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Benomyl Toxicity in Chickens

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Benomyl is a broad spectrum systemic fungicide of the benzimidazole family, registered for use on various fruits, vegetables and other agricultural crops (EPA, 1977). It is also used as a pasture dressing for the control of Pithomyces chartarum, and Epichloe typhina, the fungi responsible for facial eczema in sheep and cattle (Clare, 1969), and summer fescue toxicity in cattle (Jackson et al., 1984), respectively. Because of its extensive use, it has been widely distributed in the environment.

Benomyl binds to tubulin preventing its polymerization and thereby interfering with mitosis. The ability of benomyl to interfere with mitosis accounts for its fungicidal action (Hammershlag and Sisler, 1973). Hellman and Laryea (1990) reported its more pronounced action of inhibition of [^{3}H]thymidine incorporation in various organs of mouse. Despite its effective fungicidal action, benomyl shows low acute as well as chronic mammalian toxicity, which may be because of its low affinity for mammalian tubules and its rapid metabolism and excretion.

The oral LD_{50} of benomyl in adult rats is in excess of 10 mg/kg (Seiler, 1975). It is rapidly biotransformed by liver in rats mainly through hydroxylation and hydrolysis to methyl 5-hydroxybenzimidazole-2-ylcarbamate (Gardiner et al., 1968). Previous study in rats as a mammalian species showed that although benomyl is an inhibitor of mixed-function oxidases (MFOs), it is not a hepatotoxic agent (Dalvi, 1992). This study examines the effect of benomyl on the MFOs and liver of chickens as a representative of non-mammalian species.

MATERIALS AND METHODS

Day-old white leghorn chicks were obtained from a local

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hatchery, housed in a electrically heated unit until one month-old and then transferred to unheated units. The chicks were given feed (chick starter-grower, no 680219 FA, Nutrena Feed Division, Minneapolis, MN) and water ad libitum. Benomyl, (95% purity) was obtained from E. I. Du Pont, Wilmington, DE.

Twelve chickens of 6-10 wk age weighing 600-1000 g were randomly divided into two groups of six chickens each for one day treatment. Chickens from the first group were injected with 100 mg benomyl/kg, ip. Chickens from the second group received an equal amount of corn oil, ip, and served as a control.

In another experiment, eighteen chickens were allotted randomly to two groups. Birds from the second group were fed benomyl orally in the feed at concentrations of 4000 ppm for 15 days. The first group received a benomyl-free diet and served as a control group.

At the end of treatment period, the chickens were guillotined, and blood samples were collected at the time of sacrifice. Livers were promptly removed, weighed and perfused with ice-cold 1.15% KCl solution containing 0.05 mM EDTA. Microsomes from the livers were isolated using an ultracentrifuge (Beckman L8-80M) by the procedure described by Dalvi et al. (1975). The microsomal pellets were washed once with 1.15% KCl solution and immediately resuspended in an appropriate buffer for determining the components of the MFOs.

The cytochrome P-450 and cytochrome b5 content of the microsomes were determined by the method of Omura and Sato (1964). The activity of NADPH-cytochrome c reductase was determined by the procedure described by Dalvi and Terse (1990). The activity of benzphetamine N-demethylase was determined by the procedure reported by Dalvi et al., (1987) and aminopyrine N-demethylase according to the method described by Schenkman et al. (1967), in which formaldehyde formed from benzphetamine and aminopyrine, respectively, was measured colorimetrically. The activity of aniline hydroxylase was determined by the method of Kato and Gillette (1965), in which p-aminophenol formed from aniline was measured colorimetrically. Protein concentration in all samples was determined by Biuret method modified to include deoxycholate (Dalvi et al., 1975).

Collected blood samples were refrigerated for 1-2 hr. The samples were centrifuged at 3,000 rpm in GLC-2B Sorvall centrifuge for 15 min to separate the serum. The serum samples were analyzed for SDH, ICD and GGT by methods described in Sigma Technical Bulletin N0 50-UV,

153-UV and 418-UV, respectively.

RESULTS AND DISCUSSION

Benomyl when administered ip at 100 mg/kg to chickens, to see its effect on MFOs and liver in a single dose, showed significant induction of cytochrome P-450

Table 1. Effect of intraperitoneally administered benomyl (100 mg/kg, single dose) on hepatic microsomal electron transport components, drug metabolizing enzymes and serum enzymes in chickens.

Parameters	Control	Benomyl
Cytochrome P-450[a]	0.18 ± 0.03	0.43 ± 0.11*
Cytochrome b_5[b]	0.24 ± 0.02	0.37 ± 0.05*
NADPH-cytochrome c reductase[c]	99 ± 16	215 ± 56*
Benzphetamine N-demethylase[d]	4.82 ± 0.33	6.81 ± 0.76*
Aminopyrine N-demethylase[d]	6.08 ± 0.50	8.12 ± 0.71*
Aniline hydroxylase[e]	0.48 ± 0.01	1.62 ± 0.48*
Sorbitol dehydrogenase[f]	348 ± 66	255 ± 36
Isocitrate dehydrogenase[f]	1138 ± 61	1046 ± 115
Gamma-glutamyl transferase[g]	14.05 ± 1.54	9.58 ± 1.72

Results are expressed as the mean ± SEM, *P<0.05
[a]Expressed as nmoles cytochrome P-450/mg protein
[b]Expressed as nmoles cytochrome b_5/mg protein
[c]Expressed as nmoles of cytochrome c reduced/min/mg protein
[d]Expressed as nmoles of formaldehyde liberated/min/mg protein
[e]Expressed as nmoles of p-aminophenol liberated/min/mg protein
[f]Expressed as Sigma Units/ml
[g]Expressed as Sigma Units/L

Table 2. Effect of dietary benomyl (4000 ppm, for 15 days) on hepatic microsomal electron transport components, drug metabolizing enzymes and serum enzymes in chickens.

Parameters	Control	Benomyl
Cytochrome P-450[a]	0.206 ± 0.03	0.205 ± 0.04
Cytochrome b_5[b]	0.193 ± 0.02	0.207 ± 0.04
NADPH-cytochrome c reductase[c]	81 ± 9	64 ± 3
Benzphetamine N-demethylase[d]	4.08 ± 0.50	5.6 ± 0.68
Aminopyrine N-demethylase[d]	4.24 ± 0.54	5.84 ± 1
Aniline hydrogenase[e]	0.28 ± 0.04	0.34 ± 0.08
Sorbitol dehydrogenase[f]	415 ± 45	546 ± 97
Isocitrate dehydrogenase[f]	1196 ± 41	1402 ± 174
Gamma-glutamyl transferase[g]	21.35 ± 1	32.5 ± 11

Results are expressed as in Table 1.

(138%), cytochrome b5 (54%), NADPH-cytochrome c reductase (117%), benzphetamine N-demethylase (41%), aminopyrine N-demethylase (33%) and aniline hydroxylase (237%) activity. However, markers of the hepatotoxicity, SDH, ICD and GGT, remained unaffected (Table 1). Data from these findings show that benomyl has inducible effect on monooxygenases with no effect on hepatotoxicity in chickens at 100 mg/kg ip after 24 hr of treatment.

Chickens fed benomyl at 4000 ppm in diet for 15 days to evaluate long-term effect of benomyl, showed no significant increase in the levels of cytochrome P-450, cytochrome b5, benzphetamine N-demethylase, aniline hydroxylase, and NADPH-cytochrome c reductase. It is apparent from these results that dietary benomyl at 4000 ppm has no marked effect on MFOs in chickens.

Serum SDH, ICD and GGT, the markers of liver toxicity, also remained unchanged (Table 2) and indicated absence of hepatotoxicity of dietary benomyl.

Taken together, the present study and the previous study in rats (Dalvi, 1992) prove benomyl as a non-hepatotoxic agent in chickens and rats. Furthermore, its effect on MFOs appears to be species-dependent as benomyl at 100 mg/kg given ip showed inhibitory effect in rats and inducible effect in chickens. Route-independent effect of benomyl on MFOs was observed in rats. Our dietary study on benomyl at 4000 ppm showed no remarkable effect on MFOs in chickens. Unlike in rats, this study indicated route-dependent effect of benomyl on hepatic microsomal electron transport components and drug metabolizing enzymes in chickens. The studies suggest that short-term or long-term exposure of wild birds to benomyl or benomyl-containing earthworms (Van Gestel, 1992) may not lead to serious toxic manifestations.

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