

# Prediction of Rodent Carcinogenicity of Further 30 Chemicals Bioassayed by the U.S. National Toxicology Program

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Recently the U.S. National Toxicology Program (NTP) sponsored a comparative exercise in which different prediction approaches (both biologically and chemically based) were challenged for their predictive abilities of rodent carcinogenicity of a common set of chemicals. The exercise enjoyed remarkable scientific success and stimulated NTP to sponsor a second challenging round of tests, inviting participants to present predictions relative to the rodent carcinogenicity of a further 30 chemicals; these are currently being tested. In this article, we present our predictions based on structure-activity relationship considerations. In our procedure, first each chemical was assigned to an activity mechanism class and then, with semiquantitative considerations, was assigned a probability carcinogenicity score, taking into account simultaneously the hypothesized action mechanism and physical chemical parameters. — *Environ Health Perspect* 104(Suppl 5):1041–1044(1996)

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## Introduction

The U.S. National Toxicology Program (NTP) conducts rodent carcinogenicity bioassays whose results provide a unique opportunity to evaluate and validate methods that might serve as complements of or alternatives to the bioassays. Recently NTP sponsored a comparative exercise in which different prediction approaches (both biologically and chemically based) were challenged for their predictive ability of rodent carcinogenicity of a common set of chemicals (1,2). The comparative exercise pointed to pitfalls and strengths of the various approaches, as well as to general scientific issues relative to the prediction of rodent carcinogenicity, thus enjoying a remarkable scientific success. This stimulated NTP to sponsor a second round of tests, inviting participants to present predictions relative to the rodent carcinogenicity of a further

30 chemicals, which are currently being tested. In this article, we present our predictions based on structure-activity relationship considerations.

Since the 1960s, experience in medicinal chemistry has shown that the rigorous application of quantitative structure-activity relationship (QSAR) methods to homogeneous classes of chemicals (congeners) inducing the same type of biological activity permits the formulation of efficient quantitative models. These QSAR models contribute both to the elucidation of the action mechanisms and to the prediction of the biological activity of yet untested chemicals (3). The use of QSAR methods has been exported from medicinal chemistry, where they presently constitute a basic building block in the design of new drugs, to the study of other biological activities, including toxicity. In fact, the QSAR analyses of classes of toxic congeners have been as successful as those performed in medicinal chemistry (4–7). In addition, issues relative to the practice of risk assessment (e.g., the presence of a great number of untested chemicals in the environment) have stimulated applications (mainly related to carcinogenicity) aimed at defining general SAR or QSAR models ideally able to predict the activity of any kind of chemical. With these analyses, investigators have attempted to extend the application

of the QSAR methods beyond the limits for which they were invented, i.e., classes of congeneric chemicals (8–10).

The first comparative exercise sponsored by the NTP confirmed previous evidence pointing to limited performance of the SAR and QSAR methods for noncongeneric chemicals (the best results were 60–65% accurate). Whereas the carcinogens were correctly identified by many predictive approaches, several noncarcinogens were erroneously predicted to be positive. Moreover, the SAR and QSAR prediction systems shared the common trait of acting primarily as gross class-identifiers: they were sensitive to the presence of alerting chemical functionalities in the compounds but were not able to make gradations within each potentially harmful class (2,10). This common characteristic of the chemically based predictive approaches was indicated by the rather surprising result that the various prediction profiles were remarkably similar in spite of great differences in the principles, implementations, and degree of sophistication of the different approaches (2). In our opinion, the limited performance shown so far by the general purpose QSAR approaches is caused by—as a primary explanation—the extreme diversity of the mechanisms by which each chemical class exerts its biological activity. As a consequence, a noncongeneric QSAR model should be some supermodel incorporating various local QSAR models, each of which reflects the action mechanism of one individual chemical class. The formidable challenge of identifying and gathering sufficient data within such classes, and the mathematical complexity of such a supermodel is intuitively obvious, can be prohibitive in the generation of efficient general QSARs (10).

Ideally, one could hope to overcome these difficulties by constructing a collection of local QSARs for the individual classes of chemicals. In this way, the QSAR methods would be applied in a more rigorous manner. Such an approach faces two main difficulties: the problem of retrieving from the literature an adequate number of already bioassayed chemicals representative of each chemical class and the problem of defining the classes to which the chemicals belong. Both points are difficult to solve. In our opinion, the allocation of the chemicals is most critical, since it implies the knowledge of the action mechanisms.

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Abbreviations used: NTP, National Toxicology Program; QSAR, quantitative structure-activity relationships; SAR, structure-activity relationships.

This information can only come from experimental studies, and is not available for the great majority of the chemicals. In this article, we show how we tried to negotiate these difficulties and to outline a practical approach to the prediction of rodent carcinogenicity of the chemicals currently bioassayed by the NTP.

## Results and Discussion

To predict the rodent carcinogenicity of the 30 chemicals under consideration, we first assigned each chemical to an activity mechanism class; then, with semiquantitative considerations, we assigned a probability carcinogenicity score, simultaneously taking into account the hypothesized action mechanism and physical chemical parameters.

Because there was lack of data on mechanisms, we substituted the expert guess of the chemist's eye by exploiting our own experience and that of a number of colleagues who courteously provided advice. In the next step, for the chemicals with presumed alerting substructures we considered two physical chemical parameters: log P and  $K_c$ . Log P [calculated according to Lyman et al. (11)] is a measure of hydrophobicity: highly hydrophilic chemicals are easily excreted, whereas hydrophobic chemicals are retained in the tissues and have more possibility of exerting their harmful action. Moreover, hydrophobicity rules the interaction between the drugs and the biological receptors (3,12). Positive log P values indicate hydrophobic chemicals, and vice versa.  $K_c$  is an electrophilicity parameter whose relationship with carcinogenicity was extensively studied by Bakale (13). In the present work,  $K_c$  was estimated according to Benigni et al. (14). High  $K_c$  values are probably indicative of ability of the directly acting carcinogens (e.g., alkylating agents) to attack DNA, propensity to undergo a reductive metabolism, or general chemical reactivity. The  $K_c$  cut-off value established by Bakale is  $3.0 \times 10^{12}$ . The consideration of log P and  $K_c$  has led to the assignment of low, medium, and high carcinogenicity probability to the chemicals considered potentially harmful on the basis of structural considerations. This probability can be used as a potency estimate.

Table 1 reports our predictions in terms of + (carcinogen) and - (noncarcinogen). For the chemicals predicted as potential carcinogens, Table 2 reports the log P and  $K_c$  values and the rationale for our probability/potency estimate.

**Table 1.** Carcinogenicity predictions.

No.	Chemical	Prediction	Rationale for predictions
1	Scopolomine hydrobromide trihydrate	-	Contains an epoxide function as sole alerting moiety, but the molecular environment for the epoxide is similar to that of the noncarcinogen Endrin (15).
2	Codeine	-	No evident alerting substructures.
3	1,2-Dihydro-2,2,4-trimethylquinoline	-	Although several quinolines can induce a wide spectrum of genetic damage, this dihydro form is a basically different chemical structure without the fused-rings system and does not show any alerting substructures.
4	Nitromethane	+	Nitroaliphatics as a class are considered potentially oncogenic and mutagenic (16). Clear evidence for tetranitromethane as a carcinogen in rats and mice (15).
5	Tetrahydrofuran	+	Structurally related to the two carcinogens 1-4 dioxane and furosemide (15).
6	<i>t</i> -Butylhydroquinone	+	Structurally related to hydroquinone, a benzene metabolite; it is carcinogenic (15) and is able to form DNA adducts after activation (17,18).
7	Ethylbenzene	-	Aliphatic chain in ethylbenzene suitable for hydroxylation and then elimination (19).
8	Chloroprene	+	Structurally related to the carcinogens vinyl chloride (20) and 1,3-butadiene (15).
9	Cobalt sulfate heptahydrate	+	Several Co(II) compounds are carcinogenic (21); available evidence points to the involvement of oxidative DNA damage, mediated by the formation of various oxygen radical species (22,23).
10	D&C Yellow No. 11	+	Contains both a quinoline moiety (which is alerting per se), and a 5-atom ring, which may open and give a reactive aldehyde.
11	Isobutyraldehyde	+	Structurally related to the carcinogens acetaldehyde and formaldehyde, several low molecular weight aldehydes are mutagenic and carcinogenic (16,24,25).
12	Molybdenum trioxide	?	Whereas there is evidence for the carcinogenicity of several metal compounds (22,23), no data are available for Molybdenum; no prediction possible.
13	1-Chloro-2-propanol	-	Structurally related to the noncarcinogen 2-chloroethanol (15); alcoholic function predominantly detoxifying (19).
14	Diethanolamine	+	In presence of nitrite or oxides of nitrogen, may be nitrosated to <i>N</i> -nitrosodiethanolamine, which is carcinogenic in rats (26,27); structurally related to the carcinogen triethanolamine (15).
15	Phenolphthalein	+	5-atom ring may open and produce an alkylating carbocation.
16	Pyridine	-	Heteroaromatic pyridine ring may be <i>N</i> -hydroxylated by cytochrome P450 (28), and should not produce epoxides, as benzene does.
17	Xylene sulfonic acid, sodium salt	-	No evident alerting substructures; sulfonic group makes the molecule water soluble, thus facilitating its elimination.
18	Furfuryl alcohol	+	Structurally related to the carcinogens furan and furfural (15); may be oxidized to furfural.
19	Primaclone	+	Diazinic ring may be oxidized and give rise to phenobarbital, which is carcinogenic (29).
20	Ethylene glycol monobutyl ether	-	No evident alerting substructures.
21	Gallium arsenide	+	Arsenic carcinogenic to man, but its rodent carcinogenicity has limited evidence, and it is nonmutagenic in most short-term systems (20). This may suggest an indirect mechanism for its toxicity. In our prediction, we relied mainly on the human epidemiology data.
22	Isobutene	-	Structurally related to the noncarcinogen propylene (15).
23	Methyleugenol	-	Structurally related to the noncarcinogen eugenol (15), which is conjugated by the glucuronic acid (30).
24	Oxymetholone	+	Evidence for a role of estrogen metabolites in estrogen-induced tumorigenesis (31); oxymetholone may be activated via epoxidation.
25	Anthraquinone	-	May be hydroxylated and then eliminated.
26	Emodin	-	May be easily eliminated.
27	Citral	+	Belongs to the class of $\alpha,\beta$ -unsaturated aldehydes, which are potentially DNA-damaging agents (16).
28	Sodium nitrite	-	Very soluble salt that can be easily eliminated.
29	Cinnamaldehyde	+	Belongs to the class of $\alpha,\beta$ -unsaturated aldehydes, which are potentially DNA-damaging agents (16).
30	Vanadium pentoxide	?	Whereas there is evidence for the carcinogenicity of several metal compounds (22,23), no data are available for vanadium; no prediction possible.

**Table 2.** Chemicals predicted to be carcinogens; modulation of potency.

No.	Chemical	Physical-chemical parameters	Estimated carcinogenicity probability (potency), and rationale
4	Nitromethane	$K_e = 0.348$ $\text{Log } P = 0.08$	High, because of $\text{NO}_2$ , in spite of low $K_e$ and $\text{log } P$
5	Tetrahydrofuran	$K_e = 0.663$ $\text{Log } P = 0.46$	Low; low $K_e$ and $\text{log } P$
6	<i>t</i> -Butylhydroquinone	$K_e = 0.556$ $\text{Log } P = 2.37$	Medium; low $K_e$ and high $\text{log } P$
8	Chloroprene	$K_e = 2.808$ $\text{Log } P = 2.32$	High; high $K_e$ and $\text{log } P$
9	Cobalt sulfate heptahydrate	$K_e = \text{NA}$ $\text{Log } P = \text{NA}$	Low, because of high water solubility
10	D&C Yellow No. 11	$K_e = 3.693$ $\text{Log } P = 3.63$	High; high $K_e$ and $\text{log } P$
11	Isobutyraldehyde	$K_e = 1.009$ $\text{Log } P = 1.18$	Low; high reactivity may inactivate the chemical before it reaches the target; low $K_e$ and $\text{log } P$
14	Diethanolamine	$K_e = 0.341$ $\text{Log } P = -1.81$	Low; low $K_e$ and $\text{log } P$
15	Phenolphthalein	$K_e = 3.597$ $\text{Log } P = 0.95$	High, because of high $K_e$
18	Furfuryl alcohol	$K_e = 0.555$ $\text{Log } P = -2.62$	Low; low $K_e$ and $\text{log } P$
19	Primaclone	$K_e = 1.968$ $\text{Log } P = -1.41$	Low; low $K_e$ and $\text{log } P$
24	Oxymetholone	$K_e = 1.859$ $\text{Log } P = 3.8$	High; hydrophobic
27	Citral	$K_e = 2.137$ $\text{Log } P = 3.30$	Medium; high reactivity may diminish the chemical's amount at the target
29	Cinnamaldehyde	$K_e = 2.712$ $\text{Log } P = 1.36$	Low; very reactive

NA, not applicable. For the chemicals predicted to be carcinogenic (Table 1), the relative carcinogenicity probability was estimated based on the hypothesized action mechanism and the two physical-chemical parameters  $K_e$  and  $\text{log } P$ . The assigned numbers of the chemicals are those reported in Table 1.

## Conclusions

The procedure we followed to formulate the carcinogenicity predictions appears rather rough and approximate in comparison with the elegant quantitative methods

applied to the QSAR studies of individual chemical classes. We were able to rationalize our predictions in terms of hypothesized mechanism of action for a number of chemicals; for other chemicals, we based

our predictions on the sole structural analogy with known carcinogens and noncarcinogens. On the other hand, the field of general QSAR models is still under development, and it is not as formalized as that of QSAR for congeneric chemicals. The usefulness of the comparative exercises sponsored by the NTP is in providing stimuli and material to this research. Our participation is aimed mainly at substantiating our opinion that no real progress is possible in this area without shifting from the attempts to apply one general model to the different chemicals, to a two-phase approach consisting of categorization of the chemicals into classes with a homogeneous action mechanism, and derivation of QSAR models for the individual classes. In particular, in this work we want to validate or challenge the ability of our expert judgment to allocate the chemicals into classes.

Our choice of using only the chemical information as a basis for the predictions requires a final comment. In the first comparative exercise, activity-activity relationship (AAR) prediction methods, which use mainly biological data as input information, were also applied. These approaches were claimed to have a performance superior to those of the SAR and QSAR methods (1). According to our analyses, the difference is not so clear-cut (2). In any case, the AAR methods use costly input information, which has to be produced by both *in vitro* and *in vivo* experiments; without such information, the predictions cannot be performed. Whereas it is important to develop the AAR approaches, especially for their general scientific implications, it is also necessary to continue the search for efficient QSAR approaches, since these are the sole methods applicable when only the chemical's formula is known.

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