Bull. Environ. Contam. Toxicol. 31, 479–485 (1983)

Effect of Pyrethroid Insecticides and N-(3,5-dichlorophenyl) Dicarboximide Fungicides on Microsomal Drug-metabolizing Enzymes in the Japanese Quail (*Coturnix coturnix*)

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The liver microsomal drug-metabolizing enzymes are membrane-bound and function as a multicomponent electron transport system responsible for the metabolism of a wide variety of endogenous substrates (such as steroids, fatty acids and bile acids) and exogenous substances (such as drugs, polycyclic hydrocarbons and pesticides) (LU, 1976). A striking property of the system is the fact that these compounds not only serve as substrates, but can also function as inhibitors and/or inducers (SNYDER and REMMER, 1979). This has many implications since it can affect the metabolism and therefore the activity and/or toxicity of both endogenous and exogenous compounds. In the last decade, great progress has been made in the development of stable and highly active pyrethroid insecticides, and in the near future more widespread application of these compounds may be expected. In the same period, some N-(3,5-dichlorophenyl) dicarboximide derivatives were introduced as effective agricultural fungicides. To assess the environmental impact of these new molecules, we have undertaken studies on the effects of pesticides on drug metabolism in an avian species, the Japanese quail (*Coturnix coturnix*). The aim of the present study was to determine if pyrethroid insecticides and N-(3,5-dichlorophenyl) dicarboximide fungicides can induce the hepatic and intestinal microsomal enzymes of the quail by characterizing their effects on microsomal cytochrome P-450, NADPH-cytochrome c reductase and *in vitro* metabolism of 7-ethoxycoumarin, 7-ethoxyresorufin, aldrin and aniline.

MATERIALS AND METHODS

Animals. Female Japanese quails (about 6 weeks old) were commercially purchased and maintained one week or more in the laboratory conditions before treatment. Birds were kept in a battery equipped with wire floors and thermostatically controlled heating units, with a 20hr-daylight period. Nutritionally balanced quail diets were obtained from the CNRZ (La Minière, France). All animals were allowed free access to food and tap water. They were not starved before sacrifice.

Chemicals. The chemicals used in these experiments and their source were : cypermethrin (98.4 %) and fenvalerate (94.5 %) from Shell-Chimie, Paris ; deltamethrin (98.5 %) from Roussel-Uclaf, Paris ; iprodione (> 95 %) from Rhône-Poulenc, Paris ; permethrin (93 %) and procymidone (97 %) from Sopra, Clamart ; vinclozolin

(97.8 %) from BASF, Levallois-Perret. 7-ethoxycoumarin and 7-ethoxyresorufin were synthetized in the laboratory, according to PROUGH *et al* (1978). Other commercial chemicals were used without further purification, excepted aniline (redistilled).

Mode of intoxication. Animals were fed for 7 days on a diet containing the above compounds at 2000 ppm. The preparation of microsomal fractions and enzyme assays were performed according to RIVIERE (1983).

RESULTS

As indicated in Table 1, pyrethroid insecticides in general were found to give no or only moderate increases in hepatic cytochrome P-450 level, NADPH-cytochrome c reductase, aldrin epoxidase and 7-ethoxyresorufin dealkylase activities. On the other hand, a significative decrease in the activity of 7-ethoxycoumarin dealkylase and aniline hydroxylase was observed after pretreatment with permethrin, cypermethrin and deltamethrin. An attempt was made to distinguish between different forms of cytochrome P-450 by the use of the selective inhibitors, metyrapone and 7,8-benzoflavone (ULLRICH *et al*, 1975). As shown in table 1, the extent of inhibition was not modified by the pretreatment.

The data obtained with the N-(3,5-dichlorophenyl) dicarboximide fungicides are given in table 2. Procymidone was virtually without effect on hepatic drug-metabolizing enzymes, except a slight increase in the activity of the 7-ethoxyresorufin dealkylase. The two other fungicides were good inducers and markedly enhanced the cytochrome P-450 content (3-to 4-fold) and the activity of the 7-ethoxyresorufin dealkylase ($\simeq$ 12-fold). The increase in other microsomal enzymes was moderate and did not seen to be proportional to the level of cytochrome P-450. After exposure to vinclozolin, the activity of the 7-ethoxycoumarin dealkylase was slightly more susceptible to the inhibitory effect of metyrapone, suggesting the presence of different forms of cytochrome P-450 in control- and treated- animals.

As shown in table 3, the pretreatment of animals with pyrethroid insecticides resulted in an increase in the intestinal cytochrome P-450 (2 -to 3-fold) and in the enzyme activities (< 2-fold). The N-(3,5-dichlorophenyl) dicarboximide fungicides were also inducers of intestinal enzymes, in the order of decreasing effectiveness, vinclozolin > iprodione > procymidone.

DISCUSSION

Only a few limited studies have been devoted to the inductive effects of pyrethroid insecticides. Exposure of rats to high dosages of pyrethrum, the natural insecticide extracted from the pyrethrum flower, sligthly increased the cytochrome P-450 content and the activity of drug-metabolizing enzymes (SPRINGFIELD, 1973 ; MADHUKAR and MATSUMURA, 1979). Permethrin appeared to be a weak

TABLE 1. Effect of pyrethroid insecticides on hepatic microsomal enzymes in female Japanese Quail

	Control	Permethrin	Cypermethrin	Fenvalerate	Deltamethrin
Body weight (g)	227 ± 17[a]	255 ± 17*	242 ± 16	243 ± 11	228 ± 11
Liver weight (g)	6.1 ± 0.8	7.3 ± 0.9*	7.3 ± 1.1	7.0 ± 1.3	6.1 ± 0.5
Ratio liver weight/body weight	0.0269	0.0286	0.0302	0.0288	0.0268
Microsomal proteins (mg/g)	11.7 ± 0.4	13.0 ± 1.4	13.2 ± 1.5	13.2 ± 1.3	12.1 ± 1.2
Cytochrome P-450 (nmol/mg)	0.21 ± 0.04	0.25 ± 0.07	0.25 ± 0.11	0.27 ± 0.07	0.28 ± 0.04**
NADPH-cytochrome c reductase (nmol/mg x min)	97 ± 15	137 ± 34**	113 ± 41	116 ± 17	107 ± 12
Aniline hydroxylase (nmol/mg x min)	0.80 ± 0.17	0.58 ± 0.13*	0.54 ± 0.16*	0.88 ± 0.21	0.66 ± 0.16
Aldrin epoxidase (nmol/mg x min)	0.14 ± 0.03	0.28 ± 0.10**	0.22 ± 0.11	0.21 ± 0.05*	0.27 ± 0.09**
7-Ethoxycoumarin dealkylase (nmol/mg x min)	2.00 ± 0.48	1.20 ± 0.28**	1.14 ± 0.33**	2.07 ± 0.64	1.34 ± 0.25*
7-Ethoxyresorufin dealkylase (nmol/mg x min)	0.13 ± 0.05	0.11 ± 0.11	0.14 ± 0.04	0.13 ± 0.05	0.24 ± 0.13
7-Ethoxycoumarin dealkylase + metyrapone, 100 µM (%)	51 ± 3	51 ± 2	50 ± 3	54 ± 3	53 ± 4
7-Ethoxycoumarin dealkylase + 7,8-benzoflavone,10µM (%)	66 ± 4	70 ± 3	69 ± 6	68 ± 5	71 ± 8

a Mean ± SD (6 animals)
* Significantly different, $P < 0.05$; ** significantly different, $P < 0.01$

TABLE 2. Effect of N-(3,5-dichlorophenyl) dicarboximide fungicides on hepatic microsomal enzymes in female Japanese quail

	Control	Iprodione	Vinclozolin	Procymidone
Body weight (g)	244 ± 20	237 ± 16	247 ± 16	235 ± 16
Liver weight (g)	6.8 ± 1.0	7.7 ± 1.1	8.3 ± 1.5*	7.1 ± 0.8
Ratio liver weight/body weight	0.0279	0.0325	0.0336*	0.0302
Microsomal proteins (mg/g)	13.3 ± 1.0	13.9 ± 1.8	14.8 ± 2.7	12.4 ± 1.7
Cytochrome P-450 (nmol/mg)	0.20 ± 0.06	0.79 ± 0.21**	0.71 ± 0.20**	0.23 ± 0.06
NADPH-cytochrome c reductase (nmol/mg x min)	107 ± 19	158 ± 23**	154 ± 30**	106 ± 18
Aniline hydroxylase (nmol/mg x min)	0.71 ± 0.17	1.41 ± 0.33**	1.10 ± 0.28*	0.68 ± 0.19
Aldrin epoxidase (nmol/mg x min)	0.14 ± 0.03	0.23 ± 0.07*	0.46 ± 0.06**	0.17 ± 0.02
7-Ethoxycoumarin dealkylase (nmol/mg x min)	1.80 ± 0.44	2.90 ± 0.64**	1.81 ± 0.51	1.32 ± 0.41
7-Ethoxyresorufin dealkylase (nmol/mg x min)	0.10 ± 0.06	1.21 ± 0.31**	1.20 ± 0.46**	0.46 ± 0.21**
7-Ethoxycoumarin dealkylase + metyrapone, 100 µM (%)	54 ± 2	58 ± 3	66 ± 4	60 ± 4
7-Ethoxycoumarin dealkylase + 7,8-benzoflavone, 10 µM (%)	69 ± 2	72 ± 6	72 ± 13	68 ± 4

a Mean ± SD (6 animals)
* Significantly different, $P < 0.05$; ** significantly different, $P < 0.01$

TABLE 3. Effect of pyrethroid insecticides and N-(3,5-dichlorophenyl) dicarboximide fungicides on duodenal microsomal enzymes in female Japanese quail

	Control	Permethrin	Cypermethrin	Fenvalerate	Deltamethrin
Microsomal proteins (mg/g)	7.9 ± 1.1[a]	8.9 ± 1.0	10.0 ± 1.0*	8.8 ± 1.8	8.0 ± 1.5
Cytochrome P-450 (nmol/mg)	0.16 ± 0.04	0.42 ± 0.07**	0.28 ± 0.07**	0.28 ± 0.04**	0.28 ± 0.18*
NADPH-cytochrome c reductase (nmol/mg x min)	129[b]	139	106	140	235
7-Ethoxycoumarin dealkylase (nmol/mg x min)	0.54 ± 0.30	1.10 ± 0.35*	0.64 ± 0.14	0.94 ± 0.36	0.72 ± 0.11
7-Ethoxyresorufin dealkylase (nmol/mg x min)	0.64 ± 0.20	1.00 ± 0.26*	0.74 ± 0.21	0.92 ± 0.10*	0.94 ± 0.13*

	Control	Iprodione	Vinclozolin	Procymidone
Microsomal proteins (mg/g)	7.7 ± 1.8	8.3 ± 1.2	9.6 ± 1.5	9.0 ± 1.5
Cytochrome P-450 (nmol/mg)	0.11 ± 0.02	0.25 ± 0.07**	0.59 ± 0.16**	0.21 ± 0.09
NADPH-cytochrome c reductase	102[b]	138	141	121
7-Ethoxycoumarin dealkylase (nmol/mg x min)	0.38 ± 0.10	0.71 ± 0.21*	1.20 ± 0.51**	0.64 ± 0.33
7-Ethoxyresorufin dealkylase (nmol/mg x min)	0.54 ± 0.22	0.55 ± 0.20	0.97 ± 0.24	0.92 ± 0.55

a Mean ± SD (6 animals)
* Significantly different, $P < 0.05$; ** significantly different, $P < 0.01$
b six pooled microsomal fractions

inducer of the microsomal enzymes (CARLSON and SCHOENIG, 1980 ; LITCHFIELD, 1982). Cypermethrin was a less effective inducer of liver changes than permethrin (LITCHFIELD, 1982). Pyrethrins and pyrethroids combined with cytochrome P-450 from mouse, rabbit, sheep and rat livers to produce "type I" difference spectra (KULKARNI *et al*, 1975). The liver weight markedly increased in rats treated with DDOD, a compound closely related to vinclozolin (ITO *et al*, 1978).

The induction of drug-metabolizing enzymes depends on the animal species used. Phenobarbital, a well-known inducer of enzymes from rat liver, was not an effective inducer of avian enzymes (RIVIERE and BACH, 1982). DDT, an other classical inducer, induced rat enzymes but inhibited the activity of aniline hydroxylase in quail liver (SELL and DAVISON, 1973). On the other hand, it was recently found that an agricultural fungicide (prochloraz) was a better inducer of the hepatic cytochrome P-450 from the quail than from the rat, giving rise to a 9- and 2-fold increase, respectively (RIVIERE, 1983).

Our data clearly show that pyrethroid insecticides are very weak inducers of microsomal enzymes and, sometimes, they inhibited them. The possibility of metabolic interactions between these compounds and other toxic agents seem rather limited. At the high dosages employed, vinchlozolin and iprodione were good inducers and produced a marked increase in some components of the drug-metabolizing enzymes. Procymidone did not share the ability to induce these enzymes. We have previously demonstrated a high activity of the microsomal enzymes in the small intestine (duedenum) from the Japanese quail (RIVIERE, 1979) ; vinclozolin was the best inducer to these intestinal enzymes.

From the above observations, it appears that exposure of birds to relatively "non-toxic" pesticidal chemicals, such as fungicides, can produce considerable increase in the level of cytochrome P-450 and some drug-metabolizing enzyme activities. Further work is obviously needed to determine if these changes in the *in vitro* metabolism result in parallel changes in the susceptibility of birds to toxic compounds.

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Accepted June 1, 1983