

Prediction of Rodent Carcinogenicity Using the DEREK System for 30 Chemicals Currently Being Tested by the National Toxicology Program

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DEREK is a knowledge-based expert system for the qualitative prediction of toxicity. The DEREK system has been used to predict the carcinogenicity in rodents of the 30 chemicals in the second National Toxicology Program (NTP) carcinogenicity prediction exercise. Seven of the chemicals were predicted to be carcinogens. For 23 chemicals, there was no evidence in the DEREK knowledge base to suggest carcinogenic activity. Supplementary data from a variety of sources have been evaluated by human experts to assess confidence in each DEREK prediction. These sources included standard toxicology reference texts, genotoxicity and subchronic toxicity assay results for each chemical, as well as *Salmonella* mutagenicity and carcinogenicity data for close structural analogues. This process has led to the proposal of a number of improvements to the DEREK carcinogenicity knowledge base. — Environ Health Perspect 104(Suppl 5):1065–1073 (1996)

Key words: carcinogenicity, computer prediction, DEREK, genotoxicity, mutagenicity, National Toxicology Program, toxicity

Introduction

The ability to predict the carcinogenic potential of a chemical without recourse to long-term animal bioassays is an important tool in the effective prioritization of chemical testing programs. Benefits may also be obtained in terms of animal welfare and a reduction in the number of animals used in testing. Comparison of predictions with experimental data can additionally highlight gaps in our understanding of the mechanisms of the carcinogenic process. In the first National Toxicology Program (NTP) carcinogenicity prediction exercise, a variety of techniques were used to

prospectively predict the outcome of rodent carcinogenicity bioassays for 44 chemicals that were at that time being tested by the NTP (1). DEREK (2,3), a knowledge-based expert system for the qualitative prediction of toxicity, performed marginally better than other computer methods. For the 40 chemicals for which bioassay data are now available, 60% of the predictions made by DEREK were correct, if chemicals giving an equivocal bioassay outcome are assumed to be noncarcinogenic (2,4). In the same exercise, 75% of predictions made by human experts, using a combination of structural alerts, genotoxicity, and subchronic toxicity data, were correct (4). Ashby and Tennant (4) have suggested that, for diverse chemical sets, predictivity by any method is currently limited to a maximum of 80%, as a consequence of inadequate knowledge and uncertainties in the evaluation of experimental bioassay data. Recently, the National Institute of Environmental Health Sciences (NIEHS) has invited predictions for the outcome of rodent

carcinogenicity bioassays for a further 30 chemicals. This article describes the predictions made using the DEREK system and the subsequent evaluation of these predictions by human experts using supplementary data from a number of sources. This illustrates the intended use of DEREK as an aid to toxicological hazard assessment in conjunction with other methods.

Method

The DEREK system has been described in detail elsewhere (2,3). DEREK makes its predictions based on a series of rules contained in the DEREK knowledge base. Each rule describes the relationship between a structural feature or toxicophore and its associated toxicity. In addition to carcinogenicity, toxicological end points currently covered by the DEREK system include mutagenicity, skin sensitization, irritancy, teratogenicity, and neurotoxicity. To use the system interactively, a query structure can either be drawn at the DEREK graphical interface, retrieved from a previously saved structure file, or imported in MOLfile format. The structure is then processed, and the system compares structural features in the target compound with the toxicophores described in its knowledge base. A rule is activated or fires when the toxicophore it describes is found to be present in the structure under consideration. The toxicophore is highlighted to the user, and the toxic effect is displayed. A summary of the information used to compile the rule can be viewed if the reference screen is selected. In the case of carcinogenicity, for example, this information can additionally include details such as organ or species specificity, where sufficient supporting data exist. For query structures that fire more than one rule, each toxicophore is displayed sequentially on the screen with its associated hazard. In the event that no toxicological hazard is perceived, the system reports that no toxicophores have been identified in the structure. New DEREK rules are written following a detailed review of published sources of toxicological, mechanistic, and chemical data. The DEREK knowledge base also contains a series of rules that describe toxicophores for carcinogenic activity derived from a publication of the U.S. Food and Drug Administration (FDA) (5). These FDA rules are intended to allow regulatory concerns to be anticipated.

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Abbreviations used: DNA, deoxyribonucleic acid; FDA, U.S. Food and Drug Administration; NIEHS, National Institute of Environmental Health Sciences; NTP, National Toxicology Program; UDS, unscheduled DNA synthesis.

Structures for each of the 30 chemicals in the exercise were processed through the DEREK system using all rules in the knowledge base. Two of the chemicals (1-chloro-2-propanol and the sodium salt of xylenesulfonic acid) were known to be mixtures of positional isomers. In both cases, a single representative isomer was processed, namely 1-chloro-2-propanol and the sodium salt of 3,4-dimethyl-benzenesulfonic acid. Citral was also reported to be a mixture of *cis* and *trans* isomers, but since no DEREK rule currently differentiates between the toxicity of such geometric isomers, no account was taken of this. Other chemicals were known to contain up to 11% impurity, but since these impurities were not identified, they could not be processed. As a result, carcinogenic activity arising from the presence of unidentified impurities in the chemicals under test will not be predicted. In the event that a chemical fired one or more DEREK rules for carcinogenicity, it was predicted to be a carcinogen. No distinction was made between carcinogenicity predictions based on different rule types, although it has been suggested that the broader definition of the toxicophores that form the basis of some FDA rules may lead to more false positive predictions of carcinogenic activity compared to other rules in the DEREK carcinogenicity knowledge base (2). Otherwise, DEREK contained no evidence in its knowledge base to suggest carcinogenic activity. In some cases, this may be a consequence of the absence from the knowledge base of an as yet unidentified toxicophore. For the purposes of the current exercise, however, it was interpreted as a prediction of noncarcinogenicity.

Supplementary data from a number of sources were evaluated by human experts

to assess confidence in each DEREK prediction. By default, all predictions were initially assigned a confidence level of moderate, and this was then up- or downgraded in the light of additional information. Where supplementary data were considered to be predominantly in support of a DEREK prediction, the prediction was assigned a high level of confidence. Conversely, evidence judged to be predominantly contradictory led to a low confidence level prediction. A moderate confidence level was assigned to DEREK predictions for which the available evidence was considered either insufficient or conflicting. In all cases, confidence levels relate to the potential for rodent carcinogenicity by the given route of exposure and may not necessarily be of relevance to human health.

Genotoxicity and subchronic toxicity data for each chemical were made available to participants by NIEHS. Since many carcinogens are known to exhibit activity at least in part by a genotoxic mechanism, *in vivo* genotoxicity was considered evidence of potential carcinogenicity. In the absence of *in vivo* data, the results of *in vitro* assays, particularly the Ames test, were evaluated. Chemicals that gave a positive response in the Ames test were considered possible *in vivo* genotoxins, whereas those that gave a negative response were in general judged unlikely to be so.

Although a well-defined relationship remains to be established between carcinogenicity and the observation of toxicity in the same organ (6), significant toxicity in a subchronic study was considered a possible early indicator of potential carcinogenic activity. Particular weight was given to toxicity observed in an organ for which an increased incidence of tumors had been

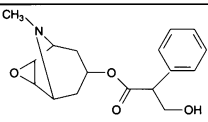
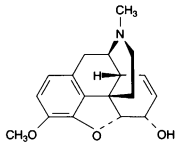
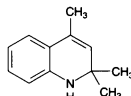
reported following administration of the compound itself, its metabolic precursor, metabolite, or close structural analogue. Such evidence was used as the basis for the prediction of the likely site of tumor formation for a number of chemicals. A comparison of the predicted tumor site with the outcome of the experimental bioassay will provide useful evidence either for or against the reasoning behind the prediction, depending on whether or not it is found to be correct. For several chemicals, lesions observed in the subchronic study were thought likely to be related to the route of exposure, and may reflect a prolonged irritant effect that could lead to nongenotoxic carcinogenicity.

Evidence from other miscellaneous sources was also evaluated. This included information contained in the DEREK reference screens and standard toxicology reference texts and genotoxicity and carcinogenicity data for structurally related compounds contained in the NTP *Salmonella* mutagenicity (7-11) and Gold carcinogenicity databases (12). DEREK predictions of mutagenicity for chemicals that were not predicted to be carcinogenic were also noted.

Results

The predictions made by the DEREK system are given in Table 1, together with associated confidence levels derived by human experts. The supplementary information used to assess the confidence in each DEREK prediction is summarized below. Except where specifically referenced, genotoxicity and subchronic toxicity data are derived from studies sponsored by the NTP, and are those supplied by NIEHS. Subchronic studies were of 90-day duration, except where stated.


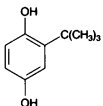
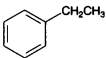

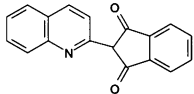


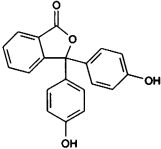

Table 1. Predictions for the 30 chemicals in the second NTP carcinogenicity prediction exercise.

Chemical					
No.	Name	Structure	CAS no.	DEREK prediction ^a	Expert confidence ^b
1	Scopolamine hydrobromide trihydrate		6533-68-2	Carcinogen	Low
2	Codeine		76-57-3	Noncarcinogen	High
3	1,2-Dihydro-2,2,4-trimethylquinoline		147-47-7	Carcinogen	Moderate

(continued)



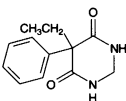

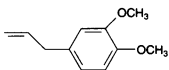
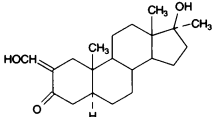
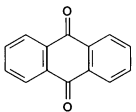
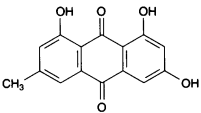
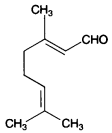
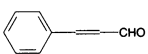
PREDICTION OF RODENT CARCINOGENICITY USING DEREK

Table 1. (continued)

Chemical					
No.	Name	Structure	CAS no.	DEREK prediction ^a	Expert confidence ^b
4	Nitromethane	$\text{CH}_3\text{—NO}_2$	75-52-5	Carcinogen	Moderate
5	Tetrahydrofuran		109-99-9	Noncarcinogen	Moderate (female mouse); high (rat, male mouse)
6	<i>t</i> -Butylhydroquinone		1948-33-0	Noncarcinogen	Low
7	Ethylbenzene		100-41-4	Noncarcinogen	Moderate
8	Chloroprene		126-99-8	Carcinogen	Moderate (mouse); high (rat)
9	Cobalt sulfate heptahydrate	$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$	10026-24-1	Noncarcinogen	Low
10	D&C Yellow No. 11		8003-22-3	Carcinogen	Moderate
11	Isobutyraldehyde		78-84-2	Noncarcinogen	Low
12	Molybdenum trioxide	MoO_3	1313-27-5	Noncarcinogen	High
13	1-Chloro-2-propanol		127-00-4	Carcinogen	Low
14	Diethanolamine	$(\text{HOCH}_2\text{CH}_2)_2\text{NH}$	111-42-2	Carcinogen	Moderate
15	Phenolphthalein		77-09-8	Noncarcinogen	Moderate
16	Pyridine		110-86-1	Noncarcinogen	Moderate (rat); high (mouse)

(continued)

Table 1. (continued)

Chemical					
No.	Name	Structure	CAS no.	DEREK prediction ^a	Expert confidence ^b
17	Xylenesulfonic acid, sodium salt		1300-72-7	Noncarcinogen	High
18	Furfuryl alcohol		98-00-0	Noncarcinogen	Low
19	Primaclone		125-33-7	Noncarcinogen	Low
20	Ethylene glycol monobutyl ether	HOCH ₂ CH ₂ OCH ₂ CH ₂ CH ₂ CH ₃	111-76-2	Noncarcinogen	Low
21	Gallium arsenide	GaAs	1303-00-0	Noncarcinogen	Moderate
22	Isobutene		115-11-7	Noncarcinogen	Moderate (rat); high (mouse)
23	Methyleugenol		93-15-2	Noncarcinogen	Low
24	Oxymetholone		434-07-1	Noncarcinogen	Low
25	Anthraquinone		84-65-1	Noncarcinogen	Moderate
26	Emodin		518-82-1	Noncarcinogen	Low
27	Citral		5392-40-5	Noncarcinogen	Moderate
28	Sodium nitrite	NaNO ₂	7632-00-0	Noncarcinogen	Low
29	Cinnamaldehyde		104-55-2	Noncarcinogen	Moderate
30	Vanadium pentoxide	V ₂ O ₅	1314-62-1	Noncarcinogen	Low

^aQualitative predictions of carcinogenic activity were made using the DEREK system; ^bA level of either high, moderate, or low confidence in each DEREK prediction was derived by human expert evaluation of supplementary data from a number of sources, including genotoxicity and subchronic toxicity data supplied to participants by NIEHS.

Supplemental Information on the Chemicals Tested by Number

1. *Scopolamine Hydrobromide Trihydrate*.

DEREK predicts scopolamine hydrobromide to be a carcinogen, since it contains an epoxide group that may lead to DNA alkylation. Although simple epoxides are generally mutagenic to *Salmonella* (7-11), scopolamine hydrobromide itself was not positive in the Ames test. This suggests a possible lack of electrophilic reactivity. Structural analogues, endrin and dieldrin, which similarly contain an epoxide fused to a bridged ring system, are also nonmutagenic in *Salmonella* (7,9). Since effects seen in the subchronic study were not judged significant, the confidence in DEREK's prediction of carcinogenicity is low.

2. Codeine. Codeine phosphate was not positive in the Ames test. No histopathological effects were observed in the subchronic study. The confidence in DEREK's prediction of noncarcinogenicity is therefore high.

3. 1,2-Dihydro-2,2,4-trimethylquinoline (monomer). DEREK predicts 1,2-dihydro-2,2,4-trimethylquinoline to be a carcinogen on the basis of the possible nitrosation by gut microflora of the secondary amine functionality, with the resulting nitrosamine leading to DNA alkylation. Since in the current case the compound is being administered by skin painting, this mechanism was not considered relevant here. A lack of genotoxic activity is supported by negative results in the Ames test (although *Salmonella* lack the ability to nitrosate) and a mouse micronucleus assay. Skin lesions, probably related to the route of exposure, were, however, observed in the subchronic study. The overall confidence in the prediction of carcinogenicity is therefore moderate, although any activity was thought unlikely to result from the mechanism suggested by DEREK.

4. Nitromethane. Nitromethane is predicted by DEREK to be a carcinogen on the basis that it is an aliphatic nitro compound. Nitromethane, was however, not mutagenic in the Ames test, and gave a negative response in a mouse micronucleus assay, indicating a lack of genotoxic activity. This is consistent with reported findings that genotoxicity may be restricted to secondary alkyl nitro compounds (13). Degeneration of the olfactory epithelium, probably related to the route of exposure, was seen in the subchronic study. The overall confidence in DEREK's prediction of carcinogenicity is therefore moderate.

5. Tetrahydrofuran. Tetrahydrofuran gave a negative response in the Ames test and a chromosome aberration assay. A mouse micronucleus assay gave negative and equivocal responses. Degeneration of the female mouse adrenal gland was observed in the subchronic study. The confidence in DEREK's prediction of noncarcinogenicity is considered high in both sexes of the rat and in the male mouse, and moderate for the female mouse.

6. *t*-Butylhydroquinone. *t*-Butylhydroquinone was not mutagenic in the Ames test, in keeping with other simple hydroquinones and their *O*-methyl derivatives in the NTP *Salmonella* mutagenicity database (7-11). The structural analogue, hydroquinone, gives a negative response in the Ames test (7), and is reported to be a rodent carcinogen, causing increased incidences of tubular cell adenomas of the kidney in male rats, of mononuclear cell leukemia in female rats, and of hepatocellular neoplasms in female mice (14). Butylated hydroxyanisole, a possible metabolic precursor of *t*-butylhydroquinone, is listed in the Gold carcinogenicity database as a rat carcinogen (12), causing an increased incidence of forestomach tumors (15). Toxic effects observed in the subchronic study include nasal hyperplasia or inflammation and mouse forestomach hyperplasia, which was probably related to the route of exposure. Confidence in DEREK's prediction of noncarcinogenicity is low, with the forestomach identified as a possible site of tumor formation.

7. Ethylbenzene. Ethylbenzene gave negative responses in the Ames test and a mouse micronucleus assay. No chemically related lesions were observed in the subchronic study. Increased tumor formation in rats following administration of ethylbenzene by gavage has been reported (16). The confidence in DEREK's prediction of noncarcinogenicity is assigned as moderate.

8. Chloroprene. DEREK predicts chloroprene to be a carcinogen because it is a halogenated alkene that may lead to DNA alkylation following metabolic epoxidation. It gave a negative response in the Ames test and for mouse micronucleus, chromosome aberration, and sister chromatid exchange assays. Hepatic lesions were observed in the rat subchronic study, together with toxicity of the respiratory tract, which was probably related to the route of exposure. No significant toxicity was observed in the mouse subchronic study. A number of chlorinated alkenes are included in the Gold carcinogenicity database. In several cases, sites of increased

tumor incidence include the liver, kidney, and respiratory tract (12). A 2-year inhalation study of chloroprene in the rat showed no evidence of carcinogenic activity (17), although a higher dose is to be used in the current bioassay. Other carcinogenicity studies of chloroprene in rodents have been considered inadequately reported for evaluation or led to no evidence of carcinogenic activity (18). The confidence in DEREK's prediction of carcinogenicity is moderate in the mouse and high in the rat. The liver and respiratory tract are identified as possible sites of tumor formation.

9. Cobalt Sulfate Heptahydrate. Cobalt sulfate was weakly mutagenic in the Ames test. It has been suggested that cobalt (II) compounds may interfere with DNA repair processes (19). Respiratory tract lesions were observed in the subchronic study, and were probably related to the route of exposure. The confidence in DEREK's prediction of noncarcinogenicity is low.

10. D & C Yellow No. 11. DEREK predicts D & C Yellow No. 11 to be a carcinogen on the basis of the presence of the quinoline ring, which may damage DNA following epoxidation. It was weakly mutagenic in the Ames test in the presence of S9 but gave a negative response in a mouse micronucleus assay. Hepatic lesions were observed in both rats and mice in the subchronic study, together with hyaline dropletlike structures in the renal tubules of the male rat, which suggests the possibility of $\alpha_{2\mu}$ -globulin nephropathy. This requires binding to the $\alpha_{2\mu}$ -globulin protein at a site of finite size (20), which was considered possibly too small to accommodate the D & C Yellow No. 11 structure. Overall, the confidence in DEREK's prediction of carcinogenicity is moderate.

11. Isobutyraldehyde. DEREK predicts isobutyraldehyde to be a mutagen, since it is an alkyl aldehyde and may exhibit electrophilic and hence DNA reactivity. It gave an equivocal response in the Ames test. Simple aldehydes, apart from formaldehyde, are typically negative in the Ames test, as evidenced by entries in the NTP *Salmonella* mutagenicity database (7-11). The subchronic study includes the observation of respiratory tract lesions, which were probably related to the route of exposure. Acetaldehyde and formaldehyde are both listed in the Gold carcinogenicity database as causing tumors of the nasal cavity (12). The confidence in DEREK's prediction of noncarcinogenicity is low,

and the respiratory tract is identified as a possible site of tumor formation.

12. Molybdenum Trioxide. Molybdenum trioxide gave a negative response in the Ames test, and no microscopic or gross pathology lesions were observed in either rats or mice in the subchronic study. Confidence in DEREK's prediction of noncarcinogenicity is therefore high.

13. 1-Chloro-2-propanol. DEREK identifies 1-chloro-2-propanol to be a carcinogen on the basis of the presence of the alkyl chloride functionality, which may lead to DNA alkylation. 1-Chloro-2-propanol may also undergo an elimination reaction leading to the formation of an epoxide. It gave a positive response in the Ames test but was negative in a mouse micronucleus assay. 2-Chloroethanol, a close structural analogue, is similarly reported to give a positive response in the Ames test and a negative result in an *in vivo* mouse micronucleus assay; it gave no evidence of carcinogenicity in a rat and mouse skin-painting carcinogenicity study (21,22). Lesions observed in the subchronic study were not considered significant. The confidence in DEREK's prediction of carcinogenicity is low.

14. Diethanolamine. Diethanolamine is identified by DEREK as a carcinogen on the basis of the possible nitrosation by gut microflora of the secondary amine functionality, with the resulting nitrosamine leading to DNA alkylation. As in the case of 1,2-dihydro-2,2,4-trimethylquinoline, such nitrosation was not considered relevant in this case since administration is by skin painting and not by a gastric route. Diethanolamine gave a negative response in the Ames test and a mouse micronucleus assay. Observations in the subchronic study included skin lesions related to the route of exposure. The overall confidence in the prediction of carcinogenicity is therefore moderate, although any activity was thought unlikely to result from the mechanism suggested by DEREK.

15. Phenolphthalein. Phenolphthalein was not mutagenic in an Ames test but gave a positive response in a mouse micronucleus assay. No histopathological lesions were seen in the rat subchronic study, and observations in the mouse study were not judged to be significant. Confidence in DEREK's prediction of noncarcinogenicity is moderate.

16. Pyridine. Pyridine was not mutagenic in the Ames test. No lesions were observed in the mouse subchronic study. In addition to changes in the liver of both

male and female rats in the subchronic study, male rat kidney lesions were also observed and were reported to be similar to those described for $\alpha_{2\mu}$ -globulin nephropathy. The pyridine structure was, however, considered probably too small for effective binding to the $\alpha_{2\mu}$ -globulin protein. The confidence in DEREK's prediction of noncarcinogenicity is high in the mouse and moderate in the rat.

17. Xylenesulfonic Acid, Sodium Salt. Xylenesulfonic acid gave a negative result in the Ames test. Minimal epidermal hyperplasia observed in the subchronic study in male rats and mice, and probably related to the route of exposure, was not considered significant. The confidence in DEREK's prediction of noncarcinogenicity is high.

18. Furfuryl Alcohol. Furfuryl alcohol was negative in the Ames test, and in chromosome aberration and sister chromatid exchange assays. Lesions of the respiratory tract, which were probably related to the route of exposure, were observed in the subchronic study. Furfuryl alcohol is readily metabolized to furoylglycine, furoic acid and furanacrylic acid via furfural in the rat (23). Furfural itself has been reported to show carcinogenic activity when administered by gavage (24). Furan is also carcinogenic (25). The confidence in DEREK's prediction of noncarcinogenicity is low.

19. Primaclone. Primaclone gave a positive response in the Ames test in the absence of S9, but the mouse micronucleus assay was negative. Hepatic lesions were observed in both rats and mice in the subchronic study, together with renal nephropathy in the male rat kidney. $\alpha_{2\mu}$ -Globulin nephropathy was considered unlikely, however, since hyaline-droplet formation was not described. Primaclone is metabolized to phenobarbital in rodents (26). Phenobarbital and its sodium salt are listed in the Gold carcinogenicity database as rat and mouse liver carcinogens (12), and may also lead to an increased incidence of thyroid tumors, following induction of thyroxine glucuronyltransferase (27). Confidence in DEREK's prediction of noncarcinogenicity is low, and the liver is identified as a possible site of tumor formation.

20. Ethylene Glycol Monobutyl Ether (2-Butoxyethanol). Ethylene glycol monobutyl ether gave a negative response in the Ames test. Lesions were observed at a number of sites in both the rat and mouse subchronic study. Ethylene glycol monobutyl ether is metabolized to

2-butoxyacetic acid (28), which is structurally similar to known peroxisome proliferators (29). Peroxisome proliferation was considered unlikely in the current instance, however, since although lesions were observed in the rat liver, these did not include the hepatocellular hyperplasia and hypertrophy that characterize this process (29). Diethylene glycol, a structural analogue of ethylene glycol monobutyl ether, was also negative in the Ames test when tested by the NTP (9) and is listed in the Gold carcinogenicity database as a rat urinary bladder carcinogen (12). The level of confidence in DEREK's prediction of noncarcinogenicity is low.

21. Gallium Arsenide. Gallium arsenide gave a negative result in the Ames test and in a mouse micronucleus assay. One of its metabolites, dimethylarsinic acid (30), is also reported to be nonmutagenic to *Salmonella* (7). Observations in the subchronic study include respiratory tract lesions, which were probably related to the route of exposure. Ashby et al. (31) have identified arsenic-containing compounds as potential carcinogens, although there is currently only limited evidence for the carcinogenicity of arsenic and its compounds in rodents (32). The level of confidence in DEREK's prediction of noncarcinogenicity is moderate.

22. Isobutene. Isobutene gave a negative response in the Ames test, but its epoxide metabolite has been reported to be mutagenic, although very high concentrations are required and the epoxide has been demonstrated to be efficiently detoxified by liver enzymes (33). No histopathological lesions were observed in the mouse subchronic study, but nasopharyngeal duct goblet cell hypertrophy was observed in the rat and was probably related to the route of exposure. A structurally related compound, propylene, was found not to be carcinogenic by inhalation (34). Confidence in DEREK's prediction of noncarcinogenicity is high for the mouse and moderate for the rat.

23. Methyleugenol. Methyleugenol gave a negative response in the Ames test and a mouse micronucleus assay, although a positive response has been reported in an *in vitro* unscheduled DNA synthesis (UDS) assay (35). It has been suggested that a lack of activity of alkenylbenzenes in the Ames test is most likely to be due to the failure of metabolic activation (35). Methyleugenol has also been reported to induce hepatic tumors in male mice treated prior to weaning (36). A number of structurally related

alkenylbenzenes are included in the Gold carcinogenicity database, with the liver a common site of increased tumor incidence (12). Eugenol, which may be formed by enzymatic *O*-demethylation of methyl-eugenol, led to an equivocal increase in hepatic neoplasms in the mouse, although it was not carcinogenic in the rat (37). Methyleugenol induced liver lesions in both rats and mice in the subchronic study, in addition to lesions of the glandular stomach, which were probably related to the route of exposure. The confidence in DEREK's prediction of noncarcinogenicity is low, and the liver is identified as a possible site of tumor formation.

24. Oxymetholone. Oxymetholone was predicted to be a mutagen by DEREK as a result of the presence of the α,β -unsaturated ketone functionality, which may exhibit electrophilic and hence DNA reactivity. It gave a negative response in the Ames test. Sites affected in the subchronic study include the mammary glands in the rat and the clitoris in the female mouse. The promotional activity of oxymetholone in rat liver carcinogenesis has been reported (38). It is an anabolic steroid with activities similar to those of testosterone, a known rodent carcinogen (39). Confidence in DEREK's prediction of noncarcinogenicity is low.

25. Anthraquinone. Anthraquinone gave a positive result in the Ames test with and without S9 but was negative in a mouse micronucleus assay. No subchronic study data are available. 1-Hydroxyanthraquinone has been reported to be a rat carcinogen (40), but no evidence was available to indicate that this was a significant metabolite of anthraquinone. In the absence of further evidence, confidence in DEREK's prediction of noncarcinogenicity is moderate.

26. Emodin. Emodin is predicted to be a mutagen by DEREK, since it is a hydroxylated anthraquinone that may exhibit DNA intercalation. Experimental evidence to support a noncovalent interaction of an oxidized metabolite of emodin with DNA has been reported (41). Emodin was mutagenic in the Ames test only in the presence of S9. It also gave a positive response in a mouse micronucleus assay. Kidney lesions were observed in both male and female rats and mice in the subchronic assay. Although some hydroxylated anthraquinones can act as tumor promoters, emodin is reported to be essentially inactive (42). The confidence in DEREK's prediction of noncarcinogenicity is low.

27. Citral. Citral is predicted to be a mutagen by DEREK because of the

presence of the α,β unsaturated aldehyde functionality, which may lead to electrophilic and hence DNA reactivity. It gave a negative response in the Ames test, although it has been suggested that the toxicity to bacteria associated with lipophilic compounds of this type may impede adequate mutagenicity testing in *Salmonella* (43). Citral also gave a negative response in a mouse micronucleus assay. No significant lesions were observed in the 14-day subchronic study, although this was not considered a strong predictor of the histopathological effects that would be observed in longer term studies. A longer study may help in the interpretation of the significance of the reported estrogenic (44) and peroxisome proliferator (29) activities of citral. In the absence of further evidence, confidence in DEREK's prediction of noncarcinogenicity is moderate.

28. Sodium Nitrite. Sodium nitrite gave a positive response in the Ames test. A positive result in an *in vivo* mouse micronucleus assay has been reported (45). Forestomach squamous epithelial hyperplasia observed in both rats and mice in the subchronic study was described as minimal to mild, and was probably related to the route of exposure. Rat forestomach squamous papilloma have, however, previously been reported in a sodium nitrite drinking-water study (46). An increased incidence of female rat liver neoplasms has also been observed in a feed, but not drinking-water, study (47). The confidence in DEREK's prediction of noncarcinogenicity is low, and the forestomach is identified as a possible site of tumor formation.

29. Cinnamaldehyde. DEREK predicts cinnamaldehyde to be mutagenic, as a result of the presence of the α,β unsaturated aldehyde functionality, which may lead to electrophilic and hence DNA reactivity. It is reported to give a negative result in the Ames test (8), although it has been suggested that the toxicity to bacteria associated with lipophilic compounds of this type may impede adequate mutagenicity testing in *Salmonella* (43). Cinnamaldehyde also gave a negative result in a mouse micronucleus assay. Forestomach hyperplasia was observed in 2- or 3-week toxicity studies, and was probably related to the route of exposure (48). A study of this duration was not, however, considered to be a strong predictor of the histopathological effects that would be observed in longer-term studies. Confidence in DEREK's prediction of noncarcinogenicity is moderate.

30. Vanadium Pentoxide. Vanadium pentoxide gave a negative response in the Ames test but a positive response in an *in vivo* mouse micronucleus assay (49). Vanadium compounds are known to interfere with mitosis and chromosome distribution, although there are currently no data to indicate that such compounds are carcinogenic in animals or man (49). Respiratory tract lesions were observed in both rats and mice in the subchronic study, and were probably related to the route of exposure. Confidence in DEREK's prediction of noncarcinogenicity is low.

Discussion

DEREK was able to make predictions for all of the chemicals in the exercise, including five inorganic compounds. Seven chemicals were predicted to be carcinogenic. Following expert evaluation of supplementary data, two predicted carcinogens, scopalamine hydrobromide and 1-chloro-2-propanol, were thought unlikely to be carcinogenic in either rats or mice, and the predictions were therefore assigned a low level of confidence. For 1,2-dihydro-2,2,4-trimethylquinoline and diethanolamine, there was a moderate level of confidence in DEREK's prediction of carcinogenicity, but carcinogenic activity was thought most likely to arise from a different mechanism to that proposed by DEREK.

A high level of confidence in both rats and mice was associated with the predictions for 3 of the 23 chemicals for which DEREK provided no evidence of carcinogenic activity. However, 11 of the 23 chemicals were thought likely to be carcinogenic, and predictions for these chemicals were therefore assigned a low level of confidence. If these compounds are found to be carcinogenic, they will identify the need for new DEREK rules. Some of the 11 chemicals, such as isobutyraldehyde and emodin, were predicted by DEREK to be mutagenic. The toxicophore associated with mutagenicity may at least in some cases also form the basis of any observed carcinogenic activity. For other chemicals, potential toxicophores were identified during the evaluation of the supplementary data. Such toxicophores include the alkenylbenzene substructure present in methyleugenol, the cobalt(II) ion present in cobalt sulfate and the vanadium atom present in vanadium pentoxide. Provided that adequate data can be found in support of these toxicophores following a detailed review of the literature, new rules describing them will be added to the DEREK carcinogenicity knowledge

base. For some chemicals, such as primaclone and ethylene glycol monobutyl ether, more work will be required before a clearly defined toxicophore can be established, if indeed these chemicals prove to be carcinogenic.

The prediction exercise has also identified possible modifications to existing DEREK rules. Currently, DEREK predicts that any nitroalkane or epoxide will be mutagenic and carcinogenic. *Salmonella* mutagenicity data for scopolamine hydrobromide and its structural analogues, however, suggest that the rule describing the mutagenicity and carcinogenicity of epoxides should perhaps be modified to exclude

structures in which the epoxide is fused to a bridged ring system. Supplementary data considered for nitromethane have also indicated that it may be appropriate to restrict the rule describing the mutagenicity and carcinogenicity of nitroalkanes to secondary, and not primary or tertiary, substrates. Additional evidence including toxicological, mechanistic, and chemical reactivity data will be sought before appropriate changes to the DEREK knowledge base are implemented.

The development of the carcinogenicity knowledge base is a current priority of the DEREK project, and follows on from the successful expansion of the knowledge

bases for other toxicological end points, such as mutagenicity and skin sensitization. Work is in progress to identify data that can be used to further support and refine existing DEREK rules, in particular the FDA rules and rules that predict mutagenic, but not carcinogenic, activity. In the latter case, evidence of genotoxic carcinogenic activity associated with the toxicophore of a given mutagenicity rule will allow the rule to be extended to include carcinogenicity as a toxicological end point. Mechanistic aspects of the carcinogenic process are also being reviewed with the aim of identifying new toxicophores for nongenotoxic carcinogenicity.

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