COMPACT and Molecular Structure in Toxicity Assessment: A Prospective Evaluation of 30 Chemicals Currently Being Tested for Rodent Carcinogenicity by the NCI/NTP

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A new series of 30 miscellaneous National Toxicology Program chemicals has been evaluated prospectively for carcinogenicity and overt toxicity by COMPACT (Computer Optimised Molecular Parametric Analysis for Chemical Toxicity: CYP1A and CYP2E1). Evaluations were also made by Hazardexpert, and for metal ion redox potentials; and these, together with COMPACT, were compared with results from the Ames test for mutagenicity in Salmonella, the micronucleus test, and 90-day subchronic rodent pathology. Seven of the 30 chemicals (nitromethane, chloroprene, xylenesulfonic acid, furfuryl alchohol, anthraquinone, emodin, cinnamaldehyde) were positive for potential carcinogenicity in the COMPACT evaluation; xylenesulphonic acid and furfuryl alchohol were only equivocally positive. Four of the 30 chemicals—scopolamine, D&C Yellow No. 11, citral, cinnamaldehyde—were positive by Hazardexpert; 6 of 30—D&C Yellow No. 11, 1-chloro-2-propanol, anthraquinone, emodin, sodium nitrite, cinnamaldehyde—were positive in the Ames test; 2 of 30—phenolphthalein and emodin—were positive in the *in vivo* cytogenetics test; and 3 of 30—molybdenum trioxide, gallium arsenide, vanadium pentoxide—were metal compounds with redox potentials of the metal/metal ion indicative of possible carcinogenicity. The overall prediction for carcinogenicity was positive for 12 of 30 chemicals: nitromethane, chloroprene, D&C Yellow No. 11, molybdenum trioxide, 1-chloro-2-propanol, furfuryl alcohol, gallium arsenide, anthraquinone, emodin, sodium nitrite, cinnamaldehyde, vanadium pentoxide). This overall prediction has been made on the basis of the results of the computer tests and from consideration of the information from bacterial mutagenicity, together with likely lipid solubility and pathways of metabolism and elimination— Environ Health Perspect 104(Suppl 5):1011–1016 (1996)

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Introduction

The key to biological activity and toxicity is inherent in the electronic structure of the chemical. Computer optimised molecular parametric analysis for chemical toxicity (COMPACT) is one method for revealing possible reasons for the toxicity of a chemical because it calculates its three-dimensional molecular structure and also its electronic structure, and thus enables its metabolic activation by the cytochromes P450 to be evaluated (*1–4*). Hence, toxicity and in particular, carcinogenicity of a chemical can be predicted (*1–4*). It is thus more sophisticated than simply identifying toxicophores by structure alert as in the Hazardexpert system (*5*), although this is useful for direct-acting agents, which are not always recognized by the COMPACT procedure. Furthermore, the influence of substituents on the active moiety of the chemical can be explored through molecular modeling techniques and quantitative structure–activity relationships (QSARs); these may augment or diminish the potency of a particular substituent and influence the metabolic profile of the parent compound (*6–8*). Investigation of the three-dimensional structure enables differentiation between structural isomers and facilitates prediction of P450 specificity, in addition to other, nongenotoxic, events such as interaction with the peroxisome proliferator and estrogen receptors (*9,10*).

The cytochromes P450 are a superfamily of enzymes involved in the Phase 1 metabolism of the majority of endogenous and exogenous chemicals (*1,11–13*). It has been established that many carcinogens act as substrates and/or inducers for cytochromes P4501A (CYP1A) (*14–16*) and P4502E (CYP2E) (*1,4,14*), which give rise to the formation of reactive intermediates, or reactive oxygen species (ROS), capable of interaction with DNA, causing miscoding, mutagenesis, cell proliferation and neoplasia (*14*). Inducers of CYP1A interact with the cytosolic Ah receptor, initiating the protein kinase C cascade, *de novo* synthesis of CYP1A, resulting in increased carcinogen activation, increased DNA replication, cell proliferation and malignancy (*14,15*). Activation by CYP2E gives rise to the production of ROS (*17*) and reactive intermediates, causing many toxic effects including cancer (*14,18*), especially in small rodents which have high sensitivity to the toxicity of ROS. Although ROS are carcinogenic per se (*14*) they may also manifest carcinogenicity by oxygenation of the chemical to a reactive intermediate which subsequently interacts with DNA. From analysis of a number of CYP2E-specific chemicals of known toxicity/carcinogenicity, it is concluded that those chemicals with ΔE values of < 15.0 are more readily oxygenated and hence activated to proximate carcinogens while those with higher ΔE values are not generally activated but may exhibit other toxicity associated with ROS generation.

From knowledge of the molecular and electronic structures of a chemical, its molecular dimensions (*overall planarity* = *area*/*depth*^{2}; collision diameter) and the energy of activation ($\Delta E = E(HOMO) - E(LUMO)$; *LUMO* = lowest unoccupied molecular orbital, *HOMO* = highest occupied molecular orbital) may be calculated. From the known characteristics of area/depth^2, diameter, and ΔE, for known carcinogenic and noncarcinogenic substrates, and for the active sites of different cytochrome P450 isoforms, it is thus possible to assign the chemical to the specific isoforms, CYP1A and CYP2E, which would activate the chemical and hence determine its potential carcinogenicity (*1,11–14*). The technique of COMPACT (CYP1A plus CYP2E) has been validated for 100 National Toxicology Program (NTP)

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Abbreviations used: COMPACT, computer optimised molecular parametric analysis for chemical toxicity; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; NTP, U.S. National Toxicology Program; QSAR, quantitative structure–activity relationships; ROS, reactive oxygen species.

chemicals of known carcinogenicity, giving a concordance with the rodent assay of 92% (*19*). In a subsequent validation study of 44 NTP chemicals, containing fewer positive carcinogens (60%) than the first validation study, concordance of the rodent assay with COMPACT (CYP1A and CYP2E) was 72%, with Hazardexpert was 70%, and with COMPACT plus Hazardexpert was 86% (*20*).

The partition coefficient (log P) of a chemical is an important mediating factor for carcinogenic potential in that it determines accessibility of the chemical to the activating enzyme, and there appears to be a range of log P values which constitute a window of optimal transport properties whereby a chemical can cross cellular boundaries to enable interaction with the P450 activating enzyme system, receptors and DNA. Moreover, the rate of metabolism of many chemicals has been related to the magnitude of their log P values. The Hazardexpert system (*5*) readily calculates log P and other values, and in addition offers structural alerts associated with carcinogenicity, mutagenicity, teratogenicity, and other forms of toxicity, such as neurotoxicity and immunotoxicity. Structure alert programs make a useful complement to COMPACT, in that direct-acting agents, that might not normally be identified by COMPACT, are readily recognized by Hazardexpert.

It has been shown that metal toxicity in rodents correlates with the magnitude of the metal ion redox potential (*2*) and this provides a means of estimating the potential carcinogenicity of metals and their compounds. Possibly, some metal toxicity is caused by the generation of oxygen radicals, and the redox potential represents a measure of the ability of the metal to bring about activation of molecular oxygen. The toxicity/carcinogenicity and oxygen radical generation of Fe^{2+}/Fe^{3+} is well documented, and the redox potential of this metal redox system ($E^o = +0.77V$ for Fe^{2+}/Fe^{3+}) is taken as an index of likely carcinogenitic. If the redox potential is high (usually positive) the indication of acute toxicity is high.

This new series of 30 NTP chemicals were thus evaluated for COMPACT (CYP1A and CYP2E), for Hazardexpert, and for metal ion redox potentials, and the results considered in the light of mutagenicity in *Salmonella, in vivo* cytogenetics, and 90-day subchronic rodent pathology, made available by NTP.

Methods

Molecular structural and electronic parameters for COMPACT analysis were determined as previously described (*3,12,16*). Criteria for P450 substrates were a high degree of molecular planarity (a/d^2) and high potential for activation by oxidative metabolism (ΔE); the plot of a/d^2 against ΔE gives a curve that separates CYP1 substrates from non-CYP1 substrates. The radius of this curve (the COMPACT radius, *CR*) is defined mathematically as:

$$CR = \sqrt{(\Delta E - 9.5)^2 + (a/d^2 - 7.8)^2}\ ;$$

substrates are positive for CYP1 if the COMPACT radius is < 5.5, and are negative if the radius is > 6.5Å.

The criteria for CYP2E substrates that have potential for ROS generation and oxidation of the substrate are, empirically, a molecular diameter of < 6.5Å, and a ΔE value of < 15.0; all CYP2E substrates that have been shown to be overtly toxic or carcinogenic have ΔE values of < 15.0. The criteria for both CYP1A and CYP2E have been slightly modified from previous definitions as the COMPACT procedure has been updated and refined (*20*).

Hazardexpert evaluations were made using the standard software (version 6.1; Compudrug Ltd., Budapest) (*5*).

Results

Only 5 of the 30 chemicals were identified as potential CYP1 substrates, and hence as potential carcinogens. Candidate chemicals are potential CYP1 substrates if they are planar (high values of area/depth^2), and have low values of ΔE, the activation energy, that is, if the COMPACT radius is < 5.5. Of the chemicals that are positive for CYP1 in COMPACT, no. 8 chloroprene (COMPACT radius = 5.7), and no. 17, xylenesulfonic acid *CR* = 6.0) are considered equivocally positive (±), and no. 25, anthraquinone (*CR* = 4.3); no. 26, emodin

Table 1. COMPACT parameters for 30 chemicals.

Chemical	Area/ Depth^2	ΔE(eV)	COMPACT radius[a]	CYP1 interaction	Molecular collision diameter	CYP2E[b] activation	Overall[c] COMPACT prediction
1. Scopolamine	1.6	15.4	8.6	–	7.9	–	–
2. Codeine	1.4	14.1	7.9	–	8.1	–	–
3. 1,2-Dihydro-2,2,4-trimethylquinoline	1.8	13.1	7.0	–	6.9	–	–
4. Nitromethane	1.4	14.8	8.3	–	4.7	+	+
5. Tetrahydrofuran	1.6	20.9	13.0	–	5.1	–	–
6. *t*-Butylhydroquinone	1.8	14.3	7.7	–	6.7	–	–
7. Ethylbenzene	2.1	16.7	9.2	–	6.0	–	–
8. Chloroprene	3.7	13.5	5.7	±	5.3	+	+
9. Cobalt sulfate	NP	NP	NP	NP	NP	NP	NP
10. D & C Yellow No. 11	2.0	13.0	6.8	–	7.8	–	–
11. Isobutyraldehyde	1.5	17.5	10.2	–	5.3	–	–
12. Molybdenum trioxide	NP	NP	NP	NP	NP	NP	NP
13. 1-Chloro-2-propanol	1.5	16.4	9.3	–	5.3	–	–
14. Diethanolamine	2.9	20.3	11.9	–	5.8	–	–
15. Phenolphthalein	2.0	13.9	7.3	–	8.1	–	–
16. Pyridine	4.3	16.2	7.6	–	5.2	–	–
17. Xylenesulfonic acid	3.7	13.9	6.0	±	6.7	–	±
18. Furfuryl alcohol	2.6	15.3	7.8	–	5.4	±	±
19. Primaclone	1.7	15.9	8.8	–	7.3	–	–
20. Ethylene glycol monobutyl ether	3.3	20.5	11.9	–	6.4	–	–
21. Gallium arsenide	NP	NP	NP	NP	NP	NP	NP
22. Isobutene	2.3	17.9	10.0	–	5.1	–	–
23. Methyleugenol	1.8	14.8	8.0	–	7.1	–	–
24. Oxymetholone	2.7	13.9	6.7	–	8.5	–	–
25. Anthraquinone	9.4	13.5	4.3	+	7.0	–	+
26. Emodin	6.6	12.3	3.1	+	7.5	–	+
27. Citral	1.5	14.2	7.9	–	6.8	–	–
28. Sodium nitrite	NP	NP	NP	NP	NP	NP	NP
29. Cinnamaldehyde	6.9	13.3	3.9	+	6.2	+	+
30. Vanadium pentoxide	NP	NP	NP	NP	NP	NP	NP

Abbreviations: NP, not predicted; +, positive; –, negative. [a]COMPACT radius, $\sqrt{(\Delta E - 9.5)^2 + (a/d^2 - 7.8)^2}$. Positive CYP1 if COMPACT radius < 5.5; ± if radius is between 5.5 and 6.5. [b]CYP2E activation, molecular collision diameter < 6.5 and ΔE < 15.0. [c]Overall COMPACT prediction is the summation of CYP1 interaction and CYP2E activation.

(*CR* = 3.1); and no. 29, cinnamaldehyde (*CR* = 3.9) are considered firmly positive (+) (Table 1).

In the COMPACT evaluation for potential CYP2E-activating substrates, criteria for positive chemicals were, molecular collision diameter < 6.5 Å and ΔE of < 15.0. Three chemicals were rated positive for CYP2E, namely, no. 4, nitromethane (diam = 4.7, ΔE = 14.8); no. 8, chloroprene (diam = 5.3, ΔE = 13.5); and no. 29, cinnamaldehyde (diam = 6.2, ΔE = 13.3) (Table 1). Furfuryl was considered equivocal for CYP2E (diam = 5.4, ΔE 15.3). Only two chemicals were positive for both CYP1 and CYP2E: chloroprene and cinnamaldehyde, although chloroprene is only equivocally positive for CYP1.

In the Hazardexpert predictions (Table 2) four chemicals were identified as potential carcinogens: scopolamine (no. 1), D & C Yellow no. 11 (no. 10), citral (no. 27), and cinnamaldehyde (no. 29); five chemicals were identified as potential mutagens: tetrahydrofuran (no. 5), *t*-butylhydroquinone (no. 6), 1-chloro-2-propanol (no. 13), phenolphthalein (no. 15), and emodin (no. 26). Only cinnamaldehyde was positive in all 3 segments, CYP1 and CYP2E of COMPACT, and the Hazardexpert prediction.

Table 2. Hazardexpert predictions for 30 chemicals.

No.	Chemical	Log P	Toxicity	Prediction of carcinogenicity
1.	Scopolamine	2.09	Carcinogen	+
2.	Codeine	2.40	Teratogen	–
3.	1,2-Dihydro-2,2,4-trimethyl quinoline	3.77	—	–
4.	Nitromethane	–0.24	—	–
5.	Tetrahydrofuran	0.50	Mutagen[a]	–
6.	*t*-Butylhydroquinone	3.05	Mutagen	–
7.	Ethylbenzene	3.06	—	–
8.	Chloroprene	1.88	—	–
9.	Cobalt sulfate		NP	NP
10.	D&C Yellow No. 11	1.96	Carcinogen	+
11.	Isobutyraldehyde	0.57	—	–
12.	Molybdenum trioxide		NP	NP
13.	1-Chloro-2-propanol	0.13	Teratogen and mutagen	–
14.	Diethanolamine	–2.73	Teratogen	–
15.	Phenolphthalein	4.53	Mutagen	–
16.	Pyridine	0.74	—	–
17.	Xylenesulfonic acid	2.81	—	–
18.	Furfuryl alcohol	0.21	—	–
19.	Primaclone	–1.17	—	–
20.	Ethylene glycol butyl ether	0.22	Teratogen	–
21.	Gallium arsenide		NP	NP
22.	Isobutene	2.08	—	–
23.	Methyleugenol	3.35	Teratogen	–
24.	Oxymetholone	4.18	Teratogen	–
25.	Anthraquinone	2.54	—	–
26.	Emodin	1.49	Mutagen	–
27.	Citral	3.24	Carcinogen	+
28.	Sodium nitrite		NP	NP
29.	Cinnamaldehyde	1.92	Carcinogen	+
30.	Vanadium pentoxide		NP	NP

Abbreviations: NP, no prediction; +, positive; –, negative. [a]Not in mammalia.

Supplemental Information on the Chemicals Tested by Number

1. Scopolamine. This compound, a hypnotic and an anesthetic, is negative in all segments of COMPACT, but Hazardexpert regards this as a probable carcinogen due to its epoxide group. The chemical is structurally similar to cocaine which is metabolized by P450 to form an *N*-oxide, and also a nitroxide via *N*-demethylation and *N*-hydroxylation, and is therefore likely to be detoxicated (*21*). Scopolamine was negative in the Salmonella assay, and we therefore regard this chemical as a noncarcinogen. Scopolamine showed no evidence of potential carcinogenicity in a 90-day subchronic rodent toxicity study.

2. Codeine. COMPACT in both segments (CYP1 and CYP2E) is negative for this compound, and Hazardexpert only regards this chemical as an uncertain teratogen; carcinogenicity is therefore most unlikely. In a 90-day rodent toxicity study no evidence of toxicity or carcinogenicity was seen.

3. 1,2-Dihydro-2,2,4-trimethylquinoline. This chemical is an antioxidant. It is negative in COMPACT; Hazardexpert is also negative and the short-term tests (micronucleus and mutagenicity) are both negative. The overall prediction for carcinogenicity is therefore negative. A 90-day skin-painting study showed no evidence of carcinogenicity, although acanthoses and hyperkeratoses were observed in both sexes of rat and mouse.

4. Nitromethane. COMPACT indicates possible carcinogenicity via CYP2E activation for this chemical. Hazardexpert does not give any indication of toxicity, as the nitro group is in an aliphatic position; it is also negative in the Ames test and in the micronucleus test. The negative log P value (–0.24) indicates that the chemical would be insufficiently lipophilic to reach the cytochrome P450 enzymes. The overall prediction is therefore that the chemical is a weak rodent carcinogen. A 90-day rodent toxicology study shows increased bone-marrow cellularity in rat and increased splenic haematopoiesis in mouse, indicating possible weak rodent carcinogenicity as predicted by COMPACT.

5. Tetrahydrofuran (THF). Although a possible CYP2E substrate, this chemical is unlikely to be activated (ΔE = 20.9) and consequently is considered to be negative in COMPACT. Hazardexpert shows this chemical to be a mutagen, but not in mammalian systems. THF was negative in the Ames and micronucleus tests, and so is predicted to be noncarcinogenic. A 90-day rodent study showed no evidence of potential carcinogenesis although slight centrilobular hepatic cytomegaly was seen in the mouse.

6. t-Butylhydroquinone. COMPACT is negative for both CYP1 and CYP2E for this chemical, but Hazardexpert indicates possible mutagenicity due to the phenolic groups. *t*-Butylhydroquinone has the potential for redox cycling, and hydroquinone itself is a known carcinogen. Structurally, the compound resembles butylated hydroxytoluene (BHT) an equivocal rodent carcinogen. However, the Ames test and *in vivo* cytogenetic studies are both negative, so there is no firm evidence for carcinogenicity. A 90-day rodent feeding study also showed no evidence of potential carcinogenicity.

7. Ethylbenzene. COMPACT gives no indication of potential carcinogenicity, Hazardexpert does the same. With the negative evidence from the short-term Ames

and cytogenicity tests, it is evident that ethylbenzene will be noncarcinogenic in rodents. A 90-day inhalation study in rodents showed no evidence of toxicity, although increased tumor formation has been reported following gavage to rats.

8. Chloroprene. This chemical, although equivocal (±) in the COMPACT CYP1 evaluation, is a clear positive in COMPACT for CYP2E specificity and activation, and therefore is predicted as a positive carcinogen by COMPACT. Hazardexpert identifies no toxicophores in the structure, and the chemical is negative in the short-term Ames and cytogenetics tests, although it is hepatotoxic. Chloroprene may form a cysteine conjugate, which could be activated via β-lyase-mediated cleavage of the cysteine conjugate, a known mechanism for carcinogenicity. By analogy with vinyl chloride, this chloroalkene would seem likely to be carcinogenic, and our overall prediction is that it would be positive. However, no evidence of potential carcinogenicity was obtained in the rodent 90-day study.

9. Cobalt Sulfate. As this is an inorganic compound, its carcinogenicity could not be predicted by the COMPACT and Hazardexpert systems. However, from the relatively low redox potential for Co/Co^{2+}, ($E^o = -0.28V$), carcinogenicity is unlikely. Many studies on cobalt compounds show them to be noncarcinogenic, and even antimutagenic. However, the 90-day rodent study showed inflammatory, metaplastic, and hyperplastic changes, but no evidence of frank carcinogenicity.

10. D & C Yellow No. 11. This compound is negative in COMPACT, but Hazardexpert predicts that it is likely to be both carcinogenic and mutagenic and there is evidence for the latter in the Ames study. Although negative in COMPACT, the prediction of carcinogenicity by Hazardexpert, and the positive Ames test give an overall positive prediction. The molecule may interact directly with DNA as seen with phenytoin, although this may occur with only one of its enantiomers. A 90-day rat study showed periportal hepatocyte degeneration, but no evidence of carcinogenicity.

11. Isobutyraldehyde. This compound is negative in both COMPACT and Hazardexpert, as well as in the Ames and micronucleus tests. The overall prediction for carcinogenicity is therefore negative. From a rat subchronic inhalation study, there is evidence of upper respiratory lesions but no evidence of potential carcinogenicity.

12. Molybdenum Trioxide. This is an inorganic compound that could not be evaluated by COMPACT or Hazardexpert. There is no evidence of potential carcinogenicity from the short-term test data, but the Mo^{3+}/Mo^{4+} redox potential is relatively high ($E^o = +0.32$ V), indicating an oxidizing agent, and potential for carcinogenicity. However, the low solubility of the compound may preclude hazardous effects, so it is considered a potential weak carcinogen. A 90-day rodent study showed no evidence of potential carcinogenicity.

13. 1-Chloro-2-propanol. COMPACT is negative for both CYP1 and CYP2E. Hazardexpert predicts teratogenicity, based on the chloroethyl moiety, and the strongly electrophilic 2-carbon could indicate a direct-acting carcinogen. The Ames test is positive, and our prediction is that the chemical is likely to be weakly carcinogenic. The 90-day rodent study showed no evidence of potential carcinogenicity.

14. Diethanolamine. This chemical is negative in COMPACT for both CYP1 and CYP2E. Hazardexpert shows weak teratogenicity based on the hydroxyethyl group, but carcinogenicity is unlikely. Diethanolamine is negative in the Ames and micronucleus tests, the overall prediction for carcinogenicity is therefore negative. The rodent subchronic toxicity study showed no evidence of potential carcinogenicity.

15. Phenolphthalein. COMPACT shows this compound to be negative for P450-mediated toxicity. Hazardexpert, however, suggests possible mutagenicity. The chemical is positive in the micronucleus test and negative in the Ames test. The overall prediction is therefore negative. A rodent 90-day feeding study showed no evidence of potential carcinogenicity.

16. Pyridine. The chemical is negative in COMPACT for both CYP1 and CYP2E; Hazardexpert also predicts no toxicity. Both the Ames and micronucleus tests were negative. The overall prediction is therefore negative. The 90-day subchronic study showed liver lesions in female rats, while males showed both liver and kidney lesions. However, no evidence of potential carcinogenicity was seen.

17. Xylenesulfonic Acid. This chemical is weakly positive in COMPACT for CYP1 and negative for CYP2E; however, Hazardexpert gives no indication of toxicity. Evidence from short-term test data, and the likelihood of rapid elimination due to the presence of the sulphonic acid group, support the view that this chemical is not likely to be a rodent carcinogen, and the overall prediction for carcinogenicity is therefore negative. The 90-day skin-painting study showed no evidence of potential carcinogenicity.

18. Furfuryl Alcohol. COMPACT for CYP1 is negative and for CYP2E is equivocal; Hazardexpert gives no indication of toxicity. The Ames and micronucleus tests are both negative; the overall prediction for carcinogenicity is therefore equivocal. A 90-day rodent inhalation study showed metaplasia and hyperplasia of the respiratory epithelium; 2-year carcinogenicity studies on the related compounds, furan and furfural showed that these chemicals were carcinogenic in liver of rats and mice.

19. Primaclone. This chemical is negative in both CYP1 and CYP2E segments of COMPACT, and Hazardexpert gives no evidence of toxicity. The chemical is considered negative in the Ames (although positive without S9 mix) and micronucleus tests, and the overall prediction is therefore negative. The 90-day rodent study showed hepatocellular hypertrophy, but no evidence of carcinogenicity.

20. Ethylene Glycol Monobutyl Ether. This chemical is negative in both CYP1 and CYP2E segments of COMPACT; Hazardexpert also predicts that it will be a teratogen but noncarcinogenic, and there is no evidence of potential genotoxicity from the *in vitro* data; so although the overall prediction for carcinogenicity is negative, this compound, being a glycol ether, is likely to be overtly toxic. The 90-day rodent study showed evidence of inflammation, and hepatic and gastric necrosis, but no frank carcinogenesis.

21. Gallium Arsenide. As this chemical is inorganic, it cannot be evaluated via the COMPACT and Hazardexpert procedures. The Ames and micronucleus tests are negative. The redox potential for Ga/Ga^{3+} is low ($E^o = -0.56V$) but for As/AsO_2- is high ($E^o = +0.25V$), indicating a likelihood of carcinogenicity. The 90-day rodent study showed inflammatory lesions, hyperplasia, and metaplasia in both rats and mice, indicative of potential carcinogenicity.

22. Isobutene. This chemical is negative in both CYP1 and CYP2E segments of COMPACT; and Hazardexpert does not indicate any toxicity, although by analogy with butadiene it can be considered a suspect carcinogen. The Ames test was negative, so the overall prediction for carcinogenesis is negative. A 90-day inhalation study gave no indication of potential carcinogenicity.

23. Methyl Eugenol. COMPACT gives no evidence of carcinogenicity for this

compound and this is in agreement with the Hazardexpert evaluation. There is support for these findings in the *in vitro* test data, and although methyl eugenol is structurally related to safrole, a known carcinogen, the overall prediction for carcinogenicity is negative. A rodent 90-day study showed no evidence of carcinogenicity.

24. Oxymetholone. Negative in both segments of COMPACT, this compound is also considered to be noncarcinogenic by Hazardexpert, although there is indication of possible teratogenicity. The compound is not mutagenic in the Ames test so the overall prediction for carcinogenicity is negative. A 90-day study shows no evidence of carcinogenicity, but published data indicate that this anabolic steroid is a promoter of rat liver carcinogenesis.

25. Anthraquinone. This chemical is highly planar and is positive for CYP1 in COMPACT but negative for CYP2E. Hazardexpert does not predict carcinogenicity, but the molecule may be reduced and generate ROS by redox cycling; it exhibits mutagenicity in the Ames test. The prediction for COMPACT is positive, and the overall prediction for potential carcinogenicity is weakly positive. No subchronic rodent studies has yet been completed.

26. Emodin. Emodin is positive for CYP1 in COMPACT, whereas Hazardexpert predicts only mutagenicity. There is evidence for mutagenicity from the Ames and micronucleus tests, and it is known that hydroxyanthraquinones are also tumor promoters. However, this hydroxylated anthraquinone will be detoxicated by conjugation. The COMPACT prediction for emodin is positive, and the overall prediction for carcinogenicity is weakly positive, because of probable detoxication by conjugation. A 90-day rodent study showed hyperplasia but no tumorigenesis.

27. Citral. This compound is negative in both segments (CYP1 and CYP2E) of COMPACT, but it is known to be a weak peroxisome proliferator and CYP4 inducer. Hazardexpert flags potential carcinogenicity due to the α,β-unsaturated aldehyde function, but the Ames and cytogenetics tests are negative. The overall prediction for carcinogenicity is negative. No 90-day rodent studies have yet been completed.

28. Sodium Nitrite. This cannot be evaluated by COMPACT as it is inorganic. The sodium ion is unlikely to be toxic, but the nitrite ion would readily give rise to the formation of nitrosamines in the gut, which may explain the indication of mutagenicity from the Ames test. As nitrosamines are markedly carcinogenic in rodents, the overall prediction for carcinogenicity is positive. A 90-day rodent study showed squamous epithelial hyperplasia of the forestomach.

29. Cinnamaldehyde. This chemical is predicted to be potentially carcinogenic by both CYP1 and CYP2E segments of COMPACT, and by Hazardexpert. There are no mutagenicity data, however, previous studies have shown that this compound is both mutagenic and carcinogenic (*22*), in agreement with the COMPACT and Hazardexpert predictions. The overall prediction of carcinogenicity is positive. A 90-day rodent gavage study showed hyperplasia of the forestomach, but no evidence of malignancy.

30. Vanadium Pentoxide. COMPACT and Hazardexpert evaluations cannot be made on this inorganic compound; the Ames and cytogenetics tests were negative. The redox potential of V^{2+}/V^{3+} is high (E^o = +0.36 V) indicating possible carcinogenesis. The overall prediction for carcinogenicity is positive. A rodent 90-day study showed epithelial hyperplasia, metaplasia, and inflammation but no tumorigenesis.

A summary of the predictions of COMPACT, the Ames and micronucleus tests, the redox potential of metals and Hazardexpert is presented in Table 3. In the overall prediction (Table 3), all prediction parameters (COMPACT, Ames, micronucleus, redox potential, Hazardexpert) are considered. The number of chemicals predicted positive for carcinogenicity is 12 of 30, of which 6 of 30 were positive in COMPACT, 3 of 30 were metal compounds considered positive for potential carcinogenicity from their metal ion redox potentials, and 3 of 30 (D&C Yellow No. 11, 1-chloro-2-propanol, and sodium nitrite) were considered positive from their mutagenicity in the Ames test, Hazardexpert, and published data.

Table 3. Summarized COMPACT and other predictions for 30 chemicals.

No.	Compound	COMPACT	Redox potential	Ames	*In vivo* cytogenetics	Hazard expert	Overall[a] prediction
1.	Scopolamine	–	–	–	–	+	–
2.	Codeine	–	–	–	–	–	–
3.	1,2-Dihydro-2,2,4-trimethyl quinoline	–	–	–	–	–	–
4.	Nitromethane	+	–	–	–	–	±
5.	Tetrahydrofuran	–	–	–	–	–	–
6.	*t*-Butylhydroquinone	–	–	–	–	–	–
7.	Ethylbenzene	–	–	–	–	–	–
8.	Chloroprene	+	–	–	–	–	+
9.	Cobalt sulfate	NP	–	–	–	NP	–
10.	D&C Yellow No. 11	–	–	+	–	+	+
11.	Isobutyraldehyde	–	–	–	–	–	–
12.	Molybdenum trioxide	NP	+	–	–	NP	+
13.	1-Chloro-2-propanol	–	–	+	–	–	±
14.	Diethanolamine	–	–	–	–	–	–
15.	Phenolphthalein	–	–	–	+	–	–
16.	Pyridine	–	–	–	–	–	–
17.	Xylenesulfonic acid	±	–	–	–	–	–
18.	Furfuryl alcohol	±	–	–	–	–	±
19.	Primaclone	–	–	+[b]	–	–	–
20.	Ethylene glycol butyl ether	–	–	–	–	–	–
21.	Gallium arsenide	NP	+	+	–	NP	+
22.	Isobutene	–	–	–	–	–	–
23.	Methyleugenol	–	–	–	–	–	–
24.	Oxymetholone	–	–	–	–	–	–
25.	Anthraquinone	+	–	+	–	–	+
26.	Emodin	+	–	+	+	–	+
27.	Citral	–	–	–	–	+	–
28.	Sodium nitrite	NP	–	+	–	NP	+
29.	Cinnamaldehyde	+	–	+	–	+	+
30.	Vanadium pentoxide	NP	+	–	–	NP	+
Positive responses (*n*)		7/30	3/30	7/30	2/30	4/30	12/30

NP, no prediction; +, positive; –, negative; ±, equivocal in COMPACT and weakly positive in the overall prediction. [a]Overall prediction is based primarily on the compact data, taking into consideration the lipophilicity of the chemical and likely rate of elimination. For compounds that are probably direct acting, predictions by Hazardexpert were given prominence, although pathways to detoxication are also taken into account. [b]Although positive without activation, this chemical gave a negative response with the S9 mix.

Those chemicals with overall prediction of potential carcinogenicity (12 of 30) are nitromethane (no. 4), chloroprene (no. 8), D&C Yellow No. 11 (no. 10), molybdenum trioxide (no. 12), 1-chloro-2-propanol (no. 13), furfuryl alcohol (no. 18), gallium arsenide (no. 21), anthraquinone (no. 25), emodin (no. 26), sodium nitrite (no. 28), cinnamaldehyde (no. 29), and vanadium pentoxide (no. 30). Those positive in COMPACT were nos. 4, 8, 17, 18, 25, 26, and 29; those positive by metal ion redox potential were nos. 12, 21, and 30.

Although we are primarily concerned in the identification of potential carcinogens, the use of COMPACT to identify substrates of CYP1A and/or CYP2E enables prediction of overt toxicity in addition to carcinogenicity, as the reactive intermediates so formed may give rise to covalent complexes with protein and RNA, as well as with DNA, and may thus initiate immunotoxicity, which can often prove fatal. The supplementation of COMPACT to include metals, by prediction of potential carcinogenicity from redox potentials and the likelihood of oxygen radical generation, together with a combination with Hazardexpert and short-term mutagenicity tests, considerably widens the scope of COMPACT predictions to include toxic metals, direct-acting carcinogens, and chemicals that effect malignancy by mechanisms other than interaction of DNA with reactive intermediates or oxygen radicals.

REFERENCES

1. Parke DV. Activation mechanisms to chemical toxicity. Arch Toxicol 60:5–15 (1987).
2. Lewis DFV. Computer-assisted methods in the evaluation of chemical toxicity. Rev Comput Chem 3:173–222 (1992).
3. Ioannides C, Lewis DFV, Parke DV. The use of computers in the safety evaluation of drugs and other chemicals. Eur J Drug Metab Pharmacokin 3:225–233 (1994).
4. Lewis DFV, Ioannides C, Parke DV. A retrospective evaluation of COMPACT predictions of the outcome of rodent carcinogenicity testing from the NTP rodent bioassay results of 40 chemicals. Environ Health Perspect 103:178–184 (1995).
5. Smithing MO, Darvas F. Hazardexpert: an expert system for predicting chemical toxicity. In: Food Safety Assessment (Finley JW, Robinson SF, Armstrong DJ, eds). Washington: American Chemical Society 1992;191–200.
6. Hansch C. Structure-activity relationships of chemical mutagens and carcinogens in QSAR. In: Environmental Toxicology, IV (Hermens JLM, Oppenhuizen A, eds). Amsterdam:Elsevier, 1991;17–29.
7. Lewis DFV, Ioannides C, Parke DV. Molecular modelling of cytochrome P4501A1: a putative access channel explains activity differences between structurally related benzo[*a*] and benzo[*e*]pyrene, and between 2- and 4-acetylaminfluorene. Toxicol Lett 71:235–243 (1994).
8. Ioannides C, Cheung Y-L, Wilson JP, Lewis DFV, Gray TJB. Importance of cytochrome P450 induction in chemical carcinogenesis: the case for 2- and 4-acetylaminofluorene. Chem Res Toxicol 6:535–541 (1993).
9. Lewis DFV, Parke MG, King RJB. Molecular modelling of the human estrogen receptor and ligand interactions based on site-directed mutagenesis and amino-acid sequence homology. J Steroid Biochem Mol Biol 25:55–65 (1995).
10. Lewis DFV, Lake BG. The interaction of some peroxisome proliferators with the mouse liver peroxisome proliferator-activated receptor (ppar): a molecular modelling and QSAR study. Xenobiotica 23:79–96 (1993).
11. Parke DV, Ioannides C. The cytochromes P448—a unique family of enzymes involved in chemical toxicity and carcinogenesis. Biochem Pharmacol 38:1313–1319 (1987).
12. Lewis DFV. Computer modelling of cytochromes P450 and their substrates: a rational approach to the prediction of carcinogenicity. Front Biotransform 7:90–136 (1992).
13. Ioannides C, Lewis DFV, Parke DV. Computer modelling in predicting carcinogenicity. Eur J Cancer Prev 2:275–282 (1993).
14. Parke DV. The cytochromes P450 and mechanisms of chemical carcinogenesis. Environ Health Perspect 102:852–853 (1994).
15. Ioannides C, Parke DV. Induction of cytochrome P4501 as an indicator of potential chemical carcinogenesis. Drug Metab Rev 25:485–501 (1993).
16. Parke DV, Ioannides C, Lewis DFV. The role of cytochromes P450 in the detoxication and activation of drugs and other chemicals. Can J Physiol Pharmacol 69:537–549 (1991).
17. Ekström G, Ingelman-Sundberg M. Rat liver microsonal NAPDH-supported oxidase activity and lipid peroxidation dependent on ethanol inducible cytochrome P450 (P450 II E1). Biochem Pharmacol 38:131–1319 (1989).
18. Halliwell B, Gutteridge JHC. Role of free radicals and catalytic metal ions in human diseases: an overview. Methods Enzymol 186: 1–85 (1990).
19. Lewis DFV, Ioannides C, Parke DV. Validation of a novel molecular orbital approach (COMPACT) to the safety evaluation of chemicals by comparison with *Salmonella* mutagenicity and rodent carcinogenicity data evaluated by the US NCI/NTP. Mutat Res 291:61–77 (1993).
20. Lewis DFV, Ioannides C, Parke DV. A retrospective evaluation of COMPACT predictions of the outcome of NTP rodent carcinogenicity testing. Environ Health Perspect 103:178–184 (1995).
21. Poet TS, Brendel K, Halpert JR. Inactivation of cytochrome P4502B protects against cocaine-mediated toxicity in rat liver slices. Toxicol Appl Pharmacol 126:26–32 (1994).
22. NIOSH. Registry of Toxic Effects of Chemical Substances (RTECS). Cincinnati, OH:National Institute of Occupational Safety and Health, 1993.