

The NIEHS Predictive-Toxicology Evaluation Project

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The Predictive-Toxicology Evaluation (PTE) project conducts collaborative experiments that subject the performance of predictive-toxicology (PT) methods to rigorous, objective evaluation in a uniquely informative manner. Sponsored by the National Institute of Environmental Health Sciences, it takes advantage of the ongoing testing conducted by the U.S. National Toxicology Program (NTP) to estimate the true error of models that have been applied to make prospective predictions on previously untested, noncongeneric-chemical substances. The PTE project first identifies a group of standardized NTP chemical bioassays either scheduled to be conducted or are ongoing, but not yet complete. The project then announces and advertises the evaluation experiment, disseminates information about the chemical bioassays, and encourages researchers from a wide variety of disciplines to publish their predictions in peer-reviewed journals, using whatever approaches and methods they feel are best. A collection of such papers is published in this Environmental Health Perspectives Supplement, providing readers the opportunity to compare and contrast PT approaches and models, within the context of their prospective application to an actual-use situation. This introduction to this collection of papers on predictive toxicology summarizes the predictions made and the final results obtained for the 44 chemical carcinogenesis bioassays of the first PTE experiment (PTE-1) and presents information that identifies the 30 chemical carcinogenesis bioassays of PTE-2, along with a table of prediction sets that have been published to date. It also provides background about the origin and goals of the PTE project, outlines the special challenge associated with estimating the true error of models that aspire to predict open-system behavior, and summarizes what has been learned to date. — Environ Health Perspect 104(Suppl 5):1001–1010 (1996)

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Definitions

Chemical bioassay. An experiment or study involving the exposure of a whole-animal test system to a test article and is conducted according to a standardized protocol so that the range and magnitude of biological responses that characterize an

end point activity, such as carcinogenicity, may be observed; the test system for the U.S. National Toxicology Program (NTP) studies normally utilizes both genders of one rat and mouse strain; the test article is usually a well-characterized, organic

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Abbreviations used: CAS RN, Chemical Abstracts Service Registry Number; CE, clear evidence; E, equivocal; EE, equivocal evidence; EQV, equivocal overall classification; LOE, level of evidence; N, none; NE, no evidence; NEG, negative overall classification; NIEHS, National Institute of Environmental Health Sciences; NP, no prediction made; NTP, U.S. National Toxicology Program; P, positive; POS, positive overall classification; PT, predictive-toxicology; PTE, Predictive-Toxicology Evaluation; PTE-1/PTE-2, first or second PTE experiment; QSAR, quantitative structure–activity relationship; SE, some evidence; STT, short-term tests; TR, Technical Report; W+, weakly positive; W+/U, weak positive or uncertain probability for being positive.

chemical, inorganic compound, mineral, polymer, or mixture.

Level of evidence (LOE). NTP assigns a LOE to each sex–species, chemical carcinogenicity experiment, as defined in each NTP Technical Report (TR). These are CE, clear evidence; SE, some evidence; EE, equivocal evidence; NE, no evidence; for older studies, they are P, positive; E, equivocal; and N, none.

Overall LOE. The LOE assigned to each sex–species experiment, combined with a classification for the overall bioassay study, using the following algorithm: *a*) If the LOE for one or more of the experiments is CE, SE, or P, then the overall classification is positive (POS); *b*) If the LOE for all of the experiments is NE or E, then the overall classification is negative (NEG); *c*) If the LOE for one or more of the experiments is EE or E and the LOE for the other experiments is NE or N, then the overall classification is equivocal (EQV); *d*) Experiments classified as inadequate study (IS) are given no consideration in arriving at the overall LOE classification.

Need for Predictive-Toxicology Models

The NTP conducts standardized chemical bioassays in rodents to identify and characterize exposures to substances that may be associated with carcinogenic or other toxicological effects on human health (1). Current regulations require that safety testing be performed in connection with the development of new chemicals or new uses of known chemicals. However, before the advent of such regulations, more chemicals came into use than can ever be tested using conventional methods. At the present time, society in general and the discipline of toxicology in particular, face the parallel tasks of performing safety evaluations that support the development of new chemical uses before human exposures are permitted and assessing the potential hazard posed by exposures to chemicals that lack safety evaluations. This situation creates an urgent need to develop PT models that

- generate predictions of known reliability or are accompanied by confidence level estimate
- identify hazardous-chemical exposures more rapidly at a lower cost than current procedures
- apply to all types of test articles, including organic, inorganic, polymeric, mineral, and mixtures

- provide information that supports sound decision making for the effective and efficient management of laboratory animal testing that is still needed by regulatory and chemical development programs
- refine and reduce reliance on the use of large numbers of laboratory animals in the conduct of chembioassays
- accelerate the performance of risk assessments and the conduct of research and development programs.

Goals of Predictive-Toxicology Research

The development of models that reliably identify the hazard for untested chemical substances, of any type, using attribute values that can be computed or obtained with minimum testing time and cost is widely recognized to be the most immediate goal of PT research.

The return of information and overall value of an NTP bioassay increases when it is included in a PTE experiment because each prediction made about its outcome represents an additional hypothesis that is tested by the bioassay. Thus, in addition to characterizing the toxicity of individual chemicals (i.e., identify hazard), standardized bioassay tests also stimulate PT research by providing both learning sets for the development of models and the means to subject model performance to hypothesis testing.

Another, less perceived, aspect of PT research has potential value that far exceeds the generation of reliable predictions per se. Some PT models are based on pattern-recognition analysis of a learning set (2–8). The learning set is a database that includes a representative number and range of classified cases, where the chemical bioactivity of each case towards a particular toxicity end point has been determined by standardized testing. Each classified case in the learning set is represented by a corresponding array of values on attributes, selected to reflect various aspects of either or both biological factors and chemical structure that may influence activity. Although “data-mining” by pattern-recognition analysis can be limited by the availability of suitable learning sets, it represents a new approach that has great potential to help discover and confirm the key factors and relationships that govern the various, multifactorial, mechanistic pathways and determine toxic effects. Thus, the ultimate value and most important goal of PT research may lie in the development of its

potential to help identify, characterize, and understand the various mechanisms or modes of action that determine the type and level of response observed when biological systems are exposed to chemicals. Because PT research can confirm existing hypotheses regarding mechanisms and stimulate the formation of new ones (9), it is complementary to and synergistic with the conduct of mechanistic studies.

The discovery aspect of PT research may also lead to an important refinement in the use of quantitative structure–activity–relationship (QSAR) models. A classical, extra thermodynamic QSAR approach (10,11) can only be applied to model chemical bioactivities governed by a unique mechanistic pathway, i.e., where chemical bioactivity is controlled by a single rate-limiting step. This limits the legitimate application of each different QSAR model, to untested chemicals that can be expected to be processed under the control of the same mechanism for which the QSAR was developed. When faced with selecting a QSAR model to study the mechanistic behavior of an untested chemical, there is no legitimate way to determine which of the many available might apply most appropriately. This uncertainty would be eliminated by the development of PT models that predict not only the activity expected for an untested chemical, but also indicate the mechanistic pathway that governs it. Thus, the output of such PT models would serve to guide the selection of QSAR models that may be used legitimately to elucidate mechanistic details and gain understanding that fosters better interpretation of the activity predicted.

Evaluation of Predictive-Toxicology Models

The advantages offered by PT research are clear; however, difficult problems remain that involve both model development and acceptance issues (12). A recent, definitive study of difficulties associated with the model confirmation problem (9) reports Verification, validation, and confirmation of numerical models of natural systems is impossible. This is because natural systems are never closed and because model results are always non-unique. Models can be confirmed by the demonstration of agreement between observation and prediction, but confirmation is inherently partial. Complete confirmation is logically precluded by the fallacy of affirming the consequent and by incomplete access to natural phenomena. Models can only be

evaluated in relative terms, and their predictive value is always open to question. The primary value of models is heuristic.

This important publication explains why it is impossible to establish confidence limits on boundaries of the feature space spanned by a PT model, which might otherwise be used to guide and restrict its application to legitimate cases. Also, because the boundaries of PT models are inexact, the legitimate range of application for PT models will always be uncertain, to some extent. The complex nature of the model confirmation problem presents a perplexing challenge to both developers and potential users; to gain acceptance and fulfill their promise, PT models must demonstrate performance accuracy that earns the confidence of would-be users.

PT-model evaluations based on cross-validation techniques (13) provide useful feedback during development of a model by analysis of a learning set of classified cases, but alone, they cannot provide the information needed to discriminate between high classification accuracy, a sign of model brittleness due to overlearning, and low prediction accuracy for unclassified cases.

The PTE Project

Overview

This project enlists the interdisciplinary resources of the entire PT community in the conduct of experiments that rigorously determine the extent to which predictions, made prospectively, agree with experimental observation. It provides objective, experimentally determined estimates for true error of model performance. It creates unique opportunities for the user and model-developer communities to jointly assess the strengths and weaknesses of various PT models and to evaluate the principles and ideas underpinning their development. More specifically, the PTE project

- identifies test sets of bioassays that focus predictive-toxicology research efforts on a common goal and thereby provides a means for the rigorous, experimental evaluation of PT models;
- provides information on NTP test results as well as samples of test-chemical to the research community,
- encourages involvement of researchers from diverse disciplines to promote the application of a wide range of alternative approaches to solving this difficult problem and to maximize the yield of what can be learned from the comparative evaluation experiments,

- disseminates information about predictions generated to encourage rigorous evaluation of PT-model performance through publication of manuscripts and sponsorship of conferences.

Origin

Tennant and Ashby (2) completed an extensive review of results from NTP standardized tests, to evaluate putative correlations between attributes for chemical substructure features and short-term test (STT) results, often used by toxicologists, because they were thought to carry information of value for predicting chemical carcinogenesis. They used heuristic techniques to analyze a large and uniform learning set, which eventually included 301 classified NTP chemical carcinogenesis bioassays, plus values on attributes obtained from various STT for mutagenicity, the most informative of which was the *Salmonella* assay (14), Ashby structural-alert assignments, histopathology results from subchronic toxicity and chronic carcinogenicity studies, plus values on ancillary attributes possibly related to chemical carcinogenesis. After publishing the last in a series of papers (2), the authors were confident that some of the knowledge gained by their in-depth analysis had relevance to the prediction of chemical carcinogenesis. They subjected their new heuristic rules and relationships to the most rigorous test possible by publishing prospective predictions for the outcome of 44 NTP chemical carcinogenesis bioassays being tested by the NTP (3). With the support and cooperation of the editor of *Mutagenesis*, others were invited to publish sets of predictions, basing them on the methods they preferred (15). A variety of researchers responded and the original set of published predictions evolved to become PTE-1.

Figure 1 illustrates how Tennant et al. used the NTP-standardized testing program to first develop their human-heuristic PT model and then to evaluate the accuracy of its performance. The flow diagram identifies the basic components needed to develop and evaluate PT models and indicates the type, source, and flow of information typical of what might be used to generate prospective predictions and organize a PTE experiment.

The "Tox testing" module in Figure 1 represents the engine that drives learning in toxicology, because it is the primary source of phenomenological observations, the foundation for learning in science. Standardized toxicity testing fosters the

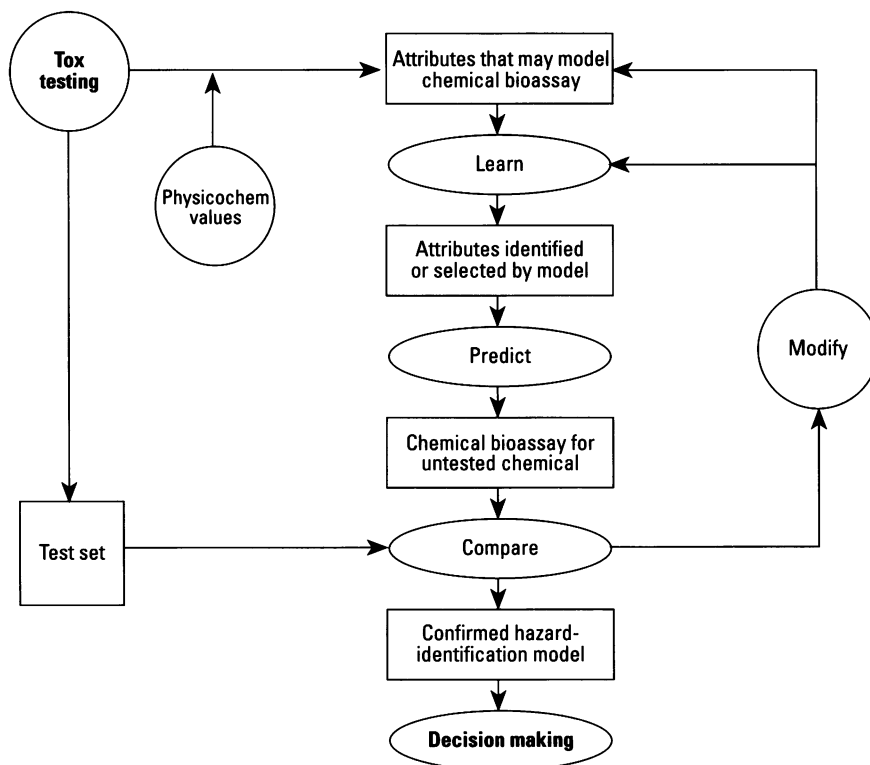


Figure 1. NTP standardized testing fuels the engine that powers the PTE project and drives learning in the young science of chemical toxicology.

healthy growth and maturation of this relatively young discipline (12) by providing learning sets that support the development of models and theories. It is important to use learning sets that include a sufficient number and variety of classified cases to adequately represent the uncertain number of multifactorial, mechanistic pathways that are associated with a complex toxicity endpoint like chemical carcinogenicity.

Figure 2 illustrates how a fully evaluated and confirmed PT model simplifies, when testing, learning, comparing, and modifying steps are no longer needed. A fully confirmed model needs only a few basic components to generate reliable predictions about hazard associated with exposure to untested chemicals. Information generated by the model is interpreted and used with confidence by decision makers.

PTE-1: Prediction Sets, Final Bioassay Results, and Workshop Conclusions

Final results for the 44 NTP bioassays that made up PTE-1 are presented in Table 1. The sets of predictions generated by PTE-1 are listed in Table 2. Several papers evaluating various aspects of the PTE-1 experiment have already been published.

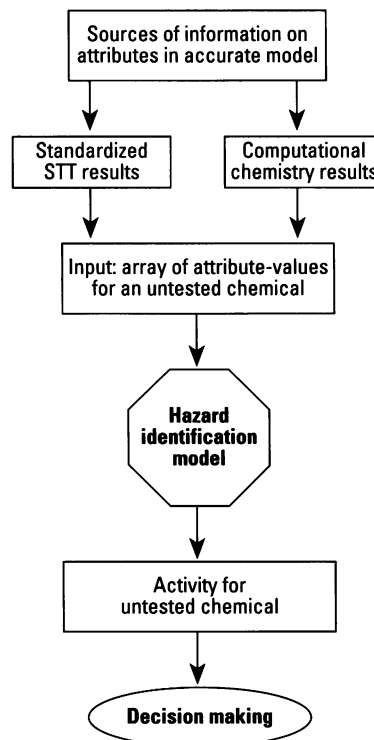


Figure 2. Flow chart of the essential components of a fully confirmed PT model.

We hope that this compilation of PTE-1 prediction sets accompanied by presentation of the final results for all 44 of the PTE-1 bioassays will inspire the publication of more papers that involve analyses of the results from this experiment to extend what has already been learned.

During 1993 the NIEHS conducted an international workshop to evaluate what had been learned from the PTE-1 collaboration. Broad consensus was evoked during discussions on some points while widely different opinions were heard on others. The workshop reached two main conclusions

(16). First, SAR-based models do not perform as accurately as models that utilize biological attributes and, second, models that used multiple attributes to represent the chemical carcinogenicity endpoint performed better than models that were based on one or two attributes.

Table 1. The 44 NTP carcinogenesis assays selected for the first predictive-toxicology evaluation experiment.

PTE1 No. ^a	NTP-chemical bioassay, test-article name ^b	CAS RN ^c	NTP TR ^d	TR peer-review ^e	ROA ^f	LOE assigned by experiment ^g				Overall classification ^h
						MR	FR	MM	FM	
1	<i>dl</i> -AMPHETAMINE SULFATE	60-13-9	387	04/90	F	NE	NE	NE	NE	NEG
2	NAPHTHALENE	91-20-3	410	03/91	I	NT	NT	NE	SE	POS
3	POLYSORBATE 80	9005-65-6	415	07/91	F	EE	NE	NE	NE	EQV
4	PROMETHAZINE HYDROCHLORIDE	58-33-3	425	12/92	G	NE	NE	NE	NE	NEG
5	RESORCINOL	108-46-3	403	03/91	G	NE	NE	NE	NE	NEG
6	γ -BUTYROLACTONE	96-48-0	406	07/91	G	NE	NE	EE	NE	EQV
7	MANGANESE (II) SULFATE MONOHYDRATE	10034-96-5	428	06/92	F	NE	NE	EE	EE	EQV
8	MONOCHLOROACETIC ACID	79-11-8	396	11/90	G	NE	NE	NE	NE	NEG
9	<i>p</i> -NITROPHENOL	100-02-7	417	07/91	D	NT	NT	NE	NE	NEG
10	TRICRESYL PHOSPHATE	1330-78-5	433	06/93	F	NE	NE	NE	NE	NEG
11	<i>o</i> -BENZYL- <i>p</i> -CHLOROPHENOL	120-32-1	424	12/92	G	NE	EE	SE	NE	POS
12	2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL	3296-90-0	452	11/94	F	CE	CE	CE	CE	POS
13	<i>t</i> -BUTYL ALCOHOL	75-65-0	436	06/94	W	SE	NE	EE	SE	POS
14	3,4-DIHYDROCUMARIN	119-84-6	423	06/92	G	SE	NE	NE	SE	POS
15	ETHYLENE GLYCOL	107-21-1	413	07/91	F	NT	NT	NE	NE	NEG
16	MERCURIC CHLORIDE	7487-94-7	408	07/91	G	SE	EE	EE	NE	POS
17	METHYLPHENIDATE HYDROCHLORIDE	298-59-9	439	06/93	F	NE	NE	SE	SE	POS
18	THEOPHYLLINE	58-55-9	N/A ⁱ	N/A	G	NE	NE	NE	NE	NEG
19	4,4-THIOBIS(6- <i>t</i> -BUTYL- <i>m</i> -CRESOL)	96-69-5	435	06/93	F	NE	NE	NE	NE	NEG
20	TRIAMTERENE	396-01-0	420	11/91	F	EE	NE	SE	SE	POS
21	5,5-DIPHENYLHYDANTOIN	57-41-0	404	06/92	F	EE	NE	NE	CE	POS
22	PENTACHLOROANISOLE	1825-21-4	414	11/91	G	SE	EE	SE	NE	POS
23	CHLORAMINATED WATER	CHLORAMINEMX	392	11/90	W	NE	EE	NE	NE	EQV
24	4,4'-DIAMINO-2,2'-STILBENEDISULFONIC ACID, DISODIUM SALT	7336-20-1	412	07/91	F	NE	NE	NE	NE	NEG
25	METHYL BROMIDE	74-83-9	385	11/90	I	NT	NT	NE	NE	NEG
26	<i>p</i> -NITROBENZOIC ACID	62-23-7	442	06/93	F	NE	SE	NE	NE	POS
27	SODIUM AZIDE	26628-22-8	389	04/90	G	NE	NE	NT	NT	NEG
28	TRIS(2-CHLOROETHYL) PHOSPHATE	115-96-8	391	04/90	G	CE	CE	EE	EE	POS
29	C.I. DIRECT BLUE 218	28407-37-6	430	12/92	F	SE	NE	CE	CE	POS
30	C.I. PIGMENT RED 3	2425-85-6	407	07/91	F	SE	SE	SE	NE	POS
31	C.I. PIGMENT RED 23	6471-49-4	411	03/91	F	EE	NE	NE	NE	EQV
32	2,4-DIAMINOPHENOL DIHYDROCHLORIDE	137-09-7	401	03/91	G	NE	NE	SE	NE	POS
33	ACETAMINOPHEN	103-90-2	394	11/90	F	NE	EE	NE	NE	EQV
34	SALICYLAZOSULFAPYRIDINE	599-79-1	457	06/95	G	SE	SE	CE	CE	POS
35	TITANOCENE DICHLORIDE	1271-19-8	399	11/90	G	EE	EE	NT	NT	EQV
36	C.I. ACID RED 114	6459-94-5	405	03/91	W	CE	CE	NT	NT	POS
37	C.I. DIRECT BLUE 15	2429-74-5	397	11/90	W	CE	CE	NT	NT	POS
38	COUMARIN	91-64-5	422	06/92	G	SE	EE	SE	CE	POS
39	2,3-DIBROMO-1-PROPANOL	96-13-9	400	06/92	D	CE	CE	CE	CE	POS
40	3,3'-DIMETHYLBENZIDINE DIHYDROCHLORIDE	612-82-8	390	04/90	W	CE	CE	NT	NT	POS
41	HC YELLOW 4	59820-43-8	419	07/91	F	EE	NE	NE	NE	EQV
42	<i>p</i> -NITROANILINE	100-01-6	418	11/91	G	NT	NT	EE	NE	EQV
43	<i>o</i> -NITROANISOLE	91-23-6	416	11/91	F	CE	CE	CE	SE	POS
44	1,2,3-TRICHLOROPROPANE	96-18-4	384	07/91	G	CE	CE	CE	CE	POS

^aSequence number assigned to the 44 NTP chemical bioassays by Tennant (3). ^bName of the bioassay-test article as presented in the title of the corresponding NTP Technical Report. ^cChemical Abstracts Service registry number unique for each chemical substance. ^dNTP Technical Report number for the bioassay. ^eDate upon which the results of each NTP chemical bioassay were peer reviewed and made public. ^fRoute of administration or exposure procedure used for the chronic studies: D, dermal or skin-pain; F, dosed feed; G, oral gavage; I, inhalation; W, dosed water. ^gLevel of evidence that is assigned by the NTP to classify the results obtained from each sex-species experiment of a chemical bioassay, as defined and presented in the front of each NTP TR: MR, male rat; FR, female rat; MM, male mouse; FM, female mouse. For theophylline, see *i*. ^hThe overall classification for each chemical bioassay was obtained by combining the LOE classifications for individual experiments, as follows: if the LOE assigned to one or more of the experiments was clear evidence (CE) or some evidence (SE), then the overall class is positive (POS); if each experiment received a LOE assignment of no evidence (NE), then the overall class is negative (NEG); if the individual experiments received LOE assignments that were a mix of equivocal evidence (EE) and NE, then the overall class is equivocal (EQV). ⁱNot applicable. The TR for chronic toxicity and carcinogenicity studies of theophylline has not been presented for peer review yet; however, NTP staff scientists have determined that these experiments produced no evidence of neoplastic effects related to dosing.

Table 2. The 13 sets of predictions made for 44 NTP carcinogenesis chemical bioassays.

PTE-1 No.	Chemical bioassay test-article	Overall classification	Tennant et al. (1)	TRIPT D-tree (2)	Benigni (3)	Weisbur-ger (4)	Bakale et al. (5)	TOP-KAT (6)	TRIPT rule set (2)	DER-EK (7)	COM-PACT (8)	Lijin-sky(4)	Multi-CASE (9)	DEREK hybrid (7)	RASH ^a (10)
1	<i>dl</i> -AMPHETAMINE SULFATE	NEG	-	-	-	-	NP	NP	+	-	-	-	-	-	-
2	NAPHTHALENE	POS	-	-	-	-	-	NP	-	-	+	-	-	-	NP
3	POLYSORBATE 80	EQV	-	-	NP	-	-	NP	-	NP	NP	-	+	NP	E
4	PROMETHAZINE-HYDROCHLORIDE	NEG	-	-	-	-	-	NP	-	-	+	+	-	-	-
5	RESORCINOL	NEG	-	+	-	-	-	-	-	-	+	-	-	-	-(E/-)
6	γ -BUTYROLACTONE	EQV	-	+	-	-	-	-	-	-	-	+	+	-	-
7	MANGANESE (II) SULFATE MONOHYDRATE	EQV	-	-	NP	-	NP	NP	-	-	NP	-	NP	-	+
8	MONOCHLOROACETIC ACID	NEG	-	-	+	-	-	-	-	+	-	-	-	+	-
9	<i>p</i> -NITROPHENOL	NEG	-	-	+	-	+	-	-	-	+	+	-	+	-
10	TRICRESYL PHOSPHATE	NEG	-	+	-	-	+	NP	+	-	+	+	+	-	-
11	<i>o</i> -BENZYL- <i>p</i> -CHLOROPHENOL	POS	+	+	W+/U	-	+	-	-	-	-	-	-	-	+
12	2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL	POS	+	+	W+/U	W+	+	-	+	-	+	-	+	-	NP
13	<i>t</i> -BUTYL ALCOHOL	POS	+	+	-	-	-	-	+	-	-	-	-	-	-(E/-)
14	3,4-DIHYDRO-COUMARIN	POS	+	+	-	-	+	-	-	-	+	+	-	-	E
15	ETHYLENE GLYCOL	NEG	+	+	-	-	-	-	+	-	-	-	-	-	-
16	MERCURIC CHLORIDE	POS	+	+	NP	-	-	NP	+	NP	NP	-	NP	NP	E
17	METHYLPHENIDATE HYDROCHLORIDE	POS	+	-	-	-	NP	-	-	+	-	-	-	+	+
18	THEOPHYLLINE ^b	NEG	+	-	-	-	-	-	-	-	+	-	-	+	+
19	4,4-THIOBIS (6- <i>t</i> -BUTYL- <i>m</i> -CRESOL)	NEG	+	+	-	-	-	-	+	-	E	-	+	-	-
20	TRIAMTERENE	POS	+	+	-	-	NP	-	+	-	+	+	+	+	-
21	5,5-DIPHENYL-HYDANTOIN	POS	+	+	W+/U	W+	-	NP	-	-	-	+	-	-	+
22	PENTACHLOROANISOLE	POS	+	+	W+/U	-	+	-	+	-	+	+	+	+	-
23	CHLORAMINATED WATER	EQV	-	-	W+/U	-	NP	NP	+	-	+	-	I	-	NP
24	4,4'-DIAMINO-2,2'-STILBENEDISULFONIC ACID, DISODIUM SALT	NEG	-	-	W+/U	-	NP	+	-	-	+	-	+	+	NP
25	METHYL BROMIDE	NEG	-	+	W+/U	W+	+	NP	+	+	-	+	+	+	NP
26	<i>p</i> -NITROBENZOIC ACID	POS	-	+	+	-	+	-	+	-	+	-	-	+	-
27	SODIUM AZIDE	NEG	-	+	NP	-	NP	NP	+	-	-	-	NP	+	-
28	TRIS(2-CHLOROETHYL) PHOSPHATE	POS	-	+	W+/U	+	+	+	+	+	-	+	+	+	-
29	C.I. DIRECT BLUE 218	POS	+	+	W+/U	-	NP	NP	+	+	+	-	+	+	-
30	C.I. PIGMENT RED 3	POS	+	+	+	-	+	+	+	+	+	+	+	+	NP
31	C.I. PIGMENT RED 23	EQV	+	+	+	-	NP	+	+	+	+	+	+	+	NP
32	2,4-DIAMINOPHENOL DIHYDROCHLORIDE	POS	+	+	W+/U	-	-	+	+	-	+	+	+	-	+
33	ACETAMINOPHEN	EQV	+	+	W+/U	-	-	-	+	-	+	-	-	+	-(E/-)
34	SALICYLAZO-SULFAPYRIDINE	POS	+	-	+	NP	NP	+	-	+	+	+	-	+	NP
35	TITANOCENE DICHLORIDE	EQV	+	+	NP	NP	+	NP	+	-	NP	-	NP	-	-
36	C.I. ACID RED 114	POS	+	+	+	NP	NP	NP	+	+	+	-	-	+	NP
37	C.I. DIRECT BLUE 15	POS	+	+	W+/U	NP	NP	+	+	NP	+	-	-	NP	NP
38	COUMARIN	POS	+	+	W+/U	NP	+	NP	+	-	+	-	-	-	+
39	2,3-DIBROMO-1-PROPANOL	POS	+	+	+	+	+	NP	+	+	-	+	+	+	+
40	3,3'-DIMETHYL-BENZIDINE DIHYDROCHLORIDE	POS	+	+	W+/U	+	-	+	+	-	+	+	+	+	+
41	HC YELLOW 4	EQV	+	+	W+/U	-	NP	- ^c	+	+	+	+	+	+	NP ^c
42	<i>p</i> -NITROANILINE	EQV	+	+	+	-	+	+	+	-	+	+	+	+	E
43	<i>o</i> -NITROANISOLE	POS	+	+	+	+	+	-	+	-	+	-	+	+	+(E/+)
44	1,2,3-TRICHLORO-PROPANE	POS	+	+	+	+	+	+	+	+	-	+	+	+	E

Abbreviations: +, positive; -, negative; NP, no prediction made; W+, weakly positive; W+U, weak positive or uncertain probability for being positive; E, equivocal. ^aseparate predictions were made for rats and mice; when the predictions were different both were entered into the table, separated by a / mark. ^bsee Table 1, footnote i. ^cThe original, published prediction was changed at the request of these authors, after information about the correct identity, structure, and CAS RN for the chemical tested was sent to all participants, along with a request for them to notify us in writing, if the new information led to a revised prediction.

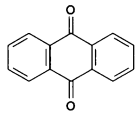


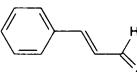
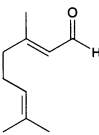
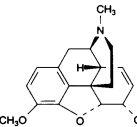
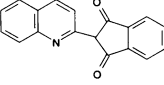
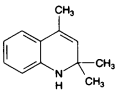
PTE-2: 30 Chemical Carcinogenicity Bioassays and 17 Prediction Sets

Table 3 identifies the 30 NTP chemical carcinogenicity bioassays incorporated into PTE-2. This table includes the 2-D structure

of each test article. The SMILES code for each test chemical is also included for those who might want to generate 3-D structures or compute physicochemical property values for them.

Table 4. tabulates the 17 sets of predictions published as part of PTE-2 to date. It provides a rapid overview of the predictions published for any of the 30 chemical carcinogenicity bioassays.

Table 3. Thirty chemical carcinogenicity bioassays for the second predictive toxicology evaluation experiment.^a

No.	Structure	Test article Molecular weight CAS registry number Smiles code	Route	MTD ^b (mg/kg/day)				NTP technical review no.	Peer review date
				Male rat	Female rat	Male mouse	Female mouse		
1		Anthraquinone; 9,10-anthracenedione 208.2 84-65-1 <chem>c(ccc1C(=O)-c2cc3)cc1C(=O)-c2cc3</chem>	Feed	72.53	82.86	844.28	817.05		
2		Chloroprene; 2-chloro-1,3-butadiene 88.54 126-99-8 <chem>ClC(=C)C=C</chem>	Inhalation	27.13	27.13	4.59	4.59	467	12/11/96
3		1-Chloro-2-propanol, technical grade 94.54 127-00-4 <chem>ClCC(O)C</chem>	Drinking water	33.88	38.22	151.83	159.22		
4		<i>trans</i> -Cinnamaldehyde; (E)-3-phenyl-2-propenal; β -phenylacrolein 132.2 14371-10-9 <chem>c(ccc1C=CC=O)cc1</chem>	Feed	483.5	552.38	1407.13	1361.75		
5		Citral; 3,7-dimethyl-2,6-octadienal 152.2 5392-40-5 <chem>O=CC=C(C)CCC=C(C)C</chem>	Feed	483.5	552.38	703.56	680.88		
6	$\text{CoSO}_4 \cdot 7 \text{H}_2\text{O}$	Cobalt sulfate heptahydrate; cobaltous sulfate 281.1 10026-24-1 <chem>CoS(=O)(=O)O.O.O.O.O.O.O.O</chem>	Inhalation	0.281	0.281	0.0475	0.0475	471	12/11/96
7		Codeine; methylmorphine 299.4 76-57-3 <chem>c(cc(c1C2(CC3)C4C=5)CC4N3C)c(OC)c1OC2C(O)C5</chem>	Feed	61.89	70.70	337.71	326.82	455	06/21/95
8		D&C Yellow No. 11; C.I. Solvent Yellow 33 273.3 8003-22-3 <chem>O=C3C(C(C4=C3C=CC=C4)=O)C(C=C2)=NC1=C2C=CC=C1</chem>	Feed	193.4	220.95	???	???	463	12/05/95
9	$(\text{HOCH}_2\text{CH}_2)_2\text{NH}$	Diethanolamine; 2,2'-iminobisethanol 105.1 111-42-2 <chem>OCCNCCO</chem>	Dermal	64	32	160	160		06/97
10		1,2-Dihydro-2,2,4-trimethylquinoline, monomer 173.3 147-47-7 <chem>c(ccc1C(=C2)C)cc1NC2(C)C</chem>	Dermal	100	100	10	10	456	06/21/95

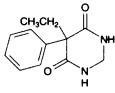

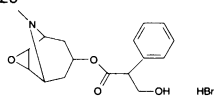

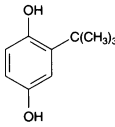

(Continued)

Table 3. (Continued).

No.	Structure	Test article Molecular weight CAS registry number Smiles code	Route	MTD (mg/kg/day)				NTP technical review no.	Peer review date
				Male rat	Female rat	Male mouse	Female mouse		
11		Emodin; 1,3,8-trihydroxy-6-methyl-9,10-anthraquinone 270.2 518-82-1 <chem>O=C1C3=C(C=C(C(=O)C=C3O)C(C2=C1C(=O)=CC(C)=C2)=O</chem>	Feed	96.7	110.48	140.71	136.18		
12		Ethylbenzene; phenylethane 106.2 100-41-4 <chem>c(ccc1CC)cc1</chem>	Inhalation	304.99	304.99	51.61	51.61	466	12/11/96
13	<chem>HOCH2CH2OCH2CH2CH2CH3</chem>	Ethylene glycol monobutyl ether; EGMBE; 2-butoxyethanol 118.2 111-76-2 <chem>OCCOCCCC</chem>	Inhalation	56.58	56.58	19.15	19.15		
14		Furfuryl alcohol; 2-furanmethanol; 2-hydroxymethylfuran 98.10 98-00-0 <chem>O(C=C1)C(=C1)CO</chem>	Inhalation	12.02	12.02	2.03	2.03		06/97
15	<chem>GaAs</chem>	Gallium arsenide; gallium monoarsenide 144.6 1303-00-0 <chem>As#Ga</chem>	Inhalation	0.094	0.094	0.016	0.016		
16		Isobutene; 2-methyl-1-propene 56.11 115-11-7 <chem>C=C(C)C</chem>	Inhalation	1719.63	1719.63	291.01	291.01		10/97
17		Isobutyraldehyde; 2-methylpropanal 72.11 78-84-2 <chem>O=CC(C)C</chem>	Inhalation	552.48	552.48	93.48	93.48	472	12/11/96
18		Methyleugenol; 1,2-dimethoxy-4-(2-propenyl)benzene 178.2 93-15-2 <chem>c(cc(c1)CC=C)c(OC)c1OC</chem>	Gavage	150	150	150	150		
19	<chem>MoO3</chem>	Molybdenum trioxide molybdenum oxide 143.9 1313-27-5 <chem>Mo(=O)(=O)=O</chem>	Inhalation	9.36	9.36	1.58	1.58	462	12/05/95
20	<chem>CH3-NO2</chem>	Nitromethane; nitrocarbol 61.04 75-52-5 <chem>O=N(=O)C</chem>	Inhalation	87.67	87.67	29.67	29.67	461	12/05/95
21		Oxymethalone; (5α,17β)-17-hydroxy-2-(hydroxymethylene)-17-methyl-androstan-3-one 332.5 434-07-1 NA	Gavage	150	100	???	???		06/98
22		Phenolphthalein; 3,3-bis(4-hydroxyphenyl)-1(3H)-isobenzofuranone 318.3 77-09-8 <chem>c(ccc1C2(-c(ccc3O)cc3)-c(ccc4O)cc4)cc1C(=O)O2</chem>	Feed	1934	2209.5	1350.84	1307.28	465	12/05/95

(Continued)

Table 3. (Continued).

No.	Structure	Test article Molecular weight CAS registry number Smiles code	Route	MTD (mg/kg/day)				NTP technical review no.	Peer review date
				Male rat	Female rat	Male mouse	Female mouse		
23		Primaclone; 5-ethylidihydro-5-phenyl-4,6-(1H,5H)-pyrimidinedione 218.3 125-33-7 <chem>c(ccc1C(C(=O)NC2)(C(=O)N2)CC)cc1</chem>	Feed	96.7	110.48	146.34	141.62	476	12/11/96
24		Pyridine; azabenzene 79.10 110-86-1 <chem>n(ccc1)cc1</chem>	Drinking water	20.85	23.52	151.83	79.61	06/97	
25		Scopolamine hydrobromide trihydrate; hyoscyne hydrobromide 438.3 6533-68-2 <chem>OCC(C(OC3CC4C2OC2C(N4C)C3)=O)C1=CC=CC=C1.Br.O.O.O</chem>	Gavage	25	25	25	25	445	06/21/95
26	NaNO_2	Sodium nitrite; nitrous acid, sodium salt; diazotizing salt 69.00 7632-00-0 <chem>O=[N+]{-}.Na{+1}</chem>	Drinking water	156.36	176.4	455.49	477.66		
27		Sodium xylenesulfonate; dimethylbenzenesulfonic acid, sodium salt 208.2 1300-72-7 NA	Dermal	240	240	727	727	464	12/05/95
28		t-Butylhydroquinone; MTBHQ; 2-(1,1-dimethylethyl)- 1,4-benzenediol 166.2 1948-33-0 <chem>O-c(ccc1O)cc1C(C)(C)C</chem>	Feed	193.4	220.95	562.85	544.7	459	06/21/95
29		Tetrahydrofuran; 1,4-epoxybutane 72.11 109-99-9 <chem>O(CC1)CC1</chem>	Inhalation	497.23	497.23	84.15	84.15	475	12/11/96
30	V_2O_5	Vanadium pentoxide; Vanadium oxide 181.9 1314-62-1 <chem>V(=O)(=O)OV(=O)=O</chem>	Inhalation	0.187	0.187	0.063	0.063		

^aNo entry indicates incomplete study; peer review not scheduled. ^bMinimally toxic dose.

Support Provided to Foster Participation in PTE Experiments

The primary purpose of a PTE experiment is to learn by focusing the intellectual resources of different research groups on a common problem. When the set of test cases for a PTE experiment is reasonably representative for the end point activity, the overall learning potential for an evaluation experiment is influenced more by the number and variety of models applied to generate predictions than by the

number of test-set bioassays. Therefore, it is important that as many predictors participate as possible.

The original announcement for PTE-2 (17) made available a package of comprehensive information that was distributed by mail or fax. Early in 1996, a page for the PTE Project was established on the Internet, as a link to the NIEHS homepage. It provides updates about the current status of the PTE-2 experiment and access to NTP database information of particular interest to PTE participants; the more important Internet addresses include:

- NIEHS Predictive-Toxicology Evaluation Project: <http://www.niehs.nih.gov/dirlecm/pte2.htm>
- NIEHS Home Page: <http://www.niehs.nih.gov/>
- NTP Home Page: <http://ntpserver.niehs.nih.gov/>
- Search Chemical Results Report (search capability provides access to virtually all NTP studies): http://ntpserver.niehs.nih.gov/cgi/iceform_Res_Stat.cgi (address subject to change)

Table 4. PTE-2 participation to date. Seventeen prediction sets for 30 NTP chemical carcinogenicity bioassays.^a

PTE-2 No.	NTP Bioassay, test-article	CAS RN	CSWG (28)	Bootman (18)	RASH (19)	CASE/ MULTICASE (20)		QSAR (21)	SHE (22)	Kerckaert et al. (22)	QSAR (23)	RL induction (7)		FALS (24)	PROGOL (6)	COMPACT (25)	Tennant & Spalding (26)	Ashby (27)	Huff et al. (28)
						OSAR (27)	MULTICASE (20)					R1 (7)	R2 (7)						
1	Anthraquinone	84-65-1	+	G+	NP-D	-	-	-	-	M-	-	NP	NP	+	+	GP+	-	-	-
2	Chloroprene	126-99-8	+	G+	+	+	+	+	NP	NP	+	+	+	-	-	NP+	+	-	+
3	1-Chloro-2-propanol	127-00-4	+	G+	+	+	+	+	+	M+	+	+	+	+	-	GP+	G+	+	+
4	Cinnamaldehyde	104-55-2	+	G+	NP-D	-	-	-	NP	NP	+	NP	NP	+	+	N-	N-	G+	+
5	Citral	5392-40-5	+	N+	E	-	-	-	NP	NP	+	-	-	+	+	N-	N-	G+	+
6	Cobalt sulfate heptahydrate	10026-24-1	+	G+	-	NP	+	+	+	M+	NP	-	NP	NP	NP	GP+	N+	E	E
7	Codeine	76-57-3	U	N+	+	+	+	-	-	-	+	NP	NP	E	-	N-	N-	-	-
8	D&C Yellow No.11	8003-22-3	NP	G+	+	-	-	+	+	M+	-	+	+	+	+	GP+	-	-	E
9	Diethanolamine	111-42-2	+	N+	+	+	+	+	+	N+	-	+	+	-	-	N-	N-	-	+
10	1,2-Dihydro-2,4-trimethyl-quinoline	147-47-7	+	-	-	-	-	-	+	N+	-	+	+	+	+	NP+	GP+	G+	+
11	Emodin	518-82-1	NP	G+	+	+	+	-	+	M+	-	+	+	+	+	GP+	N+	+	+
12	Ethylbenzene	100-41-4	NP	-	+	-	-	-	+	N+	-	NP	NP	-E	-	N-	N-	-	E
13	Ethylene glycol monobutyl ether	111-76-2	NP	-	-	-	-	-	+	N+	-	+	+	-	-	N-	N-	-	-
14	Furfuryl alcohol	98-00-0	U	-	+	+	+	+	+	N+	+	-	-	E	E	NP+	G+	+	-
15	Gallium arsenide	1303-00-0	U	G+	+	NP	+	+	NP	NP	+	+	+	NP	NP	NP	N+	+	+
16	Isobutene	115-11-7	U	G+	-	-	-	-	NP	NP	-	NP	NP	-E	-	N-	N-	-	+
17	Isobutyraldehyde	78-84-2	+	G+	+	+	+	+	+	-	-	-	-	+	+	NP+	GP+	+	+
18	Methyleugenol	93-15-2	+	G+	E	-	-	-	+	M+	-	+	+	-	-	NP+	GP+	+	+
19	Molybdenum trioxide	1313-27-5	U	G+	E	NP	NP	NP	+	N+	NP	NP	NP	NP	NP	N-	N-	-	E
20	Nitromethane	75-52-5	+	-	-	NP	+	+	+	N+	+	NP	NP	+	+	N-	N-	+	+
21	Oxymethalone	434-07-1	+	N+	E	-	-	-	+	N+	+	+	+	-	-	NP+	N+	+	+
22	Phenolphthalein	77-09-8	+	G+	E	-	-	-	+	M+	-	-	-	E	-	N-	N-	+	+
23	Primaclore	125-33-7	+	N+	+	+	+	+	+	M+	-	+	+	+	+	GP+	N+	+	+
24	Pyridine	110-86-1	U	G+	+	-	-	-	-	M-	-	+	+	+	+	NP+	N+	+	E
25	Scopolamine hydrobromide trihydrate	6533-68-2	+	-	-	-	-	-	+	N+	+	NP	NP	+	+	N-	N-	-	E
26	Sodium nitrite	7632-00-0	U	-	+	NP	-	-	+	M+	NP	-	+	NP	NP	GP+	-	-	E
27	Sodium xylenesulfonate	1300-72-7	-	-	-	+	+	-	-	-	-	-	-	-	-	N-	N-	-	-
28	t-Butylhydroquinone	1948-33-0	NP	N+	+	+	+	+	-	M-	-	+	+	-	-	N-	N-	N+	E
29	Tetrahydrofuran	109-99-9	U	-	E	-	-	-	-	-	-	-	-	+	+	N-	N-	N+	E
30	Vanadium pentoxide	1314-62-1	NP	-	-	NP	NP	+	+	N+	NP	-	-	NP	NP	NP+	N+	+	+

Abbreviations: +, positive response; P+, possible or less-probable positive response; E, equivocal response; -, no response or response unlikely; G, genotoxic; N, nonmutagenic; NP, no prediction made; Abs, abstained; U, uncertain; M, mutagenic; NP-D, dose level unavailable. ^aThe symbols used in this table refer to the overall response, or overall weight of evidence, predicted prospectively about the outcome of NTP rodent-carcinogenesis experiments that are being conducted to evaluate each test chemical. This table provides an overview of the predictions published for each of the 30 NTP chemical carcinogenicity bioassays. To achieve a desired level of uniformity, some of the predictions in the primary papers had to be transformed or translated to conform with the simple set used here. While efforts were made to avoid errors, some interpretation of the predictions published was necessary and subtle distinctions intended by different authors may have been obscured. Readers are therefore encouraged to consult the original publications for a completely reliable representation of individual prediction sets.

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