Bull. Environm. Contam. Toxicol. 23,438-444 (1979)

Cytochrome P-450 Content and NADPH-Cytochrome c Reductase Activity in Rats Treated with Carbaryl and Propoxur

Neško Nešković[1], Milenko Miloševic[2], and Radojica Kljajic[3]

[1]INEP. Department of Pesticides, Banatska 31b, P.O. Box 46, 11080 Zemun, Yugoslavia, [2]Medical Faculty, Institute of Pharmacology and Toxicology, P.O. Box 662, 11000 Beograd, Yugoslavia, and [3]Faculty of Agriculture, Department of Pesticides, Nemanjina 6, 11080 Zemun, Yugoslavia

Application of various compounds for pest control in agriculture, as well as in food industry and communal hygiene has been very intensive, and shows constant increase. Therefore, the presence of these compounds and their degradation products in the environment is increasing and, at the same time, the danger of their toxic effects upon useful organisms, including humans, is increasing as well.

Toxic effect of pesticides is demonstrated in different ways. One of the ways is their effect on some enzymes as stated by KUZ'MINSKAYA & JAKUŠKO (1959) and POKROVSKIY & NENOV (1968). Some investigations showed that the carbamate insecticides can induce changes in concentration of cytochrome P-450, haemoprotein which takes part in numerous reactions of microsomal hydroxylations as well as some microsomal enzymes (CRESS & STROTHER 1974, NEŠKOVIĆ & VITOROVIĆ 1977).

This paper reports the effect of subacute application of the most widely used carbamate insecticides carbaryl and propoxur on the NADPH-cytochrome c reductase activity and cytochrome P-450 content in rats liver.

MATERIALS AND METHODS

Male and female albino rats, 150-200 g body weight, were used. Animals were kept in the polyethylene cages (2 animals in each) at 23-25°C during the entire experiment. They were maintained on the usual diet ad libitum.

Carbaryl (6.0 and 12.0 mg/kg) and propoxur (1.0 and 2.0 mg/kg) were dissolved in sunflower oil and given orally in a volume of 2 ml/kg for periods up to 30 days. Control animals received a corresponding amount of sunflower oil. The activity of NADPH-cytochrome c reductase and cytochrome P-450 content in liver microsomes were determined on days 5, 10, 20 and 30 after the initial application of the compounds.

Microsomes were isolated from the liver of treated and control animals as described earlier (NEŠKOVIĆ et al. 1973). Protein content in microsomal fraction was determined colorimetrically according to the method described by LOWRY et al. (1951) with bovine serum albumin as the standard.

0007-4861/79/0023-0438 $0.140

Cytochrome P-450 was estimated from the absorption difference at 450 and 490 nm, by the application of extinction coeficient of $91.0^{-1}mM^{-1}$ (OMURA & SATO 1964).

NADPH-cytochrome c reductase was estimated by following the cytochrome c reduction at 550 nm according to the procedure described by MASTERS et al. (1967).

Statistical analysis of the results was performed by methods decsribed by SNEDECOR & COCHRAN (1967). Differences between two mean values were estimated using Student's t-test. The 0.05 level of probability was used as the criterion of significance.

RESULTS

After five days of treatment of female rats with carbaryl in microsomal fraction of liver a statistically significant reduction of NADPH-cytochrome c reductase activity was established (Table 1).

TABLE 1

NADPH-cytochrome c reductase activity in the liver microsomes of female rats treated with carbaryl and propoxur[a].

Treatment	Dose mg/kg	NADPH-cytochrome c reductase activity[b]			
		day 5	day 10	day 20	day 30
Control	-	57.4±3.1	62.7±5.3	51.8±3.9	49.6±4.1
Carbaryl	6.0	52.5±4.1[c]	60.3±7.1	50.4±5.0	52.8±7.1
	12.0	46.5±6.0[c]	57.5±4.2	49.9±4.4	41.0±2.9[c]
Propoxur	1.0	51.1±3.2[c]	55.9±5.1[c]	40.8±3.2[c]	47.4±3.7
	2.0	56.8±2.7	41.1±4.2[c]	40.4±3.4[c]	42.8±3.9[c]

a
The results present the mean ± S.D. There were 10 animals in each treatment group.

b
Enzyme activity is expressed in nmoles cytochrome c reduced/min per mg microsomal proteins.

c
Significantly different from control group ($P<0.05$).

In the other examination periods no statistically significant differences were observed in the activity of microsomal

NADPH-cytochrome c reductase between treated and control animals. The only exception was observed in females treated with a dose of 12.0 mg/kg for 30 days. In these animals a statistically significant decrease of enzyme activity was established.

In microsomal fraction of animals treated with propoxur statistically significant decrease of NADPH-cytochrome c reductase activity was found in nearly all examination periods. Exception were noticed in animals treated with a dose of 2.0 mg/kg for five days, and in those treated with 1.0 mg/kg for thirty days.

In males both doses of carbaryl significantly reduced the NADPH-cytochrome c reductase activity in liver during all examination periods (Table 2).

TABLE 2

NADPH-cytochrome c reductase activity in the liver microsomes of male rats treated with carbaryl and propoxur[a].

Treatment	Dose mg/kg	NADPH-cytochrome c reductase activity[b]			
		day 5	day 10	day 20	day 30
Control	-	68.0±7.1	62.1±2.9	45.8±2.1	46.4±3.1
Carbaryl	6.0	60.4±5.3[c]	53.2±3.0[c]	42.9±2.8[c]	42.1±3.2[c]
	12.0	50.6±4.7[c]	57.0±5.0[c]	41.9±4.0[c]	41.8±2.1[c]
Propoxur	1.0	56.5±5.1[c]	42.6±2.0[c]	43.3±3.1	42.5±2.7[c]
	2.0	52.3±4.9[c]	48.9±3.3[c]	48.9±6.1	39.3±4.0[c]

a
The results present the mean ± S.D. There were 10 animals in each treatment group.

b
Enzyme activity is expressed in nmoles cytochrome c reduced/min per mg microsomal proteins.

c
Significantly different from control group ($P<0.05$).

In the case of propoxur, both doses induced a statistically significant reduction of NADPH-cytochrome c reductase activity after five, ten and thirty days treatment, while the values obtained in animals treated for 20 days are at the level of control values.

Carbaryl and propoxur in female rats induced some changes

in cytochrome P-450 concentration (Table 3). In the first examination period, after five days, lower doses of both compounds caused increase in cytochrome P-450 content compared to control values. In microsomes of animals treated with propoxur for ten days a statistically significant decrease of cytochrome P-450 content was established. In other periods no statistically significant differences were noticed.

TABLE 3

Cytochrome P-450 concentration in the liver microsomes of female rats treated with carbaryl and propoxur[a].

Treatment	Dose mg/kg	Cytochrome P-450 concentration[b]			
		day 5	day 10	day 20	day 30
Control	-	0.52±0.02	0.53±0.03	0.47±0.01	0.48±0.05
Carbaryl	6.0	0.68±0.04[c]	0.55±0.03	0.45±0.03	0.44±0.05
	12.0	0.57±0.04	0.48±0.07	0.49±0.02	0.43±0.07
Propoxur	1.0	0.64±0.03[c]	0.47±0.04	0.48±0.02	0.49±0.02
	2.0	0.52±0.02	0.43±0.06[c]	0.49±0.04	0.44±0.05

a
The results present the mean ± S.D. There were 10 animals in each treatment group.

b
Cytochrome P-450 concentration is expressed in nmoles/mg of microsomal proteins.

c
Significantly different from control group ($P<0.05$).

The mean cytochrome P-450 content in microsomes isolated from liver male rats treated with carbaryl and propoxur does not differ significantly from the content found in females (Table 4).

In animals given carbaryl for five days, cytochrome P-450 content increased for 63 and 76%, independently of the dose.

Propoxur induced increase in cytochrome P-450 content of 21.6% and 23.2%. These differences are statistically significant. After 10, 20 and 30 days of treatment no statistically significant changes in cytochrome P-450 content, compared to the control values, were established.

TABLE 4

Cytochrome P-450 concentration in the liver microsomes of male rats treated with carbaryl and propoxur[a].

Treatment	Dose mg/kg	Cytochrome P-450 concentration[b]			
		day 5	day 10	day 20	day 30
Control	-	0.32±0.04	0.65±0.12	0.61±0.01	0.56±0.05
Carbaryl	6.0	0.53±0.06[c]	0.59±0.09	0.52±0.12	0.53±0.07
	12.0	0.57±0.01[c]	0.69±0.10	0.54±0.07	0.48±0.06
Propoxur	1.0	0.39±0.03[c]	0.76±0.12	0.59±0.06	0.52±0.06
	2.0	0.40±0.04[c]	0.63±0.09	0.53±0.05	0.49±0.09

a
The results present the mean ± S.D. There were 10 animals in each treatment group.

b
Cytochrome P-450 concentretion is expressed in nmoles/mg of microsomal proteins.

c
Significantly different from control group ($P<0.05$).

DISCUSSION

The effects of some pesticides, particularly the organochlorine compounds, on hepatic microsomal enzymes activity have been examined by a number of authors (WAGSTAFF & STREET 1971, HUNTER et al. 1972, PARKKI et al. 1977). On the other hand, far less is known about the effect of carbamates on liver enzymes. Nevertheless, the results indicate that these compounds can induce changes in the activity and concentration of liver enzymes (STEVENS et al. 1972, CRESS & STROTHER 1974, NEŠKOVIĆ & VITOROVIĆ 1977).

STEVENS et al. (1972) have established that some anticholinesterase insecticides, including carbaryl, induce in mice changes of aniline and hexobarbitale metabolism as a result of the effect of these insecticides on liver enzymes which take part in aniline and hexobarbitale metabolism. Moreover, CRESS & STROTHER (1974) have found a statistically significant increase of cytochrome P-450 content in liver microsomal fraction in rats fed diets with carbaryl for 14 days.

Our results (Table 1 and 2) show that carbaryl and propoxur reduce the activity of microsomal NADPH-cytochrome c

reductase in rats of both sexes. In most of the cases statistically significant changes were established. This is in agreement with our earlier results showing an inhibitory effect of some anticholinesterase insecticides on NADPH-cytochrome c reductase activity (NEŠKOVIĆ & VITOROVIĆ 1977).

At the same time, our results showed changes in cytochrome P-450 content (Table 3 and 4), but statistically significant were only present among in animals treated during five and ten days.

These results are in agreement with these obtained by CRESS & STROTHER (1974). They have found the increase of cytochrome P-450 content in microsomal liver fraction from mice after subacute application of carbaryl. KUZ'MINSKAYA & JAKUŠKO (1959) have found that the insecticides carbaryl and DDT, if applied for a longer period of time, induced a decrease of the activity of aldolase, phosphofructokinase, transketolase, glucoso-6-phosphatase and fructoso-6-phosphatdehydrase. POKROVSKIY & NENOV (1968) established the negative effect of carbaryl on some liver enzymes (tributyrase, aldolase and anilin aminotransferase) and on pancreas lipase.

As it is known, NADPH-cytochrome c reductase and cytochrome P-450 have important roles in the hydroxylation of various xenobiotics in the organism. Thus, every change in their concentration and activity may result in a change of the liver's ability to metabolize xenobiotics in the body.

From the fact that, by subacute intake of small doses of carbaryl and propoxur, as it is evident from our results, the activity and concentration of the examined liver enzymes is changed, it is clear that the liver's metabolic ability is changed as well. That interpretation is further reflected by the toxic effects of pesticides and their chemicals, as shown by CRESS & STROTHER (1974). However, it can not yet be stated for sure what causes the changes in cytochrome P-450 concentration and in NADPH-cytochrome c reductase activity. It is expected that future investigations will give answer to this question.

ACKNOWLEDGEMENT

The authors gratefully acknovledge the excellent techical assistance of Mrs. Zora Radjenović-Žagar.

REFERENCES

CRESS,C.R. and A.STROTHER: Life Sci. 14, 861 (1974).
HUNTER,J., J.D.MAXWELL, D.A.WILLIAMS, J.ROBINSON and A. RICHARDSON: Nature 237, 399 (1972).
KUZ'MINSKAYA,J.A. and V.E.JAKUŠKO: Gig.Sanit. 7, 97 (1959).
LOWRY,O.H., N.J.ROSEBROUGH, A.L.FARR and R.J.RANDAL: J.Biol. Chem. 193, 265 (1951).

MASTERS,B.S.S., C.H.WILLIAMS and H.KAMIN: Methods in Enzymology (Eds. R.W.Estabrook & M.E.Pullman), Academic Press, New York, 10, 565 (1967).
NEŠKOVIĆ,N., S.VITOROVIĆ and M.PLESNIČAR: Biochem.Pharmacol. 22, 2943 (1973).
NEŠKOVIĆ,N. and S.VITOROVIĆ: Bull.Environ.Contam.Toxicol. 18, 472 (1977).
OMURA,T. and R.SATO: J.Biol.Chem. 239, 2370 (1964).
PARKKI,M.G., J.MARNIEMI and H.VAINIO: J.Toxicol.Environ.Hlth. 3, 903 (1977).
POKROVSKIY,A.A. and P.C.NENOV: Vopr.Pitaniya 5, 56 (1968).
SNEDECOR,G.W. and W.G.COCHRAN: Statistical Methods, 6th Ed., Iowa State Univ. Press, Ames, Iowa (1967).
STEVENS,J.T., R.E.STITZEL and J.J.McPHILLIPS: Life Sci. 11, 423 (1972).
WAGSTAFF,D.J. and J.C.STREET: Toxicol.Appl.Pharmacol. 19, 10 (1971).