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The Effects of Dicofol on Induction of Hepatic Microsomal Metabolism in Rats

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In a comparative study, the induction effects of dicofol, technical Kelthane, and DDT on hepatic microsomal and cytosolic enzyme activities in rats were compared with those effects produced by phenobarbital (PhB) and β-naphthoflavone (BNF). Male rats (ca. 250 g) were injected (ip) for 4 consecutive days with 1.0 ml of vehicle containing either dicofol (1.5, 15.0, 29.5, or 59.0 m*M*, Kelthane (dicofol content equal to 29.5 or 59.0 m*M*), DDT (59.0 m*M*), or BNF (36.7 m*M*). Liver weights, microsomal protein, and cytochrome *P*-450 concentrations and microsomal and cytosolic enzyme specific activities were measured. Dicofol produced dose-related increases in all of the parameters measured except liver weight and cytosolic epoxide hydrolase activity. At a concentration of 59.0 m*M*, dicofol increased the concentrations of microsomal protein (1.7-fold) and cytochrome *P*-450 (2.9-fold), and the specific activities of cytochrome *c* reductase (1.6-fold), ethoxycoumarin *O*-deethylase (2.3-fold), aminopyrine *N*-demethylase (3.0-fold), microsomal epoxide hydrolase (2.6-fold), and glutathione *S*-transferase (2.9-fold). The induction potency of dicofol was equivalent to Kelthane, DDT, and PhB at equimolar (59.0 m*M*) concentrations of chemical. © 1987 Academic Press, Inc.

INTRODUCTION

Dicofol, the active component of Kelthane acaricide, is one of the few organochlorine pesticides that still receives widespread agricultural application. An estimated 2.0 to 2.5 million pounds of dicofol active ingredient is applied annually in the United States, primarily for mite control by the cotton and citrus industries in California, Florida, Texas, and Arizona (1).

Recent concerns about the continued agricultural use of Kelthane are that contaminants including DDT, DDD, DDE, and their isomers and analogs, collectively termed DDTr, pose a significant threat to populations of endangered species of birds as a result of effects on eggshell thinning (2). More recently, however, dicofol itself has been shown to produce significant eggshell thinning in birds (3).

Several theories have been formulated to account for the adverse effects of organohalogens on avian reproduction: (1) the induction of hepatic mixed-function oxygenase (MFO) enzymes resulting in increased metabolism of endogenous steroids and subsequent alterations in endocrine status (4), (2) the inhibition of calcium-ATPase in the shell-forming gland (5), and (3) an estrogenic action resulting in steroid hormone imbalance (6). While DDT induction of MFO has not been correlated with eggshell thinning, it has been shown (4) to effect endocrine imbalances in pigeons, resulting in disturbances in courtship behavior and nesting cycles. Alternatively, the DDT metabolite, DDE, strongly inhibits calcium-ATPase in the shell-forming gland of Pekin ducks (5). As there is no firm evidence for the metabolic conversion of dicofol to DDE (7–9), an induction phenomenon or an estrogenic effect may play a more significant role in the adverse effects of DDT analogs on shell formation than was believed previously.

While the effects of DDT on induction of hepatic drug metabolizing activity have been well characterized (10–19), those of dicofol have received only minor investiga-

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tion (10, 12–14). Currently, no data are available on the hepatic induction effects of dicolfol and Kelthane in the following areas: (1) the specific type (i.e., phenobarbital type or 3-methylcholanthrene type) of induction response elicited, (2) the relative potency of dicofol as an inducer in the isomeric combination that exists in technical Kelthane (i.e., 16% *o,p'*-dicofol and 69% *p,p'*-dicofol), and (3) the contribution of contaminants of technical Kelthane (20), including DDTr, to the induction effects of dicofol. The present research was conducted with the intention of filling these data gaps.

MATERIALS AND METHODS

Experimental design. Characterization of the type of induction response elicited by dicofol and Kelthane was performed by measuring the effects of each on rat hepatic morphology (i.e., gross liver weights and the concentrations of microsomal protein and cytochrome *P*-450) and the specific activities of cytochrome *c* reductase, aminopyrine *N*-demethylase (APND),[1] ethoxycoumarin *O*-deethylase (ECOD), microsomal epoxide hydrolase (mEH), cytosolic epoxide hydrolase (cEH), and glutathione *S*-transferase (GST), and comparing the pattern of induction responses to those elicited by phenobarbital (PhB) and β-naphthoflavone (BNF).

The relative potencies of dicofol, Kelthane, and DDT as inducers were determined by comparing the effects of equivalent doses (59.0 m*M*) of each on the indices of induction described to those effects elicited by an equivalent dose of PhB, selected as 60 mg/kg/day (i.e., 59.0 m*M*). Dicofol and DDT treatments contained the *o,p'* and *p,p'* isomers of each in proportions of 18.6 and 81.4%, respectively; analysis showed that these are the isomeric proportions in which dicofol exists in Kelthane.

The contribution of DDTr contaminants in Kelthane to the induction effects of dicofol was determined by comparing the induction responses elicited by equimolar (59.0 m*M*) doses of dicofol and Kelthane (dicofol content 59.0 m*M*).

Chemicals. Kelthane (15.8% *o,p'*-dicofol, 69.2% *p,p'*-dicofol, and 15.0% impurities including DDTr) and the *o,p* and *p,p'* isomers of dicofol (98.8%) were supplied by Rohm & Haas Co. (Spring house, PA). The *o,p'* and *p,p'* isomers of DDT (99.0%), β-naphthoflavone (98.0%), and aminopyrine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Sodium phenobarbital was obtained from J. T. Baker Chemical Co. (Phillipsburg, N.J.). Ethoxycoumarin was purchased from Sigma Chemical Co. (St. Louis, MO). [^{3}H]*cis*- and [^{3}H]*trans*-stilbene oxide were prepared by Dr. Bruce Hammock as described previously (21).

Animals and treatments. Adult male rats (Sprague–Dawley, ca. 250 g, Bantin & Kingman, Inc.) were assigned randomly to one control and nine treatment groups, six animals per group, as indicated in Table 1. Feed and water were available *ad libitum*. Treatment consisted of daily intraperitoneal (ip) injections of 1.0 ml of dose solution for 4 consecutive days. Except for sodium PhB, which was administered in sterilized saline (0.9%), all chemicals were administered in sterilized peanut oil as the vehicle. Control animals received sterilized peanut oil alone.

Preparation of microsomes. Twenty-four hours after the last treatment, the animals were terminated by cervical dislocation. Livers were immediately removed, rinsed in 150 m*M* KCl, blotted dry, and weighed. Microsomes were prepared as described previously (22). Approximately 10-g portions of liver were homogenized in 2 vol of

[1] Abbreviations used: APND, aminopyrine *N*-demethylase; ECOD, ethoxycoumarin *O*-deethylase; mEH, microsomal epoxide hydrolase; cEH, cytosolic epoxide hydrolase; GST, glutathione *S*-transferase; BNF, β-naphthoflavone; ip, intraperitoneal.

TABLE 1

Effects of Chemical Treatments on Rat Liver Morphological Indices

Treatment	Dose mg/kg	Dose m*M*	Liver weight* (g)	Microsomal protein concentration (mg protein/g liver)	Cytochrome *P*-450 concentration (nmol/mg protein)
Control	—	—	13.41 ± 0.88 (100)**	15.75 ± 1.61 (100)	0.61 ± 0.04 (100)
Phenobarbital	60.0	59.0	15.72 ± 0.50 (119)	24.00 ± 0.98‡ (158)	1.29 ± 0.10‡ (214)
β-Naphthoflavone	40.0	36.7	15.15 ± 1.00 (115)	18.41 ± 0.18 (120)	1.13 ± 0.06†,‡ (189)
Kelthane	51.4	29.5***	14.44 ± 0.71[a] (108)	26.13 ± 1.78[a]‡ (171)	1.20 ± 0.11‡,[a] (199)
	102.9	59.0***	16.07 ± 0.72[a] (122)	27.36 ± 1.51[a],‡ (179)	1.38 ± 0.13‡,[a] (229)
Dicofol	2.2	1.5	12.02 ± 0.53[a] (90)	19.99 ± 1.34[a] (131)	0.70 ± 0.03[a] (117)
	21.9	15.0	14.51 ± 0.75[b] (110)	21.75 ± 2.02‡,[a,b] (139)	1.16 ± 0.09‡,[b] (196)
	43.7	29.5	15.00 ± 0.46[b] (113)	25.78 ± 1.00‡,[b] (168)	1.33 ± 0.10‡,[b,c] (223)
	87.4	59.0	14.84 ± 0.36[b] (113)	25.65 ± 1.31‡,[b] (166)	1.70 ± 0.23‡,[c] (285)
DDT	83.7	59.0	14.43 ± 0.74 (108)	22.48 ± 0.87‡ (145)	1.26 ± 0.10‡ (210)

* Means ± SEM followed by the same letter in a column are not significantly different from the means of other dose groups within a given treatment according to Duncan's new multiple range test ($P < 0.05$).

** Mean percentage of control value.

*** Concentration of dicofol component.

‡ Mean of the treatment group is significantly different ($P < 0.05$) from that of the control group (Dunnett's *t* test).

† Cytochrome *P*-448.

ice-cold homogenization buffer (20 m*M* Tris–HCl, 1.15% KCl, pH 7.4) and centrifuged at 16,800*g* for 20 min at 0–4°C. The supernatant was centrifuged at 105,000*g* for 75 min, and the cytosolic fraction was frozen at −80°C for cytosolic enzyme measurements. The microsomal pellet was resuspended in 15 ml of wash buffer (0.4 *M* sucrose, 77 m*M* sodium pyrophosphate, pH 7.5) and recentrifuged as above. The final microsomal pellet was resuspended in a volume of 150 m*M* KCl to yield ca. 30 mg protein/ml. Microsomal protein concentrations were determined colorimetrically by the biuret method using a BSA standard (23).

Cytochromes. Cytochrome *P*-450 concentrations were measured spectrophotometrically by the carbon monoxide difference spectral method of Omura and Sato (24) in microsomes diluted with 100 m*M* Tris buffer (pH 7.4) to 1.0 mg/ml.

Enzyme activities. NADPH cytochrome *c* reductase activity was measured spectrophotometrically by the method of Pederson *et al.* (25) using concentrations of 0.1 mg/ml protein, 0.26 m*M* cytochrome *c*, and 2.58 m*M* NADPH. Specific activities of APND and ECOD were measured by standard colorimetric (41) and fluorimetric (42) assays, respectively, using substrate concentrations of 40 m*M* aminopyrine and 0.83 m*M* ethoxycoumarin. Both APND and ECOD assays were performed using microsomal protein concentrations of 0.8 mg/assay and an incubation time of 20 min.

The activities of mEH, cEH, and GST were assayed by a single-step radiometric partition method modified from Hill *et al.* (21). For mEH and GST, microsomal protein was incubated with [^{3}H]*cis*-stilbene oxide (5×10^{-5} *M*) or cytosolic protein with [^{3}H]*cis*-stilbene oxide (5×10^{-5} *M*) plus glutathione (5 m*M*), respectively, for 10 min at 37°C. For cEH, cytosol was preincubated with 0.5 m*M* diethylmaleate for 10 min at room temperature to deplete endogenous glutathione (26), and then incubated with [^{3}H]*trans*-stilbene oxide (5×10^{-5} *M*) for 10 min at 37°C. The reactions were terminated by extraction of the epoxide (mEH, cEH), or epoxide and diol (GST), with isooctane or hexanol, respectively. Reaction products were quantitated by liquid scintillation counting of an aliquot (50 μl) of the aqueous phase.

Statistical analysis. Means and standard errors were calculated for all measurements on control ($n = 5$) and treatment ($n = 5,6$) groups. Data for each measurement were subjected to one-way analysis of variance and Bartlett's test for heterogeneity of variance. The significance of the differences between treatment and control means for each measurement was determined using Dunnett's *t* test. Comparisons between equimolar treatments were made using Bonferroni's *t* test. Individual comparisons among different dose groups within a specific treatment were made using Duncan's new multiple range test.

RESULTS

Effects on liver morphological indices. There were no significant ($P < 0.05$) effects of any of the administered chemicals on the liver weights of treated rats relative to controls (Table 1). Animals receiving the three higher doses of dicofol had significantly ($P < 0.05$) greater mean liver weights than those of the low-dose dicofol group. However, this apparent dose-related effect of dicofol treatment on liver weights most likely represents an artifact, as the mean liver weight of the low-dose dicofol group was lower than that of the control group.

In all treatment groups, other than the ones receiving BNF or the lowest dose of dicofol, mean microsomal protein concentrations were significantly ($P < 0.05$) increased relative to that in the control group (Table 1). The microsomal protein concentration in animals treated with the highest dose of Kelthane (1.79 times control) was significantly ($P < 0.50$) greater than the concentration measured in animals treated with an equimolar (59.0 m*M*) dose of DDT (1.45 times control), but was not significantly greater than that produced by equimolar doses of PhB (1.58 times control) or dicofol (1.66 times control). Increasing doses of dicofol up to 43.7 mg/kg produced corresponding increases in mean microsomal protein concentrations.

Cytochrome *P*-450 concentrations were significantly ($P < 0.05$) increased in all treatment groups except for those receiving BNF or the lowest dose of dicofol. For animals treated with dicofol or Kelthane the reduced CO spectrum exhibited maximal absorbance at 450 nm. In animals treated with BNF, there was a significant shift of the reduced CO spectral maximum from an average of 450 to 448 nm. Dicofol-treated groups exhibited a dose–response trend, with increasing doses of dicofol producing correspondingly increased cytochrome *P*-450 concentrations.

Effects on liver microsomal enzyme activities. The PhB, high-dose Kelthane, and high-dose dicofol treatments resulted in significantly ($P < 0.05$) increased mean cytochrome *c* reductase activities, with values 1.5, 1.7, and 1.6 times that of the control, respectively (Table 2). For all other groups, the cytochrome *c* reductase activities were not significantly ($P < 0.05$) greater than that of the control.

Mean ECOD activities were significantly ($P < 0.05$) increased for all of the chemical treatment groups except the low-dose dicofol group. However, the response to

TABLE 2

Effects of Chemical Treatments on Rat Liver Microsomal Enzyme Specific Activities

Treatment	Dose		Mean specific activity (nmol/min mg protein)*			
	mg/kg	m*M*	Cytochrome *c* reductase	Ethoxycoumarin *O*-deethylase	Aminopyrine *N*-demethylase	Microsomal epoxide hydrolase
Control	—	—	116.0 ± 7.7 (100)**	0.44 ± 0.06 (100)	6.22 ± 0.55 (100)	5.5 ± 0.63 (100)
Phenobarbital	60.0	59.0	175.0 ± 16.8‡ (150)	0.97 ± 0.09‡ (242)	12.65 ± 1.14‡ (206)	13.2 ± 0.91‡ (260)
β-Naphthoflavone	40.0	36.7	118.5 ± 12.3 (102)	5.34 ± 0.38‡ (1,284)	7.87 ± 0.55 (132)	7.2 ± 0.51 (141)
Kelthane	51.4	29.5***	156.7 ± 14.1[a] (136)	0.92 ± 0.11‡,[a] (241)	11.96 ± 0.66‡,[a] (198)	11.2 ± 0.79‡,[a] (213)
	102.9	59.0***	196.8 ± 16.3‡,[a] (170)	1.06 ± 0.16‡,[a] (240)	15.48 ± 1.79‡,[a] (252)	13.6 ± 1.34‡,[a] (258)
Dicofol	2.2	1.5	127.9 ± 10.0[a] (110)	0.57 ± 0.07[a] (133)	9.48 ± 1.14‡,[a] (156)	7.9 ± 1.22[a] (148)
	21.9	15.0	148.8 ± 15.1[a,b] (130)	0.85 ± 0.16‡,[a,b] (195)	12.08 ± 0.73‡,[b] (196)	10.0 ± 0.57‡,[a,b] (189)
	43.7	29.5	162.0 ± 13.4[a,b] (140)	0.86 ± 0.06‡,[a,b] (214)	12.97 ± 0.52‡,[b] (213)	10.3 ± 1.37‡,[a,b] (208)
	87.4	59.0	180.3 ± 13.8‡,[b] (157)	0.93 ± 0.11‡,[b] (230)	18.19 ± 1.13‡,[c] (300)	12.7 ± 0.32‡,[b] (259)
DDT	83.7	59.0	167.0 ± 16.7 (144)	0.87 ± 0.09‡ (211)	13.72 ± 1.10‡ (221)	11.6 ± 1.38‡ (236)

* Means ± SEM followed by the same letter in a column are not significantly different from the means of other dose groups within a given treatment according to Duncan's multiple range test ($P < 0.05$).

** Mean percentage of control value.

*** Concentration of dicofol component.

‡ Mean of the treatment group is significantly different ($P < 0.05$) from that of the control group (Dunnett's *t* test).

BNF (ca. 12.8 times control) was much greater in magnitude than was the next highest response, that to PhB (2.42 times control). Mean APND activities were significantly ($P < 0.05$) increased in all treatment groups except for the group receiving BNF. The activity produced by the high-dose dicofol treatment (3.0 times control) was significantly ($P < 0.05$) greater than that produced by an equimolar (59.0 m*M*) dose of PhB (2.06 times control), but was not significantly greater than that produced by equimolar doses of Kelthane (ca. 2.5 times control) or DDT (ca. 2.2 times control).

Mean mEH activities were significantly ($P < 0.05$) increased in all treatment groups except in those which received BNF and the lowest doses of dicofol. Differences between the mEH activities produced in response to equimolar (59.0 m*M*) doses of PhB, Kelthane, dicofol, and DDT were not significantly different.

For all of the microsomal enzyme activities measured, there were dose–response-related trends in the response to dicofol treatments, with increasing doses of dicofol producing corresponding increases in the mean enzyme activities measured (Table 2).

Effects on liver cytosolic enzyme activities. None of the chemical treatments produced any significant effects on mean cEH activities (Table 3). However, there were significant ($P < 0.05$) increases in mean GST activities in response to all treatments, except that for the low-dose dicofol group. Bartlett's test showed heterogeneity of variance among GST data such that comparisons of the significance of differences between treatments and control required modification of Dunnett's *t* test for unequal variance (27). A dose–response trend was noted for Kelthane, with the mean GST activity being more responsive to the high dose (ca. 3.0 times control) than to the low dose (ca. 2.0 times control).

TABLE 3
Effects of Chemical Treatments on Rat Liver Cytosolic Enzyme Specific Activities

Treatment	Dose mg/kg	Dose mM	Mean specific activity (nmol/min mg protein)*: Cytosolic epoxide hydrolase	Mean specific activity (nmol/min mg protein)*: Glutathione S-transferase
Control	—	—	0.089 ± 0.015 (100)**	7.76 ± 0.23 (100)
Phenobarbital	60.0	59.0	0.078 ± 0.016 (87)	16.81 ± 1.45‡ (219)
β-Naphthoflavone	40.0	36.7	0.103 ± 0.017 (134)	13.66 ± 1.13‡ (176)
Kelthane	51.4	29.5***	0.092 ± 0.018[a] (113)	16.28 ± 1.64‡,[a] (210)
	102.9	59.0	0.079 ± 0.018[a] (85)	23.40 ± 2.23‡,[b] (302)
Dicofol	2.2	1.5	0.086 ± 0.009[a] (108)	11.96 ± 2.36[a] (152)
	21.9	15.0	0.067 ± 0.014[a] (78)	14.96 ± 3.03‡,[a,b] (191)
	43.7	29.5	0.068 ± 0.009[a] (83)	14.61 ± 2.63‡,[a,b] (187)
	87.4	59.0	0.086 ± 0.015[a] (116)	22.50 ± 1.96‡,[b] (288)
DDT	83.7	59.0	0.086 ± 0.013 (98)	16.37 ± 1.53‡ (212)

* Means ± SEM followed by the same letter in a column are not significantly different from the means of other dose groups within a given treatment according to Duncan's multiple range test ($P < 0.05$).
** Mean percentage of control value.
*** Concentration of dicofol component.
‡ Mean of the treatment group is significantly different ($P < 0.05$) from that of the control group (Dunnett's t test).

DISCUSSION

In this study, dicofol and its technical formulation, Kelthane, produced significant increases in microsomal protein and cytochrome P-450 concentrations, the coupled reductase (cytochrome c reductase), several cytochrome P-450-dependent monooxygenase (cytochrome P-450 monooxygenase) activities (APND and mEH) and GST activity. These observations are consistent with PhB-type induction, and are in agreement with previous reports (11, 13, 15–19) demonstrating the induction effects of dicofol and Kelthane on SER proliferation, liver weight, and cytochrome P-450 monooxygenase activities, including aldrin epoxidase, heptachlor epoxidase, APND, aniline hydroxylase, and hexobarbital oxidase. By contrast, BNF, a 3-MC-type inducer (17), produced no significant changes in any of these parameters, except for the activity of GST, which is consistent with published data (28). In addition, BNF induced the cytochrome P-448 hemoprotein and produced a marked (ca. 13-fold) increase in the activity of ECOD, both indices of 3-MC-type induction (29, 30). From the pattern of induction responses observed, it can be concluded that dicofol and Kelthane are inducers of hepatic MFO of the PhB type.

There were no significant differences between the effects of dicofol and Kelthane, containing equimolar doses of dicofol, on any of the indices of induction that were

measured (Tables 1–3). Thus, the nondicofol components of Kelthane, including DDTr which are also inducers of hepatic MFO (13, 16, 17), do not contribute significantly to the induction potency of dicofol.

The threshold for dicofol's effects on hepatic induction in rats was demonstrated to be between 2.2 and ca. 22 mg/kg (ip) under the exposure regime used in this study. Also demonstrated was the significant potency of dicofol on hepatic induction. On most indices of liver induction that were measured, dicofol was equipotent with PhB and DDT (Tables 1–3). In previous studies (16, 17, 19), dicofol has been shown to approach *p,p′*-DDT in potency on the induction of cytochrome *P*-450 monooxygenase activities in rats and mice. Abernathy *et al.* (17) demonstrated that dicofol, specific isomer not mentioned, was less potent an inducer of mouse liver aniline hydroxylase activity than were the *p,p′* and *m,p′* isomers of DDT but was more potent than the *o,p′* isomer.

In the present study, animals were treated with a mixture of isomers of dicofol (81.4% *p,p′* isomer and 18.6% *o,p′* isomer), the same proportions present in the technical formulation Kelthane. When dicofol in this isomeric composition was compared with DDT (of identical isomer composition) with respect to its effects on hepatic induction, no significant differences in potencies were observed. In mice, *p,p′*-DDT is ca. five times more potent an inducer of hepatic cytochrome *P*-450 monooxygenase activity than is *o,p′*-DDT (17). Thus, a mixture of the two isomers would be expected to exhibit less induction potency than the *p,p′* isomer, alone. It is quite possible that this wide variation in the potencies of the *p,p′* and *o,p′* isomers is not manifested in the case of dicofol and that the induction potency of the mixture of isomers does not significantly differ from either isomer alone. Alternatively, there may be species variation between rats and mice with respect to the selectivity of induction effects by different isomers of chlorinated hydrocarbons. Molecular characteristics conferring high enzyme-inducing potency to structural analogs of DDT include a high degree of electron withdrawal from the bridge carbon by electronegative groups (16). Dicofol, containing a hydroxylated bridge carbon, may actually have greater potential as an MFO inducer than does DDT, based on structural considerations. However, a higher rate of metabolism to less active metabolites such as 4,4-dichlorobenzhydrol (16) may be the reason that its actual induction potency is no greater than that of DDT.

This study has confirmed that dicofol and its technical formulation are PhB-type inducers of hepatic cytochrome *P*-450 monooxygenase activity of equal potency to DDT (of the same isomer composition). The microsomal cytochrome *P*-450 monooxygenase system is responsible for the metabolism of a wide variety of exogenous and endogenous lipophilic compounds including steroids essential for normal reproductive function (28). Induction of cytochrome *P*-450 monooxygenases by compounds such as PhB and chlorinated hydrocarbons (e.g., DDT, DDE, and dieldren) has resulted in accelerated metabolism of steroid hormones *in vitro* (4, 31, 32), and has been correlated with increased metabolism and modified action of exogenously administered steroids including progesterone, testosterone, and estradiol *in vivo* (33–37). Alterations in avian reproduction (e.g., suppressed courtship behavior, delayed breeding cycles, or failure to lay eggs entirely) in birds exposed to DDT, PCBs, or dieldren are believed to be associated with decreases in plasma estrogen levels resulting from the induction of cytochrome *P*-450 monooxygenases (4, 38, 39). These effects on reproduction may be just as important in the population declines of some species of predatory birds as is eggshell thinning (40).

It recently has been demonstrated (3) in

the ring dove that exposure to dicofol results in significant eggshell thinning. A combination of altered reproductive behavior produced by the induction of cytochrome *P*-450 monooxygenases and resultant changes in hormonal status, and direct eggshell thinning effects of dicofol, could have drastic effects on raptor populations in the wild. In light of these concerns, these authors intend to extend their study of the induction potential of dicofol to include its effects on avians.

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