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Interaction of Lindane and Carbaryl on Hepatic Microsomal Enzymes in Rats

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Formulations containing both lindane and carbaryl are used in pest control . Residues of both compounds frequently occur in foods and feeds.Lindane is a neurotoxicant producing tremors , ataxia, convulsions and prostration. Fatty changes in the liver and kidney tubule degenerations have also been noted in acute poisoning. It has been estimated that a dose of 10 mg/kg will cause signs of poisoning in men. Carbaryl is a relatively rapidly reversible inhibitor of cholinesterase and the symptoms of poisoning in animals and humans are typically cholinergic with lacrimation, salivation, miosis and convulsions. Lindane is a well-known strong inductor of microsomal enzymes (Kolmodin-Hedman et al. 1971; Pellisier and Albrecht 1976; Junqueira et al. 1986; Kumar and Dwivedi 1988). The influence of carbaryl on microsomal enzymes is dependent on several factors such as dose, species, treatment schedule. Some authors showed that carbaryl did not markedly influence the microsomal liver enzymes (Robacker et al. 1981; Lechner and Abdel-Rachman 1985), others found a decrease (Stevens et al. 1972; Nešković et al. 1979) or an increase (Cress and Strother 1974; Lee and Hong 1985) in their activity.

The aim of this study was to investigate the effects of lindane and carbaryl separately or in their combination on the hepatic microsomal enzymes in rats.

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MATERIALS AND METHODS

The following insecticides were used in this study: lindane-98% purity, obtained from the Institute of Organic Industry, Warsaw; carbaryl -99% purity obtained from the Institute of Organic Industry, Warsaw.
Male albino rats weighing 250±20 grams were given daily the following doses of insecticides : lindane 11,22 and 44 mg/kg, carbaryl 44 and 300 mg/kg and mixtures of lindane and carbaryl 11:44, 22:150 and 44:176 mg/kg . The compounds were administered orally in soy bean oil for three days. Control animals received the corresponding amount of the solvent.

The day after the administration of the last dose the animals were killed by cervical dislocation.Their livers were immediately isolated and the whole tissue homogenated in ice-cold isotonic potassium chloride - 0.01 M phosphate buffer (pH 7.4) was prepared .To obtain the postmitochondrial supernatant, the liver homogenate was centrifuged at 15,000 g for 30 min.The resultant supernatant was centrifuged at 100,000 g for 1 h in a refrigerated ultracentrifuge to isolate the microsomal fraction.The pellet of microsomes was resuspended in ice-cold 1.15% KCl - 0.01M phosphate buffer and again centrifuged as above for 30 min. The washing procedure removed most of the hemoglobin.The washed microsomes were finally suspended in 0.05M phosphate buffer (0.001M EDTA).

Using the postmitochondrial supernatant, the activities of 4-nitroanisole O-demethylase and aniline 4-hydroxylase were determined, according to procedures described by Netter and Seidel (1964) and Kato and Gillette (1965), respectively.
The content of cytochromes P-450 and b_5 in microsomal fraction was determined using the method described by Omura and Sato (1964). Protein content was determined by the method of Lowry et al.(1951). Statistical analysis was performed using Student's t-test.

RESULTS AND DISCUSSION

The effects of lindane and carbaryl separately or in combination on the content of cytochromes P-450 and b_5 are presented in Table 1. Lindane caused a significant dose-dependent increase in the content of cytochrome P-450 in liver microsomes. No significant changes in the content of cytochrome P-450 were found in the animals treated with carbaryl.

In the animals receiving mixtures of lindane and carbaryl a distinctive increase in the content of cytochrome P-450 was ascertained when compared with controls. However,in rats treated with two mixtures of lindane and carbaryl (22:150 and 44:176 mg/kg) the increase was significantly lower when compared with that in the animals given the corresponding dose of lindane alone.

In this experiment the content of cytochrome b_5 was increased only in the animals treated with the mixture of 44 mg/kg of lindane and 176 mg/kg of carbaryl.

The effects of lindane and carbaryl separately or in their combination on the activity of 4-nitroanisole O-demethylase and aniline 4-hydroxylase are presented in Table 2.

Lindane treatment increased the activity of both enzymes significantly in a dose-dependent manner. No significant changes in the activities of 4-nitroanisole O-demethylase or aniline 4-hydroxylase were noticed in the animals treated with carbaryl. Mixtures of lindane and carbaryl caused a significant increase in the activity of the enzymes when compared with controls. However, in the animals treated with a mixture containing 22 mg/kg of lindane and 150 mg/kg of carbaryl the increase in the activity of 4-nitroanisole O-demethylase was significantly lower than in rats receiving 22 mg/kg of lindane.Likewise, in the animals given the mixture containing 44 mg/kg of lindane and 176 mg/kg of carbaryl the increase in the activity of aniline 4-hydroxylase was significantly lower than that in the animals receiving 44 mg/kg of lindane.

Table 1. Content of cytochromes P-450 and b_5 in rats given lindane, carbaryl and their mixtures.

	cytochrome P-450 (nmole/mg protein)	cytochrome b_5 (nmole/mg protein)
Controls	0.97 ± 0.16	0.46 ± 0.09
Lindane (mg/kg)		
11	1.16[a] ± 0.15	0.47 ± 0.07
22	1.40[a] ± 0.09	0.51 ± 0.19
44	1.78[a] ± 0.23	0.47 ± 0.15
Carbaryl (mg/kg)		
44	0.91 ± 0.09	0.44 ± 0.04
300	1.04 ± 0.20	0.53 ± 0.08
Lindane-Carbaryl (mg/kg)		
11:44	1.23[a] ± 0.24	0.48 ± 0.08
22:150	1.13[ab] ± 0.14	0.56 ± 0.12
44:176	1.50[ac] ± 0.23	0.57[a] ± 0.07

Values presented are the mean ±SD of 8 rats/group; a-significantly different from controls: $p<0.05$; b-significantly different from animals given 22 mg/kg of lindane $p<0.05$; c- significantly different from animals given 44 mg/kg of lindane $p<0.05$.

Lindane is a powerful inducer of hepatic microsomal enzymes. Pelissier and Albrecht (1976) showed that lindane induced microsomal mono-oxygenases in the liver of rats and a synthesis of cytochrome P-450 at dietary levels of 2-20 ppm. Kolmodin-Hedman et al.(1971) found that the lowest dosages of lindane that shortened hexobarbital sleeping time were 150 mg/kg intraperitoneally once or about 0.05 mg/kg/day. Janqueira et al. (1986) found that the administration of a single intraperitoneal dose of lindane (20-80 mg/kg) to rats produced an increase in microsomal content of cytochrome P-450 of the liver. Kumar and Dwivedi (1988)

Table 2. Activity of enzymes in rats given lindane, carbaryl and their mixtures.

	4-nitroanisole O-demethylase (umole/h/100mg protein)	aniline 4-hydroxylase (ug/20min./g tissue)
Controls	1.92 ± 0.56	15.52 ± 3.03
Lindane (mg/kg)		
11	3.85[a] ± 1.44	23.79[a] ± 5.82
22	4.26[a] ± 1.63	27.03[a] ± 7.08
44	5.40[a] ± 2.49	29.05[a] + 3.33
Carbaryl (mg/kg)		
44	1.99 ± 1.01	15.84 ± 4.38
300	1.45 + 0.78	19.20 ± 8.19
Lindane-Carbaryl (mg/kg)		
11:44	4.29[a] ± 1.74	25.62[a] ± 6.06
22:150	2.45[ab] ± 1.73	23.72[a] ± 4.90
44:176	3.42[a] ± 1.49	24.95[ac] ± 4.41

Values presented are the mean ± SD of 8 rats/group; a-significantly different from controls: $p<0.05$; b-significantly different from animals given 22 mg/kg of lindane: $p<0.05$; c- significantly different from animals given 44 mg/kg of lindane: $p<0.05$.

estimated that lindane induced different hepatic cytochrome P-450 izoenzymes severalfold after four intraperitoneal doses (25 mg/kg) to rats.

Effects of carbaryl on hepatic microsomal enzymes have been examined by several authors.Nešković et al. (1979) showed that carbaryl induced the activity of microsomal NADH-cytochrome c reductase in rats of both sexes. Stevens et al. (1972) estimated that carbaryl at 0.5 the LD_{50} inhibited hydroxylation of aniline in vitro and in vivo. Cress and Strother (1974) demonstrated an

increase in cytochrome P-450 and cytochrome b_5 in mice on diets containing about 5000 ppm of carbaryl during a 14-day period. Robacker et al.(1981) found that carbaryl given orally at 100 mg/kg/day for 3 days did not affect the liver cytochrome P-450 content, NADPH-dependent reductase activity and microsomal xenobiotic metabolism in mice. Acute inhalation exposure of rats to carbaryl at 112-224 mg/m^3 prolonged pentobarbital sleeping time and decreased the liver microsomal cytochrome P-450 content and the NADPH - cytochrome c reductase activity. However, a 3-day exposure had the opposite effect on each of these parameters (Lee and Hong 1985).According to Lechner and Abdel-Rahman (1985) carbaryl is a mild inducer of the drug metabolizing enzymes in mice and rats.Following the daily treatment of 25 mg/kg of carbaryl for a period of 7 days UDP-glucuronyl transferase was significantly induced. However, aniline hydroxylase, aminopyrine demethylase and nitrobenzoate reductase enzymes were without change.

In order to investigate the interaction of lindane and carbaryl on microsomal enzymes, the compounds were administered to rats separately and in their combination. Lindane was given in doses of about 0.125, 0.25 and 0.5 of oral LD_{50}, carbaryl in doses of about 0.075 and 0.5 of oral LD_{50}.
Of the three mixtures of lindane and carbaryl used one equitoxic contained about 0.25 of LD_{50} of both compounds (22 mg/kg of lindane and 150 mg/kg of carbaryl). The other two consisted of one part of lindane and four parts of carbaryl (by weight), as they are encountered in formulations used for insect control (e.g. Gamakarbatox). In these latter mixtures lindane was used in 0.125 and 0.5 of LD_{50}.

As expected, lindane exerted a dose-dependent inducing effect on the content of cytochrome P-450 and the activity of 4-nitroanisole O-demethylase and aniline 4-hydroxylase. Unlike in many authors' reports (Stevens et al. 1972; Cress and Strother 1974; Nešković et al. 1979),no significant effects on the activity of the investigated enzymes were found in animals treated with carbaryl. All mixtures of lindane and carbaryl elevated

the activity of microsomal enzymes when compared with controls. However,the increase was lower than in animals treated with lindane alone. The two mixtures with the higher contents of carbaryl (150 and 176 mg/kg) significantly decreased the induction of microsomal enzymes caused by lindane.

Our results indicated that combined lindane and carbaryl treatments were less effective inducers of microsomal enzymes as compared to the lindane treatment alone .This means that carbaryl inhibits the biotransformation of lindane, consequently increasing its toxicity due to the increased half-life and an increased concentration at the site of critical target.

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