Pharmacokinetics and Molecular Detoxication

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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 toxicants. — 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

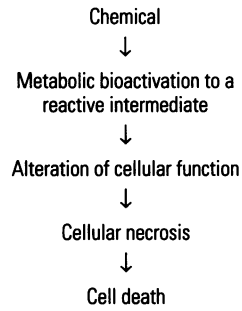

Figure 1. Hypothetical illustration of the metabolic activation of a chemical to form a toxic metabolite and the pathological consequences.

Manuscript received 15 October 1995; manuscript accepted 20 November 1995.

The authors thank the National Institutes of Health for the financial support (grants GM 36426, ES 06973, and DA 08531 to JRC).

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Abbreviations used: FMO, flavin-containing monooxygenase; CL, clearance; F, bioavailability; V, volume of distribution; Q, organ blood flow; C, concentration of chemical; AUC, area under the concentration time curve; f_u, fraction of chemical unbound; V_{max}, maximum rate of metabolism; K_m, Michaelis-Menten constant; AUMC, area under the moment curve; B, body weight; BW, brain weight; EsD, esterase D; UDPGT, uridine diphosphate glucuronosyltransferase; 2-AAF, 2-acetylaminofluorene; UDPGA, uridine-5′-diphospho-α-D-glucuronic acid.

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 phamacokinetic/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

$$C_{ss} = F \times \text{dose} / (\text{t} \times \text{CL}) \quad [1]$$

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 ($F \times$ dose) divided by the area under the systemic chemical concentration-time profile (AUC):

$$\text{CL} = F \times \text{dose} / \text{AUC}. \quad [2]$$

Substituting Equation 2 into Equation 1, one will obtain

$$C_{ss} = \text{AUC}/\text{t}. \quad [3]$$

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:

$$F = \text{AUC}_{\text{non iv}} / \text{AUC}_{\text{iv}} \quad [4]$$

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}):

$$A_{ss} = C_{ss} \times V_{ss}. \quad [5]$$

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 × time vs time profile), and AUC as defined by Benet and Galeazzi (*5*):

$$V_{ss} = \text{dose}_{\text{iv}} \times \text{AUMC} / \text{AUC}^2. \quad [6]$$

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:

$$t_{1/2} = 0.693 \times V/\text{CL}. \quad [7]$$

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., CL ≈ CL_{hep}), then the *in vivo* 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 competitve 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 (CL_{hep} with respect to blood concentration) is influenced by hepatic blood flow (Q_{hep}), the fraction of compound in blood that is free from plasma protein binding (f_u), and the intrinsic ability of the organ to eliminate a chemical (CL_{int}). For compounds that are quickly metabolized, the hepatic clearance will approach hepatic blood flow. For compounds that are slowly metabolized, hepatic clearance approximates $f_u \times \text{CL}_{\text{int}}$:

$$\text{CL}_{\text{hep}} = Q_{\text{hep}} \times f_u \times \text{CL}_{\text{int}} / (Q_{\text{hep}} + f_u \times \text{CL}_{\text{int}}). \quad [8]$$

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:

$$\text{Rate of metabolism} = V_{\max} \times \text{C} / (K_m + C) \quad [9]$$

in which V_{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:

$$\text{Rate of metabolism}/C = V_{max}/(K_m + C) = CL_{hep}/f_u. \quad [10]$$

Since identification of saturable metabolism only occurs for low extraction ratio compounds (*3*) (i.e., $f_u \times CL_{int} \approx CL_{hep}$), 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:

$$f_u \times CL_{int}/f_u \approx CL_{int} = V_{max}/(K_m + C) \approx V_{max}/(K_m). \quad [11]$$

The units for V_{max} and K_m in *in vitro* experimental conditions are generally microgram 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_{max} to describe the turnover produced by the whole liver (in the unit of μg/min) in the well-stirred model can be achieved (Table 1).

$$CL \approx CL_{hep} = Q_{hep} \times f_u \times (V_{max}/K_m)/[Q_{hep} + f_u \times (V_{max}/K_m)]. \quad [12]$$

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-term power function used in concert with the body weight are generally represented as

$$Y = a\,B^n. \quad [13]$$

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.,

$$Y = a \times B^b \times (Bw^c) \quad [14]$$

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 8.6×10^8 (*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 phramacokinetic-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 1. Typical values for constants used in *in vitro–in vivo* predictions.

Species	Total P450 (nmol) per whole liver	Hepatic blood flow, ml/min/kg
Rat	118	69
Dog	1,085	41
Man	2,320	21

Data from DJ Rance (personal communication).

Table 2. A summary of different pharmacokinetic-space–time units.

Kallynochron	W^{1-x} min
Appolysichrons	W^{y-x} min
Mesochron	24.5 apl or 5.53 kln
Syndesichrons	$B^{y-x} \times Bw^Z \times 10^2$ min

Abbreviations: kln, kallynochron; apl, appolysichrons. Reproduced with permission from Boxenbaum and Ronfeld (*8*) 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

$$CL = a B^x B w^Z \quad [15]$$

and

$$V = b B^y, \quad [16]$$

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

$$C = (D/V) e^{-kt} \quad [17]$$

$$= (D/bB^y) e^{-(a/b)(B^{x-y})t} \quad [18]$$

$$Cl/(D/B^y) = (1/b) e^{-(a/b)(t/B^{y-x})} \quad [19]$$

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^{y-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:

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

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

$$CL_{rat,\ normalized} = CL_{rat} \times CL_{man,\ microsomes} / CL_{rat,\ microsomes} \quad [21]$$

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 end points (*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*).

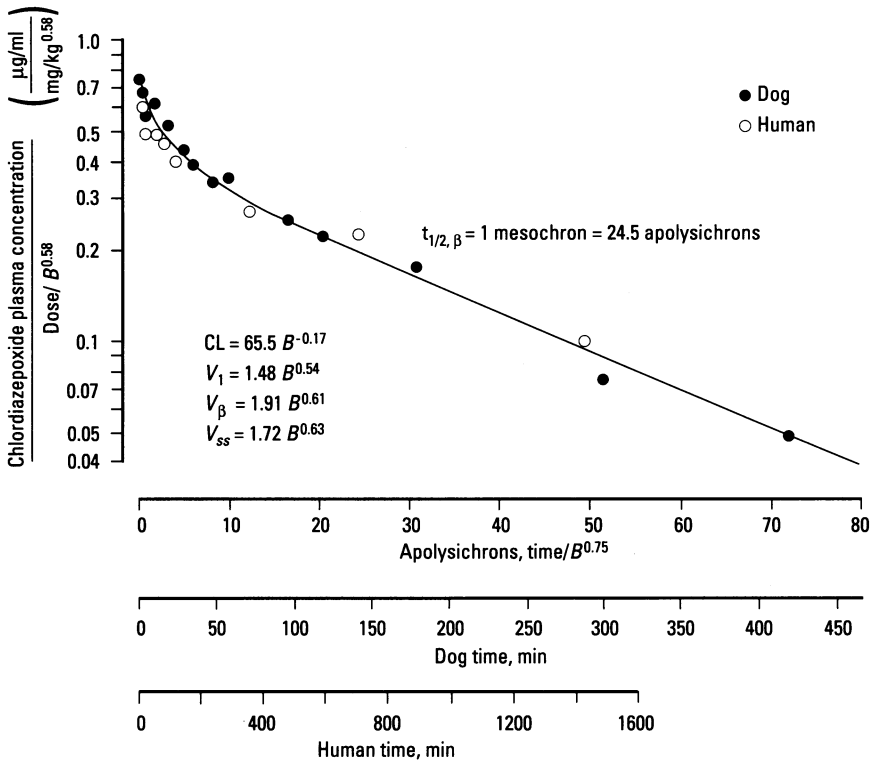


Figure 2. Complex Dedrick plot of chlordiazepoxide data. Body weight power on abscissa equals Y-X 0.58-(-0.17). Units on the abscissa (time/$B^{0.75}$) are apolysichrons. Allometric volume expressions are solved for liters. Reproduced with permission from Boxenbaum and Ronfeld (*8*).

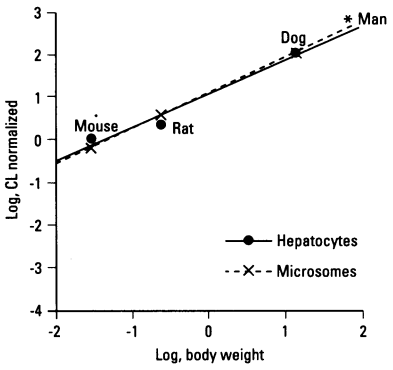


Figure 3. Allometric scaling of clearance of mafarotene normalized with *in vitro* data obtained from microsomes (- -×- -) or hepatocytes (—●—). For microsomes, $y = 0.823x + 1.035$, $r^2 = 0.999$; for hepatocytes, $y = 0.784x + 1.024$, $r^2 = 0.972$. Projected oral clearance values for man were 5.1 and 4.2 ml•min/kg, respectively. Reproduced with permission from Lave et al. (*20*). *Represents observed oral clearance in man (equal to 7.5 ml•min/kg).

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

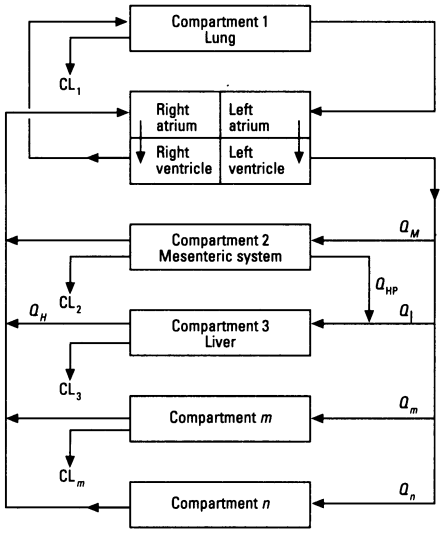


Figure 4. Schematic of a prototypical physiological model.

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 and (*S*)-nicotine *N*-1′-oxide in humans are 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 FMO3-mediated (*S*)-nicotine *N*-1′-oxide formation in the presence of human liver microsomes is probably greater that 800 μM, almost 20-fold more than that for cytochrome P4502A6 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 monooxygenase-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.

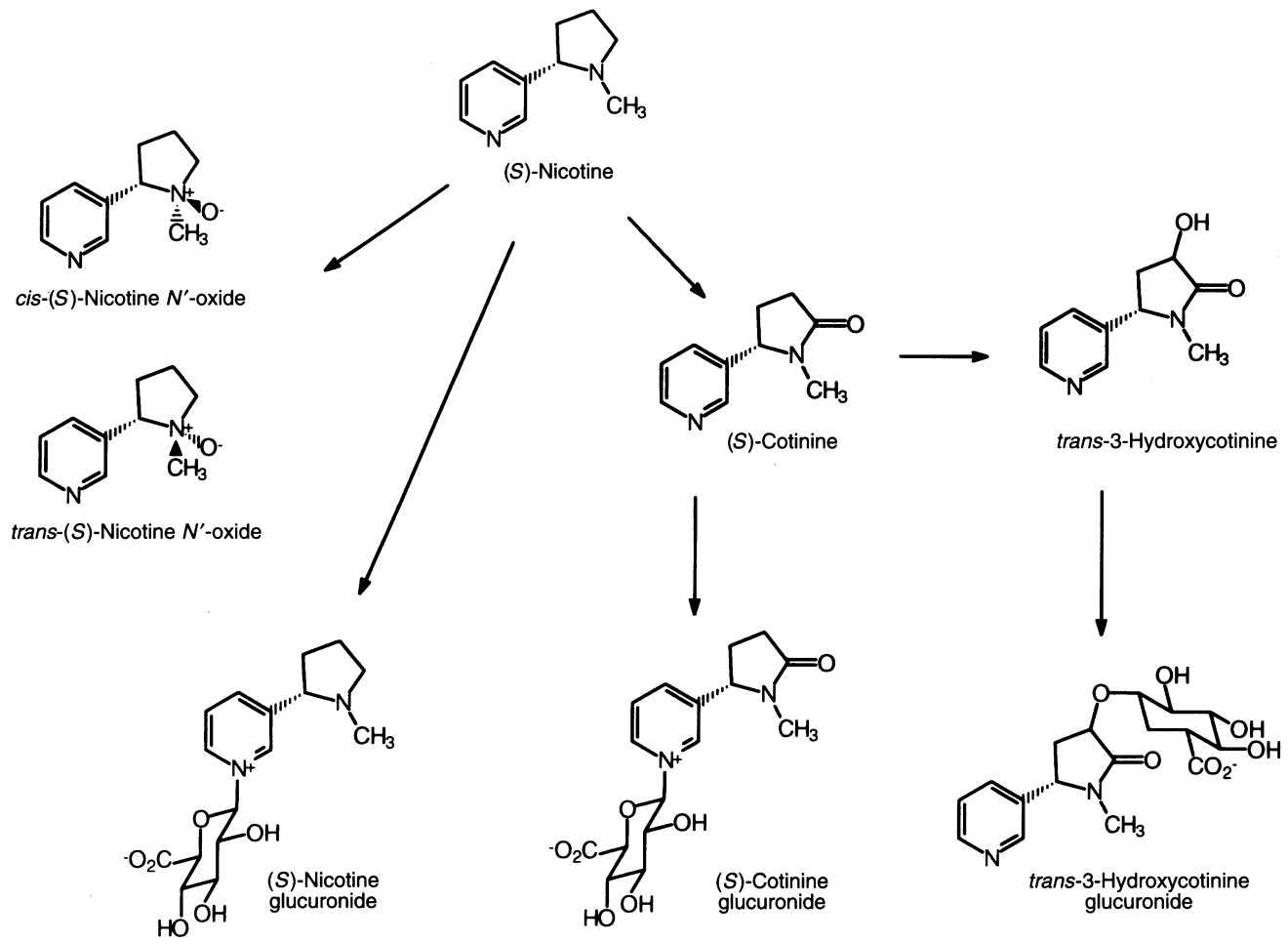


Figure 5. Scheme depicting the major (*S*)-nicotine metabolites in humans.

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 $\Delta^{1',5'}$-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 $\Delta^{1',5'}$-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 $\Delta^{1',5'}$-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 lipohilic 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 phosphorous-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*-oxygenation converts a biologically active compound into a polar, readily excreted tertiary amine *N*-oxide. FMO also catalyzes the oxygenation of organosulfur-, selenium-, phosphorous- and other heteroatom-containing chemicals that are likewise more efficiently excreted after oxygenation. 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 arylamines to the corresponding aryl hydroxylamine. Another example comes from the *N*-oxygenation 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 and xenobiotic chemicals 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 phosphonothiolate moeity 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

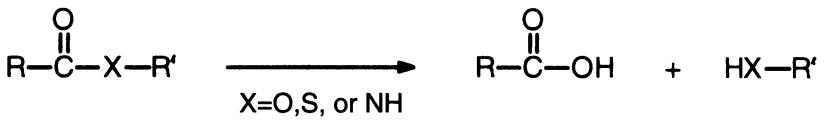


Figure 6. Hydrolysis of an ester, a thioester, or an amide to a carboxylic acid and an alcohol, a thiol, or an amine, respectively.

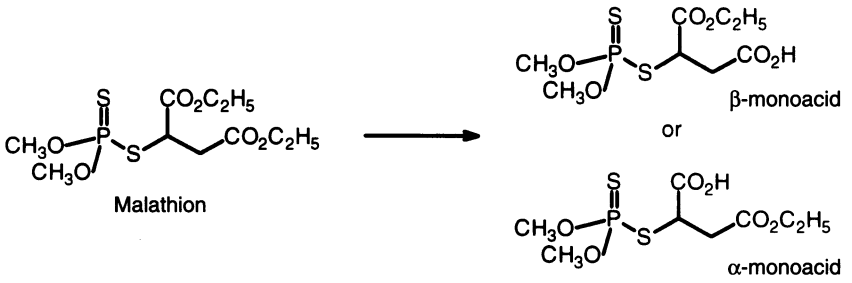


Figure 7. Hydrolysis of malathion esters.

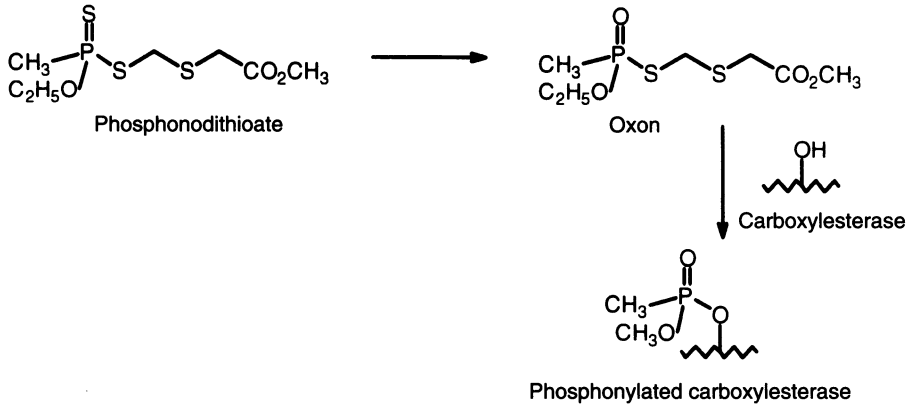


Figure 8. Oxidative desulfuration of an organothiophosphorous insecticide to the oxon.

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*).

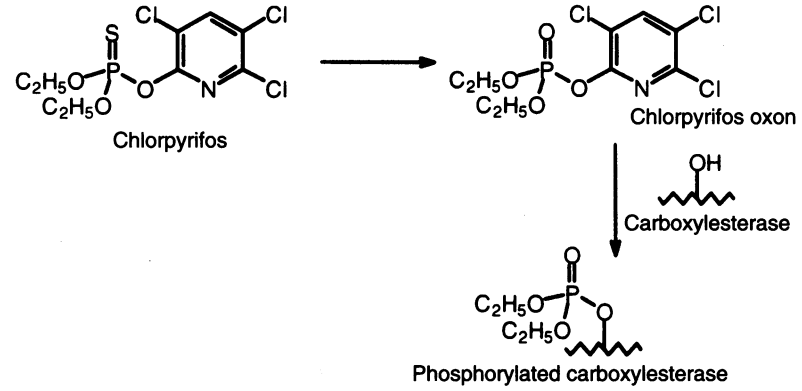


Figure 9. Oxidative desulfuration of chlorpyrifos to the oxon.

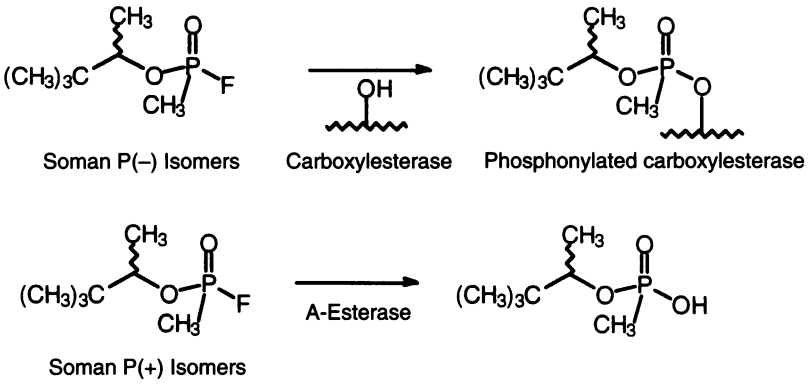


Figure 10. Phosphonylation of carboxylesterase, by soman and hydrolysis of soman by A-esterase.

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

Ester class	Chemical example	Carboxylesterase	References
Herbicides (Carboxylic esters)	Fluazifop-butyl	Human liver, skin, plasma	(*56*) (*57*)
Carbamate	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*)
Trichothecenes	T2 toxin	Rat liver	(*62–64*)
Nicotinic acid esters		Human plasma	(*65*)
Nitrosomides and nitrosocarbamates		Rat liver	(*66*)

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 benzoylecgonine, a metabolite that is largely inactive as a pyschomotor stimulant (Figure 12). The K_m value for formation of benzoylecgonine (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

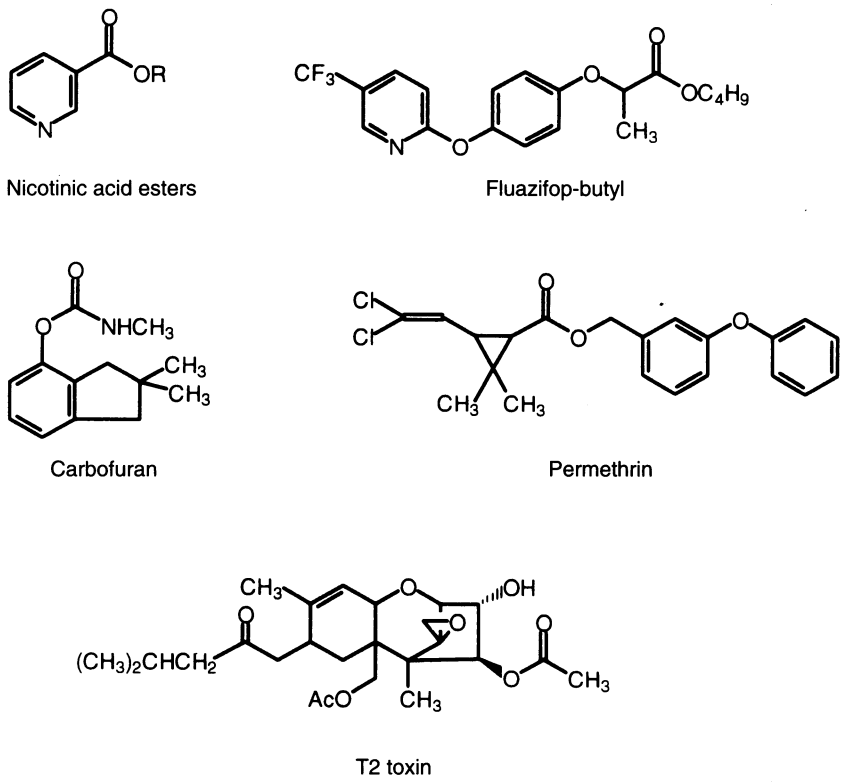


Figure 11. Some examples of environmentally important esters that are hydrolyzed by carboxylesterase.

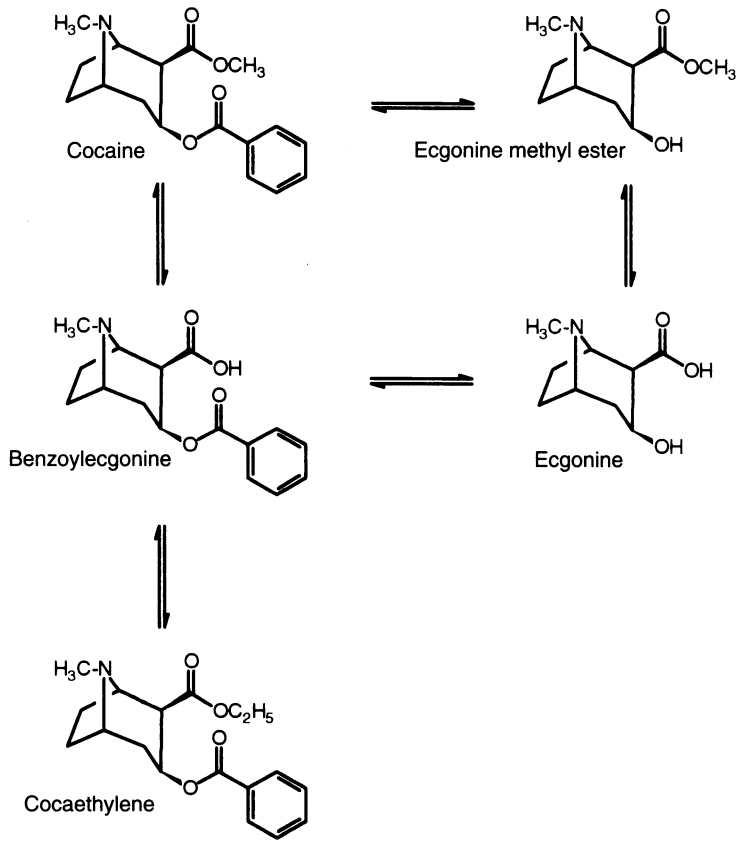


Figure 12. A scheme showing the hydrolytic metabolism and reesterification of cocaine.

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	(*68*)
Procaine	Purified from human liver, intestinal mucosa	(*68*)
Cocaine[a]	Purified from human liver	(*69*)
Meperidine	Human liver	(*70*)
Mebeverine	Human plasma	(*71*)
Lorazepam 3-acetate	Human liver	(*72*)

[a]Hydrolysis of the methyl ester of cocaine.

(-)-cocaine overdose is reported to be in the range of 30 to 460 mg/100ml, it is possible that transesterification of (-)-cocaine with ethanol constitutes a metabolic pathway contributing to increased toxicity of (-)-cocaine (*73*). Thus, in addition to the well-recognized serum cholinesterase or butyrylcholinesterase that catalyzes the hydrolysis of (-)-cocaine to ecgonine methylestester, a new human liver carboxylesterase has been discovered that hydrolyzes the methyl ester of (-)-cocaine to a pharmacologically inactive metabolite (i.e., benzoylecgonine) and a pharmacologically active transesterification product, (-)-cocaethylene (Figure 12) (*73*). Possibly the balance between (-)-cocaine ester hydrolysis and transesterification for any particular human subject may contribute to the overall susceptibility of the individual to the toxic properties of (-)-cocaine. 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, sterol 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

(*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-methylumbelliferyl was determined to be 10 μM. It is interesting to note that mercuric chloride and *p*-chloromercuribenzoate 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-methylumbelliferyl 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 intracellular 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. Paraoxonase/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 paraoxonase/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 aquisition 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

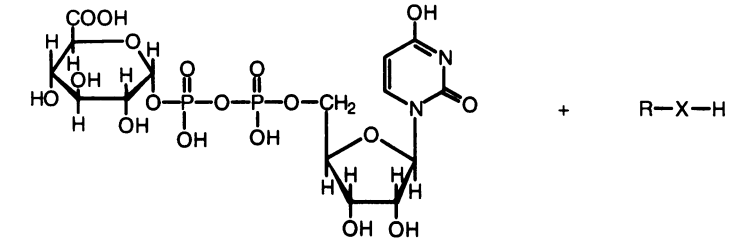
group to a large number of endogenous compounds and xenobiotics (Figure 13).

The role of glucuronidation in the detoxication of environmental chemicals and carcinogens, whether ingested, inhaled, or absorbed, is related to the other detoxication 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 physiochemical 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 hydrolases, 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 arylamines (from meat and fish in typical household cooking practices) (*106*)]; and many other compounds (Table 5).

Function of Glucuronosyltransferases in Detoxication

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



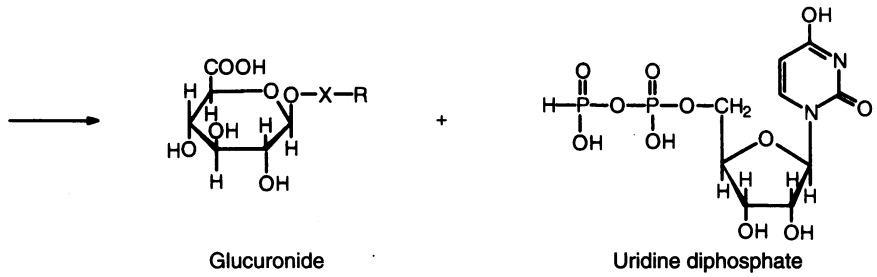


Figure 13. Conjugation of a nucleophile (i.e., R-X-H) with uridine-5′-diphospho-α-D-glucuronic acid to form the glucuronide and uridine diphosphate.

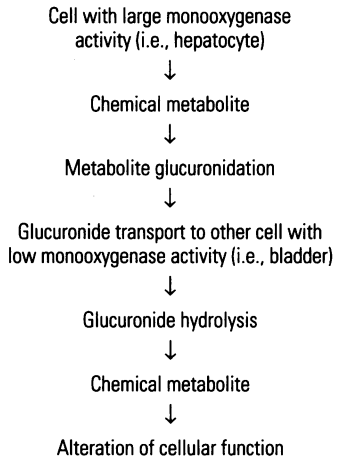


Figure 14. Hypothetical scheme depicting the metabolic bioactivation and glucuronidation of a procarcinogen, which is transported to another cell that possesses low bioactivation capacity and, nevertheless, accumulates sufficient metabolite to show significant toxicity.

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*)

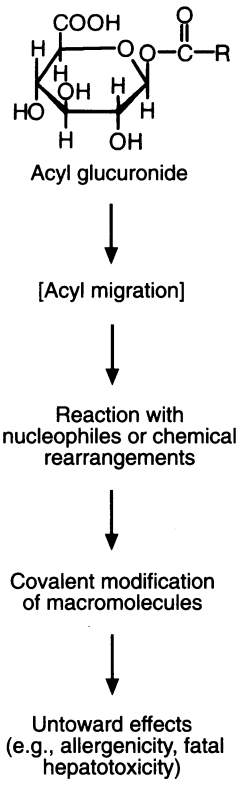


Figure 15. Hypothetical explanation of how an acyl glucuronide can participate in the covalent modification of cellular targets and eventually cause toxicity.

(*109*). In the arylamine class of compound, *N*-oxidized aromatic amines can be converted to eletrophiles via oxidation to hydroxylamines and subsequent sulfation and/or acetylation (*110*). The disposition of Phase I metabolites by glucuronidation almost certainly contributes to the detoxication 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.

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 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 C_1 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-acetylaminofluorene) to form *O*-glucuronides; thiophenols (thiophenol), and carbamic acids (diethyldithiocarbamic 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 C_1 atom of the glucuronic acid ring if the carbon atom is sufficiently nucleophilic (sulfinpyrazone, 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 Alcohols	Phenol, morphine *t*-Butanol, various steroids
S-Glucuronides	Thiophenols Thiols	Thiophenol 2-Mercaptobenzothiazole
N-Glucuronides	Aromatic amines Hydroxylamines	Aniline *N*-Hydroxy-2-naphthylamine
C-Glucuronides	1,3-Dicarbonyl compounds	Phenylbutazone, sulfinypyrazone

purifiying 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 tissure culture by transfection techniques 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 choleresis by glucuronides. In general, glucuronide excretion in bile causes choleresis of approximately 15 to 20 µl bile μmol^{-1} glucuronide (*131*). However, the phenolic drug hormol 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 glucuronosyl transferases 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 detoxication 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.

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