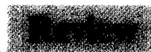
*Agric. Chem. Biotechnol.* **44**(3), 105-112 (2001)



# Insecticide Resistance in Increasing Interest

**Sung-Eun Lee[1], Jang-Eok Kim[1] and Hoi-Seon Lee***

*Research Center for Industrial Development of Biofood Materials and Institute of Agricultural & Technology, College of Agriculture, Chonbuk National University, Chonju 561-756, Korea*

*[1]Department of Agricultural Chemistry, Kyungpook National University, Taegu 702-701, Korea*

Received September 4, 2001

**Insect pests can be controlled through direct application of insecticides. Insect control by residual protectants is relatively inexpensive and has an advantage of destroying all stages of infestations. The efficacy of control is largely determined by the concentration of insecticides to which the pest species is exposed. A reduction in the period of control in the field afforded by a specific level of a protectant indicates that resistance has developed. An increase in the level of protectant is required to maintain control, and the efficacy of currently used insecticides has been severely reduced by insecticide resistance in pest species. Development of resistance to particular insecticide varies with species because insecticide resistance is often correlated with increased levels of certain enzymes, which are cytochrome P450-dependent monooxygenases, glutathione *S*-transferases and esterases. Some sections of insecticide molecules can be modified by one or more of these primary enzymes. A reduction in the sensitivity of the action site of a xenobiotic also constitutes a mechanism of resistance. Acetylcholinesterase is a major target site for insecticide action, as are axonal sodium ion channels and γ-aminobutyric acid receptors. Development of reduced sensitivity of these target sites to insecticides usually occurs. This review not only may contribute to a better understanding of insecticide resistance, but also illustrates the gaps still present for a full biochemical understanding of the resistance.**

**Key words:** *cross-resistance, esterases, glutathione S transferase, cytochrome P450-dependent monooxygenase.*

Many chemicals are used as pesticides and therapeutic agents not only to improve human health but also to reduce food shortages throughout the world. However, their continued use often results in resistance to these chemicals in microbes, plants, insects, and vertebrate pests. They can develop behavioral avoidance or physiological and biochemical mechanism to allow them to survive over the levels of the chemicals which would kill susceptible individuals. Some organisms have a natural tolerance to chemical treatments which kill other species. Resistance develops through a process of selection for rare individuals with genes, which confers an advantages to the individual in a population in the presence of a toxic chemical.

## Definition of resistance

Resistance has been defined in many different ways. Crow (1960) suggested that "resistance marks a genetic change in response to selection", a definition applicable to individual insects and populations.[1)] The World Health Organization (WHO) (1971) defined resistance as "the development of an ability in a strain of insects to tolerate doses of toxicant which would prove lethal to the majority of individuals in a normal population of the same species".[2)] Sawicki (1987) defined resistance as "a genetic change in response to selection by toxicants that may impair control in the field".[3)] Moberg (1990) has suggested a modified version of Sawicki's definition to include the phrase "at recommended application rates".[4)]

## Status of insecticide resistance in insects and mites

Georghiou (1990) listed 504 species of insect and mites for which insecticide resistance has been recorded,[5)] and also showed that the rate of increase in the number of insects and mites with resistance to pesticides was lower during the decade from 1978 to 1988 than from 1968 to 1978. However, examination on the number of classes of pesticide to which resistance was recorded indicated that there is still a steady increase in resistance to specific chemicals and that many species are now resistant to several groups of chemicals. Cyclodiene resistance was found in 291 species, DDT in 263, organophosphate in 260, carbamate in 85, and pyrethroid in 504 species.

## Cross-resistance

Cross-resistance can be defined as resistance of an organism to two or more toxicants.[5)] The phrase, "positively correlated", has been added to the definition even though a negative correlation is rare. Resistance mechanisms are generally dependent

*Corresponding author
Phone: 82-63-270-2544; Fax: 82-63-270-2550
E-mail: hoiseon@moak.chonbuk.ac.kr

on a single R-gene. Continued selection with one or more insecticides may result in accumulation of several R-genes, and resistance mechanisms. Multiple copies of a single R-gene (gene amplication) can be found.[6] In cross-resistance a single R-gene, or several R-genes, selected by a particular insecticide often confers resistance to a number of other insecticides. Oppenoorth (1985) characterized two cross-resistance patterns: parallel cross-resistance, where relative resistance to a group of insecticides was similar in several species, and criss-cross resistance, where relative resistance to several insecticides was very different in two strains of an insect.[6]

## Mechanisms conferring insecticide resistance

The resistance mechanisms available to insects may be divided into three general categories. The first category includes modified behavioral mechanisms that reduce the exposure of an insect to toxic compounds. In the second category are physiological mechanisms including altered penetration, excretion, transport or storage of insecticides by insects. The third category includes biochemical mechanisms such as the insensitivity of target sites to insecticides and enhanced detoxification by several metabolizing enzymes. Metabolism of insecticides usually results in compounds, which are more water-soluble, therefore more readily eliminated, and generally less toxic to the host insects. The detoxifying enzymes catalyze primary or secondary metabolic reactions. Primary metabolism carried out by esterase and monooxygenase enzymes is followed by the secondary metabolism, frequently by conjugation reactions including those catalyzed by glutathione *S*-transferase (GST). In this review, we concentrate on biochemical resistance mechanisms, particularly cytochrome P450-dependent monooxygenase (P450 monooxygenase).

## Biochemical mechanisms

Biochemical mechanisms relating to resistance include changes to the target sites of insecticides, which affect the interaction of the toxic compound with the site as well as altered metabolism of insecticides. Alterations to the acetylcholiesterase (AChE) enzymes of resistant insects and components of the sodium channels of the neuron system have been studied. Enzymes, which are considered to be important in metabolic mechanisms of resistance in many species, include esterases, GST, and P450-monooxygenase.

**Alteration or insensitivity of target sites.** Target sites for current insecticides are mainly limited to the insect nervous system such as cholinergic synapse and sodium channel. Primary site of action of organophosphates and carbamates is cholinergic synapse, the location of the AChE enzyme. Sodium channel is the target of numerous natural and synthetic insecticides including compounds such as DDT and pyrethroids. There have been many reports of resistance in insects resulting from changes in AChE and the sodium channel.

Acetylcholiesterase: Reduced sensitivity of AChE to organophosphates and carbamates through the inhibition of AChE is the most common type of target site alteration.[7-13] Altered AChE can contribute to the development of cross-resistance to a wide range of insecticides.[11] Apparent decreased sensitivity of AChE may also occur as a result of an increase in the amount of AChE present in the insect.[12]

Sodium channel: Knock-down resistance (*kdr*) can be related to changes in the sodium channels in the nervous system such as variation in the number of sodium channels or altered binding capacity of the channels for insecticides. A reduced density of normal sodium channels in the nerve membrane has been found in resistant strains of *Drosophila melanogster* (Meigen) and *Musca domestica* (L.).[14-16] Kasbekar and Hall (1988) used a sub-lethal dose of tetradotoxin as a sodium channel blocker to show that a wild-type strain of *D. melanogaster* with a reduced number of sodium channels has resistance to pyrethroid.[14] These data indicate that pyrethroid resistance in the *nap*$^{ts}$ strain of *D. melanogaster* may be related to the reduction in sodium channel density in the *nap*$^{ts}$ strain. Bull and Pryor (1990) have shown that houseflies resistant to a range of insecticides including permethrin have only 65 percent of saxitoxin binding sodium channels of the wild-type.[15]

However, a reduced sodium channel density was not an obligatory component of the *kdr* resistance in the housefly.[16] Tritiated saxitoxin binding studies on susceptible strains, OMS and NAIDM, and resistant strains, *super-kdr* and Learn-Pyr, revealed these strains have very similar sodium channel density. Pyrethroids have a synergistic effect on the binding of the sodium channel toxin, batrachotoxin. When the binding of [$^3$H]BTX-B was examined in the susceptible and resistant housefly strains in the presence of deltamethrin, the binding of [$^3$H]BTX-B was much lower in the resistant strains. These results suggest that modification of the pyrethroid binding site or its allosteric linkage to the batrachotoxin binding site on the sodium channel is responsible for the resistance mechanism in pyrethroid-resistant flies.[16]

γ-aminobutyric acid (GABA) receptors: Lindane and cyclodiene resistances are generally related to modification of the binding site on the GABA receptor of the neuron membrane. Studies on mechanisms of resistance in lindane-cyclodiene-resistant laboratory strains of the rust red flour beetle, *Tribolium castaneum* (Herbst), showed that differences in nerve sensitivity were the main factors contributing to resistance. In a strain ninety times more resistant to dieldrin than the susceptible strain, there was much less response by nerve tissue to dieldrin treatment than in the susceptible strain.[17]

**Esterases.** Esterases are enzymes, which catalyze the hydrolysis of esters. Carboxylesterase (CE) and phosphatase catalyze the hydrolysis of carboxylesters and phosphate esters, respectively. Many insecticides such as organophosphates, carbamates and pyrethroids contain ester groups, and could be detoxified through the hydrolysis of the ester bond. *In vitro* esterase activity is frequently determined via hydrolysis of

simple carboxylesters such as *p*-nitrophenyl acetate (*p*NPA) and α- and β-naphthylacetates (α- and β-NA). However, this CE activity measured with non-insecticidal substrates cannot hydrolyze the chemically different insecticides. CEs have been shown to be important in the degradation of organophosphates containing a carboxylester bond such as malathion. For insecticides without a carboxylester bond, esterase may be significantly resistant because the large amounts produced are sufficient to sequester the toxic dose of insecticide.[18-19)]

Relationship of esterase activity to insecticide resistance: Early studies on organophosphate (parathion and diazinon) -susceptible and -resistant strains of *M. domestica* showed lower esterase activities in the resistant houseflies in comparison with the susceptible strains.[20-21)] Activities assayed with β-NA were similar in susceptible and resistant strains, whereas the activity in resistant strains with α-NA was 15 percent of that in the susceptible strain.[21)] It was suggested that the reason for the negative correlation of esterase activity and organophosphate resistance is that an altered enzyme had been produced, which could hydrolyze organophosphates, but had reduced capacity to hydrolyze α-NA.

A positive correlation between esterase activity and insecticide resistance has been demonstrated in the peach-potato aphid *Myzus persicae* (L). Devonshire (1977) showed that increased esterase activity in this insect was caused by increased levels of a single isozyme, designated E4 on the basis of polyacrylamide gel electrophoresis profile. This enzyme hydrolyzed both α-NA and paraoxon, although the rate of the hydrolysis of α-NA was $2 \times 10^6$ faster than that of paraoxon. The enzymes from resistant strains and a susceptible strain had identical kinetic characteristics, showing that the resistance was caused by increased levels of the E4 esterase and not by a modified protein.[22)]

Dual role of esterase: There are several reports which suggest a dual role of hydrolysis and sequestration for esterases in resistance to insecticides. An organophosphate resistant strain of the green rice leafhopper, *Nephotettix cinciceps*, was found to have esterase activity, which could hydrolyze malathion, fenvalerate, and paraoxon.[18,19,23)] However, hydrolysis of paraoxon reaches a plateau within 15 min, whereas hydrolysis of malathion and fenvalerate are actually hydrolyzed. Furthermore, the reaction of paraoxon and the enzyme results in a stable intermediate, and acts in resistance to parathion through sequestration of the oxon derivative.

Byrne and Denvonshire (1991) described an *in vivo* experiment, which indicated a dual role for esterases in resistance.[24)] In studies on monocrotophos resistant tobacco whitefly, *Bemisia tabaci* (Gennadius), they examined the frequency distribution of the esterase activity in a population of resistant insects before and after treatment with cypermethrin and profenophos. Insects surviving the cypermethrin treatment had a similar pattern of esterase levels to the original population, which indicates that the cypermethrin had been hydrolyzed. Survivors treated with profenophos had a pattern of esterase levels even lower than that in a susceptible strain, an indication of the profenophos sequestration, and thus inhibition of the esterase.

Molecular biology of esterase in insecticide resistance: A series of molecular biological studies on esterases of *M. persicae* and *Culex* spp. have demonstrated the relationship between overproduction of esterasse and gene amplication or duplication. Devonshire and Sawicki (1979) proposed gene amplication as the reason for enhanced esterasse synthesis after observing an approximately twofold increase in esterase activity in each of the progressively more resistant clones of *M. persicae*.[25)] Devonshire *et al.* (1986) found that two strains of resistant aphids had much more E4 and FE4 esterase mRNAs than the susceptible aphids as determined through *in vitro* translation and immunoprecipitation with an antiserum against the enzyme E4. Full strength cDNA clones for the E4 and FE4 esterases have been isolated and characterized.[26)] Detailed analysis of the enzyme products of these two genes has been carried out. The three-dimensional structures and the amino acids at the active sites have been compared with other serine hydrolases from various sources. It seems likely that the catalytic site of these enzymes contains serine, glutamic acid, and histidine, similar to the active site of AChE.

A correlation between gene amplication and resistance has been reported for the mosquito *C. quinquefasciatus*.[27)] The organophosphate-resistant strain Tem-R, had 500-fold more B1 esterase compared with the susceptible strain, S-Lab. A cDNA coding for a 19 kDa protein sequence, which reacted with B1 esterase antiserum, was identified. This cDNA was utilized as a probe to detect the esterase B1 gene in mosquito. It was found that the resistant adults had at least 250 times more copies of the B1-coding gene than the susceptible adults.

**Glutathione *S*-transferase.** Glutathione *S*-transferase (GST) are enzymes, which catalyze the nucleophilic attack of the tripeptide glutathione (GSH) on endogenous substrates and xenobiotics. In many cases the xenobiotic substrates are plant chemicals, which may be toxic to insects feeding on the plant. GSTs are known to be involved in metabolic detoxification mechanisms of insecticides, particularly organophosphates and DDT. Although these enzymes can play a primary role in the metabolism of organophosphorus insecticides, they generally contribute to detoxification in secondary reaction mechanisms. There is no specific inhibitor for GST, which can be used in synergism studies, and the products of reactions catalyzed by this enzyme are often the same as those resulting from metabolism by monooxygenases and esterases.[28)]

Structure of glutathione *S*-transferase: The active site of GST consists of two parts: the G-site which has a very high specificity for glutathione, and the H-site, the substrate binding site.[29)] A histidine residue, known to be essential for the catalytic activity of GST, may function in the ionization of glutathione to the thiolate ion, the nucleophilic species of glutathione, which takes part in the conjugation reaction catalyzed by the enzyme. Glutathione and other compounds containing sulphydryl groups, such as cysteine and mercaptoethanol, can stabilize the enzyme, suggesting that a free-SH group is essential for the activity.[30)]

The relationship of glutathione *S*-transferase activity to insecticide resistance: Elevated GST activity is generally associated with insecticide resistance. Reidy *et al.* (1990) found that a four- to sixfold increase of GST activity assayed through conjugation with 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) was found in a cyfluthrin-resistant strain of *T. castaneum* in comparison with the activity of a susceptible strain.[31] Kinetic analysis of this cytosolic activity with CDNB showed a four- to fivefold increase in Vmax in the enzyme of the resistant strain, but no difference in Km between enzymes from the resistant and susceptible strains of the insect. Furthermore, electrophoretic analysis through SDS-PAGE showed that a single protein band was strongly stained in the GST molecular weight region of 24 to 30 kDa in extracts of the resistant beetles in comparison with the staining pattern of the susceptible beetles. Thus, increased GST activity in these resistant insects probably results from an increase in the amount of a particular GST enzyme in the insects.

Increased GST activity may be caused by the presence of higher concentrations of particular isoenzymes of GST in a resistant population. Prapanthadara *et al.* (1993) isolated seven partially purified GSTs from susceptible and resistant strains of *Anopheles gambiae* and assayed the isoenzyme for DDT-dehydrochorinase activity and CDNB conjugation.[32] They found that four isozymes, which contributed only 6 percent of total GST activity with CDNB in the susceptible strain, contributed 94 percent of the DDT metabolism observed. These four isoenzymes had 8.2-fold higher activity for DDT-dehydrochlorinase in the resistant strain in comparison with the susceptible strain.

Molecular biological study of glutathione *S*-transferase activity in insecticide resistance: Molecular biological study of GST activity in a malathion-resistant strain of *Drosophila* showed that increased levels of two GST isoenzymes, DmGST1 and DmGST2, conferred a threefold increase of total GST activity to CDNB in the resistant insects.[33] The increase of the DmGST1 isoenzyme was associated with an increase in a specific mRNA, whereas no such difference was observed in mRNA levels in resistant and susceptible insects in relation to the levels of DmGST2. Detailed studies of the two proteins showed that DmGST1 has similarities to bacterial and plant enzymes, whereas DmGST2 is characteristic of vertebrate and invertebrate enzymes. It is clear that the expression of these two isoenzymes is controlled in different ways involving structural changes in the genes and regulatory modification of gene expression.

**Cytochrome P450-dependent monooxygenase.** Cytochrome P450-dependent monooxygenases, or mixed function oxidases, are microsomal, membrane-bound enzymes located in the endoplasmic reticulum of the cell. They catalyze hydroxylation reactions in metabolism including many steps in the biosynthesis of steroids. They play an important role in the synthesis and degradation of insect hormones.[34-36]

These enzymes are implicated in the metabolism of many xenobiotics through reactions such as desulfuration, epoxidation, and hydroxylation of aliphatic and aromatic compounds, *N*-, *O*-, and *S*-dealkylation, and dehydrogenation.[37] They can oxidise insecticides to produce more soluble forms, which are readily complexed and removed. They require NADPH or NADH and molecualr oxygen for activity; one form of oxygen is inserted into the substrate and the second is released as $H_2O$. The overall mechanism includes a series of electron carriers leading to cytochrome P450 reductase and cytochrome P450, which binds the substrate into which oxygen is inserted, embeded in a phospholipid membrane.[37-38] The role of cytochrome $b_5$ in this mechanism is still unclear in most species, except for housefly.[39] Alteration to the levels or characteristics of components of the system such as the NADPH cytochrome P450 reductase and the cytochrome P450 itself may affect the rate of metabolism by the monooxygenase. These enzymes are frequently found at elevated levels in resistant insects, even though some of the oxidized products of the organophosphate insecticides are more toxic than the original insecticides. As such the role of these enzymes in resistance to organophosphate insecticides is not clear.

Components of cytochrome P450 monooxygenase system: (1) Cytochrome P450: Cytochrome P450 (P450), a hemoprotein of the cytochrome b class, has a unique absorption in the visible region, and was named based on the maximum absorption wavelength of the protein bound to carbon monoxide.[40] Omura and Sato (1964) reported that the dithionite-treated rat liver microsomes showed a difference spectrum with a peak at 450 nm, which on the other hand, was not displayed by the air-saturated P450 pigments, after the addition of carbon monoxide, indication that P450 is a reducible pigment and only the reduced form of the pigment can bind carbon monoxide.

Since then, several reports have revealed that characteristics of the P450 spectrum in the visible region are altered after the treatment with a range of organic or inorganic ligands. Three different classifications of P450 spectral change are described as types I, II, and III on the basis of spectral characteristics, in addition to the carbon monoxide-binding spectrum. Type I spectrum with oxidized P450s shows a trough at 420 nm and a maximum absorption between 385 to 390 nm. Compounds causing type I spectrum include insecticides, drugs, and other xenobiotics, which are believed to bind at a hydrophobic site close to the heme and are therefore able to affect the oxygen binding.[37] Most of these compounds are substrates of the enzyme. Ligands resulting in a type II spectrum are associated with the heme iron and frequently inhibit monooxgenase activity. Ethyl isocyanide binding results in the formation of type III spectra with reduced P450 to give two peaks at 430 and 455 nm. The relative sizes of the two peaks vary with pH, and a pH equilibrium point (with peaks of equal size) is a characteristic of a specific P450.[37]

Multiple forms of P450 have been reported in the fleshfly *S. bullata* and the blowfly *P. regina*.[41] Ion exchange chromatography of solubilized microsomes from both insects resulted in four fractions containing P450. SDS-PAGE of the solubilized

microsomes from both insects resulted in four fractions containing P450. Spectral differences indicated that different forms of P450 were present in these fractions. SDS-PAGE of the fleshfly fractions showed six hemoproteins in the expected 40 to 60 kDa molecular weight range. Failure to detect protein in this size range in the blowfly extracts suggested that these P450s were less stable than those from the fleshfly.[41]

(2) NADPH-cytochrome P450 reductase: Microsomal-bound P450 monooxygenase is dependent on the transfer of electrons from NADPH catalyzed by a flavoprotein, NADPH-cytochrome c reductase, as well as NADPH cytochrome P450 reductase or NADPH P450 reductase. In insects, cytochrome c reductase uses only NADPH as the electron donor. An antibody to the cytochrome c reductassse of southern armyworm and housefly inhibited NADPH-dependent reduction of cytochrome c or artificial electron acceptors such as dichlorophenolindophenol and ferricyanide, but did not inhibit the NADPH-dependent reduction of these electron acceptors. The antibody also inhibited microsomal epoxidation, *N*-demethylation, *O*-dealkylation, and *O*-demethylation, which indicated that NADPH cytochrome c reductase was a component of the enzyme system.[38,42]

NADPH P450 (cytochrome c) reductase was purified from adult phenobarbital-induced housefly *M. domestica*.[43] Phnobarbital is known to induce monooxygenase activity in many organisms. Insect NADPH P450 (cytochrome c) reductases are very similar in their functions, but very different in structures compared with enzymes from other organisms on examination through immunological techniques. The rabbit antibody prepared against NADPH P450 (cytochrome c) reductase from microsomes of larval midgut of the southern armyworm Spodoptera eridania showed no cross-reactivity toward rat liver NADPH (cytochrome c) reductase or *vice versa*.[38] On the other hand, the cross-reactivity of antibody prepared against the purified reductase from housefly was high for the reductase of other *Diptera* species such as *D. melanogaster* and *C. pipiens*, but was low for the enzyme from vertebrate species such as rabbit and rat liver.[43] This difference between insect reductases and mammalian reductases resulted from distinct antigenic determinants, and the enzymes had different levels of acidic and basic amino acids in the membrane-binding peptide. These differences in the amino acid residues might be related to the variable requirements for lipid-protein interaction in different species.

A comparison of RFLP-patterns of the housefly NADPH P450 (cytochrome c) reductase showed that the gene for the reductase was located on chromosome III, while the genes of other metabolic detoxifying enzymes were located on chromosome II or V.[44]

(3) Cytochrome $b_5$: In the operation of cytochrome P450-dependent monooxygenase system, two electrons from NADPH are separately transferred to cytochrome P450 by NADPH (cytochrom c) reductase, and the transfer rate of the second electron is rate-limiting. It has been suggested that cytochrome $b_5$ may also have a role as an additional electron carrier in the monooxygenase activity.

The role of cytochrome $b_5$ in insect monooxygenase activity has been examined by several authors. Scott *et al.* (1990) found that there was a 1.2- to 2.3-fold increase in cytochrome $b_5$ in seven resistant strains, Dairy and LPR (the multiple resistant strains), ASPR, Kashiwagura, and PG (the strain resistant to pyrethroid), and 3rd-Y and EPR (the organophosphate resistant strains), of housefly in comparison with a susceptible strain $S^+$.[45]

(4) Phospholipids: The components of P450 monooxygenase system are intrinsic membrane proteins, which are deeply embedded in the membrane matrix. Thus, the monooxygenase reaction may be affected by the nature of the membrane components, particularly phospholipids.[46] However, the role of lipids in monooxygenasse reactions has not been examined in detail in insects, in spite of the fact that insect microsomes have quite different phospholipid composition. Ronis *et al.* (1988) purified cytochrome P450 species from a resistant strain (Rutgers) of housefly, and the monooxygenasse activity was examined in the reconstitution system, containing purified P450 fractions, purified housefly or rat liver NADPH P450 (cytochrome c) reductase, an NADPH generating system, and a phospholipid (either dilaurylphosphatidylcholine or phosphatidylethanolamine).[47] Higher monooxygenase activity was observed using phosphatidylethanolamine as the phospholipid rather than phosphatidylcholine. It appears that a relationship may exist between the low proportion of phosphatidylcholine and higher monoxygenaseactivity in housflies. However, this cannot be applied to all insects. Purification of the cytochrome P450-dependent monooxygenase system components and sequent artificial reconstitution may allow an understanding of the influence of phospholipid constituents on the monooxygenase reaction.

Induction of insect cytochrome P450 monooxygenase activity: The induction of cytochrome P450 monooxygenases in insects by a wide range of chemicals, such as drugs, insecticides, and food antioxidants, has been well-documented. A early study of the cytochrome P450 monooxygenase induction was carried out by Perry *et al.* (1971).[48] They showed that the compounds, phenobarbital (PB) and butylated hydroxyltoluene (BHT), caused increases in P450 levels, Baygon hydroxylase and aldrin epoxidase activity of the strains of insecticide-resistant and -susceptible houseflies, whereas the monooxygenase synergist, piperonyl butoxide (PBO), resulted in reduced P450 levels.

Carino *et al.* (1992) studied the effect of phenobarbital on the expression of cytochrome P450 gene for CYP6A1 of *sbo* and Rutgers strain housflies.[49] They found that, after a 12-h treatment with drinking water containing 0.1% phenobarbital, 100- and 22-fold increases of CYP6A1 mRNA were observed for the *sbo* and Rutgers strains, respectively. The monooxygenase synergist, PBO, caused some increase in CYP6A1 mRNA, but DDT and dieldrin, both of which are known to induce P450 monooxygenase activity in houseflies, did not cause the elevation of mRNA levels. It was concluded that the

induction of cytochrome P450 monooxygenase may occur via the stimulation of a range of P450 genes.[49]

Relationship of cytochrome P450 monooxygenase activity and insecticide resistance: There are many examples in which increase levels of P450 monooxygenase activity and various components of the total system are correlated with resistance to insecticides. This is so even in situations where the products of oxidation, such as the oxon derivatives of the organophosphates, are more toxic than the insecticides themselves. In these cases it is possible that other enzyme systems are available to increase the metabolism of these compounds and prevent them from reaching their target sites.

Multiple isozymes of cytochrome P450 have been identified in an insecticide (diazinon) resistant strain, Rutgers, and a susceptible strain, NAIDM, of the housefly *M. domestica.*[50] Relative quantities of the isoenzymes differed in the two strains, and two purified P450 fractions from the Rutgers strain were much more active in aldrin epoxidation than the same two forms from the susceptible NAIDM strain.

Scott and Georghiou (1986) demonstrated that higher levels of cytochrome P450, cytochrome $b_5$, and NADPH-cytochrome c reductase activity were correlated with increased monooxygenase activity, which was shown via synergism with PBO to be the major resistance mechanism to permethrin in the resistant LPR strain of housefly.[51] They examined the relationship between a range of pyrethroid structures and resistance and found that an unsubstituted phenoxybenzyl alcohol group was always associated with resistance. They suggested that monooxygenase activity related to resistance operates through modification of the phenoxybenzyl alcohol group in the LPR strain. However, Hatano and Scott (1993) showed that extracts of the LPR stain were able to cause desulfuration of chlorpyrifos into chlorpyrifos-oxon more rapidly than extracts from the susceptible strain $S^+$.[52] They showed that the desulfuration reaction was completely inhibited by the $P450_{lpr}$ antiserum. However, there was no evidence that the $P450_{lpr}$ was involved in further metabolism of chlorpyrifos-oxon, which is more toxic than chlorpyrifos. Thus, the selection of increased $P450_{lpr}$ levels in response to pyrethroids could increase the sensitivity of the insect to chlorpyrifos by facilitating the activation of chlorpyrifos to the oxon derivative.

Increased levels of monooxygenase and GST are related to resistance to organophosphate in *Anopheles suspictus*. Hemingway *et al.* (1991) reported that the monooxygenase activity in resistant insects produced two highly active AChE inhibitors from fenitrothion, fenitrooxon, and 3-hydroxymethyl fenitrooxon.[53] They suggested that these toxic products were removed through the action of a GST, which may also be active in the dechlorination of DDT.

Overexpression of an isozyme of P450 responsible for detoxification mechanism of a certain insecticide may be caused by two different types of changes at the gene level. Waters *et al.* (1992) suggested that post-transcriptional mechanism may be responsible for mRNA stability in a resistant strain of *D. melanogaster* in comparison with a susceptible strain.[54] They prepared a cDNA clone for P450-B1, a P450 known to be important in resistance to insecticides, and showed that the P450-B1 gene in the resistant strain was structurally different from the gene in the susceptible strain. However, there was no evidence of gene amplication in the resistance strain. There was 20 to 30 times more P450-B1 mRNA in the resistant strain than in the susceptible strain, suggesting that greater stability of the mRNA may be important in the increased expression of the P450-B1 isoenzyme. It was suggested that the presence of a solitary long terminal repeat of transposable element *17.6* in the 3' untranslated region of the gene contributed to the instability of the P450-B1 mRNA from the susceptible strain. A second type of modification at the gene level has been described by Carino *et al.* (1994),[49] who found that the mRNA levels of the cytochrome P450 gene CYP6A1 in a resistant diazinon-R Rutgers strain of *M. domestica* was 10 times higher than those of susceptible strain *sbo*. This was not caused by gene amplication in the resistant strain, and there was no difference in transcript lengths between the mRNA from the resistant and susceptible strains. Difference in the CYP6A1 mRNA levels between two strains was suggested to be caused by a mutation, which affects the regulation of the CYP6A1. One or more loci located on chromosome II may be associated with the high constitutive expression of CYP6A1 in the resistant Rutgers strain.

## Conclusion

Continued use of persistant insecticides builds up resistance in insect pests. Even though successful development of new insecticides with new target sites can reduce the problem of insecticide resistance, how resistance is managed by humans should still be elucidated. Further molecular biological studies will contribute information toward a better understanding of resistance mechanisms to insecticide resistance.

**Acknowledgments.** This work was partially supported by the Brain Korea 21 project.

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