Pharmacokinetics and Molecular Detoxication

John R. Cashman,1 Beatrice Y. T. Perotti,2 Clifford E. Berkman,1 and Jing Lin1

1Pfizer Biomedical Research Institute, Seattle, Washington; 2Pfizer Incorporated, Central Research Division, Groton, Connecticut

This paper presents a comprehensive overview of the pharmacokinetic parameters used from in vivo and in vitro studies that are important in order to understand the major conceptual approaches of toxicokinetics and the disposition of environmental chemicals. In vitro biochemical information concerning the detoxication of environmental chemicals is also presented. The discussion leads to a more complete appreciation for the use of in vitro measurements for in vivo correlations. The concept of interspecies scaling in the interpolation and extrapolation of fundamental biochemical metabolic processes is illustrated with a number of examples. Additional examples of in vitro–in vivo correlations are presented in the evaluation of the impact of chemical exposure to humans. Finally, several important metabolic detoxication enzymes are presented, including the mammalian microsomal cytochrome P450 and flavin-containing monooxygenases as well as carboxylesterases and glucuronosyltransferases, to provide insight into the processes of chemical detoxication in mammalian tissue and blood. Because interspecies scaling and the pharmacokinetics of chemical disposition have already shown their usefulness in understanding some examples of chemical disposition, our summary focuses on showing the usefulness of the pharmacokinetic equations and providing confidence in using the approach for in vitro–in vivo correlations. Ultimately, the presentation may provide the reader with a conceptual framework for future evaluation of the human health risks associated with environmental toxics. — Environ Health Perspect 104(Suppl 1):23–40 (1996)

Key words: environmental chemicals, toxicokinetics, in vitro–in vivo correlations, interspecies allometric scaling, physiological modeling, (S)-nicotine, cytochrome P450, flavin-containing monooxygenase, carboxylesterases, organophosphates, glucuronidation

Introduction

For over 25 years, the concept of bioactivation of chemicals to reactive intermediates that covalently modify critical proteins and DNA, which in turn alter key cellular function, has been thoroughly established. Examples of the bioactivation of chemicals to form toxic substances following the paradigm developed many years ago (Figure 1) and refined in terms of mechanism and site of action have been reported for numerous chemicals including bromobenzene, acetaminophen, aflatoxin, polycyclic aromatic hydrocarbons, arylamines, and organophosphates to name a few (1). The purpose of this review is to present an overview as to how in vitro and in vivo pharmacokinetic parameters could be useful in understanding the conceptual framework for the disposition and detoxication of environmental chemicals. While the ability to predict which chemicals will follow the events outlined in Figure 1 is instrumental to the prediction of toxicity of a chemical, the prediction of metabolic detoxication could be equally important. In general, the details of metabolic detoxication are less appreciated than the science of metabolic bioactivation of chemicals to toxic species. This perspective will focus on the molecular basis for chemical detoxication. Where possible, the significance of metabolic detoxication to environmental chemicals will be presented. The detoxication enzymes that will be discussed in detail include cytochromes P450, flavin-containing monooxygenase (FMO), carboxylesterases and other esterases, as well as glucuronosyltransferases. As a prelude to the presentation of examples of metabolic detoxication of environmentally relevant chemicals, we will first describe some general approaches to examine the role of in vitro metabolism studies in the prediction of in vivo toxicity data.

The manifestation of clinical toxicity of chemicals is often dependent upon the peak plasma concentration achieved, the duration of exposure to the toxic entity above a particular threshold, or the amount of toxin accumulated in the body. To determine the exposure to the toxic entity and to assess the impact of such exposure, one needs to answer the following questions: How much of a chemical gets into the systemic circulation and to the site or organ of bioactivation? Where in the body will the chemical distribute? How long does the chemical remain intact in the body? How does the chemical get eliminated from the body? Is the chemical metabolized into other entities? Is the toxic effect derived from the exposed chemical or from metabolites? How much chemical in the body is needed to elicit the toxic effect? Are these toxic reactions reversible? Thus, an understanding of the absorption, distribution, and elimination of a specific chemical

Chemical

↓

Metabolic bioactivation to a reactive intermediate

↓

Alteration of cellular function

↓

Cellular necrosis

↓

Cell death

Figure 1. Hypothetical illustration of the metabolic activation of a chemical to form a toxic metabolite and the pathological consequences.
chemical will allow the prediction of the occurrence of potential toxicities.

The basic principle of toxicokinetics used in environmental science is directly derived from pharmacokinetics. Toxicokinetic parameters of a specific chemical in man can be predicted by allometrically scaling the corresponding parameters obtained in animals. These pharmacokinetic/toxicokinetic parameters in animals can be obtained from in vivo studies. The parameter clearance, can be estimated from in vitro metabolism data also. Derivation of parameters used in pharmacokinetics can be found in reviews and textbooks (2–4) and will not be described in detail here. An overview of several equations useful for calculating fundamental pharmacokinetic parameters from in vivo and in vitro data will be presented, and parameter scaling by allometry will also be introduced and contrasted to physiological modeling.

**Quantitating Exposure**

From in vivo studies in which the chemical of interest is given to animals, blood or plasma concentrations of a chemical at various time points are usually available and determined. When steady state is reached after multiple dosing or with constant infusion, the concentration ($C_{ss}$) can be predicted as

$$C_{ss} = F \times \text{dose} / (t \times \text{CL})$$

where $t$ is the dosing period, CL is the total clearance, and $F$ is the bioavailability.

The term clearance describes the rate at which a chemical is eliminated from the body. Clearance may be calculated as the available dose ($F \times \text{dose}$) divided by the area under the systemic chemical concentration-time profile (AUC):

$$\text{CL} = F \times \text{dose} / \text{AUC}.$$  

Substituting Equation 2 into Equation 1, one will obtain

$$C_{ss} = \frac{\text{AUC}}{t}.$$  

Thus, the ability to correctly predict $C_{ss}$ is dependent upon the accuracy of the measurement of AUC.

The bioavailability ($F$) of a chemical product via various routes of administration/exposure is defined as the fraction of unchanged chemical that is absorbed intact and reaches the systemic circulation following administration by any route. For an intravenous (iv) dose of a compound, bioavailability is defined as unity. For chemicals administered by other routes of administration, bioavailability is often less than unity:

$$F = \frac{\text{AUC}_{\text{non iv}}}{\text{AUC}_{\text{iv}}}$$

In many instances, the response to a particular chemical exposure, particularly toxicity, is related to the amount of a chemical in the body rather than to the systemic concentration. The amount in the body at steady state ($A_{ss}$) is the product of the systemic concentration and the steady-state apparent volume of distribution ($V_{ss}$):

$$A_{ss} = C_{ss} \times V_{ss}.$$  

The apparent volume of distribution relates the amount of chemical in the body to the concentration of the chemical in the blood or plasma. The apparent volume of distribution does not represent a real volume. Rather, it is an apparent volume that should be considered as the size of the pool of body fluids that would be required if the chemical was equally distributed throughout all portions of the body. At steady state, $V_{ss}$ reflects the sum of the volumes of all the pools into which the chemical may distribute. $V_{ss}$ can be calculated from the AUMC, area under the moment curve (i.e., an integration of the concentration vs. time profile), and AUC as defined by Benet and Galeazzi (5):

$$V_{ss} = \text{dose}_{iv} \times \text{AUMC} / \text{AUC}^2.$$  

Half-life ($t_{1/2}$) is also an extremely useful kinetic parameter for assessing exposure to a chemical. By definition, half-life is the time required for 50% of the chemical remaining in the body to be eliminated. It is the relationship between the apparent volume of distribution and the total clearance:

$$t_{1/2} = 0.693 \times \frac{V}{\text{CL}}.$$  

If the dosing interval (proportional to 1/exposure frequency) is short relative to half-life, significant accumulation will occur. Thus, the half-life parameter allows one to predict chemical accumulation within the body and quantitates the approach to plateau that occurs with periodic or constant toxicological/environmental insult.

Toxins with very short half-lives may have a smaller impact on human health because they will have a problem maintaining concentration levels at which toxicities may occur. On the other hand, chemicals with very long half-lives will accumulate in the body and possess negative characteristics in terms of toxicities. However, if toxicity occurs as a result of the formation of toxic metabolites, then it is the half-lives of such toxic metabolites that will form a relationship with the level of toxicity exposure.

Among the parameters mentioned above, clearance can sometimes be found from in vitro studies. For example, if the total clearance of a compound is related primarily to oxidative metabolism by the hepatic cytochromes P450 (i.e., $\text{CL} = \text{CL}_{\text{hep}}$), then the in vitro clearance can be predicted from data obtained from liver slices, hepatocytes, or liver microsomal incubation studies. However, it is important to note that the accuracy of this prediction will be compromised if, for example, cooperative or competitive binding to the metabolic enzyme by other substrates occurs in vivo, or substantial nonspecific binding of the substrate occurs either in vitro or in vivo.

The organ clearance of a compound can be limited by any diffusion barrier that exists between the blood and the organ of elimination. If one assumes zero diffusion barrier, as well as instantaneous and complete mixing, the simplest model that describes hepatic clearance in terms of physiologic parameters is the well-stirred model (6). The well-stirred model (Equation 8) states that hepatic clearance ($\text{CL}_{\text{hep}}$ with respect to blood concentration) is influenced by hepatic blood flow ($Q_{\text{hep}}$), the fraction of compound in blood that is free from plasma protein binding ($f_{\text{u}}$), and the intrinsic ability of the organ to eliminate a chemical ($\text{CL}_{\text{int}}$). For compounds that are quickly metabolized, the hepatic clearance will approach hepatic blood flow. For compounds that are slowly metabolized, hepatic clearance approximates $f_{\text{u}} \times \text{CL}_{\text{int}}$:

$$\text{CL}_{\text{hep}} = Q_{\text{hep}} \times f_{\text{u}} \times \text{CL}_{\text{int}} / (Q_{\text{hep}} + f_{\text{u}} \times \text{CL}_{\text{int}}).$$  

The intrinsic ability of an organ to metabolize a chemical is directly proportional to the activity of the metabolic enzymes in the organ. Such metabolic processes are characterized by Michaelis-Menten kinetics:

Rate of metabolism = $V_{\text{max}} \times C / (K_m + C)$
Molecular Detoxication

in which \( V_{\text{max}} \) the maximum rate at which metabolism can proceed, is proportional to the total concentration of enzyme. \( K_m \) is the Michaelis-Menten constant corresponding to the chemical concentration that yields 1/2 of the maximum rate of metabolism. Dividing both sides of Equation 9 by the substrate concentration in the organ (assuming the same free concentration at the metabolic site in vitro and in vivo) and in the venous blood) yields:

Rate of metabolism/C = \( \frac{V_{\text{max}}}{K_m + C} \) = CLhep/\( f_w \) \[10\]

Since identification of saturable metabolism only occurs for low extraction ratio compounds (3) (i.e., \( f_w \times CL_{\text{int}} = CL_{\text{hep}} \)), the relationship between classical enzyme kinetics and pharmacokinetics is revealed if it is assumed that the entire pool of substrate is freely available, and the substrate concentration is much lower than \( K_m \):

\[ f_w \times CL_{\text{int}} + f_w = CL_{\text{int}} = \frac{V_{\text{max}}}{(K_m + C)} = \frac{V_{\text{max}}}{K_m}. \]

The units for \( V_{\text{max}} \) and \( K_m \) in in vitro experimental conditions are generally micromolar per minute per nanomole of metabolic enzyme in the incubation medium and micrograms per milliliter, respectively. In the case of cytochrome P450 isozymes which distribution in the liver is generally considered to be homogeneous, scaling up of the \( V_{\text{max}} \) to describe the turnover produced by the whole liver (in the unit of \( \mu \text{g/min} \)) in the well-stirred model can be achieved (Table 1).

\[ CL = \frac{Q_{\text{hep}} \times f_w \times (V_{\text{max}}/K_m)}{(Q_{\text{hep}} + f_w \times (V_{\text{max}}/K_m))}. \]

Prediction of Human Pharmacokinetic Parameters from Animal Data

Once pharmacokinetic parameters are obtained via either in vitro methods or in vivo animal studies, the next crucial task is to extrapolate them to humans. Two approaches frequently used are interspecies allometric scaling and physiological modeling. Based on an entirely empirical approach, allometric scaling in general produces good data for chemicals solely or primarily eliminated from the body by processes such as biliary, renal, or pulmonary excretion. Physiological scaling requires more data, model designing, mathematical prowess, and interpretation and is therefore much more complex. In many instances, due to the lack of data, many of the initial parameters needed in the physiological model are estimated by allometric scaling. The allometric approach will be described in detail, and the physiological modeling approach will be briefly discussed.

Allometric Scaling

Interspecies scaling is a method of interpolation and extrapolation of the underlying anatomical, physiological, and biochemical similarities in mammals. Many of these similarities, or differences, are related to the size of the species. It has been recognized that many of the physiological parameters (7) that ultimately determine the values of various pharmacokinetic parameters are related across species according to their body weights raised to a certain power (7). This relationship between the rate of biological processes and body weights is linked by the metabolically active mass in a unit termed the ergosome (8). The metabolically active mass is hypothesized to correlate with the rate at which oxygen and oxidizable materials are delivered to metabolically active cells (8). The size of the metabolically active mass, or the number of ergosomes a species has, equals \( B^n \), where \( B \) is the body weight, and \( n \) is the allometric exponent (8). Thus, the rationale for normalizing data on the ordinate by a power function of body weight is based upon this premise. Allometric equations that relate a physiological variable to a one-power function used in concert with the body weight are generally represented as

\[ Y = a B^n. \]

It has also been found that, at times, the single-term power function is incapable of fully characterizing biological and pharmacokinetic processes for all mammals. Sacher (9) found that by accounting for both brain weight (Bw) and body weight using a two-term power function, i.e.,

\[ Y = a \times B^b \times (Bw)^c \]

processes such as the maximum lifespan potential can be more adequately described (9). Pharmacokinetic processes such as intrinsic clearance for compounds eliminated by the mixed function oxidases (10), when expressed relative to maximum lifespan potential instead of chronological time, also correlate well with body weight across species (11–13).

Because the allometric exponents for most biological processes, with some exceptions, are less than 1 (7), it is apparent that smaller animals will have smaller individual physiological variables than the larger animals. Yet, it was discovered that, for all species, a constant relationship exists between particular physiological variables within each species. For example, the breath:heartbeat ratio is 4 for all mammals regardless of their sizes (14), or the number of heartbeats per maximum life span is 8.6 \times 10^8 (15). In other words, smaller mammals are performing the same physiological functions in the correct relative quantities but at a much quicker pace than larger animals.

The notion of smaller animals performing the same physiological functions in the correct relative quantities but at a much quicker pace than larger animals leads to the introduction of time equivalence as well as pharmacokinetic-space time. The chronological time on the abscissa is adjusted by a power function of body weight to yield a time equivalence termed pharmacokinetic time. Pharmacokinetic time is a species-dependent unit of chronological time required to complete a species-independent pharmacokinetic event (14). Each species is endowed with its own particular ideal (mathematical) space–time continuum, which in turn is related to the organism’s total body mass (8). A summary of the different pharmacokinetic-space–time parameters is presented in Table 2 (8,16).

| Table 2. A summary of different pharmacokinetic-space–time units. |
|---------------------------------|
| **Species** | **Total P450 (nmol) per whole liver** | **Hepatic blood flow, ml/min/kg** |
| Rat | 118 | 69 |
| Dog | 1,095 | 41 |
| Man | 2,320 | 21 |
| **Data from DJ Rance (personal communication).** |

Abbreviations: kln, kallynochron; apl, appolysichrons. Reproduced with permission from Boxenbaum and Ronfeld and Boxenbaum and D'Souza (16).
When data for the ordinate and the abscissa are scaled to a power function of body weight, concentration–time profiles of chemicals administered to different species will collapse into one single profile as demonstrated in the elementary Dedrick plot (8,17–19) and the complex Dedrick plot (8). If the clearance (CL) and the volume of distribution (V) of a compound are defined by the allometric functions

\[ CL = aB^\alpha w^Z \]  \hspace{1cm} (15)

and

\[ V = bB^\beta \]  \hspace{1cm} (16)

then plasma concentration (C) after an iv bolus dose is as follows, assuming Z equals 0:

\[ C = (D/V)e^{-kt} \]  \hspace{1cm} (17)

\[ = (D/bB^\beta)e^{-(a/b)(B^\alpha)\gamma t} \]  \hspace{1cm} (18)

\[ CL/(D/bB^\beta) = (1/b)e^{-(a/b)(C/D/B^\beta)} \]  \hspace{1cm} (19)

Hence, the parameter on the ordinate will be normalized by dose per body weight raised to the power of y, and the time on the abscissa will be normalized by \( B^{\alpha-x} \) (Equation 19). The difference between the elementary Dedrick plot and the complex Dedrick plot is that, in the elementary Dedrick plot, y takes on the value 1. Examples of such superimposability between species are found in antipyrine disposition (8) using the elementary Dedrick plot and chlordiazepoxide disposition (Figure 2) with the complex Dedrick plot (8). The beauty of using allometric scaling is that pharmacokinetic parameters can be easily related from one species to another by a simple expression (8). For example:

\[ t_{1/2 \text{man}} = t_{1/2 \text{dog}} (B_{\text{man}}/B_{\text{dog}})^{x-1} \]  \hspace{1cm} (20)

Recent work by Lave et al. (20) reported that increased accuracy in the prediction of clearance of mafaroetine, a compound eliminated exclusively by metabolism, can be achieved by integrating in vitro metabolic data with in vivo pharmacokinetic data from animals. In vitro clearance from each animal species is normalized by the ratio of in vitro metabolic clearances in the corresponding animal species and man (Figure 3):

\[ CL_{\text{rat, normalized}} = \frac{CL_{\text{rat, microsomes}}}{CL_{\text{rat, microsomes}}} \]  \hspace{1cm} (21)

The in vitro metabolic clearances can be obtained from either hepatocyte or microsomal incubation data, as described by Equation 10. The normalized clearance values in animals were then extrapolated to humans using allometric scaling (Figure 3).

Allometric scaling is useful not only in estimating individual pharmacokinetic parameters (21–24) but also in estimating the entire pharmacokinetic profile (25), as well as toxicity endpoint (15). It may also offer a way to determine which larger animal to use in toxicology testing (15) and what doses to use in toxicity testing (15); allometric scaling potentially leads to the reduction of the numbers of subjects needed in a study (26).

**Physiological Modeling**

A physiological model is composed of a series of lumped components (body regions) representing organs or tissues in which concentrations are assumed to be uniform. The individual components are connected by a flow system representing the actual blood flow to and from the individual tissues. The schematic of a prototypical
physiologic model is shown in Figure 4. Each model is unique because it tries to describe various processes under different physiological constraints. Assumptions such as those related to the elimination organs, dose linearity, elimination model, protein free fraction, blood:plasma ratio, and restriction and extent of the distribution and transport processes need to be made before the development of the model. Equations describing each of the processes involved in the model are set up and solved simultaneously, yielding values for the parameters of interest.

Physiologically based pharmacokinetic modeling is useful for estimating drug disposition (27) and toxicity in different species (28), once the model is described in detail in one species. It also allows extrapolation from high dose to low dose and vice versa. As compared to the allometric approach, it has the advantages of being capable of describing situations in which nonlinearities occur, showing the differences from various routes and frequency of administration, or making a priori predictions of pharmacokinetic changes associated with various disease states, age, pregnancy (29), or drug-drug interactions. In addition, physiologically based pharmacokinetic modeling can also be used to model surrogates in tissue compartments (15).

As described above, once pharmacokinetic parameters are obtained via either in vitro methods or in vivo animal studies, the next task is to extrapolate the information to humans. Below, we provide an example of an in vitro–in vivo correlation of an environmental chemical, (S)-nicotine, to which humans are widely exposed.

**In Vitro–In Vivo Metabolic Correlations of (S)-Nicotine**

In the United States, approximately 75 million people smoke tobacco (30). Approximately 400,000 deaths occur every year in the United States from tobacco smoking, primarily from lung cancer and respiratory and heart disease (31). Recently, it has been concluded that approximately 3,000 lung cancer deaths occur in the United States annually from environmental tobacco smoke in nonsmokers (32). Passive smoking may also be responsible for some 150,000 to 300,000 cases annually of bronchitis and pneumonia in U.S. children (31). There are a large number of constituents in mainstream and sidestream smoke from cigarettes that could contribute to the untoward health effects described for smoking tobacco. (S)-Nicotine is one of the major components of tobacco and formation of (S)-cotinine in humans is useful markers of (S)-nicotine exposure because both metabolites are chemically stable and because the metabolites are formed by different enzyme systems (33).

In vitro kinetic studies showed that in the presence of adult human liver microsomes, the mean $K_m$ apparent value for formation of the iminium ion (that is subsequently converted to (S)-cotinine by the action of exogenously added aldehyde oxidase) was 39.6 μM (range 3–75 μM) (34). Interestingly, of the 13 microsome samples from which the data were obtained, three samples gave mean $K_m$ apparent values of 482 μM, over 10-fold greater than the normal mean values. The $K_m$ apparent value for the FMO3-mediated (S)-nicotine N-1'-oxide formation in the presence of human liver microsomes is probably greater than 800 μM, almost 20-fold more than that for cytochrome P450-mediated formation of (S)-nicotine $\Delta^{1,5}$-iminium ion. Thus, the relatively low percent of (S)-nicotine N-1'-oxide that is formed in vivo compared with (S)-cotinine probably reflects the fundamental kinetic properties of adult human FMO3 versus cytochrome P4502A6. In vitro data suggest that approximately 90% of the formation of (S)-nicotine $\Delta^{1,5}$-iminium ion could be explained by the action of cytochrome P4502A6. However, there may be a cytochrome P4502A6 polymorphism or differential expression in human liver preparations, and it is possible that the intersubject variability in human liver microsome samples for (S)-nicotine C-atom oxidation observed may underlie the large interindividual variability observed for (S)-nicotine metabolism in vivo. Thus, use of in vitro metabolic correlations has identified the type of cytochrome P450 responsible for iminium ion formation (i.e., CYP2A6); it has provided considerable insight into the relative amount of adult human FMO3 versus cytochrome P4502A6-mediated (S)-nicotine metabolism; and finally, it has provided understanding about the fundamental molecular basis for interindividual differences in (S)-nicotine metabolism in humans (Figure 5). Although considerable interindividual variability in both the amount of (S)-nicotine present and the type of metabolite formed [i.e., (S)-cotinine or (S)-nicotine N-1'-oxide] was observed in humans following intravenous, dermal, or free-smoking routes of administration, nevertheless, quantification of each metabolite provided information about two potentially disparate metabolic pathways [i.e., flavin-containing monoxygenase-catalyzed formation of (S)-nicotine N-1'-oxide and cytochrome P450-catalyzed (S)-nicotine $\Delta^{1,5}$-iminium ion] (33). The metabolic formation of the N-1'-oxide probably represents a detoxication pathway for (S)-nicotine, whereby the polar, highly ionized N-1'-oxide is readily excreted in the urine. On the other hand, formation of (S)-nicotine $\Delta^{1,5}$-iminium ion potentially represents a bioactivation mechanism because the iminium ion has been shown to covalently modify tissue macromolecules.

The pharmacological activity of (S)-nicotine $\Delta^{1,5}$-iminium ion is poorly understood, but it is known to be efficiently oxidized by the action of aldehyde oxidase to (S)-cotinine. (S)-Cotinine can also be further metabolized. In humans, (S)-cotinine metabolites [i.e., trans-3-hydroxy-(S)-cotinine and (S)-cotinine glucuronide] represent major metabolites of (S)-nicotine (35). Thus, the interindividual variability in formation of human (S)-nicotine metabolites (35) may be a consequence of a number of mechanisms contributing to detoxication and bioactivation including the fundamental kinetic properties of the metabolic enzymes involved and the wide individual genetic variability in the formation of additional (S)-nicotine metabolites [i.e., glucuronidation of (S)-cotinine, 3-hydroxy-(S)-cotinine, and (S)-nicotine] as well as more complicated mechanisms.
As described above, (S)-nicotine undergoes extensive Phase I oxidative metabolism in humans, and approximately 90% of a dose can be accounted for in terms of urinary metabolites. In the presence of adult human liver microsomes, cytochrome P4502A6 appears to be the major enzyme that forms (S)-nicotine Δ1,5'-iminium ion that is converted to (S)-cotinine by aldehyde oxidase (36). Because it appears that cytochrome P4502A6 and aldehyde oxidase work in concert to produce a detoxicated end product of (S)-nicotine, it is possible that humans with a deficiency of aldehyde oxidase are predisposed to an increased risk of the untoward effects of (S)-nicotine Δ1,5'-iminium ion. In addition, polymorphism leading to a reduction in the ability of a subpopulation of humans to metabolize (S)-nicotine by cytochrome P4502A6 could also result in longer half-lives of (S)-nicotine and result in increased pharmacological and behavioral effects.

While the pharmacological and neurochemical effects of increased accumulation of (S)-nicotine Δ1,5'-iminium ion in humans who are unable to oxidize the iminium ion are unknown, they are likely to be considerably more toxic than exposure to increased levels of the rapidly excreted (S)-nicotine N-1'-oxide.

Of the two (S)-nicotine N-1'-oxide diastereomers that could be formed in humans, the absolute stereoselective formation of only the trans diastereomer has been used as a selective functional probe of adult human FMO activity (33,36). Administration of a mixture of (S)-nicotine N-1'-oxide diastereomers by either free-smoking, intravenous, or dermal routes to humans showed that the N-1'-oxide was not appreciably N-1'-oxidized or reduced (33). If efficient reduction of metabolically formed (S)-nicotine N-1'-oxide to (S)-nicotine in humans occurred, it could explain the relatively low amount of N-1'-oxide formed in vivo. That (S)-nicotine N-1'-oxide reduction is not observed in vivo (33) suggests that metabolic futile cycling between nicotine N-1'-oxide and tertiary amine does not provide a reservoir for the pharmacological action of (S)-nicotine. Thus, previous reports in animals describing the reduction of (S)-nicotine N-1'-oxide to the tertiary amine is probably due to gut bacteria metabolism via the oral route of administration (37).

Cytochrome P450 Monooxygenase

The cytochrome P450 superfamily is a ubiquitous set of microsomal hemoproteins that are associated with the metabolism of xenobiotics and endogenous materials. In the human liver, approximately 20 cytochrome P450s have been characterized to some extent. In contrast, in animal liver at least 4 times that number of cytochrome P450s have been observed. Historically, the cytochrome P4502B subfamily that has
been most associated with experimental animal xenobiotic metabolism has been referred to as the phenobarbital-inducible cytochromes P450. Besides the fact that the human enzyme forms are less numerous than the animal forms, compounds often induce distinct hepatic cytochrome P450s in the animal and the human. For example, although a gene encoding human cytochrome 2B6 has been identified, a prominent role for this enzyme form in adult human liver microsomal metabolism is not likely. While an extremely large literature is available concerning the metabolic activation of chemicals by the cytochromes P450 to reactive metabolites, a number of examples of cytochrome P450-mediated detoxication of xenobiotics to less toxic materials is available. Generally, cytochromes P450 convert lipophilic compounds to oxidized metabolites that are commonly more polar and more readily excreted. Numerous examples in the literature have shown that this process can result in metabolically reactive intermediates that can covalently modify tissue macromolecules and cause cell death and ultimately tissue necrosis (Figure 1). However, cytochrome P450 can also directly catalyze the conversion of lipophilic compounds to polar, readily excreted metabolites. Many of the cytochrome P450 detoxication reactions are analogous to detoxication reactions catalyzed by the flavin-containing monooxygenases, although the microscopic enzymatic mechanism of course is completely different. For example, cytochromes P450 can catalyze the N-oxidation of tertiary amines to the tertiary amine N-oxide, the S-oxidation of sulfides (or other sulfur-containing compounds) to their corresponding sulfoxides, as well as the P-oxidation of phosphines (and other phosphorus-containing compounds) to their phosphorous oxides. Cytochromes P450 can oxidize the same substrates to the same products as the flavin-containing monooxygenase (ignoring the question of stereochemistry) and contribute to xenobiotic detoxication. Often, however, heteroatom oxidation, for example, is not the predominant reaction, and other less benign reaction products dominate for a particular substrate. For example, for a tertiary amine, one often observes extensive N-dealkylation in preference to N-oxidation, and this leads to formation of an aldehyde and an amine. It is not surprising that the flavin-containing monooxygenase and cytochromes P450 produce similar products from the same substrate, because in both cases the monooxygenases employed possess considerably potent enzyme-bound oxidizing agents.

**Flavin-containing Monooxygenase**

The action of FMO constitutes an important means of terminating the pharmacological and toxicological action of a wide number of xenobiotics including (S)-nicotine. As seen for numerous other tertiary amines, FMO-catalyzed N-oxidation converts a biologically active compound into a polar, readily excreted tertiary amine N-oxide. FMO also catalyzes the oxidation of organosulfur-, selenium-, phosphorous- and other heteroatom-containing chemicals that are likewise more efficiently excreted after oxidation. In contrast to cytochromes P450, FMO generally converts heteroatom-containing compounds to products with decreased potential for toxic or carcinogenic properties. Cytochromes P450 can oxidize nucleophilic heteroatoms to heteroatom oxides that in some cases are the same as the products from FMO, but cytochrome P450 does so considerably less efficiently than FMO. Another fundamental difference between cytochrome P450 and FMO is the apparent one- versus two-electron enzyme mechanism of oxidation of each monooxygenase, respectively. Consequently, cytochrome P450-mediated oxidation has been implicated in numerous co-oxidative and free radical processes, many of which are destructive to cellular macromolecules and tissue. The two-electron oxygenation mechanism of FMO does not preclude the enzyme from participating in metabolizing some chemicals to toxic metabolites (38). One notable example is the conversion of secondary amines to the corresponding aryl hydroxylamine. Another example comes from the N-oxidation of a tertiary amine to an unstable tertiary amine N-oxide that undergoes Cope-type elimination to produce a hydroxylamine and an olefin (38). It is possible that the hydroxylamine formed could possess untoward pharmacological properties. In principle, FMO-mediated aryl hydroxylamine formation and subsequent esterification or sulfation may contribute to the carcinogenic properties of arylamines (39). However, the contribution of this particular aspect of arylamine metabolism has not been fully addressed because many of the animal models used in carcinogenicity testing do not possess high levels of the hepatic form of FMO present in adult humans. For example, although it is recognized that there are five distinct FMO gene subfamilies encoding five forms of mammalian FMO, the most functionally prominent form of the enzyme in adult human liver (i.e., FMO3) apparently is not present to a great extent in adult rat liver, a commonly used animal for drug and chemical metabolism and toxicity studies (38). Less is known about the factors that control FMO regulation; hormone and dietary factors appear to regulate FMO expression, but this is species and tissue specific and probably occurs independently of other factors.

As discussed above, it is likely that the major form of FMO in adult human liver is distinct from that present in many experimental animals. Regardless, it has been observed that in species which are deficient in FMO, shunting of the metabolism of a toxin to the cytochrome P450 pathway occurs and makes the animal quite susceptible to the toxic properties of the chemical. On the other hand, animals with relatively high levels of FMO may efficiently detoxicate the chemical and are significantly less susceptible to the toxic properties of the toxin (40). It is believed that FMO has evolved to detoxicate nucleophilic heteroatom-containing chemicals or their metabolites present in plants that would otherwise inactivate or be metabolized by cytochrome P450 or covalently modify critical tissue macromolecules. Thus, determining a role for FMO in the detoxication of endogenous xenobiotics and drugs to which humans are exposed could constitute an important aspect of risk assessment.

**Carboxylesterases and Detoxication of Chemicals**

Carboxylesterases have long been studied with regard to their role in detoxication of drugs and environmental chemicals. To understand the role of carboxylesterases in the detoxication of chemicals in humans, a significant amount of work has been conducted using enzyme preparations from rats and other animals. The carboxylesterase enzymes used in the studies reported in the literature were obtained mainly from the liver of animals that contained the greatest activity of carboxylesterases. While hepatic enzymes from animals have provided a convenient source of a model enzyme, differences in substrate specificity have prompted researchers to use the human enzyme directly. To provide a direct supply of the enzyme from human sources, carboxylesterase (human esterase D) has been recently cloned and sequenced, and its
specific role in detoxication has begun to be examined. Notwithstanding the recent advances in the study of the recombinant human enzyme, the current understanding of the role of carboxylesterase in the detoxication of chemicals has largely been extrapolated from in vitro and in vivo studies in the rat. One caveat to correlations of this type is the fact that rats have higher endogenous levels of carboxylesterases than primates, and this difference could account for the lower sensitivity of rats to xenobiotics that are more toxic as the ester than as the hydrolysis product (41). Where possible, this review will examine the specific role of human esterase D (EsD) in the detoxication of xenobiotics. However, some information from rats (or other animals) will be presented because this information is relevant to the possible role of EsD in the detoxication of chemicals in humans.

Carboxylesterases catalyze the hydrolysis of ester, thioester, and sometimes amide bonds during the course of metabolism of xenobiotics and endogenous chemicals (Figure 6). Generally, formation of more polar readily excreted products by hydrolysis of lipophilic esters to carboxylic acids constitutes a detoxication process. While many animal and nonspecific human carboxylesterases have been reported in the literature, few highly substrate-selective human liver carboxylesterases have been described. In animals, carboxylesterases are widely distributed in different tissues. The large amount of carboxylesterase in various animal tissues in part compensates for the relatively low specific activity of the enzyme. Detoxication studies with xenobiotics have not yet been fully examined with the purified human EsD. Most of the EsD data for humans have been inferred from studies utilizing human serum or analogous carboxylesterases isolated from rats or other mammals.

Organophosphorus Compounds

One of the most studied detoxications of environmental relevance is the hydrolysis of organophosphorus insecticides. In the United States, almost 40 billion pounds of organophosphate insecticides are used every year. Thus, organophosphate insecticides comprise an important group of chemicals to which humans are exposed. Metabolism and detoxication of the malathion-type organophosphorus insecticides are among the most well studied. Generally in this class of compounds, there exists the phosphorodithionate or phosphonodithionate moiety, as well as a distal carboxylic acid ester. For such compounds, the distal carboxylic acid ester is the primary target of carboxylesterase action, which results in the hydrolysis and formation of the detoxication product containing the corresponding carboxylic acid (Figure 7) (42–44). Although malathion-type carboxyl ester hydrolysis and detoxication primarily occur in the liver and plasma, it has been shown that human carboxylesterase found in the skin also functions to detoxicate organophosphates (45). However, when malathion-type compounds are subject to metabolic activation to the corresponding oxon, the hydrolysis of the carboxylic acid does not occur—rather inhibition of the carboxylesterase by the phosphonodithiolate moiety is the preferred fate (Figure 8) (46). For the oxon metabolite, detoxication is the result of stoichiometric sequestering by the enzyme rather than a result of catalytic hydrolysis by carboxylesterase. A similar metabolic fate has been shown for simple phosphorothionates (e.g., chlorpyrifos, Figure 9) when biotransformed to the oxon in mouse or rat (47–50). It is notable that in in vivo studies in the rat, liver carboxylesterase is indeed inhibited faster than the target brain cholinesterase by paraoxon (51). This is also the case with organophosphates and organophosphites in which no metabolic activation is necessary. Such compounds have recently been examined with carboxylesterase derived from human monocytes (52); the conclusion was that organophosphates and organophosphites (with the exception of alkylphosphates) are potent inhibitors of human monocyte carboxylesterase.

Also of relevance for a role of carboxylesterase in detoxication is the molecular basis for the poisoning events with

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Hydrolysis of an ester, a thioester, or an amide to a carboxylic acid and an alcohol, a thiol, or an amine, respectively.

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Hydrolysis of malathion esters.

![Figure 8](https://example.com/figure8.png)

**Figure 8.** Oxidative desulfuration of an organothiophosphorous insecticide to the oxon.
organophosphorus nerve agents developed for chemical warfare. This is of particular importance because of the recent terrorist poisoning in Japan using sarin. It has been shown in rats that irreversible binding of soman to liver and plasma carboxylesterase accounts for a significant portion of its rapid detoxication of the soman P(-) isomers. In contrast, the P(+) isomers are largely detoxicated by A-esterases (Figure 10) (53). Thus, the proposed role of EsD in humans, on the basis of studies in the rat, is that detoxication of soman occurs when it is sequestered by EsD before it can reach target tissues containing acetylcholinesterases. For soman and other organophosphates, this represents an important part of the mechanism of carboxylesterase-mediated detoxication (54). Another example of stoichiometric sequestration of a nerve agent is the detoxication of tabun by carboxylesterase. Studies with rats showed that carboxylesterase serves as a protective mechanism against tabun by decreasing the amount available to the target site (i.e., intraneuronal acetylcholinesterase) (55).

**Detoxication of Chemicals of Environmental Concern**

Carboxylesterase has also been shown to have a role in the detoxication of various environmental chemicals. Some representative compounds are shown in Table 3. The mode of carboxylesterase-mediated detoxication of the compounds listed in Table 3 is hydrolytic cleavage of a carboxylic acid ester moiety to give the corresponding carboxylic acid and alcohol (Figure 11). Other metabolic processes can and do occur for the compounds listed in Table 3, but hydrolysis by carboxylesterase is generally looked upon as a means of producing more polar materials with considerably less toxic potential.

**Hydrolysis of Drugs by Carboxylesterases**

Carboxylesterases in the liver, gut, and other tissues have been shown to be important in the hydrolytic detoxication of certain drugs (67). Representative examples of drugs of this class that are substrates for carboxylesterases are listed in Table 4. In addition, carboxylesterases have been shown to be efficient hydrolytic catalysts for a number of physiologically important compounds including steroid and lipid esters. Recently, the characterization of a human liver carboxylesterase that hydrolyzes and transesterifies (-)-cocaine has been reported (73). The product of (-)-cocaine hydrolysis by this esterase is benzylecgonine, a metabolite that is largely inactive as a psychomotor stimulant (Figure 12). The $K_m$ value for formation of benzylecgonine (i.e., 116 μM) is much greater than the concentration of (-)-cocaine reported to be in the blood following a 100 mg intravenous dose of (-)-cocaine (i.e., 3 μM) and, presumably under such subsaturating conditions of substrate concentration, the human liver carboxylesterase would contribute to the detoxication of (-)-cocaine only if the $V_{max}$ values were sufficiently great. Interestingly, the same enzyme also possesses transesterification activity. Thus, in the presence of ethanol and (-)-cocaine, human liver carboxylesterase catalyzes the formation of cocaethylene. Although the $K_m$ value for ethanol is relatively high (i.e., 43 mM or approximately 180 mg/100 ml of blood), because the reported range of blood alcohol in individuals that have died from

---

**Table 3. Some environmental contaminant esters that are detoxicated by carboxylesterases.**

| Ester class               | Chemical example       | Carboxylesterase                          | References |
|--------------------------|------------------------|-------------------------------------------|------------|
| Herbicides               | Fluazifop-butyl        | Human liver, skin, plasma (56)            |            |
| (Carboxylic esters)      | Carbofuran             | Rat (58)                                  |            |
| Pyrethroids              | Permethrin, cypermethrin | Adult rat (59,60)                        |            |
| Acrylates                | Methyl, ethyl, butyl acrylate | Mouse nasal mucosa (61)           |            |
| Glycol monoalkyl ether acetates | Ethylene, propylene | Mouse nasal mucosa (61)                   |            |
| Trichothenes              | T2 toxin               | Rat liver (62–64)                         |            |
| Nicotinic acid esters    | *                      | Human plasma (65)                         |            |
| Nitrosomides and nitrosocarbanates | *                        | Rat liver (66)                           |            |
(-)-coclone overdose is reported to be in the range of 30 to 460 mg/100ml, it is possible that transesterification of (-)-coclone with ethanol constitutes a metabolic pathway contributing to increased toxicity of (-)-coclone (73). Thus, in addition to the well-recognized serum cholinesterase or butyrylcholinesterase that catalyzes the hydrolysis of (-)-coclone to ecgonine methyl ester, a human liver carboxylesterase has been discovered that hydrolyzes the methyl ester of (-)-coclone to a pharmacologically inactive metabolite (i.e., benzoylecgonine) and a pharmacologically active transesterification product, (-)-cocaethylene (Figure 12) (73). Possibly the balance between (-)-coclone ester hydrolysis and transesterification for any particular human subject may contribute to the overall susceptibility of the individual to the toxic properties of (-)-coclone. To date, the relative amount of esterase-catalyzed transesterification versus hydrolysis of xenobiotics has not been extensively examined. It is likely that the relative balance between these two competing metabolic pathways may represent another means of predicting the potential for toxicity of carboxyl ester-containing xenobiotics.

Cloning, Sequencing, and Tissue Localization of Human Esterase D

Human esterase D (EsD, carboxylesterase, aliesterase, EC 3.1.1.1) is one of several nonspecific esterases identified in human tissue that contribute to the hydrolytic detoxication or metabolism of xenobiotics, drugs, and enviromental chemicals. Like other human esterases (paraoxonase/arylesterase, steryl esterase, and carboxyl ester lipase), the physiological function of EsD is not well understood. EsD is, however, distinguishable from other esterases by its specificity for the hydrolysis of 4-methylumbelliferyl esters.

Table 4. Some drugs detoxicated by human carboxylesterase.

| Drug          | Carboxylesterase source                      | References |
|---------------|---------------------------------------------|------------|
| Aspirin       | Purified from human liver, intestinal mucosa | (68)       |
| Clofibrate    | Purified from human liver, intestinal mucosa | (69)       |
| Procaine      | Purified from human liver, intestinal mucosa | (69)       |
| Cocaine       | Purified from human liver                   | (69)       |
| Meperidine    | Human liver                                 | (70)       |
| Mebeverine    | Human plasma                                | (71)       |
| Lorazepam     | Human liver                                 | (72)       |

*Hydrolysis of the methyl ester of cocaine.
(74,75). The natural substrate for this enzyme has recently been identified as O-acetylated sialic acid, and one suggested endogenous role of EsD has been hypothesized to involve the reuse of sialic acids (76).

Purification of EsD has been accomplished from human erythrocytes (77–79), and the biochemical properties of the purified enzyme have been reported. EsD is an enzyme with a molecular weight of 34 kDa as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis; the $K_m$ of EsD for the substrate 4-methylumbelliferone was determined to be 10 μM. It is interesting to note that mercuric chloride and p-chloromercuri-benzoate both inhibited the purified enzyme completely at low concentrations (1.0 mM), which suggests that an SH group is necessary for the enzymatic reaction (77). In addition, purification of EsD allowed the preparation of polyclonal and monoclonal antibodies from rabbit and mouse, respectively, that have been useful for immunoquantification studies.

The EsD gene (from human erythrocytes and human liver) has now been cloned and sequenced (78.80–83) and has been localized to chromosome 13 band q14 (78,80–87). EsD has been shown to exhibit strong homology to two other esterases [i.e., acetylcholinesterase (AChE) from Torpedo californica and esterase-6 of Drosophila] (81). Homologous regions were detected about the consensus sequence serine-containing active site region. From studies of sequence homology, the active site is believed to contain the peptide isoleucine–phenylalanine–glycine–histidine–serine–methionine–glycine–glycine. The residues thought to participate in catalysis are serine, histidine, and aspartic acid (82). The human liver carboxylesterase gene has also been recently cloned and characterized (83,87,88); it showed significant sequence homology to various mammalian carboxylesterases. Furthermore, sequence analysis of human liver carboxylesterase and the genes for human cholinesterase and cholesterol esterase suggests an evolution from a common ancestral gene (88).

Although EsD was known to be present in a variety of tissues, quantification has only recently been accomplished by using the specific substrate 4-methylumbelliferone acetate (77). The results from this study showed that most EsD activity was found in the liver. Significant activity, although less than that of the liver, was also identified in the human kidney. Although little EsD enzyme activity was observed in the lung, recently a carboxylesterase from the lung (i.e., alveolar macrophage) has been purified and appears to be identical to EsD (89). The results suggest that EsD in the lung may play a role in the detoxication of inhaled xenobiotics. Carboxylesterase has also been identified in human blood monocytes (52,90). Another site that has been observed to contain carboxylesterase activity is the human skin (45), which suggests that EsD may play a role in the detoxication of absorbed xenobiotics.

Carboxylesterase activity has also been identified in human nasal tissue, suggesting a role of this tissue in the detoxication of inhaled xenobiotics as well (91). However, hydrolysis of certain inhaled esters has been correlated to lesions of the olfactory epithelium in rats due to transformation of inhaled chemicals into toxic substances rather than detoxicated metabolites (90,92). The data suggest that hydrolytic metabolism of inhaled esters in this tissue may play a significant role in the bioactivation of such substances rather than degradation and detoxication. It is possible that accumulation of some of the metabolites of inhaled esters contributes to the pathogenesis of ester-induced nasal lesions. In like fashion, it has been suggested that carboxylesterase-mediated hydrolysis of vinyl acetate generates acetaldehyde, an intracelular cross-linking agent possibly contributing to tumorigenesis (93).

The hydrolysis of lipophilic esters is a major route for the detoxication of environmental chemicals such as pesticides. Human exposure, either from occupational or passive administration, may occur via absorption by the skin or the respiratory tract. Therefore, carboxylesterase-mediated hydrolysis of xenobiotics in these tissues, as well as in the liver, may contribute to influencing the toxicity of potentially toxic esters.

**Paraoxonase/Arylesterase (A-esterase)**

It is worthy to briefly discuss the enzyme paraoxonase/arylesterase in light of the previous presentation of the metabolism of organophosphorus compounds. Pareasoxon/arylesterase has been shown to hydrolyze and therefore detoxicate many phosphate esters or phosphorothionates (i.e., organophosphorus insecticides) that have undergone metabolic activation to the oxon structure (Figure 8) before these compounds reach the target tissues containing acetylcholinesterases. Two paraoxon/arylesterases that possess the ability to hydrolyze paraoxon and a variety of organophosphorus compounds, as well as arylesters, have been identified in human serum (94–96). One was shown to be an ethylenediaminetetraacetic acid (EDTA)-sensitive esterase while the other was identified as an EDTA-insensitive paraoxonase. The highest levels of activity have been found in liver and serum. Like many other esterases, no known endogenous substrates for paraoxonase/arylesterase have been identified. It has been shown that calcium is required for paraoxonase/arylesterase, but the mechanism for the interaction with calcium is unknown at this time (97,98). Recently it has been shown that the enzyme is not a cysteine esterase as commonly thought for many years; the mechanism of mammalian paraoxonase/arylesterase must be reconsidered in light of this new finding (99).

**Conclusion**

Studies of the role of purified EsD in the metabolism and detoxication of various xenobiotics are scant compared with the information available employing other mammalian enzyme systems. It is clear, however, that EsD plays a significant role in catalyzing the detoxication of xenobiotics containing carboxylic esters. In addition, EsD also appears to play a role in the detoxication of potent anticholinesterase agents (i.e., organophosphates), not via catalysis but rather as stoichiometric scavengers (i.e., as a binding protein). With the recent acquisition of the purified human enzyme as well as the cDNA cloning and expression of the genes, a clearer understanding of the role of EsD in xenobiotic detoxication may be obtained in the near future. Furthermore, with careful in vitro studies of these purified enzymes, in vivo predictions may be made with an appreciation for in vitro–in vivo correlations.

**Glucuronidation of Xenobiotics as a Detoxication Pathway**

Formation of glucuronide conjugates of xenobiotics represents one of the most important Phase II reactions. Glucuronidation is a major detoxication pathway in all vertebrates examined, from fish (100) to human (101); glucuronide metabolites constitute some of the most significant xenobiotic-detoxicated metabolites in bile and urine. The key enzyme of the overall process known as glucuronidation is uridine diphosphate glucuronosyltransferase (UDPGT), a membrane-associated enzyme that catalyzes the transfer of the glucuronyl
group to a large number of endogenous compounds and xenobiotics (Figure 13).

The role of glucuronidation in the detoxification of environmental chemicals and carcinogens, whether ingested, inhaled, or absorbed, is related to the other detoxification pathways that have been described above: relatively lipophilic chemicals are converted to highly polar materials that are relatively efficiently excreted in the bile and urine. Glucuronosyl conjugates generally possess totally different physicochemical properties compared to those of the parent compounds. For example, the conjugates are very water soluble, are generally less toxic, and are rapidly excreted in the urine. After formation, a glucuronosyl conjugate may be immediately excreted or acted upon by hydrolyases, which leads to sequential first-pass effects on the glucuronosyl conjugate. The glucuronosyl conjugate can also reenter the liver cell and undergo enterohepatic recirculation. Glucuronides can be hydrolyzed in the gut (probably by bacteria that consume saccharides and leave the parent compound to be reabsorbed into the splanchnic circulation). Thus, pharmacokinetics and processing of glucuronide conjugates differ somewhat from those of other polar detoxication metabolites that we have discussed above. Because glucuronosyl conjugates are anions, entry into and out of hepatocytes is also dependent on the properties of the conjugate toward the organic anion transporter.

Metabolites of some environmental chemicals that are known carcinogens or toxins have been shown to be glucuronidated and detoxicated; however, there is some evidence that glucuronosyl conjugates of metabolites of toxic materials can be carriers of carcinogenic activity and exert an effect in cells quite distinct from initial glucuronidation. Thus, in cells that do not possess large polycyclic aromatic hydrocarbon hydroxylase activity, it has been observed that polycyclic aromatic hydrocarbons have significant toxicity. It is possible that carcinogens are transported from sites of high monooxygenase activity (i.e., the liver) to sites that possess low monooxygenase activity which are nevertheless selective targets (i.e., bladder) after hydrolysis of the glucuronide moiety (Figure 14). Included in the list of chemicals that could participate in this carrier phenomenon are metabolites of aromatic hydrocarbons [i.e., benzopyrene (102), benzene (103), and 2-hydroxybiphenyl (104)]; aromatic amines [2-acetylaminofluorene (105), heterocyclic amines (from meat and fish in typical household cooking practices) (106)]; and many other compounds (Table 5).

**Function of Glucuronosyltransferases in Detoxification**

The functions of UDPGTs in detoxication can be best understood in the context of overall xenobiotic metabolism. A large number of environmental chemicals, as well as endogenous lipophilic chemicals, are converted by Phase I enzymes of drug metabolism to a variety of nucleophilic and electrophilic metabolites (Figure 15) (107). It has been shown that chemically reactive, electrophilic metabolites can interact with critical cellular macromolecules which can initiate cell toxicity and play an important role in the multistage carcinogenic process (108). In many cases, electrophilic metabolites are enzymatically transformed by Phase II reactions. This is not to minimize the importance of other enzymatic and nonenzymatic metabolic processes in the bioactivation of xenobiotics to reactive intermediates. For example, in the polycyclic aromatic hydrocarbon series, phenols can be oxidized to reactive species including polyphenols, semiquinones, and quinones. Quinones may also undergo quinone–quinol redox cycles with the generation of reactive oxygen species that have also been implicated in cellular necrosis.

![Figure 13](image13.png)

**Figure 13.** Conjugation of a nucleophile (i.e., R-X-H) with uridine-5'-diphospho-α-D-glucuronic acid to form the glucuronide and uridine diphosphate.

![Figure 14](image14.png)

**Figure 14.** Hypothetical scheme depicting the metabolic bioactivation and glucuronidation of a procarcinogen, which is transported to another cell that possesses low bioactivation capacity and, nevertheless, accumulates sufficient metabolite to show significant toxicity.

**Table 5.** Environmental procarcinogens bioactivated in one cell and carried to another cell type by glucuronidation.

| Environmental chemical                          | References |
|------------------------------------------------|------------|
| Benzopyrene                                     | (102)      |
| Benzene                                        | (103)      |
| 2-Hydroxybiphenyl                                | (104)      |
| 2-Naphthylamine                                  | (104)      |
| 2-Acetylaminofluorene (2-AAF)                    | (105)      |
| 4-Aminobiphenyl                                  | (105)      |
| N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formylamide (FANFT) | (106) |
compounds may account, at least in part, for the positive correlation between cigarette smoking and the incidence of bladder cancer in humans (113). In 1941, 2-acetylaminofluorene (2-AAF), a proposed insecticide, was shown to be carcinogenic to the liver, mammary gland, and urinary bladder of rats after dietary administration (114). 2-AAF is one of the most extensively studied chemical carcinogens. The N-hydroxy metabolites of 2-AAF have been found to be more carcinogenic than the parent compound (108). It was also found that sulfation and acetylation/deacetylation reactions led to even more reactive intermediates that formed covalent adducts with DNA (107). Formation of the N-O-glucuronide of the corresponding N-hydroxy-2-AAF, N-hydroxy-2-naphthylamine, and N-hydroxy-4-aminobiphenyl have been reported. N-O-glucuronides are semistable transport forms of the aromatic amines; they are excreted via the blood into the urinary system and have the effect of detoxication (115,116). However, it is possible that the same transport form may also contribute to delivering the aromatic amine to an extrahepatic site and increasing the toxic potential of the amine at that site.

Another example of an environmental chemical, 2-hydroxybiphenyl, is widely used as an antimicrobial agent to protect edible crops, and it is possible that the human population may be exposed to it. However, it is likely that human exposure to 2-hydroxybiphenyl does not pose a significant health hazard because, at low doses, 2-hydroxybiphenyl is efficiently excreted as a glucuronide and as a sulfate ester; no significant toxicity has been observed (104,117).

**Mechanism of Glucuronosyltransferases**

Uridine-5'-diphospho-α-D-glucuronic acid (UDPGA, Figure 13) is the donating coenzyme during the glucuronidation reaction. The UDPGTs are a group of closely related membrane-bound enzymes that are responsible for the transfer of the glucuronosyl group from UDPGA to many endogenous and exogenous chemicals having nucleophilic functional groups (i.e., X in Figure 13). The mechanism of the glucuronidation catalyzed by UDPGTs is envisaged to take place via an S_N2 reaction—the nucleophilic group of the substrates attack the C1 of the pyranose acid ring of UDPGA—which results in the formation of the glucuronide (Figure 13). As shown in Figure 13, the attacking groups of the substrates should have sufficient nucleophilic character for a high rate of glucuronidation. There is a wide variety of groups that fulfill this requirement, e.g., phenols (phenol, morphine), carboxylic acids (bilirubin, valproic acid), alcohols (t-butanol, many steroids), and hydroxamic acids (N-hydroxy-2-acetylaminofluorone) to form O-glucuronides; thiophenols (thiophenol), and carbamic acids (diethylthiocarbamic acid) to form S-glucuronides; and aromatic amines (aniline), hydroxylamines (N-hydroxy-2-naphthylamine), and tertiary amines (cyproheptadine) to form N-glucuronides (Table 6). Even carbon atoms can attack the C1 atom of the glucuronic acid ring if the carbon atom is sufficiently nucleophilic (sulfinypyrazone, phenylbutazone) to form C-glucuronides (118) (Table 6). The reactivity toward forming a glucuronide will depend on the chemical structure, including both electronic and steric factors. A second requirement for a high rate of enzymatic glucuronidation is for sufficient lipid solubility of the substrate.

The resulting glucuronide is usually devoid of any significant biological and pharmacological activities. A family of homologous UDPGT isoenzymes is located in the endoplasmic reticulum of the liver, as well as in other tissues (119). Purification of the transferases catalyzing glucuronidation proved difficult because the enzyme was found to be anchored in the membrane of the endoplasmic reticulum (120); however, the difficulties involved in

**Table 6. Examples of drugs and chemicals that undergo glucuronidation.**

| Glucuronides | Functional group | Chemical examples |
|--------------|------------------|------------------|
| O-Glucuronides | Phenols | Phenol, morphine |
| | Alcohols | t-Butanol, various steroids |
| S-Glucuronides | Thiophenols | Thiophenol |
| | Thiols | 2-Mercaptobenzothiazole |
| N-Glucuronides | Aromatic amines | Aniline |
| | Hydroxylamines | N'-Hydroxy-2-naphthylamine |
| C-Glucuronides | 1,3-Dicarbonyl compounds | Phenylbutazone, sulfinypyrazone |

**Environmental Chemicals and Glucuronidation**

Aromatic amines have been widely used in the dye industry and were among the first chemicals to be recognized as human carcinogens. As early as 1895, a German physician, Ludwig Rehn (111), suggested that urinary bladder cancers found in dye workers were due to chemical exposure to certain aniline dyes. Aromatic amines such as 2-naphthylamine and 4-aminobiphenyl (Table 5) have been found in nanogram amounts in cigarettes (112). These

Figure 15. Hypothetical explanation of how an acyl glucuronide can participate in the covalent modification of cellular targets and eventually cause toxicity.

(109). In the arylamine class of compound, N-oxidized aromatic amines can be converted to electrophiles via oxidation to hydroxylamines and subsequent sulfation and/or acetylation (110). The disposition of Phase I metabolites by glucuronidation almost certainly contributes to the detoxification of metabolic products, but glucuronidation may also participate in the bioactivation of xenobiotics and transport of potentially toxic species to extrahepatic cells. Evaluation of the toxic potential of environmental chemicals must take both processes into consideration.

MOLECULAR DETOXICATION

Environmental Chemicals

[Diagram of Acyl Glucuronide]

- **Acyl glucuronide**
- **[Acyl migration]**
- **Reaction with nucleophiles or chemical rearrangements**
- **Covalent modification of macromolecules**
- **Untoward effects (e.g., allergenicity, fatal hepatotoxicity)**

**Figure 15.** Hypothetical explanation of how an acyl glucuronide can participate in the covalent modification of cellular targets and eventually cause toxicity.

| Glucuronides | Functional group | Chemical examples |
|--------------|------------------|------------------|
| O-Glucuronides | Phenols | Phenol, morphine |
| | Alcohols | t-Butanol, various steroids |
| S-Glucuronides | Thiophenols | Thiophenol |
| | Thiols | 2-Mercaptobenzothiazole |
| N-Glucuronides | Aromatic amines | Aniline |
| | Hydroxylamines | N'-Hydroxy-2-naphthylamine |
| C-Glucuronides | 1,3-Dicarbonyl compounds | Phenylbutazone, sulfinypyrazone |

**Environmental Health Perspectives • Vol 104, Supplement 1 • March 1996**
purifying homogenous preparations of a single UDPGT isoform have now largely been overcome by the use of recombinant DNA technology. The cloning of UDPGT cDNAs and their subsequent expression in eukaryotic or prokaryotic cells have proven to be useful tools for determining the structure and function of a large number of UDPGTs, especially the elusive human UDPGTs. Several UDPGT cDNA sequences (including bacteria, murine, virus, bovine, rat, and human) have been identified (120–124). The expression of UDPGT cDNA in cell culture can be used to identify the limits of substrate specificity of the individual isoenzymes and as a system for study of substrate transport, glucuronidation, and export of glucuronides in whole cells in vitro (91).

The study of UDPGTs in humans has also shown that several diseases are directly related to these enzymes. Crigler-Najjar syndrome is a familial form of severe unconjugated hyperbilirubinemia caused by a dysfunction in bilirubin glucuronidation in humans (125–127). Gilbert syndrome is a benign, mild, unconjugated hyperbilirubinemia that is found in approximately 5% of the population (128–130).

As described previously, although it seems that most xenobiotic glucuronides are detoxicated via the action of UDPGTs, glucuronidation may also lead to toxic effects in certain cases, especially at high dose levels. A well-studied example is cholerasis by glucuronides. In general, glucuronide excretion in bile causes cholerasis of approximately 15 to 20 μl bile μmol⁻¹ glucuronide (131). However, the phenolic drug morphol causes cholestasis at a high dose in the rat because harmol glucuronide is excreted to such a high extent in bile that the glucuronide precipitates and the bile channel becomes blocked (132). As discussed above, glucuronosyl conjugates can reenter the liver and hydrolyze back to the original substrate as shown in Figure 15 in an apparent metabolic cycling process. There is no doubt that this is also a factor in any glucuronide-mediated toxicity.

It is possible that glucuronosyl conjugates can become chemically reactive intermediates and participate in further metabolism, such as acyl glucuronide formation (Figure 15). For example, tolmetin, the nonsteroidal anti-inflammatory drug used for the treatment of rheumatoid arthritis may be converted to a reactive acyl glucuronide that contributes to adverse side effects observed in humans. In humans, tolmetin is primarily metabolized by oxidation on the methyl group of the phenyl ring and by conjugation of the carboxylic acid to produce the O-acyl glucuronide. The O-acyl glucuronide may hydrolyze, participate in acyl migration reactions, or covalently bind to human serum albumin. It is the irreversible binding of tolmetin glucuronides to human serum albumin that is thought to be responsible for the allergenic properties of tolmetin. It is possible that other nonsteroidal anti-inflammatory agents also possess this unfavorable property. In addition, it is likely that other carboxylic acids that participate in acyl glucuronidation may likewise produce metabolic intermediates that covalently modify important cellular proteins and produce untoward effects.

Regulation of Glucuronosyltransferases

Most investigations of the regulation of glucuronosyltransferases have been conducted in animals, even though some in vivo and in vitro studies have been done in humans (133,134). Study of the regulation of UDPGT levels related to different diets has been conducted in the rat. The results suggest that an iron-deficient diet can cause depression in UDPGT enzyme activity (135). Rats fed a diet rich in turmeric can significantly elevate UDPGT activity (136) but a vitamin E-deficient diet had no effect on the activities of UDPGT (137). Most experimental data about UDPGTs have been obtained from rats, chickens (138), rabbits (139), and monkeys (129) in vivo and in vitro, but the data and theory may be able to be applied to humans. The importance of Phase II reactions by UDPGTs in humans and other animals has also been investigated (139,140). However, in vivo studies of humans are limited, even though there are some research groups that have investigated the formation of glucuronides in humans through the analysis of urine and bile (141,142). Pharmacokinetic modeling of drug conjugates in vitro has been well established such that steady-state rate equations for the simulation of conjugation and deconjugation reactions have been developed (107). Simulations with mass-balance equations have proven useful in the understanding of conjugation and deconjugation processes in vivo (143). Through these efforts, a more quantitative interpretation of metabolic data on conjugation reactions has been obtained.

Concluding Remarks

An examination of the pharmacokinetic parameters that contribute to some of the major conceptual approaches to understanding toxicokinetics and the disposition of environmental chemicals has been presented. By the use of in vitro measurements and in vivo correlations, it is possible to predict with a good deal of certainty the extent of metabolic clearance and other important kinetic parameters. Determination of clearance invariably leads to a clearer understanding of the contribution, if any, to the toxicity of a drug or the residence time of a potentially toxic metabolite. The concept of interspecies scaling in the interpolation and extrapolation of fundamental biochemical metabolic processes has been presented. In the future, we anticipate that physiological modeling and allometric scaling will undoubtedly play an increasingly important role in predicting the toxicity of environmental chemicals in humans from studies in animals. It is likely that the use of scaling methodologies will also reduce the cost of evaluating the possible toxicity of a chemical to humans. A number of examples of important metabolic detoxification enzyme reactions have been listed to provide insight into the breadth of detoxication processes occurring in mammalian tissue; this list of metabolic processes is not exhaustive. We anticipate that the list of well-studied detoxication enzyme reactions will increase in the future. The data will provide a framework to use the pharmacokinetic information outlined here to give useful in vitro–in vivo correlations to help us understand the mechanism of action of important chemicals of environmental concern.

REFERENCES

1. Brodie BR, Reid WD, Cho AK, Sipes G, Gillette JR. Possible mechanism of liver necrosis caused by aromatic organic compounds. Proc Natl Acad Sci USA 68:160–164 (1971).

2. Welling PG, Tse FLS. Pharmacokinetics: Regulatory. Industrial, Academic Perspectives. 2d ed. New York:Marcel Dekker, 1991.
3. Benet LZ, Perotti BYT. Drug absorption, distribution, and elimination. In: Burger's Medicinal Chemistry and Drug Discovery, Vol I (Wolff ME, ed). New York:John Wiley & Sons, Inc; 1995:113-151.

4. Rowland M, Tozer TN. Clinical Pharmacokinetics: Concepts and Applications. Philadelphia:Lea & Febiger, 1989.

5. Benet LZ, Galeazzi RL. Noncompartmental determination of steady-state volume of distribution. J Pharm Sci 68:1071-1074 (1979).

6. Feng KS, Rowland M. Hepatic clearance of drugs. I. Theoretical considerations of a "well-stirred" model and a parallel tube model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on drug clearance. J Pharmacokin Biopharm 5:625-653 (1977).

7. Mordenti J, Man versus beast: pharmacokinetic scaling in mammals. J Pharm Sci 75:1028-1040 (1986).

8. Boxenbaum H, Ronfeld R. Interspecies pharmacokinetic scaling and the Dredick plots. Am J Physiol 245;R768-R775 (1983).

9. Sacher GA. Relationship of lifespan to brain weight and body weight in mammals. Ciba Found Colloq Aging 5:115-133 (1989).

10. Boxenbaum H, Fertig JB. Scaling of antipyrine intrinsic clearance of unbound drug in 15 mammalian species. Eur J Drug Metab Pharmacokin 9:177-183 (1984).

11. Gascon AR, Calvo B, Hernandez RM, Dominguez-Gil A, Pedraz J. Interspecies scaling of cimetidine-theophylline pharmacokinetic interaction: interspecies scaling in pharmacokinetic interactions. Pharm Res 11:945-950 (1994).

12. Kim SR, Chow HH, Mayersohn M. Comparative pharmacokinetics and interspecies scaling of cocaine in several mammalian species. Pharm Res 11:S-421 (1994).

13. Owens SM, Hardwick W, Blackall D, McMillan DE. Interspecies scaling of phenylcyclidine (PCP) pharmacokinetic parameters. In: 71st Annual Meeting of the Federation of American Societies for Experimental Biology, 29 March 1987, Washington, DC. Fed Proc 46:867 (1987).

14. Boxenbaum H. Interspecies scaling, allometry, physiologic time, and the ground plan of pharmacokinetics. J Pharmacokin Biopharm 10:201-227 (1982).

15. Mordenti J, Chappell W. The use of interspecies scaling in toxicokinetics In: Toxicokinetics and New Drug Development (Yacobi A, Kelly JP, Batra VK, eds). Elmsford, UK:Pergamon Press, 1989;42-96.

16. Boxenbaum H, D’Souza R. Physiological models, allometry, neoteny, space-time and pharmacokinetics. In: Pharmacokinetics: Mathematical and Statistical Approaches (Pellec A, Resigino A, eds). New York:Plenum Press, 1988;191-214.

17. Dedrick RL, Bischoff KB, Zaharko DZ. Interspecies correlation of plasma concentration history of methotrexate (NSC-740). Cancer Chemother. Rep Part 1 54:95-101 (1970).

18. Gatti G, Kahn JO, Lifson J, Williams R, Turin L, Volberding PA. Gambertoglio GJ. Pharmacokinetics of GLQ223 in rats, monkeys, and patients with AIDS or AIDS-related complex. Antimicrob Agents Chemother. 35:2531-2537 (1991).

19. Ibrahim SS, Boudinot FD. Pharmacokinetics of 2,3’-dideoxy- cytidine in rats: application to interspecies scale-up. J Pharm Pharmacol 41:829-834 (1989).

20. Lave T, Schmitt-Hoffman AH, Coassolo P, Valles B, Ubeaud G, Ba B, Brandt R, Chou RC. A new extrapolation method from animals to man: application to a metabolized compound, mafurotene. Life Sci 56:473-478 (1995).

21. Baggot JD. Application of interspecies scaling to the bispyridinium oxime HI-6. Am J Vet Res 55:689-691 (1994).

22. Gombar CT, Harrington GW, Pylipiw HM Jr, Anderson LM, Palmer AE, Reid JM, Magge PN, Burak ES. Interspecies scaling of the pharmacokinetics of N-nitrosodimethylamine. Cancer Res 50:4366-4370 (1990).

23. Jeezqel SG. Fluconazole: interspecies scaling and allometric relationships of pharmacokinetic properties. J Pharm Pharmacol 46:196-199 (1994).

24. Mordenti J, Chen SA, Moor JA, Ferraiolo BL, Green JD. Interspecies scaling of clearance and volume of distribution data for five therapeutic proteins. Pharm Res 8:1351-1359 (1991).

25. Kaul S, Barbhaiya R. Interspecies scaling of stavudine pharmacokinetics. Pharm Res 11:S-348 (1994).

26. Campbell DB. Can allometric interspecies scaling be used to predict human kinetics? Drug Inf J 28:235-245 (1994).

27. Bernareggi A, Rowland M. Physiologic modeling of cyclosporin kinetics in rat and man. J Pharmacokin Biopharm 19:21-50 (1991).

28. Andersen ME, Clewell HJ, Gargas ML, Smith FA, Reitz RH. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 87:185-205 (1987).

29. Gabrielsson JL, Johansson P, Bondesson U, Pazzlow LK. Analysis of methadone disposition in the pregnant rat by means of a physiological flow model. J Pharmacokin Biopharm 13:355-372 (1985).

30. Davila DG, Williams DE. The etiology of lung cancer. Mayo Clin Proc 68:170-182 (1993).

31. Smoking-attributable mortality and years of potential life lost: United States, 1988. Morb Mort Wkly Rep 40:62-71 (1991).

32. U.S. Environmental Protection Agency. Smoking and tobacco control. In: Monograph on the Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders, NIH Publ 93-3605. Bethesda, MD:National Cancer Institute, 1993;21-31.

33. Park SB, Jacob P III, Benowitz NL, Cashman JR. Stereoselective metabolism of (S)-(−)-nicotine in humans: formation of trans-(S)-(-)-nicotine N’-oxide. Chem Res Toxicol 6:880-888 (1993).

34. Berkman CE, Park SB, Wrighton SA, Cashman JR. In vitro–in vivo correlations of human (S)-nicotine metabolism. Biochem Pharmacol 50:565-570 (1995).

35. Benowitz NL, Jacob P III. Nicotine metabolism, pharmacokinetics and pharmacodynamics in man. In: Tobacco Smoking and (S)-Nicotine: A Neurobiological Approach (Martin WR, Van Loon GR, Iwamoto ET, Davis L, eds). New York:Plenum Press, 1987;357-373.

36. Cashman JR, Park SB, Yang Z-C, Wrighton SA, Jacob P III, Benowitz NL. Metabolism of nicotine by human liver microsomes: stereoselective formation of trans-nicotine N’-oxide. Chem Res Toxicol 5:639-646 (1992).

37. Sepkovic DW, Haley NK, Axelrad CM, Shigematsu A, LaVoie EJ. Short-term studies on the in vivo metabolism of N-oxides of nicotine in rats. J Toxicol Environ Health 18:205-214 (1986).

38. Cashman JR. Structural and catalytic properties of the mammalian flavin-containing monooxygenase. Chem Res Toxicol 14:145-181 (1991).

39. Ziegler DM, Ansher SS, Nagata T, Kadlubar FF, Jakoby WB. N-Methylation: potential mechanism for metabolic activation of carcinogenic primary alylamines. Proc Natl Acad Sci USA 85:2514-2517 (1988).

40. Mirand CL, Chung W, Reed RE, Zhao X, Henderson MC, Wang J-L, Williams DE, Buhler DR. Flavin-containing monooxygenase: a major detoxifying enzyme for the pyrrolizidine alkaloid senecionine in guinea pig tissues. Biochem Biophys Res Commun 178:546-552 (1991).

41. Chambers JP, Hartgraves SL, Murphy MR, Wayner MJ, Kuman N, Valdes JJ. Effects of three reputed carboxyesterase inhibitors upon rat serum esterase activity. Neurosci Biobehav Rev 15:85-88 (1991).

42. Imamura T, Schiller NL, Fukuto TR. Malathion and phenotho carboxyesterase activities in pulmonary alveolar macrophages as indicators of lung injury. Toxicol Appl Pharmacol 70:140-147 (1983).

43. Makhavea GF, Veselova VL, Mastrunikova TA, Shipov AE, Zhdanova GV, Ivunova FA. Inhibition of various dithio- and thiophosphates containing amino acid fragments with carboxyesterase from rat liver. Bioorg Khim 9:920-925 (1983).

44. Kawabata S, Hayasaka M, Hayashi H, Sakata M, Hatakeyama Y, Ogu N. Phenotho metabolites in human poisoning. J Toxicol Clin Toxicol 32:49-60 (1994).
38

Environmental Health Perspectives • Vol 104, Supplement 1 • March 1996

45. Heyman E, Hoppe W, Krussellman A, Tschoetschel C. Organophosphate sensitive and insensitive carboxylesterases in human skin. Chem Biol Interact 87:217–226 (1993).

46. Makhavea GF, Ivanovskaia VL, Odeova GA, Shetskova NN, Khovanskii AE. The role of esterases in the toxicity of organothiophosphorus insectoacaricides containing a fragment of mercaptoacetic acid. Bioger Khim 11:957–962 (1985).

47. Sultatos LG, Shao M, Murphy SD. The role of hepatic biotransformation in mediating the acute toxicity of the phosphorothionate insecticide chlorpyrifos. Toxicol Appl Pharmacol 73:68–76 (1985).

48. Ehrich M, Cohen SD. DVPD (dichlorvos) detoxification by binding and interactions with DDT, dieldrin, and malafoxon. J Toxicol Environ Health 3:491–500 (1977).

49. Chambers JE, Tangeng JJ, Boone S, Chambers HW. Role of detoxication pathways in acute toxicity levels of phosphorothionate insecticides in the rat. Life Sci 54:1357–1364 (1994).

50. Chambers H, Brown B, Chambers JE. Non-catalytic detoxication of six organophosphorus by rat liver homogenates. Pest Biochem Physiol 36:308–315 (1990).

51. Chambers JE, Chambers HW. Time course of inhibition of acetylcholinesterase and alyesterase following parathion and paraaxon exposure in rats. Toxicol Appl Pharmacol 103:420–429 (1990).

52. Cohen AM, Pang YM, Newcombe DS. Structural requirements for the inhibition of human monocyte carboxylesterase by organophosphorus compounds. Chem Biol Interact 80:327–338 (1991).

53. Wähländer A, Sznitciz L. Detoxification of soman in the perfused rat liver: quantitative uptake and stereospecific metabolism. Arch Toxicol 64:586–589 (1990).

54. Maxwell DM, Brecht KM, O’Neill BL. The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. Toxicol Lett 39:35–42 (1987).

55. Gupta RC, Patterson GT, Dettborn WD. Acute tabun toxicity: biochemical and histochemical consequences in brain and skeletal muscles of rat. Toxicology 46:329–341 (1987).

56. Clark NW, Scott RC, Blain PG, Williams FM. Fate of fluorozip butyl in rat and human skin in vitro. Arch Toxicol, 67:44–48 (1993).

57. McCracken NW, Blain PG, Williams FM. Human xenobiotic metabolizing esterases in liver and blood. Biochem Pharmacol 46:1125–1129 (1993).

58. Gupta RC, Kadel WL. Concerted role of carboxylesterase in the potentiation of carbofuran toxicity by iso-OMPA pretreatment. J Toxicol Environ Health 26:447–457 (1989).

59. Cantalamessa F. Acute toxicity of two pyrethrroids, permethrin and cypermethrin in neonatal and adult rats. Arch Toxicol 67:510–3 (1993).

60. Casida JE, Dufour B, Gauthier GC, Lau JT, Soderlund DM. Structure-biodegradability relationships in pyrethroid insecticides. Arch Environ Contam Toxicol 3:491–500 (1975).

61. Stott WT, Mckenna MJ. Hydrolysis of several glycol ether acetates and acrylate esters by nasal mucosal carboxylesterase in vitro. Fundam Appl Toxicol 5:399–404 (1985).

62. Johnsen H, Odden E, Lie O, Johnsen BA, Fonnum F. Metabolism of T-2 toxin by rat liver carboxylesterase. Biochem Pharmacol 35:1469–1473 (1986).

63. Fonnum F, Sterri SH, Aas P, Johnsen H. Carboxylesterases, importance for detoxification of organophosphorus anti-cholinesterases and thriclothenes. Fundam Appl Toxicol 5:329–38 (1985).

64. Wei RD, Chi FS. Modification of in vitro metabolism of T-2 toxin by esterase inhibitors. Appl Environ Microbiol 50:115–119 (1985).

65. Durrer A, Walther B, Racciatti A, Boss G, Testa B. Structure-detoxification in the relationship of hydrolysis of nicotinate esters by rat liver and brain subcellular fractions. Pharm Res 8:382–385 (1991).

66. Aukerman SL, Brundrett RB, Hartman PE. Detoxication of nitrosamides and nitrosocarboxamides in blood plasma and tissue homogenates. Environ Mutagen 6:835–849 (1984).

67. Williams FM. Clinical significance of esterases in man. Clin Pharmacokinet 10:392–403 (1985).

68. Inoue M, Morikawa M, Tsuboi M, Ito Y, Sugiura M. Comparative study of human intestinal and hepatic esterases as related to enzymatic properties and hydrolyzing activity for some pesticides and other organophosphorus compounds. Jpn J Pharmacol 73:68–76 (1995).

69. Brzenzinski MR, Abraham TL, Stone CL, Dean RA, Bosron WF. Purification and characterization of a human liver cocaine carboxylesterase that catalyzes the production of benzoyleglycine and the formation of cocaethylene from alcohol and cocaine. Biochem Pharmacol 48:1747–1755 (1994).

70. Lustig WE, Castle JR. Species differences in the hydrolysis of meperidine and its inhibition by organophosphate compounds. Fundam Appl Toxicol 11:323–332 (1988).

71. Dickinson RG, Baker PV, Franklin ME, Hooper WD. Facile hydrolysis of meverenue in vitro and in vivo: negligible circulating concentrations of the drug after oral administration. J Pharm Sci 80:952–957 (1991).

72. Liu K, Guengerich FP, Yang SK. Enantioselective hydrolysis of lorazepam 3-acetate by esterases in human and rat liver microsomes and rat brain S9 fraction. Drug Metab Dispos 19:609–613 (1991).

73. Bosron WF, Dean RA, Brzenzinski MR, Pindel EV. Human liver cocaine carboxylesterases. Biochem Pharmacol (in press).

74. Welch S, Lee J. The population distribution of genetic variants of human esterase D. Humangenetik 24:329–331 (1974).

75. Coates PM, Mestriner MA, Hopkinson DA. A preliminary genetic interpretation of the esterase isozymes of human tissue. Ann Hum Genet 39:1–20 (1975).

76. Varki A, Muchmore E, Diaz S. A acidic acid-specific O-acetyl-esterase in human erythrocytes: possible identity with esterase D, the genetic marker of retinoblastomas and Wilson disease. Proc Natl Acad Sci USA 83:882–886 (1986).

77. Lee W, Wheatley W, Benedict WT, Huang C, Lee EY-HP. Purification, biochemical characterization, and biological function of human esterase D. Proc Natl Acad Sci USA 83:6790–6794 (1986).

78. Squire J, Dryja TP, Dunn J, Goddard A, Hofmann T, Musarella M, Willard HF, Becker AJ, Gallie BL, Phillips RA. Cloning of the esterase D gene: a polymorphic gene probe closely linked to the retinoblastoma locus on chromosome 13. Proc Natl Acad Sci USA 83:6573–6577 (1986).

79. Okada Y, Wakabayashi K. Purification and characterization of esterases D-1 and D-2 from human erythrocytes. Biochem Biophys 263:130–136 (1988).

80. Lee EY-HP, Lee W. Molecular cloning of the human esterase D gene, a genetic marker of retinoblasoma. Proc Natl Acad Sci USA 83:6337–6341 (1986).

81. Sparks RS, Sparks ME, Wilson MG, Towner JW, Benedict W, Murphy AL, Young J. Regional assignment of genes for human esterase D and retinoblastoma to chromosome band 13q14. Science 208:1042–1044 (1980).

82. Ward P, Packman S, Loughman W, Sparks M, Sparks R, McMahon A, Gregory T, Ablin A. Location of the retinoblastoma susceptibility gene(s) and the human esterase D locus. J Med Genet 21:92–95 (1984).

83. Yunis JJ, Ramsey N. Retinoblastoma and subband deletion of chromosome 13. Am J Dis Child 132:161–163 (1978).

84. Duncan AMV, Morgan C, Gallie BL, Phillips RA, Squire J. Re-evaluation of the localization of esterase D and its relation to the retinoblastoma locus by in situ hybridization. Cytogenet Cell Genet 44:153–157 (1987).

85. Young LS, Lee EY-HP, To H, Bookstein R, Shew J, Donoso LA, Sery T, Giblin M, Shields JA, Lee W. Human esterase D gene: complete cDNA sequence, genomic structure, and application in the genetic diagnosis of human retinoblastoma. Hum Genet 79:137–141 (1988).

86. Long RM, Calabrese MR, Martin BM, Pohl LR. Cloning and sequencing of a human liver carboxylesterase isoenzyme. Life Sci 48:PLA-43–49 (1991).

87. Riddle PW, Richards L, Bowles MR, Pond SM. Cloning and analysis of a cDNA encoding a human liver carboxylesterase. Gene 108:289–292 (1991).
Molecular Detoxication

88. Shibata F, Takagi Y, Kitajima M, Kuroda T, Omura T. Molecular cloning and characterization of a human carboxylesterase gene. Genomics 17:76–82 (1993).

89. Mungur JS, Shi G, Mark EA, Chin DT, Gerard C, Chapman HA. A serum released by human alveolar macrophages is closely related to liver microsomal carboxylesterases. J Biol Chem 266:18382–18388 (1991).

90. Lewis LJ, Nikula KJ, Novak R, Dahl AR. Comparative localization of carboxylesterase in F344 rat, beagle dog, and human nasal tissue. Anat Rec 239:55–64 (1994).

91. Mattes PM, Mattes WB. Alpha-naphthyl butyrate carboxylesterase activity in human and rat nasal tissue. Toxicol Appl Pharmacol 114:71–76 (1992).

92. Bogdanffy MS, Kee CR, Hinchman CA, Trela BA. Metabolism of dibasic esters by rat nasal mucosal carboxylesterase. Drug Metab Dispos 19:124–129 (1991).

93. Kuykendall JR, Taylor ML, Bogdanffy MS. Cytotoxicity and DNA-protein crosslink formation in rat nasal tissues exposed to vinyl acetate are carboxylesterase-mediated. Toxicol Appl Pharmacol 123:283–292 (1993).

94. Gan KN, Smolen A, Eckerson HW, La-Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos 19:100–109 (1991).

95. Smolen A, Eckerson HW, Gan KN, Hailat N, La-Du BN. Characteristics of the genetically determined allozymic forms of human serum paraoxonase/arylesterase. Drug Metab Dispos 19:107–112 (1991).

96. Reiner E, Pawkovic E, Radic Z, Simeon V. Differentiation of esterases reacting with organophosphorus compounds. Chem Biol Interact 87:77–83 (1993).

97. Vitariu JA, Sultatos LG. The role of calcium in the hydrolysis of the organophosphate paraoxon by human serum A-esterase. Life Sci 56:125–134 (1995).

98. Fernando G, Gonzalez MC, Hernandez AF, Villanueva E, Pla A. Differences in the kinetic properties, effect of calcium and sensitivity to inhibitors of paraoxon hydrolytic activity in rat plasma and microsomal fraction from rat liver. Biochem Pharmacol 48:1559–1568 (1994).

99. Sorrenson RC, Primo-Parmo SL, Kuo C-L, Adkins S, Lockridge O, La-Du B. Reconsideration of the catalytic center and mechanism of mammalian paraoxonase/arylesterase. Proc Natl Acad Sci USA 92:7187–7191 (1995).

100. Clarke DJ, George SG, Burchell B. Glucuronidation in fish. Aquat Toxicol 20:35–56 (1991).

101. Dutton GJ. Glucuronidation of Drugs and Other Compounds. Boca Raton, FL:CRC Press, 1980.

102. Wall KL, Gao W, Koppelje JM, Kwei GY, Kauffman FC, Thomas RG. The liver plays a crucial role in the mechanism of chemical carcinogenesis due to polyacrylic acid hydrocarbons. Carcinogenesis 12:783–786 (1991).

103. Sammett D, Lee EW, Kocis JJ, Snyder R. Partial hepatectomy reduces both metabolism and toxicity of benzo[a]pyrene. J Toxicol Environ Health 5:785–792 (1979).

104. Reitz RH, Fox TR, Quast JF, Hermann EA, Watanabe PG. Molecular mechanism involved in the toxicity of orthophenylphenol and its sodium salt. Chem Biol Interact 43:99–119 (1983).

105. Cardona RA, King CM. Activation of the O-glucuronide of the carcinogen N-hydroxy-N-fluoroacetylcarbonyl by enzymatic deacetylation in vitro: formation of fluorenylamine-tRNA adducts. Biochem Pharmacol 25:1051–1056 (1976).

106. Irving CC. Influence of the argyl group on the reaction of glucuronides of N-arylacetylenedicarboxylic acids with polynucleotides. Cancer Res 37:524–528 (1977).

107. Clarke DJ, Burchell B. The uridine diphosphate glucuronosyltransferase multigene family: function and regulation. In: Handbook of Experimental Pharmacology: Conjugation and Deconjugation Reactions in Drug Metabolism and Toxicity, Vol 112 (Kauffman FC, ed). New York:Springer-Verlag, 1994:3–44.

108. Miller EC, Miller JA. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. Cancer 47:2327–2345 (1981).

109. Lorentzen RJ, TsopO. Benz(a)pyrene/benz(a)pyrenediol oxidation-reduction couples and the generation of reactive reduced molecular oxygen. Biochemistry 16:1467–1473 (1977).

110. Miller A, Miller EC. Electrophilic sulfuric acid ester metabolites as ultimate carcinogens. Adv Exp Med Biol 197:583–595 (1986).

111. Bock KW, UDP-glucuronosyltransferases and their role in metabolism and disposition of carcinogens. Adv Pharmacol 27:367–383 (1994).

112. Patrianakos C, Hoffmann D. Chemical studies on tabacco smoke, LXIV. On the analysis of aromatic amines in cigarette smoke. J Anal Toxicol 3:150–154 (1979).

113. Mommens S, Aagard J. Tobacco as a risk factor in bladder cancer. Carcinogenesis 6:335–338 (1985).

114. Wilson RH, DeEds F, Cox AJ Jr. The toxicity and carcinogenic activity of 2-aceaminofluorene. Cancer Res 1:595–608 (1941).

115. Kadubaf FF, Miller JA, Miller EC. Hepatic microsomal N-glucuronidation and nucleic acid binding of N-hydroxy arylamines in relation to urinary bladder carcinogenesis. Cancer Res 37:805–814 (1977).

116. Poupko JM, Hearl WL, Radomski J. N-Glucuronidation of N-hydroxy aromatic amines: a mechanism for their transport and bladder-specific carcinogenicity. Toxicol Appl Pharmacol 50:479–484 (1979).

117. Mira1s JC, Hamilton CM, Schindler JE, Green CE, Dabbs JE. Effect of soy bean flaxes and liquorice root extract on enzyme induction and toxicity in BCG3F1 mice. Food Chem Toxicol 31:343–350 (1993).

118. Mulder GJ, Coughtrie MWH, Burchell B, eds. Conjugation Reactions in Drug Metabolism: An Integrated Approach. Philadelphia:Taylor & Francis, 1990.

119. Harding D, Fournel-Gigleux S, Jackson MR, Burchell B. Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells. Proc Natl Acad Sci USA 85:8381–8385 (1988).

120. Visser TJ, Kaptein E, van Raaij, Glucuronidation of thyroid hormone with preference for 3',5'-triiodothyronine (reverse T3). FEBS Letters 315:65–68 (1993).

121. Jackson MR, McCarthy LR, Harding D, Wilson S, Coughtrie MWH, Burchell B. Cloning of a human liver microsomal UDP-glucuronosyltransferase cDNA. Biochem J 242:581–588 (1987).

122. Iyamagi T, Haniv M, Sagawa K, Fuji1i-Kuriyama Y, Watanabe S, Shively JE, Anan FK. Con1ng and characterization of cDNA encoding 3-methylcho1anthrene-inducible rat mRNA for UDP-glucuronosyltransferase. J Biol Chem 261:15607–15614 (1986).

123. Ralston EJ, English JJ, Dooner HK. Sequence of three bronze alleles of maize and correlation with the genetic fine structure. Genetics 119:185–197 (1988).

124. Hundle BS, O'Brien DA, Alberti M, Beyer P, Hardst JE. Functional expression of zeaxanthin glucosyltransferase from Eratsia betulola and a proposed uridine diphosphate binding site. Proc Natl Acad Sci USA 89:9321–9325 (1992).

125. Crigler JF, Najjar VA. Congenital familial non-haemolytic jaundice with kernicterus. Pediatrics 10:169–180 (1952).

126. Roy Chowdhury J, Wolkoff AW, Arias IM. Hereditary jaundice and disorders of bilirubin metabolism. In: The Metabolic Basis of Inherited Disease (Scrivner CR, ed). 6th ed. New York:McGraw-Hill, 1989:1085–1094.

127. Branchereau S, Ferry N, Myara A, Saato H, Kowai O, Trivin F, Houssin D, Danos O, Heard J. Correction of bilirubin glucuronolactontransferase in Gunn rats by gene transfer in the liver using retroviral vectors. Chirurgie 119:642–648 (1993).

128. Gilbert A, Lerouillet P. La choline simple familiale. Sem Med 21:241–245 (1901).

129. Cornelius CE. Fasting hyperbilirubinemia in Bolivian squirrel monkeys with a Gilbert's-like syndrome. Adv Vet Sci Comp Med 37:127–147 (1993).
130. Fogelfeld L, Sarova-Pinchas I, Meytes D, Barash V, Brok-Simoni F, Feigl D. Phosphofructokinase deficiency (Tarui disease) associated with hepatic glucuronyltransferase deficiency (Gilbert's syndrome): a case and family study. Israel J Med Sci 26:328–333 (1990).

131. Pang KS, Mulder GJ. A commentary: effect of flow on formation of metabolites. Drug Metab Dispos 18:270–275 (1990).

132. Krijgsheld KR, Koster HJ, Scholtens E, Mulder GJ. Cholestatic effect of harmol glucuronide in the rat. Prevention of harmol-induced cholestasis by increased formation of harmol sulfate. J Pharmacol Exp Ther 221:731–734 (1982).

133. Pandey A, Hassen AM, Benedict DR, Fitzpatrick DW. Effect of UDP-glucuronyltransferase induction on zearalenone metabolism. Toxicol Let 51:302–211 (1990).

134. Viani A, Temellini A, TUsini G, Pacifici GM. Human brain sulphotransferase and glucuronyltransferase. Hum Exp Toxicol 9:65–69 (1990).

135. Rao NJ, Jagadeesan V. Effect of long term iron deficiency on the activities of hepatic and extra-hepatic drug metabolising enzymes in Fischer rats. Comp Biochem Physiol Biochem Mol Biol 110:167–173 (1995).

136. Goud VK, Polasa K, Krishnaswamy K. Effect of turmeric on xenobiotic metabolising enzymes. Plant Foods Hum Nutr 44:87–92 (1993).

137. Iwasaki M, Iwama M, Miyata N, Iitoi Y, Kanke Y. Effects of vitamin E deficiency on hepatic microsomal cytochrome P450 and phase II enzymes in male and female rats. Int J Vitam Nutr Res 64:109–112 (1994).

138. Oka S, Terayama K, Kawaahima C, Kawasaki T. A novel glucuronyltransferase in nervous system presumably associated with the biosynthesis of HNK-1 carbohydrate epitope on glycoproteins. J Biol Chem 267:22711–22714 (1992).

139. Dulik DM, Fenselau C. Species-dependent glucuronidation of drugs by immobilized rabbit, rhesus monkey, and human UDP-glucuronitransferases. Drug Metab Dispos 15:473–477 (1987).

140. Creton EM, Sommadossi JP. Modulation of 3'-azido-3'-deoxythymidine catabolism by probenecid and acetaminophen in freshly isolated rat hepatocytes. Biochem Pharmacol 42:1475–1480 (1991).

141. Chaudhuri NK, Servando OA, Manniello MJ, Luders RC, Chao DK, Bartlett MF. Metabolism of triptolennamine in man. Drug Metab Dispos 4:372–387 (1976).

142. Fisher LJ, Thies RL, Charkowski D, Donham KJ. Formation and urinary excretion of cyproheptadine glucuronide in monkeys, chimpanzees, and humans. Drug Metab Dispos 8:422–424 (1980).

143. Brockmoller J, Roots I. Assessment of liver metabolic function. Clinical implications. Clin Pharmacokinet 27:216–248 (1994).