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# PRELIMINARY COMMUNICATIONS

## MUTAGENICITY OF 8-HYDROXYQUINOLINE AND RELATED COMPOUNDS IN THE SALMONELLA TYPHIMURIUM BIOASSAY

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8-Hydroxyquinoline, a widely used fungicidal and bactericidal agent, is weakly carcinogenic in some animal studies [1-4], but negative results have been found by others [5-8]. The contradictory opinions on the carcinogenicity of 8-hydroxyquinoline in animals may result from differences in experimental methodology and/or from the possibility that 8-hydroxyquinoline requires metabolic activation. Recently, Ames et al. [9,10] have developed Salmonella typhimurium tester strains which are highly efficient in detecting chemical mutagens and carcinogens which act as mutagens, including those activated by liver mixed-function oxidases. To date, the Ames system has demonstrated, for a variety of chemicals, a strong correlation between mutagenicity and carcinogenicity [11]. In this study, we have used the Ames bioassay to evaluate the mutagenic properties of hydroxyquinolines and quinoline and the associated requirements for metabolic activation. The results show that, in the presence of $NADP^+$ and Aroclor 1254-induced rat liver 9000 g supernatant, 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline are mutagenic in S. typhimurium TA 100.

The monohydroxyquinolines were obtained from commercial sources. Purity of the chemicals was checked by thin-layer chromatography (TLC): each compound (200 μg) was spotted on a Merck Silica gel 60 plate and developed in a closed chamber containing chloroform-acetic acid-methanol-water (65:20:10:5). Fluorescent spots were detected by ultraviolet light and, when trace amounts of impurities were noted, test compounds were purified by preparative TLC or by recrystallization. Quinoline, kindly provided by the University of California Department of Chemistry, was distilled twice before testing.

The assay for mutagenic activity is based on the ability of chemicals to revert mutant strains of S. typhimurium from a histidine requirement back to prototrophy. The methods used in this investigation are described in detail by Ames et al. [9,10] and were followed essentially without modification. Rat liver 9000 g supernatant fraction (S-9) was prepared from Aroclor 1254-induced animals [10]. Each compound, dissolved in dimethylsulfoxide, was tested on four bacterial strains, TA 98, TA 100, TA 1535 and TA 1537, at three doses (20 μg, 100 μg and 1 mg/plate), with and without the addition of 0.2 ml of S-9 to the incubation mix.

Of the compounds tested with liver S-9 in the co-factor mix, only 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline were mutagenic to S. typhimurium TA 100 (Table 1). The activity observed with quinoline is in agreement with a recent report by Ito [12]. The mutagenic effects of 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline were dose

Table 1. Mutagenicity of monohydroxyquinolines in the S. typhimurium bioassay*

| Compound | Dose/ plate (µg) | Bacterial strain and revertants/plate | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | TA 100 | | TA 98 | | TA 1535 | | TA 1537 | |
| | | (-) S-9 | (+) S-9 | (-) S-9 | (+) S-9 | (-) S-9 | (+) S-9 | (-) S-9 | (+) S-9 |
| 2-OH-quinoline | 100 | 114 | 128 | 10 | 48 | 12 | 11 | 5 | 12 |
| 4-OH-quinoline | 100 | 113 | 135 | 9 | 17 | 25 | 21 | 2 | 7 |
| 5-OH-quinoline | 100 | 111 | 439 | 8 | 49 | 8 | 14 | 8 | 17 |
| 6-OH-quinoline | 100 | 115 | 167 | 13 | 30 | 13 | 23 | 23 | 12 |
| 7-OH-quinoline | 100 | 130 | 135 | 18 | 32 | 10 | 6 | 10 | 9 |
| 8-OH-quinoline | 100 | 68 | 403 | 20 | 78 | 0 | 0 | 0 | 0 |
| Quinoline | 100 | 106 | 762 | 18 | 59 | 8 | 11 | 0 | 0 |

*The compounds listed above were tested for mutagenic activity in the four tester strains by the methods of Ames et al. [9,10]. Each compound was tested in the presence (+ S-9) and absence (- S-9) of Aroclor 1254-induced rat liver 9000 g supernatant [10]. Spontaneous reversion rates for TA 100 were 100-150 colonies/plate. Reversion rates for the other strains were 10-25 colonies/plate.

Fig. 1 Dose-response relationships of 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline to mutagenic activity. Squares = 8-hydroxyquinoline, triangles = 5-hydroxyquinoline, and circles = quinoline.

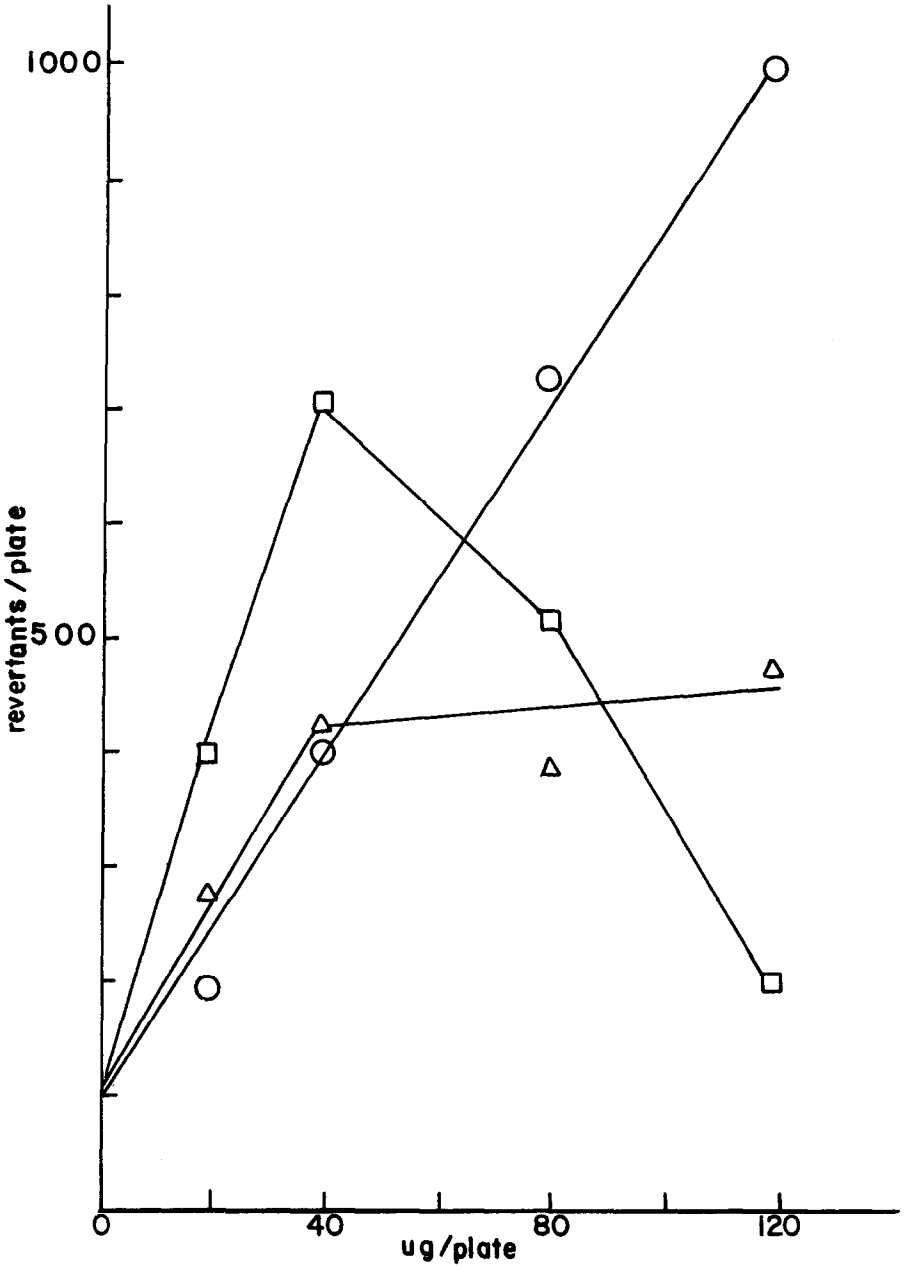

Table 2. Factors affecting the mutagenic activity of 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline*

| Compound, dose/plate | TA 100 (revertants/plate) | | | |
|---|---|---|---|---|
| | Complete medium, Aroclor S-9 | Complete medium, control S-9 | Complete (-) $NADP^+$ | Complete (+) GSH (2 mM) |
| 8-OH-quinoline (50 μg) | 507 | 145 | 87 | 116 |
| 5-OH-quinoline (100 μg) | 437 | 195 | 143 | 133 |
| Quinoline (100 μg) | 960 | 157 | 104 | 246 |

*The compounds listed above were tested for mutagenic activity toward S. typhimurium TA 100 [9,10]. The spontaneous reversion rate was approximately 150 revertants/plate. GSH = reduced glutathione.

dependent at low doses, but 8-hydroxyquinoline became toxic to the tester strain at doses above 40 µg/plate (Fig. 1). From the linear portion of the dose-effect relationship, the mutagenic specific activities of 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline were calculated to be 1-2 revertants/nmole.

The mutagenic activities of 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline required the presence of Aroclor 1254-induced liver S-9 and were expressed principally in strain TA 100. Although several compounds appeared slightly positive in TA 98 (Table 1), the number of revertant colonies was low and variable, and no clear dose-response relationships were obtained. The presence of a hydroxyl group on C-2, C-4, C-6 or C-7 of quinoline precluded activation, as these compounds were not mutagenic in TA 100. None of the compounds at the doses tested were mutagenic in tester strains TA 1535 and TA 1537.

The factors governing the activation of 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline were studied. When S-9 from untreated rats was substituted from Aroclor 1254 S-9 or when $NADP^+$ was omitted from the incubation mixture, the quinoline compounds were no longer mutagenic (Table 2). Reduced glutathione (2 mM), in the presence of a complete incubation mix, could also block the mutagenic actions of these compounds. These results are consistent with an activation mechanism involving $P_1$-450-dependent epoxidation of the quinoline ring [13,14]; however, further studies with different inducers and inhibitors are needed to explore this possibility.

These results demonstrate that 8-hydroxyquinoline and related compounds, under certain conditions of metabolic activation, are mutagenic to strain TA 100 of the Ames bioassay. The mutagenic potency of 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline can be compared to the potency of 300 chemicals tabulated by McCann *et al.* [11] in the Ames system; the activities, 1-2 revertants/nmole (Fig. 1), place the quinoline compounds at 4 orders of magnitude below aflatoxin, 2 orders of magnitude below benzo(a)pyrene, and at the same order of magnitude as benzidine. Thus, it appears that 8-hydroxyquinoline, 5-hydroxyquinoline and quinoline are mutagens of low to medium potency. Further studies are required to determine if 8-hydroxyquinoline, a widely used chemical, can, under the appropriate conditions, cause mutations in cells of higher species.

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