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Evaluation of the Mutagenic Effect of N,N-Diethylhydroxylamine in Salmonella Typhimurium TA 100

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Abstract

A recent approach suggested to suppress photosmog formation has been the addition of small quantities of N,N-diethylhydroxylamine (DEHA) to the polluted air. Thus knowledge of possible mutagenic properties of the compound became important. DEHA was investigated using the Ames Salmonella/microsome mutagenicity test. Toxicity as well as a mutagenic effect were observed at concentrations much exceeding those proposed for practical application.

It is well known that photochemical smog arises via the long chain free radical oxidation of NO to NO_2 in hydrocarbon containing atmospheres[1]. Thus one recent approach suggested for alleviating smog formation has been the addition of small quantities of free radical scavengers ("photosmog inhibitors") locally to the polluted air. Of the substances tested, mainly aromatic compounds and ammonia derivatives[2,3], N,N-diethylhydroxylamine (DEHA) has proven sufficiently active to be selected for field trials[4]. In this context the effect of DEHA exposure on living organisms is obviously of interest. Massie and Williams[5] have recently reported an insignificant change in life-span of fruit flies after exposure to DEHA at concentrations of up to 89 ppm. In judging the safety of environmental chemicals, however, mutagenic testing is of great importance. We therefore

undertook the investigations described below employing the Salmonella/microsome test.

Materials and Methods

The DEHA (EGA-Chemie, Steinheim/West-Germany) employed in these studies was of nominal 97 % purity and was tested in both original and purified form. The purification procedure consisted of a double vacuum distillation (4 torr): Three cuts were taken in the first distillation (32 ± 1^{o}, 34 ± 1^{o}, 36 ± 1^{o}) and the middle, sharply boiling cut of each of these from the second distillation used for the tests described here. Original DEHA after two weeks air oxidation was also assayed.

The mutagenic activity of the compound was assayed according to the standard method of Ames et al.[6] with the histidine requiring strain Salmonella typhimurium TA 100. This strain is sensitive to mutagens causing base-pair substitutions. The bacterial suspensions were incubated with shaking overnight before adding to the top agar. At the lower concentrations ($\leq$ 487 µmoles/plate)the reagent was dissolved in dimethylsulfoxide, while at higher concentrations it was added directly to the top agar. Based on the recommendations of de Serres and Shelby[7] concentrations in the toxic dose range were also examined. Liver microsomal fraction (S-9) was prepared from male Sprague-Dawley rats which had been injected with Aroclor 1254 five days prior to sacrifice. The experiments were repeated at least three times with and without rat liver preparation. To confirm both the reversion properties of strain TA 100 and the activity of S-9 mix, 2-aminofluorene was spot tested. The revertant colonies were screened for reversion by subculturing on biotin-supplemented minimal glucose agar without histidine.

Results and Discussion

Hydroxylamine exhibits a mutagenic effect on bacteria and in transforming DNA. The induction of chromosome aberrations in human chromosomes, Chinese hamster cells, mouse embryo cells and Vicia faba has also been described[8]. In the fluctuation test with strain his G 46 of Salmonella typhimurium a significant increase in the number of turbid tubes was observed after treatment with hydroxylamine[9]. Ames[10] described hydroxylamine as a mutagen. In a more recent publication, however, the substance was classified as non-mutagenic[11]. The difference in the results may be

caused by the use of different concentrations in the two sets of experiments. Investigations with E. coli demonstrated an increased mutagenic effect of methylhydroxylamine compared with unsubstituted hydroxylamine[12].

The data shown in Table 1 indicate that in the concentration range 0.0974 - 9.74 µmoles DEHA/plate the substance has neither a bactericidal nor a mutagenic effect. At higher concentrations the number of revertant colonies per plate increased with increasing dose. In the same concentration range decreasing survival was also observed. At concentrations exceeding 1948 µmoles/plate the toxic effect of DEHA prevented the growth of reverted cells to visible colonies. The addition of S-9 mix to the incubation system was without influence on the number of revertants indicating that no metabolic activation was involved.

Quantitatively identical results were obtained from both purified and air oxidised samples of DEHA, indicating the absence of impurity effects.

Our results demonstrate that DEHA in the concentrations proposed ($\leq$ 2 ppm) did not induce reversion in Salmonella typhimurium strain TA 100. At concentrations in the toxic range the compound was mutagenic. To exclude potential genetic hazards associated with the use of DEHA, further investigations should be carried out to test the biological effects of DEHA and especially its reaction products in the biosphere.

Table 1: Influence of DEHA on reversion of strain Salmonella typhimurium TA 100 +)

concn. (µmoles/plate)	% survival	no. of revertants per plate
0.0	100	98
0.0974	100	95
0.974	100	112
9.74	100	107
97.4	80	151
487	55	231
974	37	340
1461	17	443
1948	0.25	554

+) each value represents the mean of at least six plates

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