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Conversion of tris(8-quinolinolato-N1, O8) aluminum to 8-hydroxyquinoline and activity in bacterial reverse mutation assays

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Abstract

Tris(8-quinolinolato-N1, O8) aluminum (AlQ), an aluminum chelate of 8-hydroxyquinoline (8OHQ) is an important charge transfer molecule in semiconducting imaging devices. This study was conducted to evaluate AlQ and 8OHQ for the ability to induce reverse mutations, either in the presence or absence of mammalian microsomal enzymes, and to determine if AlQ decomposes or is metabolized to 8OHQ under assay conditions. The tester strains used in the mutation assay were *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2*uvr*A (pKM101). The assays were conducted in the presence and absence of S9. AlQ doses were 1–1000 μg per plate while 8OHQ doses were 0.947–947 μg per plate to maintain molar equivalency. Stability studies were carried out for 4 h at 37 °C under conditions designed to mimic mutation assays. Samples were analyzed by HPLC and LC/MS to tentatively identify potential metabolites of AlQ and 8OHQ. The results of the bacterial mutagenicity assay indicate that in the presence of S9, both AlQ and 8OHQ, caused increases in the mean number of revertants per plate with tester strains TA100 and WP2*uvr*A (pKM101). No increases were observed with any of the remaining tester strain/activation condition combinations. The stability study showed that AlQ degrades readily to 8OHQ under standard mutagenicity test conditions, and the positive test result with AlQ is due to the bioactivation of 8OHQ. In the presence of S9, 8OHQ is metabolized to one detectable product with molecular weight indicative of a one-oxygen insertion. 8OHQ N-oxide and 2,8-quinolinediol were ruled out as possible metabolites; 8OHQ epoxides and other quinolinediols were neither confirmed nor ruled out. Bacterial mutagenicity tests have not been shown to predict in vivo effects of 8OHQ; these assays are similarly expected to be poorly predictive of in vivo genotoxic and carcinogenic potential of AlQ.

Keywords: Tris(8-quinolinolato-N1; O8) aluminum to 8-hydroxyquinoline; Ames assay; In vitro stability; Metabolism; Mutation; *E. coli*

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1. Introduction

Organic light emitting diodes (OLEDs) are under active development for use in flat-panel image displays having potential application in a variety of consumer electronics devices. This technology uses the semiconducting and luminescent properties of certain π-conjugated organic compounds to create sharp, full-color, full-motion display images. The basic OLED cell structure consists of a stack of thin organic layers that constitute the charge transport and emitting layers. These layers are sandwiched between a metallic cathode and a transparent anode, through which light is emitted in response to the application of an appropriate voltage.

Tris(8-quinolinolato-N1, O8) aluminum (AlQ) is an electroluminescent molecule commonly used for the electron transport layer in OLED cells. This and four other charge transport materials were tested for mutagenicity, as part of a larger program to assess potential health effects of the materials used in OLED cells. Of the five materials tested, only AlQ was reported to produce a positive response in the bacterial reverse mutation assay with *Salmonella typhimurium* TA100 (unpublished data). Analysis for concentration, homogeneity and stability of the test material formulations, however, was not performed. AlQ is an aluminum chelate comprised of three molecules of 8-hydroxyquinoline (8OHQ). 8OHQ was also previously reported to produce a positive response in the Ames assay with *S. typhimurium* TA100 [1,2]. Similarities between the TA100 dose–response curves for AlQ and 8OHQ, and the requirement for metabolic activation, suggested the possibility that AlQ may decompose or undergo metabolic transformation to 8OHQ under the conditions of the mutagenicity assay. 8OHQ has the potential to be metabolized via cytochrome P450 to products that possess structural alerts for mutagenicity, such as 8-hydroxyquinoline N-oxide or various 8-hydroxyquinoline epoxides. The mutagenic activity of quinoline, for example, was suggested to be due to the formation of the water-soluble reactive quinoline-2,3-epoxide intermediate [3]. Potentially less toxic phase I metabolites, including the various quinolinediols, may also be formed in addition to various phase II metabolites such as glucuronide and sulfate conjugates. However, 8-hydroxyquinoline sulfate has been shown to induce mutagenicity in Ames bacterial tests and in cultured mammalian cells [4].

The purpose of this study was to compare the mutagenic responses of AlQ and 8OHQ in concurrent mutagenicity assays and to evaluate the stability of AlQ under the conditions of the assay. Concentrations of 8OHQ used in the mutagenicity test were 0.948 times the concentrations of AlQ used, corresponding to the expected stoichiometry of 3 mole of 8OHQ produced for every mole of AlQ degraded. Stability studies were carried out separately from the mutagenicity tests, under conditions designed to closely mimic mutation assay exposure parameters. Incubation samples were prepared by adding the test chemical to mixtures containing the appropriate tester strain, histidine/biotin or tryptophan supplement solution and a metabolic activation system containing liver microsomal enzymes (S9) or an equal volume of a similar mixture with the S9 omitted. Incubation was carried out for 4 h at 37 °C, and samples were collected periodically for analysis by high-performance liquid chromatography (HPLC). Additional analyses by liquid chromatography/mass spectrometry (LC/MS) were performed to identify potential metabolites of AlQ and 8OHQ.

2. Materials and methods

2.1. Chemicals

AlQ (purity 97.7%) was obtained from Eastman Kodak Company (Rochester, NY). 8OHQ (purity 98.0%) and 8-hydroxyquinoline N-oxide (purity 98%) were obtained from Aldrich Chemical Company (Milwaukee, WI). 8-Hydroxyquinoline glucuronide (purity 98.5%) and dimethyl sulfoxide (DMSO, purity 99.9%) were obtained from Sigma Chemical Company (St. Louis, MO). 2,8-Quinolinediol (purity ≥ 98%) was obtained from Fluka Holding AG (Buchs, Switzerland). The sources and grades of positive control materials were as follows: benzo[a]pyrene (CAS #50-32-8), Sigma Chemical Co., purity ≥ 97%; 2-aminoanthracene (CAS #613-13-8), Sigma Chemical Co., purity ≥ 90%; 2–nitrofluorene (CAS #607-57-8), Sigma Chemical Co., purity ≥ 98%; sodium azide (CAS #26628-22-8), Sigma Chemical Co., purity ≥ 99%; ICR-191 (CAS #17070-45-0), Sigma Chemical Co., purity ≥ 90%; 4–nitroquinoline-