.

compound correctly either as an initiator or as a noninitiator of the carcinogenic process. Instead of a graduated probability scale, we have used a classification schema based on – for negative; ? for uncertain; + for possible; ++ for moderate; and +++ for strong evidence based compositely on the five classes of data that we have taken to be relevant to carcinogenic initiation of cells. This class assignment will be used in combination with the promotion class, based quantitatively on potency-adjusted doses.

Promotion. The compound-specific potency for promotion will be assigned from the product of the test dose and the median value of relative potency as estimated from results published in RTECS. As described above, the CRASH program was used to compute the relative potency for each of the 30 test compounds relative to five reference compounds—B[*a*]P, nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide. These compounds were selected because they have provided consistent results in past evaluations and because they have an abundance of test data grouped in the RTECS categories of mutagenesis, reproductive toxicity, tumorigenesis, and acute toxicity—except for B[*a*]P. Four of the reference compounds are rich in test results for acute toxicity and seem well-suited to the 30 compounds to be tested. Estimates of relative potency are given in Table 2. The relative potencies within a particular column are all normalized to a potency of unity for the particular reference compound shown in the column heading.

Fewer than three matches were identified for phenophthalein and isobutene, so those relative potencies were computed by hand (*14*). In addition, sodium xylenesulfonate was based only on an acute LD_{50} value (*21*) resulting from a literature search; no test data were listed in RTECS.

Scatter plots for the test compounds versus the reference compounds, taken two by two, are shown in Figures 1 and 2. The six panels of Figure 1 illustrate that different reference compounds lead to consistent results except for compounds that have not been tested adequately. Outliers indicated by an asterisk usually result from a small number of matches involving Ames test data or other similarly based bioassays. In this analysis of 30 compounds, many of the relevant bioassay data are based on measures of acute toxicity. B[*a*]P is one of the earliest known carcinogenic compounds and has never been tested comprehensively in assays for acute toxicity. Thus, as seen in the four panels of Figure 2, the absence of acute toxicity data for B[*a*]P makes it useless in this particular application. In contrast, the estimates shown in the six panels of Figure 1 provide adequate consistency and (when corrected to a common scale associated with unit potency for B[*a*]P) will permit compound-specific estimates of the power of the promoting dosage given to both sexes and species.

The data listed in Table 2 were converted to a common scale as seen in Table 3 based on conversion factors of 1, 3.2, 8.2, 8.08, and 1 for B[*a*]P, nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide, respectively (*5*). These factors are simply the median RP values for the individual reference compounds relative to B[*a*]P.

The RP values from Table 3 were used to define a median value as shown in column 8. That value is reproduced in column

Table 2. Potency of compounds to be tested relative to reference compounds comprising B[*a*]P, nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide.[a]

Test compound	RP to benzo[*a*]pyrene	Matches	RP to nicotine	Matches	RP to cisplatin	Matches	RP to aflatoxin B_1	Matches	RP to cyclophosphamide	Matches
Scopalamine hydrobromide trihydrate	–	0	0.0113	1	0.0092	1	–	0	0.142	1
Codeine	0.0333	3	0.0958	12	0.107	9	0.048	4	1.23	8
1,2-Dihydro-2,2,4-trimethylquinoline	–	0	0.0136	2	0.0178	2	0.0024	3	0.0872	2
Nitromethane	–	0	18.8	3	0.0309	4	0.00947	3	0.157	4
Tetrahydrofuran	0.286	1	0.0175	4	0.00347	3	0.00249	4	0.0774	4
t-Butylhydroquinone	0.588	5	0.0448	4	0.027	8	0.0455	10	0.229	9
Ethylbenzene	0.0563	1	0.00281	3	0.00514	2	0.00307	4	0.0471	4
Chloroprene	40	1	0.067	4	0.141	2	0.0361	2	2.97	4
Cobalt sulfate heptahydrate	–	0	0.0859	1	0.0443	3	0.0092	1	0.477	2
D&C Yellow No. 11	0.175	4	0.00366	1	0.0014	2	0.00538	4	0.0341	6
Isobutyraldehyde	–	0	0.0296	2	0.0269	1	0.005	1	0.167	1
Molybdenum trioxide	–	0	0.0559	4	0.0282	4	0.0346	2	0.78	3
1-Chloro-2-propanol	0.00138	2	–	0	0.086	2	0.000333	2	2.77	2
Diethanolamine	0.0227	1	0.009	7	0.00991	7	0.00343	6	0.0655	7
Phenolphthalein	–	–	–	–	–	–	–	–	–	–
Pyridine	0.0358	2	0.0148	12	0.0161	8	0.00646	4	0.166	11
Sodium xylenesulfonate	–	–	–	–	–	–	–	–	–	–
Furfuryl alcohol	0.588	1	0.0224	5	0.0638	4	0.0111	4	0.856	5
Primaclone	0.0537	4	0.0148	4	0.0233	6	0.0268	6	0.62	6
Ethylene glycol monobutyl ether	0.00932	2	0.0238	10	0.0192	8	0.00693	8	0.194	10
Gallium arsenide	–	0	0.00126	1	0.0014	1	0.00202	1	0.0234	1
Isobutene	–	–	–	–	–	–	–	–	–	–
Methyleugenol	3.14	2	0.0247	3	0.0982	3	0.00407	3	0.539	5
Oxymetholone	1	1	0.24	1	–	0	0.25	1	0.509	3
Anthraquinone	1	3	0.00416	3	0.00483	6	0.00176	4	0.0537	6
Emodin	0.141	6	63	2	0.15	5	0.0382	6	5.22	5
Citral	–	0	0.0162	4	0.00545	3	0.00123	4	0.0596	4
Sodium nitrate	0.008	19	0.0603	12	0.0418	11	0.003	26	0.117	23
Cinnamaldehyde	0.0618	4	0.0225	5	0.0147	7	0.00216	9	0.55	9
Vanadium pentoxide	3.57	1	1.41	6	0.939	6	0.48	3	5.96	7

RP, relative potency. [a]Comparisons involving three or more matches were computed by the CRASH code and matches of one or two bioassays were computed by hand. Column-specific RP values are normalized to unity for the reference compound listed in the column heading.

2 of Table 4 and was used to modify the maximum test doses (MaxD) shown in columns 3 to 6 for male rats (MR), female rats (FR), male mice (MM), and female mice (FM), respectively. The potency-adjusted dosages are given in columns 7 to 10 of Table 4.

Table 5, obtained from methods described in the appendix, was designed to use the intrinsic capacity of a compound's initiation potential (column 1) and the power of the test protocol with respect to promotion, as shown in the column headings. As seen in Table 5, both considerations were used to predict the sex- and species-specific test outcomes. From the method described in the appendix, the promoting class is assigned from the median of the product of relative potency and test dosage for a database of 67 common compounds in the database of Gold et al. Classification for capacity to effectively promote carcinogenesis was assigned according to whether the potency-adjusted test dose

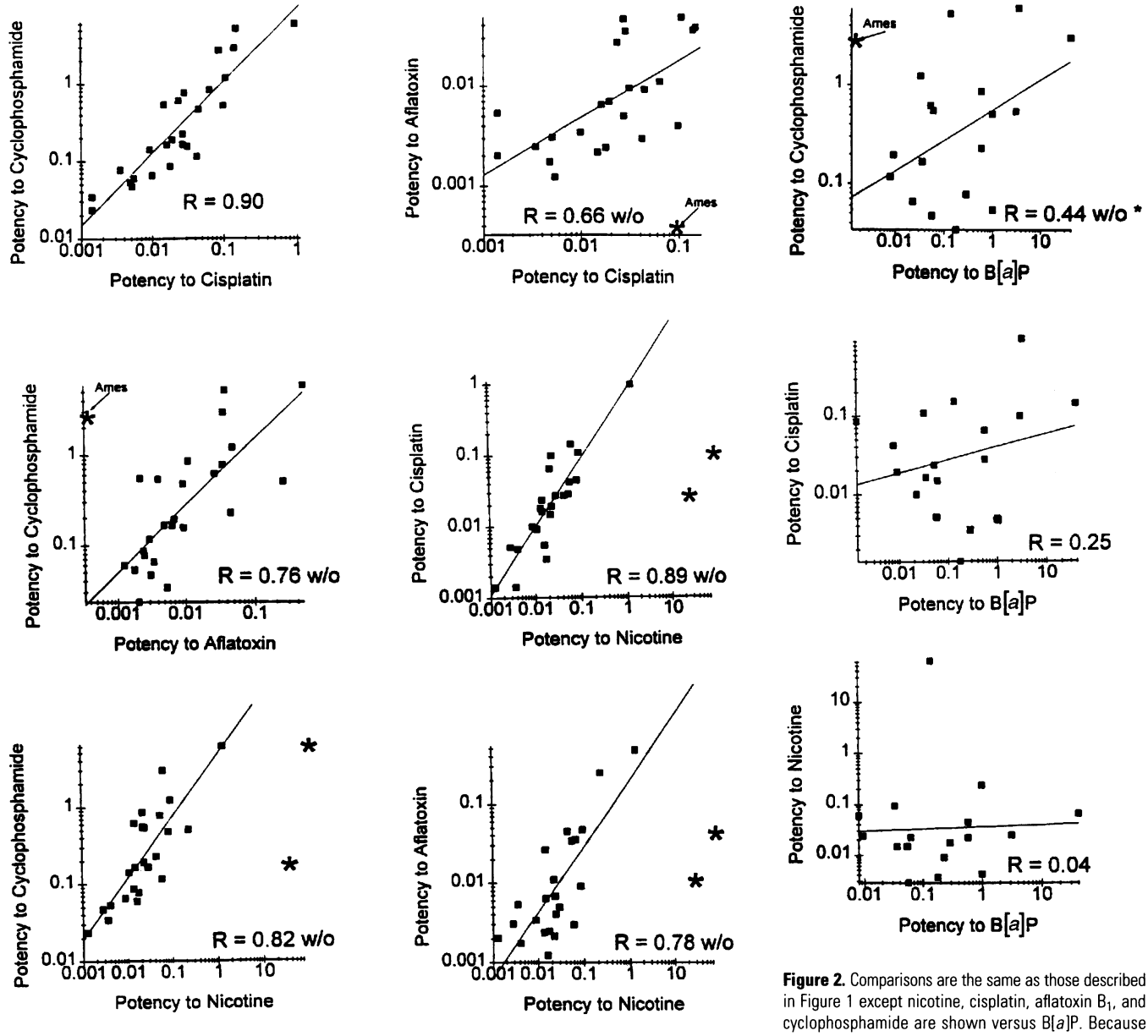

Figure 1. Scatter plots of potency of 30 compounds relative to one reference compound versus the relative potency computed by the use of a different reference compound. Panels involve nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide. Correlation coefficients are shown as "R."

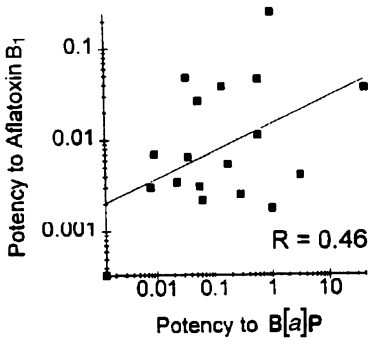

Figure 2. Comparisons are the same as those described in Figure 1 except nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide are shown versus B[*a*]P. Because B[*a*]P has inadequate test data on acute toxicity, it is not useful to analyze this particular list of 30 compounds—many of which are tested mostly by bioassays concerned with acute toxicity.

Table 3. Compound-specific relative potency estimates in Table 2, standardized to a common scale indexed by unit potency for benzo[*a*]pyrene.[a]

Test compound	Benzo[*a*]pyrene	Nicotine	Cisplatin	Aflatoxin B_1	Cyclophosphamide	Median	High/Low
Scopalamine hydrobromide trihydrate	–	0.03616	0.07544	0	0.142	0.0754	4
Codeine	0.0333	0.30656	0.8774	0.38784	1.23	0.387	40
1,2-Dihydro-2,2,4-trimethylquinoline	–	0.04352	0.14596	0.019392	0.0872	0.0654	8
Nitromethane	–	60.16	0.25338	0.0765176	0.157	0.205	800
Tetrahydrofuran	0.286	0.056	0.028454	0.0201192	0.0774	0.056	10
t-Butylhydroquinone	0.588	0.14336	0.2214	0.36764	0.229	0.229	4
Ethylbenzene	0.0563	0.008992	0.042148	0.0248056	0.0471	0.0421	6
Chloroprene	40	0.2144	1.1562	0.291688	2.97	1.16	200
Cobalt sulfate heptahydrate	–	0.27488	0.36326	0.074336	0.477	0.319	6
D&C Yellow No. 11	0.175	0.011712	0.01148	0.0434704	0.0341	0.0341	20
Isobutyraldehyde	–	0.09472	0.22058	0.0404	0.167	0.131	6
Molybdenum trioxide	–	0.17888	0.23124	0.279568	0.78	0.256	4
1-Chloro-2-propanol	0.00138	0	0.7052	0.0026906	2.77	0.354	2000
Diethanolamine	0.0227	0.0288	0.081262	0.0277144	0.0655	0.0288	4
Phenolphthalein	–	0	0	0	0	0.0303	–
Pyridine	0.0358	0.04736	0.13202	0.0521968	0.166	0.0522	5
Sodium xylenesulfonate	–	0	—	0	0	0.0162	–
Furfuryl alcohol	0.588	0.07168	0.52316	0.089688	0.856	0.523	10
Primaclone	0.0537	0.04736	0.19106	0.216544	0.62	0.191	9
Ethylene glycol monobutyl ether	0.00932	0.07616	0.15744	0.0559944	0.194	0.0762	20
Gallium arsenide	–	0.004032	0.01148	0.0163216	0.0234	0.0139	6
Isobutene	–	0	0	0	0	0.00388	–
Methyleugenol	3.14	0.07904	0.80524	0.0328856	0.539	0.539	100
Oxymetholone	1	0.768	—	2.02	0.509	0.884	4
Anthraquinone	1	0.013312	0.039606	0.0142208	0.0537	0.0396	80
Emodin	0.141	201.6	1.23	0.308656	5.22	1.23	1000
Citral	–	0.05184	0.04469	0.0099384	0.0596	0.0482	6
Sodium nitrate	0.008	0.19296	0.34276	0.02424	0.117	0.117	40
Cinnamaldehyde	0.0618	0.072	0.12054	0.0174528	0.55	0.072	30
Vanadium pentoxide	3.57	4.512	7.6998	3.8784	5.96	4.51	2

[a]Conversion factors were 3.2, 8.2, 8.08, and 1.0 for nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide, respectively.

Table 4. Potency-adjusted maximum test doses for tested animals for the 30 test compounds.[a]

No.	RP composite (B[*a*]P scale)	MaxD[b] MR	MaxD FR	MaxD MM	MaxD FM	RP×MaxD MR	RP×MaxD FR	RP×MaxD MM	RP×MaxD FM	Route	Solubility
1.	0.0754	25	25	25	25	1.885	1.885	1.885	1.885	Gav	w, alc
2.	0.387	58	67	338	327	22.446	25.929	130.806	126.549	Feed	w, alc, dil acid
3.	0.0654	100	100	10	10	6.54	6.54	0.654	0.654	SkinP	
4.	0.205	147	169	612	624	30.135	34.645	125.46	127.92	Inhal	w (ss), alc
5.	0.056	833	955	1733	1767	46.648	53.48	97.048	98.952	Inhal	w (misc), alc, ketone, HCs
6.	0.229	182	210	563	545	41.678	48.09	128.927	124.805	Feed	
7.	0.0421	511	586	1063	1083	21.5131	24.6706	44.7523	45.5943	Inhal	w (ins), org solv
8.	1.16	46	53	95	97	53.36	61.48	110.2	112.52	Inhal	org solv
9.	0.319	0.47	0.54	0.98	1	0.14993	0.17226	0.31262	0.319	Inhal	
10.	0.0341	182	210	NT	NT	6.2062	7.161	—	—	Feed	w (ins), oil, ethanol (ss)
11.	0.131	925	1061	1925	1963	121.175	138.991	252.175	257.153	Inhal	w, ethanol (misc)
12.	0.256	16	18	33	33	4.096	4.608	8.448	8.448	Inhal	w
13.	0.354	34	35	152	159	12.036	12.39	53.808	56.286	Water	w, alc
14.	0.0288	64	32	160	160	1.8432	0.9216	4.608	4.608	SkinP	w (misc), methanol
15.	0.0303	1817	2100	1351	1308	55.0551	63.63	40.9353	39.6324	Feed	w (ins), alc
16.	0.0522	21	21	152	80	1.0962	1.0962	7.9344	4.176	Water	w (misc), alc, oil, org liq
17.	0.0162	240	240	727	727	3.888	3.888	11.7774	11.7774	SkinP	
18.	0.523	20	23	42	43	10.46	12.029	21.966	22.489	Inhal	w (misc), alc
19.	0.191	91	105	146	142	17.381	20.055	27.886	27.122	Feed	w (ss)
20.	0.076	95	109	394	402	7.22	8.284	29.944	30.552	Inhal	w
21.	0.0138	0.16	0.18	0.33	0.33	0.002208	0.002484	0.004554	0.004554	Inhal	
22.	0.00388	2877	3302	5987	6106	11.16276	12.81176	23.22956	23.69128	Inhal	w (ins), alc
23.	0.539	150	150	75	75	80.85	80.85	40.425	40.425	Gav	
24.	0.884	150	100	NT	NT	132.6	88.4	—	—	Gav	is anabolic steroid
25.	0.0396	a	a	a	a	—	—	—	—	Feed	w (ins), alc
26.	1.23	45	53	68	65	55.35	65.19	83.64	79.95	Feed	w (ins), alc
27.	0.0482	909	1050	2814	2724	43.8138	50.61	135.6348	131.2968	Micro	w (ins), oil, alc (misc)
28.	0.117	155	160	456	478	18.135	18.72	53.352	55.926	Water	w, alc (ss), acid (decomp)
29.	0.072	b	b	b	b	—	—	—	—	Micro	w (dissolv), oil, alc (misc)
30.	4.51	0.31	0.36	1.96	2	1.3981	1.6236	8.8396	9.02	Inhal	w, acid, alkaline

MaxD, maximum test dose; MR, male rats; FR, female rats; MM, male mice; FM, female mice; NT, not tested; gav, gavage; skinP, skinpatch; inhal, inhalation; micro, microencapsulated chemical in feed; w, water; alc, alchol; dil, dilute; ss, slightly soluble; misc, miscible; HCs, hydrocarbons; ins, insoluble, org, organic; solv, solvent; liq, liquid; decomp, decomposes; dissolv, dissolves; —, no value for this cell. [a]Entries shown as "a" and "b" indicate that NTP test doses are not yet available for anthraquinone and cinnamaldehyde. [b]Doses are in milligram per kilogram per day.

of a particular test compound is >2σ below the mean, >1σ below the mean, within ±σ of the mean, >σ above the mean, or >2σ above the mean as given by doses shown in the headings of Table 5. The combination of promoting class and initiation class, as given in Table 5, determines the compound-specific predictions for the 2-year NTP tests of male and female populations of rats and mice, as shown in Table 6.

Results, Discussion, Conclusions

Organizers of this activity requested that each participant include qualitative prediction (+,–, or equivocal); estimate of carcinogenic potency (1 = least potent to 10 = most potent); mechanism of carcinogenicity (genotoxic or nongenotoxic); likely sites of tumor formation in both species; confidence in level of prediction; primary determinant of prediction (biological and/or chemical); and relevant comments pertaining to route of administration, exposure dose, chemical stability, solubility, alteration in gene expression, etc. The simple chemical screening tools that we have adapted from analyses of historical databases are completely inadequate for such predictive detail. We have experienced reasonable success with analyses of dose–magnitude type considerations (above). Because our concern has been for issues of risk to human health, we previously assumed that cellular initiation was a pervasive condition. In contrast, animals tested under the NTP protocols are isolated in test environments where initiation stressors are minimized. For those tests, simply assessing the promoting efficacy and ignoring initiation is generally insufficient to predict the outcome of test results.

This analysis involves the use of historical data and hypothetical models designed to test whether data from general toxicologic bioassays can be used to quantitatively (but subjectively) assign categories of carcinogenic initiation and promotion. Promotion is modeled from the product of the protocol test dosage and relative potency, as computed from RTECS data. Predictions are made for compounds currently being tested. The activity is novel with respect to most conventional approaches in the biological literature and the organizers should be commended for putting science on the line to evaluate just how much general knowledge has been accumulated from decades of research (*2*). Although the relative potency factors as used in this application seem to have a reasonably good degree of correlation with the maximum doses tested in the 2-year studies, it is still unknown whether the considerations used to evaluate the initiation potential of test compounds and the compound-specific median relative potency can be used in matrix form to predict the outcome of the 2-year test protocols to a helpful degree. Hence, discussions and conclusions should probably be left unrecorded until test results have been reported, as was the procedure for the previous 44 compounds (*22*).

To rank the carcinogenic potency of 30 compounds, a scale based on deciles was used. Placement on the scale depends on index compounds placed at the extremes. Different rankings would be expected if the ranking were organized only on the range defined by the 30 test compounds as opposed to a more general scale with saccharine, ethyl alcohol, and vinyl chloride

Table 5. Matrix for prediction of 2-year test protocols.

Initiation class	Promotion potential of treatment to rats and mice (Based on log-normal with mean [RP × MaxD] = 5 mg/kg/day, σ = 7X, and 2σ = 49X[b]				
	<0.1 mg/kg/day	0.1–0.7 mg/kg/day	0.7–35 mg/kg/day	35–245 mg/kg/day	>245 mg/kg/day
–	Negative	Negative	Negative	Negative	Equivocal
?	Negative	Negative	Negative	Equivocal	Positive
+	Negative	Negative	Equivocal	Positive	Positive
++	Negative	Equivocal	Positive	Positive	Positive
+++	Equivocal	Positive	Positive	Positive	Positive

RP, relative potency; MaxD, maximum test dose. [a]The assigned initiation class in column 1 and the promotion treatment class as listed in the row of column headings taken together determine the prediction for the outcome of the NTP 2-year testing program in both mice and rats. [b]Data from Gold et al. (*12,13*).

Table 6. Prediction of 2-year carcinogenesis test results in male rats, female rats, male mice, and female mice for protocol doses listed in Table 4.[a]

Test compound	Prediction			
	Male rats	Female rats	Male mice	Female mice
Scopalamine hydrobromide trihydrate	Negative	Negative	Negative	Negative
Codeine	Negative	Negative	Negative	Negative
1,2-Dihydro-2,2,4-trimethylquinoline	Negative	Negative	Negative	Negative
Nitromethane	Negative	Negative	Negative	Negative
Tetrahydrofuran	Equivocal	Equivocal	Equivocal	Equivocal
t-Butylhydroquinone	Positive	Positive	Positive	Positive
Ethylbenzene	Equivocal	Equivocal	Positive	Positive
Chloroprene	Positive	Positive	Positive	Positive
Cobalt sulfate heptahydrate	Negative	Negative	Negative	Negative
D&C Yellow No. 11	Positive	Positive	NT	NT
Isobutaldehyde	Equivocal	Equivocal	Positive	Positive
Molybdenum trioxide	Equivocal	Equivocal	Equivocal	Equivocal
1-Chloro-2-propanol	Equivocal	Equivocal	Positive	Positive
Diethanolamine	Negative	Negative	Negative	Negative
Phenolphthalein	Equivocal	Equivocal	Equivocal	Equivocal
Pyridine	Positive	Positive	Positive	Positive
Sodium xylenesulfonate	Negative	Negative	Negative	Negative
Furfuryl alcohol	Positive	Positive	Positive	Positive
Primaclone	Positive	Positive	Positive	Positive
Ethylene glycol monobutyl ether	Negative	Negative	Negative	Negative
Gallium arsenide	Negative	Negative	Negative	Negative
Isobutene	Negative	Negative	Negative	Negative
Methyleugenol	Positive	Positive	Positive	Positive
Oxymetholone	Equivocal	Equivocal	NT	NT
Anthraquinone	Dose = ?	Dose = ?	Dose = ?	Dose = ?
Emodin	Positive	Positive	Positive	Positive
Citral	Equivocal	Equivocal	Equivocal	Equivocal
Sodium nitrate	Positive	Positive	Positive	Positive
Cinnamaldehyde	Dose = ?	Dose = ?	Dose = ?	Dose = ?
Vanadium pentoxide	Negative	Negative	Negative	Negative

NT, not tested. [a]Test doses have yet to be determined for anthraquinone and cinnamaldehyde but according to our analysis would preferably be in the range of 17 to 880 mg/kg/day for anthraquinone and 10 to 490 mg/kg/day for cinnamaldehyde. Once the NTP test doses are determined, our prediction for carcinogenesis can be completed by potency-adjusted test doses as described in Table 5.

near the low end and aflatoxin B_1 and 2,3,7,8-TCDD at the upper end.

The median relative potencies (based on mass) for the 30 compounds varied by about 1,000-fold (i.e., 4.51/0.00388). In contrast, the more general range of the 101 compounds considered previously (*5*) varied by a millionfold based on mass units of dose and twice that based on molar doses. Respective rankings of the 30 compounds in both mass and molar units are indicated according to deciles in Table 7. Also listed in Table 7 are the decile rankings of the 30 compounds on the molar scale as defined by the list of 101 compounds. On this broader scale it is noteworthy that 27 of the 30 are above the median toxicity of category 5, and 4 of the 30 compounds are in the most toxic decile of 10.

For the NTP bioassays, extraordinary care is taken to minimize secondary sources of carcinogenic initiation and this article is our first effort to model carcinogenic initiation. Clearly, the NTP protocol requires that carcinogenic initiation be treated in a realistic manner because an on/off behavior could turn an otherwise adequate promoting dose into a negative carcinogenic test result. However, because our interest is still focused on safety for humans, we provide potency scales in both mass and molar units for all 30 of the test compounds in Table 7, whether or not the compounds are classified as carcinogens.

Table 7. Hazard ranking for carcinogenic promotion based on mass units of dose and molar units.[a]

Test compound	Mass[a]	Molar[a]	Mass[b]	Test prediction
Scopalamine hydrobromide trihydrate	5	7	7	Negative
Codeine	7	8	9	Negative
1,2-Dihydro-2,2,4-trimethylquinoline	4	5	7	Negative
Nitromethane	6	5	9	Negative
Tetrahydrofuran	4	4	7	Equivocal
t-Butylhydroquinone	6	7	9	Positive
Ethylbenzene	4	4	6	Positive
Chloroprene	9	8	10	Positive
Cobalt sulfate heptahydrate	7	8	9	Negative
D&C Yellow No. 11	3	5	6	Positive
Isobutyraldehyde	5	5	8	Positive
Molybdenum trioxide	6	7	9	Equivocal
1-Chloro-2-propanol	7	7	9	Positive
Diethanolamine	3	4	6	Negative
Phenolphthalein	3	5	6	Equivocal
Pyridine	4	4	7	Positive
Sodium xylenesulfonate	2	4	4	Negative
Furfuryl alcohol	7	7	9	Positive
Primaclone	6	7	9	Positive
Ethylene glycol monobutyl ether	5	5	7	Negative
Gallium arsenide	2	3	4	Negative
Isobutene	1	1	3	Negative
Methyleugenol	7	8	9	Positive
Oxymetholone	8	9	10	Equivocal
Anthraquinone	4	5	6	Dose ?
Emodin	9	9	10	Positive
Citral	4	5	7	Equivocal
Sodium nitrate	5	5	8	Positive
Cinnamaldehyde	5	5	7	Dose ?
Vanadium pentoxide	10	10	10	Negative

[a]Columns 2 and 3 are estimated based only on the list of 30 test compounds, but column 4 is based on a more general scale determined by ethyl alcohol and vinyl chloride near the low end and 2,3,7,8-TCDD at the high end. [b]Data from Jones et al. (*14*).

Appendix: Use of TD_{50}s and Maximum Doses Tested for 67 Compounds to Estimate the Ideal Test Dosage for Rodent Carcinogenesis Experiments: A Range-finding Hypothesis

Definitions

Potency: used to describe differential toxicity of one substance when compared with another or the effect caused by one dose relative to that of a different dosage of the same substance.

Relative potency: ratio of doses required to cause the same level of toxic effect in both frequency and severity.

Unit potency: indicates that an insult of study had identical toxicity to the test substance or dosage.

TD_{50}: dose of a substance that reduces the number of tumor-free animials by 50%.

MaxD: the highest dose tested in a 2-year study by the NTP.

Summary

Potency adjustments to both TD_{50} values and maximum doses tested in the NTP 2-year rodent carcinogenesis program seem to suggest that there may be an ideal range of test doses distributed about a potency-adjusted median of 5 mg/kg/day when expressed relative to B[*a*]P. The central $\pm\sigma$ interval would suggest an ideal range of potency-adjusted test doses between 5/7 mg/kg/day and 5×7 mg/kg/day. Protocol doses below this range may be associated with too many false negatives and protocol test doses above this range may create too many false positives. This hypothesis is developed for application to the prediction of test outcomes for 30 compounds currently scheduled for testing in the 2-year NTP rodent carcinogenesis bioassays.

Background

A previous study that evaluated the toxicity of 2,3,7,8-TCDD relative to 100 other compounds provided a list of well-tested compounds of common environmental concern (*5*). From that list, 81 compounds had published TD_{50} values (*13*) and 67 of the 81 compounds were included in the database where test doses were available (*12*).

This group of 67 compounds provides a reference database that will be standardized and used to estimate range-finding doses for the NTP 2-year rodent-testing program. The ideal target dosage for untested compounds can then be estimated from the 67 compounds.

Method

Each of the 67 compounds is compared with each of the 5 reference compounds on

the basis of a relative potency analysis, as described for the 30 test compounds analyzed above. Next, the reference-specific relative potency values are standardized to a common scale indexed by a potency of unity (i.e., 1.0) for B[*a*]P. The median of these converted values is used to characterize the potency of a particular compound. The standardized potency scale correlates well with both the TD_{50} and the maximum test doses (MaxD) for the 67 compounds. Finally, the potency-adjusted TD_{50} and MaxD values are used to plot a log-probability frequency distribution. These distributions are used to determine the central 68% of the estimates and are used as the basis for estimating the ideal test dosage. The results of that analysis can be used either for range finding of NTP test doses or to estimate if the independently determined test dose is too strong or too weak relative to the MaxD used for the central tendency (i.e., $\pm\sigma$) of the 67 compounds considered.

Results

Potency values for the 67 compounds relative to B[*a*]P, nicotine, cisplatin, aflatoxin, and cyclophosphamide are given in Table A1. The values were estimated by the CRASH personal computer program. Column-specific results are on different potency scales. A column of potency values is standardized to unit potency for the reference compound shown in the column heading. All potency values were taken as computed by the CRASH program, and therefore comparisons involving two or fewer matches were not considered.

Potency values in Table A1 were compared for the different reference compounds taken two by two, as shown in the scatter plots of Figure A1. As seen in Figure A1, estradiol is evaluated inconsistently by the various reference compounds, probably because of its hormone action. Occasionally, bis-(2-chloroethyl)ether, sodium saccharine, and vinyl chloride to a lesser degree, straggle somewhat from the central tendency. In addition, the comparisons involving B[*a*]P demonstrate more scatter because of the absence of acute toxicity data for B[*a*]P. Overall, it is clear that order-of-magnitude precision is typical between median relative potency estimates based on each of the compounds.

Using the median potency estimates for each reference compound relative to B[*a*]P, the results listed in Table A1 can be converted to a common scale as seen in Table A2. The conversion factors used were 3.2, 8.2, 8.08, and 1 for nicotine, cisplatin, aflatoxin, and cyclophosphamide, respectively. The median estimate is given in column 7 and will be used as the characteristic potency for each of the 67 compounds listed in column 2. The range of estimates is given in columns 8 and 9.

The median potency values from Table A2 for each of the 67 compounds were used to produce the scatter plots of TD_{50} or MaxD versus RP for rats and mice, as seen in Figure A2. In Figure A2, the general relationship between potency and either the TD_{50} or MaxD values seems to hold. Occasionally, hydrogen peroxide (90%), bis(chloromethyl)ether, bis-(2-chloroethyl)ether, and hormone-acting diethylstilbestrol are outliers on the scatterplots. Test results are typically based on 50 animals within a particular sex and species, so some randomness should be expected. However, beyond that randomness, there seems to be some added uncertainty for chemically reactive compounds that may bind to sites not directly related to carcinogenic mechanisms or to sites that act through hormone receptors.

The potency-adjusted TD_{50} and MaxD values were used to plot a log-probability frequency distribution, as seen in Figure A3. From Figure A3, we can see that the data seem reasonably log-normal within $\pm\sigma$ of the mean. The results appear to deviate above linearity for the tails of the distribution, but this is likely to be a result of a bias for selecting suspected hazards for testing as opposed to random selecting from the complete inventory of environmental and industrial pollutants.

Fits of the log-probability distribution to mice or rats (shown cumulatively in Figure A3) and to the combined data set are given in Table A3. As seen in Table A3, the central 68% of the estimates are within a factor of 11 for the TD_{50} data. The TD_{50} values may be intrinsically more variable than the MaxD doses because different dose–response models were used from compound to compound. This is supported to some degree by the result that 68% of the MaxD values are within a factor of 7.32 of the distribution mean.

Conclusions

Results from these comparisons suggest that potency-adjusted doses from past NTP test protocols may be used for range finding of ideal test doses for compounds scheduled for future testing. Alternatively, the potency-adjusted doses from past NTP experiments may be used to form an opinion as to whether a protocol test dose deriving from subchronic test results is within the acceptable range: too weak, so that false negative findings may result, or too strong, so that they may carry the possibility of causing false positive conclusions.

For simplicity, it is proposed that the ideal potency-adjusted test dose can be taken as 5 mg/kg/day, with a 68% confidence interval based on a factor of 7. This range is defined by the 67 compounds evaluated. For compounds tested below 5/7 mg/kg/day or above 5×7 mg/kg/day, there may be a higher frequency of false negatives and false positives. That hypothesis is applied to the 30 compounds currently scheduled for testing in the NTP rodent carcinogenesis bioassays.

Table A1. Potency values for 67 compounds relative to B[*a*]P, nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide.[a]

Test compound	RP to B[*a*]P	Matches	RP to nicotine	Matches	RP to cisplatin	Matches	RP to aflatoxin B_1	Matches	RP to cyclophosphamide	Matches
Acetamide	0.2	5	0.00141	9	0.00157	9	0.000686	9	0.014	17
Acrylonitrile	0.0794	23	0.487	12	0.058	16	0.0116	28	0.876	28
Aflatoxin B_1	8.08	74	0.496	9	1.53	24	1	Self	40	59
Aldrin	1.92	11	0.403	13	0.132	9	0.0785	19	3.11	19
Benzene	0.0324	34	0.0155	14	0.0193	16	0.0044	34	0.0696	41
Benzidine	0.57	34	0.162	5	0.0835	15	0.1	31	0.8	27
Benzo[*a*]pyrene	1	Self	0.24	9	0.0938	34	0.124	76	1.03	73
4-Biphenylamine	0.827	22	Inadequate	<3	0.149	15	0.0439	21	0.5	23
Bis(2-chloroethyl) ether	0.000312	3	Inadequate	<3	0.0003	3	Inadequate	<3	0.283	4
Bis(chloromethyl) ether	0.104	7	Inadequate	<3	0.123	3	0.511	4	1	5
Carbaryl	0.0221	10	0.0628	15	0.1	15	0.016	18	0.625	17
Carbon tetrachloride	0.417	12	0.00948	11	0.02	9	0.00109	15	0.088	15
Chlordane	0.347	3	0.0424	7	0.129	5	0.0175	9	0.8	7
Chloroambucil	3.33	17	Inadequate	<3	0.496	12	0.075	19	2.67	21
Chloroform	0.0182	8	0.0129	8	0.015	11	0.006	16	0.119	22
Cyclophosphamide	1	60	0.244	16	0.0613	46	0.03	57	1	Self
p,p'-DDD	Inadequate	<3	Inadequate	<3	Inadequate	<3	0.0222	4	0.58	5
p,p'-DDE	0.126	6	0.0568	3	0.0347	4	0.0129	9	0.467	8
DDT	0.0583	12	0.0837	13	0.183	12	0.02	21	0.957	32
Dibenz[*a,h*]anthracene	0.953	26	Inadequate	<3	3.3	7	0.112	16	3.2	11
Dibutylnitrosamine	0.0633	9	Inadequate	<3	0.0183	4	0.00204	19	0.355	15
3,3'-Dichlorobenzidine	0.194	10	Inadequate	<3	0.119	7	0.0118	10	0.26	15
1,2-Dichloroethane	0.0981	18	0.0179	9	0.014	15	0.000854	20	0.132	23
Dieldrin	1.15	8	0.51	13	0.529	10	0.146	16	3.89	14
Diethylnitrosamine	0.373	55	0.128	7	0.05	23	0.02	55	0.206	50
Diethylstilbestrol	0.562	52	0.264	12	0.15	16	0.0766	38	5.88	53
7,12-DMBA (57-97-6)	1.52	97	0.153	11	0.288	28	0.122	65	2.04	67
1,1-Dimethylhydrazine	0.319	15	0.0555	8	0.0627	15	0.0397	20	0.517	17
Dimethylnitrosamine	0.34	75	0.862	7	0.27	23	0.0498	72	0.995	61
p-Dioxane	0.0005	7	0.0128	4	0.00574	5	0.0000341	11	0.00634	10
Diphenylnitrosamine	0.0202	17	0.0063	5	0.00476	9	0.00263	17	0.11	21
ENNG (4245-77-6)	0.227	24	Inadequate	<3	2.58	6	0.0375	13	14.4	10
Estradiol	0.52	11	705	6	0.367	5	0.0918	11	13.1	14
Ethyl alcohol	0.00442	24	0.00164	22	0.00221	16	0.00041	21	0.00596	24
Ethyleimine	2.93	13	1.48	5	0.727	9	0.15	17	11.4	15
Ethylene dibromide	0.209	27	0.465	6	0.0333	15	0.00268	32	1.5	30
Ethylene thiourea	0.1	11	0.0273	5	0.0145	6	0.00281	18	0.132	23
Heptachlor	0.18	3	0.00401	3	Inadequate	<3	0.000482	8	0.214	6
Hexachlorobenzene	0.42	9	0.0169	6	0.0128	5	0.000575	10	0.149	3
Hexachlorobutadiene	0.15	7	0.334	7	0.0617	10	0.0267	14	1.45	13
Hexachloroethane	0.00304	3	0.00618	6	0.00147	3	0.000946	5	0.0349	6
Hydrazine	0.359	11	0.127	11	0.146	13	0.127	18	2.05	16
Diphenylhydrazine	0.642	6	Inadequate	<3	0.357	3	0.0146	8	0.485	9
Hydrogen peroxide (90%)	0.549	16	5.02	6	0.11	10	0.0849	20	4.8	12
Kepone	0.729	4	0.15	7	0.118	3	0.00662	7	3.49	9
Lindane	0.4	9	0.338	9	0.2	9	0.0632	17	1.01	16
3-Methylcholanthrene	1	69	1.25	3	0.175	17	0.245	38	3.32	36
Methyl hydrazine	0.0372	4	0.524	12	0.386	14	0.286	9	6.17	12
Methyl methanesulfonate	0.289	83	0.2	11	0.0606	37	0.0314	68	1.19	85
MNNG (70-25-7)	1.25	85	0.0894	9	0.348	34	0.281	74	2	59
2-Naphthylamine	0.3	35	1	5	0.04	15	0.0475	35	0.825	35
Nitrogen mustard	6.41	31	6	15	2.67	22	0.9	37	37.5	41
Phenacetin	0.0112	16	0.0109	7	0.0101	11	0.00109	19	0.0423	25
Phenobarbital	0.009	23	0.0109	7	0.0112	12	0.001	21	0.0312	26
Reserpine	8.5	10	0.733	16	0.524	15	0.245	12	9.33	26
Safrole	0.156	30	0.0207	8	0.012	15	0.00562	26	0.234	33
Sodium saccharin	0.593	13	0.011	6	0.00139	6	0.000845	17	0.0183	18
2,3,7,8-TCDD	250	19	87.8	12	203	8	54.9	25	1000	27
Tetrachloroethane	0.0282	6	0.0385	4	0.0209	8	0.00329	6	0.19	10
Tetrachloroethylene	0.00359	5	0.00227	5	0.0027	8	0.0012	6	0.0302	11
Thioacetamide	0.226	18	0.0433	4	0.0583	7	0.00208	26	0.128	21
Toxaphene	0.014	6	0.141	9	0.0892	8	0.02	15	0.807	15
Trichloroethylene	0.0434	17	0.00885	10	0.0127	11	0.00421	14	0.0651	23
2,4,6-Trichlorophenol	0.05	7	0.061	3	0.03	5	0.002	9	0.0833	10
Urethane	0.0625	48	0.0045	9	0.0103	20	0.00455	42	0.0421	43
Vinyl chloride	0.00547	4	Inadequate	<3	0.0264	4	0.000174	10	0.261	17
Vinylidene chloride	0.0309	6	0.0172	3	0.0111	5	0.0107	6	0.5	11

[a]The column-specific potency values are normalized to unit potency for the reference compounds shown in the column headings. "Matches" are the number of common bioassays used to compare the row chemical with the reference compound (column).

Table A2. Reference compounds from Table A1 converted to a common scale of relative potency.[a]

Test compound	Benzo[*a*]-pyrene (1)	Nicotine (3.2)	Cisplatin (8.2)	Aflatoxin B_1 (8.08)	Cyclophos-phamide (1)	Median	Minimum	Maximum
Acetamide	0.2	0.004512	0.012874	0.00554288	0.014	0.0129	0.004512	0.2
Acrylonitrile	0.0794	1.5584	0.4756	0.093728	0.876	0.476	0.0794	1.56
Aflatoxin B1	8.08	1.5872	12.546	8.08	40	8.08	1.5872	40
Aldrin	1.92	1.2896	1.0824	0.63428	3.11	1.29	0.634	3.11
Benzene	0.0324	0.0496	0.15826	0.035552	0.0696	0.0496	0.0324	0.158
Benzidine	0.57	0.5184	0.6847	0.808	0.8	0.685	0.5184	0.808
B[*a*]P	1	0.768	0.76916	1.00192	1.03	1	0.768	1
4-Biphenylamine	0.82	—	1.2218	0.354712	0.5	0.664	0.3547	1.22
Bis(2-chloroethyl) ether	0.000312	—	0.00246	—	0.283	0.00246	0.000312	0.283
Bis(chloromethyl) ether	0.104	—	1.0086	4.12888	1	1	0.104	4.13
Carbaryl	0.0221	0.20096	0.82	0.12928	0.625	0.201	0.0221	0.82
Carbon tetrachloride	0.417	0.030336	0.164	0.0088072	0.088	0.088	0.00881	0.417
Chlordane	0.347	0.13568	1.0578	0.1414	0.8	0.347	0.1357	1.06
Chloroambucil	3.33	—	4.0672	0.606	2.67	3	0.606	4.07
Chloroform	0.0182	0.04128	0.123	0.04848	0.119	0.0484	0.0182	0.123
Cyclophosphamide	1	0.7808	0.50266	0.2424	1	0.781	0.24	1
p,p'-DDD	—	—	—	0.179376	0.58	0.38	0.179	0.58
p,p'-DDE	0.126	0.18176	0.28454	0.104232	0.467	0.182	0.104	0.467
DDT	0.0583	0.26784	1.5006	0.1616	0.957	0.268	0.0583	1.5
Dibenz(*a,h*)anthracene	0.953	—	27.06	0.90496	3.2	2.08	0.905	27.06
Dibutylnitrosamine	0.0633	—	0.15006	0.0164832	0.355	0.107	0.0165	0.355
3,3'-Dichlorobenzidine	0.194	—	0.9758	0.095344	0.26	0.23	0.0953	0.976
1,2-Dichloroethane	0.0981	0.05728	0.1148	0.00690032	0.132	0.0806	0.0069	0.132
Dieldrin	1.15	1.632	4.3378	1.17968	3.89	1.63	1.15	4.34
Diethylnitrosamine	0.373	0.4096	0.41	0.1616	0.206	0.373	0.162	0.41
Diethylstibestrol	0.562	0.8448	1.23	0.618928	5.88	0.845	0.562	5.88
7,12-Dimethylbenz[*a*]anthracene (57-97-6)	1.52	0.4896	2.3616	0.98576	2.04	1.52	0.49	2.36
1,1-Dimethylhydrazine	0.319	0.1776	0.51414	0.320776	0.517	0.321	0.178	0.517
Dimethylnitrosamine	0.34	2.7584	2.214	0.402384	0.995	0.995	0.34	2.76
p-Dioxane	0.0005	0.04096	0.047068	0.000275528	0.00634	0.00634	0.000276	0.0471
Diphenylnitrosamine	0.0202	0.02016	0.039032	0.0212504	0.11	0.02125	0.02016	0.11
ENNG (4245-77-6)	0.227	—	21.156	0.303	14.4	7.35	0.227	21.16
Estradiol	0.52	2256	3.0094	0.741744	13.1	3.01	0.52	2256
Ethyl alcohol	0.00442	0.005248	0.018122	0.0033128	0.00596	0.00525	0.00331	0.0181
Ethyleimine	2.93	4.736	5.9614	1.212	11.4	4.74	1.21	11.4
Ethylene dibromide	0.209	1.488	0.27306	0.0216544	1.5	0.273	0.0217	1.5
Ethylene thiourea	0.1	0.08736	0.1189	0.0227048	0.132	0.0874	0.0227	0.132
Heptachlor	0.18	0.012832	—	0.00389456	0.214	0.0964	0.00389	0.214
Hexachlorobenzene	0.42	0.05408	0.10496	0.004646	0.149	0.105	0.00465	0.422
Hexachlorobutadiene	0.15	1.0688	0.50594	0.215736	1.45	0.506	0.15	1.45
Hexachloroethane	0.00304	0.019776	0.012054	0.00764368	0.0349	0.012	0.00304	0.0349
Hydrazine	0.359	0.4064	1.1972	1.02616	2.05	1.03	0.359	2.05
Diphenylhydrazine	0.642	—	2.9274	0.117968	0.485	0.564	0.118	2.93
Hydrogen peroxide (90%)	0.549	16.064	0.902	0.685992	4.8	0.902	0.549	16.06
Kepone	0.729	0.48	0.9676	0.0534896	3.49	0.729	0.0535	3.49
Lindane	0.4	1.0816	1.64	0.510656	1.01	1.01	0.4	1.64
3-Methylcholanthrene	1	4	1.435	1.9796	3.32	1.98	1	4
Methyl hydrazine	0.0372	1.6768	3.1652	2.31088	6.17	2.31	0.0372	6.17
Methyl methanesulfonate	0.289	0.64	0.49692	0.253712	1.19	0.497	0.25	1.19
MNNG (70-25-7)	1.25	0.28608	2.8536	2.27048	2	2	0.286	2.85
2-Naphthylamine	0.3	3.2	0.328	0.3838	0.825	0.384	0.3	3.2
Nitrogen mustard	6.41	19.2	21.894	7.272	37.5	19.2	6.41	37.5
Phenacetin	0.0112	0.03488	0.08282	0.0088072	0.0423	0.0349	0.00881	0.0828
Phenobarbital	0.009	0.03488	0.09184	0.00808	0.0312	0.0312	0.00808	0.0918
Reserpine	8.5	2.3456	4.2968	1.9796	9.33	4.3	1.98	9.33
Safrole	0.156	0.06624	0.0984	0.0454096	0.234	0.0984	0.0454	0.234
Sodium saccharin	0.593	0.0352	0.011398	0.0068276	0.0183	0.0183	0.00683	0.593
2,3,7,8-TCDD	250	280.96	1664.6	443.592	1000	444	250	1665
Tetrachloroethane	0.0282	0.1232	0.17138	0.0265832	0.19	0.123	0.0266	0.19
Tetrachloroethylene	0.00359	0.007264	0.02214	0.009696	0.0302	0.0097	0.00359	0.0302
Thioacetamide	0.226	0.13856	0.47806	0.0168064	0.128	0.139	0.0168	0.478
Toxaphene	0.014	0.4512	0.73144	0.1616	0.807	0.451	0.014	0.807
Trichloroethylene	0.0434	0.02832	0.10414	0.0340168	0.0651	0.0434	0.02832	0.104
2,4,6-Trichlorophenol	0.05	0.1952	0.246	0.01616	0.0833	0.0833	0.01616	0.246
Urethane	0.0625	0.0144	0.08446	0.036764	0.0421	0.0421	0.0144	0.0845
Vinyl chloride	0.00547	—	0.21648	0.00140592	0.261	0.111	0.00141	0.261
Vinylidene chloride	0.0309	0.05504	0.09102	0.086456	0.5	0.0865	0.0309	0.5

[a]Conversion to a common scale having unit potency for B[*a*]P was achieved by factors of 3.2, 8.2, 8.08, and 1.0 for nicotine, cisplatin, aflatoxin B_1, and cyclophosphamide, respectively.

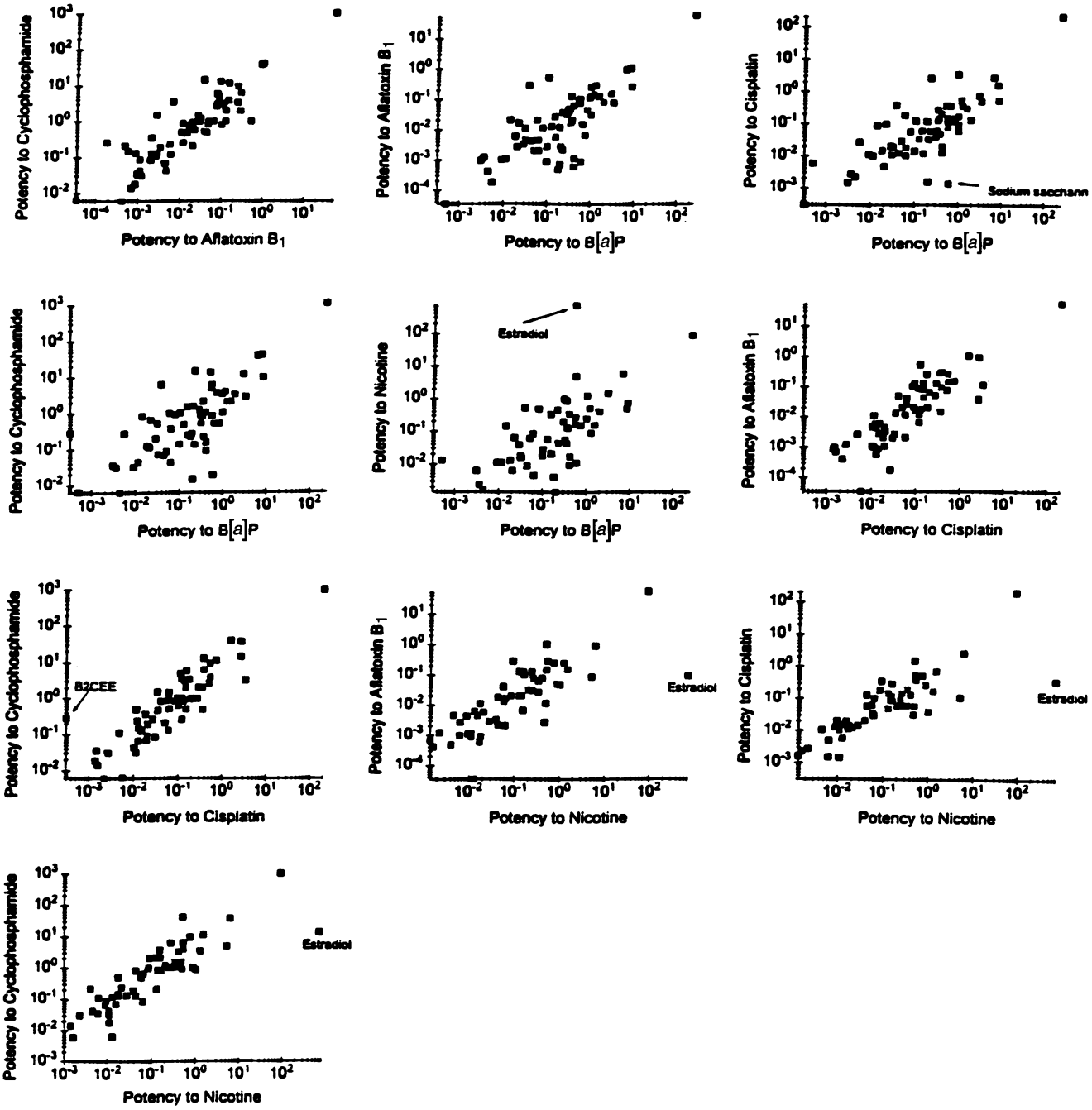

Figure A1. Scatter plots of the potency of 67 compounds relative to 5 reference compounds taken 2 at a time.

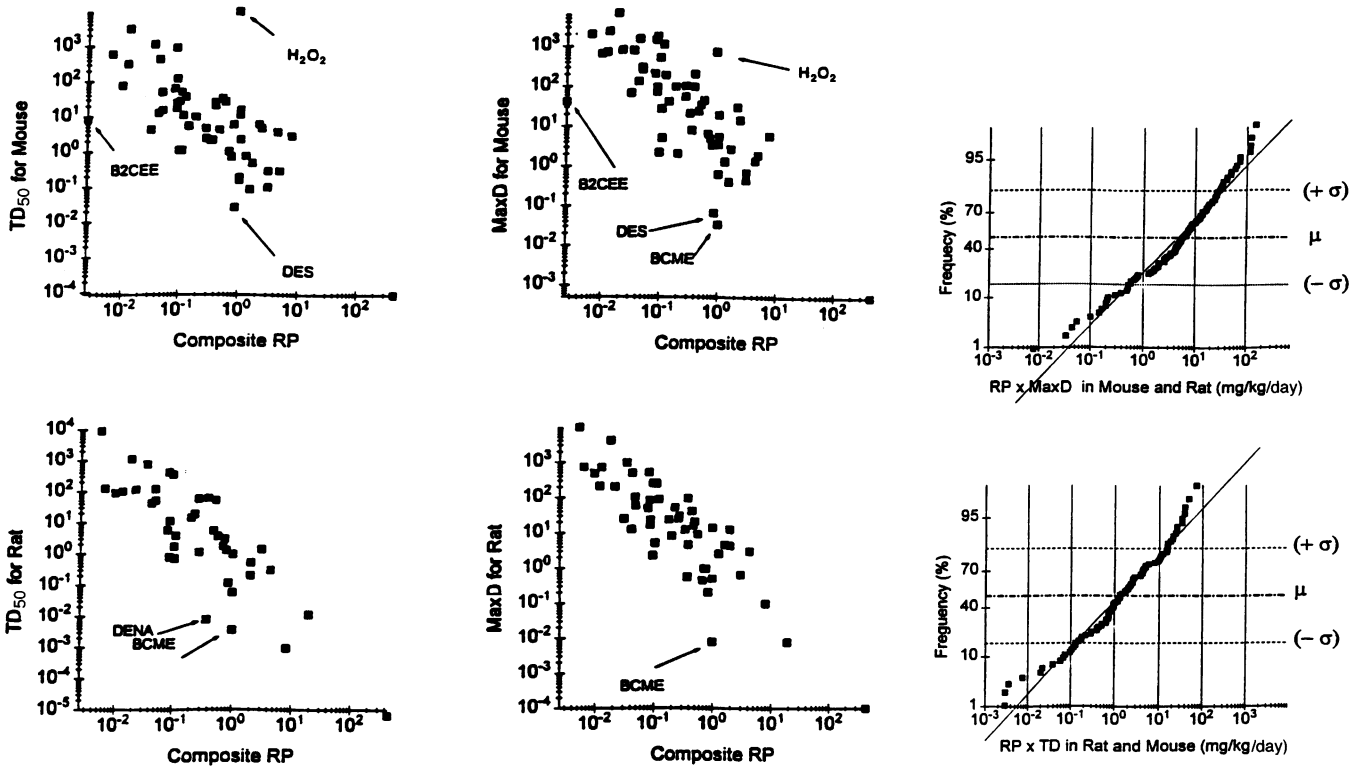

Figure A2. Median relative potency values from Table A2 were compared with the TD_{50} estimates from Gold et al. (*13*) and the maximum doses tested in the 2-year rodent carcinogenesis tests.

Figure A3. Cumulative log-normal frequency plots for potency-adjusted TD_{50} doses and maximum doses tested.

Table A3. Fitted values for a log-normal frequency distribution comparing potency-adjusted TD_{50} and maximum doses tested for rats, mice, and mice plus rats.[a]

Data fitted	Log_{10} μ	Log_{10} σ	Untransformed μ, mg/kg/day	Untransformed σ
RP × TD_{50} (mouse)	0.299	0.972	2.00	Factor of 9.38
RP × TD_{50} (rat)	0.0848	1.06	1.22	Factor of 11.5
RP × TD_{50} (both)	0.158	1.04	1.44	Factor of 11.0
RP × MaxD (mouse)	0.84	0.877	6.92	Factor of 7.50
RP × MaxD (rat)	0.499	0.822	3.16	Factor of 6.63
RP × MaxD (both)	0.617	0.865	4.75	Factor of 7.32

[a]The "untransformed" values are based on a unit potency for B[*a*]P.

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