Prediction of Rodent Carcinogenicity for 30 Chemicals

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Predictions of carcinogenic activity are made for 30 chemicals currently being assessed for rodent carcinogenicity by the U.S. National Toxicology Program. The predictions are based upon the chemical structure, the anticipated or reported mutagenicity, and the reported sub-chronic toxicity of each chemical. It is predicted that 13 chemicals will be noncarcinogenic to rodents, that 7 will be genotoxic carcinogens, and that 10 may show some evidence of presumed nongenotoxic rodent carcinogenesis. — Environ Health Perspect 104(Suppl 5):1101–1104 (1996)

Key words: carcinogenicity, National Toxicology Program, toxicity, carcinogenicity prediction, rodent bioassays

Introduction

The predictions of rodent carcinogenesis made are based upon the methods described and validated previously (*1–3*). The factors that contributed to the prediction for the present 30 chemicals are discussed below for each chemical. Structures are shown in Figure 1.

The general approach adopted involves a subjective weighing of the available indications of carcinogenic potential, together with those of its absence, for each chemical. The final decision/prediction is not, therefore, capable of strict objective definition. In that sense the predictions made here resemble the process undertaken by regulatory review groups when decisions on the potential carcinogenicity of agents have to be drawn in the absence of a complete database.

The terms *possible* and *probable* are used to qualify predictions of carcinogenic activity or inactivity. The terms have their usual relative weightings, i.e., possible indicates a soft prediction and probable a firm prediction.

The term *genotoxic* is used to imply that the chemical is able to modify the structural integrity or expression of DNA as a direct result of its intrinsic properties. Secondary effects on DNA, or no effects on DNA, are reflected in the term *nongenotoxic*. Nongenotoxic carcinogens may increase the tumor incidence in rodents by a range of secondary mechanisms, each of which must be separately interrogated for its relevance to humans. In contrast, a presumed genotoxic carcinogen is likely to present a carcinogenic hazard to humans if the human metabolism of the agent corresponds to that observed in the affected rodent species. Finally, presumed nongenotoxic carcinogens may only be effective above a threshold dose level, an assumption that is less defensible for DNA-reactive carcinogens.

This paper is part of the NIEHS Predictive-Toxicology Evaluation Project. Manuscript received 24 May 1996; manuscript accepted 29 July 1996.

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Results

The chemical structures of the 30 chemicals are shown in Figure 1. The toxicity data referred to below are as provided by the organizers of the study.

1. Scopolamine, $HBr \cdot 3H_2O$

Based upon the structure of this agent it is expected to be nonmutagenic to *Salmonella,* as observed. The epoxide substructure is probably nonelectrophilic due to its crowding by adjacent substituents. The bioassay dose levels are low, probably due to the pharmacologic activities of this chemical. It is concluded to be a probable noncarcinogen.

2. Codeine

Based upon the structure of this agent it is expected to be nonmutagenic to *Salmonella,* as observed. The alicyclic N—CH_3 group provides a soft alert to genotoxicity (formaldehyde formation or *N*-methylol formation). The low dose levels used in the bioassay probably reflect the pharmacologic activities of this chemical. It is concluded to be a probable noncarcinogen.

3. 1,2-Dihydro-2,2,4-trimethylquinoline

This chemical is a substituted (cyclic) aniline. As such it may have some potential to induce blood vessel or urinary bladder cancer in rodents. Epoxidation of the C-C double bond to yield an electrophilic epoxide is unlikely for steric reasons. The inactivity observed in the *Salmonella* assay and in the mouse bone marrow micronucleus assay does not distinguish this chemical from the carcinogen aniline. Aromatic ring hydroxylation or oxidation of one of the methyl groups offers possible routes of conjugative elimination from the test animals. The balance is concluded to favor *N*-oxidation as a potentially important route of metabolism, and as such it is concluded that this chemical is a possible genotoxic carcinogen. This prediction assumes systemic exposure following skin application. If the agent turns out to be a noncarcinogen it will probably be because the anticipated N-oxidation is only a minor route of metabolism; gauging the relative importance of competing routes of metabolism is the most difficult and most subjective aspect of carcinogen prediction.

4. Nitromethane

Some nitroalkanes are both mutagenic and carcinogenic. The precise reason for these activities is unknown. The simplicity of this structure, coupled with its inactivity in the available mutagenicity assays, is suggestive of nongenotoxicity. Inhalation of what appears to be a nasal irritant leads to the conclusion that nitromethane is a possible nongenotoxic nasal carcinogen.

5. Tetrahydrofuran

The only structural alert for this chemical is the ether oxygen atom. That functionality may lead to oxygen radical damage in exposed tissues (c.f., 1,4-dioxane). The negative mutagenicity data available do not necessarily negate this possibility. This chemical is concluded to be a possible nongenotoxic carcinogen. This conclusion is subject to the use of the inhalation route and favors an effect in nasal tissue. Radical damage to DNA is an indirect genotoxic effect. The term nongenotoxic carcinogen is used here to indicate the presumed absence of targeting of any induced mutations by adduction of the test chemical on DNA.

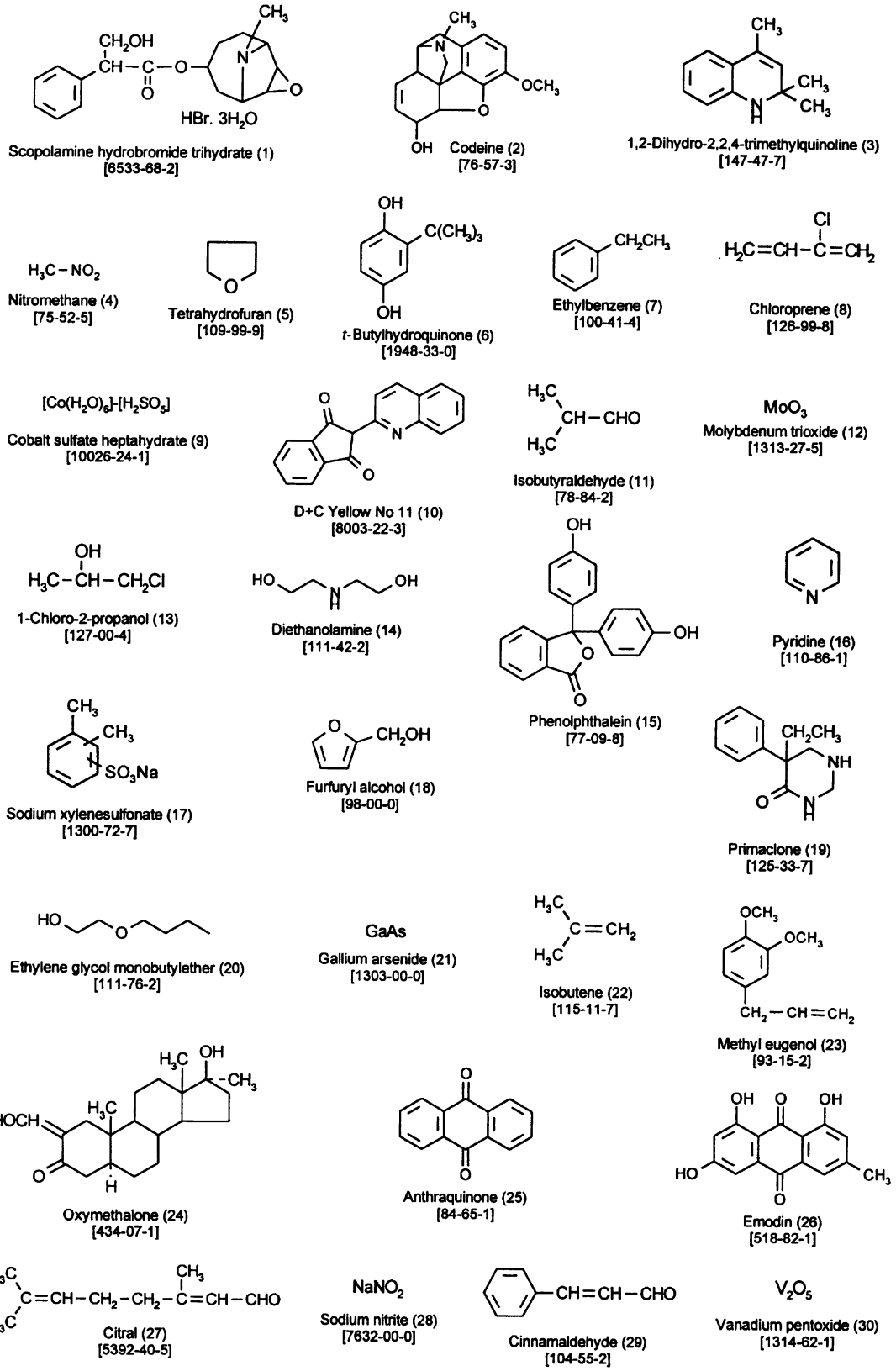

Figure 1. Structures of the 30 chemicals analyzed in this study.

6. *t*-Butylhydroquinone

This presumed nongenotoxin may affect the forestomach due to oxygen radical damage/induced cell division (c.f., other *t*-butyl phenols). It is concluded to be a possible nongenotoxic carcinogen.

7. Ethylbenzene

This is presumed to be a nongenotoxin and is a close analogue of the nonmutagenic noncarcinogen toluene. The inactivation of toluene proceeds via oxidation of the methyl group to benzyl alcohol and then to benzoic acid. The corresponding alcohol from ethylbenzene may offer the chance to form a sulphate ester of greater stability than the corresponding toluene-derived ester. Such an ester would probably be DNA-reactive. However, it is concluded that ethylbenzene is a probable noncarcinogen.

8. Chloroprene

The subchronic nasal pathology, coupled with the potential for epoxide formation, combine to indicate a genotoxic carcinogenic potential for this chemical. However, butadiene itself is mutagenic in the two genetic toxicity assays in which chloroprene is inactive. This suggests that chloroprene is not a functional analogue of butadiene within the present context. Based on the available mutagenicity data, chloroprene is classified as a probable noncarcinogen. Nonetheless, sufficient doubt remains to await the outcome of the rodent bioassay with interest.

9. Cobalt Sulfate, $7H_2O$

Cobalt is chemically related to nickel. Like some forms of nickel, this chemical is toxic to the respiratory tract. It is concluded that this chemical is a probable nongenotoxic nasal carcinogen. The word nongenotoxic is subject to the observation that nickel affects DNA methylation and genome imprinting. Perhaps the word "secondary" is better than nongenotoxic. Specifically, DNA adducts will not be the cause of any tumors observed.

10. D and C Yellow No. 11

This chemical probably is nongenotoxic. Hyalin droplets suggest a potential for male rat renal cancer. In the 90-day studies, hepatic toxicity was observed only at dose levels above the top bioassay dose level. It was not made clear at which dose levels the renal effects were seen. On the assumption that the renal effects were attenuated at the bioassay dose levels it is concluded that this chemical is a probable noncarcinogen.

11. Isobutyraldehyde

Aldehydes are usually genotoxic via reversible reaction with DNA. This aldehyde is toxic to the respiratory tract, and is concluded to be a probable respiratory tract genotoxic carcinogen.

12. Molybdenum Trioxide

There is no reason *a priori* to expect cancer, and no pathology was observed in the 90-day studies. This chemical is concluded to be a probable noncarcinogen.

13. 1-Chloro-2-propanol

Chlorohydrin is noncarcinogenic on skin application. This is the methyl analogue of chlorohydrin. Based on the mutagenicity seen in *Salmonella* (chlorohydrin itself is nonmutagenic) it is concluded that 1-chloro-2-propanol is a possible genotoxic carcinogen.

14. Diethanolamine

This chemical is predicted to be a probable noncarcinogen based on its structure and its presumed nongenotoxicity. If nitrite is present in the test diet the agent could be carcinogenic via nitrosamine formation. A referee of this article pointed out that biomethylation of the nitrogen atom in this chemical, followed by esterification of the hydroxyethyl group(s) would yield a DNA-reactive mustard. This latter idea was discounted in the present analysis; but it might be invoked to explain carcinogenicity, if such is observed for this agent.

15. Phenolphthalein

There is a potential for quinone/oxygen radical formation. The agent is active in a mouse bone marrow micronucleus assay. It is concluded to be a possible genotoxic carcinogen.

16. Pyridine

The subchronic pathology leads to the prediction of probable nongenotoxic carcinogen.

17. Xylene Sulfonic Acid

By all criteria this is considered a probable noncarcinogen.

18. Furfuryl Alcohol

There is no useful analogy with furan. Oxidation of the alcohol (CH_2OH) group to an acid, via the intermediate aldehyde, will dominate the metabolism of this chemical. Possible formation of this aldehyde (furfuraldehyde) is strengthened by the nasal pathology seen. It is concluded to be a probable genotoxic carcinogen by analogy to formaldehyde and acetaldehyde.

19. Primaclone

Based on structural similarity to phenobarbitone, and on the liver pathology reported, this chemical is concluded to be a probable nongenotoxic liver carcinogen.

20. Ethylene Glycol Monobutyl Ether

The only concerns are the potential for radical formation at the central oxygen atom and aldehyde formation at the terminal oxygen atom. The stomach pathology supports this concern. It is concluded to be a possible nongenotoxic carcinogen.

21. Gallium Arsenide

The simple link with arsenic would probably be unfounded because the carcinogenicity of arsenic is specific to oxidized species such as arsenite. We have no useful precedents by which to assess the carcinogenic potential of gallium arsenide, although some pathology was seen. This chemical is concluded to be a possible nongenotoxic carcinogen.

22. Isobutene

High dose levels produced no toxicity in the 90-day studies. One presumes that the *Salmonella* negative was observed using a dessicator protocol. If so, it is concluded that this chemical is a probable noncarcinogen.

23. Methyl Eugenol

Based on the relationship to safrole, it is concluded that this material is a possible genotoxic carcinogen. The nonmutagenicity observed is weakened by the similar inactivity of safrole in these genetic toxicity assays.

24. Oxymethalone

Based on the androgenic activities reported, it is concluded that this chemical has some nongenotoxic carcinogenic potential (c.f., mammary gland effects). The Michael center on the A-ring of the molecule may also herald cytogenetic effects. Nonetheless, a subjective appraisal is that this chemical is a probable noncarcinogen.

25. Anthraquinone

Other anthraquinones are not too relevant as they have genotoxic substituents. This chemical is predicted to be a probable noncarcinogen.

26. Emodin

Based on the 90-day pathology report, it is concluded that this material is a possible nongenotoxic carcinogen.

27. Citral

The aldehyde is potentially genotoxic due to its intrinsic elctrophilicity. Further, a Michael-reactive center exists within the molecule. Oxidation of the aldehyde, followed by conjugative elimination, will probably dominate the metabolism of this agent. It is concluded to be a probable noncarcinogen.

28. Sodium Nitrite

Nitrosamine formation from natural amino biochemicals is an imponderable factor. Limiting ridge hyperplasia is a nongenotoxic alert for stomach carcinogenicity. Nitrite ions are mutagenic in some *in vitro* assays.The balance tilts toward this chemical being a probable noncarcinogen. Given human usage of this material it is nonetheless worth confirming its noncarcinogenicity in a full bioassay.

29. Cinnamaldehyde

Dietary administration of an aldehyde causes an alert to stomach cancer. Concluded to be a possible genotoxic carcinogen.

30. Vanadium Pentoxide

Respiratory tract toxicity for this heavy metal derivative leads to the prediction of possible nongenotoxic carcinogen.

Conclusions

The above analysis represents a subjective balancing of the objective data made available to participants in the study. The classifications made can be summarized as follows:

Probable noncarcinogen: Nos. 1, 2, 7, 8, 10, 12, 14, 17, 22, 24, 25, 27, 28.

Possible nongenotoxic carcinogen: Nos. 4, 5, 6, 20, 21, 26, 30.

Probable nongenotoxic carcinogen: Nos. 9, 16, 19.

Possible genotoxic carcinogen: Nos. 3, 13, 15, 23, 29.

Probable genotoxic carcinogen: Nos. 11, 18.

Finally, greatest value will come from this predictive exercise if the quality and nature of the carcinogenicity data are displayed in a form that enables a multiple-site/trans-species/trans-gender carcinogen to be distinguished from, for example, a female mouse liver-specific carcinogen. It is clearly more important to be able to predict correctly the former than the latter. Predictive techniques have reached a level at which such resolution should be required of a system if it is to be of general use. To compare 18 out of 30 correct predictions made by one approach simply with 19 out of 30 correct predictions made by another will be little more than somewhat interesting.

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