Carcinogenicity Predictions for a Group of 30 Chemicals Undergoing Rodent Cancer Bioassays Based on Rules Derived from Subchronic Organ Toxicities

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Rodent carcinogenicities for a group of 30 chemicals which form the subject of the Second NIEHS Predictive-Toxicology Evaluation Experiment are predicted based on their subchronic organ toxicities. Predictions are made by rules learned by the rule learning (RL) induction program. — Environ Health Perspect 104(Suppl 5):1059–1063 (1996)

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Introduction

We have been using a rule induction program to analyze the relationship between rodent carcinogencinity and many features of chemicals such as responses in short-term assays and physical chemical properties. Recently we investigated the predictive strength of organ-specific toxicity for rodent carcinogenicity and noncarcinogenicity (*1*). The organ-specific toxicity was modeled by the presence or absence of 124 lesions observed at the end of subchronic studies by oral administration. Each lesion relates an organ with a morphological effect, and the 124 lesions were regrouped into a total of 32 organs and 43 morphological effects. We used the rule learning (RL) induction program to learn rules predicting rodent carcinogenicity and noncarcinogenicity from the organ-specific toxicities of 88 chemicals consisting of 60 carcinogens and 28 noncarcinogens. Four sets of rules were learned, each of which was obtained using different combinations of assumptions and features in rule formation. We applied two of the four sets of rules to a group of 30 chemicals undergoing rodent cancer bioassays, which also forms the subject of the Second NIEHS Predictive-Toxicology Evaluation (PTE-2) experiment. In the present article, we report predictions of rodent carcinogenicity and noncarcinogenicity of these 30 chemicals.

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Abbreviations used: RL, rule learning; PTE, Predictive-Toxicology Evaluation experiment; SAL, mutagenicity in *Salmonella*.

Organ-specific Toxicity of 30 Chemicals

Table 1 shows the organ-specific toxicity along with the response in the *Salmonella* mutagenicity assay (SAL) of each of the 30 chemicals. "+", "–", and "?" refer to positive, negative, and unknown responses in SAL, respectively. For each chemical, observed lesions (a pair of an organ and a morphological effect) are listed. Note that not all observed lesions were applicable because they were not among the 124 lesions on which rules were based.

Methodology: Rule Induction

The RL Program

RL (*2*) is a knowledge-based inductive rule learning program that induces one or more IF-*condition*-THEN-*class* rules from specific examples of classes. For example, to make predictions of rodent carcinogenicity, the RL program is given a set of carcinogens and noncarcinogens and induces one or more rules that classify them. RL's heuristic search can examine a much larger number of identification (or classification) criteria than can be examined by manual analysis. Also, prior domain knowledge such as facts, heuristics, or assumptions used by scientists can be included during the search to learn rules that are plausible biologically as well as statistically.

The main strength of RL is its flexibility. Given a learning problem, many problem models and assumptions can be tested. This flexibility is partly achieved through the use of a domain model, called the partial domain model, which can guide RL's rule search separately from the guidance implicit in the statistics of training examples. The domain model contains definitions of attributes to be used in representing examples and rules, a list of classes, assumptions, and constraints on rules being sought, and domain knowledge relevant to a particular problem. The values of attributes may be symbolic or numeric, or they may be binary. Constraints and domain knowledge usually take the form of preference criteria characterizing desirable properties of rules to be learned. Thus, induction in RL is guided not only by syntactic similarity and dissimilarity of features of examples but also by constraints and prior domain knowledge in the domain model.

Given a learning problem, i.e., the names of one or more target classes, a set of their examples, and a partial domain model of the problem, RL searches for rules by examining a large but limited number of combinations of features. An example is represented as a vector of attribute–value pairs, each of which describes a feature of the example. For example, the representation of methyleugenol is shown below, which means that methyleugenol is associated with degeneration of testes; necrosis, hyperplasia, and inflammation of liver; but did not cause inflammation of kidney, ..., and its rodent carcinogenicity is unknown.

((Name methyleugenol) (testes degeneration +) (liver necrosis +) (liver hyperplasia +) (liver inflammation +) (kidney inflammation –) ... (rodent ?))

The RL induction program searches for rules by generating and evaluating many

Table 1. Organ-specific toxicity of the 30 chemicals in the Second Predictive-Toxicology Evaluation experiment and their responses in the *Salmonella* mutagenicity assay.

Chemical	SAL[a]	Applicable lesions	Inapplicable lesions[b]
Anthraquinone	+	No data available	
Chloroprene	–	Degeneration, metaplasia, and inflammation in nasal cavity; necrosis and hemosiderin pigment in liver; hyperplasia in forestomach	
1-Chloro-2-propanol	+	Cytoplasmic vacuolization in liver; regeneration in kidney	Degeneration and fatty change in pancreas
Cinnamaldehyde	?	No data available	
Citral	–	Hyperplasia and metaplasia in nasal cavity	
Cobalt sulfate heptahydrate	+	Inflammation and metaplasia in larynx; inflammation in lung; metaplasia in trachea; inflammation,degeneration, and metaplasia in nasal cavity; degeneration in testes	Proliferation in larynx and lung; hyperplasia in lymph node
Codeine	?	No lesions	
D&C Yellow No.11	+	Pigmentation and degeneration in liver; pigmentation in kidney	
Diethanolamine	–	Ulceration, hyperkeratoses, and inflammation on skin; nephropathy, necrosis, and mineralization in kidney; degeneration in brain; necrosis in liver; degeneration in heart	Cytologic alteration in liver, salivary depletion in spleen, thymus, and lymph node; congestion in adrenal; and cytoplasmic alteration
1,2-Dihydro-2,2,4-trimethylquinoline	–	Inflammation and hyperkeratoses on skin; cytoplasmic vacuolization in liver	Acanthosis in skin; fibrosis in lung
Emodin	+	Nephropathy, hyperplasia, and pigmentation in kidney	Fibrosis in kidney
Ethylbenzene	–	No lesions	
Ethylene glycol monobutyl ether	–	Regeneration, hemosiderin pigment, necrosis, and degeneration in liver; hematopoiesis and hemosiderin pigment in spleen; degeneration and hemosiderin pigment in kidney; hyperplasia in bone marrow; necrosis, ulceration, inflammation, and hyperplasia in forestomach; degeneration in testes	
Furfuryl alcohol	–	Metaplasia, hyperplasia, degeneration, exudate, and inflammation in nasal cavity	Hypertrophy in nasal cavity
Gallium arsenide	–	Hemosiderin pigment in spleen; inflammation and hyperplasia in lung; hemosiderin pigment and regeneration in liver; metaplasia in larynx; hyperplasia in bone marrow; hyperplasia in lymph node	Atrophy in testes
Isobutene	–	No applicable lesions	Hypertrophy in nasal cavity
Isobutyraldehyde	–	Necrosis, inflammation, degeneration, and metaplasia in nasal cavity; inflammation and metaplasia in larynx; inflammation and metaplasia in trachea; necrosis in spleen; degeneration in testes	Depletion in spleen; osteodystrophy in bone
Methyleugenol	–	Inflammation, degeneration, and necrosis in stomach glandular; pigmentation, necrosis, hyperplasia, and inflammation in liver; atrophy in uterus; degeneration in testes	Cytologic alteration in liver; hypertrophy in adrenal; cytoplasmic alteration
Molybdenum trioxide	–	No lesions	
Nitromethane	–	Degeneration in nasal cavity; degeneration in sciatic nerve; degeneration in spinal cord; hematopoiesis in spleen	
Oxymethalone	–	Degeneration in heart; regeneration and mineralization in kidney; cytoplasmic vacuolization in adrenal; atrophy in ovary	Hyperplasia in mammary gland; hydrometra in uterus
Phenolphthalein	–	Cellular depletion in bone marrow	Necrosis and pigmentation in bone marrow; atrophy in testes
Primaclone	+	Hypertrophy in liver; nephropathy and regeneration in kidney; hematopoiesis in spleen	Cytoplasmic alteration in adrenal
Pyridine	–	Degeneration, hypertrophy, inflammation, and pigmentation in liver; nephropathy in kidney	
Scopolamine hydrobromide trihydrate	–	No applicable lesions	Degeneration in adrenal
Sodium nitrite	+	Hyperplasia in forestomach; hematopoiesis in spleen; degeneration in testes	
Sodium xylenesulfonate	–	Hyperplasia in skin	
t-Butylhydroquinone	–	Hyperplasia and inflammation in nasal cavity; pigmentation in spleen; mineralization in kidney; hyperplasia in skin; hyperplasia in forestomach	
Tetrahydrofuran	–	Inflammation in forestomach; atrophy in uterus	Acanthosis in forestomach; degeneration in adrenal
Vanadium pentoxide	–	Hyperplasia and inflammation in lung; inflammation, hyperplasia, and metaplasia in nasal cavity	Exudate and fibrosis in lung

[a] +, –, and ? indicate positive, negative, or unknown response, respectively, in the *Salmonella* mutagenicity assay. [b] Inapplicable lesions are lesions that were observed among the 30 chemicals but were not used in learning rules.

combinations of features. It starts with single features and successively specializes rules by adding new features or specializing values associated with features. The choice and order of feature combination are in general dependent on their concordance with training data. For example, a combination of features with higher positive predictive values may be evaluated prior to other combinations of features. On the other hand, it is also possible to provide RL with a set of assumptions that guide it such that specific types of rules are excluded or included. For example, in predicting rodent carcinogenicity of nongenotoxic chemicals, rules including responses in short-term assays were preferred (*3*). Thus, the plausibility of a rule is determined by its performance (how accurately it classifies examples) and its concordance with assumptions, constraints, and domain knowledge.

The result of rule search is a set of IF-*condition*-THEN-*class* rules, where condition is a conjunction ("AND") of features. For example, the following rule uses two features to predict rodent carcinogenicity:

IF (liver +) AND
(kidney +) THEN (rodent C),

which is interpreted as if a chemical causes any morphological effect on liver and kidney, then it is classified as a carcinogen. Such IF-THEN rules are very easy to understand, unlike numerical weights and nodes in a neural network; and the comprehensibility of rules permits the facile verification of rules by experts. Unless a learning problem is simple enough to classify all training examples with a single rule, RL finds a disjunctive set of rules, each of which classifies a subset of training examples. Such rules are then used collectively to make predictions on new cases.

RL is a descendant of the Meta-DENDRAL system (*4*), which specialized in finding rules of mass spectrometry in chemistry. However, unlike the Meta-DENDRAL, RL is a general purpose learning program that can be applied to many problems in different domains. RL has been applied successfully in several real-world problems, including predicting rodent carcinogenicity with short-term assays (*3*), predicting human developmental toxicity based on the results of animal toxicity assays (*5*), triggering design in high energy physics (*6*), and analyzing massive quantities of data on infant mortality (*7*). RL is written in C programming language. An export version of RL is available on request. However, the export version does not include new features that are under further development and some of which were used in the present study.

Rules

In our previous study, which investigated the relationship between organ-specific toxicity and rodent carcinogenicity, we reported four sets of rules, two of which were learned using the responses in SAL in addition to organ-specific toxicity (*1*). Of the four sets learned in the previous study, we used two sets to make predictions for the 30 chemicals presented in the second PTE experiment. Both rule sets were learned using only the organ-specific toxicity and did not include responses in SAL. Since these two rule sets correspond to the first and third rule sets reported previously (*1*), we will refer to them as R1 and R3, respectively. Both rule sets were learned from 88 chemicals, of which 60 were carcinogens and 28 were noncarcinogens. None of the 30 chemicals in the second PTE experiment were included in the training data. The main differences between R1 and R3 are the assumptions the RL induction program used in evaluating and learning rules. For R1, RL gave greater weight to liver and kidney toxicities than to other organs, while RL gave equal weights to all organs for rules in R3. In other words, when a chemical caused an effect in liver or kidney, it was taken more seriously (for carcinogenicity) than an effect in another organ. The choice of kidney and liver was made because it was found that they were the two organs most indicative of carcinogenicity, and the battery of these two organs was even more accurate in classifying the 88 chemicals. The details of assumptions as well as the analysis have been described (*1*).

Tables 2 and 3 show rules in R1 and R3, respectively. Both rule sets contain eight rules, three of which predict rodent carcinogenicity. For each rule, its condition and the class it predicts are shown along with three statistics. #C and #NC refer to the number of carcinogens and noncarcinogens in the training data that were covered by a rule. CF refers to a certainty factor. For a rule predicting rodent carcinogenicity, CF is calculated by $(\#C-0.5)/(\#C+\#NC)$; similarly, for a rule predicting noncarcinogenicity, CF is obtained by $(\#NC-0.5)/(\#C+\#NC)$.

Predictions

Table 4 shows the predictions for the 30 chemicals in the second PTE experiment

Table 2. Eight rules in rule set R1 learned by the RL induction program.[a]

Rule no.	Rule condition IF	Class THEN	Coverage #C	Coverage #NC	Coverage CF
1-1	(Kidney +) and (degeneration –)	C	18	1	0.92
1-2	(Liver +) and (syncytial-alteration –)	C	27	2	0.91
1-3	(Kidney +) and (nasal cavity –)	C	33	3	0.90
1-4	(Liver –), (regeneration –) and (inflammation +)	NC	6	12	0.64
1-5	(Kidney –), (liver –) and (hyperplasia +)	NC	2	6	0.69
1-6	(Liver –), (regeneration –) and (bone-marrow +)	NC	1	3	0.62
1-7	(Liver –), (nasal cavity +) and (necrosis +)	NC	1	4	0.70
1-8	(Kidney –) and (brain +)	NC	2	4	0.58

[a]#C, #NC, and CF refer to the number of carcinogens and noncarcinogens (in the training data) covered by each rule and a certainty factor, respectively.

Table 3. Eight rules in rule set R3 learned by the RL induction program.[a]

Rule no.	Rule condition IF	Class THEN	Coverage #C	Coverage #NC	Coverage CF
3-1	(Liver +)	C	29	4	0.86
3-2	(Kidney +)	C	35	5	0.86
3-3	(Spleen +)	C	12	2	0.82
3-4	(Liver –), (regeneration –) and (inflammation +)	NC	6	12	0.64
3-5	(Kidney –), (liver –) and (hyperplasia +)	NC	2	6	0.69
3-6	(Karyomegaly –), (brain +) and (degeneration –)	NC	0	3	0.83
3-7	(Spleen –) and (cellular depletion +)	NC	1	3	0.62
3-8	(Nasal cavity +), (exudate –) and (necrosis +)	NC	1	4	0.70

[a]#C, #NC, and CF refer to the number of carcinogens and noncarcinogens (in the training data) covered by each rule and a certainty factor, respectively.

Table 4. Predictions made by rule sets, R1 and R3.[a]

CAS	Chemical	Predictions SAL	R1	R3
84-65-1	Anthraquinone	+	?[a]	?
126-99-8	Chloroprene	–	C	C
127-00-4	1-Chloro-2-propanol	+	C	C
104-55-2	Cinnamaldehyde	?	?	?
5392-40-5	Citral	–	NC	NC
10026-24-1	Cobalt sulfate heptahydrate	+	NC	NC
76-57-3	Codeine	?	?	?
8003-22-3	D&C Yellow No. 11	+	C	C
111-42-2	Diethanolamine	–	C	C
147-47-7	1,2-Dihydro-2,2,4-trimethylquinoline	–	C	C
518-82-1	Emodin	+	C	C
100-41-4	Ethylbenzene	–	?	?
111-76-2	Ethylene glycol monobutyl ether	–	C	C
98-00-0	Furfuryl alcohol	–	NC	NC
1303-00-0	Gallium arsenide	–	C	C
115-11-7	Isobutene	–	?	?
78-84-2	Isobutyraldehyde	–	NC	NC
93-15-2	Methyleugenol	–	C	C
1313-27-5	Molybdenum trioxide	–	?	?
75-52-5	Nitromethane	–	?	C
434-07-1	Oxymethalone	–	C	C
77-09-8	Phenolphthalein	–	NC	NC
125-33-7	Primaclone	+	C	C
110-86-1	Pyridine	–	C	C
6533-68-2	Scopolamine hydrobromide trihydrate	–	?	?
7632-00-0	Sodium nitrite	+	NC	C
1300-72-7	Sodium xylenesulfonate	–	NC	NC
1948-33-0	*t*-Butylhydroquinone	–	C	C
109-99-9	Tetrahydrofuran	–	NC	NC
1413-62-1	Vanadium pentoxide	–	NC	NC

[a]? indicates an inability to predict.

Table 5. Rules in each set that provide evidence of carcinogenicity or noncarcinogenicity.

CAS	Chemical	Rules matching each chemical[a] R1 C	R1 NC	R2 C	R2 NC
84-65-1	Anthraquinone	–	–	–	–
126-99-8	Chloroprene	2	–	1	8
127-00-4	1-Chloro-2-propanol	1,2,3	–	1,2	–
104-55-2	Cinnamaldehyde	–	–	–	–
5392-40-5	Citral	–	5	–	5
10026-24-1	Cobalt sulfate heptahydrate	–	4,5	–	4,5
76-57-3	Codeine	–	–	–	–
8003-22-3	D&C Yellow No. 11	2,3	–	1,2	–
111-42-2	Diethanolamine	2,3	–	1,2	–
147-47-7	1,2-Dihydro-2,2,4-trimethylquinoline	2	–	1	–
518-82-1	Emodin	1,3	–	2	–
100-41-4	Ethylbenzene	–	–	–	–
111-76-2	Ethylene glycol monobutyl ether	2,3	–	1,2,3	–
98-00-0	Furfuryl alcohol	–	4,5	–	4,5
1303-00-0	Gallium arsenide	2	–	1,3	–
115-11-7	Isobutene	–	–	–	–
78-84-2	Isobutyraldehyde	–	5,7	3	5,8
93-15-2	Methyleugenol	2	–	1	–
1313-27-5	Molybdenum trioxide	–	–	–	–
75-52-5	Nitromethane	–	–	3	–
434-07-1	Oxymethalone	3	–	2	–
77-09-8	Phenolphthalein	–	6	–	7
125-33-7	Primaclone	1,2,3	–	1,2,3	–
110-86-1	Pyridine	2,3	–	1,2	–
6533-68-2	Scopolamine hydrobromide trihydrate	–	–	–	–
7632-00-0	Sodium nitrite	–	5	3	5
1300-72-7	Sodium xylenesulfonate	–	5	–	5
1948-33-0	*t*-Butylhydroquinone	1	4,5	2,3	4
109-99-9	Tetrahydrofuran	–	4	–	4
1413-62-1	Vanadium pentoxide	–	4,5	–	4,5

– indicates no matching rules. [a]Rules are referred to by rule numbers shown in Tables 2 and 3.

made by the two rule sets, R1 and R3. The table also contains the responses in the *Salmonella* mutagenicity assay. The model of organ toxicity from which RL learned rules is not exactly equal to the model under which the organ toxicities of the 30 chemicals in the PTE were observed. In fact, in Table 1, we already indicated that some lesions caused by some chemicals were not applicable because there were no matching lesions in the 124 lesions on which the rules were based.

Neither R1 nor R3 makes predictions for all 30 chemicals; R1 and R3 made predictions for 22 and 23 chemicals, respectively. Of the 22 chemicals predicted by R1, 13 were predicted to be carcinogens and 9 were to be noncarcinogens. R3 predicted 15 of 23 chemicals to be carcinogens and 8 to be noncarcinogens. R1 and R3 agreed on predictions for 21 chemicals but disagreed on two chemicals, nitromethane and sodium nitrite. While R1 was not able to make any predictions for nitromethane, R3 predicted it to be a carcinogen. Also, while R1 predicted sodium nitrate to be a noncarcinogen, R3 predicted it to be a carcinogen.

The rules in each set that match each chemical are shown in Table 5. Rules are referred to by the numbers assigned to them in Tables 2 and 3. For example, in R1, chloroprene matches rule-2 (which predicts carcinogenicity) and no rules predicting noncarcinogenicity. In other words, rule-2 in R1 provides the evidence that chloroprene is a carcinogen, and there is no evidence that it is a noncarcinogen. On the other hand, in R3, while evidence for carcinogenicity is provided by rule-1, rule-8 also provides the evidence for noncarcinogenicity. However, since the certainty (CF) of rule-1 is greater than that for rule-8, chloroprene was predicted to be a carcinogen.

Let us look at the two chemicals, nitromethane and sodium nitrite, for which the predictions of R1 and R3 did not agree. While there are no rules in R1 matching nitromethane, in R3, rule-3 matches the chemical. Thus, R1 did not make a prediction for nitromethane, and R3 predicted it to be a carcinogen because of the evidence provided by the training data, i.e., there were 12 (of 60) carcinogens that caused spleen toxicity but only 2 (of 28) noncarcinogens that caused spleen toxicity. For sodium nitrite, R1 predicted it to be a noncarcinogen because of the evidence for noncarcinogenicity provided by rule-5, i.e., a chemical is more likely to be a noncarcinogen if it does not affect liver and kidney but causes hyperplasia on other organs. On the other hand, R3 predicted sodium nitrate to be a carcinogen because the evidence for carcinogenicity provided by rule-3 (i.e., the presence of spleen toxicity) is greater than the evidence for noncarcinogenicity provided by rule-5 (i.e., no effects on liver and kidney and the presence of hyperplasia on other organs). In other words, despite the fact that sodium nitrite did not cause liver or kidney toxicity, it is predicted to be a carcinogen by R3 due to the lesion in the spleen.

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