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THE METABOLISM OF INSECTICIDES: THE ROLE OF MONOOXYGENASE ENZYMES

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INTRODUCTION

Although cytochrome P-450-dependent xenobiotic-metabolizing monooxygenase systems have been investigated extensively and reviewed frequently, this is not true of their role in insecticide metabolism. In the majority of the investigations reported, the substrates of choice are drugs, carcinogens, or compounds related to them. The last comprehensive review of the oxidative metabolism of insecticides was published in 1980, with the review of the literature extending only through 1978 (1). Since that time it has become apparent that the FAD-containing monooxygenase is of considerable importance (2) in insecticide metabolism and that its role relative to that of cytochrome P-450 needs to be redefined. Co-oxidation during prostaglandin synthesis (3) has also emerged as a new metabolic route and our knowledge of some other aspects of insecticide oxidation has been extended.

Even though insecticides are subject to the whole array of phase I and phase II xenobiotic-metabolizing enzymes, the role of monooxygenases is of primary and critical importance. In addition to their role in detoxication, their production of highly reactive intermediates plays an important part in activation reactions and hence in both acute and chronic toxicity. Since the monooxygenases are a common locus for the metabolism of many types of xenobiotics,

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most of which can act as inducers or inhibitors as well as substrates, they are the most important locus for interactions between different compounds (4). Their role as activating systems in genotoxicity testing has also been extended to include insecticides (5). In insects, the target organisms, they play a role in resistance, hormone metabolism, and polyphagy (6) in addition to their role in the detoxication and activation of xenobiotics.

This review consists of aspects of the topic on which our information has been extended since the last comprehensive review (1). Due to space limitations, citation of the literature is selective rather than comprehensive.

MONOOXYGENASE SYSTEMS

Despite considerable effort by many investigators, the mechanism of action of cytochrome P-450 has not been completely elucidated. However, the current status of our knowledge is summarized in Figure 1. It is apparent that insecticides, while acting as substrates or inhibitors, may also play a role in lipid peroxidation and in the generation of active oxygen species. We now know that cytochrome P-450 exists as a number of isozymes (7) that vary in their distribution between species, strains, and organs as well as in their substrate specificities, physical properties, and inducibility, although the importance of this multiplicity in insecticide metabolism is not yet clear. Many of the studies to date have been carried out on induced isozymes, but recently we have purified several constitutive forms from the livers of untreated mice (8) and are currently investigating their specificity vis-a-vis insecticide substrates (9). Multiplicity is also known in insects (10), although in this case the isozymes have been less well defined. It is apparent, however, that neither aryl hydrocarbon hydroxylase activity (11), nor cytochromes found in insect microsomes with λ_{max} lower than 450 nm (12), should be equated with the cytochrome P-448 induced in mammalian liver by polycyclic aromatic hydrocarbons.

The basic distribution of cytochrome P-450 between cell types, organs, strains, and species has been known for some time, but less is known about these aspects as they relate to insecticide metabolism. Most previous studies have been carried out on rodent liver or insects (1), but recently some emphasis has been placed on fish and birds. In the former case, this includes the demonstration of aldrin epoxidation in carp *(Cyprinus carpio)*, grass carp *(Ctenopharyngodon idella)*, tilapia *(Tilapia aurea)*, and trout *(Salmo trutta)*, as well as parathion desulfuration in the carp (13) and the oxidation of diazinon by five species of marine and saltwater fish: carp, rainbow trout *(Salmo gairdneri)*, channel catfish *(Ictalurus punctatus)*, dace *(Tribolodon hakonensis)*, and yellow tail *(Seriola quinqueradiata)* (14).

An extensive study of wild sea birds and one land bird (15, 16) showed the oxidation of the cyclodienes, aldrin, and HCE by liver microsomes from the

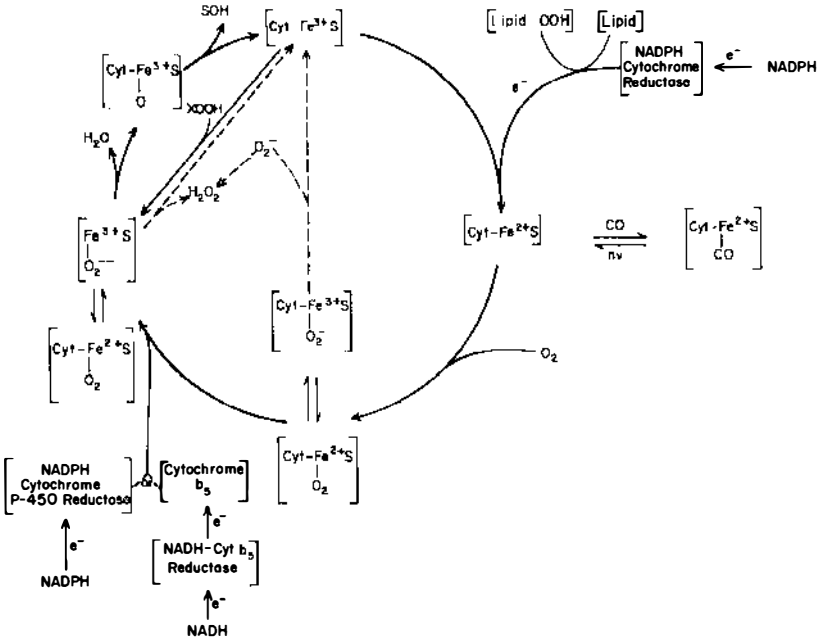

Figure 1 Reaction sequence of the cytochrome P-450-dependent monooxygenase system.

puffin *(Fratercula arctica)*, the razorbill *(Alca torda)*, the guillemot *(Uria aalge)*, the shag *(Phalacrocorax aristotelis)*, the cormorant *(Phalocrocorax carbo)*, the Manx shearwater *(Puffinus ridibundus)*, and the rook *(Corvus frugilegus)*. Aldrin epoxidation has also been demonstrated in several organs of Japanese quail (17), and naphthalene has been shown to be oxidized to 1-naphthol and the 1,2-diol by liver microsomes of the pigeon (18). Both oxidative dearylation and oxidative desulfuration of parathion was demonstrated in liver microsomes of four species of wild birds: the barn-owl *(Tyto alba)*, the blackbird *(Turdus merula)*, the African bulbul *(Pycnonotus capensis)*, and the house sparrow *(Passer domesticus)* (19).

The status of cytochrome P-450-dependent monooxygenase systems in insects has recently been reviewed (6, 10, 12).

The other microsomal monooxygenase of importance is the FAD-containing monooxygenase. This enzyme, first described as an amine oxidase and later shown to oxidize a variety of sulfur compounds, is widely distributed in vertebrate species and occurs in several tissues (20). It has not to date been identified in invertebrates. The mechanism of action is illustrated in Figure 2. It can be seen that the oxidizable xenobiotic is the third substrate to combine with the enzyme (NADPH, then oxygen, and then the oxidizable substrate). According to the proposed mechanism, it appears that V_{max} should be similar for all

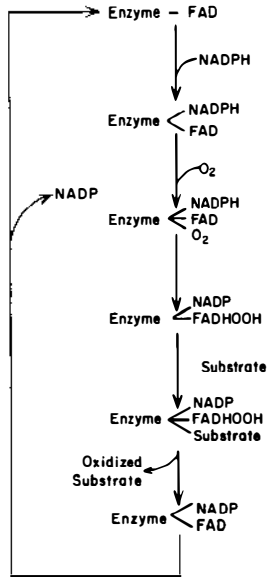

Figure 2 Reaction sequence of the FAD-containing monooxygenase.

substrates, with K_m being variable (20). In the case of insecticides, the low solubility in water frequently makes determination of kinetic constants difficult.

CYTOCHROME P-450 REACTIONS

Desulfuration and Ester Cleavage

The enzymatic substitution of a sulfur atom by an oxygen atom typifies bioactivation of all phosphorothioate and phosphorodithioate insecticides. Initial studies (21) on the characterization of parathion desulfuration by mammalian liver implicated cytochrome P-450, as they indicated that the activity resides in microsomes, requires NAD(P)H and molecular oxygen, and is inhibited by CO, SKF 525-A, methylenedioxyphenyl compounds, and substituted imidazoles.

Conclusive evidence for the monooxygenase nature of the parathion activation was provided by the use of reconstituted systems consisting of homogeneous hepatic microsomal cytochrome P-450 from rat or rabbit liver, NADPH cytochrome P-450 reductase, NADPH, and phospholipid (21). The reaction mechanism proposed for the formation of paraoxon from parathion includes initial donation of a singlet oxygen atom to the sulfur atom of parathion to yield a compound analogous to S-oxide. One of the four resonance forms of the proposed intermediate rearranges internally to form a cyclic P-S-O intermediate. This resultant phosphooxathiiran then undergoes a cyclic electron shift with the loss of elemental sulfur, forming paraoxon.

Parathion is metabolized in vitro by the microsomal monooxygenase system to paraoxon, diethyl phosphorothioic acid, diethyl phosphoric acid, and *p*-nitrophenol. Similar metabolic patterns consisting of both activation by desulfuration and detoxication by oxidative ester cleavage have now been demonstrated by in vitro studies on several organophosphorus (OP) insecticides (1). These observations led to the question of whether these two reactions are catalyzed by the same enzyme or each is catalyzed by a separate system involving different cytochrome P-450 species. Neal and co-workers (21) have proposed that all of these products originate non-enzymatically from the enzymatically produced phosphooxathiiran intermediate. Both molecular oxygen and oxygen from water participate in metabolite production, and the accessibility of water to the active site of cytochrome P-450 and the *cis* or *trans* position of the sulfine with respect to the *p*-nitrophenyl group seem to determine their relative proportions. Whether this hypothesis is applicable to the metabolism of other OP insecticides under different experimental conditions (1) is not yet clear.

In addition to the monooxygenase-catalyzed dearylation of phosphorothionate insecticides, oxidative disruption of the acid-anhydride bond of phosphates has been observed in a few cases (1), although in general oxons are considered poor substrates for the microsomal monooxygenase system. Recently, an unusual substrate specificity in the oxidative dearylation of paraoxon analogs was described (22). The dearylation process in question is a typical monooxygenase reaction, since it requires NADPH and oxygen and is inhibited by CO, SKF 525-A, and piperonyl butoxide. Although methyl-, ethyl-, n-butyl-, and n-amyl-paraoxon were not readily metabolized, considerable dearylation of n-propyl paraoxon was observed. When the two alkyl substituents were not identical, only the compounds with an n-propyl group were metabolized. Dearylation was also observed with analogs containing certain branched chains or modified ethyl groups. This unusual substrate specificity is associated with the presence of a 3-carbon chain or its steric equivalent. The reaction mechanism is probably different from that for the monooxygenase-catalyzed dearylation of phosphorothioates, since *p*-nitrophenol production from the paraoxon analogs is not accompanied by release of the corresponding dialkyl phosphate.

A number of reports are available indicating the role of species, strain, tissues, sex, and other factors on desulfuration and ester cleavage of OP insecticides (1, 13, 14, 23–35).

Epoxidation and Aromatic Hydroxylation

As early as 1951, the accumulation of a metabolite was observed in different tissues of rats treated with chlordane. The isolation and identification of the metabolite as an epoxide was reported later. Since then, as indicated in our earlier review (1), at least 75 animal species capable of epoxidation of organ-

ochlorine insecticides have been reported in the literature. Now the list is further extended by 26 animal species (13, 15, 16, 36–42).

The epoxidation of aldrin can be quantified with high sensitivity and reproducibility by gas chromatography, requires minimal amounts of liver microsomes or liver tissue, and the product, dieldrin, is stable and rather refractive to further metabolism. As a result, this reaction may prove a useful index for the routine clinical evaluation of the status of the human liver endoplasmic reticulum. Two reports on aldrin epoxidase activity using needle biopsy samples of liver from monkey (43) and human (44) have already appeared. The obvious utility of this monooxygenase reaction is now being exploited in studies using isolated hepatocytes from rodents and humans (45, 46).

Reports indicating that epoxidation can be observed in vitro when liver microsomes and cyclodiene insecticides are incubated in the presence of NADPH and air first appeared in 1965. The monooxygenase nature of the epoxidation reaction was later confirmed by the demonstration of obligatory requirements for reduced pyridine nucleotides, NADPH-cytochrome P-450 reductase, and molecular oxygen. Further supportive evidence for the involvement of cytochrome P-450 comes from the fact that epoxidation proceeds most actively with microsomes compared to other subcellular fractions and is significantly inhibited by several classical specific inhibitors of the monooxygenase system: CO, methylenedioxyphenyl compounds, substituted imidazoles, SKF-525A, and metyrapone (1).

The most conclusive evidence for the monooxygenase nature of the epoxidation reaction comes from reconstitution studies utilizing purified cytochrome P-450. Yu & Terriere (47) isolated six chromatographically distinct forms of microsomal cytochrome P-450 (A_1, A_2, B_1, B_2, C_1, and C_2) from NAIDM and Rutger's strains of housefly. In reconstitution experiments they used these fractions, rat liver NADPH-cytochrome P-450 reductase, and a synthetic phospholipid. The highest activity reported was for the B_1 and C_1 fractions of Rutger's and NAIDM houseflies respectively. Levi & Hodgson (9) investigated aldrin epoxidation in cytochrome P-450 systems reconstituted from uninduced mouse liver. Of five cytochrome P-450 fractions designated A_1, A_2, B_1, B_2, and B_3, according to the order of elution from DEAE cellulose columns, the A_1 fraction showed the highest activity in epoxidation of aldrin to dieldrin. A_2 and B_3 fractions also showed some activity, while B_1 and B_2 were inactive. A report on the evaluation of aldrin epoxidation in monooxygenase systems reconstituted from purified rat liver microsomal cytochrome P-450 or P-448 is also available (48). Either phenobarbital or 3-methylcholanthrene-pretreated rats were used. Considering the turnover numbers, the cytochrome P-448-dependent rate of aldrin epoxidation was less than 3% of the cytochrome P-450-dependent activity when the highest value determined for cytochrome P-448 was compared to the lowest value obtained in the

cytochrome P-450 system. The apparent Km for aldrin in the complete P-450 system was 7 μM and the maximum value reported for turnover number was 2.2 mol. dieldrin/min/mol. cytochrome P-450. A marked stimulation by ethanol, significant inhibition by SKF 525-A, and no effect of 7,8-benzoflavone on cytochrome P-450-dependent epoxidase activity was noted, while ethanol and 7,8-benzoflavone caused significant inhibition in the P-448 system. These results clearly indicate that aldrin is a selective substrate for phenobarbital-induced cytochrome P-450 as compared to the P-448 species induced by 3-methylcholanthrene.

In rat liver microsomes (49), the singlet oxygen quencher 1,4-diazabicyclo (2, 2, 2) octane inhibited epoxidation, while guanosine had no effect. While tiron and superoxide dismutase are known to inhibit reactions mediated by superoxide anion, the former stimulated aldrin epoxidation and the latter had no effect. The participation of neither H_2O_2 nor $OH\cdot$ was examined and the oxygen species responsible for aldrin epoxidation remains far from being clearly established.

Besides aldrin, heptachlor, isodrin, and photoaldrin can also serve as substrates for microsomal epoxidase activity. Except for chlordene, epoxidation occurs by oxygen attack at the C_6 and C_7 position in the substrate molecule. Methylated analogs of aldrin, however, do not undergo epoxidation. With chlordene, formation of 2,3-epoxide was noted with fish and mouse liver microsomes. Apart from this, an additional route leading to the formation of hydroxyepoxide via a hydroxychlordene intermediate has also been observed with pig liver and housefly microsomes. Although these observations tend to suggest that epoxidation occurs only if a reactive double bond is present in the substrate molecule, this becomes questionable when the results of studies with chlordane, a member of the cyclodiene family with a saturated ring, are considered. Both α- and β-chlordane isomers undergo initial desaturation to an intermediate 1-exo-2-dichlorochlordene that is subsequently epoxidized to oxychlordane. Subsequently, another pathway was proposed (50) in which chlordane undergoes reductive dehalogenation to give dihydroheptachlor or dihydrochlordene, which are oxidized to their respective epoxides after desaturation.

Lindane and other isomers of hexachlorocyclohexane produce 2,4,6-trichlorophenol as the major oxidation product when metabolized by either housefly or rat liver microsomes in the presence of oxygen and NADPH (51). The reaction was inhibited by CO; the order of reactivity was $\delta > \epsilon > \alpha > \lambda > \beta$. A direct insertion of oxygen into the cyclohexane ring to yield pentachlorocyclohexanone-*gem*-chlorohydrins was proposed as the reaction mechanism. The labile chlorohydrins are subsequently converted to corresponding cyclohexanones, which undergo two-step dehydrochlorinations via their enol forms to yield 2,4,6-trichloro-phenol. Mouse hepatic microsomal enzymes

were shown to convert 1,1-bis(p-chlorophenyl) ethylene, an mammalian metabolite of DDT, into 1,1-bis(p-chlorophenyl)-1,2-ethanediol (52). The putative epoxide intermediate, however, was not isolated.

The susceptibility of aromatic rings to oxidative attack by microsomal monooxygenase systems is well known, but there are relatively few examples involving insecticides, although naphthalene was a early example of arene oxide formation. This reaction has recently been studied in birds (18). The relative scarcity of examples of arene oxide formation is probably because various ring substituents offer more favorable sites for hydroxylation. Carbaryl metabolism does, however, proceed via this pathway. Aerobic incubation of carbaryl with NADPH fortified liver microsomes yields 4,5-hydroxy-1-naphthyl methylcarbamate and 5,6-dihydro-5,6-dihydroxy-1-napthyl methylcarbamate. The production of these metabolites of carbaryl was envisioned to proceed via an epoxide intermediate. Apart from carbaryl, ring hydroxylation is also known to occur in some other carbamate insecticides such as Landrin®, propoxur, Tsumacide®, and Pyramat®. In none of these cases has the postulated epoxy intermediate been isolated. Ring hydroxylation is rarely observed with OP insecticides and usually represents a minor pathway. Available reports include ring hydroxylation of phosmet, the thiophenol moiety of fonofos and its chloro analog (1).

Several synthetic pyrethroids undergo ring hydroxylation of the alcohol moiety before or after ester cleavage, yielding a variety of metabolites. The hydroxylation rates are usually higher with *cis* than with *trans* isomers and the preferred sites of hydroxylation vary in different animal species (53–55). For example, both *cis*- and *trans*-permethrin give rise to 4'-hydroxy metabolites with microsomal preparations from carp or trout and to 6-hydroxy derivatives with mouse or housefly preparations. Hydroxylation at the 2' position is observed only with the *cis* isomer and only with mouse liver microsomes. Both 4' and 5-hydroxy derivatives are produced from cypermethrin by mouse liver microsomes. Kadethrin and decamethrin were shown to be hydroxylated at the 4' position by microsomal oxidases.

Aliphatic Hydroxylation

Aliphatic C-H bonds not adjacent to hetero atoms are susceptible to initial oxidative attack, yielding stable alcohols that may in certain cases be the subject of further oxidation or conjugation. Several studies have firmly established that aliphatic hydroxylation of alkyl side chains represents the predominant route for oxidative biotransformation of carbamate insecticides. The chemical nature of the metabolites produced depends upon the kind and number of substituents. Thus, oxidation of the methyl group may stop at the alcohol step, as in case of Tsumacide®, or may proceed to the carboxy analog with compounds such as Banol. In carbamates such as in Landrin®, Bux®, UC-

10854, RE-11775, RE-5365, carbofuran, and N-(2-toluenesulfenyl) carbofuran, there may be simple hydroxy analog formation (1).

Aliphatic hydroxylation of alkyl ring substituents of some OP insecticides, such as O, O, O-tri-*o*-tolyphosphate (TOCP) and O, O, O-tri-(*p*-ethylphenyl)-phosphate (TPEP), results in activation, while oxidation of the 3-methyl group of fenitrothion to a carboxyl group leads to detoxication. The side chain on the heteroaromatic ring of diazinon and diazoxon also undergoes hydroxylation to produce hydroxydiazinon and isohydroxydiazinon, metabolites B and C. The involvement of cytochrome P-450 is suggested from the observed inhibition by CO, piperonyl butoxide, and substituted imidazoles. All oxidation products with intact ester linkages exhibit anticholinesterase activity.

For some time, epoxides were thought to be the terminal residues of cyclodiene insecticides such as aldrin. However, several studies have indicated the production of small amounts of 2-hydroxy, 4-hydroxy derivatives or Klein's metabolite from dieldrin following attack by microsomal monooxygenases. Similar to dieldrin, the rat hepatic microsomal system has also been found to catalyze conversion of endrin to four monohydroxylated products. Related cyclic compounds vulnerable to hydroxylation by the microsomal monooxygenase systems include dihydroaldrin, dihydroisodrin, HCE, photoisodrin, chlordene, dehydrochlordene, photoheptachlor, toxaphene, and methylated analogs of aldrin and dieldrin (1, 56, 57).

Extensive investigations on the in vitro metabolism of pyrethroids were carried out by Casida, Elliott, Miyamoto, and co-workers [cited in (1)]. On the basis of recovered radiocarbon in various metabolites, hydroxylation of the *trans* methyl group of the isobutyl side chain in the acid moiety represents the major pathway of metabolism with compounds like allethrin, while that of *cis* methyl group appears to be a minor site. This oxidation results in the sequential formation of the corresponding hydroxymethyl, aldehyde, and carboxylic acid. These or similar pathways have been found to be operative in the case of natural pyrethroids as well as in that of synthetic compounds.

Heterocyclic Ring Hydroxylation

Heterocyclic ring hydroxylation represents a rare reaction in insecticide metabolism and is best studied in the case of nicotine. Earlier reports indicated that rabbit liver microsomes can catalyze hydroxylation at the α-carbon of the pyrrolidine ring in the nicotine molecule. This biotransformation required NADPH and oxygen, and the hydroxynicotine produced was further oxidized by aldehyde oxidase to cotinine. Cotinine formation, but not the disappearance of nicotine, was inhibited by SKF 525-A and KCN. Reconstitution studies (58) have provided further evidence for the participation of cytochrome P-450 in nicotine oxidation. The reaction depends on cytochrome P-450, NADPH cytochrome P-450 reductase, and NADPH. Despite the several known metabo-

lites of nicotine (1, 59), the authors monitored the disappearance of substrate to quantitate activity. Heterocyclic ring hydroxylation has also been shown as an intermediate metabolic step in the metabolism of anabasine analogs and an experimental insecticide, R-16661 (1).

Dealkylation

The oxidative cleavage of alkyl groups attached to electronegative heteroatoms such as nitrogen and oxygen is a metabolic reaction frequently encountered with insecticides. Since all the commercial carbamate insecticides contain N-alkyl groups in their molecules, the N-dealkylation reaction first reported by Hodgson & Casida (60, 61) assumes major significance in their metabolism. Using rat liver microsomes, the enzymatic splitting of the C-N bond in 4-nitrophenyl-N,N-dimethylcarbamate was found to require NADPH and molecular oxygen. The reaction was inhibited by classical inhibitors of the monooxygenase system such as piperonyl butoxide and SKF 525-A.

From the mechanistic point of view, the initial attack may occur either at the carbon attached to the nitrogen or at the nitrogen itself. Thus, initial α-carbon oxidation directly yields a carbinolamine that ultimately rearranges to liberate a carbonyl compound and the dealkylated amine, while prior N-oxidation leading to carbinolamine formation represents another route. The experimental evidence, however, is strongly in favor of C-oxidation leading to N-hydroxylalkyl metabolites. Although dimethyl as well as monomethyl carbamates serve as substrates for the monooxygenase-catalyzed N-dealkylation reaction, only one methyl group of dimethylcarbamates participates in hydroxylation, yielding an N-methylcarbamate, and there is no evidence for the further oxidation of the remaining N-methyl group to yield the primary carbamate. In some cases, the N-hydroxymethyl derivatives are sufficiently stable to be isolated following either in vivo or in vitro oxidation (1).

In contrast to carbamates, in which N-dealkylation usually results in detoxication, oxidation of certain OP insecticides such as scradan and dimefox via postulated N-oxide intermediates leads to the formation of highly potent anticholinesterase agents, while detoxication involving carbinolamine intermediates occurs with others. Some data on the N-dealkylation of cotinine and nicotine are also available (1, 32, 62, 63).

The O-dealkylation of OP triesters to the corresponding diester is a major mechanism of detoxication and is seen in both insects and mammals. It differs from other O-dealkylations in that the oxygen attack is on an ester linkage rather than on an ether. The reaction mechanism is analogous to N-dealkylation except that the hydroxy or diol intermediates of an O-dealkylation reaction are extremely unstable and undergo spontaneous rearrangement to liberate the dealkylated substrate. Paraoxon serves as a substrate for microsomal monooxygenase-catalyzed O-deethylation and leads to the production of either etha-

nol, acetaldehyde, or acetate in different animal species. Although this reaction has been extensively studied with a number of OP triesters, the obligatory requirement for cytochrome P-450 remains uncertain, at least in the case of chlorfenvinphos (1).

Although O-dealkylation is a rather common reaction in the case of OP triesters (1,64), its occurrence in the metabolism of other classes of insecticides appears rare. Several reports (1, 65, 66) indicate that one or both *para* substituted methoxy groups of methoxychlor are readily cleaved off by liver microsomes in the presence of NADPH and air. The same degradative pathway was implicated in the metabolism of ethoxychlor, in the formation of 2-hydroxyphenyl N-methylcarbamate from propoxur, and in the oxidation of 3-methyoxy group of rotenone (1).

Dehydrogenation

Desaturation, a novel reaction mechanism first discovered during in vitro enzymatic conversion of α- and β-chlordane to 1,2-dichlordene, is now firmly established as a typical monooxygenase reaction catalyzed by microsomal cytochrome P-450. The necessary evidence for the oxidative dehydrogenation was presented in the case of lindane. The reaction required NADPH and molecular oxygen and was inhibited by CO and SKF 525-A (67). Lack of inhibition by KCN further differentiates this dehydrogenase system from the cytochrome b_5-dependent fatty acid desaturase system. In the proposed mechanism, the oxenoid, generated from oxygen and the enzyme(s), abstracts two hydrogen atoms from the substrate to yield water and the alkene directly. It now appears that chlordane isomers and hexachlorocyclohexene also serve as substrates for microsomal enzymes isolated from the housefly (49, 51, 68) and the rat (49–51, 69). Recently dehydrogenation, dehydrochlorination, and droxylation were found to be involved in the metabolism of lindane by human liver microsomes. The rates were found to be comparable to those reported for rats and insects despite considerable quantitative variation between individuals (70).

INSECTICIDE METABOLISM DURING PROSTAGLANDIN BIOSYNTHESIS

In addition to the cytochrome P-450-dependent monooxygenase system, coupling of the N-dealkylation of an insecticide to prostaglandin biosynthesis has recently been described (3). The reconstituted prostaglandin endoperoxide synthetase involves two enzyme activities, fatty acid cyclooxygenase that catalyzes bis-dioxygenation to produce the hydroperoxy endoperoxide PGG_2, and hydroperoxidase, the reduction of PGG_2 to PGH_2. A variety of xenobiotics that can provide the requisite pair of electrons for the last step of the reaction are

themselves co-oxidized. Ram seminal vesicle microsomes, when incubated in the presence of air and arachidonic acid, effected the N-dealkylation of aminocarb as measured by formaldehyde production. Besides aminocarb, several mono- and di-methyl substituted model compounds were found to be substrates. However, S- or O-dealkylation was not observed with the compounds tested. The N-demethylation rate was comparable to that observed with NADPH-dependent hepatic microsomal cytochrome P-450-mediated monooxygenase reactions and detectable amounts of neither cytochrome P-450 nor NADPH-dependent dealkylation were present. Although the reaction mechanism is not yet precisely understood, the proposed dehydrogenase mechanism of N-dealkylation for the prostaglandin synthetase coupled system is expected to be very different from that suggested for the cytochrome P-450 system. Since prostaglandin biosynthesis occurs in many cell types in various tissues such as lung, kidney, and skin, xenobiotic metabolism via this alternate pathway could conceivably play a significant role in determining the susceptibility of target organs lacking monooxygenase activity.

FAD-CONTAINING MONOOXYGENASE REACTIONS

Sulfoxidation

In the presence of NADPH and oxygen, thioether-containing OP insecticides are oxidized by the FAD-containing monooxygenase purified from pig liver microsomes. The stoichiometry between NADPH and substrate consumed is 1:1 and the product, in the case of phorate and disulfoton, is the sulfoxide (71, 72). The sulfoxides are optically active and further oxidation to the sulfone is not apparent. n-Octylamine, which inhibits cytochrome P-450 and activates the FAD-containing monooxygenase, increases the rate of sulfoxidation. Structural changes around the thioether sulfur that affect nucleophilicity or cause steric hindrance tend to decrease the rate of sulfoxidation. Although neither thiono nor thiolo sulfur atoms of phosphorodithioates are attacked, substitution of either by oxygen decreases thioether oxidation. O,O-dimethyl compounds that contain thioether sulfur are not oxidized as readily as their O,O-diethyl analogs.

The (−) isomer of profenofos is activated to a more potent cholinesterase inhibitor by an oxidative attack on the thiolo sulfur that, in the case of the less toxic (+) isomer, results in detoxication (73). We have recently shown (Levi & Hodgson, unpublished information) that this reaction is catalyzed primarily by cytochrome P-450, with only a small contribution due to the FAD-containing monooxygenase. Furthermore, one of the cytochrome P-450 isozymes purified from the hepatic microsomes of uninduced mice (8) is more active in this regard than the others.

Sulfur-containing carbamates are not as effectively metabolized by the

FAD-containing monooxygenase as OP insecticides but some, including thiofanox, aldicarb, methiocarb, and croneton, are oxidized at appreciable rates (71, 72).

Methiochlor and its analog containing one $-SCH_3$ and one $-OCH_3$ group are both readily oxidized by the pig liver FAD-containing monooxygenase (2), the product presumably being the monosulfoxide, since one mole of NADPH is consumed per mole of substrate oxidized. This leaves unanswered the question of the origin of the sulfones known to be formed in vivo.

Many microsomal sulfur oxidations have been attributed to cytochrome P-450 (1) and, by comparison with non-insecticidal substrates, it appears probable that this may often be the case. The relative importance of these two metabolic routes for common substrates is presently unknown. We have addressed this problem in the case of the hepatotoxicant thiobenzamide, and we find that the relative contributions vary from species to species and organ to organ (74). In the mouse their contributions are approximately equal in the liver, while in the lung 85% of thiobenzamide oxidation proceeds via the FAD-containing enzyme.

In view of the well-known formation in vivo of sulfones (1) and the apparent inability of the FAD-containing monooxygenase to carry insecticide oxidations beyond the sulfoxide, it can be speculated that the second step is catalyzed by cytochrome P-450.

In this regard it is interesting that, although methidathion is not a substrate for the FAD-containing monooxygenase, the S-methyl derivative of its hydrolysis product is. This compound, which is formed in vivo, is metabolized to the sulfoxide by the FAD-enzyme, but further oxidation to the sulfone is apparently mediated by the cytochrome P-450-dependent system (27).

Desulfuration

The FAD-containing monooxygenase catalyzes the oxidation of fonofos and phenyl fonofos. Reaction stoichiometry, product identification, and the formation of a potent cholinesterase inhibitor all indicate that the reaction is an oxidative desulfuration producing the oxon as the principal product (75). Previous studies on fonofos activation (76–79) all implicate the cytochrome P-450-dependent monooxygenase system and propose a mechanism involving a cyclic phosphooxathiran intermediate similar to that proposed for parathion (21). Since previous studies on fonofos were carried out with microsomal preparations and, since both the FAD-dependent monooxygenase and the cytochrome P-450-dependent monooxygenase system are NADPH and O_2 dependent, the relative roles of the two systems were not defined. It is clear, however, that cytochrome P-450 is responsible for the oxidative desulfuration of phosphorothioates, such as parathion, since this reaction has been carried out with purified, reconstituted cytochrome P-450 (21). It is possible that phos-

phonates, such as fonofos and its phenyl analogs, are metabolized by both systems. Lee et al (78) showed that retention of configuration, considered to be consistent with the above mechanism for cytochrome P-450, was the principal stereochemical course for the activation of fonofos isomers by rat liver microsomes. They speculated that the 21–28% inversion observed was due to a second mechanism involving an initial attack on the phosphorus atom.

The phosphorodithioate analog of fonofos is neither a substrate nor an inhibitor for the FAD-containing monooxygenase. It is not understood why, with this enzyme, oxidative desulfuration occurs with phosphonates but not with phosphorothioates. Although the former lack p^{π}-d^{π} bonding due to the absence of free electrons in the carbon atom covalently bound to the phosphorus, in contrast to the oxygen atom in the latter, the significance of this in the reaction mechanism is as yet unknown.

Indirect support for an attack on phosphorus is provided by the observation that this enzyme also catalyzes the oxidation of the phosphines, diphenylmethylphosphine and the CNS depressant 3-dimethylamino-propyldiphenylphosphine, the products being diphenylmethylphosphine oxide in the former and both the P-oxide and the N,P-dioxide in the latter (2). Since diphenylmethylphosphine does not contain either sulfur or nitrogen, the attack is presumably on the phosphorus atom. Moreover, the pentavalent sulfide of diphenyl methylphosphine is metabolized by this enzyme to its oxygen analog, the phosphine oxide. These two observations, taken together, increase the probability that the activation of fonofos and other phosphonates is via an attack on the phosphorus atom.

Amine Oxidation

Tetram and its analogs are also substrates for the FAD-containing monooxygenase and the product appears to be the corresponding N-oxide (71, 72).

MONOOXYGENASES AND NEWER INSECTICIDES

The following are some brief remarks concerning monooxygenation of some of the newer insecticides or compounds of potential importance in insecticide development. They include synthetic pyrethroids, juvenile hormones and hormone analogs, anti-juvenoids, and an insect growth regulator, diflubenzuron.

Pyrethroids

Although oxidation of pyrethroids is well known and many of the reactions, and the structural basis for them, have been reviewed either in reference (1) or in the section on cytochrome P-450 reactions above, it is now clear that hydrolysis is the most important route for the metabolism of the majority of the synthetic pyrethroids (53, 80–82). Inhibition of the esterases involved, by other

insecticides or related compounds, raises the possibility of synergistic interactions (82).

The rapid and complete debromination of tralomethrin and thalocythrin to yield deltamethrin and cypermethrin is apparently non-enzymatic, but the two products and their subsequent enzymatic hydrolysis products are subject to oxidative metabolism (83, 84).

Hormones and Hormone Analogs

The final step in the biosynthesis of juvenile hormones is epoxidation, and monooxygenases are involved in this and in their deactivation in insects. It is apparent, however, that epoxide hydrolase and esterase action are more important in their metabolic deactivation in insects. The low toxicity of juvenile hormones and hormone analogs to mammals, however, may be due to the rapidity with which they are oxidized in mammalian preparations. These and other aspects have recently been reviewed (10).

Anti-juvenoids, the precocenes, appear to function as suicide substrates for the monooxygenases of the corpus allatum, blocking hormone production and causing necrosis of the gland. Although the postulated epoxide has not been isolated, the resultant diol has. The early studies leading to these conclusions have recently been reviewed (10). Because of the potential for applied use this area has been one of intense interest. Recently (85), synthetic analogs of precocenes, such as substituted isopentenylphenols, have been synthesized whose mode of action is indistinguishable from the natural precocenes. However, precocenes are known to be hepatotoxic (86) and nephrotoxic (87) in rats, although at high dose levels, effects possibly mediated through oxidative formation of reaction intermediates similar to those formed in the corpus allatum. If this is the case, the selective difference in sensitivity between the insect gland and the mammalian tissue is remarkable.

Insect Growth Regulators

Diflubenzuron [1-(2,6-difluorobenzoyl)-3-3(4-chlorophenyl)urea], a compound that affects chitin formation in insects, is one of the growth regulators currently of much interest as an insecticide. On the basis of in vivo studies in houseflies (88, 89) it is known to produce a number of metabolites characteristic of monooxygenase activity, including 2,6-difluorobenzamide, 2,6-difluorobenzoic acid, 4-chlorophenyl urea, and several ring hydroxylated species. The monooxygenase nature of this metabolism is supported by sesamex inhibition (89).

Similar pathways are known in domestic animals, but frequently the bulk of the administered dose is excreted in the feces unchanged (90). In chickens diflubenzuron is particularly refractory to metabolism, the small amount broken down by microsomal metabolism being apparently the result of non-oxidative pathways (91).

INDUCTION

Effect of Induction

Induction or inhibition of the cytochrome P-450-dependent monooxygenase system following chemical pretreatment of animals might be expected to be reflected in an increased or decreased monooxygenase activity in vitro. This does appear to be the case in several animal species (1). In addition, phenobarbital induces cyclodiene epoxidase activity in three species of blow fly (39), rat (92), and mouse hepatocytes in culture (45) as well as induces cytochrome P-450 in susceptible houseflies (93). Liver microsomal aldrin epoxidase is significantly increased in nestling and adult barn owls by Aroclor-1254 (94) and in rats by either toxaphene or (non)polar extracts of toxaphene (95). On the other hand, 3-methylcholanthrene (92) or α- or β-endosulfan (96) exposure lowers hepatic aldrin epoxidase activity. In blow flies, β-naphthoflavone induction of aldrin epoxidase was found to be relatively minor (39), while this inducer had no effect on mouse hepatocytes in culture (45). There is evidence, in addition to that previously summarized (10), that aldrin epoxidase in insects can also be induced by the allelochemicals present in plants (38, 40, 41).

A significant induction of in vitro liver microsomal metabolism of OP compounds via O-dealkylation and dearylation pathways has been reported when rats or mice were pretreated with either phenobarbital (30), halobenzenes (97), DDE (98), or different pesticides (31, 99).

Induction studies also suggest that constitutive and phenobarbital-induced forms of hepatic microsomal enzymes, which can be inhibited by α-naphthoflavone, participate in nicotine oxidation (100). After either 3-methylcholanthrene or β-naphthoflavone treatment nicotine oxidase activity per cytochrome P-448 molecule was actually decreased but the specific activity of the enzyme remained unchanged. Female rats, fed on a diet containing either Aroclor-1254, phenobarbital, or β-naphthoflavone for one week, followed by oral lindane administration, showed induction of liver microsomal lindane dehydrogenase activity by Aroclor-1254 and phenobarbital, while β-naphthoflavone pretreatment impaired the enhancement of dehydrogenase activity (69).

Insecticides as Inducers

Subsequent to previous reviews (1, 31, 99) a number of reports have appeared on induction by insecticides. Generally they fall into three areas: effects on the LD_{50} of other insecticides; effects of kepone, mirex, and related compounds on liver function; general induction of xenobiotic-metabolizing enzymes and its possible consequences. Space limitations preclude a complete review but a brief summary with selected references follows.

Dieldrin was shown to protect against the acute toxicity of chlorfenvinphos,

the effect due to increased metabolism (101), while large doses of carbaryl provided only marginal protection against the acute effects of parathion and propoxur (102).

Kepone (chlordecone) is a potent inducer of microsomal cytochrome P-450 and monooxygenase activity (99, 103). In addition, it causes a dramatic enhancement of the hepatotoxicity of haloalkanes such as carbon tetrachloride (104). However, it is not immediately obvious that the latter activity is related to enzyme induction (104, 105). The related compound, mirex, is a more potent inducer than kepone, although it does not cause the enhancement of haloalkane toxicity. It is, in fact, the most potent inducer of cytochrome P-450 among pesticides (99). The inducing effect of mirex and related compounds on various aspects of xenobiotic metabolism have frequently been demonstrated (99, 106–108).

Many insecticides have been shown to induce cytochrome P-450 and associated monooxygenase activities (99). These investigations continue to be extended and recent studies include the following: the effect of lindane on CF1 mice (109); induction of cytochrome P-450 by methylenedioxyphenyl synergists such as piperonyl butoxide and the possible role of the methylene carbon (110, 111); the possible role of induction by dieldrin (112), toxaphene, and carbaryl (113) on the expression of carcinogenicity caused by other compounds; an attempt to classify pesticide inducers according to the pattern of activities induced (114, 115).

INHIBITION

Inhibition of Insecticide Metabolism

Although much of the current literature on monooxygenase inhibition concerns compounds related to insecticides, this topic is beyond the scope of this review. Either the compounds are not themselves synergists or the inhibited reactions do not involve insecticides. For example, much of the work on methylenedioxyphenyl compounds concerns safrole and isosafrole. A comprehensive review of these aspects, which are of great importance in consideration of the mode of action of synergists, is in press (116).

Some findings directly related to insecticide action are summarized below. Pretreatment with piperonyl butoxide was found to result in different levels of inhibition of desulfuration and hydroxylation of azinphosmethyl and azinphosethyl by mouse hepatic microsomes (24). Piperonyl butoxide was also shown to block the metabolism in vivo of the hormone analog methoprene in the imported fire ant, *Solenopsis invicta* (117). In the housefly, juvenile hormone I was shown to be a competitive inhibitor for the formation of the metabolite-inhibitory complex formed by piperonyl butoxide (118), a finding that tends to confirm that monooxygenases are involved in juvenile hormone

inactivation and that the sometimes reported hormone-like activity of methylenedioxyphenyl compounds may be due to their ability to block hormone degradation. Several naturally occurring methylenedioxyphenyl compounds have been shown to affect insecticide metabolism. This includes the effect of myristicin on parathion and paraoxon metabolism (119) and the effect of dillapiole (2,3-dimethoxy-4,5-methyldioxyallylbenzene) and several of its synthetic analogs on pyrethrin toxicity (120).

5,6-Dichloro-1,2,3-benzothiadiazole has been shown to form a metabolite-inhibitory complex with cytochrome P-450 (121), a finding that presumably explains its activity as an insecticide synergist.

Insecticides as Inhibitors

In vitro, insecticides are frequently competitive inhibitors of the metabolism of related compounds, but these interactions are usually marginal in vivo. They will not be discussed further. Of greater interest is the ability of some insecticides to inactivate cytochrome P-450 during the course of their metabolism and as a result affect the metabolism and toxicity of other compounds.

Parathion inactivates the microsomal cytochrome P-450-dependent monooxygenase system when administered in vivo or when incubated in vitro with microsomes NADPH and O_2 (21, 122). This metabolism-dependent inactivation is brought about by the interaction of an active metabolite (probably singlet sulfur) with the heme iron, which results in a loss of heme. Other interactions include the formation of hydrodisulfide (R-S-SH) linkages with cysteine residues in the protein moiety of cytochrome P-450. These latter reactions are apparently of less importance in the inactivation mechanism (21, 122).

This inactivation mechanism probably also explains the results of in vitro studies that have shown that aldrin epoxidation, parathion oxidation (25, 26), and chlorpyrifos desulfuration (123) are inhibited by parathion, as well as the effect of fenitrothion on monooxygenases in weanling rats (124).

Phenolphthalein, halogenated fluoresceins, and di- and triphenylmethane derivatives have also been shown to be inhibitors of aldrin epoxidase (125).

SUMMARY AND CONCLUSIONS

In summary, it can be said that advances have been made in understanding the range and significance of monooxygenase reactions involving insecticides in both target and non-target species and that some specific aspects emerge that should command the attention of insecticide toxicologists and others in the immediate future.

The role of co-oxidation of insecticides during prostaglandin synthesis has just emerged as an issue but, if we can extrapolate from other xenobiotics

(126), we can predict with some confidence that it will assume major proportions.

It is also apparent that both activation and detoxication reactions are catalyzed by the FAD-containing monooxygenase and that many of these reactions were formerly attributed to the cytochrome P-450-dependent monooxygenase system. Since any particular substrate may be oxidized by either or both of these two routes, it is essential that studies be conducted to define their relative contributions to xenobiotic oxidation in microsomal preparations and ultimately in vivo.

The area of interactions and the mechanisms behind them is also of importance, from the public health point of view in the case of multiple exposures, and from the practical viewpoint in the case of insecticide synergists.

While the above aspects are not the only ones of future importance, they should contribute to the ultimate goal of insecticide toxicology: practical, safe use of chemicals for the control of insect pests.

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