Cytochromes P450 and Species Differences in Xenobiotic Metabolism and Activation of Carcinogen

David F.V. Lewis, Costas Ioannides, and Dennis V. Parke

School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, United Kingdom

The importance of cytochrome P450 isoforms to species differences in the metabolism of foreign compounds and activation of procarcinogens has been identified. The possible range of P450 isozymes in significant variations in toxicity exhibited by experimental rodent species may have a relevance to chemical risk assessment, especially as human P450s are likely to show changes in the way they metabolize xenobiotics. Consequently, in the safety evaluation of chemicals, we should be cautious in extrapolating results from experimental animal models to humans. This paper focuses on examples in which species differences in P450s lead to significant alterations in carcinogenic response, and includes a discussion of the current procedures for toxicity screening, with an emphasis on short-term tests. *Key words*: carcinogenicity, cytochromes P450, metabolic activation, species differences, xenobiotic metabolism. *Environ Health Perspect* 106:633–641 (1998). [Online 4 September 1998]
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There is much current concern (*1–6*) over the suitability of animal species as surrogates for *Homo sapiens* in chemical safety evaluation; one reason for this stems from the finding that, compared to the fairly large number (>400) of known rodent carcinogens (*7–11*), only a relatively small number of compounds (~20–30) have been shown to be carcinogenic in humans (*12,13*). This has led to the view that small mammalian species—such as those used experimentally in, for example, the rodent bioassay—are more susceptible to carcinogenicity than are human populations. Although the reasons for this disparity may be due to the fact that rodent carcinogenicity test procedures involve life-span studies with the chemical administered at its maximum tolerated dose (MTD), whereas human exposure is not normally under controlled conditions, the variation in body size could represent one factor in differential tumor susceptibility, as has been shown for diethylnitrosamine in various animal species (*14*). Furthermore, there are many examples of marked species differences (*15*) in the toxicity of foreign compounds, with one particular animal model representing a closer paradigm to *Homo sapiens* than another depending on the chemical concerned. Although there may be many reasons for these differences, it is likely that some may be traced to variations in primary metabolism between species in which the cytochrome P450 (CYP) mono-oxygenases are mainly involved (*16–19*).

Xenobiotic metabolism is generally regarded as proceeding via two stages, Phase I and Phase II; the initial oxidative stage in Phase I is followed by conjugation by a second class of enzyme, usually referred to as a conjugase. Over 90% of Phase I metabolism is mediated by cytochromes P450, a superfamily of heme-thiolate enzymes of which over 750 individual members have now been characterized in terms of gene or protein sequence data (*20–23*); only 36 human P450s have been sequenced to date, whereas in the rat, 47 P450s are known from isolated cDNAs, etc. In mammals, exogenous metabolism occurs mainly in the liver, which also represents the major site of P450 proteins, although smaller concentrations are found in other organs and tissues. Apart from those isoforms primarily associated with endobiotic metabolism, hepatic microsomal P450s comprise members of families CYP1, CYP2, and CYP3, of which the CYP2 family possesses five main subfamilies, CYP2A through to CYP2E. The CYP1 family contains two major isozymes, CYP1A1 and CYP1A2, which appear to be highly conserved across most animal species in terms of primary sequence homology, as is the CYP2E subfamily (*19,22,24*). The major xenobiotic-metabolizing P450 isoforms in five mammalian species are shown in Table 1.

It is generally recognized that induction of P450s associated with metabolic activation (e.g., CYP1A and CYP2E) is also regarded as indicative of potential toxicity/carcinogenicity (*25–27*). However, CYP1A1, an isoform most closely associated with the activation of procarcinogens [such as benzo(*a*)pyrene] in small rodents and readily inducible by polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is poorly expressed in human liver (*28*) and, consequently, is not likely to be of relevance to human hepatocarcinogenicity. Nevertheless, CYP1A1 is expressed in human lung (*28*) and also has been shown to be readily induced by procarcinogenic PAHs, including those present in cigarette smoke and exhaust emissions, thus constituting a potentially important factor in pulmonary carcinoma. CYP1A2, however, is involved in the metabolic activation of polyaromatic and heterocyclic amines and amides, such as those formed during the cooking of meat products; this form is both present and inducible in human liver (*28*) as well as in liver of experimental animals species. Consequently, although CYP1A and CYP2E subfamilies are well conserved across mammalian species, variations in their expression/induction and its consequences [reviewed by Lewis (*23*)] can explain some of the examples of species differences in the metabolism and activation of potentially toxic chemicals such as butadiene (*29*). CYP2E, for example, is inducible by low molecular weight compounds such as benzene, ethanol, acetone, carbon tetrachloride, and dichloromethane; it appears that many of the toxic consequences of CYP2E induction are due to the associated generation of reactive oxygen species (ROS) (*27,30*). There is evidence to suggest that small rodents such as the mouse (*31*) are more susceptible to the toxic/carcinogenic effects of ROS production often associated with CYP2E induction (*32*). Although there is some relationship between ROS-mediated toxicity and body weight (*33*) in which species life span could also be a factor (*34*), the increased susceptibility of small rodents to ROS relative to the situation in *Homo sapiens* may be due to the fact that glutathione

Address correspondence to D.F.V. Lewis, School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK.
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(GSH) is used as a radical scavenger in rodents, thus leading to a rapid depletion of their cellular GSH on exposure to ROS-generating chemicals (*35*) but not in humans, where epoxide hydrolase is employed in order to conserve GSH levels.

The xenobiotic-metabolizing P450s can, in fact, be regarded as a double-edged sword in respect to activation and detoxication of chemicals, as some forms (particularly CYP1A1/2 and CYP2E1) are able to mediate in the activation of procarcinogens (*19,36*) as shown in Table 2, whereas others are largely associated with detoxifying metabolism (*21,37*). Moreover, it is possible to trace species differences in xenobiotic metabolism to variations in P450 levels, their inducibilities, and the existence of different P450 isoforms of the same protein family or subfamily in various mammalian species (*19,23*). However, it is difficult to make meaningful comparisons between xenobiotic metabolism, and hence toxic activation/detoxication of carcinogens, and other foreign compounds, in humans and experimental animal species due, in part, to the differences between the P450s involved and the way in which their levels are affected by factors such as age, sex, diet, genetics, pathophysiological status, tissue specificity, and environment, for example [reviewed by Gibson and Skett (*38*)].

Laboratory-bred rodent species such as the rat and mouse tend to be genetically pure strains and therefore are unlikely to be subject to the considerable interindividual variation in P450 complement that is apparent in human ethnogeographical populations (*39*). Furthermore, there is little comparison even between rats and mice with respect to chemical carcinogenicity (*40*) and in the metabolic pathways for both activation and detoxication. Moreover, there are notable sex and strain differences with respect to the way in which chemicals are metabolized (and therefore activated or detoxified) by the same rodent species (*41*). Pure laboratory strains may exhibit such differences in metabolic activation as a result of diet and/or environment because these factors can lead to significant changes in P450 complement and inducibility, in addition to alterations in other enzymes and proteins relevant to toxic activation (*24*). However, in some cases, the metabolism of a particular chemical may be similar between, for example, one rodent species and *Homo sapiens*, whereas there are also many examples of significant species differences. Tamoxifen, a therapeutic agent used in the treatment of breast cancer, is a known carcinogen in rodents and other experimental animal species, but it is generally regarded as being largely safe in humans (*42*). The reason for the species difference is mainly due to the way in which tamoxifen is metabolized in humans as opposed to the situation in rats and

Table 1. P450 isoforms in various mammalian species[a]

CYP	Human	Monkey	Rabbit	Rat	Mouse
1A	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2
2A	2A6, 2A7	–	2A10, 2A11	2A1, 2A2, 2A3	2A4, 2A5
2B	2B6	2B17	2B4, 2B5	2B1, 2B2, 2B3	2B9, 2B10, 2B13
2C	2C8, 2C9, 2C18, 2C19	2C20, 2C37	2C1, 2C2, 2C3, 2C4, 2C5, 2C14, 2C15, 2C16	2C6, 2C7, 2C11, 2C12, 2C13, 2C22, 2C23, 2C24	2C29
2D	2D6	2D17	–	2D1, 2D2, 2D3, 2D4, 2D5	2D9, 2D10, 2D11, 2D12, 2D13
2E	2E1	2E1	2E1, 2E2	2E1	2E1
3A	3A4, 3A5	3A8	3A6	3A1, 3A2, 3A9	3A11, 3A13, 3A16
4A	4A9, 4A11	–	4A4, 4A5, 4A6, 4A7	4A1, 4A2, 4A3, 4A8	4A10, 4A12, 4A14

CYP, cytochrome P450 subfamily; –, no information available to date. Data from Nelson et al. (*22*).
[a]These are found predominantly, but not exclusively, in the liver of the relevant species.

Table 2. Known carcinogens activated by human P450 enzymes

CYP1A1	CYP1A2	CYP2A6	CYP2E1	CYP3A4
BaP	PhIP	DMN	Benzene	Aflatoxin B1
PhIP	Glu-P-1	DEN	Styrene	Aflatoxin G1
PAHs	Glu-P-2	NNK	Acrylonitrile	Sterigmatocystin
	IQ	NNN	Vinylcarbamate	BaP-7,8-diol
	MeIQ	NNAL	Vinylchloride	Estradiol
	MeIQx		Vinylbromide	6-Aminochrysene
	Trp-P-2		Ethylcarbamate	Senecionine
	4-Aminobiphenyl		Trichloroethene	MOCA
	2-Naphthylamine		Carbon tetrachloride	Tris-(2,3-DPP)
	2-Aminofluorene		Chloroform	
	2-AAF		DMN	
	NNK		DEN	
			NNK	
			NNN	
			NNAL	
			Butadiene	

Abbreviations: BaP, benzo(*a*)pyrene; PAHs, polyaromatic hydrocarbons; PhIP, 2-amino-1-methyl-6-phenylimidazo-(4,5-*b*)pyridine; Glu-P-1, 2-amino-6-methyl-dipyrido(1,2-*a*:3,2′-*d*)imidazole; Glu-P-2, 2-aminodipyrido(1,2-*a*:3,2′-*d*)imidazole; IQ, 2-amino-3-methylimidazo(4,5-*f*)quinoline; MeIQ, 2-amino-3,5-dimethylimidazo(4,5-*f*)quinoline; MeIQx, 2-amino-3,5-dimethylimidazo(4,5-*f*)quinoxaline; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido(4,3-*b*)indole; 2-AAF, 2-acetylaminofluorene; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; DMN, *N*-nitrosodimethylamine; DEN, *N*-nitrosodiethylamine; NNN, nornitrosonicotine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; MOCA, 4,4′-methylene bis(2-chloroaniline); DPP, dibromopropylphosphate.
Data from Guengerich (*96*.)

Table 3. Current procedures for chemical safety evaluation

Category	Tests
Long-term tests	Rodent two-species carcinogenicity bioassay (2 years) Repeated dose toxicity study (1 year) 90-Day exposure 14-Day exposure
Short-term tests	Reverse mutation assay (Ames test for mutagenicity) Micronucleus test (induction of micronuclei in bone marrow) Chromosomal aberration Sister-chromatid exchange Acute toxicity study Physicochemical measurements (log P_{oct}, water solubility, pKa, melting and boiling points, vapor pressure) Enzyme induction study (ENACT procedure)
Computer-based tests	Electronic structure calculation (via molecular orbital procedures) Quantitative structure–activity relationship (QSAR) study Calculation of log P_{oct}, pKa, log D and molecular weight (Pallas System) Structural alert analysis (DEREK system, CASE, TOPKAT, etc.) Evaluation of P450-mediated activation (COMPACT system)

Abbreviations: DEREK, deductive estimate of risk from existing knowledge; TOPKAT, toxicity prediction by computer-assisted technology; ENACT, enzyme activation in chemical toxicity; CASE, computer-automated structure evaluation; COMPACT, computer-optimized molecular parametric analysis of chemical toxicity.

mice. Tamoxifen is readily metabolized by CYP2C9 and CYP3A4 isoforms in *Homo sapiens*, which leads to its relatively rapid elimination in human subjects. However, different P450 families and subfamilies appear to be involved in the metabolic activation of tamoxifen in rodents; this may involve the formation of an epoxide (a highly reactive electrophilic species), although other activating pathways exist, which are also P450-mediated, such as ethyl group hydroxylation and subsequent formation of a carbonium ion intermediate that is able to interact covalently with DNA, thus giving rise to genotoxicity and, eventually, to carcinogenesis (*42*).

In addition to these differences in the P450s themselves, the levels of the various isoforms differ markedly between species and are also subject to different regulatory mechanisms. Furthermore, experimental animals are maintained in a strictly controlled environment under specific dose regimens and dietary restrictions (*43–45*), which is in complete contrast to the situation experienced by the majority of humans. As an individual's complement of the drug-metabolizing P450s is subject to their degree of medication, lifestyle, pathophysiological state, and level of involuntary exposure to potentially toxic chemicals in the environment, in addition to other factors such as diet, age, sex, ethnicity, and possible in-born metabolic defects (i.e., genetic polymorphism), it is probably unwise to attempt any extrapolation from the effects observed in experimental animals to a likely scenario encountered in human metabolism. However, recent advances in molecular biological techniques that enable the stable expression of human P450s in heterologous systems, together with test procedures for genotyping an individual's P450 complement, point the way forward to future chemical safety evaluation whereby medical practitioners could be in a position to advise on specific treatments for disease states and other conditions based on a knowledge of the patients' enzyme activities and metabolic competence (*23*).

Current Procedures for Chemical Safety Evaluation

A variety of methods (*46–52*) have been developed for the risk assessment and safety evaluation of chemicals destined for human exposure and can be broadly classified into three main categories: long-term or life-span studies, short-term procedures, and computer-based tests, as shown in Table 3.

Long-term Test Procedures

The rodent carcinogenicity bioassay (*53*) represents the traditional method for the screening of chemicals for potential toxicity and involves the use of two rodent species (rat and mouse) as surrogates for humans. The rodent bioassay comprises life-span studies on both sexes of rat and mouse, usually at three dose levels, based on the MTD (*53*). Ideally, for a chemical to be regarded as carcinogenic in at least one segment of the rodent assay, there should be a dose-dependent increase in tumor burden relative to the control group. However, for various reasons, this criterion is not rigidly adhered to, as can be appreciated from a careful inspection of the National Toxicology Program (NTP) compilations of carcinogenicity bioassays, for example. Furthermore, the site of tumor incidence (or multiplicity) appears to be another factor in determining whether a chemical is regarded as a carcinogen because unusual or rare cancer sites tend to be given less weight than those that occur, for example, in the liver or kidney.

Due to differences in the metabolism of certain chemicals between rats and mice, there is often little or no concordance for rat and mouse carcinogenicity data (*40*). Moreover, gender variations in the xenobiotic metabolizing enzymes are the likely cause of the low correlation between sexes within the same rodent species, and the sex differences in P450s has been well established in the rat and mouse. It is thus possible to rationalize some of the sex and species differences in xenobiotic metabolism and carcinogenic activation/detoxication in terms of altered levels of P450 isozymes, their variation in inducibility in different organs and tissues of different mammals, and their isoform specificities, which exhibit either a dominance or preference in different sexes and species.

A further genetic factor must be considered when attempting to extrapolate findings based on rodent studies to the human condition. This is the additional complication of genetic polymorphism in human ethnogeographical populations, some of which pertain to P450 enzymes such as CYP2D6 (*54*) and CYP2E1 (*30*), for example. It is not possible to identify chemicals that may be inadequately cleared in certain percentages of human subjects on the basis of experimental findings arising from tests carried out in rodents or, indeed, in any other mammalian species, including other primates. Furthermore, P450 isozymes from the same family or subfamily but in different species may metabolize the same chemical differently, even though the P450s concerned may be highly homologous, because even a single amino acid change can dramatically alter the enzyme's substrate specificity or regioselectivity of metabolism as in CYP2A, for example (*55,56*).

Two chemicals that may serve as examples are coumarin and butadiene; both of these are metabolized differently between rodent species and are activated in one species but not in the other. Coumarin is metabolized in the rat via 3,4-epoxidation, which represents an activating pathway because coumarin is a carcinogen in this species (*55*). However, in the mouse, coumarin undergoes 7-hydroxylation, which is considered to be a detoxifying pathway. As far as butadiene is concerned, the opposite situation occurs (*29*). In the mouse, this chemical is a carcinogen via P450-mediated activation to the mono- and diepoxide, whereas, in the rat, butadiene is noncarcinogenic because the diepoxide is not formed. It is possible to trace both of these species differences to alterations in the P450s involved in the metabolism of the two chemicals; interestingly, in these examples, the situation in humans resembles that shown in the mouse rather than in the rat, although there are many other cases in which the opposite is found and others in which neither rodent species mimics the human situation.

The main reason for these species differences in carcinogenicity probably involves the relevant P450 isozymes that metabolize the compounds concerned. For coumarin, the major route of metabolism in humans, i.e., the 7-hydroxylation, is catalyzed by P4502A6 (CYP2A6), whereas the analogous mouse ortholog is CYP2A5, which mediates the same metabolic pathway. However, in the rat, the CYP2A1 isoform facilitates formation of the carcinogenic 3,4-epoxide, and this significant species variation can be explained by molecular modeling of the relevant enzymes' active sites (*55*). Similarly, the species differences in butadiene metabolism can be rationalized by active site modeling of the CYP2E1 enzymes from rats, mice, and humans (*55*).

It can be argued that the use of small rodents as surrogates for humans is scientifically flawed due to fundamental differences in the metabolizing enzymes in the mammalian species. The <50% concordance between rat and mouse and between male and female in each species serves to diminish confidence in the rodent bioassay; there is also little correlation with human epidemiological studies for carcinogenicity. The enormous expense of the 2–3 year rodent bioassay (approaching $1 million per chemical) provides an additional incentive to investigate complementary short-term and noninvasive methods for the screening of foreign compounds.

Short-term Test Procedures

Partly as a result of the escalating costs of life-span and other long-term studies,

together with concerns over their essentially ritualistic nature, an increasing number and variety of short-term *in vivo* and *in vitro* procedures have been developed over the past 30 years or more (*57–61*). A selection of these short-term tests are listed in Table 3; these bear relatively lower costs in comparison with life-span rodent bioassays and other long-term tests.

These short-term procedures tend to focus on monitoring individual stages that lead the development of carcinogenicity and other related toxic responses to chemical insult. For example, the Ames test for bacterial mutagenicity has been used for some time as a guide to the genotoxic nature of a particular chemical in mammalian systems (*62*). The modification known as an S9 mix represents an attempt to more effectively reproduce the likely activation/detoxication mechanisms that could occur in mammals. Although this significant improvement of the technique, which makes use of mammalian microsomal P450s induced by Arochlor 1254, can be expected to give rise to satisfactory concordances with, for example, rodent carcinogenicity, the concordance between the two procedures is disappointingly low, around 55% overall and, in a recent study (*63*), correlations were less than 40% between mutagenic potency (revertants per mM/l/plate) and carcinogenic potency (increased tumor risk per mM/kg/day). However, these correlations could be improved to just over 60% when fewer chemicals were considered (*63*).

Nevertheless, the low cost and straightforwardness of the Ames test has led to its general adoption as a standard technique for assessing the potential genotoxicity of chemicals destined for human exposure. Indeed it is useful and informative to establish whether the carcinogenicity of a compound is made manifest via a genotoxic mechanism, either by directly reacting with DNA (*48,64,65*) or as a result of enzymic activation; the comparison between Ames tests conducted with or without S9 will facilitate this, and if both are negative, the chemical concerned can then be regarded as a nongenotoxic carcinogen. The ways in which carcinogenicity may be in evidence without genotoxicity include receptor-mediated events such as those produced by peroxisome proliferators, by estrogenic compounds, and by chemicals such as TCDD, which are potent inducers of cytochrome P4501 (CYP1) via high-affinity binding to the cytosolic aryl hydrocarbon (Ah) receptor (*26*). In fact, the estrogen receptor and peroxisome proliferator-activated receptor are both members of the steroid hormone receptor superfamily and are involved in promoting cell growth, whereas the Ah receptor is another type of nuclear receptor, which is also associated with increased cell division following translocation to the nucleus of its ligand-bound complex. All of these receptors, however, bind to specific DNA response elements in the regulatory (noncoding) regions of particular genes associated with the production of enzymes and other proteins involved in cell proliferation (*23,38*).

ENACT (enzyme activation in chemical toxicity), a short-term procedure related to the modified Ames test (i.e., involving an activation system), is associated with P450 enzyme induction (*27*); in contrast to the *in vitro* Ames test, ENACT uses a small number of animals to assess the ability of a chemical to raise the levels of some key P450 isozymes that have been associated with toxic effects. By measuring the extent of induction at differing dose levels, it is possible to establish whether the particular increase in the P450 concerned is dose dependent, such that dose–response relationships can be formulated. This is useful for establishing the inducing ability of the chemical, thus providing a possible estimate of threshold exposure levels in humans.

There is satisfactory agreement between the Ames test (with S9 mix) and enzyme induction studies in some cases (*25,26*), whereas either or both of these procedures are sometimes in line with carcinogenicity data for certain chemicals. There are also examples where the results of these techniques can be explained in terms of the molecular or electronic structures of the compounds themselves (*66*). Furthermore, both the Ames and ENACT tests produce numerical values that allow the data to be analyzed via quantitative structure–activity relationship (QSAR) studies, whereby correlations are sought between biological activity and one or more descriptor variables that may come from either a physicochemical measurement or a computer-assisted evaluation of the chemicals' molecular and/or electronic structures (*67–75*). Such QSAR procedures, which also involve the use of statistical methods for assessing correlations between what may be a relatively large number of parametric variables, can be extremely helpful in both formulating a rational description of potency differences within series of related chemicals and in providing a means for predicting the likely activity/toxicity of untested compounds (*68,69,76*). There are several descriptor variables often found to be involved in the explanation of differences in toxicity and other forms of biological activity for many classes of chemicals (*68*): octanol–water partition coefficient (log P_{oct}); water solubility; acid–base dissociation constant (pKa); molecular weight; vapor pressure; molar refractivity; molar polarizability; superdelocalizability; molecular orbital energies (HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital); dipole moment; molecular electrostatic potential/electrostatic isopotential (MEP/EIP); and electron densities/atomic charges. As far as the oil/water partition coefficient (and the related octanol–water partition coefficient; P_{oct}) is concerned, there are numerous examples of the use of this estimate of compound lipophilicity being correlated with changes in biological potency for large numbers of structurally related series of chemicals (*77,78*). However, the frontier orbital energy, E(LUMO), frequently appears in QSAR expressions involving chemical toxicity; there are also instances where the E(HOMO) value, or the combined parameter ΔE [ΔE = E(LUMO) - E(HOMO)] correlate closely with both carcinogenicity and other forms of toxicity, particularly those associated with P450-mediated events (*76*).

Computer-based Tests

Use of computers has made a huge impact on virtually all aspects of modern living. However, the employment of computer technology in toxicity screening has always been a controversial subject for various reasons (*79*), including a considerable suspicion of computer-generated data by many toxicologists and the difficulty of directly relating molecular structural parameters to a biological end point, which can often involve several sequential stages and may be susceptible to a variety of largely external factors such as environment, diet, and dose regimen, for example.

The attraction of using computers in toxicity testing is obvious to most scientists involved in the safety evaluation of chemicals destined for human exposure and for hazard identification of substances in the environment. There are several advantages of computer-based procedures: they are rapid, relatively inexpensive, and always reproducible, and they allow researchers to avoid the need for actual chemical synthesis and any form of experimentation (*80*). However, care needs to be exercised in interpretating computer-generated results; for example, the outcome of QSAR analyses can, under certain circumstances, be misleading in terms of overinterpretation of inadequate or insufficient data. Nevertheless, as a tool used with other noninvasive *in vitro* procedures, some computer methods (see Table 4) for toxicity testing can be extremely helpful in prescreening chemicals and for prioritization

of large numbers of compounds for further evaluation using short-term procedures (*81*). Moreover, there have been many examples which demonstrate that computer-based evaluations can explain the findings of biological studies, including the rodent carcinogenicity bioassay; in some cases, these are able to provide a satisfactory level of prediction, which can be as high as about 70%. Indeed, the employment of a battery of computer tests and other short-term procedures could prove extremely useful as an alternative to the life-span rodent bioassay. Many groups of investigators worldwide are currently involved in developing a variety of computer methods for the prediction of carcinogenicity (*69,81*).

Table 4. Comparison between four computer-based methods of toxicity evaluation

Methods and characteristics	COMPACT	DEREK	HazardExpert	TOPKAT
Molecular structure	3D	2D only	2D only	2D only
Electronic structure	+	–	–	Empirical charge calculations only
Metabolism	+	–	+	–
Log P	–	–	+	+
pKa	–	–	+	+
Molecular interactions	+	–	–	–
QSAR	+	–	–	+
Correlation with toxicity	~70%	Not known	Not known	95% in some cases
Hardware	Any	VAX/Unix	PC	PC
Cost	$1,000 per chemical	Not known	$15,000	$15,000/module
No. of atoms	350	64	Limited by PC	Limited by PC
Knowledge base	P450 interactions	FDA	EPA	QSAR/NCI
Publications	>30	>5	>5	>10
Years in use	10	7	7	10
Commercial	No	Yes	Yes	Yes

Abbreviations: 3D, three dimensional; 2D, two dimensional; +, calculated by method; –, not calculated by method; QSAR, quantitative structure–activity relationship; FDA/NCI, Food and Drug Administration/National Cancer Institute; PC, personal computer. Most carcinogens are associated with known P450 activation; therefore, the COMPACT should have a high success rate in identifying P450-mediated toxicity. DEREK (*91*) and HazardExpert should identify direct-acting carcinogens and also other forms of toxicity not detected by COMPACT. Consequently, these methods work well in combination with COMPACT (*81*). HazardExpert (*92*) has some advantages over DEREK, as it calculates log P and pKa, it runs on a PC, and is available to academics at a considerably reduced cost. TOPKAT (*93*) performs well for certain classes of chemicals within its database, but is expensive for academics because there is no educational discount offered.

Species Differences Relating to P450 Variations

A potential difficulty emerges, however, when one has to consider the possible effects of species differences in metabolism and toxic response of chemicals. Extrapolation of experimental data from small rodents and other mammalian test species to *Homo sapiens* is not always straightforward, although there can be an approximately linear relationship between activity and species body weight; more frequently, such relationships tend to be nonlinear but may, nevertheless, be amenable to statistical analysis. Nonlinear relationships between, for example, Phase 1 metabolism and species body weight are often due to orthologous P450s in those species, differential distribution of the P450 isoforms responsible for bioactivation, and/or a variation in mechanism that may involve different P450 families or subfamilies. Such considerations can be addressed, albeit partially, by molecular modeling of the P450 isozymes involved in chemical metabolism, but such procedures are in the early stages of development (*23*). However, the metabolism of tamoxifen in *Homo sapiens*, for example, can be explained by modeling the interaction of this substrate within the active sites of the P450s involved, thus demonstrating the potential for a technique that can predict the likely metabolic fate of an untested chemical even before synthesis. The success of procedures such as these for exploring all metabolic and toxic possibilities for a new compound, which may be intended for use as a therapeutic agent, requires the detailed structural analysis of every enzyme or protein that could be involved in metabolism or toxic activation; this necessitates carrying out crystallographic and/or nuclear magnetic resonance (NMR) studies on at least one protein in each class such that a reasonable homology model can be constructed. Benigni and coworkers (*82,83*) have stressed that the importance of the biological activity data is often overlooked in QSAR studies because scientific scrutiny tends to become focused on the structural descriptors and their statistical significance. The normally wide variation and margins of error in the biological data are usually disregarded in the search for apparently highly significant correlations.

For drug metabolism via P450 isozymes, the following examples serve to illustrate the importance of considering the metabolic pathways and enzymes involved. First, the metabolism of hexobarbital has been studied in various mammalian species, and the results are shown in Table 5. In this case, the half-life of the drug ($t_{1/2}$) exhibits a good correlation (r = 0.83) with body weight (W) for the five species, provided that the raw data are converted into their logarithmic form. The relationship (shown in Fig. 1) then becomes as follows:

$$\log t_{1/2} = 0.323\ (\pm 0.127) \log W + 1.994,$$

where n = 5; standard error (SE) = 0.336; r = 0.8256; F (variance ratio) = 6.42.

Hexobarbital is metabolized by P450s of the CYP2B and CYP2C subfamilies in the rat and humans, so it may be reasonable to assume that these are also involved the mouse, rabbit, and dog, in which barbiturates tend to exhibit induction of both subfamilies, with a preference for CYP2B (*84*). Although CYP2B isozymes are inducible in both rodents and in rabbits, this is not the case in *Homo sapiens*; in humans, the level of the CYP2B6 ortholog is extremely low in hepatic tissue (*85*) and is apparently poorly induced by phenobarbital and other barbiturate drugs, which is in complete contrast with the situation in rats, mice, and rabbits (*84*). Such considerations may explain the relatively long half-life in humans where the CYP2C subfamily of isozymes, particularly CYP2C19, appear to be the major catalysts of hexobarbital metabolism (*86*).

Table 5. Metabolic half-lives ($t_{1/2}$) of hexobarbital in five mammalian species

Species	$t_{1/2}$ (min)	Body weight (kg)
Mouse	19	0.025
Rabbit	60	2.5
Rat	140	0.20
Dog	260	5.0
Human	360	68.0

Data from Pratt and Taylor (*94*).

Second, omeprazole metabolism has been studied in several mammalian species, and the clearance data are presented in Table 6. In *Homo sapiens*, the major route of metabolism appears to be a methyl hydroxylation step, which is primarily catalyzed by CYP2C19, a known polymorphic isoform; however, CYP3A4 is also involved in omeprazole metabolism, and being the main component of human liver P450, this pathway represents an important secondary route of omeprazole clearance in humans (*87*).

There is significant variation in the complement of P450 isoforms between *Homo sapiens* and other mammalian species. For example, CYP2B is well conserved between rodents and rabbits, but is poorly expressed in human liver. In contrast, CYP3A represents the major component of

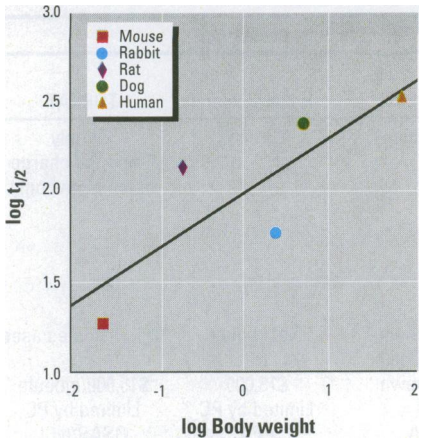

Figure 1. A plot of log half-life ($t_{1/2}$) versus log body weight (kg) for hexobarbital metabolism in five mammalian species.

Table 6. Species variation in omeprazole clearance

Species	Intrinsic clearance (l/min)	Body weight (kg)
Rat	0.52	0.25
Rabbit	0.96	2.0
Dog	3.40	15.0
Human	3.65	67.0

Data from Okudaira et al. (*95*).

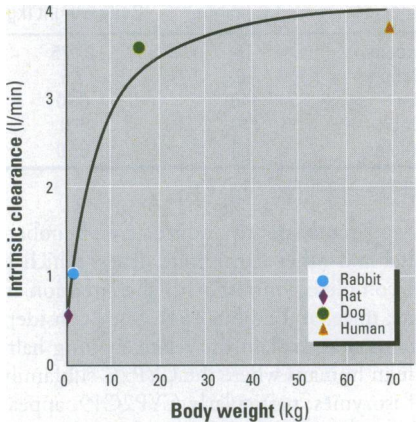

Figure 2. A plot of intrinsic clearance (l/min) against body weight (kg) for omeprazole metabolism in four mammalian species.

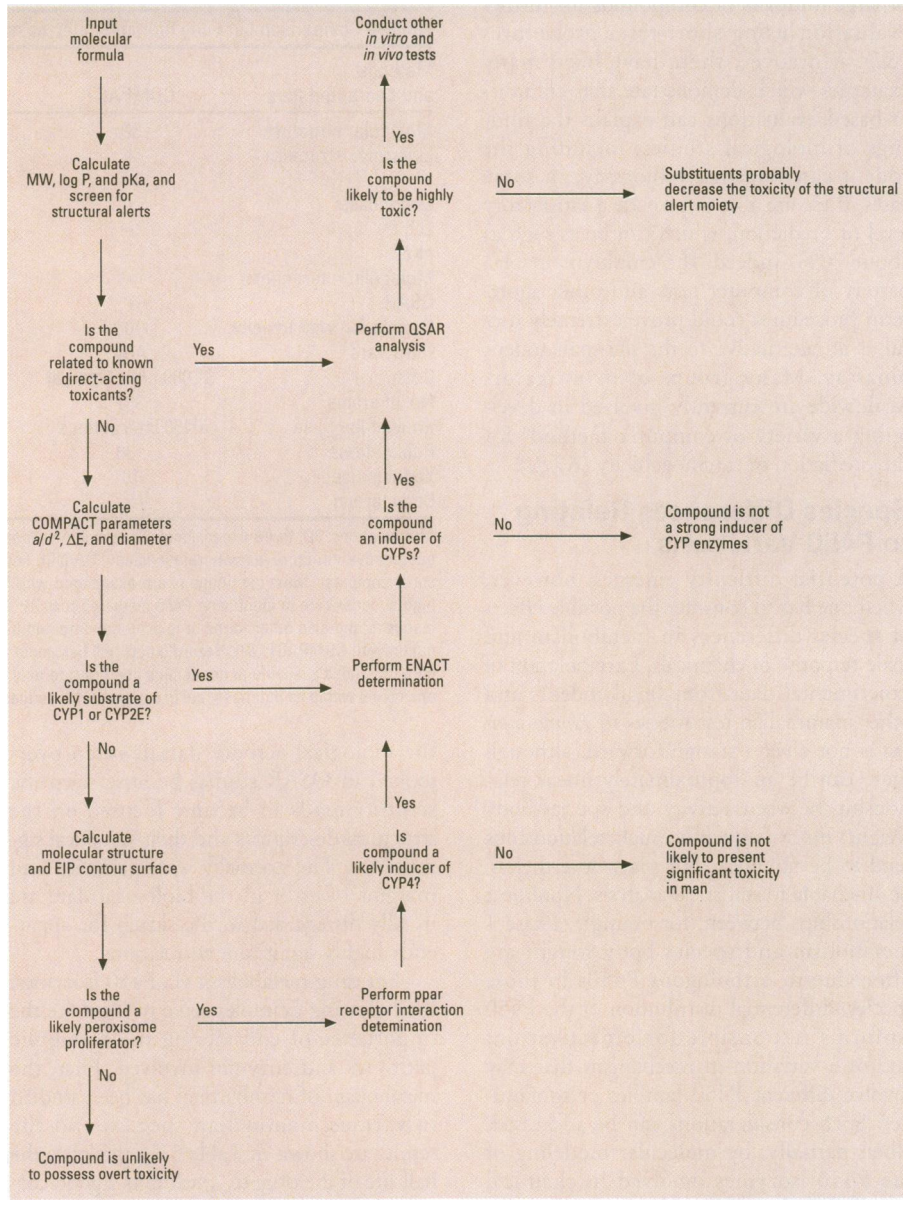

Figure 3. A decision tree approach to the safety evaluation of chemicals. Abbreviations: MW, molecular weight; a/d^2, area/depth^2; EIP, electrostatic isopotential; QSAR, quantitative structure–activity relationship; ppar, peroxisome proliferator-activated receptor.

human hepatic P450 although the levels of this subfamily are generally quite low in other species, especially in experimental animals. The CYP2C subfamily in humans possesses distinctly different isoforms that exhibit varying substrate specificity, and human CYP2C19 is subject to genetic polymorphism. Consequently, although hexobarbital is likely to be metabolized in the same position in most mammalian species via CYP2B and/or CYP2C subfamily mediation, the metabolism of omeprazole and tamoxifen, for example, is more complex, with several Phase 1 pathways being mediated by different P450s. The data presented in Tables 5 and 6 indicate that there is a general relationship between rate of metabolism and body weight, but because of the different P450s involved and their altered levels between species, the relevant metabolic rate changes from animals to humans depend on the nature of the chemical being metabolized.

For omeprazole intrinsic clearance (CL_{int}) in the four mammalian species, a log–log relationship with body weight also holds, but the best fit with the data is given by the following:

$$CL_{int} = 4.077\ (\pm 0.355)\ W/(4.635 + W),$$

where $n = 5$; SE = 0.034; $r = 0.988$; $F = 81.8$.

Figure 2 presents this relationship for the species in question; the relationship is approximately linear with body weight for the three animal species but exhibits a marked nonlinearity when the human data are considered. The increased rate of omeprazole clearance in man is probably due to the mediation of CYP3A4, as this represents such a major component of the hepatic P450 complement, in contrast with other mammalian species in which enzymes of this family constitute only a small percentage of total P450 in the liver. Although the main route of omeprazole metabolism is through the CYP2C19 isoform in *Homo sapiens*, this isozyme represents only a relatively minor proportion of the human hepatic P450 complement.

Conclusions

Consequently, the extrapolation of animal data to humans is not always simple and

straightforward due to species differences in the P450 enzymes present, including their active site differences, which may even occur within enzymes of the same family or subfamily. A review by Smith (*16*) provides further examples of such species differences in metabolism that can also be explained in terms of P450s and describes many important aspects of pharmacokinetics. In particular, the relevance of log $D_{7.4}$ (D is the distribution coefficient) values were shown to be potentially useful for differentiating between substrates of different P450 isozymes, primarily CYP3A, CYP2C, and CYP2D isoforms, as these constitute the P450 enzymes in human liver that metabolize the large majority of currently used pharmaceuticals, although log $D_{7.4}$ is also of importance in the context of Phase II metabolism.

The log $D_{7.4}$ parameter is, in fact, the lipophilicity (log P) of a chemical that is ionization-corrected for a pH medium of 7.4, as this represents the most commonly used value for physiological pH. The expressions relating these quantities for compounds that are ionizable can be represented in the following way:

$$\log D_{7.4} = \log P - \log (1 + 10^{7.4-pKa}) \text{ for acids}$$
$$\log D_{7.4} = \log P - \log (1 + 10^{pKa-7.4}) \text{ for bases}$$

Consequently, it is relatively straightforward to evaluate the log $D_{7.4}$ for any compound from the neutral log P value and pKa, provided that such information is available. It is important to use log $D_{7.4}$ instead of log P for the estimation of compound lipophilicity because ionization will diminish the observed lipophilic character of a chemical, provided that the compound contains groupings that may become partially ionized at physiological pH. Because log P is one of the major physicochemical parameters used in the safety evaluation of chemicals destined for human exposure, the potential for ionization is extremely relevant, particularly where P450-mediated metabolism is possible; this can provide an early indication of probable P450 isoform specificity. Furthermore, lipophilicity of a chemical plays an important role in its biological fate in almost all species, including *Homo sapiens*, because this factor determines the bioavailability and bioaccumulation effects of the compound itself, and biological half-lives are often closely related to lipophilic potential (*88*). Therefore, from a compilation of short-term test data, physiochemical parameters, and molecular structural information, it should be possible to assess the likely risk to human health from exposure to a given substance; such procedures are currently under development in this laboratory and elsewhere. Figures 3 and 4 represent examples of how a decision tree approach may be applied to both chemical safety evaluation and P450 specificity, respectively.

The decision tree approach to toxicity screening presented in Figure 3 has been constructed on the basis of several studies on predicting rodent carcinogenicity as conducted in this laboratory. We have proposed that this may also be of benefit in human risk assessment (*89*). Figure 4 represents a decision tree for determining the likely P450 isoform specificity of a given chemical; this is based on extensive studies on the structural characteristics of substantial numbers of P450 substrates and inducers (*90*). Although there are differences in the levels of P450 families and subfamilies, including tissue distribution, between humans and other animal species, the decision tree in Figure 4 can be employed across mammalian species because the particular P450 substrate specificities tend to remain relatively well-conserved when one compares specificities for individual P450 families and subfamilies. We hope that workers in the field will find these decision trees of use in the screening of chemicals for potential toxicity, where possible species differences in metabolism may be attributable in part to the relative activities of orthologous P450 isoforms.

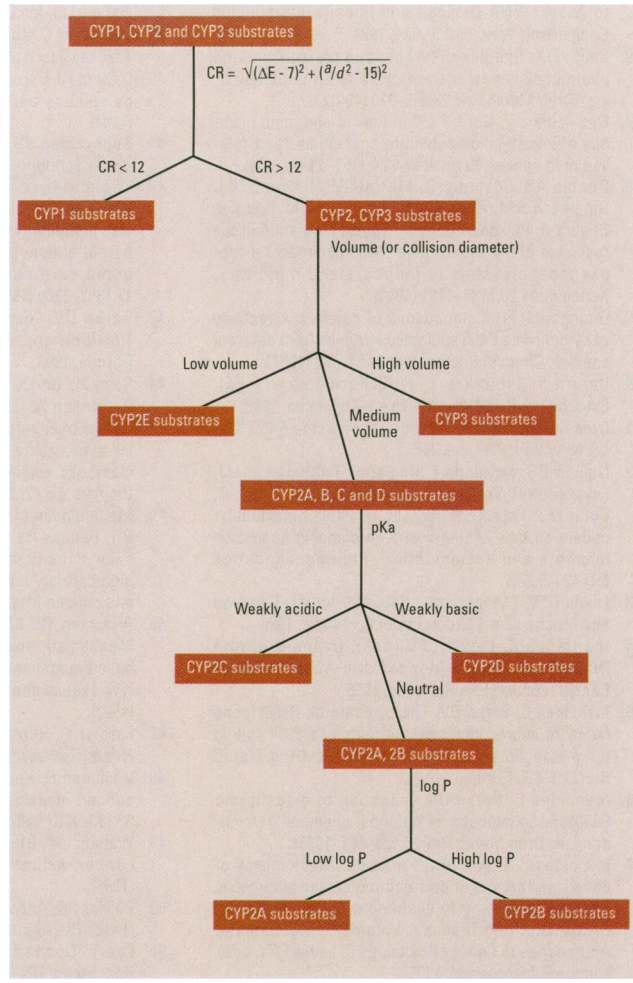

Figure 4. A proposed decision tree for predicting (CYP) P450 substrate specificity. CR, compact radius. By and large, the logP values of P450 substrates are greater than zero, although there are exceptions, such as some CYP2D and CYP2E substrates. CYP2D6 substrates usually possess a protonatable nitrogen atom 5–7 Å from the site of metabolism. CYP2C9 substrates generally possess a hydrogen bond donor/acceptor atom 5-8 Å from the site of metabolism. Some compounds can be substrates for more than one P450, especially where there is overlapping specificity. This scheme does not necessarily apply to steroids and other endogenous chemicals, which can be substrates of several P450s.

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