Acta Biochimica Polonica
Vol. 40 No. 1 1993

The effect of 4,4-dimethylmorpholine chloride (Stymulen) on the Ames tester strains of *Salmonella typhimurium*

Małgorzata Kobierska-Szeliga, Małgorzata Bilbin and Hanna Czeczot

Department of Biochemistry, Institute of Biopharmacy, Medical School, S. Banacha 1, 02-097 Warsaw, Poland

4,4-dimethylmorpholine chloride (Stymulen; Instytut Przemysłu Organicznego, Warszawa, Poland) a pesticide of the dimethylmorpholine class, is widely used in agriculture, growth regulator of grain. Thus, man may be exposed to Stymulen both through direct occupational contact and by consuming food containing residues of that pesticide. The data on genetic properties of Stymulen are very fragmentary.

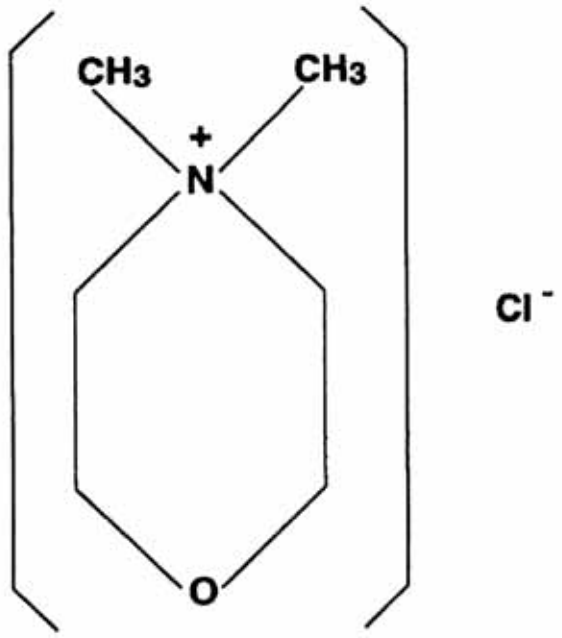

Fig. 1. Chemical structure of 4,4-dimethylmorpholine chloride (Stymulen)

Many pesticides have been evaluated for possible mutagenicity. Since most of them are also carcinogens it seemed important to determine the ability of Stymulen to induce mutations in the *Salmonella* test system of Ames.

Mutagenicity of Stymulen was tested using *S. typhimurium* TA1535, TA1538, TA97, TA98, TA100, TA102 and TA104 according to Ames *et al.* [1]. Stymulen was tested in the non-toxic concentrations ranging from 1 to 500 μg/plate, with and without the liver microsomal fraction S9 prepared from the rats treated with Aroclor 1254 according to Ames [1].

The average concentration of protein in the S9 fraction, determined according to Lowry *et al.* [2] was 38 mg/ml (36 - 42 mg/ml). Positive mutagenesis controls with 4-NQO – (TA97, TA98 without S9 metabolic activation), MMS (TA100 without metabolic activation), MMC–mitomycin C (TA102 without metabolic activation) and 2-aminofluorene (TA97, TA98, TA100 with S9 metabolic activation) were also performed. Mutagenicity assays were carried out in triplicate; the plates were incubated for 72 h at 37 °C, and the number of colonies (reverse mutations his^- to his^+ form) were counted.

The effect of Stymulen on the number of revertants of *S. typhimurium* strains without (–S9) and with (+S9) rat liver microsomal fraction is shown in Table 1.

[1] Abbreviations: 2-AF, 2-aminofluorene; MMC, mitomycin C; MMS, methyl methanesulfonate; 4-NQO, 4-nitroquinoline-*N*-oxide

Table 1

The effect of Stymulen in the Salmonella tester strains TA1535, TA1538, TA97, TA98, TA100, TA102, TA104 without and with metabolic activation

Stymulen µg/plate	Presence of S9 mix [b]	Number of revertants *his*$^+$ per plate ± S.D. [a]						
		TA 1535	TA 1538	TA 97	TA 98	TA 100	TA 102	TA 104
0 control	–	12 ± 6	21 ± 5	140 ± 44	27 ± 9	161 ± 46	454 ± 53	545 ± 92
1	–	12 ± 7	25 ± 4	167 ± 80	24 ± 9	165 ± 40	505± 108	451 ± 51
10	–	12 ± 8	18 ± 4	122 ± 47	27 ± 12	152 ± 31	466 ± 67	583 ± 207
100	–	10 ± 3	15 ± 4	133 ± 40	19 ± 7	155 ± 43	372 ± 111	600 ± 191
500	–	14 ± 4	16 ± 7	95 ± 30	24 ± 7	145 ± 35	414 ± 101	509 ± 129
positive control [c]	–	NT	NT	920 ± 65	480 ± 50	2450 ± 120	6455 ± 130	NT
0 control	+	11 ± 5	53 ± 23	114 ± 46	49 ± 24	189 ± 37	591 ± 63	615 ± 136
1	+	13 ± 6	61 ± 29	112 ± 33	56 ± 13	180 ± 16	564 ± 80	467 ± 168
10	+	11 ± 8	56 ± 22	123 ± 33	56 ± 23	172 ± 35	566 ± 101	541 ± 172
100	+	13 ± 8	62 ± 21	148 ± 36	54 ± 16	204 ± 49	457 ± 69	591 ± 198
500	+	13 ± 7	67 ± 12	106 ± 40	64 ± 21	193 ± 21	546 ± 48	499 ± 121
positive control [c]	+	NT	NT	1520 ± 80	5380 ± 72	2970 ± 150	NT	NT

[a] Average of 15 plates

[b] S9 mix from Aroclor 1254 pretreated rat liver (50 µl/ml mix)

[c] positive controls:

–, without S9 metabolic activation: 4-NQO 10 µg/plate for TA97, TA98; MMS 1 µl/plate for TA100; MMC 0.5 µg/plate for TA102

+, with metabolic activation: 2-AF (2-aminofluorene) 10 µg/plate for TA97, TA98, TA100; NT, not tested

Stymulen and its metabolites found by the microsomal S9 fraction showed non mutagenic activity in the tester strains of *S. typhimurium*. These results do not exclude the possibility of mutagenicity of Stymulen in other test *in vitro* and *in vivo* used for genotoxicity screening.

REFERENCES

1. Ames, B.N., Mc Cann, J. & Yamasaki, E. (1975) *Mutation. Res.* **129,** 299 - 310.
2. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) *J. Biol. Chem.* **193,** 265 - 275.