Studies on *N*-Nitroso Derivatives of *N*-Methylcarbamate Insecticides (III)

Mutagenicity of *N*-Nitroso Derivatives of 3-Methylphenyl *N*-Methylcarbamate, 3,4-Dimethylphenyl *N*-Methylcarbamate and Naphthyl *N*-Methylcarbamate for *Salmonella typhimurium*

(Received September 19, 1977)

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N-Methylcarbamate insecticides, 3-methylphenyl *N*-methylcarbamate, 3,4-dimethylphenyl *N*-methylcarbamate and naphthyl *N*-methylcarbamate, and their *N*-nitroso derivatives were tested for mutagenic activity to *Salmonella typhimurium* TA 98 and TA 100 in the presence and absence of metabolic activation system (rat liver microsomal preparation, "S-9 mix"). The nitroso derivatives were mutagenic for strain TA 100 without S-9 mix but not for strain TA 98, indicating that the nitroso derivatives were potent base-change mutagens. The mutagenic activity of nitroso derivatives for strain TA 100 was found to disappear in the presence of S-9 mix. Neither strain TA 100 nor strain TA 98 responded to *N*-methylcarbamate insecticides with or without S-9 mix.

1. Introduction

Studies on the reaction of *N*-methylcarbamate insecticides with nitrite *in vitro* have suggested that a part of the carbamate insecticides ingested might be converted to *N*-nitroso derivatives in a human stomach.^{1),2)}

Eisenbrand *et al.*³⁾ reported the carcinogenicity of naphthyl *N*-methyl-*N*-nitrosocarbamate (NO-NAC) derived from naphthyl *N*-methylcarbamate (NAC). Uchiyama *et al.*⁴⁾ investigated the mutagenicity of the nitrosated *N*-methylcarbamate insecticides on the repair test using *Bacillus subtilis* and on the mutation test employing *Escherichia coli* B/r WP2 *try*⁻Hcr⁺. The mutagenicity of NO-NAC were also investigated in various systems.⁵⁾⁻⁸⁾ Recent works on the mutagenicity of chemical carcinogens have demonstrated a close relationship between carcinogenicity and mutagenicity.^{9),10)} The mutation test developed by Ames *et al.* is widely used as a screening test for possible carcinogens.⁹⁾

In the present paper, the mutagenic activity

of 3-methylphenyl *N*-methyl-*N*-nitrosocarbamate (NO-MTMC), 3, 4-dimethylphenyl *N*-methyl-*N*-nitrosocarbamate (NO-MPMC) and NO-NAC was tested by the method of Ames *et al.*⁹⁾ using *Salmonella typhimurium* TA 100 and TA 98 in the presence and absence of the metabolic activation system (S-9 mix). The mutation was detected by determining the reversion from *his*⁻ to *His*⁺.

2. Materials and Methods

2.1 Chemicals

3-Methylphenyl *N*-methylcarbamate (MTMC), 3, 4-dimethylphenyl *N*-methylcarbamate (MPMC), NAC, NO-MTMC, NO-MPMC and NO-NAC were prepared in the same manner as described in the previous paper.¹¹⁾ Nitroso derivatives were stored at -20°C.

2.2 Mutation test

The strains TA 100 and TA 98 of *S. typhimurium* were kindly supplied by Prof. Ames (University of California, Berkeley, Calif.). The mutation assay was carried out as described by Ames *et al.*⁹⁾

Table 1. Composition of S-9 Mix

Component	Quantity per 0.5 ml
S-9 Fraction	150 μ l
NADPH	2.3 μ mol
Glucose-6-phosphate	2.3 μ mol
MgCl ₂	4.0 μ mol
KCl	16.5 μ mol
Sodium phosphate buffer (pH 7.4)	50 μ mol

with some modifications.¹²²⁻¹⁴²

For metabolic activation, "S-9 mix was prepared with "S-9 fraction" and other components (Table 1). The S-9 fraction was the supernatant obtained from the homogenate of PCB treated rat liver (Wistar rat, male) by the centrifugation at 9000 g for 20 min.

The compounds tested were freshly dissolved in dimethyl sulfoxide at the desired concentrations. To a tube containing 0.1 ml of solution of test

Table 2. Summarized Data on the Mutagenicity of *N*-Methylcarbamate Insecticides and Their *N*-Nitroso Derivatives for *S. typhimurium*

Compound	Chemical structure	Mutagenicity			
		TA 98		TA 100	
		with S-9 mix	without S-9 mix	with S-9 mix	without S-9 mix
NO-MPMC		—	—	—	+
NO-MTMC		—	—	—	+
NO-NAC		—	—	—	+
MPMC		—	—	—	—
MTMC		—	—	—	—
NAC		—	—	—	—

When the number of revertants (His⁺) was markedly observed more than 50 in TA 98, and more than 200 in TA 100, the mutagenic activity of these compounds was judged as positive (+).

compound was added 0.5 ml of S-9 mix and then 0.1 ml of the culture of bacterial tester strain. This mixture was pre-incubated at 37°C for 20 min, and mixed with 2 ml of melted top agar at 45°C containing 0.1 μ mol of biotin and 0.1 μ mol

of histidine (trace amounts of histidine are necessary for mutagenesis to occur). The mixture was poured onto a minimal-glucose agar plate.¹⁰⁾ In tests on the mutagenicity of compounds without metabolic activation, 0.5 ml of 100 mM sodium

Fig. 1. Effects of the concentration of *N*-nitrosated carbamate insecticides on mutagenicity for *S. typhimurium* TA 98 and TA 100
 ●—●, with S-9 mix; ○—○, without S-9 mix. The ordinate shows total number of His⁺ revertants per plate and the abscissa shows μ moles of compounds added per plate.

phosphate buffer (pH 7.4) was added instead of S-9 mix. After the incubation at 37°C for 2 days, the revertant colonies were counted.

3. Results and Discussion

The mutagenicity of NO-MTMC, NO-MPMC, NO-NAC, MTMC, MPMC and NAC was tested on TA 98 and TA 100. The results of mutation tests are shown in Table 2. Figure 1 shows the relations between the numbers of revertant colonies per plate and the concentrations of *N*-nitrosated carbamate insecticides. The nitrosocarbamates were found to be potent mutagens.

The nitrosocarbamates were mutagenic for *S. typhimurium* TA 100 but not for TA 98, indicating that the mutations were due to base-pair substitutions.⁹⁾ The mutagenicity was found to be in the following order of effectiveness, NO-MPMC < NO-NAC < NO-MTMC (at 5 μ mol/plate of doses).

The nitrosocarbamates showed mutagenic activity in the absence of metabolic activation system. This confirmed the view that unlike nitrosamines, most nitrosamides did not require enzymic activation to convert them to alkylating agents, which were the presumed active mutagens.¹⁰⁾

The mutagenic activity of nitrosocarbamates for TA 100 disappeared with S-9 mix. This suggested that the *N*-nitrosated carbamate insecticides could be changed metabolically to non-mutagens, though the possibility that the mutagens might be inactivated by other causes, such as the adsorption of mutagens to protein in S-9 mix, could not be ignored.

The *N*-methylcarbamate insecticides were non-mutagenic even at a high dose (5000 μ mol/plate) in the presence and absence of metabolic activation system, though it was reported that carbamate insecticides as well as their nitroso derivatives gave positive results in chromosomal aberration

tests on Chinese hamster cells *in vitro*.¹⁷⁾

This work was supported by Cancer Research Fund from the Ministry of Health and Welfare (1976) and Cancer Research Fund from the Ministry of Education, Science and Culture (1976).

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