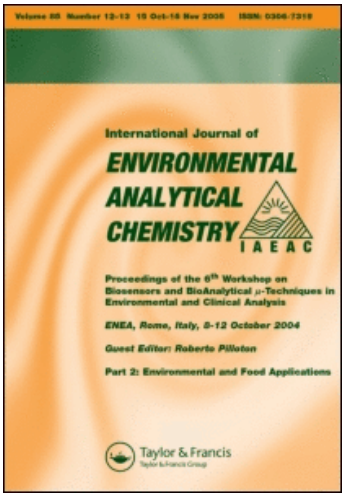

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Occupational Exposure Control by Simultaneous Determination of N-methylcarbamates and Organophosphorus Pesticide Residues in Human Urine

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On-column transesterification with methanol was applied for the gas chromatographic determination of N-methylcarbamates extracted from human urine. Transesterification conversion efficiencies of N-methylcarbamates dioxacarb, carbofuran and OMS-22, calculated from the amount of the on-column produced O-methyl-N-methylcarbamate (DMC), were 96, 77 and 76% with detection limits of 8, 10 and 10 ng, respectively.

In the investigated concentration range of 0.2–3 μg/ml of urine the extraction efficiencies with methylene chloride were independent of the initial concentration of N-methylcarbamate added to urine samples of non-exposed persons. The recoveries and rel. S.D. were 74 ± 11, 64 ± 8 and 79 ± 12% for dioxacarb, carbofuran and OMS-22, respectively.

The procedure was applied for the gas chromatographic determination of carbofuran and its metabolites containing the N-methylcarbamic group extracted from urine samples of occupationally exposed persons in a pesticide formulating plant. The level of extracted N-methylcarbamates and the concentration of degradation products of organophosphorus pesticides detected in the urine of the same persons were correlated with the blood and plasma cholinesterase activities. Although the determination of DMC includes only a smaller part of the excreted N-methylcarbamate, a simultaneous determination of both carbamates and organophosphorus residues made it possible to distinguish the cause of depression in cholinesterase activity, indicating early and specifically the exposure to a particular group of agents hazardous to health.

KEY WORDS: N-methylcarbamates, carbofuran, organophosphorus pesticides, urine, cholinesterase activity, occupational exposure.

INTRODUCTION

The pursuit of carbamates in water, soil, food and biological tissues and fluids is becoming increasingly important in connection with the evaluation of both environmental and occupational hazards associated with the use of these chemicals as herbicides, insecticides and pharmaceuticals.

Occupational exposure is frequently controlled by means of a decrease in cholinesterase activity in blood and/or plasma, a parameter reflecting not only the absorption of carbamates but of organophosphorus pesticides as well. Pesticide uptake can be proved directly by determining the parent agent or specific metabolites or residues excreted in urine, the choice being dependent on the persistence of the compound or on the course of metabolic degradation.

In the case of carbamates only a negligible excretion of intact parent compounds should be expected. The available data show that only 1% of methylpenthynolcarbamate was recovered in human urine after an oral dose of 300 mg.[1]

Radiochemical investigations carried out in different animal species with either ring-C^{14} or carbonyl-C^{14} radiolabelled carbamates established that over 90% of the former but less than 40% of the latter carbamate was excreted with urine.[2–4] These data justify the selection of 1-naphthol, a product of carbaryl hydrolysis, as an indicator of occupational exposure.[5] For the same purpose on the basis of the extensive investigations of carbofuran metabolism, not only in mammals but also in insects and plants,[4,6,7] a monitoring programme was conceived. It included the determination of carbofuran-phenol and 3-keto-carbofuran in human urine. Carbaryl and propaxur resorption was followed by the determination of 1-naphthol and isopropoxyphenol.[8]

By a different approach total carbofuran residues were determined in field treated lettuce,[9] soil,[10] tobacco,[11] and various plants,[12] namely by the on-column conversion of N-methylcarbamates to methyl-N-methylcarbamate.[13] In this work we applied the analogous procedure for the gas chromatographic determination of carbofuran and its metabolites containing the N-methylcarbamic group extracted from the urine of occupationally exposed persons in a pesticide formulating plant. The obtained carbamate results and the amount of organophosphorus pesticide residues detected in the urine of the same persons were tentatively discussed with the aim to differentiate the causes for depressed cholinesterase activity.

The linearity range, reproducibility and detection limits for gas chromatographic determination not only of carbofuran but also of OMS-

22 and dioxacarb have been checked in view of a foreseen project for the prospective production of carbamates.

EXPERIMENTAL

Materials†

Methylisocyanate, purum, >98%, Fluka AG, Buchs, Switzerland.
Dioxacarb, purity 99%, Chromos-Katran-Kutrilin, Zagreb, Yugoslavia.
OMS-22, technical, Hercules Powder Company, Wilmington, U.S.A.
Carbofuran, purity 99.6%; O,O-Diethyl phosphate, Lot. No. 7302, purity 98%; O,O-Diethyl phosphorodithioate potassium salt, Lot. No. 5731; O,O-Diethyl phosphorothionate potassium salt, Lot. No. 5732; all obtained from the U.S. Environmental Protection Agency (EPA), Research Triangle Park, N.C., U.S.A.

Instruments

a) Varian Aerograph Series 2800 (columns I and II) and 1400 (column III) gas chromatographs equipped with Alkali Flame Ionization Detectors (AFID) having a Rb_2SO_4 salt tip.

†List of compounds used in this work:

Pesticide		Compound analysed	
Common name	Chemical name	Chemical name	Abbreviation
Dioxacarb	2-(1,3-dioxolan-2-yl)-phenyl-N-methylcarbamate	O-methyl--N-methylcarbamate	DMC
OMS-22	2-chloro-3-isopropyl-phenyl-N-methylcarbamate		
Carbofuran	2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methylcarbamate		
Quinalphos	O,O-diethyl-O-(2-quinoxalyl) phosphorothionate	O,O-diethyl phosphate	DEP
		O,O-diethyl phosphorothionate potassium salt	DETPK
Phosalone	S-(6-chloro-2-oxobenzoxazolin-3-yl) methyl diethyl phosphorothiolothionate		
		O,O-diethyl phosphorodithioate potassium salt	DEDTPK

b) Gas chromatographic columns: glass 1.8 m × 2 mm i.d., packed with 5% Carbowax 20 M on 80/100 mesh Gas Chrom Q; the first 15 cm of the column was packed with 160 mesh regular glass beads treated by dipping them into the methanolic solution of 10^{-2} M NaOH and then air drying (column I); glass 1.8 × 2 mm i.d., packed with 4% SE-30 + 6% OV-210 on 80/100 mesh Gas Chrom Q (column II); glass 1.5 m × 2 mm i.d., packed with 25% Triton X-305 on 80/100 mesh Chromosorb W-AW/DMCS (column III).

c) Operating conditions: temperatures—column I 115°C, column II 120°C, column III 140°C, injector 205°C and detector 220°C (Varian 2800), injector 190°C and detector 235°C (Varian 1400); nitrogen carrier gas flow 30 ml/min., air flow 235 ± 10 ml/min and hydrogen flow 35 ± 3 ml/min.

d) O-methyl-N-methylcarbamate (DMC) was identified with a gas chromatograph-mass spectrometer system (GC-MS). A Perkin-Elmer Sigma 3 gas chromatograph was coupled with a Kratos MS 25 mass spectrometer and Data System DS 50 S. The ionization source temperature was 200°C and electron energy 70 eV. The glass column was packed with 20% Triton X-305 on 80/100 mesh Chromosorb W-AW/DMCS and heated at 130°C. Helium was used as a carrier gas.

Determination of transesterification conversion efficiencies of carbamates, response linearity of AFID and identification of DMC by GC-MS

A definite amount of the methanolic solution of carbamate pesticides and 50 μl of 10^{-1} M methanolic NaOH were made up with methanol to 1 ml to give a theoretical yield of 1.5–30 μg DMC/ml. The samples were analysed on gas chromatographic column I.

A sample solution for GC-MS characterization was prepared in the following way: to 10 ml of boiling 2 M NaOH in methanol 25 ml of the methanolic solution of OMS-22 (2.37 mg/ml) was added. The cooled sample was evaporated and the remaining oil extracted with 5 ml of hexane.

Analysis of urine samples

Simulated samples, prepared by adding definite amounts of carbamate pesticides or organophosphorus salts to the urine of non-exposed persons, were treated by one of the following procedures:

a) Determination of N-methylcarbamate pesticides To a 10-ml aliquot of

a urine sample 0.5 ml of the water solution of carbamate (or deionized water in the case of blank) and 5 ml of methylene chloride were added. After mixing for 1 min on a Vortex mixer the organic layer was transferred into a centrifuge tube, 0.5 g of sodium sulphate was added and the sample centrifuged at 425 G for 3 min. An aliquot of 3.5 ml of a methylene chloride layer was transferred into a test tube with a ground stopper, evaporated to 1 ml under a stream of nitrogen, mixed with 3 ml of methanol and evaporated again to less than 1 ml. To that sample, to make the final volume of 1 ml, 50 μl of 10^{-1} M NaOH in methanol and methanol were added just before gas chromatographic analysis (column I).

The recoveries were determined in relation to the standard DMC solution prepared by mixing equivalent amounts of methylisocyanate and methanol at room temperature. A dilution series of standard solutions was prepared in methanol.

b) Determination of organophosphorus pesticide metabolites The urine samples were treated according to the method for the determination of DETPK and DEDTPK described by Drevenkar and co-workers[14] (Procedure I) and by the method of Blair and Roderick[15] for the determination of DEP, DETPK and DEDTPK. The gas chromatographic analysis of DEP methylated derivative was performed on column III and of DETPK and DEDTPK methylated derivatives on column II. The standards were prepared by adding the known amounts of DEP, DETPK and DEDTPK water solutions to 5-ml urine samples of non-exposed persons. Subsequently either of the two methods was used.

The urine samples collected from persons occupationally exposed to carbamates and organophosphorus pesticides were treated by both the (*a*) and (*b*) procedures.

c) Persistence of carbofuran in urine samples and in deionized water A definite amount of aqueous solution of carbofuran was added to 150 ml of urine samples collected from four different non-exposed persons or to deionized water to make the final concentration of 1.23 μg/ml. The samples were stored at 4°C in dark glass bottles for 10 days. The carbofuran concentration in urine and water samples was determined immediately after addition and then after 1, 2, 4, 7, 8, 9, and 10 days according to procedure (*a*).

Cholinesterase activity

Blood and plasma cholinesterase activities were measured as described by Ellman and co-workers.[16]

RESULTS AND DISCUSSION

Determination of carbamates in urine

As a selective and direct procedure for the control of persons occupationally exposed to N-methylcarbamates we introduced the on-column transesterification with methanol in the presence of a catalytic amount of NaOH[13] for the gas chromatographic determination of their residues extracted from human urine.

Transesterification conversion efficiencies of N-methylcarbamates dioxacarb, carbofuran and OMS-22 were calculated from the amount of DMC produced by the on-column reaction of carbamate with methanol. Figure 1 shows typical gas chromatograms of a transesterification product of N-methylcarbamates, the identity of which was confirmed by GC-MS analysis (Figure 2). No interferences were observed.

For the quantitative evaluation of gas chromatograms the peak heights were measured and correlated with the appropriate standard DMC solution. Several determinations were performed by injecting the same quantities of carbamate solution. The efficiency of N-methylcarbamates conversion to DMC (Table I) did not depend on the injected quantity of

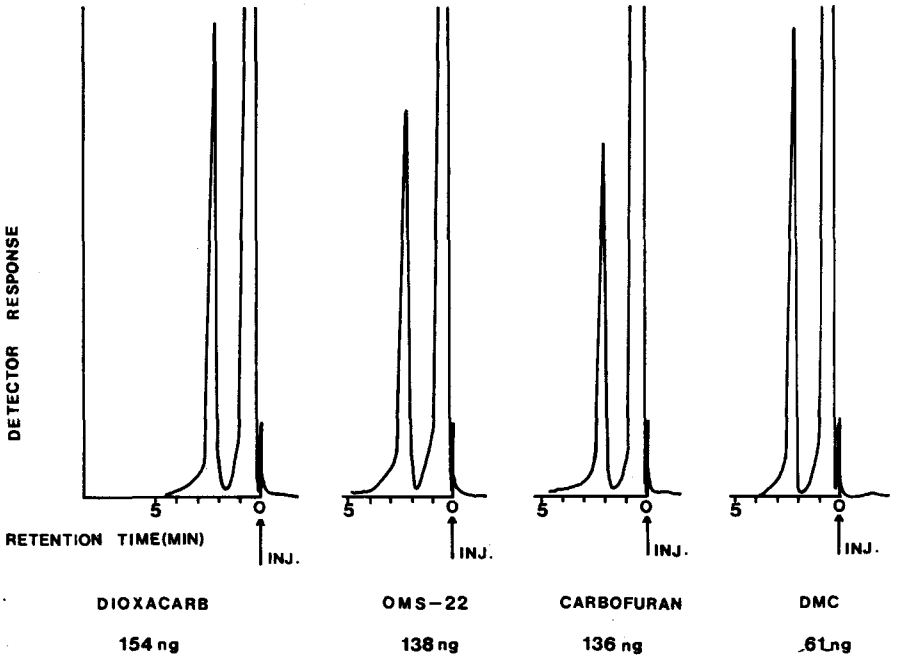

FIGURE 1 Gas chromatograms of tested carbamates after on-column transesterification with methanol and of DMC standard solution.

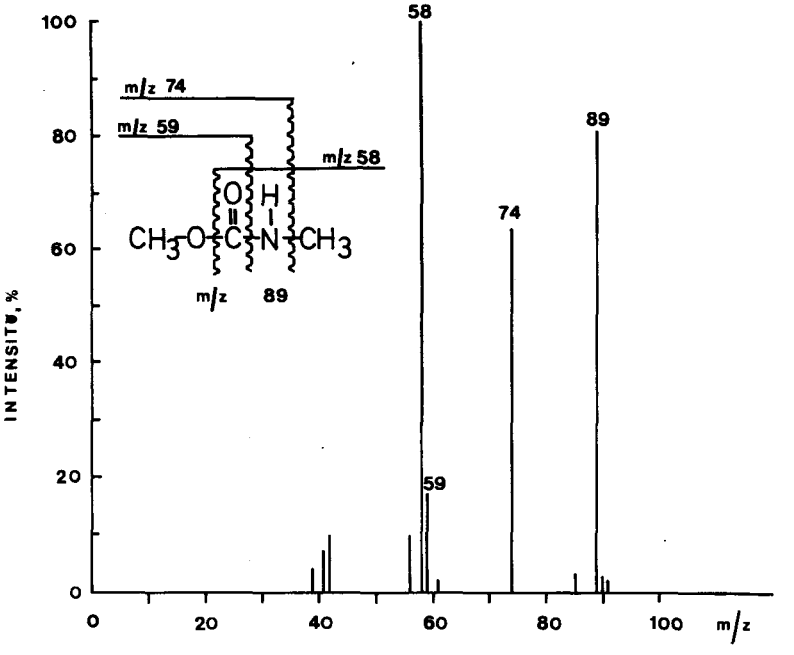

FIGURE 2 DMC mass spectrum.

TABLE I

Linearity of response, transesterification conversion efficiency and detection limit for dioxacarb, carbofuran, OMS-22 and DMC analysed by gas chromatography

	DMC	Dioxacarb	Carbofuran	OMS-22
Concentration range, μg DMC/ml	1.7–88	1.3–38.3	2.0–31.1	1.4–31.5
Slope, b	0.09114	0.08893	0.07017	0.06907
Standard error of the slope, s_b	0.00167	0.00211	0.00238	0.00171
Variance of the regression, s^2	0.08434	0.04760	0.03888	0.01608
Number of determinations, N	14	25	22	21
Conversion efficiency, %		96	77	76
Detection limit, ng	3	8	10	10

the compound and it was nearly quantitative even at the nanogram level. The results are in accordance with the results of Moye[13] who has made a survey of conversion efficiency of several N-methylcarbamates, including furadan (carbofuran). The solvent, injector temperature, injector packing and NaOH concentrations were optimized for mobam (4-benzothienyl N-methylcarbamate) conversion. To relative standard deviation in response to a 36-ng mobam standard of 1.7% for 6 consecutive injections was obtained. The conversion efficiency of mobam was 81% and of furadan 87%.

The calibration curves (Figure 3) for the quantitative gas chromatographic determination of dioxacarb, carbofuran and OMS-22 were constructed by taking the ratio of sample to standard peak height as the ordinate in order to eliminate possible variations in experimental conditions. For each N-methylcarbamate a sample of a concentration theoretically yielding 13 μg/ml of DMC after on-column transesterification was taken as the standard.

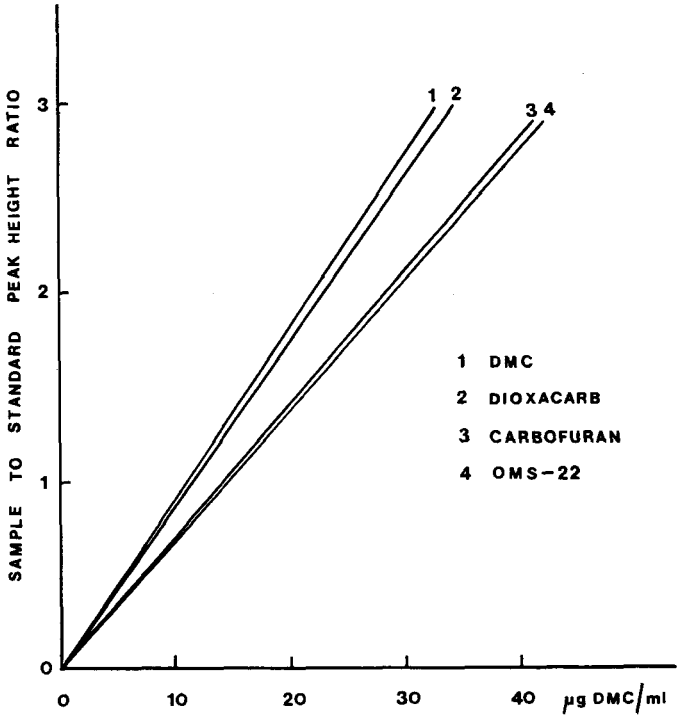

FIGURE 3 Calibration curves for the GC determination of carbamates by on-column transesterification with methanol. Standard: 13 μg DMC/ml of the sample.

The linearity and reproducibility ranges and detection limits obtained for the studied N-methylcarbamates are tabulated in Table I.

The known amounts of dioxacarb, carbofuran and OMS-22 were added to urine samples of non-exposed persons in concentrations ranging from 0.2 to 3 μg/ml in order to construct the calibration curves for the determination of urinary excreted N-methylcarbamates in humans (Figure 4). The extraction was carried out with methylene chloride. The recoveries and detection limits are shown in Table II. In the investigated concentration range the extraction efficiency was found to be independent of the initial concentration of N-methylcarbamates in urine samples. The

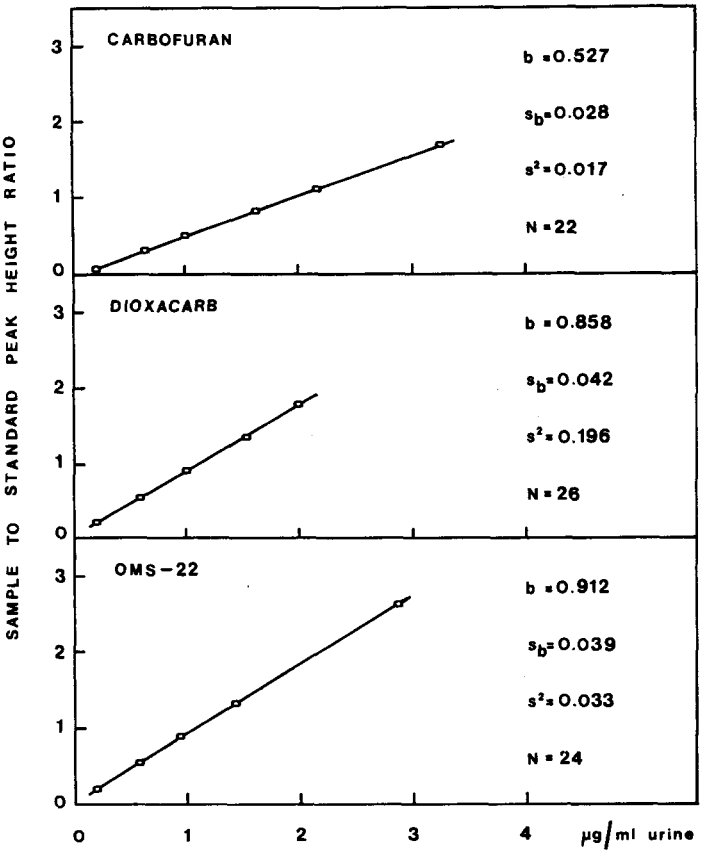

FIGURE 4 Calibration curves and linearity ranges for the quantitative determination of carbofuran, dioxacarb and OMS-22 added to urine of non-exposed persons. Standard: methanolic solution of DMC; concentrations: 4.198 μg/ml for carbofuran and 2.098 μg/ml for dioxacarb and OMS-22. b = slope; s_b = standard error of the slope; s^2 = variance of the regression; N = number of determinations.

TABLE II

Recovery range and detection limit for dioxacarb, carbofuran and OMS-22 added to urine of different non-exposed persons

Pesticide	Concentration in urine (μg/ml)	Recovery %±S.D. (no. of determinations)	Detection limit (μg/ml urine)
Dioxacarb	0.205–3.084	74±11 (30)	0.205
Carbofuran	1.234	64± 8 (56)	0.210
OMS-22	0.191–2.868	79±12 (26)	0.190
Mixture:			
Dioxacarb 1	0.072–1.079	60±12 (30)	
Carbofuran 1	0.071–1.058		
OMS-22 1	0.067–1.004		

mean recoveries and standard deviations of dioxacarb and OMS-22 were therefore calculated from all extraction experiments performed with different initial concentrations of these compounds in urine. The results of experiments in which carbofuran persistence in urine was checked were taken for the same calculation, because of a large number of implied data. No interfering peaks were obtained on the chromatogram if the samples of blank urine were subjected to the same procedure.

The experiments carried out by dissolving the carbamates in deionized water showed that there are no significant differences in extraction recoveries in relation to urine samples. Also, the extraction efficiency was not dependent on the pH value of the aqueous carbamate solution in the range $3 < pH < 9$.

The persistence of free N-methylcarbamate in urine was checked by addition of known amounts of carbofuran to urine samples of four non-exposed persons. The samples were stored at 4°C. During 10 days no change in initial concentrations was observed showing that this interval between the collecting and analysis of urine samples does not affect the results as far as the parent pesticide is concerned. The same results were obtained if carbofuran was added to deionized water.

Occupational exposure control

A comparative study of blood cholinesterase inhibition versus organophosphorus pesticide residues in urine in a group of workers from a pesticide formulating plant showed that the rate of urinary excretion of residues can hardly be correlated with the decrease in whole blood and

plasma cholinesterase activity. In this particular case the uptake of quinalphos was pursued by determining its residues DEP and DETPK (Table III). The small amounts of DEDTPK, also detected in most samples, were a consequence of work with phosalone one or two days earlier.

Urine samples were collected before and at the end of the working day at 30-day intervals during three months of the most intense pesticide formulation i.e. May, June and July. The last sampling was carried out in September on the first working day immediately after a one-month vacation. The blood samples were collected on the same days before the beginning of work.

TABLE III

Urinary excretion of DEP, DETPK and DEDTPK at the beginning (a) and at the end (b) of the working day and blood plasma cholinesterase activities in persons occupationally exposed to organophosphorus pesticides

		ng/ml of urine						Cholinesterase activity (%)	
		DEP		DETPK		DEDTPK			
Workers	Month[a]	*a*	*b*	*a*	*b*	*a*	*b*	blood	plasma
	5	592	308	292	187	285	151	77	58
A	6	99	96	94	73	49	ND	84	58
	7	1896	656	349	381	61	48	79	61
	5	232	86	83	139	ND	43	74	70
B	6	ND	ND	37	ND	ND	ND	73	70
	7	247	106	83	103	54	43	80	73
	5	119	ND	ND	ND	ND	<20	85	82
C	6	ND	ND	ND	ND	ND	ND	68	NM
	7	113	ND	68	ND	ND	ND	68	65
	5	193	193	82	112	83	59	88	88
D	6	ND	67	45	63	37	38	93	83
	7	213	ND	100	128	ND	ND	86	87
	5	ND	ND	44	57	ND	26	86	100
E	6	ND	ND	36	68	ND	41	46	76
	7	357	168	197	181	<20	ND	72	76
	5	228	134	ND	65	ND	60	83	56
F	6	206	73	81	<50	42	<20	34	42
	7	447	238	203	139	57	<20	59	48
	5	680	319	163	181	201	192	88	77
G	6	NM	NM	NM	NM	NM	NM	NM	NM
	7	1807	1245	759	1752	50	78	68	45

[a]5 = May, 6 = June, 7 = July.
ND = not detected.
NM = not measured.

The highest concentrations of organophosphorus pesticide residues were found in the urine sample of worker G collected in July. They were accompanied by the highest decrease in cholinesterase activity (Table III). On the first working day after the one-month vacation only small amounts of residues were detected at the end of work (158 ng DEP/ml, 123 ng DETPK/ml and 37 ng DEDTPK/ml) but the whole blood and plasma cholinesterase activities were still decreased—28% and 65% respectively.

In workers E and F the lower urinary residue excretion in June was accompanied by a higher decrease in enzyme activity, while in July a higher residue excretion and increased cholinesterase activity were determined in the same persons (Table III). After vacation in the urine sample of the worker F collected at the end of the work on the first working day only 67 ng DEDTPK/ml was detected and his blood and plasma cholinesterase activities were increased—77% and 92% respectively.

In the urine samples of other workers no organophosphorus pesticide residues were found after vacation and their blood and plasma cholinesterase activities were between 80–100% of the pre-exposure value.

It seems that the amount of residues in urine do not always correlate with cholinesterase inhibition and that the additional impacts should be taken into account for an adequate consideration. No attempt was made to study this further and neither were blood cholinesterases analysed with respect to enzyme variants.

Simultaneous determination of as many pesticide residues as possible provides a more reliable indication of the past and present exposure and of the uptake of the parent pesticide. In a pesticide formulating plant the workers alternately work with different organophosphorus pesticides and some less toxic carbamates in the time intervals of 3–5 days. Moreover, both groups of pesticides are often formulated simultaneously on the same premises so that the blood cholinesterase activity in the exposed persons is affected by both classes of compounds.

The uptake of carbofuran in a group of workers from a pesticide formulating plant was evaluated by gas chromatographic determination of DMC originating from the parent N-methylcarbamate and its metabolites containing the N-methylcarbamic group extracted from their urine samples. The levels of N-methylcarbamates extracted from urine and the concentrations of the degradation products of organophosphorus pesticides, DETPK and DEDTPK, detected in the urine of these individuals were correlated with whole blood and plasma cholinesterase activities.

Simultaneously with the urine samples of occupationally exposed

persons five urine samples of non-exposed persons were analysed. Neither DMC nor DETPK and DEDTPK were detected in any of these samples.

Urine samples were collected at the beginning and at the end of working hours during three working days. Blood samples were collected before the beginning of work on the first and on the third day.

The DMC results are given in Table IV. The DMC concentration in urine samples on the first and second day was below 100 ng/ml. This value was also the detection limit. The exceptions were the urine sample of worker No. 6 on the first day and samples of workers Nos. 2, 4 and 8 on the second day with concentrations between 100 and 220 ng DMC/ml. No DMC was detected in urine samples on the third day. Cholinesterase activity was significantly decreased in all exposed individuals (Table IV).

The results of urine analysis for the metabolites of organophosphorus pesticides DETPK and DEDTPK are shown in Table V. During 3 days DETPK was found in all urine samples and DEDTPK was detected in the sample of worker No. 8. DEDTPK was also detected in urine sample of worker No. 7 on the second and third day.

Assuming that carbofuran metabolism in humans follows the same general pathways as in mammals,[4,7] by the extraction applied only a part of the non-conjugated carbofuran metabolites—i.e. species containing the N-methylcarbamic group—can be isolated from urine. The experiments in rats showed that more than 90% of the radioactivity in the urine of rats treated with carbofuran-C^{14} could not be extracted with an organic

TABLE IV

Urinary excretion of DMC at the beginning (*a*) and at the end (*b*) of the work and blood and plasma cholinesterase activities in occupationally exposed persons

	1st day			2nd day		3rd day			
	Cholinesterase activity (%)		DMC (ng/ml)	DMC (ng/ml)		Cholinesterase activity (%)		DMC (ng/ml)	
Workers	blood	plasma	*a*	*a*	*b*	blood	plasma	*a*	*b*
1	35	60	ND	<100	<100	—	—	—	—
2	55	75	<100	ND	220	41	54	ND	ND
3	39	56	<100	ND	<100	39	56	ND	ND
4	34	89	<100	122	109	66	87	ND	ND
5	74	96	<100	ND	<100	51	40	ND	ND
6	58	44	130	<100	<100	27	21	ND	ND
7	72	91	<100	<100	100	32	13	ND	ND
8	42	42	ND	184	<100	71	46	ND	ND

ND = not detected.

TABLE V

Urinary excretion of DETPK and DEDTPK at the beginning (*a*) and at the end (*b*) of work in occupationally exposed persons

	1st day Concentration (ng/ml)		2nd day Concentration (ng/ml)				3rd day Concentration (ng/ml)			
	DETPK	DEDTPK	DETPK		DEDTPK		DETPK		DEDTPK	
Worker	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	24	ND	25	67	ND	ND	—	—	—	—
2	23	ND	40	49	ND	ND	55	50	ND	ND
3	107	ND	138	184	ND	ND	98	155	ND	ND
4	49	ND	38	61	ND	ND	42	33	ND	ND
5	52	ND	54	84	ND	ND	49	84	ND	ND
6	207	ND	79	245	ND	ND	223	122	ND	ND
7	52	ND	66	100	21	38	181	130	52	43
8	131	46	124	125	85	47	232	98	84	32

ND = not detected.

solvent.[4] Only 3.5%, 0.08%, and 1.06% of 3-hydroxy-carbofuran, 3-keto-carbofuran and carbofuran, respectively of total C^{14} in urine after 24 h were obtained as organo-extractable metabolites. After acid hydrolysis of the water-soluble conjugates less than 10% of the metabolites remained in the aqueous layer after chloroform extraction. 3-Keto-carbofuran-phenol accounted for over 50% of the total water-soluble C^{14} in the rat urine.

This explains the relatively small amounts of DMC in the methylene chloride extract of non-hydrolysed urine samples of the exposed workers. A simultaneous analysis of hydrolysed samples has not yet been performed because of the limited number of urine samples available and the interruption of carbofuran production, but it will be included in further investigation of occupational exposure.

The sampling time was far from optimum but the analysis of 24-h urine samples was not suitable for the routine control of occupationally exposed persons because of sampling difficulties. Therefore only samples taken immediately before and after work hours were analysed as it was recommended for organophosphorus residues.[14] Besides, the activities during leave or leisure hours were unknown and so were the rate of spontaneous reactivation and/or cholinesterase resynthesis.

Although the DMC determination includes only a smaller part of the excreted N-methylcarbamate it was possible to distinguish the causes of cholinesterase activity decrease by simultaneously determining

organophosphorus residues. The illustrative examples are workers Nos. 2, 4, 6 and 8 in whose urine samples DMC values exceeded the detection limit, i.e. over 100 ng/ml.

In the urine of worker No. 2 only small amounts of DMC and DETPK were detected on the first day. The initially decreased cholinesterase activity (blood 55%, plasma 75%) might be due to insufficient reactivation of the enzyme inhibited during previous exposure. A further decrease in activity on the third day (blood 41%, plasma 54%) was caused in the first place by carbamate absorption.

In worker No. 4 the initially decreased blood cholinesterase activity (34%) was accompanied by the presence of both DMC and DETPK in the urine sample on the first and on the second day. On the third day blood cholinesterase activity was increased (66%) and only DETPK was detected in urine.

The initial low cholinesterase activity in worker No. 6 (blood 58%, plasma 44%) was a consequence of absorption of both organophosphorus and carbamate pesticides. A further decrease in activity (blood 27%, plasma 21%) on the third day when no DMC was detected in urine, was obviously due to prolonged exposure to organophosphorus pesticides resulting in increased DETPK excretion.

Worker No. 8 is an example of depressed cholinesterase activity at the beginning of the experiment (blood and plasma 42%) which was caused by exposure to organophosphorus pesticides. On the second day a short increase in urinary DMC concentration and at the beginning of the third day the increased DETPK excretion were determined. No considerable changes were detected in plasma enzyme activity during these days and the activity in blood rose on the third day (71%). A further decrease in enzyme activity due to organophosphorus pesticide absorption could be expected later on.

The determination of urinary residue excretion indicates early and specifically the exposure to a particular group of agents hazardous to health and should be a stimulus for well-timed changes in further working regime, i.e. in more efficient measures of protection.

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