Predicting Chemical Carcinogenesis in Rodents

Joseph T. Wachsman, Douglas W. Bristol, Judson Spalding, Michael Shelby, and Raymond W. Tennant
National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 USA

Identification of a chemical as a human carcinogen usually comes from epidemiological studies, which are frequently initiated and/or confirmed by experiments with laboratory animals. For some time now, we have relied heavily upon the rodent bioassay to assess the potential hazards of chemicals in humans. This assay, although of great value, is expensive, requires 3–4 years to provide results and, consequently, permits the testing of only a handful of chemicals each year. We also have available a host of predictive methods which draw upon various kinds of information and test results, including standardized short-term *in vivo* biological assays, physicochemical properties of chemicals, disposition and metabolism studies, subchronic organ toxicity studies, structure–activity relationships, and rules generated by either human expert intuition or computer-learning methods. Can we effectively use this knowledge to decrease our dependence on the 2-year rodent bioassay, prioritize those chemicals for which the bioassay would still be desirable, and still adequately protect public health?

Table 1. Prediction set of 44 chemicals

No.	Chemical	CAS no.	Technical report no.
1	Amphetamine sulfate[b]	60-13-9	387
2	Naphthalene[c]	91-20-3	410
3	Polysorbate 80[c]	9005-65-6	415
4	Promethazine HCl[b]	58-33-3	425
5	Resorcinol[b]	108-46-3	403
6	g-Butyrolactone	96-48-0	406
7	Manganese sulfate monohydrate	10034-96-5	428
8	Monochloroacetic acid	79-11-8	396
9	*p*-Nitrophenol	100-02-7	417
10	Tricresyl phosphate	1330-78-5	433
11	*o*-Benzyl-*p*-chlorophenol[c]	120-32-1	424
12	2,2-Bis(bromomethyl)-1,3-propanediol[a]	3296-90-0	
13	t-Butyl alcohol[a]	75-65-0	436
14	3,4-Dihydrocoumarin	119-84-6	423
15	Ethylene glycol[b]	107-21-1	413
16	Mercuric chloride[c]	7487-94-7	408
17	Methylphenidate HCl[c]	298-59-9	439
18	Theophylline[a]	58-55-9	
19	4,4'-Thiobis(6-t-butyl-m-cresol)[b]	96-69-5	435
20	Triamterene[c]	396-01-0	420
21	Diphenylhydantoin	57-41-0	404
22	Pentachloroanisole	1825-21-4	414
23	Chloramine	10599-90-3	392
24	4,4'-Diamino-2,2'-stilbenedisulfonic acid	7336-20-1	412
25	Methyl bromide	74-83-9	385
26	*p*-Nitrobenzoic acid[c]	62-23-7	442
27	Sodium azide[b]	26628-22-8	389
28	Tris(2-chloroethyl)phosphate[c]	115-96-8	391
29	CI Direct blue 218[b]	28407-37-6	430
30	CI Pigment red 3[b]	2425-85-6	407
31	CI Pigment red 23[c]	6471-49-4	411
32	2,4-Diaminophenol 2HCl[c]	137-09-7	401
33	4-Hydroxyacetanilide[c]	103-90-2	394
34	Salicylazosulfapyridine[a]	599-79-1	
35	Titanocene dichloride[c]	1271-19-8	399
36	CI Acid red 114[b]	6459-94-5	405
37	CI Direct blue 15[b]	2429-74-5	397
38	Coumarin	91-64-5	422
39	2,3-Dibromo-1-propanol[b]	96-13-9	400
40	3,3'-Dimethylbenzidine 2HCl[b]	612-82-8	390
41	HC Yellow 4[c]	59820-43-8	419
42	*p*-Nitroaniline[c]	100-01-6	418
43	*o*-Nitroanisole[b]	91-23-6	416
44	1,2,3-Trichloropropane[b]	96-18-4	384

[a]Bioassay incomplete at the time of the workshop.
[b]Chemicals with high agreement among the predictions and unambiguous bioassay results.
[c]Chemicals with poor agreement among the predictions and weak positive or equivocal bioassay results.

An International Workshop on Predicting Chemical Carcinogenesis in Rodents was convened to address these questions. The workshop was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, 24–25 May 1993. Approximately 150 people attended the workshop, including participants from the United Kingdom, France, Italy, and Japan; their interests ranged from active involvement in the predictive process to a desire for a more basic understanding of the mechanisms of carcinogenesis.

The foundation for the workshop was provided by a publication of Tennant et al. (*1*) that made prospective predictions on the potential rodent carcinogenicity of 44 chemicals (Table 1) that were then in the process of being tested by the National Toxicology Program (NTP). Tennant et al. used a variety of different kinds of information including mutagenicity, toxicity, and subchronic pathology for the predictive process and proposed that the NTP bioassay be used as an experimental tool for validating predictive methods. The editor of Mutagenesis then invited other investigators to make predictions on the same set of 44 chemicals, using whatever factors, assay systems, or programs they deemed appropriate. This invitation resulted in the peer-reviewed publication of predictive papers from seven additional groups (*2–8*), as well as the informal communication of two other sets of predictions.

A major goal of the workshop was to evaluate the state of the art of prediction of rodent carcinogenesis, including an examination of the strengths and limitations of the different predictive methods. The question of what types of additional information could improve the predictive process was a recurring theme at the conference. Of equal importance was a consideration of the strengths and limitations of the NTP bioassay, the ultimate standard for evaluating predictive methods. Finally,

Address correspondence to J. T. Wachsman, MD B3-09, NIEHS, PO Box 12233, Research Triangle Park, NC 27709 USA.

it was suggested that the cumulative information derived from the various predictive methods could be incorporated into the process of selecting chemicals for the 2-year rodent bioassay.

Two-year bioassay results for 40 of the 44 chemicals in the prediction experiment were available at the time of the workshop, and it was considered unlikely that the results from the 4 additional chemicals (Table 1) would significantly influence the general conclusions. It was apparent from the start of the workshop that there was a substantial number of chemicals (14) for which there was overall agreement among the predictions and the unambiguous 2-year bioassay results (Table 1). This group included bioassay results that were either positive (trans-species or multiple site) or negative. Bioassay results that were indicative of weak (single site, single species) or equivocal carcinogenic activity were poorly correlated with many of the predictive methods. This group contained 15 chemicals with highly diverse structural and biological properties (Table 1). It was also determined that the 40 chemicals examined, were, in most ways, representative of the complete NTP database of 450 chemicals. A possible exception to this was the somewhat higher percentage of equivocal bioassay results among the 40 chemicals than that seen in the more comprehensive NTP database.

On the whole, the human expert approach performed best, and it would appear that the more extensive and varied the information base used, the more accurate the predictive process. Approaches attempting to predict carcinogenesis primarily from chemical structure, while ignoring a cross-section of biological information, did not perform as well. A possible exception to this generalization was the method that exclusively used experimentally determined electrophilicity (k_e) as a predictor. It did surprisingly well, perhaps because electrophilicity itself may be a measure of metabolic reactivity. It was suggested that some of the predictive systems would perform better if the results generated by the computer were to be evaluated by a human expert.

Several speakers analyzed the types of problems encountered with the NTP bioassay. One example was the nature of equivocal calls. Because equivocal results are uninformative, it is desirable to minimize their occurrence. Furthermore, we do not know how reproducible equivocal results are, or for that matter, the reproducibility of any of the NTP bioassay results. It is likely that under ideal testing circumstances, including the use of increased numbers of animals and histopathological sampling of tissues, chemicals giving equivocal results will be found to consist of a mixture of negative, weak positive, and uncertain carcinogens. The feasibility of predicting equivocal bioassay results was discussed, but not resolved.

It was stated repeatedly that the predictive process would benefit from further studies on the mechanisms of carcinogenesis. It was suggested that we prioritize chemicals for the rodent bioassay not only on the basis of our need to know if they are carcinogens, but also on their utility in providing mechanistic insights. Of course, it would be of great value to have data on the metabolism, distribution, and bioavailability of each chemical, as well as to know which chemicals interact with cell receptors (plasma membrane, cytosolic, nuclear) or induce a stress response (heat shock proteins, reactive oxygen species, inductions of cytochrome P450s, etc.), which chemicals are inflammatory or mitogenic, and which chemicals can alter patterns of gene expression.

In summary, the predictions of carcinogenicity by human experts were the most accurate overall, apparently because they used a broad range of information about biological responses and chemical properties to develop a total weight of evidence for each prediction. Methods that were generally based on more highly specialized information such as molecular configuration or electronegativity did not fare as well. Despite the different approaches, there was excellent agreement among the predictions made for some chemicals that produced unambiguous bioassay results, whether for carcinogenic chemicals like 2,3-dibromo-1-propanol and 3,3'-dimethylbenzidine or noncarcinogenic chemicals like resorcinol and amphetamine sulfate. The information currently available from predictive systems can support decisions involving testing priorities and the regulation of chemicals.

The discipline of predicting chemical toxicity and carcinogenesis is in its infancy. However, progress has been made over the past 5 years, and there is every reason to believe that the development of this scientific discipline will continue. It is hoped that the predictive process will improve the methods for selecting test chemicals, lead to a reduction in the use of laboratory animals, continue to protect the public health, and at the same time encourage new approaches to our understanding of the mechanisms of carcinogenesis.

International Workshop on Predicting Chemical Carcinogenesis

Scientific Program Committee

John Ashby, Douglas Bristol (chair), Michael Shelby, Judson Spalding, Stanley Stasiewicz, Raymond Tennant, Joseph Wachsman (coordinator)

Predictors

R. Tennant, J. Spalding, S. Stasiewicz, J. Ashby, H. Borgstedt, D. Sanderson, C. Earnshaw, J. Langowski, K. Enslein, V. Gombar, B. Blake, H. Rosenkranz, G. Klopman, D. Lewis, C. Ionnides, D. Parke, T. Jones, M. Morris, C. Easterly, G. Bakale, R. McCreary, R. Benigni

Discussants

C. Barrett, W. Farland, J. Goodman, L. Kier, W. Lijinsky, J. Popp, A. Richard, E. Zeiger

Other Presenters

J. Dunnick, S. Eustis, J. Haseman, M. Shelby, B. Schwetz

References

1. Tennant RW, Spalding J, Stasiewicz S, Ashby J. Prediction of the outcome of rodent carcinogenicity bioassays currently being conducted on 44 chemicals by the National Toxicology Program. Mutagenesis 5:3–14(1990).
2. Enslein K, Blake BW, Borgstedt HH. Prediction of probability of carcinogenicity for a set of ongoing NTP bioassays. Mutagenesis 5: 305–306(1990).
3. Rosenkranz HS, Klopman G. Prediction of carcinogenicity in rodents of chemicals currently being tested by the US National Toxicology Program: Structure-activity correlations. Mutagenesis 5:425–432(1990).
4. Lewis DFV, Ioannides C, Parke DV. A prospective toxicity evaluation (COMPACT) on 40 chemicals currently being tested by the National Toxicology Program. Mutagenesis 5:433–435 (1990).
5. Benigni R. QSAR prediction of rodent carcinogenicity for a set of chemicals currently bioassayed by the US National Toxicology Program. Mutagenesis 6:423–425(1991).
6. Jones TD, Easterly CE. On the rodent bioassays currently being conducted on 44 chemicals: a RASH analysis to predict test results from the National Toxicology Program. Mutagenesis 6:507–514(1991).
7. Sanderson DM, Earnshaw CG. Computer prediction of possible toxic action from chemical structure; the DEREK system. Hum Exp Toxicol 10:261–273(1991).
8. Bakale G, McCreary RD. Prospective k_e screening of potential carcinogens being tested in rodent bioassays by the US National Toxicology Program. Mutagenesis 7:91–94(1992).