Mutagenecity of Nitroso Derivatives of N-Methylcarbamate Insecticides in Microbiological Method

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A great variety of pesticides has become detectable in our environment with their widespread usage, even though the actual concentration is quite low. Possible products which can be derived by the interaction of these pesticides with each other or with other contaminants such as nitrite in food additives must be considered as having an unfavorable environmental impact on human life.

The mutagenic actions of N-nitroso compounds are well-established. Recently the formation of a potent mutagenic compound, nitrosocarbaryl, by the interaction of N-methylcarbamate insecticide carbaryl with sodium nitrite in acidic media was reported (ELESPURU et al. 1973, 1974, SIEBERT and EISENBRAND 1974), which should be noted as a new parameter in the toxicological evaluation of carbamates.

Among the many pesticides being used today N-methylcarbamates are holding a special position, since they serve as possible precursors of nitroso compounds in the physiological conditions.

In the present paper, eight kinds of N-methylcarbamate insecticides were chemically converted to corresponding N-nitroso compounds. The mutagenic activities of these were tested by means of two microbiological assay systems including the "rec-assay" procedure (KADA et al. 1972). All of the N-nitroso-N-methylcarbamates employed here exhibited a fairly strong mutagenesity. The relationship between chemical structure and mutagenic activity was partly clarified.

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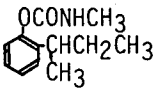
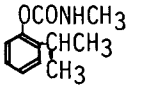
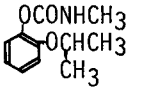
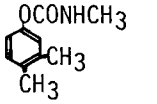
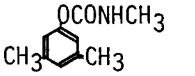
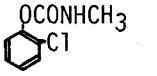
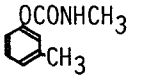
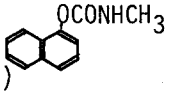
EXPERIMENTAL

Nitroso derivatives of N-methylcarbamates: Eight kinds of N-methylcarbamates listed in Table I were employed. These are gifts from Society of Agricultural Chemical Industry, Nihonbashi, Tokyo.

TABLE I

Carbamate Insecticides Investigated

Registered Abbreviation	Chemical Structure	Systematic Name	Trade Name*
BPMC		2-sec-Butylphenyl N-methylcarbamate	Bassa
MIPC		2-Isopropylphenyl N-methylcarbamate	Mipsin
PHC		2-Isopropoxyphenyl N-methylcarbamate	Suncide
MPMC		3,4-Xylyl N-methylcarbamate	Meobal
XMC		3,5-Xylyl N-methylcarbamate	Maqbarl
CPMC		2-Chlorophenyl N-methylcarbamate	Hopcide
MTMC		3-Tolyl N-methylcarbamate	Tsumacide
NAC (carbaryl)		1-Naphthyl N-methylcarbamate	Denapon

*Trade name in Japan

Each N-methylcarbamates dissolved in glacial acetic acid was nitrosated with $NaNO_2$ at a molar ratio of 1:2. Aqueous $NaNO_2$ solution was added dropwise over one hour with stirring at 0°. Reaction mixture was kept standing for an additional one hour at 0° and thereafter at room temperature overnight. The mixture was then poured into water and extracted with ether. The extract was washed with H_2O, saturated $NaHCO_3$ solution and again with H_2O. The ether layer was dried over Na_2SO_4 and freed from the solvent under reduced pressure to remain raw crystal or brown oil, which was redissolved in a small amount of benzene and subsequently subjected to column chromatography on silica gel.

The first fraction eluted with benzene was collected and freed from solvent to obtain a pale yellow oil except N-nitrosocarbaryl which was recrystallized from ether. They are all positive to Gries reagent. Each product was confirmed to be sufficiently pure without any further purification and identified as the anticipated nitroso compound by elementary analyses, IR and NMR spectra. The yields were around 30 %. The physico-chemical data obtained from each nitroso derivative were shown in Table II.

TABLE II

Properties of Nitrosocarbamates

Compd.	Formula	m.p. °C	Analysis(%) Calcd. (Found) C	H	N	Cl	IR*2 C=O cm^{-1}	NMR*4 NCH_3 (3H,s) ppm
NO-BPMC	$C_{12}H_{16}O_3N_2$	-*1	61.00 (61.81)	6.83 (6.83)	11.86 (11.54)		1763	3.27
NO-MIPC	$C_{11}H_{14}O_3N_2$	-	59.45 (59.23)	6.35 (6.19)	12.60 (12.59)		1765	3.20
NO-PHC	$C_{11}H_{14}O_4N_2$	-	55.45 (55.70)	5.92 (5.90)	11.76 (11.71)		1769	3.30
NO-MPMC	$C_{10}H_{12}O_3N_2$	-	57.68 (57.58)	5.81 (5.76)	13.46 (13.41)		1762	3.24
NO-XMC	$C_{10}H_{12}O_3N_2$	-	57.68 (57.90)	5.81 (5.80)	13.46 (13.37)		1763	3.25
NO-CPMC	$C_8H_7O_3N_2Cl$	-	44.76 (45.12)	3.29 (3.28)	13.05 (12.63)	16.52 (16.15)	1767	3.25
NO-MTMC	$C_9H_{10}O_3N_2$	-	55.66 (56.20)	5.19 (5.23)	14.43 (14.43)		1762	3.26
NO-NAC	$C_{12}H_{12}O_3N_2$	68-69	62.60 (62.70)	4.38 (4.31)	12.17 (12.23)		1744*3	3.22

*1: oily material
*2: liquid film
*3: KBr tablet
*4: $CDCl_3$, TMS as an internal standard

Assay for mutagenesity 1) Back mutation method: An auxotrophic mutant of *Escherichia coli* B/r WP-2 try⁻ was employed. Cells grown overnight at 37° in the medium containing glucose, mineral salts and 10 μg/ml of L-tryptophan were plated on agar plate containing glucose, salts and 1 μg/ml of L-tryptophan. Approximately 4×10^8 cells were added in one plate and reverted mutants to tryptophan independence were scored after incubation at 37° for 48 hours. Mutagenic activity was determined by adding the varied amount of nitrosocarbamates dissolved in DMSO into the hole in the center of seeded agar lawn. Significant increase of appearance of tryptophan independent mutant was recorded as plus in Fig. 1.

2) Rec-assay method: In general, DNA-damage provoking agents are mutagenic. Utilizing the wild and DNA-recombination-lacking cell strains, the damage on DNA was assayed according to the method devised by KADA et al. (1972). *Baccillus subtilis* Marburg 17A of recombination-capable strain and *Bacillus subtilis* Marburg 45T of recombination-lacking strain having the highest sensitivity to frameshift mutagens were employed. This repair test was performed on Difco nutrient agar supplemented with 3 % of yeast extract for 18 hours at 37°. The result showing at least 3 mm inhibition zone in 45T in contrast to zero mm in 17A at any concentration tested was judged as plus in Fig. 2.

RESULTS AND DISCUSSION

N-Methylcarbamate insecticides employed in this work are used regularly today in Japan. Systematic, common and trade names as well as the chemical structure are indicated in Table I. Results in Figs. 1 and 2 were expressed in terms of plus or minus for the various amounts of nitroso-carbamates added in each plate. Original, non-nitrosated, carbamates did not show any positive results in either method, even in the highest concentration of 10 mg/plate.

The order of mutagenic potency could be alterable depending on the difference of methods or cell strains. In the inactivation of transforming DNA using *Haemophilus influenzae*, ELESPURU et al. (1974) reported that N-nitroso-carbaryl is fifteen times more effective than MNNG. In the present experiments, nitroso-CPMC and nitroso-MTMC could induce back mutation at much less concentration than nitrosocarbaryl and revealed approximately the same effect with MNNG, though no attempt was made to distinguish between two types of back mutation to prototrophy either by true reverse mutations at the tryptophan locus or by the induction of ochre(UAA) suppressors (BRIDGES et al. 1968).

Compound*1	Amount of nitrosocarbamate added (μg/plate) 100	50	10	5	1	0.5	0.1	0.05
NO-CPMC	+	+	+	+	+	+	-	
NO-MTMC	+	+	+	+	+	-		
NO-XMC	+	+	+	+	-			
NO-NAC	+	+	+	+	-			
NO-MPMC	+	+	+	-				
NO-PHC	+	+	-					
NO-MIPC	+	+	-					
NO-BPMC	+	+	-					
MNNG*2	+	+	+	+	+	+	-	

Fig. 1. Assay of Mutagenesity of N-Nitrosated Carbamate Insecticides in *Escherichia coli* B/r WP-2 try⁻

*1: Chemical structures were indicated in Table I.
*2: N-methyl N'-nitro-N-nitrosoguanidine

Compound*1	Amount of nitrosocarbamate added (μg/plate) 100	50	10	5	1	0.5	0.1	0.05	0.01	0.005	0.001
NO-BPMC	+	+	+	+	+	+	+	+	+	+	-
NO-MIPC	+	+	+	+	+	+	+	+	+	-	
NO-PHC	+	+	+	+	+	+	+	+	-		
NO-MPMC	+	+	+	+	+	+	-				
NO-XMC	+	+	+	+	+	+	-				
NO-CPMC	+	+	+	+	+	+	-				
NO-MTMC	+	+	+	+	+	-					
NO-NAC	+	+	+	+	-						
MNNG*2	+	+	-								
MMC*3	+	+	+	+	+	+	-				

Fig. 2. Activity of N-Nitrosated Carbamate Insecticides in "Rec-assay" Utilizing *Bacillus subtilis* Marburg 45T and 17A

*1: Chemical structures were indicated in Table I.
*2: N-methyl N'-nitro-N-nitrosoguanidine
*3: Mitomycin C

On the contrary, the rec-assay method depicted the extremely high potencies of the nitrosocarbamates. All of eight nitrosocarbamates exhibited far stronger DNA-damaging than MNNG and most of them were comparable to or more potent than mitomycin C.

It is quite interesting that nitroso-BPMC and nitroso-MIPC were the most effective compounds when tested by rec-assay, whereas these two induced minimum mutation in *E. coli* system. Further, the reverse response was found that nitroso derivatives of CPMC and MTMC which were the most potent in *E. coli* system showed a relatively mild effect on the rec-assay system.

The chemical structure characteristic of those derivatives showing extraordinally high response to rec-assay is rather massive branched side chain of ortho-position of phenylcarbamates, while simple and mono-substituted methyl and chloro derivatives brought the greater induction of back mutation. N-Nitroso derivatives derived from dimethyl substituted MPMC and XMC occupied the intermediate place in both assay methods. The reason for such structure-activity relationship remains obscure.

It must be noted that all of these N-methylcarbamates which are generally accepted as "safe" pesticides could be converted to nitroso compounds and the nitroso derivatives are all potent mutagens in any case. Since carbamates have been widely used and nitrite is a common constituent of human saliva (SANDER and SCHWEINSBERG 1972), the stomach can provide the acidic condition preferable for nitrosation. However, at present, carbamate insecticides should not produce any significant amount of nitroso derivatives at the levels which are normally in the environment. Nevertheless, the possible hazard to man still remains when occupational exposure is taken into consideration.

REFERENCES

BRIDGES,B.A.,J.LAW and R.J.MUNSON: Molec. Gen. Genetics 103, 266 (1968).

ELESPURU,R.K. and W.LIJINSKY: Food Cosmet. Toxicol. 11, 807 (1973).

ELESPURU,R.,W.LIJINSKY and J.K.SETLOW: Nature 247, 386 (1974).

KADA,T.,K.TUTIKAWA and Y.SADAIE: Mutation Res. 16, 165 (1972).

SANDER,J. and F.SCHWEINSBERG: Zbl. Bakt. Hyg. 156, 299 (1972).

SIEBERT,D. and G.EISENBRAND: Mutation Res. 22, 121 (1974).