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Neonicotinoid Metabolism: Compounds, Substituents, Pathways, Enzymes, Organisms, and Relevance

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ABSTRACT: Neonicotinoids are one of the three principal insecticide chemotypes. The seven major commercial neonicotinoids are readily biodegraded by metabolic attack at their *N*-heterocyclylmethyl moiety, heterocyclic or acyclic spacer, and *N*-nitroimine, nitromethylene, or *N*-cyanoimine tip. Phase I metabolism is largely dependent on microsomal CYP450 isozymes with situ selectivity in hydroxylation, desaturation, dealkylation, sulfoxidation, and nitro reduction. Cytosolic aldehyde oxidase is a nitroreductase for some neonicotinoids. Phase II metabolism involves methylation, acetylation, and formation of glucuronide, glucoside, amino acid, and sulfate- and glutathione-derived conjugates. Some neonicotinoids act as proinsecticides with metabolism to more potent nicotinic agonists. Pest resistance is more commonly due to synergist-reversible CYP450 detoxification than to nAChR mutants or variants. Metabolites in some cases contribute to mammalian hepatotoxicity and carcinogenesis and in others to enhanced plant vigor and stress shields. These relationships explain much of neonicotinoid comparative toxicology and provide the basis for continued and improved safety and effectiveness of this chemotype.

KEYWORDS: Aldehyde oxidase, clothianidin, CYP450, imidacloprid, insecticide metabolism, neonicotinoids, thiamethoxam

INTRODUCTION

Two decades of increasing use of neonicotinoids have established their important role in protecting crops and pets from pest insect attack.[1−11] The 7 major commercial neonicotinoids consist of 3 structural components (**A**, **B**, **C**) and 12 unique substituents (Figure 1). Thorough metabolism studies were made for imidicloprid (IMI),[12−16] nitenpyram (NIT),[16] thiacloprid (THI),[17,18] acetamiprid (ACE),[16, 19] clothianidin (CLO),[20−22] thiamethoxam (TMX),[23,24] and dinotefuran (DIN)[25] to define their distribution, persistence, and metabolic fate as required for registration and establishment of tolerance values. These studies on individual compounds usually compared rats, goats, hens, and plants with the same general pathways in each organism. In addition, comparative studies of compounds within the neonicotinoid chemotype are of interest in evaluating primary and secondary toxicity mechanisms, residues, and resistance. Our laboratory at Berkeley therefore examined the metabolism of this set of seven neonicotinoids in mice[26,27] and spinach[28] with findings that serve as the basis for much of this review. References for specific products or reactions in the figures and schemes that follow are generally given or referred to in the literature cited above.

Neonicotinoids act at the nicotinic acetylcholine receptor (nAChR) agonist site, where their potency and effectiveness are determined primarily by the structural features of the overall molecule.[5,8] In contrast, the individual substituents determine biodegradability and the metabolites formed. It is therefore appropriate to consider neonicotinoid metabolism substituent by substituent followed by the pathways observed for the molecules as a whole. The substituent reactions are gleaned from the compound pathways that follow.

COMPOUNDS AND SUBSTITUENTS

***N*-Heterocyclylmethyl Moieties (Figure 2).** *N*-Methylene hydroxylation of each neonicotinoid oxidatively cleaves the molecule at **A**−**B** into the amine moiety and heterocyclic aldehyde, which is reduced in small part to the alcohol but mostly oxidized to the acid. The pyridinyl (**A1**) and thiazolyl (**A2**) chlorine substituents are partially displaced by glutathione (GSH), leading ultimately to the *N*-acetylcysteine and *S*-methyl metabolites. 6-Hydroxypyridinyl-3-carboxylic acid and the corresponding *N*-methylpyridone are also formed. The tetrahydrofuryl moiety (**A3**) is hydroxylated at each ring methylene with extensive ring-opening.

Heterocyclic or Acyclic Spacer (Figure 3). The IMI imidazolidine moiety (**B1**) is hydroxylated at either one or both of the methylene substituents without ring-opening, and there is also desaturation to the olefin and conversion to the desethano acyclic compound. The sequence of these reactions has been suggested as shown in one system[29] but not firmly assigned on a general basis. The NIT spacer (**B2**) is *N*-demethylated, *N*-deethylated, or both. The THI thiazolidine (**B3**) undergoes methylene hydroxylation or desaturation or, alternatively, via the sulfoxide, yields the ring-opened methyl sulfoxide or sulfonic acid. The spacer moieties of ACE (**B4**) and CLO or DIN (**B5**) undergo facile *N*-demethylation with remethylation for desmethyl-CLO reverting to CLO once again. The oxadiazine moiety of TMX (**B6**) is *N*-demethylated or cleaved after hydroxylation of the OCH_2 moiety to the corresponding CLO and *N*-desmethyl-CLO. The hydroxyoxadiazine, *N*-hydroxymethyl, or *N*-formyl intermediates have not been reported.

***N*-Nitroimine, Nitromethylene, or *N*-Cyanoimine (Figure 4).** The electronegative *N*-nitro tip (=N—NO_2) (**C1**), a critical feature for selective binding to the insect nAChR,

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neonicotinoid	compound abbrev.	structural components
chloropyridinyl (CP)		
imidacloprid	IMI	A1 B1 C1
nitenpyram	NIT	A1 B2 C2
thiacloprid	THI	A1 B3 C3
acetamiprid	ACE	A1 B4 C3
clorothiazolyl (CT)		
clothianidin	CLO	A2 B5 C1
thiamethoxam	TMX	A2 B6 C1
tetrahydrofuryl (THF)		
dinotefuran	DIN	A3 B5 C1

A heterocyclylmethyl moiety
B heterocyclic or acyclic spacer
C $=NNO_2$, $=CHNO_2$ or =NCN tip

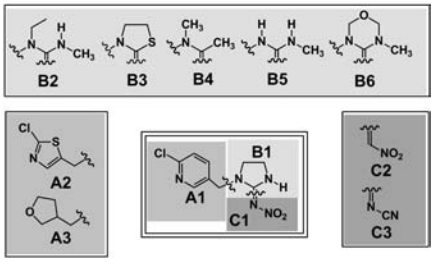

Figure 1. Seven commercial neonicotinoids consisting of 3 structural components (**A**, **B**, **C**) and 12 unique substituents (bottom).

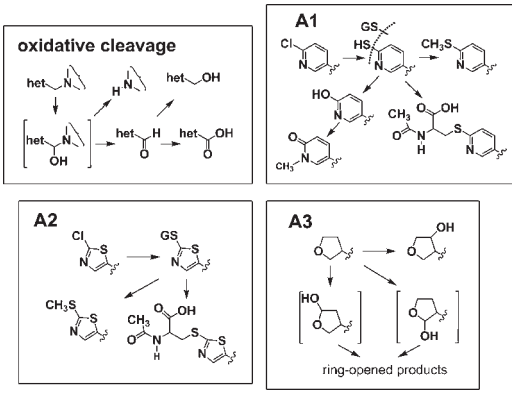

Figure 2. Metabolic cleavage of the *N*-heterocyclylmethyl moiety (**A**-**B**) and further modification of the chloropyridinyl (**A1**-**CP**), chlorothiazolyl (**A2**-**CT**), and tetrahydrofuryl (**A3**-**THF**) substituents.

is reduced to the *N*-nitroso- and *N*-aminoguanidines (=N—NO and $=N—NH_2$) and also yields the corresponding =NH (guanidine) and =O (urea) by unknown pathways. The *N*-aminoguanidine reacts with methylglyoxal or a pyruvate derivative to give the methyltriazinone.[30] The nitromethylene ($=CH—NO_2$) tip (**C2**) is converted to the cyano derivative[31] and carboxylic acid, which undergoes decarboxylation.[32] The *N*-cyanoimine (=N—CN) moiety (**C3**) is cleaved to the guanidine or hydrolyzed to the *N*-carbamoylimine.

PATHWAYS

Chloropyridinyl Compounds. The substituent reactions above can be combined to give the IMI metabolites observed (Figure 5). The 4-hydroxy, 4-*O*-glucuronide, 5-hydroxy, 4,5-dihydroxy, olefin, and desethano compounds are formed with the unchanged *N*-nitroguanidine functionality. In another set of metabolites the *N*-nitroguanidine is reduced to the *N*-nitroso-guanidine and *N*-aminoguanidine with cleavage to the guanidine and hydrolysis to the urea (observed also as the *N*-glucoside and *N*-gentiobioside in spinach). A portion of the $=N—NH_2$ compound is converted to the methyltriazinone. The imidazolidine and imidazoline cleavage products are observed from IMI and the corresponding olefin, respectively. NIT (Figure 6) undergoes facile *N*-deethylation and *N*-demethylation, and the nitromethylene is converted to the cyano, carboxylic acid, and decarboxylated derivatives. The cleaved amine fragment is observed per se and as the glucuronide. THI (Figure 7) is converted to the 4-hydroxy, olefin, and sulfoxide derivatives. The sulfoxide cleaves to the ring-opened compound, which undergoes methylation with sulfoxidation to the methyl sulfoxide. Alternatively, the cleaved sulfenic acid is oxidized to the sulfonic acid. Cleavage or hydrolysis of the N—CN bond gives the descyano and *N*-carbamoylimine derivatives, respectively. ACE (Figure 8) undergoes *N*-demethylation and hydrolysis of the *N*-cyano substituent to the *N*-carbamoylimine of both. Cleavage to *N*-(6-chloropyridinyl-3-methyl), *N*-methylamine, or *N*-(6-chloropyridinyl-3-methyl)amine is followed by acetylation. The *N*-descyano compound is also observed, as are the *N*-methylene cleavage products of ACE and its *N*-desmethyl derivative. Additional metabolites on direct administration of 6-chloropyridinyl-3-carboxylic acid (Figure 9) are formed by GSH conjugation at the 6-position and cleavage to give the *S*-methyl, *S*-mercapturic acid, and hydroxy derivatives as glycine and aspartate conjugates and the phenyl sulfate. *N*-Methylpyridonecarboxylic acid is also formed. 6-Chloropyridinyl-3-carboxylic acid gives a variety of amino acid conjugates in spinach and is also methylated in part. The glucoside and gentiobioside of 6-chloropyridinyl-3-methanol are also observed in spinach

Chlorothiazolyl Compounds. TMX initially undergoes any one of three reactions: demethylation to desmethyl-TMX; nitro reduction to =N—NO followed by the =N—H and =O series for both TMX and desmethyl-TMX; or cleavage of the oxadiazine to give CLO (Figure 10). The metabolites of CLO (also observed from TMX) are primarily *N*-demethylation (and remethylation) products with nitro reduction to =N—NO and cleavage to =N—H and =O with or without demethylation (Figure 11). Four substituted guanidines and an amine from **A**−**B** cleavage are found in mice and spinach. The chlorothiazolyl moiety (Figure 12) undergoes a variety of conjugation reactions with glycine and glucuronic acid, and the 2-chlorothiazolyl-5-methylamine is acetylated. Although the GSH conjugate by replacement of chlorine in the 2-position is not observed, it proceeds to yield the methylthio and mercapturate derivatives of 2-chlorothiazolyl-5-carboxylic acid and the glycine conjugate of the former compound.

Tetrahydrofuryl Compound. DIN (Figure 13) gives the most complicated pathway because of the $=N—NO_2$ reduction and cleavage and *N*-demethylation combined with the ring-opening reactions of the tetrahydrofuryl moiety. In some series the $=N—NO_2 \rightarrow =N—NO \rightarrow =N—NH_2$ plus =N—H and =O sequences are all observed. Tetrahydrofuryl hydroxylation at the 2- or 5-position with ring-opening gives a large series of cyclic and acyclic metabolites, respectively, again with various degrees of $=N—NO_2$ modification. Additional metabolites are tetrahydrofurylmethylamine alone or acetylated, tetrahydrofurylcarboxylic acid and 3-hydroxytetrahydrofurylcarboxylic acid free and glycine conjugates, and the guanidine and *N*-nitroguanidine from **A**−**B** cleavage.

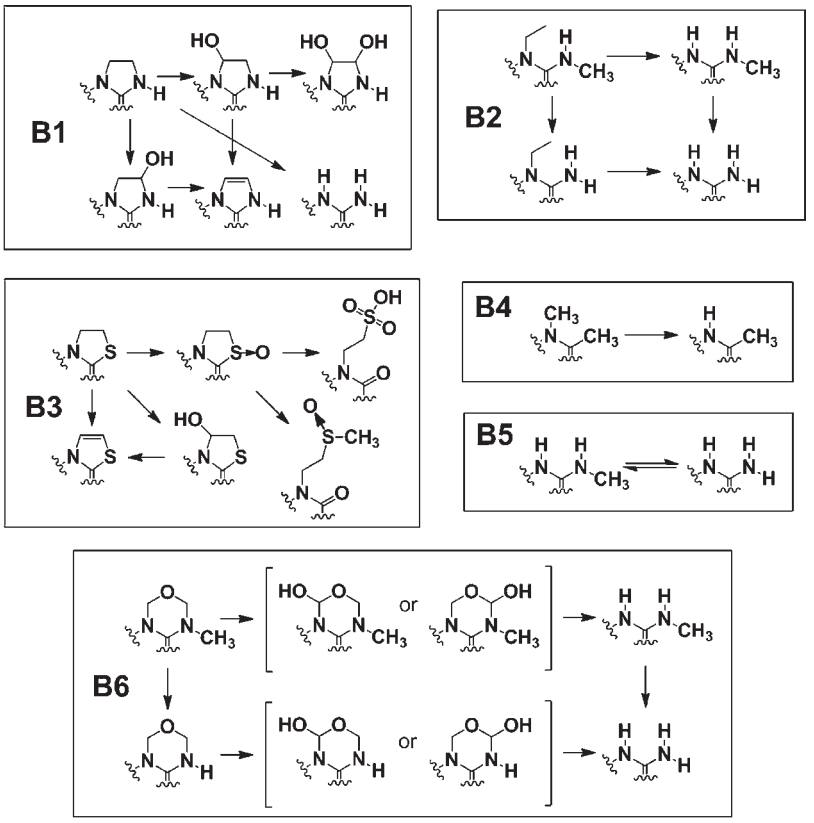

Figure 3. Metabolic modification of the heterocyclic or acyclic spacer substituents for IMI (**B1**), NIT (**B2**), THI (**B3**), ACE (**B4**), CLO and DIN (**B5**), and TMX (**B6**).

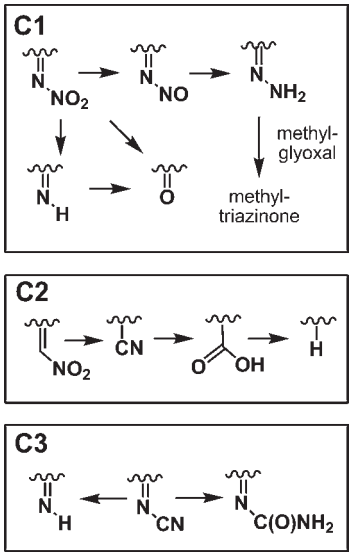

Figure 4. Metabolic modification of the *N*-nitroimine (**C1**), nitromethylene (**C2**), or *N*-cyanoimine (**C3**) tip.

■ ENZYMES AND MICROBIAL OR INSECT SYSTEMS

CYP450s. Microsomal CYP450s carry out most of the phase I reactions considered above with isozyme situ specificity. CYP2D6 is selective for IMI nitro reduction, and CYP3A4 is selective for 5-hydroxylation with some olefin formation.[33] CYP3A4 is also very effective for TMX conversion to CLO.[34] IMI resistance in *Drosophila melanogaster* correlates with *Cyp6g1* overexpression and cross-resistance to DDT.[35] A binding site model has been presented for C5 hydroxylation of IMI by *Bemisia tabaci* CYP6CM1vQ (Figure 14).[36]

Aldehyde Oxidase (AOX). Reduction of the IMI nitro group is only due in part to microsomal CYP450 with NADPH.[37] The cytosolic fraction of liver is also involved and does not require NADPH. More specifically, cytosolic aldehyde oxidase (AOX) serves as a neonicotinoid nitroreductase when assayed with a cosubstrate such as *N*-methylnicotinamide.[38] *N*-Nitroso-IMI is an irreversible AOX inhibitor and may therefore give product inhibition on IMI metabolism.[39] There is a large magnitude of species specificity in liver cytosolic AOX metabolism of IMI, with rabbit most active, monkey, human, mouse, cow, and rat intermediate, and dog, chicken, and cat least effective.[38] Rabbit liver AOX is most active on CLO and desmethyl-TMX, with DIN and IMI intermediate, and NIT and TMX most slowly reduced.[31] The activity of insect AOX[40] in neonicotinoid metabolism has not been reported.

Microorganisms. IMI is not metabolized by microorganisms in bovine rumen fluid, which readily reduces the nitro group of several nitroaromates.[37] Microbial metabolism of pesticides is of special interest because of environmental implications and possible application to bioremediation of contaminated soils. *Stenotrophomonas maltophilia* GGMCCL.1788 hydroxylates IMI in the 5-position[41] and THI in the 4-position[42] and *N*-demethylates ACE.[43] *Pseudomonas* sp.1G converts IMI and TMX to their *N*-nitrosoguanidine, guanidine, and *N*-carbamoylimine metabolites.[44] *Rhodotorula mucilaginosa* strain IM-2 converts ACE to the *N*-acetyl derivative of 6-chloropyridinyl-3-methylamine and hydrolyzes THI to the corresponding

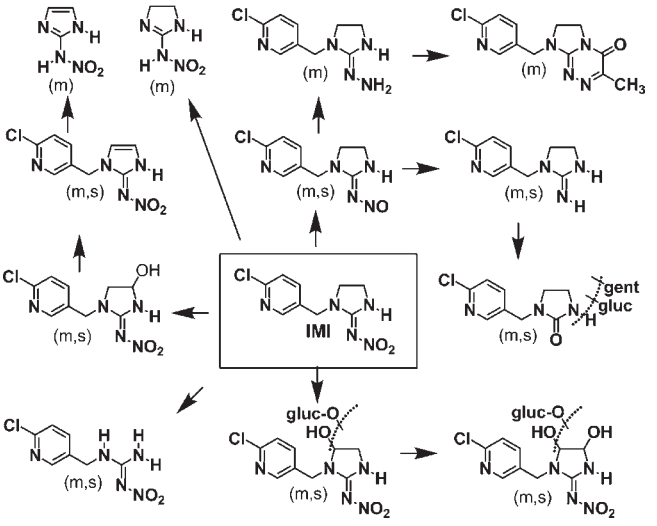

Figure 5. Metabolic pathways of IMI in mice (m) and spinach (s).

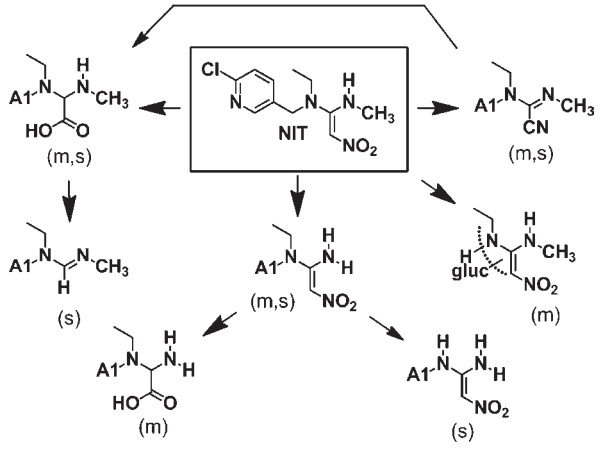

Figure 6. Metabolic pathways of NIT in mice (m) and spinach (s).

N-carbamoylimine.[45] The toxicology of the metabolites is an important factor in the effectiveness of detoxification.

Insects. The first steps have been taken in defining the metabolic fate of neonicotinoids in insects. *Musca domestica* oxidizes nithiazine (the starting point for neonicotinoid chemistry) at the nitromethylene carbon[32] and metabolizes IMI mostly to the corresponding olefin.[46] *Leptinotarsa decemlineata* also converts IMI to the olefin.[47] *B. tabaci* oxidizes IMI to 5-hydroxy-IMI.[48] *Apis mellifera* converts IMI to 5-hydroxy-IMI and the olefin[49,50] and *N*-demethylates ACE.[51] *Spodoptera frugiperda* larvae convert TMX to CLO.[24] Some of the metabolites retain high insecticidal activity or agonist potency, for example, the olefin from IMI [52] and CLO from TMX.[24] The importance of neonicotinoid metabolism in species specificity and resistance is considered below.

RELEVANCE

Phase I and Phase II Metabolites (Figure 15). Each neonicotinoid has multiple sites of initial metabolic attack (phase I) and ultimately forms a series of conjugates (phase II) for excretion from animals or retention in plants. There are some common metabolites for all CP neonicotinoids and others for the CT neonicotinoids, allowing identification of the chemotype involved as residues or in accidental exposure or intentional poisoning. The primary concern

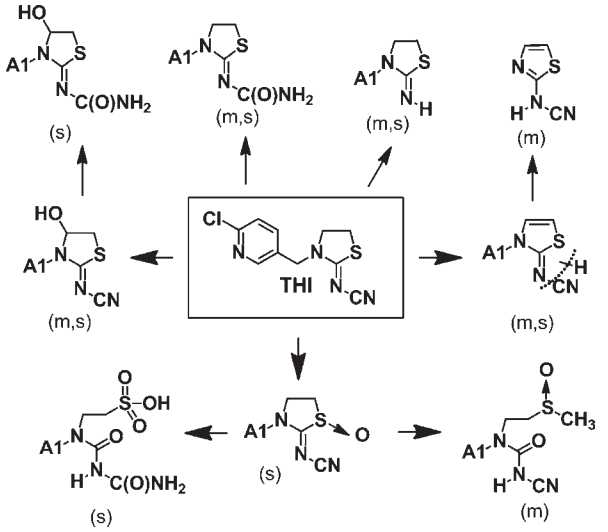

Figure 7. Metabolic pathways of THI in mice (m) and spinach (s).

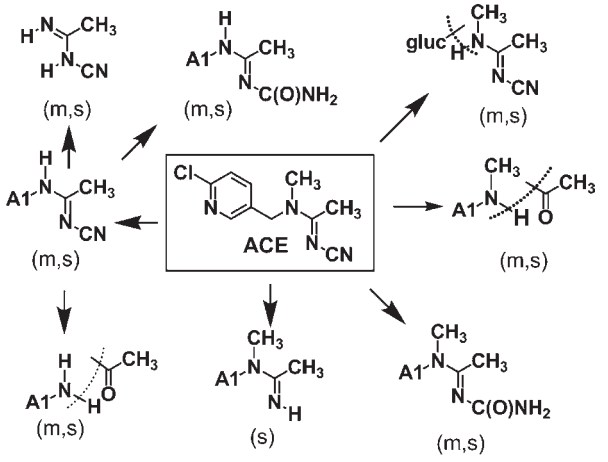

Figure 8. Metabolic pathways of ACE in mice (m) and spinach (s).

is to identify and evaluate the phase I metabolites that may be bioactive, whereas the conjugation reactions are generally detoxifications except possibly the *N*-methylation.

Nicotinic Receptor Potency. Neonicotinoid and nicotinoid agonist action and toxicity are generally related to their binding affinities at the relevant nAChRs.[5,8] Thus, nAChR potency is a good predictor of neonicotinoid toxicity if the relevant nAChR is assayed and the effects of metabolic activation or detoxification are minimized with a highly effective synergist.[53] Correspondingly, small modifications in molecular structure alter both the potency and nAChR subtype selectivity.[54] As one illustration, the change from a *N*-nitroimine or *N*-cyanoimine to their guanidine metabolites, that is, from a neonicotinoid to a nicotinoid, involves a dramatic shift from insect-selective to vertebrate-selective action (Table 1). From another standpoint, the failure to find a correlation between agonist potency and toxicity indicates either that the target site assay is not relevant or that activation and detoxification are not adequately blocked or accounted for. As an example, TMX either has a binding mode different from that of most neonicotinoids in some insect receptors[59] or undergoes metabolic activation to CLO[24] as considered below.

Proneonicotinoids (Figure 16). Several neonicotinoids are known or proposed to be proinsecticides undergoing metabolic

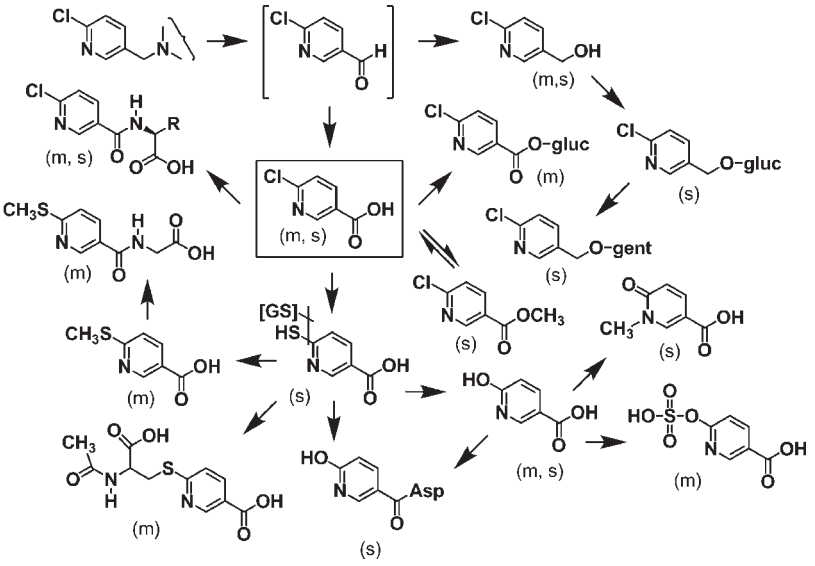

Figure 9. Metabolic pathways of the chloropyridinyl moiety in mice (m) and spinach (s).

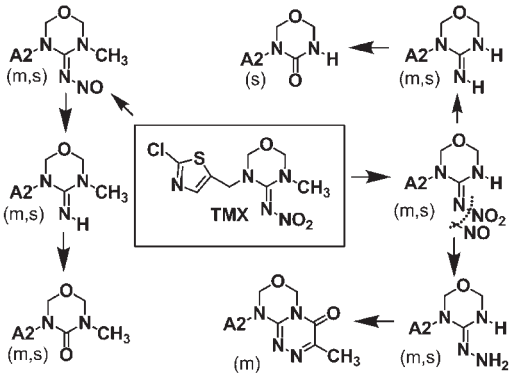

Figure 10. Metabolic pathways of TMX in mice (m) and spinach (s).

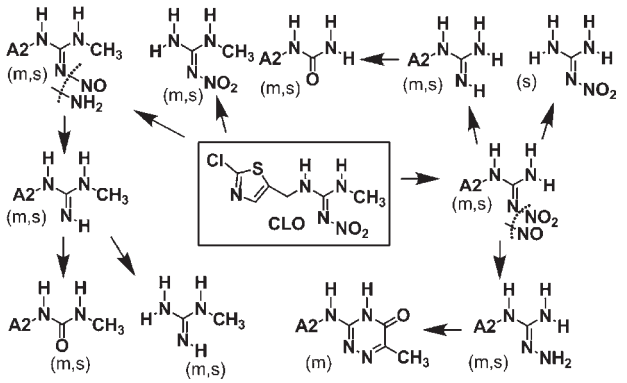

Figure 11. Metabolic pathways of CLO in mice (m) and spinach (s).

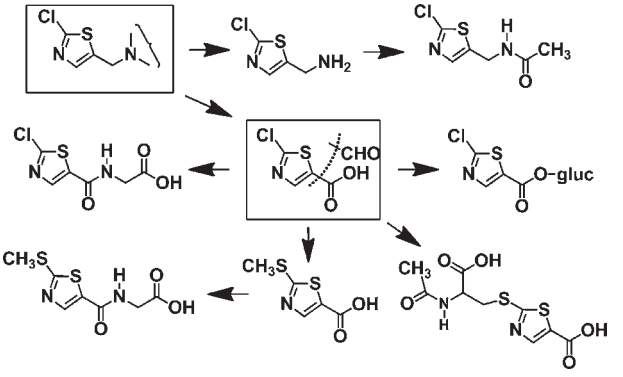

Figure 12. Metabolic pathways of the chlorothiazolyl moiety in mice (m) and spinach (s).

activation or chemical modification to the ultimate active agents. One type is the masked IMI derivatives including *N*-methyl-IMI, which is oxidatively *N*-demethylated to IMI,[60,61] and the oxodioxolylmethyl analogues, which hydrolytically decompose to IMI.[62] Interestingly, bis-IMI with a six or seven methylene tether acts directly rather than as an IMI precursor.[63,64] A second type is the masked CLO derivatives including TMX, which is oxidatively metabolized by CYP450s to *N*-desmethyl-TMX and CLO, both potent insecticides or agonists.[24,34] Other compounds presumed to be pro-CLO derivatives are the TMX analogues with the $-CH_2OCH_2-$ moiety replaced by $-CH_2SCH_2-$, $-CH_2NHCH_2-$, and $-CH_2C(OCH_3)_2CH_2-$ undergoing chemical or metabolic conversion to CLO.[65,66] A third class of potential proneonicotinoids is the masked nitromethylene compounds with Mannich bases as the primary example. These compounds can act directly[67−69] or after acidic or enzymatic cleavage, reverting to the nitromethylene starting compounds (e.g., CH-IMI).[70,71]

Synergist Action and Resistance. Two standard CYP450 inhibitors, piperonyl butoxide and particularly *O*-propyl *O*-(2-propynyl) phenylphosphate (PPP), strongly synergize the toxicity of neonicotinoids to *M. domestica*,[53] establishing the importance of oxidative detoxification in limiting insecticidal activity. Two commercial fungicides (triflumizole and propiconazole) acting as CYP450 and ergosterol biosynthesis inhibitors strongly synergize the toxicity of ACE and THI but not IMI to *A. mellifera*.[50] Fungicides of this type, with tebuconazole as the example, do not increase THI lethality in bees under field conditions.[72] Neonicotinoid resistance or cross-resistance attributable to enhanced detoxification is reported in *Drosophila*,[35] *Bemisia*,[6] and *Leptinotarsa*.[47]

Safety. Two types of bioactivity are of interest, that is, nAChR-active metabolites and others that might contribute to

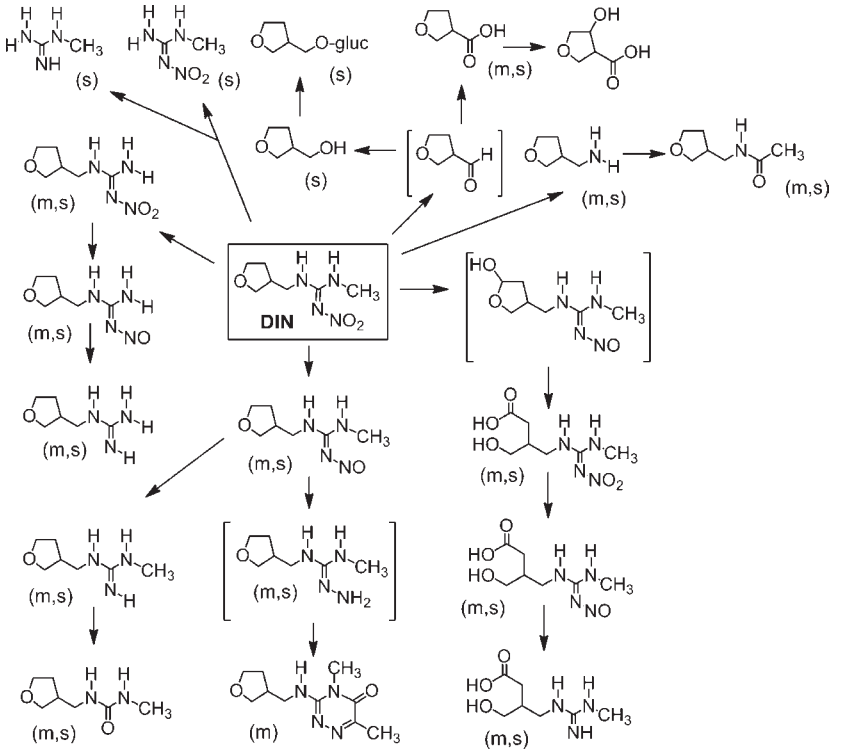

Figure 13. Metabolic pathways of DIN in mice (m) and spinach (s). The pathways shown do not include certain combinations observed for *N*-demethylation, 2-hydroxylation, 5-hydroxylation, and aminoguanidine and methyltriazinone formation.

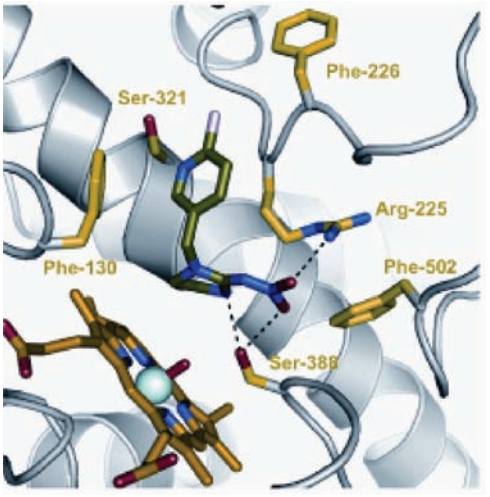

Figure 14. Binding site model for C5 hydroxylation of IMI by *Bemisia tabaci* CYP6CM1vQ. The heme is shown in the lower left. Important predicted binding residues are Phe-130, Phe-226, Ser-388, and Arg-225. Reproduced with permission from ref 36. Copyright 2009 Elsevier.

secondary toxic effects. The desnitro and descyano metabolites with their ionized guanidinium functionality are nAChR agonists at the mammalian α4β2 receptor and toxicants as derived from IMI, IMI-olefin, THI, and THI-olefin with potencies similar to that of nicotine (Table 1). The only secondary effects of neonicotinoid metabolites extensively studied are the hepatotoxicity and hepatocarcinogenicity in mice (but not mutagenicity) of *N*-desmethyl-TMX with its activity synergized by *N*-desmethyl-CLO inhibiting inducible nitric oxide synthase[73−75] (Figure 17).

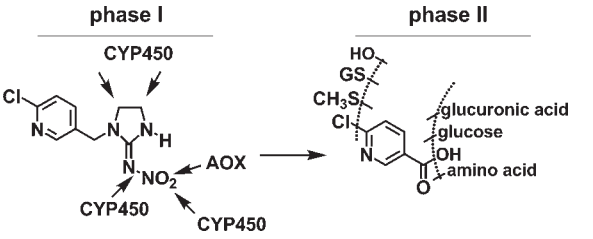

Figure 15. Phase I enzymes and phase II reactions.

Table 1. IC_{50} Values and Selectivity Ratios of Neonicotinoids and Their Guanidine Metabolites for Insect and Vertebrate α4β2 nAChRs

		IC_{50}[a] (nM)		
compound	tip	insect	vertebrate	selectivity ratio vertebrate:insect
neonicotinoids				
IMI	NNO_2	4.6	2600	565
IMI-olefin	NNO_2	1.7	1700	1000
THI	NCN	2.7	860	319
THI-olefin	NCN	2.7	430	159
guanidine metabolites				
IMI	NH	1530	8.2[b]	0.005
IMI-olefin	NH		23	
THI	NH	200	4.4	0.022
THI-olefin	NH	185	1.4	0.008

[a] Inhibitor concentration for 50% inhibition. Data from refs 55−58. [b] IC_{50} 7.0 nM for (−)-nicotine.[56]

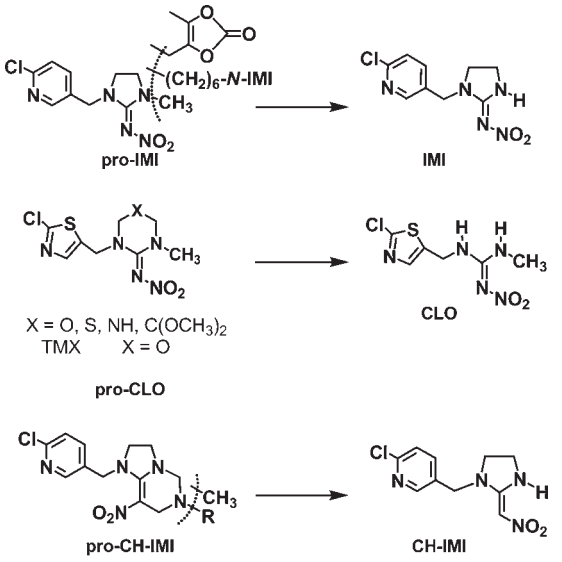

Figure 16. Proneonicotinoids.

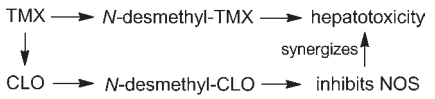

Figure 17. Proposed mechanism for metabolite-induced TMX hepatotoxicity in mice. *N*-Desmethyl-CLO inhibition of inducible nitric oxide synthase (iNOS) lowers nitric oxide level and synergizes *N*-desmethyl-TMX hepatotoxicity.

Plant Vigor and Stress Shield. IMI, TMX, CLO, and several other neonicotinoids are reported to enhance plant growth not only by controlling pest insects but also by acting on the plant directly, in some cases conferring drought tolerance and induced resistance against microbial pathogens.[76,77] The mechanistic relationship between the IMI, TMX, and CLO effects is unknown. Some or all of the IMI action is attributed to the metabolite 6-chloropyridinyl-3-carboxylic acid.[76]

Future Considerations. The current neonicotinoids were introduced into the market between 1991 and 2002,[3,7,11] primarily to control sucking insect pests by systemic action. Resistance has already reduced their effectiveness for many of the early uses. New compounds are needed for resistant pest strains and refractory species (e.g., many lepidopterous larvae and other chewing insects), perhaps bringing new metabolic enzymes or pathways into play. In any case, the wide diversity of neonicotinoid substituents provides opportunities for metabolic selectivity and programmed persistence. These developments must be achieved without compromising the selective toxicity for pests relative to people that has led to the wide acceptance of neonicotinoids as safe and effective insecticides.

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ABBREVIATIONS USED

ACE, acetamiprid; AOX, aldehyde oxidase; CLO, clothianidin; CP, chloropyridinyl; CT, chlorothiazoyl; DIN, dinotefuran; GSH, glutathione; IMI, imidacloprid; nAChR, nicotinic acetylcholine receptor; NIT, nitenpyram; PPP, *O*-propyl *O*-(2-propynyl) phenylphosphonate; THF, tetrahydrofuryl; THI, thiacloprid; TMX, thiamethoxam

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