Chiral substances possess a unique architecture such that, despite sharing identical molecular formulas, atom-to-atom linkages, and bonding distances, they cannot be superimposed. Thus, in the environment of living systems, where specific structure-activity relationships may be required for effect (e.g., enzymes, receptors, transporters, and DNA), the physiochemical and biochemical properties of racemic mixtures and individual stereoisomers can differ significantly. In drug development, enantiomeric selection to maximize clinical effects or mitigate drug toxicity has yielded both success and failure. Further complicating genetic polymorphisms in drug disposition, stereoselective metabolism of chiral compounds can additionally influence pharmacokinetics, pharmacodynamics, and toxicity. Optically pure pharmaceuticals may undergo racemization in vivo, negating single enantiomer benefits or inducing unexpected effects. Appropriate chiral antidotes must be selected for therapeutic benefit and to minimize adverse events. Enantiomers may possess different carcinogenicity and terogenicity. Environmental toxicology provides several examples in which compound bioaccumulation, persistence, and toxicity show chiral dependence. In forensic toxicology, chiral analysis has been applied to illicit drug preparations and biological specimens, with the potential to assist in determination of cause of death and aid in the correct interpretation of substance abuse and “doping” screens. Adrenergic agonists and antagonist, nonsteroidal anti-inflammatory agents, SSRIs, opioids, warfarin, valproate, thalidomide, retinoic acid, N-acetylcysteine, carnitine, penicillamine, leucovorin, glucarpidase, pesticides, polychlorinated biphenyls, phenyletheramides, and additional compounds will be discussed to illustrate important concepts in “chiral toxicology.”

Key Words: antidotes; chiral; ecotoxicology; forensic toxicology; stereoisomer; teratology.

As mechanisms of toxicity are elucidated, precise structure-activity relationships have become more apparent. This review introduces the concept of “chiral toxicology”—the diverse impact of chirality on multiple aspects of toxicology. An historical overview and review of chemical principles guide an exposition of chiral illustrations in drug development, stereoselective metabolism and toxicity, chiral antidotes, carcinogenicity, reproductive toxicology, environmental toxicology, and forensic toxicology.

HISTORY

The study of chirality and symmetry has spanned the disciplines of chemistry, biology, and physics. Early work by Arago (1811) and Biot (1812a,b) demonstrated the effects of cut crystals on the plane of polarized light—different crystals rotated light left or right. Biot later showed that natural organic products, such as oil of turpentine, sugar solutions, camphor, and tartaric acid could produce similar rotation. In 1848, Pasteur recrystallized the racemic sodium ammonium salt of tartaric acid and noted two different crystal forms. Using tweezers, he separated them, and after redissolved them, found that they rotated polarized light differently (Pasteur, 1848). Subsequently, in 1857 Pasteur made the first observation of biological enantioselectivity, when he noted bacterial capacity to ferment only dextro-tartaric acid (Gal, 2008; Pasteur, 1848). In 1874, Van’t Hoff, recipient of the first Nobel Prize in Chemistry, and Le Bel independently outlined the relationship between three-dimensional molecular structure and optical activity and the concept of the asymmetric carbon atom (Le Bel, 1874; van’t Hoff, 1874). In 1891, Fisher performed the unbelievable feat of identifying the 16 different spatial configurations of aldohexose (C₆H₁₂O₆), the most prominent member being D-glucose (Fischer, 1891). He created eponymous Fisher projections to represent their three-dimensional structure (and was awarded the second Nobel Prize in Chemistry).

The physiological and toxicological significance of chiral compounds was soon explored. Dextro-cocaine was found to
have greater activity and more rapid onset, and shorter duration than levo-cocaine (Ehrlich and Einhorn, 1894; Poulson, 1890). Differences in atropine (racemic hyoscyamine) and levo-hyoscyamine on pupillary, cardiac, and salivary activity and effects on frog spinal cord reflexes were described in 1903 (Cushny, 1903). Differences in toxic dose were reported for nicotine isomers in 1904 (Pictet and Rotschy, 1904) and for camphor isomers in 1910 (Grove, 1910). Exploring endogenous compounds, Abderhalden and Müller (1908) described the significant differential vasopressor effects of levo- and dextro-epinephrine. Therapeutically, Frey (1918) reported that quinidine, the isomer of quinine, was more effective in treating dysrhythmias.

CHEMICAL PRINCIPLES

Definitions and Terms

Isomers are compounds with the same molecular formula but different structural formulas or different spatial arrangement (Fig. 1A). Methoxybenzene (anisole), phenylmethanol (benzyl alcohol), 2-methylphenol (ortho-cresol), 3-methylphenol (meta-cresol), and 4-methylphenol (para-cresol) are examples of constitutional or structural isomers. Although they share the same molecular formula (C7H8O) and molar mass, their atoms are stereoisomers arising from compounds with identical molecular formulas, atom-to-atom linkages, and bonding distances; all are “chiral” compounds.

Cis- and trans- isomers are stereoisomers arising from compounds with restricted rotation (e.g., containing double bonds or some alicyclic compounds). Cis-diastereomers have substituent groups projecting in the same direction; trans-diastereomers have substituents oriented in opposing directions (Fig. 2A). Conformational isomers (conformers) are stereoisomers due to bond rotation. Acetylcholine is an example of a rotamer (conformer that differs by restricted rotation about only a single bond): the gauche forms are conformers of each other (Fig. 2B) (International Union of Pure and Applied Chemistry [IUPAC], 1997). The 1,4-benzodiazepines typify ring inversion isomers. The seven-membered nonplanar ring can take on two possible “boat” formations that are conformational isomers of each other (Fig. 2C) (Paizs and Simonyi, 1999). Rotational barriers induced by large substituent groups may prevent rotation at low energy levels. Polychlorinated biphenyls (PCBs) are a good example of atropisomers—stereoisomers resulting from hindered rotation about single bonds, where the steric strain barrier to rotation is high enough to allow for the isolation of the separate conformers (e.g., Fig. 8C) (IUPAC, 1997).

Several terms are commonly utilized to describe enantiomer proportions within a given mixture (Fig. 3). The enantiomer ratio (ER = E1/E2) and enantiomer fraction [EF = E1/(E1 + E2)] convey similar information, but must be interpreted appropriately. The enantiomer excess [ene. = |E1 - E2|, where E1 + E2 = 1 (or 100%)] is defined as the absolute difference between the mole or weight fraction of each enantiomer, and is commonly expressed as a percentage (IUPAC, 1997). A racemate (racemic mixture) represents the unique case of a perfect 1:1 (equimolar, enene. = 0) mixture of enantiomers. Thus, it yields no optical rotation. Enantiomer proportions may change over time under certain experimental, physiological, or environmental conditions.

Nomenclature

Confusion arises from the multiple methods of naming chiral entities. The oldest d- (dextro, symbolized as “+”), l-(levor, symbolized as “-”) terminology arose to describe how each specific enantiomer affected polarized light (Pasteur, 1848). When linearly polarized light passes through and interacts with an enantiomer sample, it undergoes “optical rotation.” If the enantiomer rotates the plane of polarized light to the right, it is termed dextro-rotation (+). If it rotates the plane to the left, it is levo-rotation (−) (Fig. 4A). Enantiomer’s dextro- or levo-rotation are equal in absolute magnitude but opposite in direction. Importantly, the dextro- or levo-rotation by each enantiomer has no correlation with the actual assignment of its atoms in space, and thus no relation to d/l or R/S naming conventions. For this reason, a compound’s (±) optical activity may be provided alongside its d/l or R/S name. Fisher developed the d/l system as a means to describe carbohydrate stereoisomers (Fischer, 1891). It is also commonly applied to amino acids. “D” or “L” is assigned by relating a molecule to (chiral) glyceraldehyde. The molecule is written in a Fischer projection. One aligns a given molecule with the carbon in the highest oxidation state superiorly. At the chiral center closest to the bottom, if the substituent (e.g., OH) projects to right, it is classified as the D-isomer. If the substituent projects to left, it is the L-isomer (Fig. 4B). The Cahn-Ingold-Prelog (CIP) System (R- [rectus], S- [sinister] convention) or “absolute configuration” is assigned by assigning “priority” to the
groups attached to the stereocenter (by highest atomic number of the most proximate substituent and additional rules) (Cahn et al., 1956, 1966; Prelog and Helmchen, 1982). One then “looks down” the substituent with the lowest priority to determine if the decreasing order of the remaining groups occurs in a clockwise (R) or counterclockwise (S) fashion (Fig. 4C). R/S assignment does not always parallel D/L assignment. The E- (entgegen), Z- (zusammen) system describes more complex cis-trans configurations. CIP priorities are assigned to the substituents at each end of a rotational barrier. The (Z)-isomer has higher priorities groups on the same side; the (E)-isomer has two groups with the higher priorities on opposite sides (Fig. 4D). Endo- and exo-designations are applied to substituents of molecules which contain bridges. In endo-isomers, the group is towards (cisto) the longest bridge; exo-isomers are oriented away from the longest bridge (e.g., Fig. 7A). M (minus) and P (plus) designations for ring conformers such as diazepam are assigned on the basis of the characteristic torsion angle of C2-C3-N4-C5 of the diazepam ring (Fig. 2C) (Paizs and Simonyi, 1999). Confusingly, P (plus, right-handed, clockwise, Δ)- and M (minus, left-handed, counterclockwise, Λ) designations are also applied to supramolecular systems that are helical, propeller, or screw-shaped (Fig. 4E) (IUPAC, 1997).

CHIRAL PHARMACOLOGY

Drug Development

FDA initial guidance on chiral drugs was set forth in 1992, as the differential actions and toxicities of enantiomers became more evident, and as the technology for chiral drug development and detection advanced (U.S. Food and Drug Administration, 1992). Identified chiral-specific issues included appropriate manufacturing controls (exclusion of diastereomeric impurities), product stability (racemization during storage), pharmacokinetic evaluations and quantifications that accounted for chiral differences (different dose-response curves), and correct interpretation of data when the pharmacokinetic properties of isomers in animal models differed from humans. According to the guidelines, composition of a chiral drug had to be known when applied in pharmacological, toxicological, and clinical studies.

In part because of the burdens associated with determining the profiles and toxicities of mixed compounds, racemates have...
virtually disappeared from development as new molecular entities. Single enantiomer or achiral drugs now dominate newly approved drugs in the United States and abroad (Agranat et al., 2002; Caner et al., 2004). Additionally, drugs previously granted patent protection and marketed as racemates are candidates for a “chiral switch” (i.e., development as single or paired enantiomers (in the case of diasteromic mixtures), which permits additional years of market exclusivity (Agranat et al., 2002)). Economic forces (e.g., market share) and favorable clinical profiles have driven successful chiral switches such as esomeprozole, levofloxacine, and escitalopram (Fig. 6A), and racemic veterinarian compounds (medetomidine) have been adapted for human use ((S)-dexametomidine) (Table 1). Antiviral analogues (abacavir, didanosine, lamivudine, stavudine, vidarabine, zidovudine, etc.) are produced as single isomers such that their ring substitutions mimic the 2R,5R architecture of the “natural” nucleosides (adenosine, cytidine, guanosine, thymidine, etc.). Compounds may contain atypical amino acid isomers or their derivatives (e.g., D-serine in goserelin). Other examples of current chiral pharmaceuticals from a range of pharmaceutical classes are provided in Table 1. These comprise drugs in which the enantiomer was developed primarily, “chiral switches,” and drugs offered only as enantiomers because the other isomer has no clinical effect or adverse effects in vivo (e.g., Levodopa and levothyroxine).

Ideally, therapeutic activity would reside in one enantiomer and adverse effects in the other. Unfortunately, there is a range of possibilities, and the combined actions of the individual enantiomers may actually make the racemate or enantiomer combinations desirable. For example, when racemic dobutamine is administered, the nonselective β1 and β2 agonism by the l-dobutamine enantiomer is moderated by the a1 agonism of d-dobutamine to yield a clinical effect of apparent β1-selectivity (Majerus et al., 1989; Ruffolo, 1987). Alternatively, an individual enantiomer may retain the racemate’s undesirable activities, lose desired activity, or present new

FIG. 2. (A) Cis-trans isomerism arising from the different position of atoms (or groups) around a reference point (platinum atom), a carbon-carbon double bond, and a cyclic structure (cycloalkane). (B) Acetylcholine conformers (rotamers) The gauche forms (seen in Newman projection) are conformers. (C) Two conformations of diazepam which interconvert via ring inversion.
Distinctions between the terms used to convey enantiomer proportions. In a mixture composed of 75% of enantiomer 1 (E₁) and 25% of enantiomer 2 (E₂), the enantiomer ratio \( \text{ER} = E_1/E_2 \) = 75%/25% = 3.0, the enantiomer fraction \( \text{EF} = E_1/(E_1 + E_2) \) = 75%/75% + 25% = 0.75 (or 75%), and the enantiomer excess \( \text{e.e.} = |E_1 - E_2|, \) where \( E_1 + E_2 = 1 \) (or 100%) = 75% - 25% = 0.5 (or 50%).

To complicate matters, chiral selection can be short-circuited through chiral inversion—the conversion of one enantiomer into its opposite (e.g., Fig. 7B) (Ali et al., 2007; Wsol et al., 2004). In vivo, this normally occurs with the assistance of an enzyme catalyst. Drugs from a variety of therapeutics classes that undergo chiral inversion have been reviewed (Ali et al., 2007; Wsol et al., 2004). Ibuprofen and other arylpropionic acid derivatives are well-described molecules capable of chiral inversion (Hao et al., 2005; Wsol et al., 2004). (S)-ibuprofen inhibits COX1 and COX2 equally. The (R)-enantiomer weakly inhibits COX1 and has no effects on COX2. However, an (R)-metabolite, (R)-ibuprofenoyl-CoA, actually has greater COX2 inhibition. A majority of (R)-ibuprofenoyl-CoA undergoes unidirectional conversion to active (S)-ibuprofen (Khininiicki et al., 1991; Wsol et al., 2004). (R)-ibuprofen thus functions as a pro-drug and contributes to therapeutic effects (Neupert et al., 1997). In contrast, ketorolac (acetic acid derivative) displays species-specific interconversion—71% in mice and 12% in rats. Human inter-conversion is minimal—0% \( R(+)\)-ketorolac to \( S(-)\)-ketorolac and 6% \( S(-)\)-ketorolac to \( R(+)\)-ketorolac (Mroszczyk et al., 1996). Thus, animal models might overestimate or underestimate efficacy if significant interconversion occurs. Age-dependent and enantiomer-specific elimination occurs with ketorolac—clearance in children is twice that of adults and clearance of active (S)-enantiomer is four times that of inactive (R)-isomer (Kaufman et al., 1999). This would suggest that higher weight-adjusted doses would be required to achieve comparable plasma concentrations of the active isomer in children. Nonstereospecific assays would also tend to misconstrue the duration of effect.

Biological Implications: Metabolism and Clinical Effects

Biological systems are chiral entities. Humans are primarily composed of l-amino acids and d-carbohydrates. Protein secondary structure includes right-handed (P-) coiled alpha helices, and most DNA is similarly in a right-handed spiral configuration (termed “B-DNA,” as opposed to left-handed “Z-DNA”) (Dickerson et al., 1982). Tertiary structures create unique three-dimensional binding, catalytic, and stabilization domains (Fig. 5). On the macroscopic scale, during normal growth and development both the human cardiac and gastrointestinal systems have specific rotational patterns, and additionally, left-right “mirror” symmetry evolves. Unique structural-activity relationships thus proceed from specific architecture constraints at multiple systemic levels. Therefore, in a chiral environment, stereoisomers might experience selective absorption, protein binding, transport, enzyme interactions and metabolism, receptor interactions, and DNA binding. Thus, each stereoisomer or isomeric mixture can have different pharmacokinetic, pharmacodynamic, therapeutic, and adverse effect profiles. A given structure’s capacity to accommodate chiral disparities will influence the magnitude and type of difference in effects (if any) observed between enantiomers. For example, one enantiomer might be completely unable to complex with a particular receptor or enzyme or lose precise alignment at a catalytic site, whereas at a different molecule, no impairment might occur (Fig. 5). These consequences of stereospecificity have been reported for multiple pharmaceutical classes including antibiotic, cardiovascular, chemotherapeutic, psychotropic, pulmonary, and rheumatic drugs (Baker and Prior, 2002; Hutt and O’Grady, 1996; Kean et al., 1991; Mehvar et al., 2002; Ranade et al., 2002; Smith et al., 2005; Wsol et al., 2004).
Absorption/carriers. The drug efflux transporter P-glycoprotein, which participates in drug absorption, distribution, and excretion, is regulated stereospecifically. For example, R-cetirizine upregulates P-glycoprotein expression, while S-cetirizine down-regulates it (Shen et al., 2007). P-glycoprotein is enantioselectively inhibited by the levo-isomer of mefloquine, which can affect the transport of P-glycoprotein substrates such as cyclosporine and vinblastine (Lu et al., 2001; Pham et al., 2000). The human reduced folate carrier is stereospecific for the natural (6S) stereoisomer of 5-formyl tetrahydrofolate (leucovorin) and the antifolate methotrexate (Matherly and Hou, 2008; Narawa et al., 2007). Stereospecific transport contributes to methyl mercury central nervous system (CNS) toxicity. Methyl mercury binds cysteine to generate methyl mercury-cysteine [CH$_3$Hg-S-CH$_2$-CH(NH$_2$)COOH]. The structure’s mimicry of methionine [CH$_3$-S-CH$_2$-CH$_2$-CH(NH$_2$)COOH] permits L-type large neutral amino acid.

**FIG. 4.** Stereoisomer naming conventions. (A) According to optical activity: incoherent light is passed through a polarizer lens and then through a tube (polarimeter tube) containing a sample of the enantiomer. The degree of rotation of polarized light by an enantiomer is determined by an analyzer—a rotatable polarizer lens—in conjunction with a detector. Dextro-rotary (+) compounds rotate the polarized light plane to the right (clockwise), levo-rotary (−) compounds to the left (anti-clockwise). (B) The d/l (Fischer) naming convention for glyceraldehydes—the asterix denotes the substituent determining D- or L-assignment. (C and D) The CIP R/S- and E/Z-naming conventions according to priority of substituents. (E) The IUPAC $P,M$-naming conventions of helical, propeller, or screw-shaped structures.
carrier-mediated transport across the blood brain barrier (Clarkson et al., 2007). Methyl mercury-L-cysteine uptake significantly exceeds that of methyl mercury-D-cysteine (Kerper et al., 1992; Mokrzan et al., 1995). At certain concentrations, S\(^{(-)}\)-bupivacaine has a vasoconstrictor effect absent in the R\(^{(+)}\)-isomer, which results in drug remaining at the injection site and a longer duration of analgesia (Aps and Reynolds, 1978). Levobupivicaine (Chirocaine), which did not carry the “black box” warning for cardiotoxicity required of racemic bupivacaine, has been discontinued in the United States (U.S. Food and Drug Administration, 2008).

Protein binding. Chirality may influence the basic pharmacological property of protein binding. Albumin has species-specific, stereo-specific binding preferences (Pistolozzi and Bertucci, 2008). Despite diazepam’s rapid interconversion (Fig. 2C), its M-form prevails when bound to albumin. Bilirubin, which is achiral in solution due to rapid interconversion of its M- and P-forms, binds albumin in the P-form (Pistolozzi and Bertucci, 2008). Human albumin prefers the active S-enantiomer of ketoprofen and has stereoselectivity to other non-steroidal anti-inflammatory drugs (NSAIDs). Human albumin also displays stereoselective binding to warfarin (Fitos et al., 2002; Tatsumi et al., 2007). Albumin binds S\(^{(+)}\)-chloroquine more avidly than R\(^{(-)}\)-chloroquine, whereas alpha-L-acid glycoprotein binds the R-enantiomer more tightly (Augustijns and Verbeke, 1993; Ofori-Adjei et al., 1986). Alpha-L-acid glycoprotein has stereospecific affinity for R\(^{(-)}\)-disopyramide, S\(^{(-)}\)-verapamil, and R\(^{(+)}\)-propranolol.

### Table 1

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Racemate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armodafinil (Nuvigil)</td>
<td>Modafinil (Provigil)</td>
</tr>
<tr>
<td>Betamethasone (many)</td>
<td>N.A. (16(^{S}) isomer of dexamethasone)</td>
</tr>
<tr>
<td>Cisatracurium (Nimbex)</td>
<td>Atracurium (Tracrium)</td>
</tr>
<tr>
<td>Desloratadine (Clarinex)</td>
<td>Loratadine (Claritin)</td>
</tr>
<tr>
<td>Dexamethasone (many)</td>
<td>N.A. (16(^{R}) isomer of Betamethasone)</td>
</tr>
<tr>
<td>Dexchlorpheniramine (Polaramine, non-US)</td>
<td>Chlorpheniramine (Chlor-Trimeton, etc.)</td>
</tr>
<tr>
<td>Dexametomidine (Precedex(^{®}))</td>
<td>Medetomidine (Dormitor, veterinary)</td>
</tr>
<tr>
<td>Dexamethylphenidate (Focalin)</td>
<td>Methylphenidate (Ritalin, etc.)</td>
</tr>
<tr>
<td>Dextroamphetamine (Dexedrine)</td>
<td>Amphetamine (Adderall)</td>
</tr>
<tr>
<td>Dextromethorphan (many)</td>
<td>N.A.</td>
</tr>
<tr>
<td>Escitalopram (Lexapro)</td>
<td>Citalopram (Celexa)</td>
</tr>
<tr>
<td>Esomeprazole (Nexium)</td>
<td>Omeprazole (Prilosec)</td>
</tr>
<tr>
<td>Ezopiclone (Lunesta)</td>
<td>Zopiclone (sold outside United States)</td>
</tr>
<tr>
<td>Levalbuterol (Xopenex)</td>
<td>Albuterol (Proventil, Ventolin, etc.)</td>
</tr>
<tr>
<td>Levobetaxolol (Betaxon)</td>
<td>Betaxolol (Betopic)</td>
</tr>
<tr>
<td>Levobunolol (Betagen)</td>
<td>N.A.</td>
</tr>
<tr>
<td>Levocabastine (Livostin)</td>
<td>N.A.</td>
</tr>
<tr>
<td>Levocetirizine (Xyzal)</td>
<td>Cetirizine (Zyrtec)</td>
</tr>
<tr>
<td>Levodopa (with carbipoda as Atamet, Sinemet)</td>
<td>N.A.</td>
</tr>
<tr>
<td>Levofloxacin (Levaquin)</td>
<td>Ofloxacin (Flloxin)</td>
</tr>
<tr>
<td>Levonorgestrel (Alesse, Seasonale, Plan B, etc.)</td>
<td>Norgestrel (Lo/Ovral, Cryselle, etc.)</td>
</tr>
<tr>
<td>Levorphanol (Levo-Dromoran)</td>
<td>N.A.</td>
</tr>
<tr>
<td>Levothyraxine (Synthroid, N.A.</td>
<td></td>
</tr>
<tr>
<td>Levoxyl, etc.)</td>
<td></td>
</tr>
<tr>
<td>Technetium Tc99m Bicisate (Neurolite)</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

**FIG. 5.** (A) An hypothetical enantiomer (Enantiomer\(_1\)) interacting with a hypothetical enzyme structure (Target\(_1\)), where domain A’ is a catalytic site, B’ is a hydrophobic pocket, and C’ and D’ are areas of steric hindrance. Enantiomer\(_1\) can effectively bind within the structure’s architecture and achieve appropriate orientation at the catalytic site. (B) Regardless of what rotational position Enantiomer\(_2\) assumes, it is incapable of appropriately aligning with either the active or stabilization domains (i.e., to avoid the steric hindrance imposed by D’ on C, A is displaced away from the catalytic site A’, and B–B’ interactions are not as efficient). (C) If a structure’s architecture is less constrained (e.g., Target\(_2\)), then effective interactions may occur.
(Hanada et al., 2000), and preferably binds the P-conformer of diazepam (Fitos et al., 2007). Correct interpretation of pharmacokinetic data and pharmacokinetic model constructs may be compromised if species-specific and enantiomer-specific differences in protein binding are not accounted for. The implications of stereoselective protein binding, bioequivalence, and the employment of stereospecific assay techniques are reviewed by Brocks (2006), Srinivas (2004), and others. In an organism provided a racemic “drug,” nonstereospecific assays would attribute an observed effect to the total free “drug” even if isomers had markedly dissimilar binding properties (e.g., protein binding of 65% in isomer R and 35% in isomer S would yield apparent “drug” binding of 50%). The correct clinical or adverse effect dose-response relationship would be misinterpreted if R/S isomer activity differed. As only free or unbound drug participates in elimination or receptor interaction, observed clearance, apparent volume of distribution, tissue distribution, and duration of effect might be further misconstrued, particularly as isomer ratios changed over time. Dose calculations and dosing intervals derived from a test species could be incorrect if humans or other species displayed alternative stereo-specific binding preferences (particularly of an active isomer) (Tocco et al., 1990). Thus, considering each stereoisomer as a distinct chemical entity refines pharmacokinetic models of racemates and mixtures.

Metabolism and elimination. As might be expected for enzymatic processes, the biotransformation reactions (e.g., hydrolysis, reduction, oxidation, and conjugation) may demonstrate isomeric preference. (S,S)-hydroxybupropion is stereoselectively active at dopamine transporters, norepinephrine transporters, and nicotinic acetylcholine receptors (Damaj et al., 2004). At therapeutic concentrations, CYP2B6-mediated hydroxylation of (S)-bupropion to metabolically active (S,S)-hydroxybupropion is significantly greater than (R)-bupropion, leading to greater apparent oral clearance and lower plasma concentrations (Kharasch et al., 2008). (S,S)-hydroxybupropion is formation-rate-limited, whereas (R,R)-hydroxybupropion and racemic hydroxybupropion are elimination-rate-limited; thus, CYP2B6 phenotypic variability, inhibition or induction, or overdose might alter the clinical consequences of bupropion ingestion (Kharasch et al., 2008). Hepatic, jejunal mucosa, and platelet sulfation of R(−)-salbutamol (albuterol) is approximately ten times greater than the S(+)-isomer (Walle et al., 1993). The (S)-enantiomer of carvedilol undergoes stereoselective oxidation by cytochrome P450 (CYP) 2D6 and CYP1A2 in liver and stereoselective glucuronidation in liver and intestine, which is at least partly responsible for stereoselective presystemic clearance (Ishida et al., 2008). CYP2C19 preferential metabolism of S(−)-lansoprazole is further influenced by polymorphism status (homozygous and heterozygous extensive metabolizers, and poor metabolizers), such that the R/S ratios for the lansoprazole AUC in these polymorphisms is 12.7, 8.5, and 5.8, respectively, after oral dosing (Miura, 2006). Similarly, systemic R/S enantiomer exposures to fluoxetine, metoprolol, pantoprozole, and trimipramine are altered according to CYP2D6 or CYP2C19 status (Brocks, 2006). CYP2D6 stereoselectively catalyses the O-demethylation of (R)-venlafaxine (Eap et al., 2003). Stereoselective drug metabolism and elimination has been reported for a numerous other compounds: ketamine, whose R(−)-ketamine inhibits the more rapidly clearing S(+)-ketamine; (S)-pentoxifylline conversion to its M1 metabolite; tramadol N-demethylation to S(−)-O-demethyltramadol; renal tubular secretion of dextro-cetirizine; and clearance of verapamil isomers (Garcia Queglas et al., 2007; Ihmsen et al., 2001; Nicklasson et al., 2002; Schwartz et al., 1994; Strolin Benedetti et al., 2008; Williams and Wainer, 2002). From a therapeutic standpoint, protease-resistant, cell-penetrating peptides are being developed for drug delivery through incorporation of L-amino acids (Pujals et al., 2008).

Receptor (target) interactions. There are multiple examples of varied receptor types with chiral dependence. Vision itself requires cis-trans isomerism (Fig. 2A). 11-cis-retinal isomerizes to all-trans-retinal upon light absorption (Fig. 7C depicts analogous cis-trans isomers of retinoic acid in clinical use). This produces a conformational change within rhodopsin to yield activated metarhodopsin II, with subsequent triggering of the G-protein second messenger cascade and transmission of the photoactivation signal (Ahuja et al., 2009). Levo-carvone is perceived as spearmint, whereas dextro-carvone provides a caraway aroma (Williams and Wainer, 2002). R(+) -limonene, an essential oil employed as a food and cosmetic additive, solvent, and cleaning product generates a pleasant lemon-orange scent; S(−)-limonene smells like turpentine (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1999). Both may produce allergic reactions (Heydorn et al., 2003). Dextromethorphan primarily provides an anti-tussive effect, although levomethorphan, a noncommercially available DEA schedule 2 compound, has classic, potent systemic opioid effects. The conformers of acetylcholine (Fig. 2B) are physiologically relevant. It appears that the anti and gauche forms demonstrate variable affinity to nicotinic and muscarinic receptor subtypes, and the anti form is the preferential conformation for acetylcholinesterase catalysis (Edvardsen and Dahl, 1991; Lin et al., 2007; Vistoli et al., 2007). D-Serine coagonizes the N-methyl-D-aspartate receptor (Wolsker et al., 2008). The antiepileptic S(+) -vigabatrine irreversibly inhibits gamma-aminobutyric acid (GABA) transaminase, whereas the R-enantiomer is without activity (Gidal et al., 1999). Complicating matters, a xenobiotic may have stereospecificity at certain receptors, but not others (Fig. 5). S(−)-propranolol exhibits approximately 100 times greater antagonism than R(+) -propranolol at β1, β2, and β3 receptors (Popp et al., 2004; Ranade and Somberg, 2005). S(−)-propranolol is also up to four times more bioavailable and has a longer half-life, with stereoselective binding to human alpha 1-acid glycoprotein...
Chiral Pharmaceuticals: Clinical Toxicological Correlations

Citalopram. Escitalopram, the active enantiomer, is a commercially successful chiral derivation from racemic citalopram (Fig. 6A). CYP 3A4 and CYP 2C19, and CYP 2D6 demethylate of escitalopram to (S)-desmethylcitalopram and (S)-didemethylcitalopram. There is wide inter- and intrapatient variability in escitalopram serum concentrations (Reis et al., 2007). Because of its serotonergic activity, escitalopram retains the ability to cause hypotension, serotonin syndrome, and restless leg syndrome (Covyeou and Jackson, 2007; Forest Pharmaceuticals, Inc. 2009; Grover et al., 2007; Huska et al., 2007; Nahshoni et al., 2004; Olsen et al., 2004; Page et al., 2008; Vari and Beckson, 2007) Both the S- and racemic citalopram prolong the QT equally and slightly more than placebo, and the manufacturer reports both QT prolongation and rare cases of torsade de pointes (Forest Pharmaceuticals, Inc. 2009). QT prolongation may be delayed for up to 10-h postingestion in escitalopram overdose, mandating prolonged cardiovascular monitoring (Yuksel et al., 2005). Thus, clinical superiority of escitalopram appeared undemonstrated in a review: despite the ability to take a lower absolute daily dose, the adverse effect profiles and cost are effectively similar (Anonymous, 2002).

Methadone. Methadone use has been associated with prolonged QT interval and torsades de pointes (Ehret et al., 2006; Pearson and Woosley, 2005). Clinician unawareness of this risk led to pretreatment and ongoing electrocardiogram screening guidelines for patients prescribed methadone (Krantz et al., 1983). Opioid activity resides in the methadone R(-)-enantiomer; temperature-dependent hERG potassium channel inhibition resides primarily with the S(+)-enantiomer (IC50 of 2 μM) (Fig. 6B) (Eap et al., 2007). Methadone is primarily metabolized by N-demethylation to an inactive metabolite EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene) (Gerber et al., 2004). In a complex fashion, stereoselective cytochrome P450 enzymes, primarily CYP3A4, CYP2B6, and CYP2C19, and to a lesser extent CYP2C9 and CYP2D6, convert methadone to EDDP and other inactive metabolites (Gerber et al., 2004; Totah et al., 2007). At CYP3A4, both R(-) and S(+)-enantiomers are metabolized equally, but when given as a racemate, there is mutual competitive inhibition, which results in equal decreased clearance. At therapeutic concentrations of each individual enantiomer, CYP2B6 metabolizes (S)-methadone faster; at higher concentrations, the (R)-enantiomer reaction proceeds faster. However, with the racemate, the (R)-enantiomer has virtually no effect on (S)-methadone metabolism, where as (R)-metabolism is significantly retarded by (S)-, indicating stereo-preference for (S)-methadone. At CYP2C19, with less overall metabolism, just the opposite occurs, (R) is more preferentially metabolized; again (S) and (R) inhibit each other’s metabolism. 2B6 genotype status—specifically the *6/*6 slow metabolizer—has been associated with a reduced ability to metabolize (S)-methadone, a higher mean heart-rate–corrected QT, and an increased risk of prolonged QTc (Eap et al., 2007). A separate pharmacokinetic study evaluating peak and trough methadone levels in patients with various 2B6 genotypes also found (S)-enantiomer concentrations could be achieved which could significantly impair repolarization (Crettol et al., 2005). Thus, genetic polymorphisms, coupled with dose-dependent stereochernistry might underlie the clinical toxicity seen with methadone administration.

Warfarin. Warfarin has significant, divergent interpatient metabolism and dosing requirements. Patients display a wide dosing range to achieve the narrow therapeutic window...
between overcoagulation (risking hemorrhagic complications) and undercoagulation (risking thrombotic complications). Warfarin is available only as a racemic mixture, although the S(−)-isof orm is significantly more potent (Fig. 6C) (Scott, 1993). Metabolic hydroxylation occurs at various locations depending on the enantiomer (Au and Rettie, 2008; Kaminsky and Zhang, 1997; Park, 1988). S(−)-warfarin metabolism occurs via CYP2C9 (to 7-hydroxywarfarin, major contribution), CYP2C9 (to 6-hydroxywarfarin, moderate contribution); CYP3A4 (to dehydrowarfarin, minor contribution); and ketoreductase [to (S,S)-warfarin alcohol 2 minor contribution]. In comparison, R(+) -warfarin metabolism proceeds by CYP1A2 (to 6- and 8-hydroxywarfarin, moderate contribution); CYP2C19 (to 8-hydroxywarfarin, moderate contribution); CYP3A4 (to 10-hydroxywarfarin, moderate contribution); CYP3A4 (to dehydrowarfarin, minor contribution); and ketoreductase [to (R,S)-warfarin alcohol 1, moderate contribution]. Suppression or acceleration of the various CYP enzymes involved by exogenous drugs can thus affect the clearance of both (R)- and (S)-isoforms. Moreover, because the (R)-enantiomer inhibits the metabolism of (S)-warfarin at CYP2C9, impaired metabolism of (R)-warfarin may cause increased levels of the active (S)-isof orm. Complexity further increases when known genetic heterogeneity is superimposed upon interacting isomer and xenobiotic effects. CYP2C9 *2 and *3 variants cause 30% and 80% reduced activity and require lower warfarin doses. Additionally, a variable polymorphic pharmacodynamic contribution from the vitamin K epoxide reductase complex subunit (VKORC1) may also alter the R/S ratio and contribute to warfarin resistance (Au and Rettie, 2008; Osman et al., 2007; Rettie and Tai, 2006). The failure of a CYP2C9 and VKORC1-only genotype-guided warfarin dosing strategy to show benefit might in part be due to inattention to these additional stereospecific interactions (Anderson et al., 2007).

CHIRAL ANTIDOTES

Dextrose (D-glucose)

Dextrose is a common antidote routinely administered to reverse hypoglycemia induced by organic or toxicological cause (anti-diabetics, ethanol, quinine, salicylates, etc.). Most glucose transporters, which are critical to growth, development, and health, have a high affinity for D-glucose, and generally negligible affinity for L-glucose (Fig. 4B, D/L conventions) (Cunningham et al., 2006). L-Glucose cannot be metabolized and does not show increased uptake in response to insulin in human volunteers. Other diverse downstream effects of D-glucose, such as upregulation of adenosine transport in aortic smooth muscle cells, decrease in lymphocyte intracellular ionized magnesium, and fibroblast premature replicative senescence do not occur if L-glucose is substituted (Blazer et al., 2002; Delva et al., 2002; Leung et al., 2005; MacLean et al., 2001). In vitro, glucose isomers alter hematocrit and blood viscosity differently, due to erythrocyte uptake specificity for D-glucose (Buhler et al., 2001). Under conditions of excess solute-free water, D-glucose causes erythrocyte swelling, as water follows D-glucose intracellularly; under initial isotonic conditions, excluded L-glucose results in erythrocyte shrinkage. L-Glucose does taste as sweet as D-glucose, presumably by presenting the same glyco phore (two-dimensional surface part) to the taste receptor (Shallenberger, 1997). However, L-glucose is a significant laxative, which is likely secondary to its limited oral and intestinal absorption (the three-dimensional structure conferring transporter specificity), with consequential induction of osmotic diarrhea (Fine et al., 1993; Kimura et al., 2002; Raymer et al., 2003). D-Glucose is also generally favored over other D-glucose epimers such as D-mannose or D-galactose. Similarly sodium-coupled glucose transporters show a much lower affinity for D-galactose than D-glucose (Scheepers et al., 2004).

N-acetyl-L-cysteine (L-NAC)

N-acetylcysteine provides an effective means of prevention and treatment of acetaminophen-induced hepatotoxicity, even in cases of delayed presentation following overdose (Keays et al., 1991; Smilkstein et al., 1988). L-NAC is also utilized to prevent contrast induced nephropathy (Massicotte, 2008). Only the L-form is antidotally useful. In animal experiments, the L-isomer, which is derived from physiologic L-cysteine, prevents hepatotoxicity and provides prolonged elevations of hepatic glutathione (Wong et al., 1986a). The nonphysiologic D-isomer cannot increase glutathione stores or prevent hepatotoxicity, despite increasing acetaminophen sulfuration (Corcoran and Wong, 1986; Wong et al., 1986b). L-NAC also has demonstrable extra-hepatic benefits—improving cardiac index and systemic mean oxygen delivery despite decreasing systemic vascular resistance (Harrison et al., 1991). Interestingly, only the L-isomer was capable of mediating vascular tone—blunting tolerance to the hypotensive effect of glyceryl trinitrate in rats (Newman et al., 1990). L-NAC was twice as effective at inhibiting cell cycle progression and topoisomerase-IIa activity (Gordina et al., 1998). This would be protective by interrupting cell division in the setting of inadequate substrates or growth factors. L-NAC, but not D-NAC, provides protection against ultraviolet light induced DNA damage in cultured fibroblasts and retinal damage in vivo (Busch et al., 1999; Morley et al., 2003). The unnatural D-NAC also resists enzymatic degradation, limiting the useful liberation of cysteine (De Flora et al., 1995; Sarnstrand et al., 1995).

L-Carnitine

L-(R)-carnitine is primarily used in the treatment of valproate (VPA) toxicity (Lheureux et al., 2005; Russell, 2007). It is also suggested for treatment of drug-associated mitochondrial toxicity (e.g., from nucleoside analogs) and anthracycline
cardiotoxicity (Claessens et al., 2003; Delaney et al., 2007; Zeidan et al., 2002). Brain carnitine uptake is stereospecific for L-carnitine (Huth et al., 1981), and the acetylated L-form can serve as a precursor for releasable glutamate (Tanaka et al., 2003). Although both D- and L-carnitine reduce the incidence of murine ammonia-induced seizures, only L-carnitine lowered ADP and AMP levels (Matsuoka and Igisu, 1993). Compared with D-carnitine, L-carnitine also improved cardiac metabolic function, oxygen consumption, and mechanical efficiency by moderating free fatty acid metabolism (Liedtke et al., 1981, 1982). The D-isomer is considered biologically inactive and harmful (Hathcock and Shao, 2006). It competitively depletes serum and cardiac and skeletal muscles of L-carnitine (Arancio et al., 1989; Ayala, 1995; Rebouche, 1983; Tsoko et al., 1995), and competitively inhibits L-carnitine intestinal uptake and renal reabsorption (Gross and Henderson, 1984). Use of racemic D,L-carnitine was associated with myasthenialike syndromes (Bazzato et al., 1981; Clair et al., 1984; Rossini et al., 1981) and cardiac arrhythmias, which disappeared after L-carnitine administration. Toxic cardiac effects of D-carnitine have been described in patients with renal failure on long-term hemodialysis and in doxorubicin cardiotoxicity. Neither the D-isomer nor the racemate should be antidotally administered.

Physostigmine

Antidotal use of physostigmine (eserine) dates from 1864, when it was reported to reverse severe poisoning secondary to atropine ingestion (Nickalls and Nickalls, 1988). Physostigmine, a carbamate inhibitor, is derived from the seed (Calabar bean) of the vine Physostigma venenosum Balfour, and was used in the ancient trial by ordeal (Fraser, 1863). Although its nonspecific analeptic properties are no longer considered useful in sedative-hypnotic or tricyclic overdose, physostigmine is currently recommended as a diagnostic and therapeutic agent for antimuscarnic poisoning (Burns et al., 2000). Naturally available 3αS-(−)-physostigmine is over 100 times more effective in inhibiting acetylcholinesterase and butyrylcholinesterase in tissue, erythrocytes, and serum in humans and animal models (Atack et al., 1989; Barak et al., 2009; Brossi, 1985, Brossi et al., 1986; Chen et al., 1992; Hill and Newkome, 1969; Petcher and Pauling, 1973; Yu et al., 1997). This stereoselectivity was recently demonstrated to depend upon asymmetric interactions within the acetylcholinesterase active center hydrophobic pocket, which is distinct from the catalytic site (Barak et al., 2009). Unexpectedly, at dosages which insignificantly inhibited acetylcholinesterase, (−)-physostigmine protects against lethal sarin exposures and inhibits sarin-induced motor endplate postjunctional damage and myopathy (Harris et al., 1990; Kawabuchi et al., 1988). This sarin-protective mechanism is apparently due to independent (−)-physostigmine antagonism at the nicotinic receptor. Physostigmine binding at nicotinic receptors is close to, but distinct from the acetylcholine binding site on the α-subunit (Pereira et al., 2002). At low doses, (−)-physostigmine functions as an ineffective receptor agonist, whereas at higher doses it produces marked channel blockade (Militante et al., 2008). In addition to antidotal considerations, a more complete understanding the site-specific properties of stereoisomers of physostigmine and its carbamate analogues (e.g., at acetylcholinesterase vs. the acetylcholine receptor) aims to further drug development efforts to treat Alzheimer’s disease and myasthenia gravis.

Leucovorin

Leucovorin (folinic acid, 5-formyltetrahydrofolic acid) is provided as rescue therapy to patients receiving high doses of the antimitabolite antifolate methotrexate (MTX) and is used to counteract MTX toxicity (Bleyer, 1977; Flombaum and Meyers, 1999; Smith and Nelson, 2008). Endogenously produced as l-leucovorin, it had been commercially available only as the racemate until 2008, when levoleucovorin [(6S)-leucovorin] received FDA approval (Spectrum Pharmaceuticals, 2008). Only the active S-form in racemic leucovorin is metabolized to reduced folates (tetrahydrofolate, 5-CH3-tetrahydrofolate, 10-CHO-tetrahydrofolate, and 5,10-CH2-tetrahydrofolate) (Bunni and Priest, 1991). During intravenous administration of the racemate, the active l-form (6S) conversion into 5-methyl-tetrahydrofolate is rapid, whereas the inactive isomer is slowly eliminated by renal excretion (Schilsky and Ratain, 1990). Oral bioavailability of leucovorin is poor above 40 mg and is negligible for the d-(6R)-form (Bleyer, 1989; Schilsky and Ratain, 1990). The renal tubular cell is the only cell where the inactive form is transported actively; however, under circumstances of high or frequent intravenous racemic leucovorin doses, d-leucovorin can compete with and inhibit levoleucovorin passive transmembrane transport (Bleyer, 1989). Compared with the d-isomer, levoleucovorin is 20 times more effective in inhibiting carrier-mediated membrane transport of methotrexate and 100-times more effective in preventing MTX growth inhibition in murine tumor cells (Sirotnak et al., 1979). As it is entirely active, levoleucovorin is prescribed at one-half of the usual racemic dose (Spectrum Pharmaceuticals, 2008). Levoleucovorin at this dose appears to provide as efficacious rescue treatment as the racemate in high dose MTX chemotherapy (Goorin et al., 1995; Jaffe et al., 1993).

Glucarpidase

Glucarpidase (carboxypeptidase G2, CPDG2) is undergoing evaluation as an antidote for methotrexate toxicity. This bacterially derived enzyme cleaves glutamate residues from methotrexate to render it inactive. Carboxypeptidase’s affinity for methotrexate is 10- to 15-fold higher than for leucovorin; its affinity for folate and 5-methyltetrahydrofolate (levocovorin’s active metabolite) are similar (Albrecht et al., 1978; European Medicines Agency [EMEA], 2008; Sherwood et al., 1985).
Active levo-(6S)-leucovorin is inactivated about 50% faster than nonphysiologic dextro-(6R)-leucovorin by glucarpidase (Hempel et al., 2005). The cleavage site is distinct from the chiral carbon. Fifteen minutes after CPDG2 administration, median leucovorin and active 5-methyltetrahydrofolate concentrations dropped by 8% and >97% (Widemann et al., 1998). Remaining leucovorin was likely in the inactive d-form (Widemann and Adamson, 2006). In healthy volunteers, glucarpidase reduced active leucovorin and activated levo-5-methyltetrahydrofolate exposures by 50 and 100% despite a 2-h window between drug administration (European Medicines Agency [EMEA], 2008). Human in vivo glucarpidase activity against active leucovorin and activated levo-5-methyltetrahydrofolate has been shown to persist for at least 26 h (European Medicines Agency [EMEA], 2008). Because of this stereoselective destruction of active leucovorin and its metabolite, many protocols separate leucovorin administration from glucarpidase administration by 2–4 h.

Dexrazoxane

Following extravasation of anthracycline drugs, dexrazoxane [S(+)-1,2-bis(3,5-dioxopiperazin-1-yl)propane, ICRF-187] is used to diminish tissue damage and need for surgical excision of necrosis (Mouridsen et al., 2007). Its mechanism of action appears to involve reversible inhibition of topoisomerase II and inhibition by its metabolite, an ethylenediaminetetraacetic acid analog, of free radical formation via iron removal from the iron-doxorubicin complex (Hasinoff and Aoyama, 1999; Reeves, 2007). For this reason, dexrazoxane is also used to limit anthracycline-associated cardiomyopathy (van Dalen et al., 2008). Dexrazoxane was developed due to the limited solubility of racemic razoxane (ICRF-159) (Zhang et al., 1994). In vitro, both dexrazoxane and its stereoisomer levrazoxane (ICRF-186) are equally cytotoxic and inhibitory toward DNA topoisomerase II (Hasinoff et al., 1995). However, the enzyme dihydroprymidine amidohydrolase in liver and kidney stereoselectively catalyzes the first ring-opening step in dexrazoxane versus levrazoxane, and can subsequently open the second ring only in dexrazoxane, which is required for activity (Hasinoff, 1994; Hasinoff and Aoyama, 1999) At lower doses, corresponding to those used clinically, dexrazoxane improved survival in anthracycline treated animals, and at some doses additionally demonstrated increased cardioprotection and a decrease in immune effector cells (Hasinoff and Aoyama, 1999; Zhang et al., 1994). These differences disappeared at higher doses.

**D-Penicillamine**

Penicillamine is used in the treatment of copper poisoning and Wilson’s disease and has the advantage of oral availability. D-(S)- and L-(R)-penicillamine chelate copper equally. Penicillamine is second- or third-line therapy for lead and mercury poisoning. Differential toxicity of the L- and D-forms was reported as early as 1948—L-penicillamine inhibited growth and produced generalized seizures in rats (Wilson and Du Vigneaud, 1948). The L-enantiomer was also associated with impaired L-amino acid intestinal absorption (Wass and Evered, 1970), optic neuritis (Kean et al., 1991; Tu et al., 1963), nephrotic syndrome (Sternlieb, 1966) and pyridoxine antagonism (Williams, 1990). Historically, in the United Kingdom, only the d-form obtained from hydrolysis of penicillin was used, whereas U.S. manufacturers originally synthesized penicillamine from racemic valine (Walsh, 1992). Cases of thrombocytopenia and leukopenia became less prevalent when United States use of the racemic drug was discontinued (Williams, 1990).

**CARCINOGENICITY AND MUTAGENICITY**

DNA’s unique three-dimensional structure may interact with certain compounds stereospecifically (e.g., as previously detailed with cisplatin). Nucleoside analog chemotherapeutic agents (clofarabine, cytarabine, fludarabine, gemcitabine, etc.) intentionally imitate native nucleoside 2R,5SR structure in order to inhibit replication. Rats treated with (S)-N’-nitrosonomicotine, a tobacco-specific carcinogenic nitrosamine, generated three to five times higher levels of metabolite-DNA adducts than with (R)-N’-nitrosornomicotine in the esophagus, a known site of tumorgenesis (Lao et al., 2007). Epoxide transformations, in particular, provide the opportunity for stereospecific carcinogenicity or mutagenicity. P450 enzymes convert the dietary and respirable dust contaminant aflatoxin B1 to aflatoxin B1-8,9-epoxide, the toxicant. Exo-aflatoxin B1-8,9-epoxide interacts with DNA (forming guanine adducts in particular) and is least 500-fold more potent than the endo-stereoisomer (Fig. 7A) (Stewart et al., 1996). Similarly, P450 enzymes convert styrene to styrene 7,8-epoxide, also present in two enantiomeric forms. The R(+)-enantiomer is more mutagenic than the S(−)-enantiomer in S. typhimurium TA100 strains, with the racemic mixture intermediate between the two (Pagano et al., 1982). R(+)-styrene 7,8-epoxide depletes glutathione to a greater extent than S-styrene oxide (Carlson et al., 2006). Benzo[a]pyrene, a polycyclic aromatic hydrocarbon implicated in tumorogenesis, is stereospecifically metabolized by cytochrome P450 to the reactive ultimate toxicant (+)-7R,8S-dihydriodiol-9S,10R-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, which subsequently reacts to primarily form (+)-trans-guanine adducts (Mocquet et al., 2007). Nucleotide excision repair of the induced DNA lesions is also carried out in a stereospecific manner. 1,3-Butadiene is metabolized by P450 2E1 to butadiene monooxide, and subsequently undergoes epoxidation and epoxide hydrolysis to generate (R,R)- or (S,S)-butadiene diol-epoxide isomers. In an in vivo bacterial expression system, the (R,R)-enantiomer induced base pair A to G mutations exclusively, whereas adducts of the (S,S)-enantiomer were exclusively A to C mutations (Carnical
Chiral factors are implicated in the actual teratogenic mechanism of several compounds. Stereospecific maternal or fetal enzymes, fetal receptors, or transplacental transport mechanisms might all contribute to toxicity. Several examples follow where stereospecific mechanisms of human teratogenicity are supported. Animal embryologic toxicity of compounds primarily considered to be environmental pollutants is contained in a following section.

**REPRODUCTIVE TOXICITY**

Thalidomide

Thalidomide apparently works by tumor necrosis factor-alpha inhibition, inhibition of angiogenesis, and other mechanisms. \(R^+(+)-\)thalidomide was reported to be responsible for sedative effects (Eriksson et al., 2000; Hoglund et al., 1998), whereas \(S^-(−)-\)thalidomide and its derivatives were reported to be teratogenic (Fig. 7B) (Blaschke et al., 1979; Heger et al., 1994). It was further proposed that the thalidomide tragedy could have been avoided if the single \(R^+(+)-\)enantiomer had been used. Irrespective of the fact that an earlier study in an appropriate animal model demonstrated equivalent teratogenic potential of both isomers, which was greater than the racemate (Fabro et al., 1967), chiral inversion occurs with thalidomide (Reist et al., 1998). Humans interconvert \((S^−)-\) and \((R^+)-\)thalidomide enantiomers (Fig. 7B) rapidly with both oral and intravenous dosing (Eriksson et al., 2001). Albumin, hydroxyl ions, phosphate, and amino acids appear to mediate this effect (Reist et al., 1998). Therefore, even if single enantiomers of thalidomide were provided, the ensuing enantiomeric mixture could contribute to toxicity (Agranat et al., 2002). Similar inter-conversion has been demonstrated for thalidomide analogues (e.g., lenalidomide, EM 12, CC-4047), although certain substitutions can confer optical stability (Schmahl et al., 1988; Teo et al., 2003; U.S. Food and Drug Administration, 2005; Yamada et al., 2006). On the basis of the animal study demonstrating teratogenicity of both thalidomide isomers and evidence of human chiral inversion, exposure to either thalidomide enantiomer or unstable thalidomide derivatives during the period of sensitivity (days 20–36 after conception; Dencker and Eriksson, 1998) would risk fetal harm. Susceptibility might additionally be influenced by genetic polymorphisms which alter thalidomide metabolism (Ando et al., 2002) in the maternal/fetal unit.

Retinoic Acids

Following a 1979 landmark report, oral isotretinoin revolutionized the treatment of recalcitrant cystic and conglobate acne (Peck et al., 1979). Alitretinoin (9-cis retinoic acid for topical treatment of cutaneous Kaposi’s sarcoma lesions) and isotretinoin (13-cis retinoic acid) are isomers of tretinoin (all-trans retinoic acid) (Fig. 7C). Teratogenic effects are presumed to be mediated in part via transformation of alitretinoin and isotretinoin to \(R^+(+)\) retinoic acid (Adams, 1993). Variations in teratogenicity among the isomers also likely arise secondary to species-specific, stereoselective tissue transport (Collins and Mao, 1999). cis-isomerization of the retinoic acid side chain reduces the teratogenicity (Collins and Mao, 1999). However, transplacental exposures of therapeutic doses of isotretinoin were sufficient to produce well described, severe teratogenic effects—including spontaneous abortion; craniofacial, cardiac, thymic, and CNS malformations; motor and sensory deficiencies; and sex-susceptible cognitive impairment (Lammer et al., 1985; McCaffery et al., 2003). Persistent...
fetal exposures led to the creation of one of the most stringent risk management prescribing programs in the United States (iPLEDGE) (Honein et al., 2007). Excess exogenous retinoids disrupt the precise retinoic acid concentrations at specific stages of embryonic development which are required for induction of anterior-posterior development of the brain, dorsal-ventral development of the spinal cord, and some sexual dimorphic traits (McCaffery et al., 2003). Structure does confer receptor specificity—retinoic acid receptors (RARs) bind both all-trans and 9-cis retinoic acid, whereas only 9-cis retinoic acid binds to retinoid X receptors, which partner with RARs, vitamin D receptors, thyroid hormone receptors, peroxisome proliferator–activated receptors, and others (Germain et al., 2006a, b). The low (1–2%) percutaneous absorption of topical 0.05% tretinoin does not significantly increase systemic retinoid plasma concentrations above the range of natural endogenous levels (Thielitz and Gollnick, 2008). Although isolated case reports suggested a link between topical tretinoin exposure and fetal congenital abnormalities, several studies (215:430, 106:389, and 94:133 case-controls), failed to detect any effect (Jick et al., 1993; Loureiro et al., 2005; Shapiro et al., 1997). The U.S. FDA pregnancy classification for topical tretinoin (up to 0.1%) (category C), reflects this decreased perceived risk compared with the oral formulation (category X). While not U.S. FDA approved, systemic absorption of topical isotretinoin gel (up to 0.1%) is negligible despite repeated application (Thielitz and Gollnick, 2008). Although formal studies are lacking, daily application of topical alitretinoin gel (0.1%) (pregnancy category D) for up to 60 weeks did not yield detectable 9-cis-retinoic acid metabolites or 9-cis-retinoic acid plasma concentrations above those in untreated healthy volunteers (Eisai, Inc., 2007). Factors increasing dermal systemic absorption could alter teratogenic potential of the topical formulations.

Valproic Acid

Fetal VPA syndrome produces a consistent facial phenotype, systemic and orthopedic involvement, CNS dysfunction, and altered physical growth and cognitive development. It is thought to occur due to exposure at weeks 20–24 as the neural tube is closing. VPA and its S-derivatives appear to inhibit histone deacetylase, which leads to increased unfolding of chromatin and increased gene expression (Eikel et al., 2006). In particular, pattern-forming, homeobox A1 (Hoxa1) mRNA is produced in excess and outside of the normal developmental periods following VPA and 4-yn-VPA exposure (Stodgell et al., 2006). The VPA metabolite R(+) and S(−)-enantiomers of 2-n-propyl-4- pentenoic acid (4-en-VPA) and 2-n-propyl-4-pentyenoic acid (4-yn-VPA) significantly differ in teratogenicity (Nau et al., 1991). Almost four times more teratogenic than VPA, (S)-4-yn-VPA is 7.5 times more teratogenic than the (R)-isomer, and 1.9 times more teratogenic than the racemate, despite similar neurotoxicity (Hauck and Nau, 1992). Whole embryo cultures confirmed that (S)-4-yn-VPA produced dose-dependent dysmorphogenesis and embryo death in contrast to (R)-4-yn-VPA (Andrews et al., 1995).

ENVIRONMENTAL TOXICOLOGY

Many environmental contaminants are chiral, including organophosphorus compounds, organochlorines, pyrethroids, PCBs, polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), fipronil, and pharmaceutical contaminants. Degradation of these compounds, as well as compound bioaccumulation, persistence, and toxicity often show chiral dependence.

Organophosphorus Compounds

If the three substituent atoms are different, then the phosphorous atom is a chiral center (Fig. 8A). In geographically distinct areas, enantioselective degradation of organophosphorus compounds occurs (Lewis et al., 1999).

**FIG. 8.** (A) Enantiomers of substituted organophosphorus compounds (X ≠ Y ≠ Z). (B) Chiral centers of the pyrethroids permethrin, cypermethrin, cyfluthrin, and bifenthrin—the asterix (*) denotes chiral atoms. If the R1 and R2 substituents differ (e.g., in bifenthrin), then additional E- and Z-isomers are possible. If R3 contains a nonhydrogen substituent group such as CN, an alternative chiral carbon (#) is present. (C) PCB 139 (2,2′,3,4,4′,6-hexachlorobiphenyl)—one of several PCBs with stable atropisomers which have stereospecific effects in vivo. PCB atropisomers have generally been identified on the basis of optical activity, as assignment of absolute configuration is difficult.
Some bacteria contain phosphotriesterases capable of catalyzing the cleavage of the phosphate-oxygen bond and degrading the toxic organophosphate esters. Differences in geography, temperature, and deforestation can cause these bacteria to switch which enantiomer is preferentially degraded (Lewis et al., 1999; Wong, 2006). As might be anticipated from the previous discussion of acetylcholinesterase stereoselectivity in relation to physostigmine, significant enantiomer- and species-specific acetylcholinesterase susceptibility has been demonstrated for several organophosphorus insecticides. This occurs in such diverse species as Daphnia magna (water flea, the standard model of environmental toxicity), the electric eel, and humans (Nillos et al., 2007; Zhou et al., 2007). Therefore, chirality is an important aspect in ascertaining the environmental impact of organophosphorus compounds.

The stereospecificity seen in organophosphorus insecticides extends to chemical warfare nerve agents. Certain enantiomers are significantly more toxic, and show prolonged in vivo persistence due to variable stereospecific protein binding (Yeung et al., 2008). Variable acetylcholinesterase inhibition has been demonstrated for tabun, sarin, soman (with two chiral centers), and VX (Benschop and De Jong, 1988). These stereospecific differences might be anticipated given selectivity of the active site previously described for carbamates.

**Pyrethroids**

The pyrethroids, sodium channel opener insecticides derived from chrysanthemum flowers, have at least 2 chiral centers. Those with cyano-substitution at the α-carbon may have an additional chiral center (Fig. 8B). This generates 4 or 8 possible stereoisomers. Pyrethroids may isomerize slowly at the asymmetric α-carbon atom in polar solvents and in the presence of light (Wong, 2006). The trans-diastereomers of β-cypermethrin and β-cyfluthrin are selectively degraded in alkaline soils (Li et al., 2008). Pesticide-degrading bacteria selectively metabolize cis-bifenthrin and permethrin (Liu et al., 2005a). In geographically distinct separate soil samples, enantioselective microbial-mediated degradation was frequently observed for cis-bifenthrin, permethrin, and cyfluthrin; the rate was dependent upon variations in the microbial content, soil type, pH, and environmental conditions (Qin et al., 2006). Stereospecific toxicity has been observed for the pyrethroids. The (1R)-isomers of cycloprothrin were significantly more larvical than the (1S) enantiomers (Jiang et al., 2008). The two enantiomers of cis-bifenthrin and permethrin with the (1R) configuration, that is, (1R)-cis and (1R)-trans, were substantially more toxic to “bystander” water fleas (Ceriodaphnia dubia and Daphnia magna, an important food sources for many larger aquatic organisms) (Liu et al., 2005b). Only two of the eight cypermethrin and cyfluthrin enantiomers, (1R)-cis-alpha-S and 1R-trans-alpha-S, had significant activity, with 10–100 times more toxicity. Levometa-cyhalothrin was over 162 times more acutely toxic than dextro-lambda-cyhalothrin to zebrafish (Xu et al., 2008a). It was also more embryocidal and teratogenic. Cis-permethrin exposed mice had significant reproductive toxicity including reduced epididymal sperm counts and motility, and a decrease in testes and plasma testosterone levels not seen with the trans-isomer, which appeared to have faster metabolism (Zhang et al., 2008). Preliminary data also suggests that (1S)-cis-bifenthrin is a much more significant estrogen disruptor than the (1R)-enantiomer. (Wang et al., 2007).

**Polychlorinated Biphenyls, Dioxins, Dibenzofurans, and Organochlorines**

PCBs were used as coolants and lubricants in transformers, capacitors, and other electrical equipment. Although U.S. domestic production was banned in 1977, they remain present in numerous hazardous waste sites. In humans, exposure to PCBs has been linked to chloracne, ocular and pulmonary symptoms in adults, neurobehavioral and immunological changes in children, teratogenicity, and hepatic carcinoma (Aoki, 2001). Additional thymic, CNS, and reproductive toxicities exist in animals (Aoki, 2001; Tanabe, 1988). The 209 theoretical PCB cogener is named and numbered sequentially according to a variety of sources: IUPAC nomenclature, Chemical Abstracts Service number, and the original Ballschmiter and Zell enumeration as modified by several authors (Mills et al., 2007). Seventy-eight PCBs demonstrate axis chirality (existing as rotational nonsuperimposable isomers); 19 are stable atropisomers in biota (Fig. 8C) (Haglund, 1996). Several PCB atropisomers display enantio-specific P450 enzyme interactions (detailed in Haglund, 1996; Kania-Korwel et al., 2008). PCBs also demonstrate species-specific accumulation—for example, in pelicans, seals, and polar bears (Karasek et al., 2007; Wiberg et al., 1998). Furthermore, metabolite fractions are different from the original food-source, indicating enanti-selective formation, metabolism, transport, or clearance. Moreover, tissue-specific and organ-specific enantiomer-selective retention has been shown in rats. Compared with adipose and liver tissue, rat lungs had reversed enantiomer preferences for PCB 149 metabolites (Larsson et al., 2002). Enrichment of specific PCB 95, PCB 149, and PCB 132 isomers is reported in human liver samples (Chu et al., 2003). The concentration of at least five relevant PCB congeners are enriched in milk and dairy products from cows, goats, and ewes in a dairy-product and species-specific manner (Bordajandi and Gonzalez, 2008). This is presumed to occur through species-dependent metabolism as well as further transformations by microorganisms during the fermentation and ripening process. Lastly, levo-PCB 136 was recently shown to enantioselectively enhance ryanodine binding at ryanodine type 1 and 2 receptors and increase calcium flux, raising the possibility that chiral PCBs may have additional toxicological mechanisms (Pessah et al., 2009).
In addition to the multiple PCB isomers, 456 of 837 possible methylsulfonyl-PCB-derivatives are chiral (Nezel et al., 1997). The mercapturic acid pathway forms methyl sulfone metabolic products of PCBs (MeSO2-CB) (Karasek et al., 2007). Microbial reductive dechlorination— the major degradation mechanism for PCBs under anaerobic conditions—occurs in an enantiospecific manner for certain PCBs (e.g., PCB 91 and PCB 95) (Pakdeesusuk et al., 2003). Examining the specific isomers thus helps elucidate the contribution of biological activity (natural attenuation) versus chemical, distribution, and transport processes in contaminated ecosystems (Nezel et al., 1997). Although atmospheric PCB contributions are racemic, certain PCBs are nonracemic in water, suspended particulate matter, sediments and phytoplankton (which absorb PCBs by passive diffusion and lack the ability to metabolize them [Asher et al., 2007]). As discharge of water from a previously contaminated upstream source increased, the enantiomer fraction of PCBs was obtained down-stream diverged from the racemate, leading to the identification of transport of contaminated sediment as the major PCB source. Thus, the concept of chiral fingerprinting or “chiral signatures” of PCBs can now being applied to differentiate among PCBs source contamination from upstream river-water, storm-water runoff, sewer overflows, and atmospheric deposition from urban-generated PCBs (Asher et al., 2007).

As early as 1969 it was noted that the stereoisomers of cyclodiene insecticides could produce different toxicity. Mice fed endrin at 5 ppm suffered a 33% mortality, whereas dieldrin (its stereoisomer) was no different than control (Good and Ware, 1969). Both compounds reduced the litter size of offspring. Similar to the multiple PCB congeners, PCDD molecules can exist in 75 possible planar isomers. Isomer toxicity varies greatly by factors of 1000 to 10,000 (Boening, 1998). Isomeric analysis of agricultural soils permits source determination of PCDDs and PCDFs (municipal incinerators versus agrochemical impurities) (Xu et al., 2008b). Depending on soil type and location, preferential degradation of either enantiomer of o,p’-DDT may occur (Aigner et al., 1998; Li et al., 2006). The (−)-o,p’-DDT enantiomer is an active estrogen mimic at the human estrogen receptor, whereas (+)-o,p’-DDT was negligible (Hoekstra et al., 2001). Thus, the environmental impact of organochlorines must be approached from a geo-local, species-specific, and organ-specific perspective.

**Fipronil**

Fipronil, a racemic phenylpyrazole pesticide, is a noncompetitive GABA receptor antagonist available in the United States since 1996. Humans exposed to fipronil may have headache, nausea, and seizures, and prolonged GABA<sub>A</sub> receptor blockade has been demonstrated in mammalian brains (Li and Akk, 2008). Fipronil is also used in commercial pet products. Fipronil’s environmental fate is sediment-dependent: preferential transformation of S(+) fipronil occurs in anoxic sulfidogenic sediment, whereas preferential transformation of R(−)-enantiomer in methanogenic anoxic sediment (Jones et al., 2007). In a geographically disparate area, under flooded (anaerobic) conditions, (S)-fipronil was preferentially degraded in methanogenic soil samples, whereas no selectivity was noted under aerobic conditions, a difference attributed to alternative pH, temperature, soil water content, organic matter content or microbial populations (Tan et al., 2008). Determination of the persistent enantiomer is important to assess environmental risk. Although highly selective to insect nerve cells, “aquatic bystanders” are susceptible to species-specific enantiomer toxicity of fipronil. *C. dubia* is selective affected by the (S)-enantiomer (Wilson et al., 2008). Similarly, crayfish are significantly more sensitive to the (S)-enantiomer, whereas larval grass shrimp are significantly more sensitive to the (R)-enantiomer (Overmyer et al., 2007). Highlighting the economic importance of understanding these complex enantiomer relationships, the introduction of rice seed treated with fipronil produced major losses in Louisiana crayfish harvests, and degradation products were found to persist for several years (Bedient et al., 2005). Fipronil is rapidly biotransformed by the rainbow trout, which selectively transform the (S)-enantiomer. The sulfone metabolite has a thrice-greater half-life and thus may bioaccumulate (Konwick et al., 2006). Future environmental assessment of fipronil will have to account for local conditions, enantio-selective food-source toxicity, and bioaccumulation.

**Pharmaceutical Contaminants**

Pharmaceuticals may contaminate the environment following unchanged parent compound elimination from humans or animals or active disposal in solid or liquid waste streams. Pharmaceutical residues persist nearly year round in some major waterways (Comeau et al., 2008; Sacher et al., 2008). However, relatively little work has been done on chiral aspects of these xenobiotics or their significance. The NSAIDs and ibuprofen in particular can be found in many rivers and lakes; the active (S)-enantiomer was preferentially degraded in lake water (Buser et al., 1999). Nonpharmacologically active R(−)-ibuprofen was degraded more rapidly by microorganisms in biofilm reactors than the pharmacologically active isomer (Winkler et al., 2001). In aquatic toxicity studies, growth and feeding of *P. promelas* (fathead minnow) were more adversely affected by S-fluoxetine than R-fluoxetine, whereas water fleas did not have stereospecific susceptibility (Stanley et al., 2007). The enantiomeric fraction of R(+)-propranolol decreases following wastewater treatment, and thus water samples which contained racemic mixtures of propranolol confirmed suspected discharges of untreated sewage (Fono and Sedlak, 2005). Similar studies have examined atenolol and metoprolol isomers of wastewater effluent (Nikolić et al., 2006). (R)-propranolol has greater effects on reproduction than either the racemate or the (S)-isomer at the highest tested dose in water fleas, whereas growth was affected more by (S)-propranolol in *P. promelas*,
similar to mammals (Stanley et al., 2006). Residues of 4-
methylbenzylidene camphor, an organic ultraviolet filter used
in personal care sunscreens products, could be modified by
enantioselective biodegradation and in lakes and in fish (Buser
et al., 2005).

**FORENSIC TOXICOLOGY**

In forensics, chiral principles can be applied to both to drug/
product seizures and to biological samples. It has been
estimated that over half of illicit compounds possess at least
one chiral center (Mile, 2005). Truxilline, a tropine alkaloid, is
present as 11 stereoisomers in the coca leaf. Resolution of these
and other stereoisomers of cocaine-associated impurities can
provide a manufacturing fingerprint for law-enforcement
intelligence and strategic purposes (Tagliaro et al., 2007).
Illicitly produced heroin is commonly “cut” with sugars; thus,
the chiral analysis of these in addition to other excipients
(phenacetin, caffeine, etc.) may provide important information
for forensic purposes (Lurie, 1998; United Nations Office on
Drugs and Crime, 2005). Chiral analysis can be applied to
other drug seizures. For example, presumed methamphetamine
seizures might demonstrate levo-ephedrine and dextro-
methamphetamine, which would be consistent with levo-ephedrine
as the precursor material. Such analysis can be extended to
other phenylethylamine derivatives such as “ecstasy” (com-
monly 3,4-methylenedioxyamphetamine) and N,N-dime-
ythylamphetamine to provide clues to precursor materials and
synthesis pathways (Lee et al., 2007; Tagliaro and Bortolotti,
2008). Due to the very different U.S. DEA scheduling of
racemic methorphan (CII, not clinically available in the United
States), levomethorphan (CII, not clinically available in the
United States) and dextromethorphan (unscheduled), forensic
determination of methorphan isomers seizures must be
performed in order to support illegality (Lurie and Cox, 2005).

Chiral principles must be applied to correctly interpret the
significance of “positive” biological samples from employ-
ment or post-mortem drug testing. As an example, certain
amphetamine or methamphetamine enantiomer compositions
may or may not be appropriate following a reported history of
ingestion (Fig. 9). Methamphetamine is metabolized unidi-
rectionally to amphetamine (and 4-hydroxyamphetamine) (Jirovsky et al., 1998). d-Methamphetamine is metabolized
more rapidly than the l-enantomer; thus, the enantiomer ratio
will change over time (Cody, 2002). Pharmacodynamic
differences include induction of higher systolic blood pressures
and more prolonged and psychologically desirable effects with
the d-isomer (Mendelson et al., 2006). Finding methamphet-
amine would undermine a claim of medical legitimacy in xenobiotics composed of or directly metabolized to amphetamine—
for example, one would expect to find a mixture of d- and
l-amphetamine, but not methamphetamine in individuals
consuming Adderall [d,l-amphetamine salts (3:1), DEA
schedule II] (Barr Laboratories, 2007). Similarly, Dexedrine
dextroamphetamine sulfate, DEA schedule II) should only
result in serum concentrations of d-amphetamine, and only
d-amphetamine should be detected following ingestion of
Vyvanse (lisdexamfetamine dimesylate, DEA schedule II), a
dextroamphetamine pro-drug (Fig. 9). Vicks Vapor Inhaler
(unscheduled) contains 50 mg of l-methamphetamine (per
inhaler). Forensic specimens should contain only the l-isomer
and the l-amphetamine metabolite. Conversely, Desoxyn
(d-(S)-methamphetamine hydrochloride, DEA schedule II)—
prescribed for attention deficit disorder with hyperactivity and
exogenous obesity—should yield solely the d-isomers. Didrex
(d-benzphetamine, DEA schedule III) is an anti-obesity drug
marketed in the United States. Metabolism to d-methamphet-
amine and separate metabolism to d-amphetamine result in
much higher d-amphetamine to d-methamphetamine ratios
(mean = 2.4) than normally seen in methamphetamine abuse
(without generation of l-isomers) (Cody, 2002). Elderly
(selegiline hydrochloride, unscheduled), used in the treatment
of Parkinson’s disease, is manufactured only as the l-isormer. A
certain percentage is metabolized to (only) l-methamphetamine
and l-amphetamine. Clobenzorex is an anorexic drug now
illegal in US, but abused in professional sports. It is available
abroad (as Asenlix, Dinintel, and Finedal) as part of un-
approved diet regimens utilized by U.S. residents (U.S. Food
and Drug Administration, 1987). Approximately 5% is
converted to racemic amphetamine (Baden et al., 1999).
Fenproporex (DEA schedule IV) is appetite suppressant used
outside U.S. FDA action occurred in 2006 because the
“Brazilian Diet Pill” contained this compound and resulted
in workplace drug testing “failures” (U.S. Food and Drug
Administration, 2006). Roughly 25–35% is converted to
racemic amphetamine. Methamphetamine and amphetamine
metabolites of mephedrine (CIV), another anorectic marketed
primarily in Europe, may persist beyond the parent compound
(Engel et al., 1986; Musshoff, 2000). Gewodin is an over the
counter antipyretic/analgesic multi-ingredient product available
in Germany, Taiwan, and elsewhere, containing acetamino-
phen, propyphenazone (isopropylphenazone), caffeine, and
famprofazone (Musshoff and Kraemer, 1998). Stereoselective
famprofazone metabolism leads to significantly more
l-methamphetamine production than l-amphetamine or the
d-isomers (Rodriguez et al., 2004). With wide interindividual
variability, the calcium channel antagonist and anti-anginal
prenylamine (unscheduled) metabolizes to significantly more
d-metabolites (and d-amphetamine) than l-amphetamine
(Kraemer et al., 2003; Liu and Liu, 2002). Amphetaclear
(INN: amfeclaral) is a phenethylamine derivative which was
patented in 1960 as an amphetamine pro-drug with prolonged
duration of action (Cavallito, 1960; Kolliker and Oehme,
2004). Metabolism yields chloral hydrate plus d-amphetamine
or d,l-amphetamine, depending on the method of preparation
(Fig. 9). Other compounds which metabolize to methamphet-
amine or amphetamine include amphetaminil (unscheduled),
dimethylamphetamine (CI), ethylamphetamine (CI), fenacrine (unscheduled), fenethylline (CI), furfuril (unscheduled), and mesocarb (unscheduled) (Cody, 2002; Liu and Liu, 2002; Musshoff, 2000). Thus, a detailed history of reported xenobiotic ingestion and appropriate isomer analysis may support or refute a claim of legitimate pharmaceutical ingestion or aid in the determination of cause of death.

Chiral analysis has been applied to screen athletes for exogenous administration of androgens since 1983 (Bowers, 2008). The endogenous steroid profile of testosterone (T) to its enantiomer epitestosterone (E)—the urinary T/E ratio—occurs in populations in two modal distributions in both sexes at about 0.1 and 1.0 (Bowers, 2008). Rare cases of physiologically high T/E ratios (between 6 and 12) and low T/E ratios (due to deletion polymorphism in the uridine diphospho-glucuronosyl transferase 2B17 gene) are reported (Dehennin, 1994; Schulze et al., 2008). According to the rules of the World Anti-Doping Agency, an E/T glucuronide ratio equal to or exceeding 4 to 1 is an atypical result suggestive of exogenous androgenic steroid administration and requires further investigation (World

![FIG. 9. Pharmaceuticals that contain or are metabolized to amphetamine isomers and methamphetamine isomers. Solid lines indicate that no metabolism is required; dotted lines indicate that metabolism occurs. Amphelcral, clobenzorex, fenproporex, and prenylamine are metabolized to both d-(S)- and l-(R)-amphetamine.](image-url)
Anti-Doping Agency, 2007). T/E ratios can be coupled with evaluation of $^{13}$C/$^{12}$C ratios (as pharmaceutically produced anabolic steroids exhibit a depleted ratio on account of their plant origin) to provide additional evidence of a doping offense (Piper et al., 2008).

CONCLUSION

Chiral considerations are relevant to diverse aspects of toxicology and pharmacology. The additional complexity permits a more complete and precise understanding of toxicological pathophysiology. Evaluation of chiral compounds must take into account three-dimensional structure-activity relationships, which may take on varied importance at different receptor types. Local conditions species and tissue differences and population polymorphisms may additionally influence enantiomer ratios and xenobiotic effects.

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