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Toxicological Study of Carbaryl in Rats

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bstract □ The apparent effectiveness of 1-naphthyl--methyl carbamate (carbaryl) against a wide variety insects motivated the study of its mammalian xicity. In this toxicological study of carbaryl, mature ale rats inhaled carbaryl at a mean concentration of 2mg, 168 mg and 224 mg/m³ for one hour. After halation, pentobarbital sleeping time, NADPH-cyto-rome c reductase activity, cytochrome p-450 and otein content in liver microsomes, various tissue sidues, cholinesterase inhibition in plasma and histo-ithological findings at autopsy were observed. The ntobarbital sleeping time was prolonged in rats haled with carbaryl for one day while the sleeping ne was shortened in the 3 days inhaled group. The anges of cytochrome p-450 content and NADPH-tochrome c reductase activity exhibited biphasic sponse showing the decrease in the one day inhaled oup and the increase in the 3 days inhaled group. he marked depression of plasma ChE activity was served in rats inhaled with carbaryl at 112 mg/m³, wever no more progressive effect was observed at e higher concentration of the compound. The main servations in histopathological finding were ciliary tachment, epithelial swelling and subepithelial flammatory cellular infiltration in trachea due to e irritation.

eywords □ Carbaryl, β-NADPH, Cytochrome c, BA, Apparatus of Inhalation System, Sleeping ne, Alveolar hemorrhage, Ciliary detachment.

Carbaryl is the common representative of the -methylcarbamates used for insecticidal appli-ition. The mode of action of carbaryl, like ie organophosphates, is reversible inhibition of cholinesterase[1-7] and the signs and symptoms are typically cholinergic with lacrimation, salivation, miosis, convulsions and death[8].

Numerous studies have been published on the toxicity of carbaryl in mammals. It has been shown by Vandekar[9] and Plestina *et al.*[10] that there was a good correlation between degree of ChE inhibition and intensity of symptoms as determined by the ChE activity in brain and plasma of animals poisoned by a series of monomethyl carbamates including carbaryl given by intravenous route in male rats.

As a result, over-exposure to carbaryl might be expected to give rapid inhibition in early stage. Also, Mount[11,12] has conducted the carbaryl-induced toxicity test in experimental animals, with determination of carbaryl tissue levels and ChE activity, and were able to establish critical diagnostic parameters.

Neskovic[13] reported that the NADPH-cytochrome c reductase activity was decreased significantly in carbaryl-treated rats and cytochrome p-450 content was increased simultaneously in male rats fed diets containing carbaryl.

In the meantime, Dikshith *et al.*[14] demonstrated that the activity of ATPase and glucose-6-phosphatase in liver of the carbaryl-dosed rats showed a slight increase and Lockard[15] reported that carbaryl rapidly inhibited DNA, RNA and protein synthesis in L-2 cells from the rat lungs.

Marked functional and structural changes

have been detected in the endocrine glands of rats given carbaryl. Shtenberg[16] and Pipy[17] showed that carbaryl inhibited the phagocytic activity of the reticulo-endothelial system, whereas Robel[18] reported carbaryl reduced locomotor activity.

The histopathological findings of carbaryl toxicity include cloudy swelling of the convoluted and loop tubules[19], widespread fatty degeneration[20,21] of the liver, interstitial myocarditis[22] and hemorrhagic areas in the lungs.

The afore-mentioned toxicological reports on carbaryl were conducted by administering the drug by *per os* and/or intravenous route in experimental animals, however few studies on toxicity concerning inhalation through the respiratory tract have been reported. As carbaryl is a spraying pesticide, poisoning by inhalation is a frequent occurrence due to crop dusting.

In view of this fact, an attempt was made to investigate the toxicological effect of carbaryl when this agent is inhaled in rats.

EXPERIMENTAL METHODS

Materials

Carbaryl, standard (Gasukuro Kogyo Inc.), Nicotinamide (GR,E,Merck), β-NADPH (Tetrasodium salt, Sigma), Cytochrome c (from horse heart, Sigma), Acetylthiocholine iodide (Boehringer Manheim), 5,5'-Dithiobisnitrobenzoic acid (Boehringer Manheim), Trimethylamine (TMA, Sigma), CO gas(Matheson Gas Product, U.S.A.), Heptafluorobutyric anhydride (HFBA, Sci-Eq, Tokyo Kasei), Florisil (60/100, for column chromatography, Kanto Chemical), Albumin (from bovine serum, Sigma) were used.

Apparatus

UV spectrophotometer (Varian, Cary 219), Gas chromatograph (Varian, Vista 4600 series) Refrigerated centrifuge(Sorvall RC-5B), OMN mixer and homogenizer (Sorvall), Wrist-actic shaker (Burrel corporation) were used.

Single and Repeated Exposure

Male rats of Sprague-Dawley strain weighin about 200 g were fed a laboratory chow an water *ad libitum*. The rats were housed in wir cages and acclimatized for a week before ex perimentation.

The inhalation chamber was as designed b the authors and illustrated in Fig. 1~2. 24 hours before experimentation, the animals in 1 of the groups were intoxicated by inhalatio in the chamber with carbaryl (technical grade 99% of 110 μm average particle size, fo 60 minutes at a concentration of 112 mg/m³, 16 mg/m³, and 224 mg/m³, respectively.

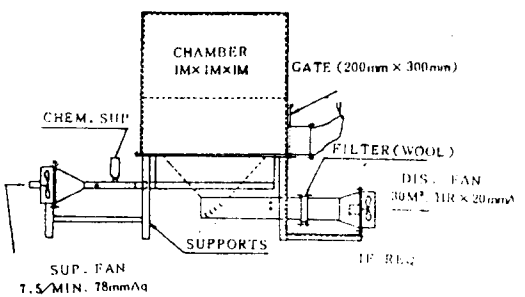

Fig. 1. Apparatus of Inhalation System(lateral view)

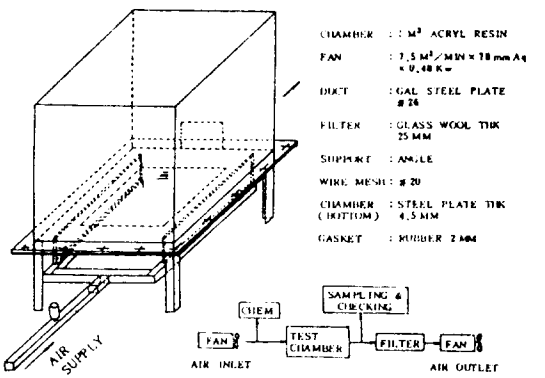

Fig. 2. Apparatus of Inhalation System

The mean concentration of carbaryl in the chamber was calculated by determining the static pressure of the fan and collecting the dust over 10 times with the filter from discharge duct.

Determination of the Sleeping Time

Barbiturate sleeping time was determined by injecting the animals intraperitoneally with pentobarbital sodium, 20 mg/kg, and the time from loss until recovery of the righting reflex was designated as sleeping time.

Measurements of NADPH-cytochrome c reductase activity, Cytochrome P-450 and Protein Content

(a) **The isolation of liver microsomal fraction**

At sacrifice the animasl following intoxication were lightly anesthetized with ether and the liver was perfused[23-25] *in situ* with 0.9 % NaCl solution.

The liver was excised, finely chopped with a razor, and homogenized with 0.25 M sucrose solution in a Potter Elvehjem Tissue Grinder and the 10~20 % of liver homogenate was then differentially centrifuged as shown in Fig. 3 according to the methods of Cinti *et al.*[23] that were modified from the Kamath method[26].

The supernatant was centrifuged at 12,000 xg for 10 min in a Sorvall RC-5B refrigerated centrifuge, and 8 mM $CaCl_2$ solution was added to the post-mitochondrial supernatant to obtain complete sedimentation of microsomes and then centrifuged at 27,000 xg for 15 min a Sorvall HB-4 rotor.

The pellet was resuspended in an equal volume of 0.15 M KCl and resedimented at 27,000 xg for 15 min. Thus, resultant pellets were used as a microsomal fraction.

(b) **Quantitative determination of cytochrome P-450 content**

Concentrations of cytochrome P-450 in isolated microsomes were determined by differencial spectrophotometry according to the procedure described by Omura and Sato[24] and Takashi *et al*[27]. respectively; microsomal suspension was prepared by adding 0.2 M phosphate buffer (10^{-3} M EDTA, pH 7.4) to the microsomal pellet, and then CO was bubbled through the suspension for about 1 min. The sample was then divided equally between two cuvettes and allowed to stand for 3 min. The baseline was recorded, then a small amount of sodium dithionite was added to the contents in the sample cuvette, and the spectrum was obtained 1 min

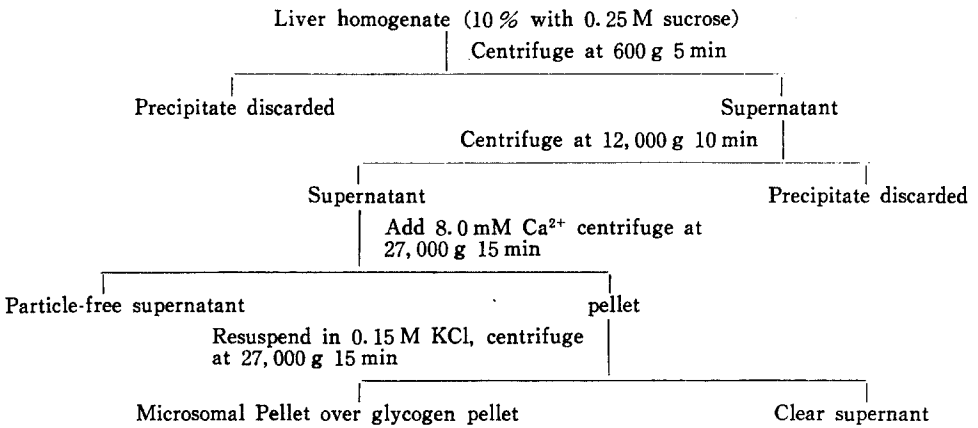

Fig. 3. Schematic Representation of Procedure for the Rapid Isolation of Microsomes. All steps are carried out at 0~4°C.

Table I. Composition of the Incubation Medium for NADPH-cytochrome c reductase

	Solution I		Solution II		Solution III	
Components	NADPH	5.7 mg	cytochrome c	3.68 mg	KCN	9.75 mg
	KCN	9.75 mg			nicotinamide	366 mg
	nicotinamide	366 mg				
Total Volume	100 ml with 0.05M phosphate buffer(10^{-3}M EDTA, pH 7.6)		1 ml with water		100 ml with 0.05 M phosphate buffer (10^{-3}M EDTA, pH7.6)	

later.

The concentration of cytochrome P-450 was calculated from the spectrum using the molar extinction difference of 104 mM^{-1} cm^{-1} for the absorption difference between peak position at 450 nm and 500 nm.

(c) **Estimation of NADPH-cytochrome c reductase activity**

The activity of microsomal NADPH-cytochrome c reductase was estimated by the procedure of Mazel[28] based on methods of Master *et al.*[29] and 0.05 M phosphate buffer (10^{-3} M EDTA, pH 7.6) was used as suspending medium for determination of NADPH-cytochrome c reductase activity. The incubation mediums were prepared as shown in Table I.

In a sample cuvette, 2 ml of Solution I was incubated and in reference cuvette, 2 ml of Solution III was incubated at 25°C for 8 min and at the end of 8 min, 0.5 ml of Solution II was added to each cuvette and incubated for additional 2 min. The 0.5 ml of microsomal suspension was added to each cuvette and mixed rapidly. The cuvettes were placed into the compartment of the recording spectrophotometer, maintained at 25°C, and recorded the change in optical density at 550 nm between the blank and sample cuvette during the first 3~4 min when the reaction rate was linear.

The activity of NADPH-cytochrome c reductase was calculated from the molar extinction coefficient of 19.1 mM^{-1} cm^{-1} for the difference between reduced and oxidized cytochrome c.

(d) **Determination of microsomal protein**

Determination of protein in microsomal fraction were carried out by the method of Lowry *et al.*[30] with bovine serum albumin as the standard.

Analysis of Carbaryl

Tissue and blood extraction and cleanup for carbaryl analysis were a modification of the procedure described by Lawrence[31] and Mount *et al.*[32] Tissue samples were extracted with acetone and centrifuged in refrigerated-centrifuge for removal of fatty material and freshly drawn blood was placed immediately into methylene chloride to halt carbaryl's breakdown. (Fig. 1)

Extraction and Cleanup

About 1g of tissues, excluding blood, was weighed and homogenized with 10ml of acetone in Sorvall homogenizer for 4 min. Approximately 5 g of sodium sulfate was added into the homogenate in 50 ml of centrifuge tube. Caps were secured and the tubes shaken for 25 min and then centrifuged at −20°C. One ml of the sample was used in the extraction of blood, no sodium sulfate was added, methylene chloride was used as the extracting solvent, and was shaken for 10 min in centrifuge tubes, then centrifuged.

All supernatants were evaporated at 30°C using rotary vacuum evaporator. The column was prepared by adding 1g of Florisil to the

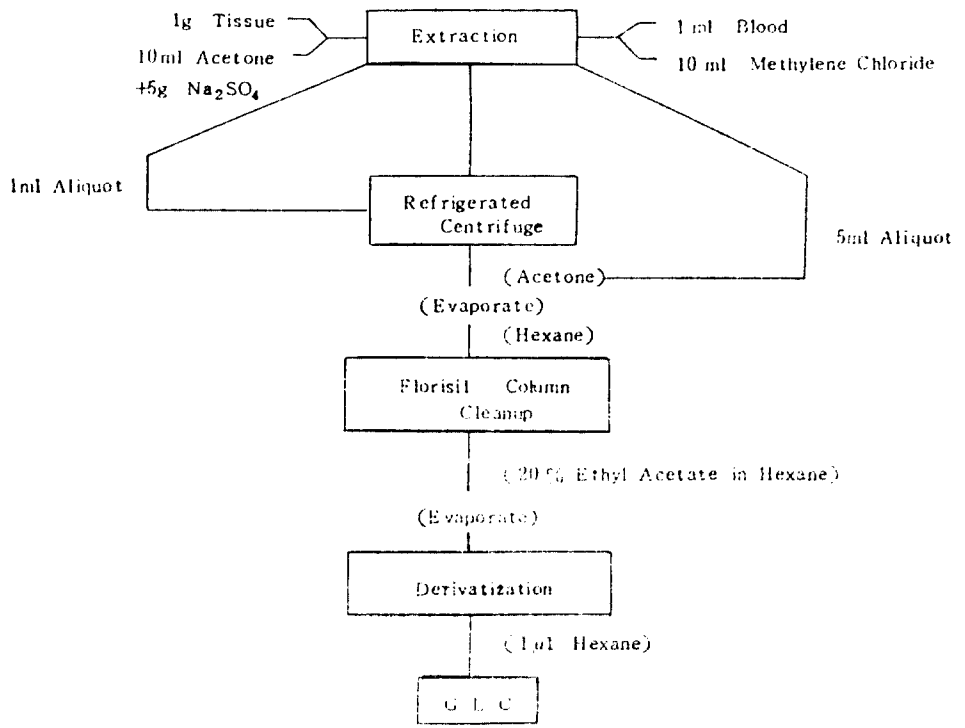

Fig. 1. The Analyzing Procedure of Carbaryl in Tissue and Blood.

Florisil column containing hexane and sodium sulfate was added to about 15ml of upper layer. The residue dissolved in hexane was transferred to the Florisil column and eluted with 15 ml of 20 % ethyl acetate in hexane and the solvent was then evaporated just to dryness.

Derivatization

Following evaporation, 0.02 ml of heptafluorobutyric anhydride and 0.5 ml of 0.1 M TMA solution was added to the residue in the vial containing carbaryl. The vial was immediately capped, shaken, and allowed to set for 30 min in room temperature, then 4.5 ml of hexane and 10 ml of distilled water was added. The vial was shaken vigorously for 10 seconds and centrifuged. After centrifugation, an aliquot of the hexane layer was injected into the gas chromatograph. At that time, heptachlor (0.05 mg/ml) was used as an internal standard.

Gas Chromatography

GC analysis were performed on a gas chromatograph equipped with a Ni electron-capture detector, a recording integrator (Vista 401 data system) and the chromatographic conditions were as follows:

Column: 3% OV-17 on chromosorb W-HP (80~100 mesh) 2 mm X 2 m, glass column
Column temp: 160°C
Carrier gas: N_2, 35 ml/min
Injector port: 220°C
Detector temp: 230°C

Determination of Plasma Cholinesterase Activity

Cholinesterase activity of plasma was measured using the basic procedure of Ellman *et al.*[33] and acetylthiocholine was used as the substrate.

The production rate of yellow anion of 2-nitro-5-mercaptobenzoate by the continuous reaction of thiocholine with 5,5'-dithiobisnitrobenzoic acid (DTNB) was measured at 405 nm in a spectrophotometer and the degree of enzyme activity was indicated as units/l.

Histopathological Methods

Portions of tissue were taken for micropathological examination from each of the controls and carbaryl-treated rats. Tissue sections were fixed in 8.0 % neutral buffered formalin solution and embedded in paraffin, sectioned[14,19] at 5 μm thickness and stained in the conventional manner with hematoxylin and eosin.

RESULTS AND DISCUSSION

The effects of carbaryl on the activity of drug metabolizing enzyme and changes of protein content.

Barbiturate sleeping time was determined by injecting pentobarbital sodium intraperitoneally with according to the method of Cress *et al.*[34].

The NADPH-cytochrome c reductase activity, cytochrome P-450 and protein content in liver microsomes were determined to observe the effect of different carboryl concentrations in inhalation chamber.

The contents of cytochrome P-450 were 1.27, 1.17 and 1.12 nmol/mg protein according to the carbaryl cencentration in the chamber, and the rates of decrease were 7.30, 14.60 and 18.25 % in comparison with control value of 1.37 nmol/mg protein. The NADPH-cytochrome c reductase activities were 184.34, 174.79 and 153.17 n mol cytochrome c reduced/min/mg protein with the decreasing rate of 6.08, 10.95 and 21.96 respectively, in comparison with the control value of 196.28 n mol/cytochrome c reduced/min/mg protein (Table II).

Hodgson *et al.*[35] have shown that many inhibitors of mammalian mixed-function oxidase activity can also act as inducers on longer exposure.

In this experiment, repeated administration of carbaryl during a 3-day period for 1 hour per day at a mean concentration of 168 mg/m^3 resulted in an increase of cytochrome P-450 content at a rate of 12.05 %, in comparison with control group (Table III).

Similar results were observed by Neskovic[13] that the increase was about 36 % in male rats and 8.4 % in female rats respectively when the rats were fed with diets containg 2,000 ppm

Table II. Effect of Carbayl on Hepatic Microsomal Cytochrome P-450 Contents and NADPH-cytochrome c reductase Activities in Rats

Group	Microsomal cytochrome P-450		NADPH-cytochrome c reductase	
	n mol/mg protein	% decrease	n mol cyt. c reduced /min/mg protein	% decrease
Control	1.37±0.05[a](7)[b]	0	196.28±29.81(6)	0
Carbaryl				
112mg/m^3	1.27±0.05 (9)	7.30	184.34±24.64(6)	6.08
168mg/m^3	1.17±0.06[c](10)	14.60	174.79±11.17(9)	10.95
224mg/m^3	1.12±0.04[c] (9)	18.25	153.17±11.32(7)	21.96

a : Mean±S.E.
b : The number in parentheses indicates the number of animals.
c : Statistically significant ($p<0.05$)

Table III. Change of Cytochrome P-450 Contents in Liver Microsomal Fraction of Rats Inhaled with Carbaryl for 1 or 3 days

Treatment	Days of Administration			
	1		3	
	n mol/mg protein	% decrease	n mol/mg protein	% increase
Control	1.37±0.05[a](7)[b]	0	1.24±0.05(8)	0
Carbaryl 168mg/m³	1.17±0.06(10)[c]	14.60	1.41±0.04(9)[c]	12.05

a : Mean±S.E.
b : The number in parentheses indicates the number of animals.
c : Statistically significant ($p<0.05$)

Table IV. Change of Protein Concentrations in Liver Microsomal Fraction of Rats

Group	n[b]	Hepatic Microsomal Protein	
		mg/g of liver	% increase
Control	9	21.94±0.62[a]	0
Carbaryl			
112mg/m³	8	23.18±0.98	5.65
168mg/m³	10	23.87±0.77	8.80
224mg/m³	9	25.21±0.76[c]	14.90

a : Mean±S.E.
b : The numbers of animals.
c : Statistically significant ($p<0.05$)

f carbaryl during a 2-month period.

The protein contents in microsomal fraction were increased in proportion to the carbaryl concentration in the chamber compared with control group as shown in Table IV, and repeated inhalation for 3 days at a concentration of 168mg/m³ also increased the protein contents with the increasing rate of 11.5%,

Therefore, these facts suggest that protein synthesis in liver microsome is performed continuously.

Consequently, carbaryl exhibited an inhibitory effect on drug metabolizing enzyme in the early stage and inducing effect in the late stage. Also, significant increase in hepatic microsomal protein suggested that carbary acted as a phenobarbital type inducer in the first stage and the action appeared to be a biphasic response.

This finding is considered to be similar to the report by Hodgson *et al.* maintaining that temporary decrease of cytochrome P-450 in the early stage is due to some of the anticholinesterase pesticides can inhibit the initial microsomal mixed-functional oxidase activity.

On the other hand, Stevens *et al.*[36] described that the barbiturate sleeping time can be used as an index of change in the rate of drug metabolism, because the hypnotic effect of barbi-

Table V. Pentobarbital Sleeping Time in Rats Inhaled with Carbaryl for 1 or 3 days

Treatment	Days of Administration			
	1		3	
	Minutes±S.E.	% increase	Minutes±S.E.	% decrease
Control	430.0±1.9(8)[a]	0	43.0±1.9(8)	0
Carbaryl 168mg/m³	49.9±1.7(7)[b]	16.0	33.6±3.7(7)[b]	21.9

a : The number in parentheses indicates the number of animals.
b : Statistically significant ($p<0.05$)

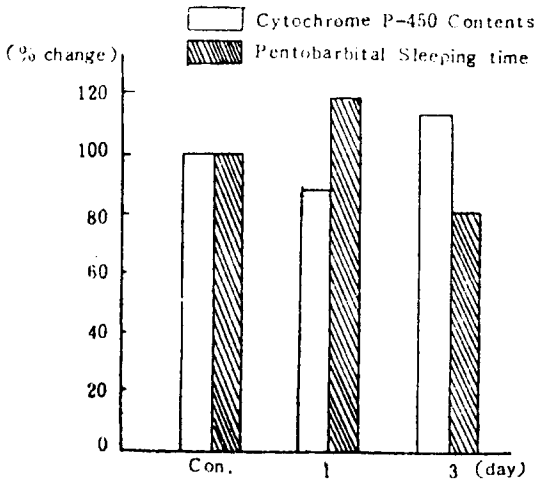

Fig. 5. Effect of Daily Inhalation of Carbaryl on Cytochrome P-450 Content and Pentobarbital

turate is terminated solely by hepatic microsomal metabolism.

The sleeping time by pentobarbilal sodium was prolonged to 49.9 min in rats inhaled with carbaryl for one day in comparison with 43.0 min of control groups as shown in Table V, while the sleeping time was shortened to 33.(min in the 3 days inhaled group.

This appears to confirm the results obtaine by Stevens *et al.*[36] and Cress *et al.*[34] wh determined the hexobarbital and pentobarbita sleeping time, respectively for the carbaryl treated rats. The histogram of variation betwee sleeping time and cytochrome P-450 content i shown in Fig 5.

It may be concluded that carbaryl exhibite biphasic response in that it inhibits the dru metabolizing enzyme in the early stage an induces the enzyme activity in the later stage.

Carbary Residues and Changes of ChE Activit in Plasma.

Table VI presents the carbaryl residues i tissues. Gas chromatograms of the derivatize carbaryl standard and the derivatized carbaryl from liver are illustrated in Fig. 6 and 7.

The highest tissue level of carbaryl was see in heart and the concentrations in tissue were

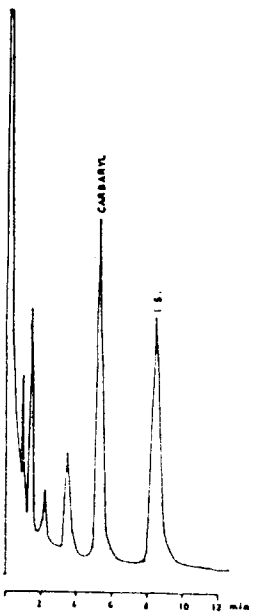

Fig. 6. Chromatogram of the Derivatized Carbaryl Standard.

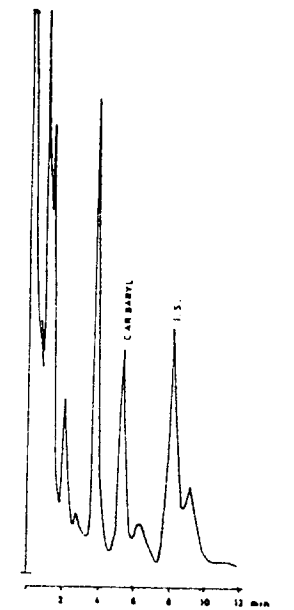

Fig. 7. Chromatogram of the Derivatized Carbaryl from Liver of Poisoned Rat.

Table VI. Distribution of Carbaryl in Various Tissues and Blood

Group	Residue (Mean±S.E.), ppm				
	Heart	Kidneys	Lungs	Liver	Blood
Control	ND	ND	ND	ND	ND
Carbaryl					
112mg/m³	0.18±0.04(2)[a]	0.34±0.04(6)	0.27±0.03(7)	0.15±0.04(4)	0.03±0.00(5)
168mg/m³	0.53±0.14(4)	0.38±0.09(6)	0.5 ±0.08(5)	0.21±0.06(3)	0.25±0.07(5)
224mg/m³	0.77±0.05(2)	0.59±0.06(5)	0.48±0.05(4)	0.48±0.13(3)	0.34±0.07(6)

a : The numbers in parentheses indicate the number of animals.

decreased in the order of kidneys, lungs, and liver. The distribution and concentration of carbaryl in tissue differed with routes of administration.

Houston *et al.*[37] reported that different routes of administration of same amount of a ^{14}C-labelled does (0.5 mg/kg) of carbaryl to male rats showed that oral administration resulted in lower plasma carbamate levels than those achieved by jugular vein injection, due to a liver first-pass effect.

The average particle size of carbaryl used in this experiment was 110 μm, and their site of deposition is highly dependent upon the size of particles[8]. Particles greater than 5 μm are usually deposited in the nasopharyngeal and consequently, some particles are supposed to pass to the gastrointestinal tract.

Mount *et al.*[11,32] reported that the concentration in tissue was highest in liver and decreased in the order of heart and brain after dosing carbaryl orally to rats. Also Strother *et al.*[38] reported that the concentrations of radio-labelled carbaryl in each organs were in the order of liver, adipose, kidneys and heart after administering ^{14}C-carbaryl intraperitoneally to rats. Besides, Johnson[39] *et al.* has maintained that the greater part of residual carbaryl remained in the skin when carbaryl was applied directly to the leghorn, using a polyethylene duct dispenser in such a manner as to assure through contact with the skin.

Judging from this result, the pattern of diposition of residual carbaryl in tissues due to inhalation varied from that of *per os* and/or intraperitoneal injection and was found to be characteristic.

The inhibition of the ChE activity in plasma was proportional to its concentration as shown in Table VII.

The mechanism of anticholinesterase action of carbamates is inhibition of the true cholinesterase enzyme and the intensity of the cholinergic effects produced after carbamate intoxication correlated with the degree of cholinesterase inhibition[40] as reported by Vandekar

Table VII. Effect of Carbaryl on the Activities of Plasma ChE

Group	n	ChE Activity (unit/l) Mean±S.E.	% depression
Control	4	445.74±8.29	0
Carbaryl			
112mg/m³	7	246.567±9.66[a]	44.66
168mg/m³	6	238.51±21.71[a]	46.49
224mg/m³	8	225.80±11.68[a]	49.34

a : Statistically significant ($p<0.05$)

et al.[9] who found a good correlation between the ChE depression and the intensity of the symptoms.

In this experiment, the marked depression of plasma ChE activity was observed with 44.66% of depression in rats inhaled with carbaryl for 60 min at 112 mg/m^3, however no further effect was found at an increased concentration of 224 mg/m^3 with 49.30% of depression. Although the toxicity of carbaryl varied in depending on the route of administration, there was a positive corelation between degree of ChE inhibition and dose of carbaryl. Thus, ChE activity in the plasma or red blood cell is used to evaluate exposure to carbamates[11].

The plasma ChE depression was 49.34% after inhaling carbaryl for 60 min at a concentration of 224 mg/m^3 and similar results were observed by Mount *et al.*[11] who observed that in rats, the plasma ChE depressions were 53% and 50% at 60 min after dosing in the 450 mg/kg and 800 mg/kg, respectively. Meanwhile, Plestina *et al.*[10] reported that the ChE depression was 59.8% at 60 min after administering propoxur intramuscularly in rats in the 2 mg/kg. Judging from these results, it can be recognized that there has been a difference between the degree of ChE depression and toxic dose.

Pathological Findings

The histopathological findings following carbaryl exposure revealed a slight advanced findings from that of the controls according to their concentrations. The findings for 29 cases were generalized, because the group inhaled at the concentration of 112 mg/m^3 showed almost no significant difference from that of 224 mg/m^3, and the results are listed in Table VIII.

Apparently at an early stage, capillary congestions in lungs and kidneys, central venous and sinusoidal congestion in liver were observed.

Table VIII. Summary of Histopathological Findings.

Organs	Histopathologic Findings
Lungs	1) Capillary congestion 2) Alveolar hemorrhage 3) Interstitial hemorrhage 4) Emphysema
Liver	1) Sinusoidal congestion 2) Central venous congestion 3) Vacuolar change
Kidneys	1) Capillary congestion 2) Proximodistal tubular interstitial hemorrhage 3) Proximal tubular swelling
Trachea	1) Ciliary detachment 2) Epithelial swelling 3) Subepithelial infiammatory cellular infiltration

On the other hand, Dikshith *et al.*,[14] maintained that oral administration of carbaryl (200 mg/kg for 3 days a week) for a period of 90 days did not produce any significant histological changes in liver and kidneys. But results similar to ours were reported by Boyd *et al.*[20] (single dose, 744 mg/kg) and El-Dakroury *et al.*[41].

These are generally considered to be early stages of general finding of sudden uneffected death. Also, in the lungs, alveolar (Fig. 8) and interstitial hemorrhage (Fig. 9) appeared and these changes were similar to those reported by Carpenter *et al.*,[19] who treated 50% carbaryl wet powder for 4 hours at a mean concentration of 390 mg/m^3. These are thought to be the direct irritation of the lung due to inhalation and severe congestion.

In the liver, vacuolar change (Fig. 10) was revealed, which is considered to be the same as fatty degeneration. Nir *et al.*,[22] have reported

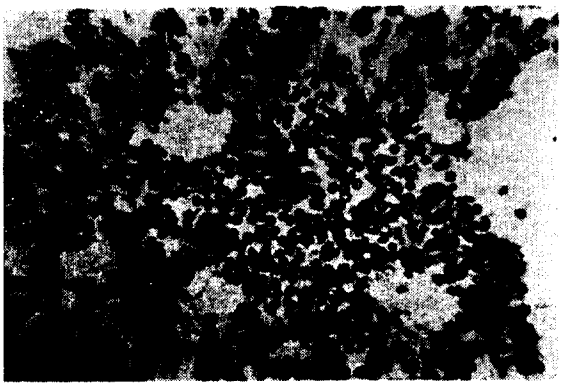

Fig. 8. Alveolar hemorrhage.
H & E, ×100

Fig. 9. Interstitial hemorrhage of the lung
H & E, ×400

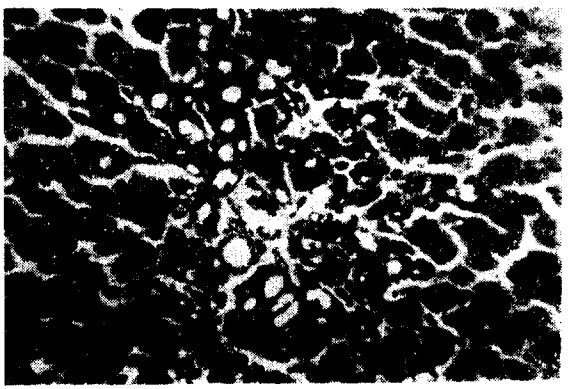

Fig. 10. Vacuolar infiltration(fat change) of the liver. H & E, ×400

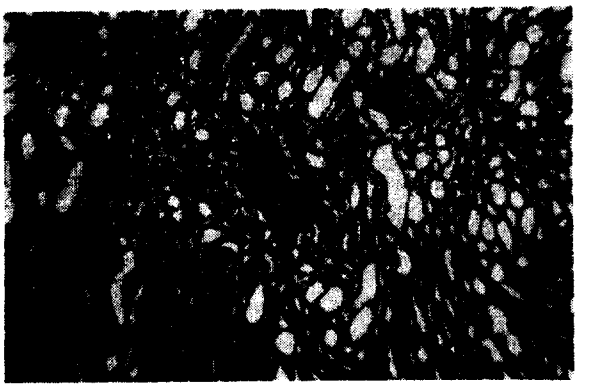

Fig. 11. Proximodistal tubular interstitial hemorrhage. H & E, ×400

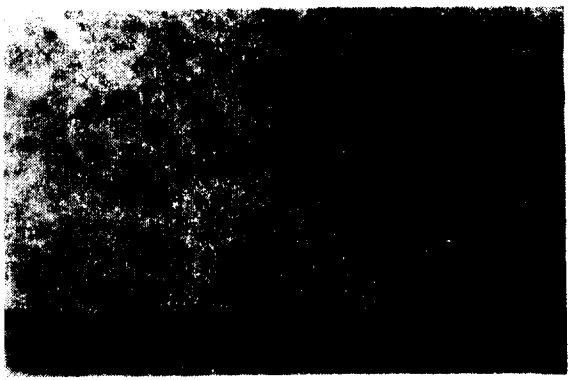

Fig. 12. Proximal tubular swelling.
H & E, ×400

Fig. 13. Ciliary detachment and epithelial swelling of the trachea. H & E, ×400

the same observation after administering carbaryl to leghorn at the concentration of 180 mg/kg, for 60 days.

In the mean time, the main observations in the kidneys were proximdistal tubular interstitial hemorrhage (Fig. 11) and proximal tubular swelling (Fig. 12).

In general, the tubular interstitial hemorrhage is observed when kidney is exposed to the toxic substance. Proximal tubular swelling was observed here similar to that reported by Carpenter *et al.*,[19] but these are considered to be transitory nature and not as early signs of toxic degeneration.

In the trachea, ciliary detachment, epithelial swelling and subepithelial inflammatory cellular infiltration (Fig. 13) were observed and these are considered to be caused by the inhalation of certs toxic material.

Inasmuch as little information was available concerning the detailed effects of carbaryl on trachea due to inhalation, it further studies on the pathological changs occuring on longer exposure.

CONCLUSIONS

Male rats of Sprague-Dawley strain were inhaled carbaryl at a concentration of 112 mg/m^3, 168 mg/m^3 and 224 mg/m^3 for 60 min and the barbiturate sleeping time, cytochrome P-450 content, NADPH-cytochrome c reductase activity, various tissue residues and ChE activity were also measured. Histopathological observation at autopsy were performed. The results obtained from these experiments were as follows:

The pentobarbital sleeping time was prolonged in rats inhaled with carbaryl for one day, while it was shortened in the 3 days inhaled group.

The changes of cytochrome P-450 content and NADPH-cytochrome c reductase activity showed the decrease in the early stage in comparison with the control group, while the cytochrome P-450 content was increased in the 3 days inhaled group showing the biphasic response.

The tissue levels of carbaryl was highest in heart and decreased in the order of kidneys lungs, liver and blood.

The marked depression of plasma ChE activity was observed in rats inhaled with carbaryl at 11 mg/m^3, however, no further effect was observed at the higher concentration of the compound.

The observations in the histopathological finding were capillary congestions in lungs and kidneys, central venous and sinusoidal congestion in liver. In trachea, ciliary detachment, epithelia swelling and subepithelial inflammatory cellular infiltration were revealed.

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